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(54) Title: CELL PERMEABLE PEPTIDOMIMETIC MACROCYCLES

(57) Abstract: The present invention provides peptidomimetic macrocycles and methods for selecting peptidomimetic macrocycles and methods of using such peptidomimetic macrocycles for the treatment of disease.



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CELL PERMEABLE PEPTIDOMIMETIC MACROCYCLES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/342,472, filed May 27, 2016, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Peptides are becoming increasingly important in pharmaceutical applications. However, linear and unmodified peptides often suffer from poor metabolic stability, poor membrane permeability, and promiscuous binding due to conformational flexibility. To improve these properties, researchers have generated α -macrocyclic peptides utilizing a variety of crosslinkers, including disulfide (Jackson et al. (1991), J. Am. Chem. Soc. 113:9391-9392), thioether (Brunel et al. (2005), Chem. Commun. (20):2552-2554), diester (Caporale et al. (2010), J. Med. Chem., 53: 8072-8079), lactam (Phelan et al. (1997), J. Am. Chem. Soc. 119:455-460; Taylor (2002), Biopolymers 66: 49-75), triazole (Cantel et al. (2008), J. Org. Chem., 73: 5663), and hydrocarbon (Blackwell et al. (1998), Angew. Chem. Int. Ed. 37:3281-3284; Schafmeister et al. (2000), J. Am. Chem. Soc. 122:5891-5892). An increasing number of studies have reported the identification of hydrocarbon-stapled peptides for intracellular targets that exhibit potent target binding, *in vitro* efficacy and, in some cases, *in vivo* proof-of-concept (Walensky et al. (2004), Science, 305: 1466-1470; Takada et al. (2012), Sci Transl Med, 4 (148): 148ra117; Hao et al., Cancer Cell (2013), 23 (5): 583-593; Chang et al. (2013), Proc. Natl. Acad. Sci. USA 110(36):E3445-3454).

[0003] Positive charge and hydrophobicity have been described characteristic features of most cell permeable peptides including the hydrocarbon stapled peptides. However, while amphipathic α -helical peptides carrying positive charge may have enhanced cell penetration, such peptides also may cause cell lysis (i.e., loss of plasma membrane integrity due to irreversible membrane pore formation). Cell lysis by amphipathic α -helical peptides carrying positive charges may be attributed to the nonspecific cell membrane insertion activity of the peptides (Li et al. (2009), 48: 8712; Muppidi et al. (2011), Bioorg. Med. Chem. Lett. 21:7412-7415). Thus, there is a significant need for improved method to produce peptides or peptidomimetics that have good cell permeability but do not cause membranolysis. The present invention addresses this and other needs in the art.

SUMMARY OF THE INVENTION

[0004] The present invention provides biologically active peptidomimetic macrocycles. The present invention also provides methods for selecting biologically active peptidomimetic macrocycles. The present invention also provides methods for treating or controlling a disease or condition by

administering biologically active peptidomimetic macrocycles. In some embodiments, the biologically active peptidomimetic macrocycle is an biologically active peptidomimetic macrocycle with enhanced cell permeability.

[0005] In one aspect a method is provided for selecting a peptidomimetic macrocycle (*e.g.*, a peptidomimetic macrocycle with enhanced cell permeability), the method comprising: a) synthesizing a peptidomimetic macrocycle comprising a polypeptide with a crosslinker connecting a first amino acid and a second amino acid; b) determining if the peptidomimetic macrocycle fulfills two or more conditions (*e.g.*, 3, 4, 5, 6, 7, 8, 9, or 10) selected from a group consisting of: a length of the peptidomimetic macrocycle ranges from 10-24 amino acids, a molecular weight of the peptidomimetic macrocycle ranges from about 500 to about 1500 Da, a percent polar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent nonpolar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent hydrophobic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent β -branched amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent aromatic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a von Heijne value of the peptidomimetic macrocycle ranges from 2 to 9, a net charge carried by the peptidomimetic macrocycle ranges from -4 to +2, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the C-terminus of the peptidomimetic macrocycle ranges from -4 to +2, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the N-terminus of the peptidomimetic macrocycle ranges from -4 to +2, a ratio of charged amino acids over uncharged amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10, a positively charged amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a negatively charged amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a charged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, an uncharged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10, a tyrosine content of the peptidomimetic macrocycle ranges from 5% to 50%, an alanine content of the peptidomimetic macrocycle ranges from 5% to 50%, a tryptophan content of the peptidomimetic macrocycle ranges from 5% to 50%, a non-natural amino acid content of the peptidomimetic macrocycle ranges from 5% to 50%, a number of halo-substituents of the peptidomimetic macrocycle ranges from 1 to 20, a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine of the peptidomimetic macrocycle ranges from 1 to 20, a ratio of fluorine atoms to chlorine atoms of the peptidomimetic

macrocycle ranges from 0.1 to 10, a presence of a carboxylic acid with a substituent in the capping groups of the peptidomimetic macrocycle, a presence of an amine with a substituent in the capping groups of the peptidomimetic macrocycle, and a partition coefficient ($\log P_{\text{octanol/water}}$ at 37 °C) of the peptidomimetic macrocycle ranges from -5.0 to 5.0; and c) selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle (*e.g.*, a peptidomimetic macrocycle with enhanced cell permeability) if the peptidomimetic macrocycle fulfills two or more conditions in (b).

[0006] In some embodiments, the synthesizing comprises synthesizing at least 2 peptidomimetic macrocycles. In some embodiments, the synthesizing comprises synthesizing at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, or at least 1000 peptidomimetic macrocycles.

[0007] In some embodiments, the method further comprises determining an aqueous solubility of the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the aqueous solubility indicates a lack of precipitated polypeptide.

[0008] In some embodiments, the aqueous solubility of the peptidomimetic macrocycle is determined by evaluating the turbidity of a solution comprising the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the turbidity of the aqueous solubility indicates a lack of precipitated polypeptide.

[0009] In some embodiments, the method further comprises calculating an amphipathicity value of the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the amphipathicity of the peptidomimetic macrocycle falls in a range that is optimal for cell permeability.

[0010] In some embodiments, the selected peptidomimetic macrocycle is soluble, does not have off-target effects, or a combination thereof.

[0011] In some embodiments, the selected peptidomimetic macrocycle comprises a first C-terminal amino acid that is hydrophobic. In some embodiments, the selected peptidomimetic macrocycle comprises a second C-terminal amino acid that is hydrophobic. In some embodiments, the selected peptidomimetic macrocycle comprises a third C-terminal amino acid that is hydrophobic. In some embodiments, the selected peptidomimetic macrocycle comprises a fourth C-terminal amino acid that is hydrophobic. In some embodiments, the selected peptidomimetic macrocycle comprises a fifth C-terminal amino acid that is hydrophobic. In some embodiments, the selected peptidomimetic

macrocycle comprises a sixth C-terminal amino acid that is hydrophobic. In some embodiments, the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, 3, 4, 5, 6, 7, or 8 amino acids that are C-terminal to the second amino acid connected to the crosslinker. In some embodiments, the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, 3, 4, 5, or 6 hydrophobic amino acids that are C-terminal to the second amino acid connected to the crosslinker. In some embodiments, the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, or 3 glutamines that are C-terminal to the second amino acid connected to the crosslinker. In some embodiments, the amino acid that is hydrophobic is a small hydrophobic amino acid. In some embodiments, the amino acid that is hydrophobic is an alanine, a D-alanine, or an Aib.

[0012] In some embodiments, the selected peptidomimetic macrocycle is a helical polypeptide. In some embodiments, the selected peptidomimetic macrocycle comprises an α -helix. In some embodiments, the selected peptidomimetic macrocycle comprises an amphipathic α -helix.

[0013] In some embodiments, the first amino acid connected to the crosslinker or the second amino acid connected to the crosslinker is an α,α -disubstituted amino acid. In some embodiments, the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are α,α -disubstituted amino acids.

[0014] In some embodiments, the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by two amino acids. In some embodiments, the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by three amino acids. In some embodiments, the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by six amino acids.

[0015] In some embodiments, the crosslinker spans 1 turn of an α -helix of the peptidomimetic macrocycle. In some embodiments, the crosslinker spans 2 turns of an α -helix of the peptidomimetic macrocycle. In some embodiments, the length of the crosslinker is from about 5 Å to about 9 Å per turn of an α -helix of the peptidomimetic macrocycle.

[0016] In some embodiments, the selected peptidomimetic macrocycle provides a therapeutic effect.

[0017] In some embodiments, an ability of the selected peptidomimetic macrocycle to penetrate cell membranes by an energy-dependent process is improved relative to a corresponding uncrosslinked peptidomimetic macrocycle. In some embodiments, an ability of the selected peptidomimetic macrocycle to penetrate cell membranes by an energy-independent process is improved relative to a

corresponding uncrosslinked peptidomimetic macrocycle. In some embodiments, the energy-dependent process is primary active transport, secondary transport, endocytosis, or a combination thereof. In some embodiments, the energy-dependent process is active transport. In some embodiments, the energy-independent process is passive diffusion, passive transport, facilitated diffusion, filtration, or a combination thereof. In some embodiments, the energy-independent process is passive transport.

[0018] In some embodiments, the selected peptidomimetic macrocycle binds to MDM2 with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to MDM2 or MDMX with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to MDMX with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to a PB1 peptide binding site of a PA protein with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to a PB2 peptide binding site of a PB1 protein with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to viral polymerase, for example, a RNA-dependent RNA polymerase with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle inhibits an influenza RNA-dependent RNA polymerase. In some embodiments, the virus is influenza virus. In some embodiments, the peptidomimetic macrocycle is capable of competing with the binding of a peptide of the sequence MDVNPTLLFLKVPAQ or MERIKELRNLM to the viral RNA-dependent RNA polymerase. In some embodiments, the selected peptidomimetic macrocycle binds to MCL-1, BCL-X_L, BCL-2, or a combination thereof with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to MCL-1 with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to BCL-X_L with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle binds to BCL-2 with a K_D value of 1×10^{-7} M or less. In some embodiments, the selected peptidomimetic macrocycle has an IC₅₀ value of 100 nM or less to a target protein. In some embodiments, the selected peptidomimetic macrocycle has an EC₅₀ value of 100 μM or less. In some embodiments, the selected peptidomimetic macrocycle has an IC₅₀ value of 10 nM or less to a target protein. In some embodiments, the selected peptidomimetic macrocycle has an EC₅₀ value of 10 μM or less. In some embodiments, the selected peptidomimetic macrocycle has an IC₅₀ value of 1 nM or less to a target protein. In some embodiments, the selected peptidomimetic macrocycle has an EC₅₀ value of 1 μM or less. In some embodiments, the selected peptidomimetic macrocycle has a penetration efficiency value of 100 or less. In some embodiments, the selected peptidomimetic macrocycle has a penetration efficiency

value of 10 or less. In some embodiments, the selected peptidomimetic macrocycle has a penetration efficiency value of 1 or less.

[0019] In one aspect, provided herein is a peptidomimetic macrocycle selected according to a method described herein, wherein the peptidomimetic macrocycle penetrates cell membranes by an energy-dependent process and binds to a target protein with a K_D value of 1×10^{-7} M or less, wherein the intracellular target is not MDM2 or MDMX. In some embodiments, the energy-dependent process comprises primary active transport, secondary transport, or endocytosis. In some embodiments, the energy-dependent process comprises active transport.

[0020] In one aspect, provided herein is a peptidomimetic macrocycle selected according to a method described herein, wherein the peptidomimetic macrocycle penetrates cell membranes by an energy-independent process and binds to a target protein with a K_D value of 1×10^{-7} M or less, wherein the target protein is not MDM2 or MDMX. In some embodiments, the energy-independent process comprises passive diffusion, passive transport, facilitated diffusion, or filtration. In some embodiments, the energy-independent process comprises passive transport.

[0021] In one aspect, provided herein is a method of treating or controlling a disease or condition, comprising administering an effective amount of a peptidomimetic macrocycle selected according to a method described herein, to a subject in need thereof.

[0022] In some embodiments, the method comprises: a) synthesizing a peptidomimetic macrocycle comprising a polypeptide with a crosslinker connecting a first amino acid and a second amino acid; b) determining if the peptidomimetic macrocycle fulfills two or more conditions (*e.g.*, 3, 4, 5, 6, 7, 8, 9, or 10) selected from a group consisting of: a length of the peptidomimetic macrocycle ranges from 10-24 amino acids, a molecular weight of the peptidomimetic macrocycle ranges from about 500 to about 1500 Da, a percent polar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent nonpolar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent hydrophobic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent β -branched amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a percent aromatic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a von Heijne value of the peptidomimetic macrocycle ranges from 2 to 9, a net charge carried by the peptidomimetic macrocycle ranges from -4 to +2, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the C-terminus of the peptidomimetic macrocycle ranges from -4 to +2, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the N-terminus of the peptidomimetic macrocycle ranges from -4 to +2, a ratio of charged amino acids over uncharged amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10, a positively charged amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a negatively charged

amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a charged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, an uncharged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%, a ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10, a tyrosine content of the peptidomimetic macrocycle ranges from 5% to 50%, an alanine content of the peptidomimetic macrocycle ranges from 5% to 50%, a tryptophan content of the peptidomimetic macrocycle ranges from 5% to 50%, a non-natural amino acid content of the peptidomimetic macrocycle ranges from 5% to 50%, a number of halo-substituents of the peptidomimetic macrocycle ranges from 1 to 20, a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine of the peptidomimetic macrocycle ranges from 1 to 20, a ratio of fluorine atoms to chlorine atoms of the peptidomimetic macrocycle ranges from 0.1 to 10, a presence of a carboxylic acid with a substituent in the capping groups of the peptidomimetic macrocycle, a presence of an amine with a substituent in the capping groups of the peptidomimetic macrocycle, and a partition coefficient ($\log P_{\text{octanol/water}}$ at 37 °C) of the peptidomimetic macrocycle ranges from -5.0 to 5.0; c) selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle (*e.g.*, a peptidomimetic macrocycle with enhanced cell permeability) if the peptidomimetic macrocycle fulfills two or more conditions in (b); and d) administering an effective amount of the enhanced peptidomimetic macrocycle selected in c), to a subject in need thereof.

[0023] In one aspect, provided herein is a peptidomimetic macrocycle selected according to a method described herein, wherein the peptidomimetic macrocycle comprises two non-natural amino acids connected by a macrocycle-forming crosslinker and an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence of Table 3a or 3b.

[0024] In one aspect, provided herein is a peptidomimetic macrocycle selected according to a method described herein, wherein the peptidomimetic macrocycle comprises two non-natural amino acids connected by a macrocycle-forming crosslinker and an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence of Table 4a or 4b.

[0025] In some embodiments, the target protein is an intracellular protein. In some embodiments, the target protein is a viral polymerase RNA-dependent RNA polymerase. In some embodiments, the target protein is MCL-1, BCL-X_L, BCL-2, or a combination thereof.

INCORPORATION BY REFERENCE

[0026] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0028] **Figure 1** shows (top) amino acids 1 (14) to 16 (29) of a stapled (i.e. crosslinked) wild type (WT) p53 (SP-153) in a helix wheel; and (bottom) amino acids 1 to 14 of a stapled peptide of a p53 mimetic (SP-18) in a helix wheel. The sequences of SP-153 and SP-18 are shown in **Table 1a**.

[0029] **Figure 2** shows a plot of penetration efficiency vs reverse-phase HPLC retention time (RT) for cross-linked polypeptides derived from the MDM2/X-binding helix of p53. Cell permeability is calculated as $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$, and enhanced penetration efficiency corresponds to a lower value for $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$.

[0030] **Figure 3** shows a plot of viable SJSA-1 cells (%) vs. log concentration (μM) of indicated peptide after incubation of the cells with the peptide for 72 hr in 10% serum.

[0031] **Figure 4** shows (top) amino acids 1 to 21 of a stapled peptide of a BIM mimetic (SP-203) derived from amino acids 146 to 166 of a stapled wild type (WT) BIM peptide (SP-202) in a helix wheel; and (bottom) amino acids 1 to 19 of a stapled peptide of a BIM mimetic (SP-208) derived from amino acids 146 to 164 of a stapled wild type (WT) BIM peptide (SP-202) in a helix wheel. The sequences of SP-203 and SP-208 are shown in **Table 4a**.

[0032] **Figure 5** shows a plot of penetration efficiency vs reverse-phase HPLC retention time (RT) for cross-linked polypeptides derived from the MCL-1/ BCL-X_L/BCL-2-binding helix of BIM. Penetration efficiency is calculated as $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$, and enhanced penetration efficiency corresponds to a lower value for $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$.

[0033] **Figure 6** shows a plot of penetration efficiency vs von Heijne (VH) value for cross-linked polypeptides derived from the MCL-1/ BCL-X_L/BCL-2-binding helix of BIM. penetration efficiency is calculated as $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$, and enhanced penetration efficiency corresponds to a lower value for $1000 \cdot [\text{EC}_{50} (\mu\text{M}) / \text{IC}_{50} (\text{nM})]$.

[0034] **Figure 7** shows a plot of viable Raji cells (%) vs. log concentration (μM) of the indicated peptides after incubation of the cells with the peptide for 48 hr in 5% serum.

[0035] **Figure 8** shows (top) amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-180) derived from amino acids 1 to 15 of a wild type (WT) PB1 sequence of an influenza virus (MDVNPTLLFLKVPAQ) in a helix wheel; and (bottom) amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-188) derived from amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-180) in a helix wheel. The sequences of SP-180 and SP-188 are shown in **Table 3a**.

[0036] **Figure 9** shows (top) amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-180) derived from amino acids 1 to 15 of a wild type (WT) PB1 sequence of an influenza virus (MDVNPTLLFLKVPAQ) in a helix wheel; and (bottom) amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-192) derived from amino acids 1 to 15 of a stapled peptide of a PB1 mimetic (SP-180) in a helix wheel. The sequences of SP-180 and SP-192 are shown in **Table 3a**.

DETAILED DESCRIPTION OF THE INVENTION

[0037] As used herein, the term “macrocycle” refers to a molecule having a chemical structure including a ring or cycle formed by at least 9 covalently bonded atoms.

[0038] As used herein, the term “peptidomimetic macrocycle” or “crosslinked polypeptide” refers to a compound comprising a plurality of amino acid residues joined by a plurality of peptide bonds and at least one macrocycle-forming linker which forms a macrocycle between a first naturally-occurring or non-naturally-occurring amino acid residue (or analog) and a second naturally-occurring or non-naturally-occurring amino acid residue (or analog) within the same molecule, and these terms include pharmaceutically acceptable salts of such compounds. Peptidomimetic macrocycle include embodiments where the macrocycle-forming linker connects the α -carbon of the first amino acid residue (or analog) to the α -carbon of the second amino acid residue (or analog). The peptidomimetic macrocycles optionally include one or more non-peptide bonds between one or more amino acid residues and/or amino acid analog residues, and optionally include one or more non-naturally-occurring amino acid residues or amino acid analog residues in addition to any which form the macrocycle. A “corresponding uncrosslinked polypeptide” when referred to in the context of a peptidomimetic macrocycle is understood to relate to a polypeptide of the same length as the macrocycle and comprising the equivalent natural amino acids of the wild-type sequence corresponding to the macrocycle.

[0039] As used herein, the term “stability” refers to the maintenance of a defined secondary structure in solution by a peptidomimetic macrocycle as measured by circular dichroism, NMR or another biophysical measure, or resistance to proteolytic degradation *in vitro* or *in vivo*. Non-limiting examples of secondary structures contemplated in this invention are α -helices, 3_{10} helices, β -turns, and β -pleated sheets.

[0040] As used herein, the term “helical stability” refers to the maintenance of α -helical structure by a peptidomimetic macrocycle as measured by circular dichroism or NMR. For example, in some embodiments, a peptidomimetic macrocycle exhibits at least a 1.25, 1.5, 1.75 or 2-fold increase in α -helicity as determined by circular dichroism compared to a corresponding uncrosslinked macrocycle.

[0041] The terms “cell permeability” and “cell penetrability” and “penetration efficiency” are used interchangeably to refer to the ability of a molecule (*e.g.*, a peptidomimetic macrocycle or peptide) to cross a cell membrane regardless of mechanism, whether by an energy-dependent process or an energy-independent process. Energy dependent processes include endocytosis, which is a process where eukaryotic cells internalize segments of plasma membrane, cell-surface receptors and other components from the extracellular fluid. Endocytosis mechanisms may include but are not limited to receptor mediated endocytosis, pinocytosis and phagocytosis. Energy independent processes include diffusion or osmosis. Penetration efficiency can be calculated as $1000 * [EC_{50} (\mu M) / IC_{50} (nM)]$, and enhanced penetration efficiency corresponds to a lower value for $1000 * [EC_{50} (\mu M) / IC_{50} (nM)]$.

[0042] The term “amino acid” refers to a molecule containing both an amino group and a carboxyl group. Suitable amino acids include, without limitation, both the D-and L-isomers of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. The term amino acid, as used herein, includes, without limitation, α -amino acids, natural amino acids, non-natural amino acids, and amino acid analogs.

[0043] The term “ α -amino acid” refers to a molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the α -carbon.

[0044] The term “ β -amino acid” refers to a molecule containing both an amino group and a carboxyl group in a β configuration. The abbreviation “b-” prior to an amino acid represents a β -configuration for the amino acid.

[0045] The term “naturally occurring amino acid” refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

[0046] The following table shows a summary of the properties of natural amino acids:

Amino Acid	3-Letter Code	1-Letter Code	Side-chain Polarity	Side-chain charge (pH 7.4)	Hydropathy Index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	-4.5
Asparagine	Asn	N	polar	neutral	-3.5

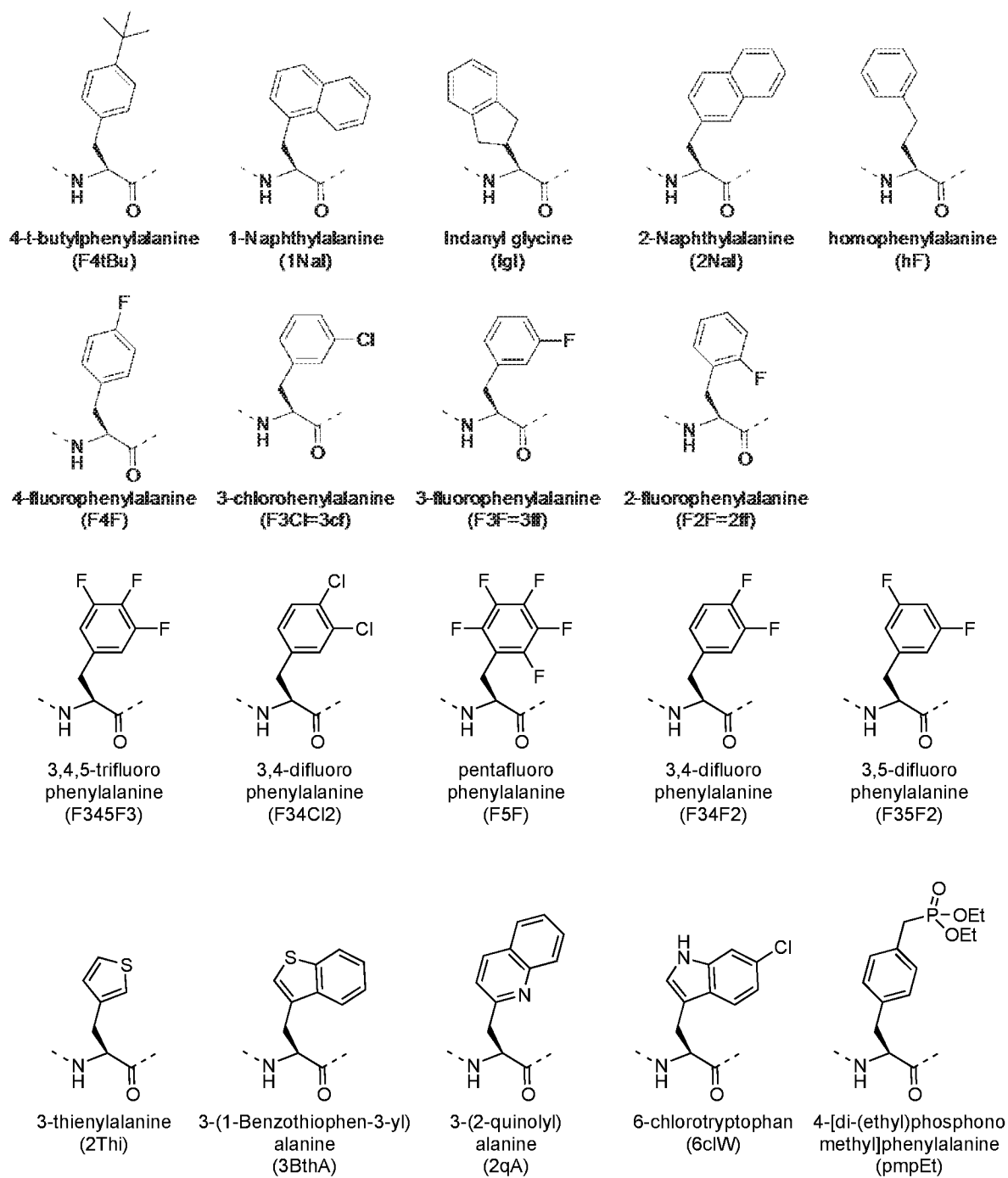
Aspartic acid	Asp	D	polar	negative	-3.5
Cysteine	Cys	C	polar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	-3.5
Glutamine	Gln	Q	polar	neutral	-3.5
Glycine	Gly	G	nonpolar	neutral	-0.4
Histidine	His	H	polar	positive(10%) neutral(90%)	-3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	-3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	-1.6
Serine	Ser	S	polar	neutral	-0.8
Threonine	Thr	T	polar	neutral	-0.7
Tryptophan	Trp	W	nonpolar	neutral	-0.9
Tyrosine	Tyr	Y	polar	neutral	-1.3
Valine	Val	V	nonpolar	neutral	4.2

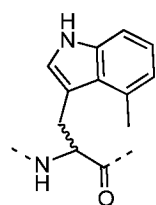
[0047] “Hydrophobic amino acids” include small hydrophobic amino acids and large hydrophobic amino acids. “Small hydrophobic amino acids” are glycine, alanine, proline, and analogs thereof. “Large hydrophobic amino acids” are valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, tyrosine, and analogs thereof. “Polar amino acids” are serine, threonine, asparagine, glutamine, cysteine, tyrosine, and analogs thereof. “Charged amino acids” include positively charged amino acids and negatively charged amino acids. “Positively charged amino acids” include lysine, arginine, histidine, and analogs thereof. “Negatively charged amino acids” include aspartate, glutamate, and analogs thereof. “Side chain hydrogen bonding amino acids” include charged side chain hydrogen bonding amino acids and uncharged side chain hydrogen bonding amino acids. “Charged side chain hydrogen bonding amino acids” include lysine, arginine, histidine, aspartate, glutamate, and analogs thereof. “Uncharged side chain hydrogen bonding amino acids” include asparagine, cysteine, glutamine, methionine, serine, threonine, tryptophan, tyrosine, and analogs thereof.

[0048] The term “amino acid analog” refers to a molecule which is structurally similar to an amino acid and which can be substituted for an amino acid in the formation of a peptidomimetic macrocycle. Amino acid analogs include, without limitation, β -amino acids and amino acids where the amino or carboxy group is substituted by a similarly reactive group (e.g., substitution of the primary amine with a secondary or tertiary amine, or substitution of the carboxy group with an ester).

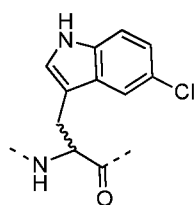
[0049] The term “non-natural amino acid” refers to an amino acid which is not one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter

abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V. Non-natural amino acids or amino acid analogs include, without limitation, structures according to the following:

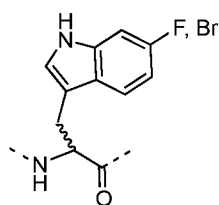




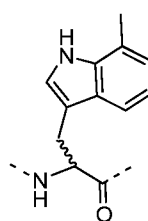
D- or L- 4-methyl
tryptophan
(dl4mW)



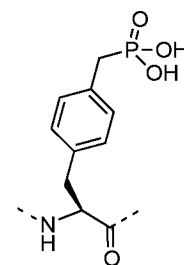
D- or L- 5-chloro
tryptophan
(dl5cIW)



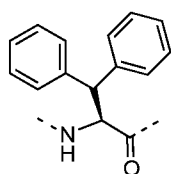
D/L-6-fluorotryptophan &
D/L-6-bromotryptophan
(ddl6fW & dl6brW)



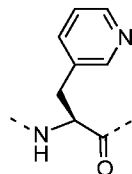
D/L-7-methyl
tryptophan
(dl7mW)



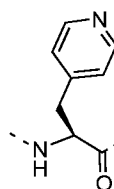
4-(phosphonomethyl)
phenylalanine
(Pmp)



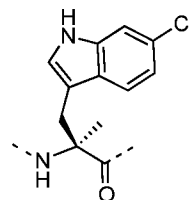
3,3-diphenyl-alanine
(Dip)



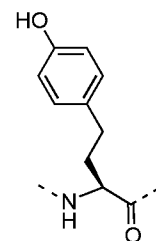
3-pyridyl-alanine
(3PaI)



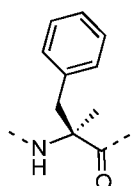
4-pyridyl-alanine
(4PaI)



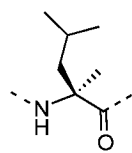
α-methyl-6-chloro
tryptophan
(Me6cIW)



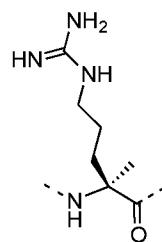
homotyrosine
(hY)



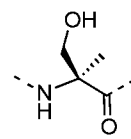
α-methyl-phenylalanine
(Amf)



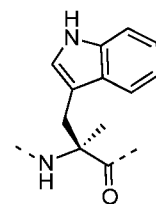
α-methyl-leucine
(Aml)



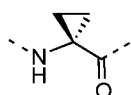
α-methyl-arginine
(Amr)



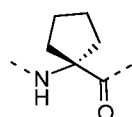
α-methyl-serine
(Ams)



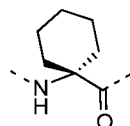
α-methyl-tryptophan
(Amw)



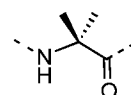
1-amino-cyclopropane-
1-carboxylic acid
(Ac3c)



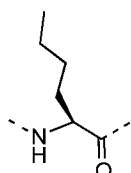
1-amino-cyclopentane-
1-carboxylic acid
(Ac5c)



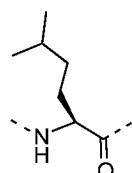
1-amino-cyclohexane-
1-carboxylic acid
(Ac6c)



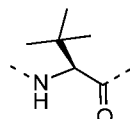
α-amino
isobutyric acid
(Aib)



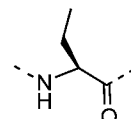
Norleucine
(Nle)



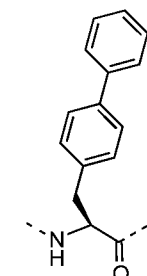
Homoleucine
(hL)



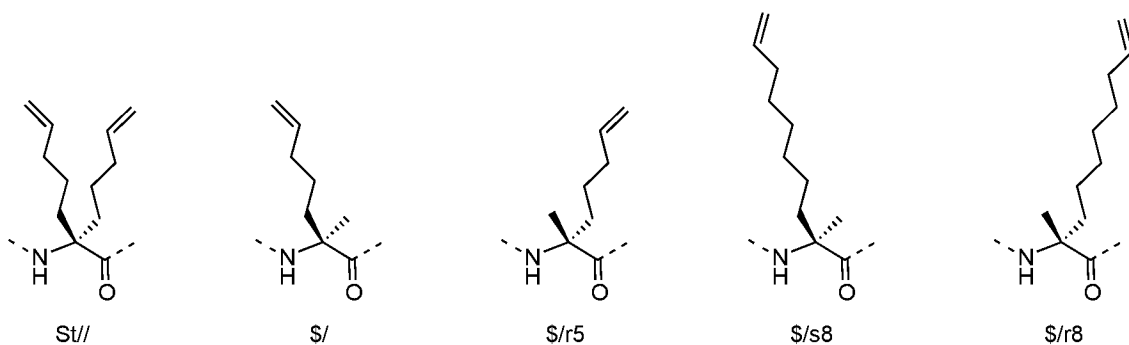
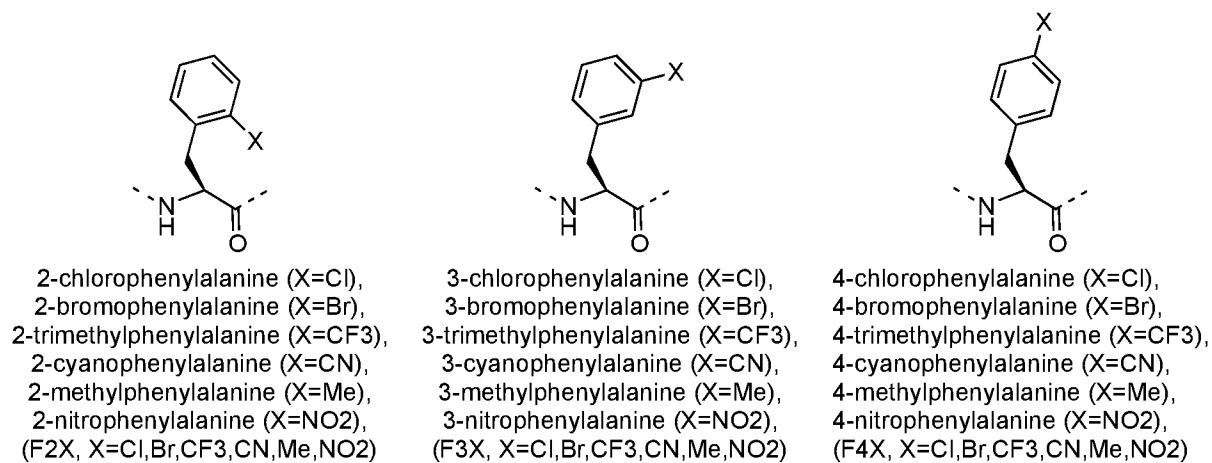
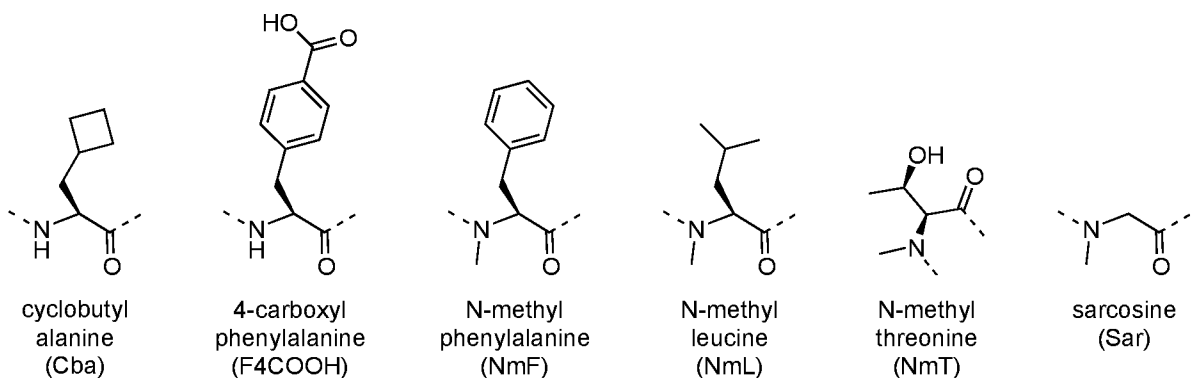
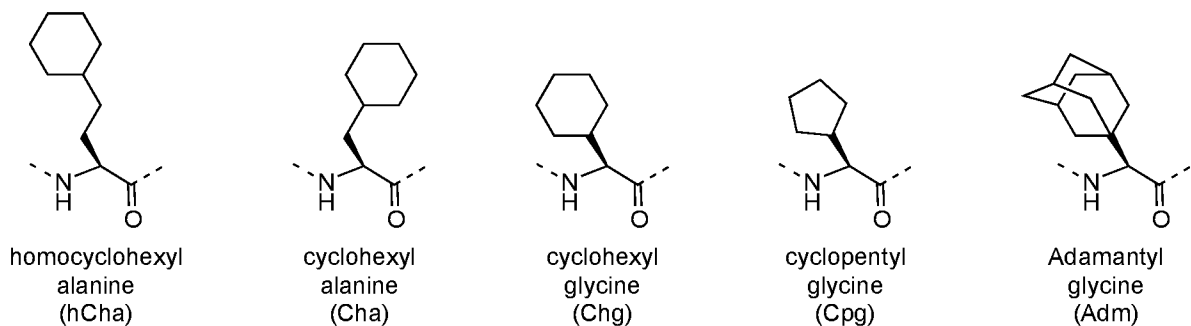
tert-Butyl glycine
(Tle)

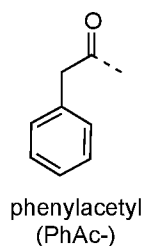
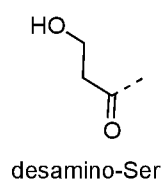
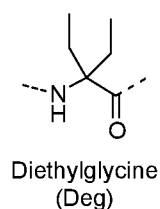
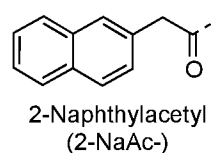
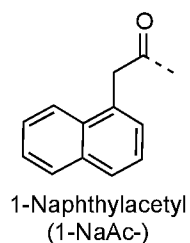
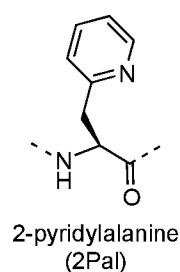
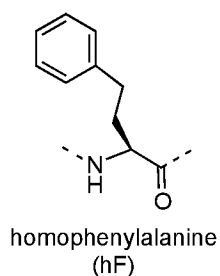
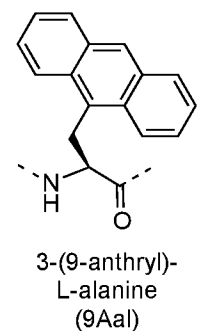
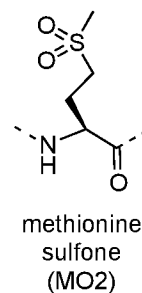
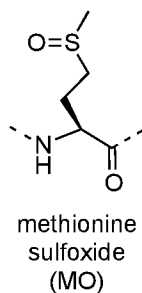
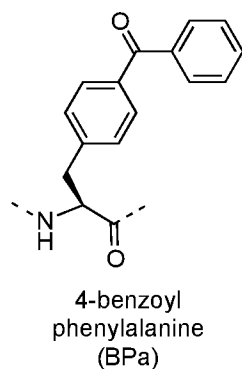
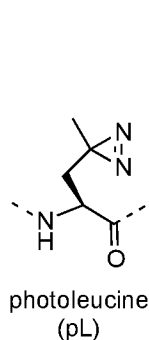
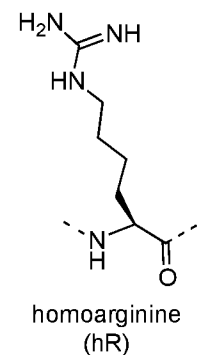
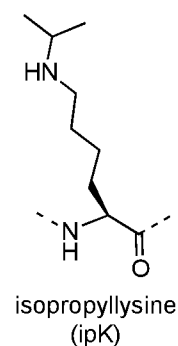
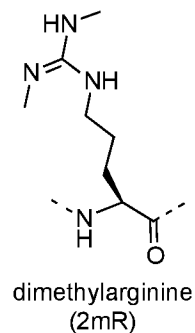
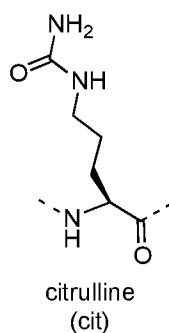
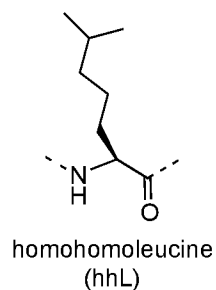
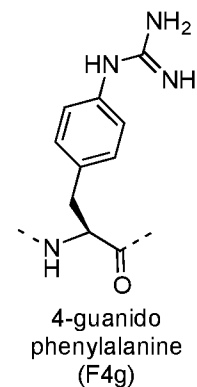
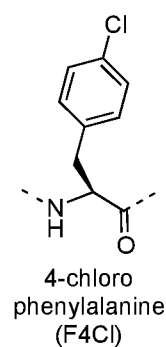
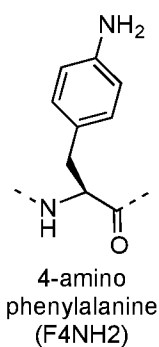
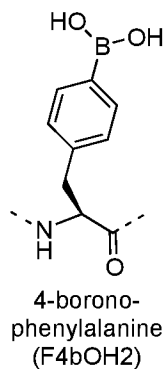
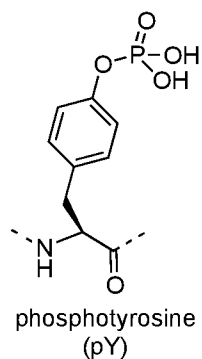


aminobutyric acid
(Abu)



biphenylalanine
(Bip)





[0050] Amino acid analogs include β -amino acid analogs. Examples of β -amino acid analogs include, but are not limited to, the following: cyclic β -amino acid analogs; β -alanine; (R)- β -phenylalanine; (R)-1,2,3,4-tetrahydro-isoquinoline-3-acetic acid; (R)-3-amino-4-(1-naphthyl)-butyric acid; (R)-3-amino-4-(2,4-dichlorophenyl)butyric acid; (R)-3-amino-4-(2-chlorophenyl)-butyric acid; (R)-3-amino-4-(2-cyanophenyl)-butyric acid; (R)-3-amino-4-(2-fluorophenyl)-butyric acid; (R)-3-amino-4-(2-furyl)-butyric acid; (R)-3-amino-4-(2-methylphenyl)-butyric acid; (R)-3-amino-4-(2-naphthyl)-butyric acid; (R)-3-amino-4-(2-thienyl)-butyric acid; (R)-3-amino-4-(2-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-(3,4-dichlorophenyl)butyric acid; (R)-3-amino-4-(3,4-difluorophenyl)butyric acid; (R)-3-amino-4-(3-benzothienyl)-butyric acid; (R)-3-amino-4-(3-chlorophenyl)-butyric acid; (R)-3-amino-4-(3-cyanophenyl)-butyric acid; (R)-3-amino-4-(3-fluorophenyl)-butyric acid; (R)-3-amino-4-(3-methylphenyl)-butyric acid; (R)-3-amino-4-(3-pyridyl)-butyric acid; (R)-3-amino-4-(3-thienyl)-butyric acid; (R)-3-amino-4-(3-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-(4-bromophenyl)-butyric acid; (R)-3-amino-4-(4-chlorophenyl)-butyric acid; (R)-3-amino-4-(4-cyanophenyl)-butyric acid; (R)-3-amino-4-(4-fluorophenyl)-butyric acid; (R)-3-amino-4-(4-iodophenyl)-butyric acid; (R)-3-amino-4-(4-methylphenyl)-butyric acid; (R)-3-amino-4-(4-nitrophenyl)-butyric acid; (R)-3-amino-4-(4-pyridyl)-butyric acid; (R)-3-amino-4-(4-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-pentafluorophenylbutyric acid; (R)-3-amino-5-hexenoic acid; (R)-3-amino-5-hexynoic acid; (R)-3-amino-5-phenylpentanoic acid; (R)-3-amino-6-phenyl-5-hexenoic acid; (S)-1,2,3,4-tetrahydro-isoquinoline-3-acetic acid; (S)-3-amino-4-(1-naphthyl)-butyric acid; (S)-3-amino-4-(2,4-dichlorophenyl)butyric acid; (S)-3-amino-4-(2-chlorophenyl)-butyric acid; (S)-3-amino-4-(2-cyanophenyl)-butyric acid; (S)-3-amino-4-(2-fluorophenyl)-butyric acid; (S)-3-amino-4-(2-furyl)-butyric acid; (S)-3-amino-4-(2-methylphenyl)-butyric acid; (S)-3-amino-4-(2-naphthyl)-butyric acid; (S)-3-amino-4-(2-thienyl)-butyric acid; (S)-3-amino-4-(2-trifluoromethylphenyl)-butyric acid; (S)-3-amino-4-(3,4-dichlorophenyl)butyric acid; (S)-3-amino-4-(3,4-difluorophenyl)butyric acid; (S)-3-amino-4-(3-benzothienyl)-butyric acid; (S)-3-amino-4-(3-chlorophenyl)-butyric acid; (S)-3-amino-4-(3-cyanophenyl)-butyric acid; (S)-3-amino-4-(3-fluorophenyl)-butyric acid; (S)-3-amino-4-(3-methylphenyl)-butyric acid; (S)-3-amino-4-(3-pyridyl)-butyric acid; (S)-3-amino-4-(3-thienyl)-butyric acid; (S)-3-amino-4-(3-trifluoromethylphenyl)-butyric acid; (S)-3-amino-4-(4-bromophenyl)-butyric acid; (S)-3-amino-4-(4-chlorophenyl)-butyric acid; (S)-3-amino-4-(4-cyanophenyl)-butyric acid; (S)-3-amino-4-(4-fluorophenyl)-butyric acid; (S)-3-amino-4-(4-iodophenyl)-butyric acid; (S)-3-amino-4-(4-methylphenyl)-butyric acid; (S)-3-amino-4-(4-nitrophenyl)-butyric acid; (S)-3-amino-4-(4-pyridyl)-butyric acid; (S)-3-amino-4-(4-trifluoromethylphenyl)-butyric acid; (S)-3-amino-4-pentafluorophenylbutyric acid; (S)-3-amino-5-hexenoic acid; (S)-3-amino-5-hexynoic acid; (S)-3-

amino-5-phenylpentanoic acid; (S)-3-amino-6-phenyl-5-hexenoic acid; 1,2,5,6-tetrahydropyridine-3-carboxylic acid; 1,2,5,6-tetrahydropyridine-4-carboxylic acid; 3-amino-3-(2-chlorophenyl)-propionic acid; 3-amino-3-(2-thienyl)-propionic acid; 3-amino-3-(3-bromophenyl)-propionic acid; 3-amino-3-(4-chlorophenyl)-propionic acid; 3-amino-3-(4-methoxyphenyl)-propionic acid; 3-amino-4,4,4-trifluoro-butyric acid; 3-aminoadipic acid; D- β -phenylalanine; β -leucine; L- β -homoalanine; L- β -homoaspartic acid γ -benzyl ester; L- β -homoglutamic acid δ -benzyl ester; L- β -homoisoleucine; L- β -homoleucine; L- β -homomethionine; L- β -homophenylalanine; L- β -homoproline; L- β -homotryptophan; L- β -homovaline; L-N ω -benzyloxycarbonyl- β -homolysine; N ω -L- β -homoarginine; O-benzyl-L- β -homohydroxyproline; O-benzyl-L- β -homoserine; O-benzyl-L- β -homothreonine; O-benzyl-L- β -homotyrosine; γ -trityl-L- β -homoasparagine; (R)- β -phenylalanine; L- β -homoaspartic acid γ -t-butyl ester; L- β -homoglutamic acid δ -t-butyl ester; L-N ω - β -homolysine; N δ -trityl-L- β -homoglutamine; N ω -2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl-L- β -homoarginine; O-t-butyl-L- β -homohydroxy-proline; O-t-butyl-L- β -homoserine; O-t-butyl-L- β -homothreonine; O-t-butyl-L- β -homotyrosine; 2-aminocyclopentane carboxylic acid; and 2-aminocyclohexane carboxylic acid.

[0051] Amino acid analogs include analogs of alanine, valine, glycine or leucine. Examples of amino acid analogs of alanine, valine, glycine, and leucine include, but are not limited to, the following: α -methoxyglycine; α -allyl-L-alanine; α -aminoisobutyric acid; α -methyl-leucine; β -(1-naphthyl)-D-alanine; β -(1-naphthyl)-L-alanine; β -(2-naphthyl)-D-alanine; β -(2-naphthyl)-L-alanine; β -(2-pyridyl)-D-alanine; β -(2-pyridyl)-L-alanine; β -(2-thienyl)-D-alanine; β -(2-thienyl)-L-alanine; β -(3-benzothienyl)-D-alanine; β -(3-benzothienyl)-L-alanine; β -(3-pyridyl)-D-alanine; β -(3-pyridyl)-L-alanine; β -(4-pyridyl)-D-alanine; β -(4-pyridyl)-L-alanine; β -chloro-L-alanine; β -cyano-L-alanine; β -cyclohexyl-D-alanine; β -cyclohexyl-L-alanine; β -cyclopenten-1-yl-alanine; β -cyclopentyl-alanine; β -cyclopropyl-L-Ala-OH • dicyclohexylammonium salt; β -t-butyl-D-alanine; β -t-butyl-L-alanine; γ -aminobutyric acid; L- α,β -diaminopropionic acid; 2,4-dinitro-phenylglycine; 2,5-dihydro-D-phenylglycine; 2-amino-4,4,4-trifluorobutyric acid; 2-fluoro-phenylglycine; 3-amino-4,4,4-trifluorobutyric acid; 3-fluoro-valine; 4,4,4-trifluoro-valine; 4,5-dehydro-L-leu-OH • dicyclohexylammonium salt; 4-fluoro-D-phenylglycine; 4-fluoro-L-phenylglycine; 4-hydroxy-D-phenylglycine; 5,5,5-trifluoro-leucine; 6-aminohexanoic acid; cyclopentyl-D-Gly-OH • dicyclohexylammonium salt; cyclopentyl-Gly-OH • dicyclohexylammonium salt; D- α,β -diaminopropionic acid; D- α -aminobutyric acid; D- α -t-butylglycine; D-(2-thienyl)glycine; D-(3-thienyl)glycine; D-2-aminocaproic acid; D-2-indanylglycine; D-allylglycine•dicyclohexylammonium salt; D-cyclohexylglycine; D-norvaline; D-phenylglycine; β -aminobutyric acid; β -aminoisobutyric acid; (2-bromophenyl)glycine; (2-methoxyphenyl)glycine; (2-methylphenyl)glycine; (2-thiazoyl)glycine; (2-thienyl)glycine; 2-amino-

3-(dimethylamino)-propionic acid; L- α,β -diaminopropionic acid; L- α -aminobutyric acid; L- α -t-butylglycine; L-(3-thienyl)glycine; L-2-amino-3-(dimethylamino)-propionic acid; L-2-aminocaproic acid dicyclohexyl-ammonium salt; L-2-indanylglycine; L-allylglycine•dicyclohexyl ammonium salt; L-cyclohexylglycine; L-phenylglycine; L-propargylglycine; L-norvaline; N- α -aminomethyl-L-alanine; D- α,γ -diaminobutyric acid; L- α,γ -diaminobutyric acid; β -cyclopropyl-L-alanine; (N- β -(2,4-dinitrophenyl))-L- α,β -diaminopropionic acid; (N- β -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-D- α,β -diaminopropionic acid; (N- β -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-L- α,β -diaminopropionic acid; (N- β -4-methyltrityl)-L- α,β -diaminopropionic acid; (N- β -allyloxycarbonyl)-L- α,β -diaminopropionic acid; (N- γ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-D- α,γ -diaminobutyric acid; (N- γ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-L- α,γ -diaminobutyric acid; (N- γ -4-methyltrityl)-D- α,γ -diaminobutyric acid; (N- γ -4-methyltrityl)-L- α,γ -diaminobutyric acid; (N- γ -allyloxycarbonyl)-L- α,γ -diaminobutyric acid; D- α,γ -diaminobutyric acid; 4,5-dehydro-L-leucine; cyclopentyl-D-Gly-OH; cyclopentyl-Gly-OH; D-allylglycine; D-homocyclohexylalanine; L-1-pyrenylalanine; L-2-aminocaproic acid; L-allylglycine; L-homocyclohexylalanine; and N-(2-hydroxy-4-methoxy-Bzl)-Gly-OH.

[0052] Amino acid analogs include analogs of arginine or lysine. Examples of amino acid analogs of arginine and lysine include, but are not limited to, the following: citrulline; L-2-amino-3-guanidinopropionic acid; L-2-amino-3-ureidopropionic acid; L-citrulline; Lys(Me)₂-OH; Lys(N₃)-OH; N δ -benzyloxycarbonyl-L-ornithine; N ω -nitro-D-arginine; N ω -nitro-L-arginine; α -methyl-ornithine; 2,6-diaminoheptanedioic acid; L-ornithine; (N δ -1-(4,4-dimethyl-2,6-dioxo-cyclohex-1-ylidene)ethyl)-D-ornithine; (N δ -1-(4,4-dimethyl-2,6-dioxo-cyclohex-1-ylidene)ethyl)-L-ornithine; (N δ -4-methyltrityl)-D-ornithine; (N δ -4-methyltrityl)-L-ornithine; D-ornithine; L-ornithine; Arg(Me)(Pbf)-OH; Arg(Me)₂-OH (asymmetrical); Arg(Me)₂-OH (symmetrical); Lys(ivDde)-OH; Lys(Me)₂-OH • HCl; Lys(Me₃)-OH chloride; N ω -nitro-D-arginine; and N ω -nitro-L-arginine.

[0053] Amino acid analogs include analogs of aspartic or glutamic acids. Examples of amino acid analogs of aspartic and glutamic acids include, but are not limited to, the following: α -methyl-D-aspartic acid; α -methyl-glutamic acid; α -methyl-L-aspartic acid; γ -methylene-glutamic acid; (N- γ -ethyl)-L-glutamine; [N- α -(4-aminobenzoyl)]-L-glutamic acid; 2,6-diaminopimelic acid; L- α -aminosuberic acid; D-2-aminoadipic acid; D- α -aminosuberic acid; α -aminopimelic acid; iminodiacetic acid; L-2-aminoadipic acid; threo- β -methyl-aspartic acid; γ -carboxy-D-glutamic acid γ,γ -di-t-butyl ester; γ -carboxy-L-glutamic acid γ,γ -di-t-butyl ester; Glu(OAll)-OH; L-Asu(OtBu)-OH; and pyroglutamic acid.

[0054] Amino acid analogs include analogs of cysteine and methionine. Examples of amino acid analogs of cysteine and methionine include, but are not limited to, Cys(farnesyl)-OH, Cys(farnesyl)-

OMe, α -methyl-methionine, Cys(2-hydroxyethyl)-OH, Cys(3-aminopropyl)-OH, 2-amino-4-(ethylthio)butyric acid, buthionine, buthioninesulfoximine, ethionine, methionine methylsulfonium chloride, selenomethionine, cysteic acid, [2-(4-pyridyl)ethyl]-DL-penicillamine, [2-(4-pyridyl)ethyl]-L-cysteine, 4-methoxybenzyl-D-penicillamine, 4-methoxybenzyl-L-penicillamine, 4-methylbenzyl-D-penicillamine, 4-methylbenzyl-L-penicillamine, benzyl-D-cysteine, benzyl-L-cysteine, benzyl-DL-homocysteine, carbamoyl-L-cysteine, carboxyethyl-L-cysteine, carboxymethyl-L-cysteine, diphenylmethyl-L-cysteine, ethyl-L-cysteine, methyl-L-cysteine, t-butyl-D-cysteine, trityl-L-homocysteine, trityl-D-penicillamine, cystathionine, homocystine, L-homocystine, (2-aminoethyl)-L-cysteine, seleno-L-cystine, cystathionine, Cys(StBu)-OH, and acetamidomethyl-D-penicillamine.

[0055] Amino acid analogs include analogs of phenylalanine and tyrosine. Examples of amino acid analogs of phenylalanine and tyrosine include β -methyl-phenylalanine, β -hydroxyphenylalanine, α -methyl-3-methoxy-DL-phenylalanine, α -methyl-D-phenylalanine, α -methyl-L-phenylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 2,4-dichloro-phenylalanine, 2-(trifluoromethyl)-D-phenylalanine, 2-(trifluoromethyl)-L-phenylalanine, 2-bromo-D-phenylalanine, 2-bromo-L-phenylalanine, 2-chloro-D-phenylalanine, 2-chloro-L-phenylalanine, 2-cyano-D-phenylalanine, 2-cyano-L-phenylalanine, 2-fluoro-D-phenylalanine, 2-fluoro-L-phenylalanine, 2-methyl-D-phenylalanine, 2-methyl-L-phenylalanine, 2-nitro-D-phenylalanine, 2-nitro-L-phenylalanine, 2,4,5-trihydroxy-phenylalanine, 3,4,5-trifluoro-D-phenylalanine, 3,4,5-trifluoro-L-phenylalanine, 3,4-dichloro-D-phenylalanine, 3,4-dichloro-L-phenylalanine, 3,4-difluoro-D-phenylalanine, 3,4-difluoro-L-phenylalanine, 3,4-dihydroxy-L-phenylalanine, 3,4-dimethoxy-L-phenylalanine, 3,5,3'-triiodo-L-thyronine, 3,5-diiodo-D-tyrosine, 3,5-diiodo-L-tyrosine, 3,5-diiodo-L-thyronine, 3-(trifluoromethyl)-D-phenylalanine, 3-(trifluoromethyl)-L-phenylalanine, 3-amino-L-tyrosine, 3-bromo-D-phenylalanine, 3-bromo-L-phenylalanine, 3-chloro-D-phenylalanine, 3-chloro-L-phenylalanine, 3-chloro-L-tyrosine, 3-cyano-D-phenylalanine, 3-cyano-L-phenylalanine, 3-fluoro-D-phenylalanine, 3-fluoro-L-phenylalanine, 3-fluoro-tyrosine, 3-iodo-D-phenylalanine, 3-iodo-L-phenylalanine, 3-iodo-L-tyrosine, 3-methoxy-L-tyrosine, 3-methyl-D-phenylalanine, 3-methyl-L-phenylalanine, 3-nitro-D-phenylalanine, 3-nitro-L-phenylalanine, 3-nitro-L-tyrosine, 4-(trifluoromethyl)-D-phenylalanine, 4-(trifluoromethyl)-L-phenylalanine, 4-amino-D-phenylalanine, 4-amino-L-phenylalanine, 4-benzoyl-D-phenylalanine, 4-benzoyl-L-phenylalanine, 4-bis(2-chloroethyl)amino-L-phenylalanine, 4-bromo-D-phenylalanine, 4-bromo-L-phenylalanine, 4-chloro-D-phenylalanine, 4-chloro-L-phenylalanine, 4-cyano-D-phenylalanine, 4-cyano-L-phenylalanine, 4-fluoro-D-phenylalanine, 4-fluoro-L-phenylalanine, 4-iodo-D-phenylalanine, 4-iodo-L-phenylalanine, homophenylalanine, thyroxine, 3,3-diphenylalanine, thyronine, ethyl-tyrosine, and methyl-tyrosine.

[0056] Amino acid analogs include analogs of proline. Examples of amino acid analogs of proline include, but are not limited to, 3,4-dehydro-proline, 4-fluoro-proline, cis-4-hydroxy-proline, thiazolidine-2-carboxylic acid, and trans-4-fluoro-proline.

[0057] Amino acid analogs include analogs of serine and threonine. Examples of amino acid analogs of serine and threonine include, but are not limited to, 3-amino-2-hydroxy-5-methylhexanoic acid, 2-amino-3-hydroxy-4-methylpentanoic acid, 2-amino-3-ethoxybutanoic acid, 2-amino-3-methoxybutanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-amino-3-benzyloxypropionic acid, 2-amino-3-benzyloxypropionic acid, 2-amino-3-ethoxypropionic acid, 4-amino-3-hydroxybutanoic acid, and α -methylserine.

[0058] Amino acid analogs include analogs of tryptophan. Examples of amino acid analogs of tryptophan include, but are not limited to, the following: α -methyl-tryptophan; β -(3-benzothienyl)-D-alanine; β -(3-benzothienyl)-L-alanine; 1-methyl-tryptophan; 4-methyl-tryptophan; 5-benzyloxy-tryptophan; 5-bromo-tryptophan; 5-chloro-tryptophan; 5-fluoro-tryptophan; 5-hydroxy-tryptophan; 5-hydroxy-L-tryptophan; 5-methoxy-tryptophan; 5-methoxy-L-tryptophan; 5-methyl-tryptophan; 6-bromo-tryptophan; 6-chloro-D-tryptophan; 6-chloro-tryptophan; 6-fluoro-tryptophan; 6-methyl-tryptophan; 7-benzyloxy-tryptophan; 7-bromo-tryptophan; 7-methyl-tryptophan; D-1,2,3,4-tetrahydro-norharman-3-carboxylic acid; 6-methoxy-1,2,3,4-tetrahydronorharman-1-carboxylic acid; 7-azatryptophan; L-1,2,3,4-tetrahydro-norharman-3-carboxylic acid; 5-methoxy-2-methyl-tryptophan; and 6-chloro-L-tryptophan.

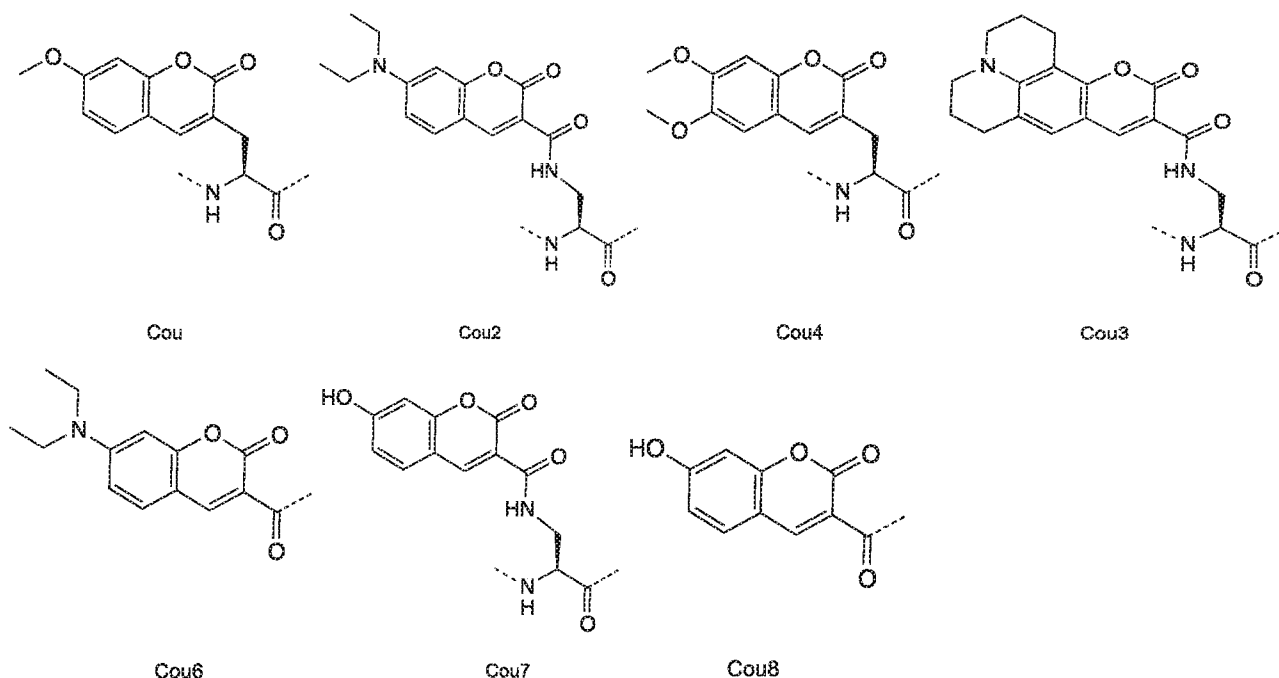
[0059] In some embodiments, amino acid analogs are racemic. In some embodiments, the D isomer of the amino acid analog is used. In some embodiments, the L isomer of the amino acid analog is used. In other embodiments, the amino acid analog comprises chiral centers that are in the R or S configuration. In still other embodiments, the amino group(s) of a β -amino acid analog is substituted with a protecting group, *e.g.*, tert-butyloxycarbonyl (BOC group), 9-fluorenylmethyloxycarbonyl (Fmoc), tosyl, and the like. In yet other embodiments, the carboxylic acid functional group of a β -amino acid analog is protected, *e.g.*, as its ester derivative. In some embodiments the salt of the amino acid analog is used.

[0060] A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of a polypeptide without abolishing or substantially abolishing its essential biological or biochemical activity (*e.g.*, receptor binding or activation). An “essential” amino acid residue is a residue that, when altered from the wild-type sequence of the polypeptide, results in abolishing or substantially abolishing the polypeptide’s essential biological or biochemical activity.

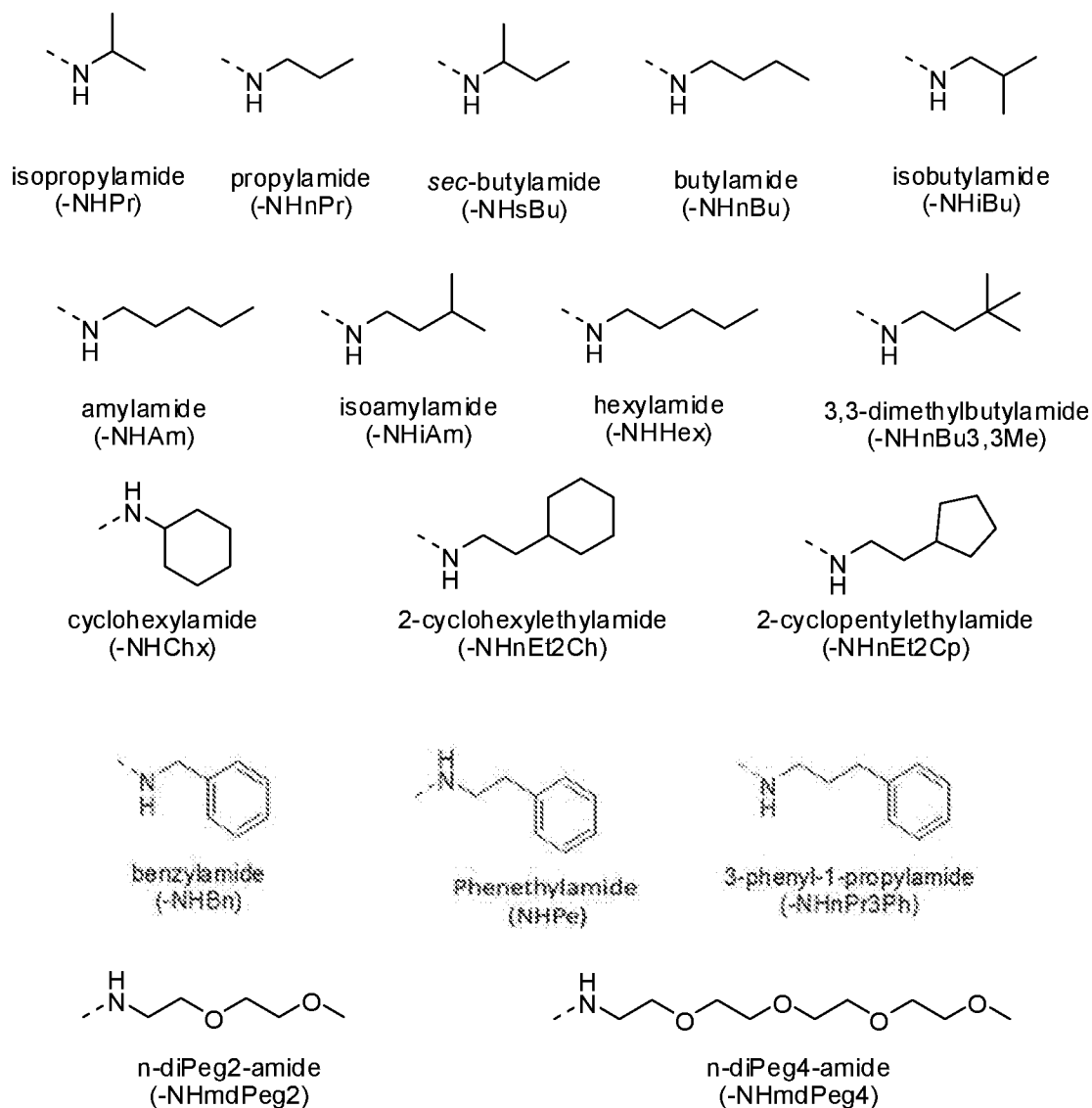
[0061] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having

similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, K, R, H), acidic side chains (*e.g.*, D, E), uncharged polar side chains (*e.g.*, G, N, Q, S, T, Y, C), nonpolar side chains (*e.g.*, A, V, L, I, P, F, M, W), β -branched side chains (*e.g.*, T, V, I) and aromatic side chains (*e.g.*, Y, F, W, H). Thus, a predicted nonessential amino acid residue in a polypeptide, *e.g.*, is replaced with another amino acid residue from the same side chain family. Other examples of acceptable substitutions are substitutions based on isosteric considerations (*e.g.*, norleucine for methionine) or other properties (*e.g.*, 2-thienylalanine for phenylalanine, or 6-Cl-tryptophan for tryptophan).

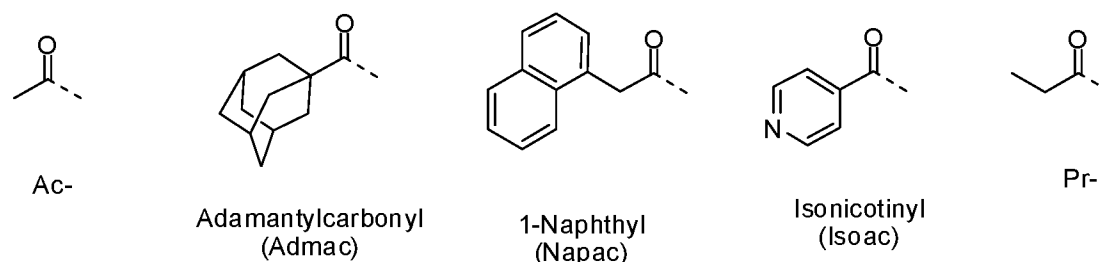
[0062] Amino acids designated as “Cit” represent citrulline. Amino acids designated as “Cou4”, “Cou6”, “Cou7” and “Cou8”, respectively, represent the following structures:

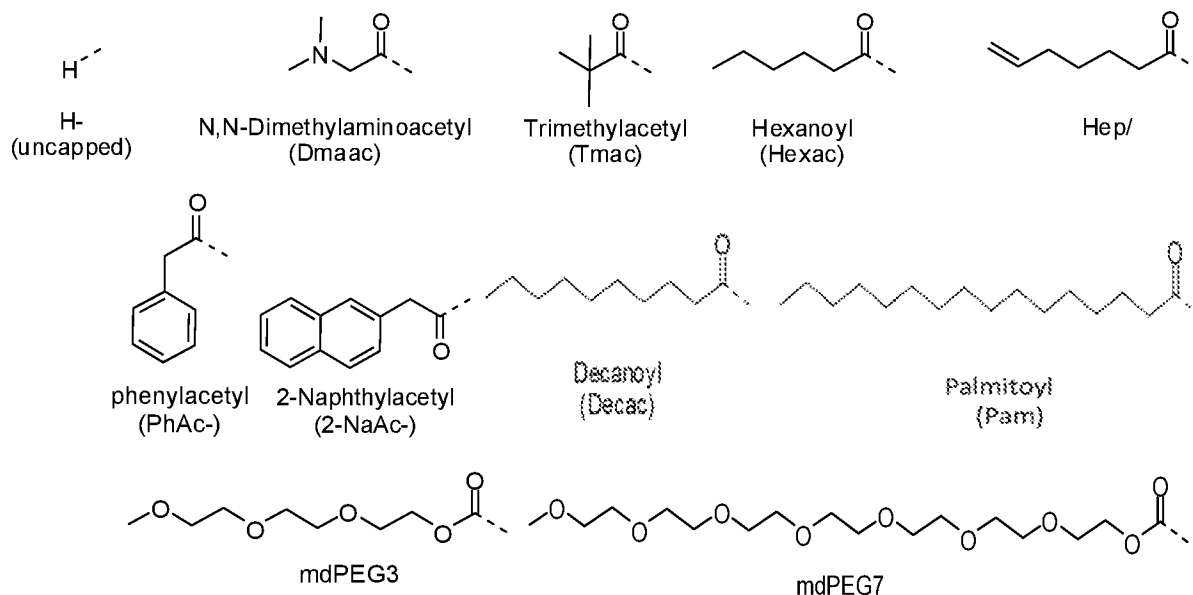


[0063] The term “capping group” refers to the chemical moiety occurring at either the carboxy or amino terminus of the polypeptide chain of the subject peptidomimetic macrocycle. The capping group of a carboxy terminus includes an unmodified carboxylic acid (*i.e.* –COOH) or a carboxylic acid with a substituent. For example, the carboxy terminus can be substituted with an amino group to yield a carboxamide at the C-terminus. Various substituents include but are not limited to primary, secondary, and tertiary amines, including pegylated secondary amines. Representative secondary amine capping groups for the C-terminus include:



[0064] The capping group of an amino terminus includes an unmodified amine (i.e. $-\text{NH}_2$) or an amine with a substituent. For example, the amino terminus can be substituted with an acyl group to yield a carboxamide at the N-terminus. Various substituents include but are not limited to substituted acyl groups, including C_1 - C_6 carbonyls, C_7 - C_{30} carbonyls, and pegylated carbamates. Representative capping groups for the N-terminus include, but are not limited to, 4-FBzl (4-fluoro-benzyl) and the following:





[0065] The term “member” as used herein in conjunction with macrocycles or macrocycle-forming linkers refers to the atoms that form or can form the macrocycle, and excludes substituent or side chain atoms. By analogy, cyclodecane, 1,2-difluoro-decane and 1,3-dimethyl cyclodecane are all considered ten-membered macrocycles as the hydrogen (–H) or fluoro substituents or methyl side chains do not participate in forming the macrocycle.

[0066] The symbol “” when used as part of a molecular structure refers to a single bond or a *trans* or *cis* double bond.

[0067] The term “amino acid side chain” refers to a moiety attached to the α -carbon (or another backbone atom) in an amino acid. For example, the amino acid side chain for alanine is methyl, the amino acid side chain for phenylalanine is phenylmethyl, the amino acid side chain for cysteine is thiomethyl, the amino acid side chain for aspartate is carboxymethyl, the amino acid side chain for tyrosine is 4-hydroxyphenylmethyl, etc. Other non-naturally occurring amino acid side chains are also included, *e.g.*, those that occur in nature (*e.g.*, an amino acid metabolite) or those that are made synthetically (*e.g.*, an α,α di-substituted amino acid).

[0068] The term “ α,α di-substituted amino acid” refers to a molecule or moiety containing both an amino group and a carboxyl group bound to a carbon (the α -carbon) that is attached to two natural or non-natural amino acid side chains.

[0069] The term “polypeptide” encompasses two or more naturally or non-naturally-occurring amino acids joined by a covalent bond (*e.g.*, an amide bond). Polypeptides as described herein include full length proteins (*e.g.*, fully processed proteins) as well as shorter amino acid sequences (*e.g.*, fragments of naturally-occurring proteins or synthetic polypeptide fragments).

[0070] The term “first C-terminal amino acid” refers to the amino acid which is at (i.e. closest to) the C-terminus. The term “second C-terminal amino acid” refers to the amino acid attached at the N-terminus of the first C-terminal amino acid.

[0071] The term “macrocyclization catalyst” or “macrocycle-forming catalyst” as used herein refers to any catalyst which can be used to prepare a peptidomimetic macrocycle by mediating the reaction between two reactive groups. Reactive groups can be, for example, an azide and alkyne, in which case macrocyclization catalysts include, without limitation, Cu catalysts such as catalysts which provide a reactive Cu(I) species, such as CuBr, CuI or CuOTf, as well as Cu(II) salts such as Cu(CO₂CH₃)₂, CuSO₄, and CuCl₂ that can be converted in situ to an active Cu(I) catalyst by the addition of a reducing agent such as ascorbic acid or sodium ascorbate. Macrocyclization catalysts can additionally include, for example, Ru catalysts known in the art such as Cp*RuCl(PPh₃)₂, [Cp*RuCl]₄ or other Ru catalysts which can provide a reactive Ru(II) species. In other cases, the reactive groups are terminal olefins. In such embodiments, the macrocyclization catalysts or macrocycle-forming catalysts are metathesis catalysts including, but not limited to, stabilized, late transition metal carbene complex catalysts such as Group VIII transition metal carbene catalysts. For example, such catalysts are Ru and Os metal centers having a +2 oxidation state, an electron count of 16 and pentacoordinated. In other examples, catalysts have W or Mo centers. Various catalysts are disclosed in Grubbs et al., “Ring Closing Metathesis and Related Processes in Organic Synthesis” Acc. Chem. Res. 1995, 28, 446-452, U.S. Pat. No. 5,811,515; U.S. Pat. No. 7,932,397; U.S. Application No. 2011/0065915; U.S. Application No. 2011/0245477; Yu et al., “Synthesis of Macrocyclic Natural Products by Catalyst-Controlled Stereoselective Ring-Closing Metathesis,” Nature 2011, 479, 88; and Peryshkov et al., “Z-Selective Olefin Metathesis Reactions Promoted by Tungsten Oxo Alkylidene Complexes,” J. Am. Chem. Soc. 2011, 133, 20754. In yet other cases, the reactive groups are thiol groups. In such embodiments, the macrocyclization catalyst is, for example, a linker functionalized with two thiol-reactive groups such as halogen groups.

[0072] The term “halo” or “halogen” refers to fluorine, chlorine, bromine or iodine or a radical thereof.

[0073] The term “alkyl” refers to a hydrocarbon chain that is a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C₁-C₁₀ indicates that the group has from 1-10 (inclusive) carbon atoms in it. In the absence of any numerical designation, “alkyl” is a chain (straight or branched) having 1-20 (inclusive) carbon atoms in it.

[0074] The term “alkylene” refers to a divalent alkyl (i.e. -R-).

[0075] The term “alkenyl” refers to a hydrocarbon chain that is a straight chain or branched chain having one or more carbon-carbon double bonds. The alkenyl moiety contains the indicated number

of carbon atoms. For example, C₂-C₁₀ indicates that the group has from 2-10 (inclusive) carbon atoms in it. The term “lower alkenyl” refers to a C₂-C₆ alkenyl chain. In the absence of any numerical designation, “alkenyl” is a chain (straight or branched) having 2-20 (inclusive) carbon atoms in it.

[0076] The term “alkynyl” refers to a hydrocarbon chain that is a straight chain or branched chain having one or more carbon-carbon triple bonds. The alkynyl moiety contains the indicated number of carbon atoms. For example, C₂-C₁₀ indicates that the group has from 2-10 (inclusive) carbon atoms in it. The term “lower alkynyl” refers to a C₂-C₆ alkynyl chain. In the absence of any numerical designation, “alkynyl” is a chain (straight or branched) having 2-20 (inclusive) carbon atoms in it.

[0077] The term “aryl” refers to a monocyclic or bicyclic aromatic ring system wherein 0, 1, 2, 3, 4, or more atoms of each ring are substituted by a substituent. Exemplary aryls include 6-carbon monocyclic or 10-carbon bicyclic aromatic ring systems. Examples of aryl groups include phenyl, naphthyl and the like. The term “arylalkoxy” refers to an alkoxy substituted with aryl.

[0078] “Arylalkyl” refers to an aryl group, as defined above, wherein one of the aryl group’s hydrogen atoms has been replaced with an alkyl group (*e.g.*, a C₁-C₅ alkyl group) as defined above. Representative examples of an arylalkyl group include, but are not limited to, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 2-ethylphenyl, 3-ethylphenyl, 4-ethylphenyl, 2-propylphenyl, 3-propylphenyl, 4-propylphenyl, 2-butylphenyl, 3-butylphenyl, 4-butylphenyl, 2-pentylphenyl, 3-pentylphenyl, 4-pentylphenyl, 2-isopropylphenyl, 3-isopropylphenyl, 4-isopropylphenyl, 2-isobutylphenyl, 3-isobutylphenyl, 4-isobutylphenyl, 2-sec-butylphenyl, 3-sec-butylphenyl, 4-sec-butylphenyl, 2-t-butylphenyl, 3-t-butylphenyl and 4-t-butylphenyl.

[0079] “Arylamido” refers to an aryl group, as defined above, wherein one of the aryl group’s hydrogen atoms has been replaced with one or more -C(O)NH₂ groups. Representative examples of an arylamido group include 2-C(O)NH₂-phenyl, 3-C(O)NH₂-phenyl, 4-C(O)NH₂-phenyl, 2-C(O)NH₂-pyridyl, 3-C(O)NH₂-pyridyl, and 4-C(O)NH₂-pyridyl,

[0080] “Alkylheterocycle” refers to an alkyl group (*e.g.*, a C₁-C₅ alkyl group), as defined above, wherein one of the C₁-C₅ alkyl group’s hydrogen atoms has been replaced with a heterocycle. Representative examples of an alkylheterocycle group include, but are not limited to, -CH₂CH₂-morpholine, -CH₂CH₂-piperidine, -CH₂CH₂CH₂-morpholine, and -CH₂CH₂CH₂-imidazole.

[0081] “Alkylamido” refers to an alkyl group (*e.g.*, a C₁-C₅ alkyl group), as defined above, wherein one of the alkyl group’s hydrogen atoms has been replaced with a -C(O)NH₂ group. Representative examples of an alkylamido group include, but are not limited to, -CH₂C(O)NH₂, -CH₂CH₂C(O)NH₂, -CH₂CH₂CH₂C(O)NH₂, -CH₂CH₂CH₂CH₂C(O)NH₂, -CH₂CH₂CH₂CH₂CH₂C(O)NH₂, -CH₂CH(C(O)NH₂)CH₃, -CH₂CH(C(O)NH₂)CH₂CH₃,

-CH(C(O)NH₂)CH₂CH₃, -C(CH₃)₂CH₂C(O)NH₂, -CH₂CH₂NHC(O)CH₃,
-CH₂CH₂NHC(O)CH₂CH₃, and -CH₂CH₂NHC(O)CH=CH₂.

[0082] “Alkanol” refers to an alkyl group (*e.g.*, a C₁-C₅ alkyl group), as defined above, wherein one of the alkyl group’s hydrogen atoms has been replaced with a hydroxyl group. Representative examples of an alkanol group include, but are not limited to, -CH₂OH, -CH₂CH₂OH, -CH₂CH₂CH₂OH, -CH₂CH₂CH₂CH₂OH, -CH₂CH₂CH₂CH₂CH₂OH, -CH₂CH(OH)CH₃, -CH₂CH(OH)CH₂CH₃, -CH(OH)CH₃ and -C(CH₃)₂CH₂OH.

[0083] “Alkylcarboxy” refers to an alkyl group (*e.g.*, a C₁-C₅ alkyl group), as defined above, wherein one of the alkyl group’s hydrogen atoms has been replaced with a -COOH group. Representative examples of an alkylcarboxy group include, but are not limited to, -CH₂COOH, -CH₂CH₂COOH, -CH₂CH₂CH₂COOH, -CH₂CH₂CH₂CH₂COOH, -CH₂CH(COOH)CH₃, -CH₂CH₂CH₂CH₂CH₂COOH, -CH₂CH(COOH)CH₂CH₃, -CH(COOH)CH₂CH₃ and -C(CH₃)₂CH₂COOH.

[0084] The term “cycloalkyl” as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups wherein the cycloalkyl group additionally is optionally substituted. For example a cycloalkyl can be saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, 3 to 8 carbons, and or 3 to 6 carbons, Some cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0085] The term “heteroaryl” refers to an aromatic monocyclic, bicyclic, or tricyclic ring system having 1 or more heteroatoms. For example, a heteroaryl includes an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, the heteroatoms selected from O, N, or S (*e.g.*, carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of O, N, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, 4 or more atoms of each ring are substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinoliny, indolyl, thiazolyl, and the like.

[0086] The term “heteroarylalkyl” or the term “heteroaralkyl” refers to an alkyl substituted with a heteroaryl. The term “heteroarylalkoxy” refers to an alkoxy substituted with heteroaryl.

[0087] The term “heteroarylalkyl” or the term “heteroaralkyl” refers to an alkyl substituted with a heteroaryl. The term “heteroarylalkoxy” refers to an alkoxy substituted with heteroaryl.

[0088] The term “heterocyclyl” refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic,

1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, the heteroatoms selected from O, N, or S (*e.g.*, carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of O, N, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring are substituted by a substituent. Examples of heterocyclyl groups include piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

[0089] The term “substituent” refers to a group replacing a second atom or group such as a hydrogen atom on any molecule, compound or moiety. Suitable substituents include, without limitation, halo, hydroxy, mercapto, oxo, nitro, haloalkyl, alkyl, alkaryl, aryl, aralkyl, alkoxy, thioalkoxy, aryloxy, amino, alkoxycarbonyl, amido, carboxy, alkanesulfonyl, alkylcarbonyl, and cyano groups.

[0090] In some embodiments, the compounds of this invention contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are included unless expressly provided otherwise. In some embodiments, the compounds of this invention are also represented in multiple tautomeric forms, in such instances, the invention includes all tautomeric forms of the compounds described herein (*e.g.*, if alkylation of a ring system results in alkylation at multiple sites, the invention includes all such reaction products). All such isomeric forms of such compounds are included unless expressly provided otherwise. All crystal forms of the compounds described herein are included unless expressly provided otherwise.

[0091] As used herein, the terms “increase” and “decrease” mean, respectively, to cause a statistically significantly (*i.e.* $p < 0.1$) increase or decrease of at least 5%.

[0092] As used herein, the recitation of a numerical range for a variable is intended to convey that the variable is equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable is equal to any integer value within the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable is equal to any real value within the numerical range, including the end-points of the range. As an example, and without limitation, a variable which is described as having values from 0 to 2 takes the values 0, 1 or 2 if the variable is inherently discrete, and takes the values 0.0, 0.1, 0.01, 0.001, or any other real values ≥ 0 and ≤ 2 if the variable is inherently continuous.

[0093] As used herein, unless specifically indicated otherwise, the word “or” is used in the inclusive sense of “and/or” and not the exclusive sense of “either/or.”

[0094] The term “on average” represents the mean value derived from performing at least three independent replicates for each data point.

[0095] The term “biological activity” encompasses structural and functional properties of a macrocycle. Biological activity is, *e.g.*, structural stability, α -helicity, affinity for a target, resistance to proteolytic degradation, cell penetrability, intracellular stability, *in vivo* stability, or any combination thereof.

[0096] The term “binding affinity” refers to the strength of a binding interaction, for example between a peptidomimetic macrocycle and a target. Binding affinity can be expressed, for example, as an equilibrium dissociation constant (“ K_D ”), which is expressed in units which are a measure of concentration (*e.g.* M, mM, μ M, nM, etc.). Numerically, binding affinity and K_D values vary inversely, such that a lower binding affinity corresponds to a higher K_D value, while a higher binding affinity corresponds to a lower K_D value. Where high binding affinity is desirable, “improved” binding affinity refers to higher binding affinity *i.e.* lower K_D values.

[0097] The term “ratio of binding affinities” refers to the ratio of dissociation constants (K_D values) of a first binding interaction (the numerator), versus a second binding interaction (denominator). Consequently, a “reduced ratio of binding affinities” to Target 1 versus Target 2 refers to a lower value for the ratio expressed as $K_D(\text{Target 1})/K_D(\text{Target 2})$. This concept can be due either to a decrease in the K_D value for Target 1 or an increase in the value for the K_D value for Target 2.

[0098] The term “*in vitro* efficacy” refers to the extent to which a test compound, such as a peptidomimetic macrocycle, produces a beneficial result in an *in vitro* test system or assay. *In vitro* efficacy can be measured, for example, as an “ IC_{50} ” or “ EC_{50} ” value, which represents the concentration of the test compound which produces 50% of the maximal effect in the test system.

[0099] The term “ratio of *in vitro* efficacies” or “*in vitro* efficacy ratio” refers to the ratio of IC_{50} or EC_{50} values from a first assay (the numerator) versus a second assay (the denominator). Consequently, an improved *in vitro* efficacy ratio for Assay 1 versus Assay 2 refers to a lower value for the ratio expressed as $IC_{50}(\text{Assay 1})/IC_{50}(\text{Assay 2})$ or alternatively as $EC_{50}(\text{Assay 1})/EC_{50}(\text{Assay 2})$. This concept can also be characterized as “improved selectivity” in Assay 1 versus Assay 2, which can be due either to a decrease in the IC_{50} or EC_{50} value for Target 1 or an increase in the value for the IC_{50} or EC_{50} value for Target 2.

[00100] The term “solubility” used herein refers to a property of a molecule to dissolve in a solvent to form a homogeneous solution.

[00101] The term “turbidity” used herein refers to a measure of the degree to which an aqueous solution loses its transparency due to the presence of suspended particles.

[00102] The term “net charge” used herein refers to the integral sum of positive and negative particles that make up a polypeptide.

[00103] The term “tyrosine content” used herein refers to the percentage of tyrosine residues in a polypeptide amino acid sequence. In some embodiments, tyrosine content can be total tyrosine content. In certain embodiment, tyrosine content can be L-tyrosine content. In certain embodiments, tyrosine content can be m-tyrosine content. In other embodiments, tyrosine content can be o-tyrosine content.

[00104] The term “alanine content” used herein refers to the percentage of alanine residues in a polypeptide amino acid sequence. In some embodiments, alanine content can be total alanine content. In certain embodiment, alanine content can be L-alanine content. In other embodiments, alanine content can be D-alanine content.

[00105] The term “tryptophan content” used herein refers to the percentage of tryptophan residues in a polypeptide amino acid sequence. In some embodiments, tryptophan content can be total tryptophan content. In certain embodiment, tryptophan content can be L-tryptophan content. In other embodiments, tryptophan content can be D-tryptophan content.

[00106] A “von Heijne value”, calculated using the method of Hessa *et al.* (*Nature*: 433, 377-381 (2005)), can be used as an indicator of a variety of properties of a polypeptide, including hydrophobicity and membrane integration efficiency.

[00107] The term “partition coefficient” used herein refers to a particular ratio of the concentrations of a solute between the two solvents. For example, a partition coefficient of a peptide can be a ratio of the concentrations of the peptide between octanol and water, $\log P_{\text{octanol/water}}$, e.g., at 37 °C. The logarithm of the partition coefficient is $\log P$, which can be measured using the shake-flask method (Dearden *et al.*, *Molecular Informatics*: 7(3), 133-144, 1988).

[00108] The term “HPLC retention time” used herein refers to the time needed to elute a polypeptide from an HPCL column.

[00109] The term “amphipathicity” used herein refers to a property of a molecule to be both hydrophilic (water-soluble) and lipophilic (lipid-soluble). An amphipathic molecule can comprise a polar group and a nonpolar group.

[00110] The details of one or more particular embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

Peptidomimetic Macrocycles

[00111] Described herein are stably cross-linked peptides modeled after the N-terminal transactivation domain of p53 (“p53 peptidomimetic macrocycles”). These cross-linked peptides contain at least two modified amino acids that together form an intramolecular cross-link that can help to stabilize the α -helical secondary structure of a portion of p53 that is thought to be important

for binding of p53 to MDM2 and for binding of p53 to MDMX. Accordingly, a cross-linked polypeptide described herein can have improved biological activity relative to a corresponding polypeptide that is not cross-linked. The p53 peptidomimetic macrocycles are thought to interfere with binding of p53 to MDM2 and/or of p53 to MDMX, thereby liberating functional p53 and inhibiting its destruction. The p53 peptidomimetic macrocycles described herein can be used therapeutically, for example to treat cancers and other disorders characterized by an undesirably low level or a low activity of p53, and/or to treat cancers and other disorders characterized by an undesirably high level of activity of MDM2 or MDMX. The p53 peptidomimetic macrocycles can also be useful for treatment of any disorder associated with disrupted regulation of the p53 transcriptional pathway, leading to conditions of excess cell survival and proliferation such as cancer and autoimmunity, in addition to conditions of inappropriate cell cycle arrest and apoptosis such as neurodegeneration and immune deficiencies. In some embodiments, the p53 peptidomimetic macrocycles bind to MDM2 (*e.g.*, GenBank® Accession No.: 228952; GI:228952) and/or MDMX (also referred to as MDM4; GenBank® Accession No.: 88702791; GI:88702791).

[00112] **Table 1a** shows a list of peptidomimetic macrocycles derived from the MDM2/MDMX-binding helix of p53 that were prepared. **Table 1b** shows a list of selected peptidomimetic macrocycles from **Table 1a**. SP-153 was prepared by including an i, i+7 crosslink to the sequence of LP-1. SP-18 was prepared from the sequence of SP-153. SP-147 was prepared from the sequence of SP-18. (**FIG. 1**).

[00113] **Table 2a** shows a list of peptidomimetic macrocycles derived from the MDM2/MDMX-binding helix of p53 that were prepared. **Table 2b** shows a list of selected peptidomimetic macrocycles from **Table 2a**. A partial staple scan was performed on the linear peptide p-CF₃-Phe7-D-PMI-β. SP-166, a potent and selective MDM2 antagonist, was prepared by including an i, i+7 crosslink to the sequence of p-CF₃-Phe-7-D-PMI-b. SP-166 exhibited SJSA-1 sarcoma cell killing activity at a single digit micromolar concentration (EC₅₀= 1.5 mM). (**FIG. 3**). SP-172 was prepared by increasing the alanine content to 35% while maintaining the net charge and VH score by adding four alanine residues to the C-terminus of SP-166. SP-172 exhibited improved SJSA-1 sarcoma cell killing activity (EC₅₀= 0.15 mM) compared to SP-166 (**FIG. 3**). SP-172 exhibited similar cell killing activity as SP-147.

[00114] Peptidomimetic macrocycles can also be prepared that target or interact with proteins that a virus needs for infection or replication within a host cell. Such viruses may be, for example, influenza viruses belonging to Orthomyxoviridae family of viruses. This family also includes Thogoto viruses and Dhoriviruses. There are several types and subtypes of influenza viruses known, which infect humans and other species. Influenza type A viruses infect people, birds, pigs, horses,

seals and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes and named on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). For example, an “H7N2 virus” designates an influenza A subtype that has an HA7 protein and an NA2 protein. Similarly an “H5N1” virus has an HA 5 protein and an NA1 protein. There are 16 known HA subtypes and 9 known NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses, and H3N8 also has recently been shown to cause illness in dogs.

[00115] Antiviral agents according to the invention can be used to protect high-risk groups (hospital units, institutes caring for elderly, immuno-suppressed individuals), and on a case by case basis. A potential use for antiviral agents is to limit the spread and severity of the future pandemics whether caused by avian H5N1 or other strains of influenza virus. Avian influenza A viruses of the subtypes H5 and H7, including H5N1, H7N7, and H7N3 viruses, have been associated with high pathogenicity, and human infection with these viruses have ranged from mild (H7N3, H7N7) to severe and fatal disease (H7N7, H5N1). Human illness due to infection with low pathogenicity viruses has been documented, including very mild symptoms (*e.g.*, conjunctivitis) to influenza-like illness. Examples of low pathogenicity viruses that have infected humans include H7N7, H9N2, and H7N2.

[00116] Influenza B viruses are usually found in humans but can also infect seals. Unlike influenza A viruses, these viruses are not classified according to subtype. Influenza B viruses can cause morbidity and mortality among humans, but in general are associated with less severe epidemics than influenza A viruses. Although influenza type B viruses can cause human epidemics, they have not caused pandemics.

[00117] Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. These viruses can also infect dogs and pigs. These viruses are not classified according to subtype.

[00118] Influenza viruses differ from each other in respect to cell surface receptor specificity and cell tropism, however they use common entry pathways. Charting these pathways and identification of host cell proteins involved in virus influenza transmission, entry, replication, biosynthesis, assembly, or exit allows the development of general agents against existing and emerging strains of influenza. The agents may also prove useful against unrelated viruses that use similar pathways. For example, the agents may protect airway epithelial cells against a number of different viruses in addition to influenza viruses.

[00119] In one embodiment the targeted virus is an adenovirus. Adenoviruses most commonly cause respiratory illness; symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection. Acute respiratory disease (ARD), first recognized among military recruits during World War II, can be caused by adenovirus infections during conditions of crowding and stress. Adenoviruses are medium-sized (90-100 nm), nonenveloped icosahedral viruses containing double-stranded DNA. There are 49 immunologically distinct types (6 subgenera: A through F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body. Some adenoviruses, such as AD2 and Ad5 (species C) use clathrin mediated endocytosis and macropinocytosis for infectious entry. Other adenoviruses, such as Ad3 (species B) use dynamin dependent endocytosis and macropinocytosis for infectious entry.

[00120] In one embodiment the targeted virus is a respiratory syncytial virus (RSV). RSV is the most common cause of bronchiolitis and pneumonia among infants and children under 1 year of age. Illness begins most frequently with fever, runny nose, cough, and sometimes wheezing. During their first RSV infection, between 25% and 40% of infants and young children have signs or symptoms of bronchiolitis or pneumonia, and 0.5% to 2% require hospitalization. Most children recover from illness in 8 to 15 days. The majority of children hospitalized for RSV infection are under 6 months of age. RSV also causes repeated infections throughout life, usually associated with moderate-to-severe cold-like symptoms; however, severe lower respiratory tract disease may occur at any age, especially among the elderly or among those with compromised cardiac, pulmonary, or immune systems. RSV is a negative-sense, enveloped RNA virus. The virion is variable in shape and size (average diameter of between 120 and 300 nm), is unstable in the environment (surviving only a few hours on environmental surfaces), and is readily inactivated with soap and water and disinfectants.

[00121] In one embodiment the targeted virus is a human parainfluenza virus (HPIV). HPIVs are second to respiratory syncytial virus (RSV) as a common cause of lower respiratory tract disease in young children. Similar to RSV, HPIVs can cause repeated infections throughout life, usually manifested by an upper respiratory tract illness (*e.g.*, a cold and/or sore throat). HPIVs can also cause serious lower respiratory tract disease with repeat infection (*e.g.*, pneumonia, bronchitis, and bronchiolitis), especially among the elderly, and among patients with compromised immune systems. Each of the four HPIVs has different clinical and epidemiologic features. The most distinctive clinical feature of HPIV-1 and HPIV-2 is croup (*i.e.*, laryngotracheobronchitis); HPIV-1 is the leading cause of croup in children, whereas HPIV-2 is less frequently detected. Both HPIV-1 and -2

can cause other upper and lower respiratory tract illnesses. HPIV-3 is more often associated with bronchiolitis and pneumonia. HPIV-4 is infrequently detected, possibly because it is less likely to cause severe disease. The incubation period for HPIVs is generally from 1 to 7 days. HPIVs are negative-sense, single-stranded RNA viruses that possess fusion and hemagglutinin-neuraminidase glycoprotein “spikes” on their surface. There are four serotypes types of HPIV (1 through 4) and two subtypes (4a and 4b). The virion varies in size (average diameter between 150 and 300 nm) and shape, is unstable in the environment (surviving a few hours on environmental surfaces), and is readily inactivated with soap and water.

[00122] In one embodiment the targeted virus is a coronavirus. Coronavirus is a genus of animal virus belonging to the family Coronaviridae. Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and a helical symmetry. The genomic size of coronaviruses ranges from approximately 16 to 31 kilobases, extraordinarily large for an RNA virus. The name “coronavirus” is derived from the Latin corona, meaning crown, as the virus envelope appears under electron microscopy to be crowned by a characteristic ring of small bulbous structures. This morphology is actually formed by the viral spike peplomers, which are proteins that populate the surface of the virus and determine host tropism. Coronaviruses are grouped in the order Nidovirales, named for the Latin nidus, meaning nest, as all viruses in this order produce a 3’ co-terminal nested set of subgenomic mRNAs during infection. Proteins that contribute to the overall structure of all coronaviruses are the spike, envelope, membrane and nucleocapsid. In the specific case of SARS a defined receptor-binding domain on S mediates the attachment of the virus to its cellular receptor, angiotensin-converting enzyme 2.

[00123] In one embodiment the targeted virus is a rhinovirus. Rhinovirus is a genus of the Picornaviridae family of viruses. Rhinoviruses are the most common viral infective agents in humans, and a causative agent of the common cold. There are over 105 serologic virus types that cause cold symptoms, and rhinoviruses are responsible for approximately 50% of all cases. Rhinoviruses have single-stranded positive sense RNA genomes of between 7.2 and 8.5kb in length. At the 5’ end of the genome is a virus-encoded protein, and like mammalian mRNA, there is a 3’ poly-A tail. Structural proteins are encoded in the 5’ region of the genome and nonstructural at the end. This is the same for all picornaviruses. The viral particles themselves are not enveloped and are icosahedral in structure.

[00124] Any secondary structure of a viral protein (or of a host cell protein involved in viral infectivity) can form the basis of the methods. For example, a viral protein comprising a secondary structure which is a helix may be used to design peptidomimetic macrocycles based on the helix.

[00125] In one embodiment, the peptidomimetic macrocycle is designed based on the PB1 or PB2 sequence of an influenza virus. The PB1 sequence is highly conserved across all known strains of influenza A virus, which may result in less drug resistance should than that observed with the current standard of care. An alignment of the first 25 N-terminal amino acids of PB1 from the NCBI data bank's 2,485 influenza A virus strains (Ghanem, 2007) demonstrates the remarkable sequence conservation in the PA interaction domain of PB1. Therefore, antiviral therapies based on the PB1 sequence may block most, if not all, influenza A virus strains. Additionally, sequence modification of a peptidomimetic macrocycle based on these few variations in PB1 may enable an antiviral cocktail of PB1 inhibitors to eliminate resistance due to escape mutants.

[00126] **Table 3a** shows a list of peptidomimetic macrocycles derived from the PA-binding helix of PB1 that were prepared. **Table 3b** shows a list of selected peptidomimetic macrocycles from **Table 3a**. SP-185 and SP-188 were prepared by increasing the length and alanine content (%) of the SP-180 sequence. These modifications led to a five-fold increase in antiviral activity compared to that of SP-180. SP-192 was prepared by incorporating an i, i+7 crosslink instead of the i, i+4 crosslink of SP-180. SP-192 exhibited improved anti-viral activity ($EC_{50} = 4.5$ mM) compared to that of SP-180.

[00127] In some embodiments of the invention, the peptide sequence is derived from the BCL-2 family of proteins. The BCL-2 family is defined by the presence of up to four conserved BCL-2 homology (BH) domains designated BH1, BH2, BH3, and BH4, all of which include α -helical segments (Chittenden *et al.* (1995), *EMBO* 14:5589; Wang *et al.* (1996), *Genes Dev.* 10:2859). Anti-apoptotic proteins, such as BCL-2 and BCL-X_L, display sequence conservation in all BH domains. Pro-apoptotic proteins are divided into "multidomain" family members (*e.g.*, BAK, BAX), which possess homology in the BH1, BH2, and BH3 domains, and "BH3-domain only" family members (*e.g.*, BID, BAD, BIM, BIK, NOXA, PUMA), that contain sequence homology exclusively in the BH3 amphipathic α -helical segment. BCL-2 family members have the capacity to form homo- and heterodimers, suggesting that competitive binding and the ratio between pro- and anti-apoptotic protein levels dictates susceptibility to death stimuli. Anti-apoptotic proteins function to protect cells from pro-apoptotic excess, *i.e.*, excessive programmed cell death. Additional "security" measures include regulating transcription of pro-apoptotic proteins and maintaining them as inactive conformers, requiring either proteolytic activation, dephosphorylation, or ligand-induced conformational change to activate pro-death functions. In certain cell types, death signals received at the plasma membrane trigger apoptosis via a mitochondrial pathway. The mitochondria can serve as a gatekeeper of cell death by sequestering cytochrome c, a critical component of a cytosolic complex which activates caspase 9, leading to fatal downstream proteolytic events. Multidomain proteins such as BCL-2/BCL-X_L and BAK/BAX play dueling roles of guardian and executioner at the

mitochondrial membrane, with their activities further regulated by upstream BH3-only members of the BCL-2 family. For example, BID is a member of the BH3-domain only family of pro-apoptotic proteins, and transmits death signals received at the plasma membrane to effector pro-apoptotic proteins at the mitochondrial membrane. BID has the capability of interacting with both pro- and anti-apoptotic proteins, and upon activation by caspase 8, triggers cytochrome c release and mitochondrial apoptosis. Deletion and mutagenesis studies determined that the amphipathic α -helical BH3 segment of pro-apoptotic family members may function as a death domain and thus may represent a critical structural motif for interacting with multidomain apoptotic proteins. Structural studies have shown that the BH3 helix can interact with anti-apoptotic proteins by inserting into a hydrophobic groove formed by the interface of BH1, 2 and 3 domains. Activated BID can be bound and sequestered by anti-apoptotic proteins (*e.g.*, BCL-2 and BCL-X_L) and can trigger activation of the pro-apoptotic proteins BAX and BAK, leading to cytochrome c release and a mitochondrial apoptosis program. BAD is also a BH3-domain only pro-apoptotic family member whose expression triggers the activation of BAX/BAK. In contrast to BID, however, BAD displays preferential binding to anti-apoptotic family members, BCL-2 and BCL-X_L. Whereas the BAD BH3 domain exhibits high affinity binding to BCL-2, BAD BH3 peptide is unable to activate cytochrome c release from mitochondria *in vitro*, suggesting that BAD is not a direct activator of BAX/BAK. Mitochondria that over-express BCL-2 are resistant to BID-induced cytochrome c release, but co-treatment with BAD can restore BID sensitivity. Induction of mitochondrial apoptosis by BAD appears to result from either: (1) displacement of BAX/BAK activators, such as BID and BID-like proteins, from the BCL-2/BCL-X_L binding pocket, or (2) selective occupation of the BCL-2/BCL-X_L binding pocket by BAD to prevent sequestration of BID-like proteins by anti-apoptotic proteins. Thus, two classes of BH3-domain only proteins have emerged, BID-like proteins that directly activate mitochondrial apoptosis, and BAD-like proteins, that have the capacity to sensitize mitochondria to BID-like pro-apoptotics by occupying the binding pockets of multidomain anti-apoptotic proteins. Various α -helical domains of BCL-2 family member proteins amenable to the methodology disclosed herein have been disclosed (Walensky *et al.* (2004), *Science* 305:1466; and Walensky *et al.*, U.S. Patent Publication No. 2005/0250680, the entire disclosures of which are incorporated herein by reference).

[00128] **Table 4a** shows a list of peptidomimetic macrocycles derived from the MCL-1/ BCL-X_L /BCL-2-binding helix of BIM that were prepared. **Table 4b** shows a list of selected peptidomimetic macrocycles from **Table 4a**. SP-203 was prepared by incorporating an i, i+7 crosslink into the sequence of the linear peptide LP-2. SP-208 was prepared by removal of the two terminal arginine residues and an alanine substitution at position 13 of SP-203. SP-355 was prepared by a homoleucine substitution at position 9 and a F4F at position 17 of SP-208.

[00129] **Table 1a** - Peptidomimetic macrocycles derived from the MDM2/MDMX-binding helix of p53.

SP#	Sequence	Calc. (M + 2) / 2	Found mass
LP-1	Ac-LSQETFSDLWKLLPEN-NH ₂		
153	Ac-LSQETF\$8DLWKLL\$EN-NH ₂		
1	Ac-LTF\$8AYWAQL\$A-NH ₂	758.93	758.97
2	Ac-F\$8AYWSAL\$A-NH ₂	631.36	1261.60
3	Ac-F\$8FYWEAc3cL\$A-NH ₂	696.38	1392.14
4	Ac-F\$8VYWEAc3cL\$A-NH ₂	672.38	1343.67
5	Ac-F\$8AYWOAL\$A-NH ₂	644.88	1289.13
6	Ac-LTF\$8AYWAQL\$AANlea-NH ₂	886.52	887.08
7	Ac-LTF\$8AYWAQL\$AAIA-NH ₂	886.52	866.80
8	Ac-ETF\$8EHWAQL\$AAIA-NH ₂	910.50	910.85
9	Ac-ITF\$8EHWAQL\$AAIA-NH ₂	902.52	903.17
10	Ac-LTF\$8HYWALL\$S-NH ₂	792.46	792.49
11	Ac-LTF\$8AEWAQL\$SAA-NH ₂	820.96	820.39
12	Ac-LTF\$8HYWAAL\$S-NH ₂	771.43	771.48
13	Ac-LTF\$8AYWAQL\$AANleA-NH ₂	886.52	886.89
14	Ac-LTF\$8HYWAAbuL\$S-NH ₂	778.44	778.51
15	Ac-LTF34F2\$8EYWAQhL\$SAA-NH ₂	891.97	891.88
16	Ac-LTF34F2\$8EYWAQL\$SAA-NH ₂	884.97	884.48
17	Ac-LTF3CI\$8EYWAQL\$SAA-NH ₂	883.96	883.96
18	Ac-LTF\$8EYWAQCba\$SAA-NH ₂	872.97	873.39
19	Ac-LTF\$8AYWSQL\$S-NH ₂	774.93	774.96
20	Ac-LTF\$8AYWRQL\$A-NH ₂	801.47	801.52
21	Ac-LTF\$8AYWRAL\$S-NH ₂	780.96	780.95
22	Ac-LTF\$8AYWAQhL\$SAA-NH ₂	844.98	844.81
23	Ac-LTF\$8AYWRAL\$A-NH ₂	772.96	773.03
24	Ac-LTF\$8HYWAQL\$S8S-NH ₂	799.95	799.97
25	Ac-ETF\$8EYWAQCba\$AWNlea-NH ₂	987.02	987.06
26	Ac-LTF\$8HYWAHL\$S-NH ₂	804.45	804.48
27	Ac-STF\$8AYWAQL\$S-NH ₂	753.91	753.94
28	Ac-LTF\$8HYWAQL\$H-NH ₂	824.96	824.98
29	Ac-ETF\$8AYWAQL\$S-NH ₂	774.91	774.96
30	Ac-LTF\$8EYWAQCpg\$SAA-NH ₂	872.97	872.86
31	Ac-LTF\$8AYWAQCba\$AANleA-NH ₂	892.52	892.64
32	Ac-LTF\$8AYWEQL\$S-NH ₂	795.94	795.90
33	Ac-LTF\$8EYWAQCha\$SAA-NH ₂	886.99	886.85
34	Ac-LTF\$8EYWAQL\$S-NH ₂	795.94	795.97
35	Ac-LTF\$8AYWAQL\$E-NH ₂	787.94	787.98
36	Ac-LTF\$8AYWHQL\$S-NH ₂	799.95	799.97
37	Ac-LTF\$8VYWAQL\$SVS-NH ₂	874.00	873.90
38	Ac-LTF\$8AYWKQL\$S-NH ₂	795.46	795.53
39	Ac-LTF\$8VYWAQL\$SVA-NH ₂	866.00	865.90
40	Ac-LTF\$8AYWOQL\$S-NH ₂	788.46	788.50
41	Ac-LTF\$8AYWRQL\$S-NH ₂	809.47	809.51
42	Ac-LTF\$8VYWAQL\$SAS-NH ₂	859.98	859.91
43	Ac-HTF\$8HYWAQL\$S-NH ₂	811.93	811.96
44	Ac-LTF\$8HHWAQL\$S-NH ₂	786.94	786.94
45	Ac-LTF\$8AYWAQL\$SWA-NH ₂	895.49	895.28
46	Ac-LHF\$8HYWAQL\$S-NH ₂	817.95	818.02

SP#	Sequence	Calc. (M + 2) / 2	Found mass
47	Ac-LTF\$r8AYWAQL\$SLA-NH ₂	859.00	858.87
48	Ac-ITF\$r8EYWAQL\$S-NH ₂	795.94	795.38
49	Ac-F\$r8AY6clWQAL\$AA-NH ₂	704.37	703.84
50	Ac-LTF\$r8AYWAAL\$AAIa-NH ₂	858.01	857.84
51	Ac-ETF\$r8EYWAQL\$SAA-NH ₂	874.95	874.34
52	Ac-LTF\$r8AEWAAL\$SAA-NH ₂	792.45	791.90
53	Ac-LTF\$r8EYWAQL\$AAFa-NH ₂	932.51	932.87
54	Ac-LTF\$r8EYWAQL\$AAVa-NH ₂	908.51	908.31
55	Ac-LTF\$r8AYWVAL\$SAA-NH ₂	823.48	824.08
56	Ac-LTF\$r8AF4coohWAQL\$AAIa-NH ₂	900.51	900.24
57	Ac-LTF\$r8EYWLQL\$S-NH ₂	816.96	817.33
58	Ac-LTF\$r8AF4coohWAQL\$SAA-NH ₂	851.97	851.40
59	Ac-LTF\$r8AYWLQL\$S-NH ₂	787.96	787.39
60	Ac-LTF2C1\$r8HYWAQL\$S-NH ₂	816.93	816.99
61	Ac-LTF\$r8AYWAQL\$AALa-NH ₂	886.52	886.33
62	Ac-LTF\$r8AHWAAL\$SAA-NH ₂	796.46	796.88
63	Ac-LTF\$r8AHWAQL\$AAIa-NH ₂	873.51	874.13
64	Ac-LTF\$r8HYWWQL\$S-NH ₂	857.47	857.50
65	Ac-LTF\$r8HY2NalAQL\$S-NH ₂	805.45	805.52
66	Ac-LTF\$r8AYWAQL\$AAAa-NH ₂	865.49	865.89
67	Ac-LTF%cs7HYWAQL%c7S-NH ₂	818.91	818.99
68	Ac-LTF\$r8AHWAQL\$AHIa-NH ₂	906.53	906.87
69	Ac-LTF2CN\$r8HYWAQL\$S-NH ₂	812.44	812.47
70	Ac-LTF3C1\$r8HYWAQL\$S-NH ₂	816.93	816.99
71	Ac-LTF\$r8EHWAQL\$AALa-NH ₂	902.52	902.90
72	Ac-LTF\$r8EHWAQL\$AHIa-NH ₂	935.53	937.03
73	Ac-ITF\$r8FYWVQL\$AAIa-NH ₂	938.55	939.16
74	Ac-LTF\$r8AYWAQL\$AAWa-NH ₂	923.01	922.81
75	Ac-ITF\$r8EYWVQL\$AHFA-NH ₂	979.54	979.35
76	Ac-LTF\$r8AYWCouQL\$S-NH ₂	853.95	854.61
77	Ac-ITF\$r8FHWAQL\$AEFA-NH ₂	957.52	958.12
78	Ac-LTF\$r8HYWAc5cQL\$S-NH ₂	819.96	820.02
79	Ac-LTF\$r8AYWAQL\$AAAAa-NH ₂	901.01	901.42
80	Ac-LTF\$r8HYWAQhL\$S-NH ₂	806.95	807.00
81	Ac-LTF%cs9HYWAQL%c9S-NH ₂	832.93	832.97
82	Ac-LTF\$r8HYWAQCha\$S-NH ₂	819.96	820.02
83	Ac-F\$r8AYWEAc3cL\$AF-NH ₂	731.90	731.96
84	Ac-LTF\$r8AEWAQL\$AAIa-NH ₂	869.51	869.87
85	Ac-LTF\$r8AYWAQL\$SAibAA-NH ₂	880.50	880.34
86	Tmac-LTF\$r8EYWAQhL\$SAA-NH ₂	895.01	895.41
87	Ac-LTF\$r8CouYWAQL\$S-NH ₂	853.95	854.70
88	Ac-STF\$r8AYWAQL\$S-NH ₂	753.91	753.94
89	Ac-LTF\$r8AYWAEL\$S-NH ₂	767.43	767.41
90	Ac-LTF\$r8AYWAQL\$E-NH ₂	787.94	787.98
91	Ac-LTF\$r8AYWAQL\$AAFa-NH ₂	903.51	903.28
92	Ac-LTF\$r8EYWAQL\$AAIa-NH ₂	915.52	915.34
93	Ac-LT3Pal\$r8HYWAQL\$S-NH ₂	800.44	800.49
94	Ac-LTF\$r8AYWAQL\$AAVa-NH ₂	879.51	879.30
95	Dmaac-LTF\$r8EYWAQhL\$SAA-NH ₂	895.50	896.06
96	Ac-LTF\$r8HYWAQChg\$S-NH ₂	812.95	812.99
97	Ac-LTF\$r8HYWAQF\$S-NH ₂	816.94	816.99
98	Ac-LTF\$r8HYWAQCba\$S-NH ₂	805.95	805.96

SP#	Sequence	Calc. (M + 2) / 2	Found mass
99	Ac-LTF\$8HYWAQCpg\$S-NH ₂	805.95	805.96
100	Ac-LTF3CN\$8HYWAQL\$S-NH ₂	812.44	812.47
101	Ac-F\$8RYWEAc3cL\$A-NH ₂	700.90	700.95
102	Ac-LTF4F\$8HYWAQL\$S-NH ₂	808.94	809.00
103	Ac-LTF2F\$8HYWAQL\$S-NH ₂	808.94	809.00
104	Ac-LTF3F\$8HYWAQL\$S-NH ₂	808.94	809.00
105	Ac-LTF35F2\$8HYWAQL\$S-NH ₂	817.94	817.95
106	Ac-ETF\$8VYWAQL\$SLA-NH ₂	880.99	880.85
107	Ac-ETF\$8VYWAQL\$SSA-NH ₂	917.49	917.34
108	Ac-LTF34F2\$8HYWAQL\$S-NH ₂	817.94	817.95
109	Ac-LTF\$8EF4coohWAQCba\$AAIa-NH ₂	935.52	935.64
110	Ac-LTF\$8AYWAQL\$SVS-NH ₂	859.98	859.84
111	Ac-LTF\$8AYWAQL\$SVG-NH ₂	844.98	844.81
112	Ac-F\$8KYWEAc3cL\$A-NH ₂	686.89	686.97
113	Ac-LTF\$8AYWAQL\$SAS-NH ₂	845.97	845.85
114	Ac-ETF\$8VYWAQL\$SAA-NH ₂	859.97	859.84
115	Ac-ETF\$8VYWAQL\$SAa-NH ₂	859.97	859.76
116	Ac-ETF\$8VYWAQL\$SVA-NH ₂	873.98	873.82
117	Ac-LTF\$8EYWAQCba\$SAFA-NH ₂	946.51	946.55
118	Ac-LTF34F2\$8EYWAQCba\$SANIeA-NH ₂	947.51	947.57
119	Ac-F\$8AY6clWEAL\$A-NH ₂	669.34	1338.56
120	Ac-LTF\$8EF4coohWAQCba\$SANIeA-NH ₂	943.51	943.59
121	Ac-LTF\$8EYWSQCba\$SANIeA-NH ₂	937.51	937.58
122	Ac-LTF\$8VYWAQL\$SAa-NH ₂	851.99	851.84
123	Ac-LTF%r8AYWAQL%S-NH ₂	767.94	768.00
124	Ac-LTF\$4rn6HYWAQL\$4a6S-NH ₂	827.45	827.50
125	Ac-LTF\$8AYWAQL\$AAIa-NH ₂	886.52	887.09
126	Ac-ETF\$8VYWAQL\$SVS-NH ₂	881.98	881.89
127	Ac-ETF\$8KYWAQL\$SSA-NH ₂	932.00	931.92
128	Ac-ETF\$8AYWAQCba\$AAANIeA-NH ₂	900.50	900.59
129	Ac-ETF\$8VYWAQL\$SSNa-NH ₂	881.47	881.30
130	Ac-LTF\$8AYWVQL\$S-NH ₂	780.95	781.62
131	Ac-LTF\$8VYWAQL\$SSNa-NH ₂	873.49	873.38
132	Ac-ETF\$8VYWAQL\$SSa-NH ₂	867.96	867.83
133	Ac-LTF\$8VYWAQL\$SSa-NH ₂	859.98	859.47
134	Ac-F\$8AAWRAL\$Q-NH ₂	648.39	648.48
135	Ac-TF\$8AAWAAL\$Q-NH ₂	656.38	1311.62
136	Ac-TF\$8AAWRAL\$A-NH ₂	670.40	670.46
137	Ac-LTF\$8AY6clWAQL\$SAA-NH ₂	854.95	855.35
138	Ac-LTF\$8FYWAQL\$A-NH ₂	796.95	797.33
139	Ac-LTF\$8AYWAQL\$AAAibA-NH ₂	872.50	872.92
140	Ac-LTF\$8EYWAQL\$A-NH ₂	787.94	788.10
141	Ac-ALTF\$8AYWAQL\$AAANIeAA-NH ₂	957.55	957.94
142	Ac-LTF\$8AYWAQL\$AAAAAa-NH ₂	936.53	936.94
143	Ac-ETF\$8EYWAQL\$AWNlea-NH ₂	981.02	981.46
144	Ac-LTF\$8EYWAQL\$AWNlea-NH ₂	973.04	973.50
145	Ac-ETF\$8EYWAQL\$AAAAAa-NH ₂	973.51	
146	Ac-LTF\$8EYWAQL\$AAAAAa-NH ₂	1001.05	
147	Ac-LTF\$8EYWAQL\$AAAAAa-NH ₂	965.53	
148	Ac-LTF\$8AYWSQL\$AAANIeA-NH ₂	894.51	894.75
149	Ac-LTF\$8AHWAQL\$S-NH ₂	753.93	754.31
150	Ac-FTF\$8FYWAQL\$S-NH ₂	821.94	822.41

SP#	Sequence	Calc. (M + 2) / 2	Found mass
151	Ac-LTF\$8AYWAQL\$AAAAAAa-NH ₂	972.05	972.50
152	Ac-LTF\$8AYWAQF\$S-NH ₂	783.93	784.20

Table 1b - Selected peptidomimetic macrocycles derived from the MDM2/MDMX-binding helix of p53.

SP#	L	RT*	Ala (%)	IC ₅₀ MDM2 (nM)	SJSA-1 EC ₅₀ (μM)**	Solubility (mg/mL)
1	12	74.2	25	140.7	6	≤ 1
13	15	91.5	33	29.02	1.12	3
142	17	≥ 100	47	30.77	0.18	4.5
151	18	≥ 100	50	10	0.1	5

* Normalized and calculated according to Example 11 (*see* table and equation).

**10% serum, 72 hr

L = length in amino acids; RT= retention time; Ala= alanine content

Table 2a - Peptidomimetic macrocycles that inhibit the MDM2/MDMX and p53 interaction.

SP#	Sequence	Calc. (M + 2)/2	Found Mass
159	Ac-tawyanfekllr-NH ₂	776.92	777.46
160	Ac-tawyanf4CF3ekllr- NH ₂	810.91	811.41
161	Ac-t\$5vya\$5f4CF3ekllr-NH ₂		844.25
162	Ac-tawy\$5nf4CF3e\$5llr-NH ₂		837.03
163	Ac-tawya\$5f4CF3ek\$5lr-NH ₂		822.97
164	Ac-tawyanf4CF3e\$5lr\$5a-NH ₂		908.35
165	Ac-t\$8wyanf4CF3e\$5llr-NH ₂		858.03
166	Ac-tawy\$5nf4CF3ekll\$5a-NH ₂	878.97	879.86
167	Ac-tawya\$5f4CF3ekllr\$5a-NH ₂		936.38
172	Ac-tawy\$5nf4CF3ekll\$5aaaa-NH ₂		

Table 2b - Selected peptidomimetic macrocycles that inhibit the MDM2/MDMX and p53 interaction.

SP#	Ch	L	VH	RT (min)*	Ala (%)	K _i MDM2 (nM)	SJSA-1 EC ₅₀ (μM)**
159	1	12	9.8	5.53	17	19251.34	>30
160	1	12	9.8	6.52	17	48.16	ND
166	0	13	6.3	7.99	15	2.92	1.5
172	0	17	6.7	8.74	35	10.9	0.34

* See Example 11 table

**10% serum, 72 hr

Ch = net charge; L = length in amino acids; VH= von Heijne; RT= retention time; Ala= alanine content

Table 3a - Prepared peptidomimetic macrocycles derived from the PA-binding helix of pB1.

SP#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
174	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	K	V	Aib	A	Q		-NH ₂
175	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	K	A	Aib	A	Q		-NH ₂
176	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	K	V	Aib	A	A		-NH ₂
177	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	K	V	P	Aib	Q		-NH ₂
178	Ac-	F	D	V	N	\$	T	L	L	\$	L	K	V	Aib	A	Q		-NH ₂
179	5-FAM-Ba	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Aib	A	Q		-NH ₂
180	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Aib	A	Q		-NH ₂
181	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Aib	A	Q	A	-NH ₂
182	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	A	A	Q	A	-NH ₂
183	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	A	A	A	A	-NH ₂
184	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	S	A	Q	A	-NH ₂
185	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	A	Q	A	-NH ₂
186	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	E	A	Q	A	-NH ₂
187	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	S	A	A	A	-NH ₂
188	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	A	A	A	-NH ₂
189	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	E	A	A	A	-NH ₂
190	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Aib	A	Q	A	-NH ₂
191	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Aib	A	Q	A	-OH
192	Ac-	Nle	D	V	N	\$r8	T	L	L	F	L	A	\$	A	A	Q		-NH ₂
193	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	Aib	Q	A	-NH ₂
194	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	A	Q	Aib	-NH ₂
195	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	Aib	A	A	-NH ₂
196	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	A	A	Aib	-NH ₂
197	Ac-	Nle	D	V	N	\$	T	L	L	\$	L	A	V	Q	A	Aib	A	-NH ₂
198	Ac-	Nle	D	V	N	\$r8	T	L	L	F	L	A	\$	A	A	Q	A	-NH ₂
199	Ac-	Nle	D	V	N	\$r8	T	L	L	F	L	A	\$	A	A	A	A	-NH ₂
200	Ac-	Nle	D	V	N	\$r8	T	L	L	F	L	A	\$	Q	A	Q	A	-NH ₂
201	Ac-	Nle	D	V	N	\$r8	T	L	L	F	L	A	\$	Q	A	A	A	-NH ₂

Table 3b - Selected peptidomimetic macrocycles derived from the PA-binding helix of pB1.

SP#	Crosslink	Ch	L	VH	RT (min)*	Ala (%)	CPL, EC ₅₀ (μM)**
180	i, i+4	-1	15	4.7	10.68	13	23
185	i, i+4	-1	17	7.2	8.02	24	4.1
188	i, i+4	-1	17	5.1	9.54	29	6.1

SP#	Crosslink	Ch	L	VH	RT (min)*	Ala (%)	CPE, EC ₅₀ (μM)**
193	i, i+4	-1	17	7		18	<6.1***
194	i, i+4	-1	17	7	8.47	18	<6.1***
195	i, i+4	-1	17	4.9	10.01	24	<6.1***
196	i, i+4	-1	17	4.9	10.18	24	<6.1***
197	i, i+4	-1	17	4.9	10.26	24	<6.1***
192	i, i+7	-1	15	4.9	10.18	20	4.5
198	i, i+7	-1	17	5.1	10.92	29	<4.5***
199	i, i+7	-1	17	3	13.03	35	<4.5***
200	i, i+7	-1	17	7.2	8.86	24	<4.5***
201	i, i+7	-1	17	5.1	10.83	29	<4.5***

* See Example 11 table

** By Neutral Red assay (Influenza A Virus H1N1 California/07/2009)

*** Predicted

Ch = net charge; L = length in amino acids; VH= von Heijne; RT= retention time; Ala= alanine content

Table 4a - Prepared peptidomimetic macrocycles derived from the MCL-1/BCL-X_L/BCL-2-binding helix of BIM

SP#		I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Calc.(M + 2) / 2	Found mass
LP2	Ac-	I	W	I	A	Q	E	L	R	R	I	G	D	E	F	N	A	Y	Y	A	R	R	-NH ₂	
202	Ac-	I	W	I	A	Q	E	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A	R	R	-NH ₂	
203	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A	R	R	-NH ₂	1344.74
204	Ac-	I	W	I	A	Q	E	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A	R	R	-NH ₂	1373.56
205	Ac-		W	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1103.12
206	Ac-			I	A	Q	A	L	R	\$r	I	G	D	A	F	A	\$	Y	Y	A			-NH ₂	988.45
207	Ac-			I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	A	A			-NH ₂	963.94
208	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1159.87
209	Ac-		W	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1102.94
210	Ac-			I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1010.06
211	Ac-			I	A	A	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	981.86
212	Ac-			I	A	Q	A	L	A	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	967.45
213	Ac-			I	A	Q	A	L	R	\$r	I	A	D	A	F	N	\$	Y	Y	A			-NH ₂	1016.93
214	Ac-			I	A	Q	A	L	R	\$r	I	G	D	A	A	N	\$	Y	Y	A			-NH ₂	971.89
215	Ac-			I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	A	Y	A			-NH ₂	963.94
216	Ac-	I	\$	I	A	Q	\$	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A			-NH ₂	1185.17
217	Ac-	I	W	I	A	Q	A	L	R	%r	I	G	D	A	F	N	%	Y	Y	A			-NH ₂	1160.14
218	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	E	F	A	\$	Y	Y	A			-NH ₂	1167.14
219	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	Q	A	N	\$	Y	Y	A			-NH ₂	1150.13
220	FITC-Ba	I	W	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1368.67
221	5-FAM-Ba	I	W	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1353.18
222	5-FAM-Ba	I	W	I	A	Q	A	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A			-NH ₂	1382.99
223	Ac-	I	A	I	A	Q	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1102.12
224	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A			-NH ₂	1188.64
225	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1188.15
226	Ac-	I	W	I	A	A	A	L	R	\$r	I	G	D	E	F	N	\$	Y	Y	A			-NH ₂	1160.13
227	Ac-	I	W	I	A	A	A	L	R	\$r	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1159.64
228	Ac-	I	W	I	A	A	A	L	R	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1131.13
229	Ac-	I	W	I	A	Q	A	L	R	\$r	I	G	D	A	F	A	\$	Y	Y	A			-NH ₂	1138.14
230	Ac-	I	W	I	A	Q	A	L	Cit	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1160.98
231	Ac-	I	W	I	A	Q	A	L	Cit	\$r	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1188.64
232	Ac-	I	W	I	A	Q	A	L	H	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1150.12
233	Ac-	I	W	I	A	Q	A	L	H	\$r	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1178.63
234	Ac-	I	W	I	A	Q	A	L	Q	\$r	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1145.62
235	Ac-	I	W	I	A	Q	A	L	Q	\$r	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1174.13

SP#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Calc.(M + 2) / 2	Found mass	
236	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	A	N	\$	Y	Y	A			-NH ₂	1121.62	1122.5
237	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	I	N	\$	Y	Y	A			-NH ₂	1142.65	1143.59
238	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	Q	I	N	\$	Y	Y	A			-NH ₂	1171.16	1171.9
239	Ac-	I	W	I	A	Q	A	A	R	\$r8	I	G	D	A	A	N	\$	Y	Y	A			-NH ₂	1100.6	1101.5
240	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	A	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.83
241	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	A	D	Q	F	N	\$	Y	Y	A			-NH ₂	1195.16	1196.23
242	Ac-	I	W	I	A	Q	A	L	R	\$r8	A	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1138.62	1139.61
243	Ac-	I	W	I	A	Q	A	L	R	\$r8	A	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1167.13	1168.11
244	Ac-	I	W	I	A	Q	A	L	R	\$r8	F	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1176.63	1177.63
245	Ac-	I	W	I	A	Q	A	L	R	\$r8	F	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1205.14	1205.94
246	Ac-	I	W	F	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1176.63	1177.63
247	Ac-	I	W	F	A	Q	A	L	R	\$r8	I	G	D	Q	F	N	\$	Y	Y	A			-NH ₂	1205.14	1206.13
248	Ac-	I	W	I	A	Q	A	L	A	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1117.11	1118.15
249	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	N	A	F	N	\$	Y	Y	A			-NH ₂	1159.15	1159.63
250	Ac-	I	W	I	A	Q	A	A	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1138.62	1139.2
251	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	Q	F	A	\$	Y	Y	A			-NH ₂	1166.65	1167.3
252	Ac-	I	W	Cha	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1179.65	1180.15
253	Ac-	I	W	hhL	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1173.65	1174.39
254	Ac-	I	W	Adm	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1198.66	1199.28
255	Ac-	I	W	hCha	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1186.66	1186.98
256	Ac-	I	W	hF	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1183.64	1184.48
257	Ac-	I	W	Igl	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1190.65	1190.41
258	Ac-	I	W	F4CF3	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1210.62	1211.31
259	Ac-	I	W	F4tBu	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1204.66	1205.39
260	Ac-	I	W	2Nal	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1201.64	1202.2
261	Ac-	I	W	Bip	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1214.65	1215.43
262	Ac-	I	W	I	A	Q	A	Cha	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1179.65	1180.22
263	Ac-	I	W	I	A	Q	A	hhL	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1173.65	1174.4
264	Ac-	I	W	I	A	Q	A	Adm	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1198.66	1199.05
265	Ac-	I	W	I	A	Q	A	hCha	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1186.66	1187.25
266	Ac-	I	W	I	A	Q	A	hAdm	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1205.67	1206.4
267	Ac-	I	W	I	A	Q	A	hF	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1183.64	1184.29
268	Ac-	I	W	I	A	Q	A	Igl	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1190.65	1190.4
269	Ac-	I	W	I	A	Q	A	F4CF3	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1210.62	1210.94
270	Ac-	I	W	I	A	Q	A	F4tBu	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1204.66	1205.29
271	Ac-	I	W	I	A	Q	A	2Nal	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1201.64	1202.15

SP#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		Calc.(M + 2) / 2	Found mass
272	Ac-	I	W	I	A	Q	A	A	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1214.65	1214.91
273	Ac-	I	W	I	A	Q	A	L	R	\$r8	Cba	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1165.64	1166.07
274	Ac-	I	W	I	A	Q	A	L	R	\$r8	hL	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.37
275	Ac-	I	W	I	A	Q	A	L	R	\$r8	Cha	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1179.65	1180.22
276	Ac-	I	W	I	A	Q	A	L	R	\$r8	Tba	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.18
277	Ac-	I	W	I	A	Q	A	L	R	\$r8	hhL	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1173.65	1173.93
278	Ac-	I	AmW	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.18
279	Ac-	I	Aib	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1109.13	1109.46
280	Ac-	AmL	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.27
281	Ac-	I	W	AmL	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1137.37
282	Ac-	I	W	I	Aib	Q	A	L	R	\$r8	I	G	AmD	A	F	N	\$	Y	Y	A			-NH ₂	1173.65	1173.93
283	Ac-	I	W	I	A	Aib	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1138.14	1138.32
284	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	AmD	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.37
285	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	F4F	A			-NH ₂	1160.64	1161.45
289	Ac-	I	W	Tba	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.37
290	Ac-	I	W	hL	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1166.65	1167.37
291	Ac-	I	W	Chg	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1172.65	1173.47
292	Ac-	I	W	Ac6c	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1165.64	1166.44
293	Ac-	I	W	Ac5c	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1158.63	1159.32
294	Ac-	E	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1139.11	1139.52
295	Ac-	R	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1152.64	1153.49
296	Ac-	K	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1138.63	1138.97
297	Ac-	H	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1143.12	1143.87
298	Ac-	S	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1118.1	1118.8
299	Ac-	Q	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1138.62	1139.24
300	Ac-	A	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1110.1	1110.75
301	Ac-	Aib	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1117.11	1117.78
302	Ac-	F	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1148.12	1148.96
303	Ac-	I	D	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1095.6	1096.32
304	Ac-	I	R	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1116.14	1116.95
305	Ac-	I	H	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1106.62	1107.24
306	Ac-	I	S	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1081.6	1181.98
307	Ac-	I	N	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1095.11	1095.58
308	Ac-	I	L	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1094.63	1095.3
309	Ac-	I	F	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1111.62	1112.33
310	Ac-	I	2Nal	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1136.63	1137.3

SP#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		Calc.(M + 2) / 2	Found mass
311	Ac-	I	W	I	S	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1139.13	1139.89
312	Ac-	I	W	I	L	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1152.15	1152.94
313	Ac-	I	W	I	F	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1169.14	1169.86
314	Ac-	I	W	I	A	L	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1152.15	1152.84
315	Ac-	I	W	I	A	A	A	L	K	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1117.13	1117.97
316	Ac-	I	W	I	A	A	A	L	R	\$r8	I	Abu	D	A	F	N	\$	Y	Y	A			-NH ₂	1145.14	1145.9
317	Ac-	I	W	I	A	A	A	L	R	\$r8	I	V	D	A	F	N	\$	Y	Y	A			-NH ₂	1152.15	1152.94
318	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	E	A	F	N	\$	Y	Y	A			-NH ₂	1138.14	1138.87
319	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	G	N	\$	Y	Y	A			-NH ₂	1086.1	1086.89
320	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	W	N	\$	Y	Y	A			-NH ₂	1179.14	1180.04
321	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	hF	N	\$	Y	Y	A			-NH ₂	1166.65	1167.46
322	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F4CF3	N	\$	Y	Y	A			-NH ₂	1193.63	1194.38
323	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F4tBu	N	\$	Y	Y	A			-NH ₂	1187.67	1188.36
324	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	2Nal	N	\$	Y	Y	A			-NH ₂	1184.65	1185.5
325	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	Bip	N	\$	Y	Y	A			-NH ₂	1197.65	1198.54
326	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	D	\$	Y	Y	A			-NH ₂	1131.62	1132.4
327	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	E	\$	Y	Y	A			-NH ₂	1138.63	1139.02
328	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	Q	\$	Y	Y	A			-NH ₂	1138.14	1138.84
329	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	S	\$	Y	Y	A			-NH ₂	1117.62	1118.5
330	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	H	\$	Y	Y	A			-NH ₂	1142.64	1143.25
331	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	L	Y	A			-NH ₂	1106.14	1107.05
332	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1113.63	1114.27
333	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1134.65	1135.33
334	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Cha	A		-NH ₂	1154.66	1155.31
335	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	hF	A		-NH ₂	1158.65	1159.5
336	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	W	A		-NH ₂	1171.15	1171.78
337	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	2Nal	A		-NH ₂	1176.65	1177
338	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	D		-NH ₂	1153.12	1153.77
339	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	E		-NH ₂	1160.13	1160.8
340	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	Q		-NH ₂	1159.64	1160.26
341	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	S		-NH ₂	1139.13	1139.47
342	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	H		-NH ₂	1164.14	1165.05
343	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	R		-NH ₂	1173.66	1174.4
344	Ac-	I	W	I	A	A	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	K		-NH ₂	1159.66	1160.26
345	Ac-	I	W	I	A	Q	A	Aml	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Y	A		-NH ₂	1166.65	1167.18
346	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	AmD	A	F	N	\$	Y	Y	Y	A		-NH ₂	1166.65	1167.46

SP#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Calc.(M + 2)/ 2	Found mass
347	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	F4F	Y	A			-NH ₂	1161.26
348	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	Y	Y	Alb			-NH ₂	1167.46
349	Ac-	I	W	I	A	Q	A	A	Cit	\$r8	I	G	D	A	F	N	\$	Y	Y	A			-NH ₂	1139.71
350	Ac-	I	W	I	A	Q	A	L	Cit	\$r8	I	G	N	A	F	N	\$	Y	Y	A			-NH ₂	1160.4
351	Ac-	I	W	I	A	Q	A	L	Cit	\$r8	I	G	D	A	A	N	\$	Y	Y	A			-NH ₂	1122.87
352	Ac-	I	W	I	A	Q	A	L	Cit	\$r8	I	G	D	A	V	N	\$	Y	Y	A			-NH ₂	1136.47
353	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	A	F	N	\$	A	Y	A			-NH ₂	1113.9
354	Ac-	I	W	I	A	Q	A	L	R	\$r8	hL	G	D	A	F	N	\$	F4F	Y	A			-NH ₂	1168.57
355	Ac-	I	W	I	A	Q	A	L	R	\$r8	hL	G	D	A	F	N	\$	Y	F4F	A			-NH ₂	1168.2
356	Ac-	I	W	I	A	Q	A	L	R	\$r8	hL	G	D	A	F	N	\$	F4F	F4F	A			-NH ₂	1169.59
357	Ac-	A	W	I	A	A	A	L	R	\$r8	hL	G	D	A	F	N	\$	Y	F4F	A			-NH ₂	1118.89
358	Ac-	A	W	I	A	A	A	L	R	\$r8	hL	G	D	A	F	N	\$	A	F4F	A			-NH ₂	1072.92
359	Ac-	I	W	I	A	Q	A	A	R	\$r8	hL	G	D	A	F	N	\$	F4F	F4F	A			-NH ₂	1148.59
286	Ac-	I	\$r8	I	A	Q	A	L	R	St	I	G	D	E	F	N	\$s8	Y	Y	A			-NH ₂	1199.74
287	Ac-	I	W	I	A	\$	A	L	R	St	I	G	D	E	F	N	\$s8	Y	Y	A			-NH ₂	1207.7
288	Ac-	I	W	I	A	Q	A	L	R	\$r8	I	G	D	E	F	N	St	Y	Y	A	\$r5	A	-NH ₂	1307.42

Table 4b - Selected peptidomimetic macrocycles derived from the MCL-1/ BCL-X_L/BCL-2-binding helix of BIM.

SP#	Ch	L	VH	RT (min)*	Ala (%)	IC ₅₀ (nM)		Raji Cell Viability EC ₅₀ (μM)**
						MCL-1	BCL-X _L	
204	0	21	18.9	9.07	9.5	ND	9.2	>30
203	1	21	16.5	10.56	14.3	10.6	3.9	>30
208	0	19	9.1	15.07	21	8.4	22.4	6.6
355	0	19	8.3	17.69	21	27.0	13.0	0.7

* See Example 11 table

**5% serum, 48 hr

Ch = net charge; L = length in amino acids; VH= von Heijne; RT= retention time; Ala= alanine content

[00130] In the sequences shown above and elsewhere, the following abbreviations are used: “Nle” represents norleucine, “Aib” represents 2-aminoisobutyric acid, “Ac” represents acetyl, and “Pr” represents propionyl. Amino acids represented as “\$” are α -Me S5-pentenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. Amino acids represented as “\$r5” are α -Me R5-pentenyl-alanine olefin amino acids connected by an all-carbon comprising one double bond. Amino acids represented as “\$s8” are α -Me S8-octenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. Amino acids represented as “\$r8” are α -Me R8-octenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. “Ahx” represents an aminocyclohexyl linker. The crosslinkers are linear all-carbon crosslinker comprising eight or eleven carbon atoms between the α -carbons of each amino acid. Amino acids represented as “\$/” are α -Me S5-pentenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/r5” are α -Me R5-pentenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/s8” are α -Me S8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/r8” are α -Me R8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “Amw” are α -Me tryptophan amino acids. Amino acids represented as “Aml” are α -Me leucine amino acids. Amino acids represented as “Amf” are α -Me phenylalanine amino acids. Amino acids represented as “2ff” are 2-fluoro-phenylalanine amino acids. Amino acids represented as “3ff” are 3-fluoro-phenylalanine amino acids. Amino acids represented as “St” are amino acids comprising two pentenyl-alanine olefin side chains, each of which is crosslinked to another amino acid as indicated. Amino acids represented as “St/” are amino acids comprising two pentenyl-alanine olefin side chains that are not crosslinked. Amino acids represented as “%St” are amino acids comprising two pentenyl-alanine olefin side chains, each of which is crosslinked to another amino acid as indicated via fully saturated hydrocarbon crosslinks. Amino acids represented as “Ba” are β -alanine. The lower-case character “e” or “z” within the designation of a crosslinked amino acid (*e.g.* “\$er8” or “\$zr8”) represents the configuration of the double bond (*E* or *Z*, respectively). In other contexts, lower-case letters such as “a” or “f” represent D amino acids (*e.g.* D-alanine, or D-phenylalanine, respectively). Amino acids designated as “NmW” represent N-methyltryptophan. Amino acids designated as “NmY” represent N-methyltyrosine. Amino acids designated as “NmA” represent N-methylalanine. Amino acids designated as “Sar” represent sarcosine. Amino acids designated as “Cha” represent cyclohexyl alanine. Amino acids designated as “Cpg” represent cyclopentyl glycine. Amino acids designated as “Chg” represent cyclohexyl glycine. Amino acids designated as “Cba” represent cyclobutyl alanine.

Amino acids designated as “F4I” represent 4-iodo phenylalanine. Amino acids designated as “F3Cl” represent 3-chloro phenylalanine. Amino acids designated as “F4cooh” represent 4-carboxy phenylalanine. Amino acids designated as “F34F2” represent 3,4-difluoro phenylalanine. Amino acids designated as “6clW” represent 6-chloro tryptophan. The designation “iso1” or “iso2” indicates that the peptidomimetic macrocycle is a single isomer.

Methods of Selection

[00131] The properties of peptidomimetic macrocycles are assayed, for example, by using the methods described below. In some embodiments, a selected peptidomimetic macrocycle has improved biological properties relative to a corresponding polypeptide lacking the substituents described herein. “Selecting” refers to the process of identifying polypeptides with enhanced cell permeability, *e.g.*, helical peptidomimetic macrocycles.

[00132] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on one or more properties of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a length of the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a molecular weight (MW) of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a polar amino acids (*e.g.*, S, T, N, Q, C, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a nonpolar amino acids (*e.g.*, A, V, L, I, P, F, M, W, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a hydrophobic amino acids (*e.g.*, G, A, P, V, L, I, F, M, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a β -branched amino acids (*e.g.*, T, V, I, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an aromatic amino acids (*e.g.*, Y, F, W, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a von Heijne value of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a net charge carried by the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected

based on a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the N-terminus of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a positively charged amino acids (*e.g.*, K, R, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a negatively charged amino acids (*e.g.*, D, E, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, N, C, Q, M, S, T, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a charged side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an uncharged side chain hydrogen bonding amino acids (*e.g.*, N, C, Q, M, S, T, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of charged side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, and analogs thereof) over uncharged side chain hydrogen bonding amino acids (*e.g.*, N, C, Q, M, S, T, W, Y, and analogs thereof) in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a tyrosine content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an alanine content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a tryptophan content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a non-natural amino acid content in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a number of halo-substituents in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of fluorine atoms to chlorine atoms in the amino acid

sequence of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a presence or an absence of a carboxylic acid with a substituent in the capping groups of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a presence or an absence of an amine with a substituent in the capping groups of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an amphipathicity of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a partition coefficient of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a solubility of the polypeptide. In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a reverse-phase HPLC retention time of the polypeptide.

[00133] In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a length of the amino acid sequence of the polypeptide, a molecular weight (MW) of the polypeptide, a polar amino acids content in the amino acid sequence of the polypeptide, a nonpolar amino acids content in the amino acid sequence of the polypeptide, a hydrophobic amino acids content in the amino acid sequence of the polypeptide, a β -branched amino acids content in the amino acid sequence of the polypeptide, an aromatic amino acids content in the amino acid sequence of the polypeptide, a von Heijne value of the polypeptide, a net charge carried by the polypeptide, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus of the polypeptide, a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the N-terminus of the polypeptide, a ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the polypeptide, a positively charged amino acids content in the amino acid sequence of the polypeptide, a negatively charged amino acids content in the amino acid sequence of the polypeptide, a side chain hydrogen bonding amino acids content in the amino acid sequence of the polypeptide, a charged side chain hydrogen bonding amino acids content in the amino acid sequence of the polypeptide, an uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the polypeptide, a ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the polypeptide, a tyrosine content in the amino acid sequence of the polypeptide, an alanine content in the amino acid sequence of the polypeptide, a tryptophan content in the amino acid sequence of the polypeptide, a non-natural amino acid content in the amino acid sequence of the polypeptide, a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of

the polypeptide, a ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the polypeptide, a presence or an absence of a carboxylic acid with a substituent in the capping groups of the polypeptide, a presence or an absence of an amine with a substituent in the capping groups of the polypeptide, an amphipathicity of the polypeptide, a partition coefficient of the polypeptide, a solubility of the polypeptide, a reverse-phase HPLC retention time of the polypeptide, or any combination thereof.

[00134] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length of the polypeptide. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 10-24 amino acids. For example, the length of the selected peptidomimetic macrocycle is 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 23 amino acids, or 24 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 10-23, 10-22, 10-21, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, or 10-12 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 11-24, 12-24, 13-24, 14-24, 15-24, 16-24, 17-24, 18-24, 19-24, 20-24, 21-24, or 22-24 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 11 amino acids to 23 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 11-22, 11-21, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, or 11-13 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 12-23, 13-23, 14-23, 15-23, 16-23, 17-23, 18-23, 19-23, 20-23, or 21-23 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 12 amino acids to 22 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 12-21, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, or 12-14 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 13-22, 14-22, 15-22, 16-22, 17-22, 18-22, 19-22, or 20-22 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 13 amino acids to 21 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 13-20, 13-19, 13-18, 13-17, 13-16, or 13-15 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 14-21, 15-21, 16-21, 17-21, 18-21, or 19-21 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 14 amino acids to 20 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 14-19, 14-18, 14-17, or 14-16 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 15-20, 16-20, 17-20, or 18-20 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 15 amino acids to 19 amino acids. For example, the length of the selected

peptidomimetic macrocycle ranges from 15-18 or 15-17 amino acids. For example, the length of the selected peptidomimetic macrocycle ranges from 16-19 or 17-19 amino acids. In some embodiments, the length of the selected peptidomimetic macrocycle ranges from 16 amino acids to 18 amino acids. For example, the length of the selected peptidomimetic macrocycle is 17. In some embodiments, the length of the selected peptidomimetic macrocycle is 14. In some embodiments, the length of the selected peptidomimetic macrocycle is 15. In some embodiments, the length of the selected peptidomimetic macrocycle is 16. In some embodiments, the length of the selected peptidomimetic macrocycle is 17. In some embodiments, the length of the selected peptidomimetic macrocycle is 18. In some embodiments, the length of the selected peptidomimetic macrocycle is 19. In some embodiments, the length of the selected peptidomimetic macrocycle is 20. In some embodiments, the length of the selected peptidomimetic macrocycle is 21.

[00135] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the molecular weight of the polypeptide. In some embodiments, the MW of the selected peptidomimetic macrocycle ranges from about 500 to about 1500 Da. For example, the MW of the selected peptidomimetic macrocycle ranges from about 500 to about 600 Da, from about 500 to about 700 Da, from about 500 to about 800 Da, from about 500 to about 900 Da, from about 500 to about 1000 Da, from about 500 to about 1100 Da, from about 500 to about 1200 Da, from about 500 to about 1300 Da, from about 500 to about 1400 Da, from about 500 to about 1500 Da, from about 600 to about 700 Da, from about 600 to about 800 Da, from about 600 to about 900 Da, from about 600 to about 1000 Da, from about 600 to about 1100 Da, from about 600 to about 1200 Da, from about 600 to about 1300 Da, from about 600 to about 1400 Da, from about 600 to about 1500 Da, from about 700 to about 800 Da, from about 700 to about 900 Da, from about 700 to about 1000 Da, from about 700 to about 1100 Da, from about 700 to about 1200 Da, from about 700 to about 1300 Da, from about 700 to about 1400 Da, from about 700 to about 1500 Da, from about 800 to about 900 Da, from about 800 to about 1000 Da, from about 800 to about 1100 Da, from about 800 to about 1200 Da, from about 800 to about 1300 Da, from about 800 to about 1400 Da, from about 800 to about 1500 Da, from about 900 to about 1000 Da, from about 900 to about 1100 Da, from about 900 to about 1200 Da, from about 900 to about 1300 Da, from about 900 to about 1400 Da, from about 900 to about 1500 Da, from about 1000 to about 1100 Da, from about 1000 to about 1200 Da, from about 1000 to about 1300 Da, from about 1000 to about 1400 Da, from about 1000 to about 1500 Da, from about 1100 to about 1200 Da, from about 1100 to about 1300 Da, from about 1100 to about 1400 Da, from about 1100 to about 1500 Da, from about 1200 to about 1300 Da, from about 1200 to about 1400 Da, from about 1200 to about 1500 Da, from about 1300 to about 1400 Da, from about 1300 to about 1500 Da, or from about 1400 to about 1500 Da. In some embodiments, the MW of the selected

peptidomimetic macrocycle ranges from about 600 to about 1300 Da. In some embodiments, the MW of the selected peptidomimetic macrocycle ranges from about 700 to about 1400 Da. In some embodiments, the MW of the selected peptidomimetic macrocycle ranges from about 800 to about 1500 Da.

[00136] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a polar amino acids (*e.g.*, A, S, T, N, Q, C, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the polar amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected

peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the polar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00137] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a nonpolar amino acids (*e.g.*, A, V, L, I, P, F, M, W, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the nonpolar amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%,

from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the nonpolar amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00138] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the hydrophobic amino acids (*e.g.*, G, A, P, V, L, I, F, M, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the hydrophobic amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%,

56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some

embodiments, the hydrophobic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00139] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the β -branched amino acids (*e.g.*, T, V, I, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the β -branched amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected

peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the β -branched amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00140] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the aromatic amino acids (*e.g.*, Y, F, W, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the aromatic amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35%

to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the aromatic amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00141] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the von Heijne value of the polypeptide. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 2 to 9. For example, the von Heijne value of the selected peptidomimetic macrocycle is 2, 3, 4, 5, 6, 7, 8, or 9, along with all values in between. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 2-8, 2-7, 2-6, 2-5, 2-4, or 2-3. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 3-9, 4-9, 5-9, 6-9, 7-9, or 8-9. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 3 to 8. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 3-7, 3-6, 3-5, or 3-4. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4-8, 5-8, 6-8, or 7-9.

[00142] In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4 to 7. For example, the von Heijne value of the selected peptidomimetic macrocycle

ranges from 4-6 or 4-5. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 5-7 or 5-6. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4-6. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4-5. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5-5.5, including 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, and 5.5 along with all values in between. In some embodiments, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5-9.5. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5-8.5, 4.5-7.5, 4.5-6.5, 5.5-9.5, 5.5-8.5, 5.5-7.5, 5.5-6.5, 6.5-9.5, 6.5-8.5, 6.5-7.5, 7.5-9.5, or 7.5-8.5.

[00143] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the net charge carried by a peptide. For example, peptidomimetic macrocycles carrying a high number of negative charges may have poor cell permeability. A peptidomimetic macrocycle carrying a high number of positive charges may have good cell permeability, but may cause undesired cell damage (*e.g.*, cell lysis). A selected peptidomimetic macrocycle can carry a net charge such that the polypeptide is cell permeable, but not damaging to cells (*e.g.*, does not cause cell lysis). In some embodiments, the net charge of the selected peptidomimetic macrocycle ranges from -4 to +2, including -4, -3, -2, -1, 0, +1, and +2. In some embodiments, the net charge of the selected peptidomimetic macrocycle ranges from -3 to +1, including -3, -2, -1, 0 and +1. In some embodiments, the net charge of the selected peptidomimetic macrocycle ranges from -2 to 0, including -2, -1, and 0. In some embodiments, the net charge of the selected peptidomimetic macrocycle is zero or negative. In some embodiments, the net charge of the selected peptidomimetic macrocycle is not positive. In some embodiments, the net charge of the selected peptidomimetic macrocycle is zero or is not positive. In some embodiments, the net charge of the selected peptidomimetic macrocycle is -2. In some embodiments, the net charge of the selected peptidomimetic macrocycle is -1. In some embodiments, the net charge of the selected peptidomimetic macrocycle is 0.

[00144] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the polypeptide. For example, peptidomimetic macrocycles carrying a high number of negative charges at the C-terminus and/or the N-terminus may have poor cell permeability. A peptidomimetic macrocycle carrying a high number of positive charges the C-terminus and/or the N-terminus may have good cell permeability, but may cause undesired cell damage (*e.g.*, cell lysis). A selected peptidomimetic macrocycle can carry a net charge at the C-terminus and/or the N-terminus such that the polypeptide is cell permeable, but not damaging to cells (*e.g.*, does not cause cell lysis).

In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle ranges from -4 to +2, including -4, -3, -2, -1, 0, +1, and +2. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle ranges from -3 to +1, including -3, -2, -1, 0 and +1. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle ranges from -2 to 0, including -2, -1, and 0. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is zero or negative. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is not positive. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is zero or is not positive. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is -2. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is -1. In some embodiments, the net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at (*e.g.*, closest to) the C-terminus and/or the N-terminus of the selected peptidomimetic macrocycle is 0.

[00145] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of charged amino acids (*e.g.*, K, R, H, D, E, and analogs thereof) over uncharged amino acids in the amino acid sequence of the polypeptide. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. For example, the ratio of charged amino acids over uncharged amino acids of the selected peptidomimetic macrocycle can be 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 or 10.0 along with all values in between. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.2, from 0.2 to 0.4, from 0.4 to 0.6, from 0.6 to 0.8, from 0.8 to 1, from 1 to 2, from 2 to 4, from 4 to 6, from 6 to 8, or from 8 to 10. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.5. In

some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.5 to 1. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1 to 5. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.1. For example, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2, at least about 0.3, at least about 0.4, at least about 0.5, at least about 0.6, at least about 0.7, at least about 0.8, at least about 0.9, at least about 1, at least about 1.5, or at least about 2. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.3. In some embodiments, the ratio of charged amino acids over uncharged amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.4.

[00146] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the positively charged amino acids (*e.g.*, K, R, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the positively charged amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80% along with all values in between. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to

75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the positively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00147] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the negatively charged amino acids (*e.g.*, D, E, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the negatively charged amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%,

38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the

negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the negatively charged amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00148] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, N, C, Q, M, S, T, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the side chain hydrogen bonding amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the side chain

hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00149] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a charged side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the charged side chain hydrogen bonding amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%,

from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the charged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00150] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an uncharged side chain hydrogen bonding amino acids (*e.g.*, N, C, Q, M, S, T, W, Y, and analogs thereof) content in the amino acid sequence of the polypeptide. In some embodiments, the uncharged

side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 80%. For example, the uncharged side chain hydrogen bonding amino acids content of the selected peptidomimetic macrocycle can be 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, along with all values in between. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 15% to 55%, from 15% to 60%, from 15% to 65%, from 15% to 70%, from 15% to 75%, from 15% to 80%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 20% to 55%, from 20% to 60%, from 20% to 65%, from 20% to 70%, from 20% to 75%, from 20% to 80%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 25% to 55%, from 25% to 60%, from 25% to 65%, from 25% to 70%, from 25% to 75%, from 25% to 80%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 30% to 55%, from 30% to 60%, from 30% to 65%, from 30% to 70%, from 30% to 75%, from 30% to 80%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 35% to 55%, from 35% to 60%, from 35% to 65%, from 35% to 70%, from 35% to 75%, from 35% to 80%, from 40% to 45%, from 40% to 50%, from 40% to 55%, from 40% to 60%, from 40% to 65%, from 40% to 70%, from 40% to 75%, from 40% to 80%, from 45% to 50%, from 45% to 55%, from 45% to 60%, from 45% to 65%, from 45% to 70%, from 45% to 75%, from 45% to 80%, from 50% to 55%, from 50% to 60%, from 50% to 65%, from 50% to 70%, from 50% to 75%, from 50% to 80%, from 55% to 60%, from 55% to 65%, from 55% to 70%, from 55% to 75%, from 55% to 80%, from 60% to 65%, from 60% to 70%, from 60% to 75%, from 60% to 80%, from 65% to 70%, from 65% to 75%, from 65% to 80%, from 70% to 75%, from 70% to 80%, or from 75% to 80%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 30% to 50%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 40% to 60%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 50% to 70%. In some

embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. For example, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 30%. In some embodiments, the uncharged side chain hydrogen bonding amino acids content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 40%.

[00151] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of charged side chain hydrogen bonding amino acids (*e.g.*, R, K, D, E, H, and analogs thereof) over uncharged side chain hydrogen bonding amino acids (*e.g.*, N, C, Q, M, S, T, W, Y, and analogs thereof) in the amino acid sequence of the polypeptide. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. For example, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids of the selected peptidomimetic macrocycle can be 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 or 10.0, along with all values in between. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.2, from 0.2 to 0.4, from 0.4 to 0.6, from 0.6 to 0.8, from 0.8 to 1, from 1 to 2, from 2 to 4, from 4 to 6, from 6 to 8, or from 8 to 10. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.5. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.5 to 1. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen

bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1 to 5. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.1. For example, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2, at least about 0.3, at least about 0.4, at least about 0.5, at least about 0.6, at least about 0.7, at least about 0.8, at least about 0.9, at least about 1, at least about 1.5, or at least about 2. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.3. In some embodiments, the ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.4.

[00152] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a tyrosine content of the polypeptide. In some embodiments, the alanine content of the selected peptidomimetic macrocycle ranges from 5% to 50%. For example, the tyrosine content of the selected peptidomimetic macrocycle can be 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50%, along with all values in between. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 10%, from 5% to 15%, from 5% to 20%, from 5% to 25%, from 5% to 30%, from 5% to 35%, from 5% to 40%, from 5% to 45%, from 5% to 50%, from 10% to 15%, from 10% to 20%, from 10% to 25%, from 10% to 30%, from 10% to 35%, from 10% to 40%, from 10% to 45%, from 10% to 50%, from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 40% to 45%, from 40% to 50%, or from 45% to 50%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 30%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 10% to 35%. In

some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 40%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. For example, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 10%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the tyrosine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 25%.

[00153] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on an alanine content of the polypeptide. In some embodiments, the alanine content of the selected peptidomimetic macrocycle ranges from 5% to 50%. For example, the alanine content of the selected peptidomimetic macrocycle can be 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50%, along with all values in between. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 10%, from 5% to 15%, from 5% to 20%, from 5% to 25%, from 5% to 30%, from 5% to 35%, from 5% to 40%, from 5% to 45%, from 5% to 50%, from 10% to 15%, from 10% to 20%, from 10% to 25%, from 10% to 30%, from 10% to 35%, from 10% to 40%, from 10% to 45%, from 10% to 50%, from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 40% to 45%, from 40% to 50%, or from 45% to 50%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 30%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 10% to 35%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 40%. In some embodiments, the alanine content in the amino acid

sequence of the selected peptidomimetic macrocycle is at least about 5%. For example, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 10%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the alanine content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 25%.

[00154] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a tryptophan content of the polypeptide. In some embodiments, the tryptophan content of the selected peptidomimetic macrocycle ranges from 5% to 50%. For example, the tryptophan content of the selected peptidomimetic macrocycle can be 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50%, along with all values in between. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 10%, from 5% to 15%, from 5% to 20%, from 5% to 25%, from 5% to 30%, from 5% to 35%, from 5% to 40%, from 5% to 45%, from 5% to 50%, from 10% to 15%, from 10% to 20%, from 10% to 25%, from 10% to 30%, from 10% to 35%, from 10% to 40%, from 10% to 45%, from 10% to 50%, from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 40% to 45%, from 40% to 50%, or from 45% to 50%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 30%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 10% to 35%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 40%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. For example, the tryptophan content in the amino acid sequence of the selected peptidomimetic

macrocycle is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 10%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the tryptophan content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 25%.

[00155] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a non-natural amino acid content of the polypeptide. In some embodiments, the non-natural amino acid content of the selected peptidomimetic macrocycle ranges from 5% to 50%. For example, the non-natural amino acid content of the selected peptidomimetic macrocycle can be 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50%, along with all values in between. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 10%, from 5% to 15%, from 5% to 20%, from 5% to 25%, from 5% to 30%, from 5% to 35%, from 5% to 40%, from 5% to 45%, from 5% to 50%, from 10% to 15%, from 10% to 20%, from 10% to 25%, from 10% to 30%, from 10% to 35%, from 10% to 40%, from 10% to 45%, from 10% to 50%, from 15% to 20%, from 15% to 25%, from 15% to 30%, from 15% to 35%, from 15% to 40%, from 15% to 45%, from 15% to 50%, from 20% to 25%, from 20% to 30%, from 20% to 35%, from 20% to 40%, from 20% to 45%, from 20% to 50%, from 25% to 30%, from 25% to 35%, from 25% to 40%, from 25% to 45%, from 25% to 50%, from 30% to 35%, from 30% to 40%, from 30% to 45%, from 30% to 50%, from 35% to 40%, from 35% to 45%, from 35% to 50%, from 40% to 45%, from 40% to 50%, or from 45% to 50%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5% to 30%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 10% to 35%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 15% to 40%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. For example, the non-natural amino acid content in the amino acid sequence of the

selected peptidomimetic macrocycle is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 5%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 10%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 15%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 20%. In some embodiments, the non-natural amino acid content in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 25%.

[00156] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a number of halo-substituents (*e.g.*, F, Cl, Br, or I) in the amino acid sequence of the polypeptide. In some embodiments, the number of halo-substituents of the selected peptidomimetic macrocycle ranges from 1 to 20. For example, the number of halo-substituents of the selected peptidomimetic macrocycle can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, along with all values in between. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 1 to 10, from 1 to 15, from 1 to 20, from 2 to 3, from 2 to 4, from 2 to 5, from 2 to 10, from 2 to 15, from 2 to 20, from 3 to 4, from 3 to 5, from 3 to 10, from 3 to 15, from 3 to 20, from 4 to 5, from 4 to 10, from 4 to 15, from 4 to 20, from 5 to 10, from 5 to 15, from 5 to 20, from 10 to 15, from 10 to 20, or from 15 to 20. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-5. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5-10. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-10. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 1. For example, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least about 15. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 3. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 4. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected

peptidomimetic macrocycle is at least 5. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 10.

[00157] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the polypeptide. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine of the selected peptidomimetic macrocycle ranges from 1 to 20. For example, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine of the selected peptidomimetic macrocycle can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, along with all values in between. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 1 to 10, from 1 to 15, from 1 to 20, from 2 to 3, from 2 to 4, from 2 to 5, from 2 to 10, from 2 to 15, from 2 to 20, from 3 to 4, from 3 to 5, from 3 to 10, from 3 to 15, from 3 to 20, from 4 to 5, from 4 to 10, from 4 to 15, from 4 to 20, from 5 to 10, from 5 to 15, from 5 to 20, from 10 to 15, from 10 to 20, or from 15 to 20. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-5. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5-10. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-10. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 1. For example, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least about 15. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 3. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 4. In some embodiments, the number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 5. In some embodiments, the

number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine in the amino acid sequence of the selected peptidomimetic macrocycle is at least 10.

[00158] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the polypeptide. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 10. For example, the ratio of fluorine atoms to chlorine atoms of the selected peptidomimetic macrocycle can be 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 or 10.0, along with all values in between. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.2, from 0.2 to 0.4, from 0.4 to 0.6, from 0.6 to 0.8, from 0.8 to 1, from 1 to 2, from 2 to 4, from 4 to 6, from 6 to 8, or from 8 to 10. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.1 to 0.5. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 0.5 to 1. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1 to 5. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.1. For example, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2, at least about 0.3, at least about 0.4, at least about 0.5, at least about 0.6, at least about 0.7, at least about 0.8, at least about 0.9, at least about 1, at least about 1.5, or at least about 2. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.2. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.3. In some embodiments, the ratio of fluorine atoms to chlorine atoms in the amino acid sequence of the selected peptidomimetic macrocycle is at least about 0.4.

[00159] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a number of halo-substituents (*e.g.*, F, Cl, Br, or I) in the amino acid sequence of the polypeptide. In some embodiments, the number of halo-substituents of the selected peptidomimetic macrocycle ranges from 1 to 20. For example, the number of halo-substituents of the selected peptidomimetic macrocycle can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, along with all values in between. In some embodiments, the number of halo-substituents in the amino acid

sequence of the selected peptidomimetic macrocycle ranges from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 1 to 10, from 1 to 15, from 1 to 20, from 2 to 3, from 2 to 4, from 2 to 5, from 2 to 10, from 2 to 15, from 2 to 20, from 3 to 4, from 3 to 5, from 3 to 10, from 3 to 15, from 3 to 20, from 4 to 5, from 4 to 10, from 4 to 15, from 4 to 20, from 5 to 10, from 5 to 15, from 5 to 20, from 10 to 15, from 10 to 20, or from 15 to 20. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-5. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 5-10. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle ranges from 1-10. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 1. For example, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least about 15. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 2. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 3. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 4. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 5. In some embodiments, the number of halo-substituents in the amino acid sequence of the selected peptidomimetic macrocycle is at least 10.

[00160] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a presence or an absence of a carboxylic acid with a substituent in the capping groups of the polypeptide. In some embodiments, the peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the presence of a carboxylic acid with a substituent in the capping groups of the polypeptide. In some embodiments, the peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the absence of a carboxylic acid with a substituent in the capping groups of the polypeptide. In some embodiments, the peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the presence of an amine with a substituent in the capping groups of the polypeptide. In some embodiments, the peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the absence of an amine with a substituent in the capping groups of the polypeptide.

[00161] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on a partition coefficient (*e.g.*, $\log P$) of the peptide. For example, the $\log P$ of the selected peptidomimetic macrocycle can be measured as $\log P_{\text{octanol/water}}$ at 37°C. In some embodiments, the

$\log P_{\text{octanol/water}}$ of the selected peptidomimetic macrocycle ranges from -5.0 to 5.0, including -5.0, -4.0, -3.0, -2.0, -1.0, 0, 1.0, 2.0, 3.0, 4.0, and 5.0, along with all values in between. In some embodiments, the $\log P_{\text{octanol/water}}$ of the selected peptidomimetic macrocycle ranges from -5 to 5.0, for example, from -5 to -4, from -5 to -3, from -5 to -2, from -5 to -1, from -5 to 0, from -5 to 1, from -5 to 2, from -5 to 3, from -5 to 4, from -5 to 5, from -4 to -3, from -4 to -2, from -4 to -1, from -4 to 0, from -4 to 1, from -4 to 2, from -4 to 3, from -4 to 4, from -4 to 5, from -3 to -2, from -3 to -1, from -3 to 0, from -3 to 1, from -3 to 2, from -3 to 3, from -3 to 4, from -3 to 5, from -2 to -1, from -2 to 0, from -2 to 1, from -2 to 2, from -2 to 3, from -2 to 4, from -2 to 5, from -1 to 0, from -1 to 1, from -1 to 2, from -1 to 3, from -1 to 4, from -1 to 5, from 0 to 1, from 0 to 2, from 0 to 3, from 0 to 4, from 0 to 5, from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 3, from 2 to 4, from 2 to 5, from 3 to 4, from 3 to 5, or from 4 to 5. In some embodiments, the $\log P_{\text{octanol/water}}$ of the selected peptidomimetic macrocycle ranges from -2 to 2. In some embodiments, the $\log P_{\text{octanol/water}}$ of the selected peptidomimetic macrocycle is at least -5, for example, at least -4, at least -3, at least -2, at least -1, at least 0, at least 1, at least 2, at least 3, at least 4, or at least 5.

[00162] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length and the von Heijne value of the polypeptide. For example, the length of the selected peptidomimetic macrocycle ranges from 10 amino acids to 24 amino acids, from 11 amino acids to 23 amino acids, from 12 amino acids to 22 amino acids, from 13 amino acids to 21 amino acids, from 14 amino acids to 20 amino acids, from 15 amino acids to 19 amino acids, or from 16 amino acids to 18 amino acids, and the von Heijne value of the selected peptidomimetic macrocycle ranges from 2 to 9, from 3 to 8, from 4 to 7, from 4 to 6, or from 4 to 5. For example, the length of the selected peptidomimetic macrocycle is 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, or 21 amino acids, and the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5 to 5.5. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, and a von Heijne value ranging from 4 and 7.

[00163] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length and the alanine content of the polypeptide. For example, the length of the selected peptidomimetic macrocycle ranges from 10 amino acids to 24 amino acids, from 11 amino acids to 23 amino acids, from 12 amino acids to 22 amino acids, from 13 amino acids to 21 amino acids, from 14 amino acids to 20 amino acids, from 15 amino acids to 19 amino acids, or from 16 amino acids to 18 amino acids, and the alanine content of the selected peptidomimetic macrocycle ranges from 15% to 50%, including 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%,

44%, 45%, 46%, 47%, 48%, 49%, and 50% along with all values in between. For example, the length of the selected peptidomimetic macrocycle is 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, or 21 amino acids, and the alanine content of the selected peptidomimetic macrocycle ranges from 25% to 40%, including 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, and 40% along with all values in between. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, and an alanine content ranging from 25% to 40%.

[00164] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length and the net charge of the polypeptide. For example, the length of the selected peptidomimetic macrocycle ranges from 10 amino acids to 24 amino acids, from 11 amino acids to 23 amino acids, from 12 amino acids to 22 amino acids, from 13 amino acids to 21 amino acids, from 14 amino acids to 20 amino acids, from 15 amino acids to 19 amino acids, or from 16 amino acids to 18 amino acids, and the net charge of the selected peptidomimetic macrocycle ranges from -3 to 1, including -3, -2, -1, 0 and 1. For example, the length of the selected peptidomimetic macrocycle is 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, or 21 amino acids, and the net charge of the selected peptidomimetic macrocycle ranges from -3 to 1, including -3, -2, -1, 0 and 1. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, and a net charge ranging from -2 to 0.

[00165] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the von Heijne value and the net charge of the polypeptide. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 2 to 9, from 3 to 8, from 4 to 7, from 4 to 6, or from 4 to 5, and the net charge of the selected peptidomimetic macrocycle ranges from -3 to 1, including -3, -2, -1, 0 and 1. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5 to 5.5, including 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, and 5.5 along with all values in between, and the net charge of the selected peptidomimetic macrocycle ranges from -3 to 1, including -3, -2, -1, 0 and 1. For example, the selected peptidomimetic macrocycle has a von Heijne value ranging from 4 and 7, and a net charge ranging from -2 to 0.

[00166] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the von Heijne value and the alanine content of the polypeptide. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 2 to 9, from 3 to 8, from 4 to 7, from 4 to 6, or from 4 to 5, and the alanine content of the selected peptidomimetic macrocycle ranges from 15% to 50%, including 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%,

29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, and 50% along with all values in between. For example, the von Heijne value of the selected peptidomimetic macrocycle ranges from 4.5 to 5.5, including 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, and 5.5 along with all values in between, and the alanine content of the selected peptidomimetic macrocycle ranges from 25% to 40%, including 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, and 40% along with all values in between. For example, the selected peptidomimetic macrocycle has a von Heijne value ranging from 4 and 7, and an alanine content ranging from 25% to 40%.

[00167] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on length, von Heijne value and alanine content of the polypeptide. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, a von Heijne value ranging from 4 and 7, and an alanine content ranging from 25% to 40%.

[00168] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the von Heijne value and the net charge of the polypeptide. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, a von Heijne value ranging from 4 and 7, and a net charge ranging from -2 to 0.

[00169] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the von Heijne value, the net charge, and the alanine content of the polypeptide. For example, the selected peptidomimetic macrocycle has a von Heijne value ranging from 4 and 7, a net charge ranging from -2 to 0, and an alanine content ranging from 25% to 40%.

[00170] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length, the net charge, and the alanine content of the polypeptide. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, a net charge ranging from -2 to 0, and an alanine content ranging from 25% to 40%.

[00171] A peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the length of its amino acid sequence, its von Heijne value, its net charge, and the alanine content of its amino acid sequence. For example, the selected peptidomimetic macrocycle has a length ranging from 14 amino acids to 20 amino acids, a von Heijne value ranging from 4 and 7, a net charge ranging from -2 to 0, and an alanine content ranging from 25% to 40%.

[00172] In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on the reverse-phase HPLC retention time of the polypeptide.

[00173] In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on amphipathicity of the polypeptide.

[00174] In some embodiments, a peptidomimetic macrocycle with enhanced cell penetrability can be selected based on solubility of the polypeptide, for example if the selected peptidomimetic macrocycle is determined to be soluble based on visual examination of the turbidity of a solution of the polypeptide.

Assays

Assay to Determine α -helicity

[00175] In solution, the secondary structure of polypeptides with α -helical domains can reach a dynamic equilibrium between random coil structures and α -helical structures, often expressed as a “percent helicity”. Thus, for example, α -helical domains can be predominantly random coils in solution, with α -helical content usually under 25%. Peptidomimetic macrocycles with optimized linkers, on the other hand, can possess, for example, an α -helicity that is at least two-fold greater than that of a corresponding uncrosslinked polypeptide. In some embodiments, macrocycles can possess an α -helicity of greater than 50%. To assay the helicity of peptidomimetic macrocycles, the compounds are dissolved in an aqueous solution (*e.g.* 50 mM potassium phosphate solution at pH 7, or distilled H₂O, to concentrations of 25-50 μ M). Circular dichroism (CD) spectra are obtained on a spectropolarimeter (*e.g.*, Jasco J-710) using standard measurement parameters (*e.g.* temperature, 20°C; wavelength, 190-260 nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 sec; bandwidth, 1 nm; path length, 0.1 cm). The α -helical content of each peptide is calculated by dividing the mean residue ellipticity (*e.g.* $[\Phi]_{222\text{obs}}$) by the reported value for a model helical decapeptide (Yang *et al.* (1986), *Methods Enzymol.* 130:208)).

Assay to Determine Melting Temperature (T_M)

[00176] A peptidomimetic macrocycle comprising a secondary structure such as an α -helix exhibits, for example, a higher melting temperature than a corresponding uncrosslinked polypeptide. Typically peptidomimetic macrocycles exhibit T_M of $> 60^\circ\text{C}$ representing a highly stable structure in aqueous solutions. To assay the effect of macrocycle formation on melting temperature, peptidomimetic macrocycles or unmodified peptides are dissolved in distilled H₂O (*e.g.* at a final concentration of 50 μ M) and the T_M is determined by measuring the change in ellipticity over a temperature range (*e.g.* 4 to 95 $^\circ\text{C}$) on a spectropolarimeter (*e.g.*, Jasco J-710) using standard parameters (*e.g.* wavelength 222nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 sec; bandwidth, 1 nm; temperature increase rate: 1 $^\circ\text{C}/\text{min}$; path length, 0.1 cm).

Protease Resistance Assay

[00177] The amide bond of the peptide backbone is susceptible to hydrolysis by proteases, thereby rendering peptidic compounds vulnerable to rapid degradation *in vivo*. Peptide helix formation, however, typically buries the amide backbone and therefore can shield it from proteolytic cleavage.

The peptidomimetic macrocycles can be subjected to *in vitro* trypsin proteolysis to assess for any change in degradation rate compared to a corresponding uncrosslinked polypeptide. For example, the peptidomimetic macrocycle and a corresponding uncrosslinked polypeptide are incubated with trypsin agarose and the reactions quenched at various time points by centrifugation and subsequent HPLC injection to quantitate the residual substrate by ultraviolet absorption at 280 nm. Briefly, the peptidomimetic macrocycle and peptidomimetic precursor (5 mcg) are incubated with trypsin agarose (Pierce) (S/E ~125) for 0, 10, 20, 90, and 180 min. Reactions are quenched by tabletop centrifugation at high speed; remaining substrate in the isolated supernatant is quantified by HPLC-based peak detection at 280 nm. The proteolytic reaction displays first order kinetics and the rate constant, k , is determined from a plot of $\ln[S]$ versus time ($k = -1 \times \text{slope}$)

Ex Vivo Stability Assay.

[00178] Peptidomimetic macrocycles with optimized linkers possess, for example, an *ex vivo* half-life that is at least two-fold greater than that of a corresponding uncrosslinked polypeptide, and possess an *ex vivo* half-life of 12 hr or more. For *ex vivo* serum stability studies, a variety of assays can be used. For example, a peptidomimetic macrocycle and a corresponding uncrosslinked polypeptide (2 mcg) are incubated with fresh mouse, rat and/or human serum (2 mL) at 37 °C for 0, 1, 2, 4, 8, and 24 hr. To determine the level of intact compound, the following procedure can be used: The samples are extracted by transferring 100 μ L of sera to 2 mL centrifuge tubes followed by the addition of 10 μ L of 50 % formic acid and 500 μ L acetonitrile and centrifugation at 14,000rpm for 10 min at 4 ± 2 °C. The supernatants are then transferred to fresh 2 mL tubes and evaporated on Turbovap under N_2 < 10 psi, 37 °C. The samples are reconstituted in 100 μ L of 50:50 acetonitrile:water and submitted to LC-MS/MS analysis.

In vitro Binding Assays.

[00179] To assess the binding and affinity of peptidomimetic macrocycles and peptidomimetic precursors to acceptor proteins, a fluorescence polarization assay (FPA) is used, for example. The FPA technique measures the molecular orientation and mobility using polarized light and fluorescent tracer. When excited with polarized light, fluorescent tracers (*e.g.*, FITC) attached to molecules with high apparent molecular weights (*e.g.* FITC-labeled peptides bound to a large protein) emit higher levels of polarized fluorescence due to their slower rates of rotation as compared to fluorescent tracers attached to smaller molecules (*e.g.* FITC-labeled peptides that are free in solution).

[00180] For example, fluoresceinated peptidomimetic macrocycles (25 nM) are incubated with the acceptor protein (25-1000 nM) in binding buffer (140 mM NaCl, 50 mM Tris-HCl, pH 7.4) for 30 min at room temperature. Binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (*e.g.* Perkin-Elmer LS50B). K_D values can be determined by

nonlinear regression analysis using, for example, GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). A peptidomimetic macrocycle shows, in some embodiments, similar or lower K_D than a corresponding uncrosslinked polypeptide.

In vitro Displacement Assays To Characterize Antagonists of Peptide-Protein Interactions.

[00181] To assess the binding and affinity of compounds that antagonize the interaction between a peptide and an acceptor protein, a fluorescence polarization assay (FPA) utilizing a fluoresceinated peptidomimetic macrocycle derived from a peptidomimetic precursor sequence is used, for example. The FPA technique measures the molecular orientation and mobility using polarized light and fluorescent tracer. When excited with polarized light, fluorescent tracers (*e.g.*, FITC) attached to molecules with high apparent molecular weights (*e.g.* FITC-labeled peptides bound to a large protein) emit higher levels of polarized fluorescence due to their slower rates of rotation as compared to fluorescent tracers attached to smaller molecules (*e.g.* FITC-labeled peptides that are free in solution). A compound that antagonizes the interaction between the fluoresceinated peptidomimetic macrocycle and an acceptor protein will be detected in a competitive binding FPA experiment.

[00182] For example, putative antagonist compounds (1 nM to 1 mM) and a fluoresceinated peptidomimetic macrocycle (25 nM) are incubated with the acceptor protein (50 nM) in binding buffer (140 mM NaCl, 50 mM Tris-HCl, pH 7.4) for 30 min at room temperature. Antagonist binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (*e.g.* Perkin-Elmer LS50B). K_D values can be determined by nonlinear regression analysis using, for example, GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

[00183] Any class of molecule, such as small organic molecules, peptides, oligonucleotides or proteins can be examined as putative antagonists in this assay.

Assay for Protein-ligand binding by Affinity Selection-Mass Spectrometry

[00184] To assess the binding and affinity of test compounds for proteins, an affinity-selection mass spectrometry assay is used, for example. Protein-ligand binding experiments are conducted according to the following representative procedure outlined for a system-wide control experiment using 1 μ M peptidomimetic macrocycle plus 5 μ M hMDM2. A 1 μ L DMSO aliquot of a 40 μ M stock solution of peptidomimetic macrocycle is dissolved in 19 μ L of PBS (Phosphate-buffered saline: 50 mM, pH 7.5 Phosphate buffer containing 150 mM NaCl). The resulting solution is mixed by repeated pipetting and clarified by centrifugation at 10,000g for 10 min. To a 4 μ L aliquot of the resulting supernatant is added 4 μ L of 10 μ M hMDM2 in PBS. Each 8.0 μ L experimental sample thus contains 40 pmol (1.5 μ g) of protein at 5.0 μ M concentration in PBS plus 1 μ M peptidomimetic macrocycle and 2.5% DMSO. Duplicate samples thus prepared for each concentration point are incubated for 60 min at room temperature, and then chilled to 4 °C prior to size-exclusion

chromatography-LC-MS analysis of 5.0 μL injections. Samples containing a target protein, protein–ligand complexes, and unbound compounds are injected onto an SEC column, where the complexes are separated from non-binding component by a rapid SEC step. The SEC column eluate is monitored using UV detectors to confirm that the early-eluting protein fraction, which elutes in the void volume of the SEC column, is well resolved from unbound components that are retained on the column. After the peak containing the protein and protein–ligand complexes elutes from the primary UV detector, it enters a sample loop where it is excised from the flow stream of the SEC stage and transferred directly to the LC-MS via a valving mechanism. The $(M + 3H)^{3+}$ ion of the peptidomimetic macrocycle is observed by ESI-MS at the expected m/z , confirming the detection of the protein-ligand complex.

Assay for Protein-ligand K_D Titration Experiments.

[00185] To assess the binding and affinity of test compounds for proteins, a protein-ligand K_D titration experiment is performed, for example. Protein-ligand K_D titrations experiments are conducted as follows: 2 μL DMSO aliquots of a serially diluted stock solution of titrant peptidomimetic macrocycle (5, 2.5, ..., 0.098 mM) are prepared then dissolved in 38 μL of PBS. The resulting solutions are mixed by repeated pipetting and clarified by centrifugation at 10 000g for 10 min. To 4.0 μL aliquots of the resulting supernatants is added 4.0 μL of 10 μM hMDM2 in PBS. Each 8.0 μL experimental sample thus contains 40 pmol (1.5 μg) of protein at 5.0 μM concentration in PBS, varying concentrations (125, 62.5, ..., 0.24 μM) of the titrant peptide, and 2.5% DMSO. Duplicate samples thus prepared for each concentration point are incubated at room temperature for 30 min, then chilled to 4 $^{\circ}\text{C}$ prior to SEC-LC-MS analysis of 2.0 μL injections. The $(M + H)^{1+}$, $(M + 2H)^{2+}$, $(M + 3H)^{3+}$, and/or $(M + Na)^{1+}$ ion is observed by ESI-MS; extracted ion chromatograms are quantified, then fit to equations to derive the binding affinity K_D as described in “*A General Technique to Rank Protein-Ligand Binding Affinities and Determine Allosteric vs. Direct Binding Site Competition in Compound Mixtures.*” Annis, D. A.; Nazef, N.; Chuang, C. C.; Scott, M. P.; Nash, H. M. *J. Am. Chem. Soc.* 2004, 126, 15495-15503; also in “*ALIS: An Affinity Selection-Mass Spectrometry System for the Discovery and Characterization of Protein-Ligand Interactions*” D. A. Annis, C.-C. Chuang, and N. Nazef. In *Mass Spectrometry in Medicinal Chemistry*. Edited by Wanner K, Höfner G: Wiley-VCH; 2007:121-184. Mannhold R, Kubinyi H, Folkers G (Series Editors): *Methods and Principles in Medicinal Chemistry*

Assay for Competitive Binding Experiments by Affinity Selection-Mass Spectrometry

[00186] To determine the ability of test compounds to bind competitively to proteins, an affinity selection mass spectrometry assay is performed, for example. A mixture of ligands at 40 μM per component is prepared by combining 2 μL aliquots of 400 μM stocks of each of the three

compounds with 14 μL of DMSO. Then, 1 μL aliquots of this 40 μM per component mixture are combined with 1 μL DMSO aliquots of a serially diluted stock solution of titrant peptidomimetic macrocycle (10, 5, 2.5, ..., 0.078 mM). These 2 μL samples are dissolved in 38 μL of PBS. The resulting solutions were mixed by repeated pipetting and clarified by centrifugation at 10 000g for 10 min. To 4.0 μL aliquots of the resulting supernatants is added 4.0 μL of 10 μM hMDM2 protein in PBS. Each 8.0 μL experimental sample thus contains 40 pmol (1.5 μg) of protein at 5.0 μM concentration in PBS plus 0.5 μM ligand, 2.5% DMSO, and varying concentrations (125, 62.5, ..., 0.98 μM) of the titrant peptidomimetic macrocycle. Duplicate samples thus prepared for each concentration point are incubated at room temperature for 60 min, then chilled to 4 $^{\circ}\text{C}$ prior to SEC-LC-MS analysis of 2.0 μL injections. Additional details on these and other methods are provided in "A General Technique to Rank Protein-Ligand Binding Affinities and Determine Allosteric vs. Direct Binding Site Competition in Compound Mixtures." Annis, D. A.; Nazef, N.; Chuang, C. C.; Scott, M. P.; Nash, H. M. *J. Am. Chem. Soc.* **2004**, *126*, 15495-15503; also in "ALIS: An Affinity Selection-Mass Spectrometry System for the Discovery and Characterization of Protein-Ligand Interactions" D. A. Annis, C.-C. Chuang, and N. Nazef. In Mass Spectrometry in Medicinal Chemistry. Edited by Wanner K, Höfner G: Wiley-VCH; **2007**:121-184. Mannhold R, Kubinyi H, Folkers G (Series Editors): Methods and Principles in Medicinal Chemistry.

Binding Assays in Intact Cells.

[00187] It is possible to measure binding of peptides or peptidomimetic macrocycles to their natural acceptors in intact cells by immunoprecipitation experiments. For example, intact cells are incubated with fluoresceinated (FITC-labeled) compounds for 4 hr in the absence of serum, followed by serum replacement and further incubation that ranges from 4-18 hr. Cells are then pelleted and incubated in lysis buffer (50mM Tris [pH 7.6], 150 mM NaCl, 1% CHAPS and protease inhibitor cocktail) for 10 min at 4 $^{\circ}\text{C}$. Extracts are centrifuged at 14,000 rpm for 15 min and supernatants collected and incubated with 10 μL goat anti-FITC antibody for 2 hr, rotating at 4 $^{\circ}\text{C}$ followed by further 2 hr incubation at 4 $^{\circ}\text{C}$ with protein A/G Sepharose (50 μL of 50% bead slurry). After quick centrifugation, the pellets are washed in lysis buffer containing increasing salt concentration (e.g., 150, 300, 500 mM). The beads are then re-equilibrated at 150 mM NaCl before addition of SDS-containing sample buffer and boiling. After centrifugation, the supernatants are optionally electrophoresed using 4%-12% gradient Bis-Tris gels followed by transfer into Immobilon-P membranes. After blocking, blots are optionally incubated with an antibody that detects FITC and also with one or more antibodies that detect proteins that bind to the peptidomimetic macrocycle.

Cellular Penetrability Assays.

[00188] A peptidomimetic macrocycle is, for example, more cell penetrable compared to a corresponding uncrosslinked macrocycle. Peptidomimetic macrocycles with optimized linkers possess, for example, cell penetrability that is at least two-fold greater than a corresponding uncrosslinked macrocycle, and often 20% or more of the applied peptidomimetic macrocycle will be observed to have penetrated the cell after 4 hours. To measure the cell penetrability of peptidomimetic macrocycles and corresponding uncrosslinked macrocycle, intact cells are incubated with fluorescently-labeled (*e.g.* fluoresceinated) peptidomimetic macrocycles or corresponding uncrosslinked macrocycle (10 μ M) for 4 hr in serum free media at 37 °C, washed twice with media and incubated with trypsin (0.25%) for 10 min at 37°C. The cells are washed again and resuspended in PBS. Cellular fluorescence is analyzed, for example, by using either a FACSCalibur flow cytometer or Cellomics' KineticScan ® HCS Reader.

Cellular Efficacy Assays.

[00189] The efficacy of certain peptidomimetic macrocycles is determined, for example, in cell-based killing assays using a variety of tumorigenic and non-tumorigenic cell lines and primary cells derived from human or mouse cell populations. Cell viability is monitored, for example, over 24-96 hr of incubation with peptidomimetic macrocycles (0.5 to 50 μ M) to identify those that kill at $EC_{50} < 10$ μ M. Several standard assays that measure cell viability are commercially available and are optionally used to assess the efficacy of the peptidomimetic macrocycles. In addition, assays that measure Annexin V and caspase activation are optionally used to assess whether the peptidomimetic macrocycles kill cells by activating the apoptotic machinery. For example, the Cell Titer-glo assay is used which determines cell viability as a function of intracellular ATP concentration.

In Vivo Stability Assay.

[00190] To investigate the *in vivo* stability of the peptidomimetic macrocycles, the compounds are, for example, administered to mice and/or rats by IV, IP, PO or inhalation routes at concentrations ranging from 0.1 to 50 mg/kg and blood specimens withdrawn at 0', 5', 15', 30', 1 hr, 4 hr, 8 hr and 24 hr post-injection. Levels of intact compound in 25 μ L of fresh serum are then measured by LC-MS/MS as above.

In vivo Efficacy in Animal Models.

[00191] To determine the anti-oncogenic activity of peptidomimetic macrocycles *in vivo*, the compounds are, for example, given alone (IP, IV, PO, by inhalation or nasal routes) or in combination with sub-optimal doses of relevant chemotherapy (*e.g.*, cyclophosphamide, doxorubicin, etoposide). In one example, 5×10^6 RS4;11 cells (established from the bone marrow of a patient with acute lymphoblastic leukemia) that stably express luciferase are injected by tail vein in NOD-SCID mice 3 hr after they have been subjected to total body irradiation. If left untreated, this form of

leukemia is fatal in 3 weeks in this model. The leukemia is readily monitored, for example, by injecting the mice with D-luciferin (60 mg/kg) and imaging the anesthetized animals (*e.g.*, Xenogen *In Vivo* Imaging System, Caliper Life Sciences, Hopkinton, MA). Total body bioluminescence is quantified by integration of photonic flux (photons/sec) by Living Image Software (Caliper Life Sciences, Hopkinton, MA). Peptidomimetic macrocycles alone or in combination with sub-optimal doses of relevant chemotherapeutics agents are, for example, administered to leukemic mice (10 days after injection/day 1 of experiment, in bioluminescence range of 14-16) by tail vein or IP routes at doses ranging from 0.1mg/kg to 50 mg/kg for 7 to 21 days. Optionally, the mice are imaged throughout the experiment every other day and survival monitored daily for the duration of the experiment. Expired mice are optionally subjected to necropsy at the end of the experiment. Another animal model is implantation into NOD-SCID mice of DoHH2, a cell line derived from human follicular lymphoma, that stably expresses luciferase. These *in vivo* tests optionally generate preliminary pharmacokinetic, pharmacodynamic and toxicology data.

Clinical Trials.

[00192] To determine the suitability of the peptidomimetic macrocycles for treatment of humans, clinical trials are performed. For example, patients diagnosed with cancer and in need of treatment can be selected and separated in treatment and one or more control groups, wherein the treatment group is administered a peptidomimetic macrocycle, while the control groups receive a placebo or a known anti-cancer drug. The treatment safety and efficacy of the peptidomimetic macrocycles can thus be evaluated by performing comparisons of the patient groups with respect to factors such as survival and quality-of-life. In this example, the patient group treated with a peptidomimetic macrocycle can show improved long-term survival compared to a patient control group treated with a placebo.

In vitro Testing for Inhibition of Influenza Replication

[00193] This influenza antiviral evaluation assay examines the effects of compounds at designated dose-response concentrations. See also Noah, J.W., W. Severson, D.L. Noah, L. Rasmussen, E.L. White, and C.B. Jonsson, *Antiviral Res*, 2007. **73**(1): p. 50-9. Madin Darby canine kidney (MDCK) cells are used in the assay to test the efficacy of the compounds in preventing the cytopathic effect (CPE) induced by influenza infection. Either Ribavirin or Tamiflu is included in each run as a positive control compound. Subconfluent cultures of MDCK cells are plated into 96-well plates for the analysis of cell viability (cytotoxicity) and antiviral activity (CPE). Drugs are added to the cells 24 hr later. At a designated time, the CPE wells also receive 100 tissue culture infectious doses (100 TCID₅₀s) of titered influenza virus. 72 hr later the cell viability is determined. The effective compound concentrations which reduce viral-induced CPE by 25% (IC₂₅), 50% (IC₅₀), and 90%

(IC₉₀) are calculated by regression analysis with semi-log curve fitting. Cell viability is assessed using CellTiter-Glo (Promega). The toxic concentration of drug that reduces cell numbers by 50% and 90% (TC₅₀ and TC₉₀, respectively) are calculated as well. Selectivity (therapeutic) indices (SI = TC/IC) are also calculated.

In vivo Testing for Inhibition of Influenza Replication

[00194] *In vivo* testing of compounds can be performed, including testing on mammals such as rats or ferrets. Because ferrets (*Mustela putorius furo*) are naturally susceptible to infection with human influenza A and B viruses and their disease resembles that of human influenza, these animals have been widely used as a model for influenza virus pathogenesis and immunity studies. See Sidwell, R.W. and D.F. Smee, *Antiviral Res*, 2000. **48**(1): p. 1-16; and Colacino, J.M., D.C. DeLong, J.R. Nelson, W.A. Spitzer, J. Tang, F. Victor, and C.Y. Wu, *Antimicrob Agents Chemother*, 1990. **34**(11): p. 2156-63. Ferrets are also the model of choice for the study of avian influenza virus H5N1 pathogenesis in mammals. See also Zitzow, L.A., T. Rowe, T. Morken, W.-J. Shieh, S. Zaki, and J.M. Katz, *Pathogenesis of Avian Influenza A (H5N1) Viruses in Ferrets*. 2002. p. 4420-4429. The activities of the PB1 Stapled Peptides can be compared to Ribavirin or Oseltamivir as a positive control.

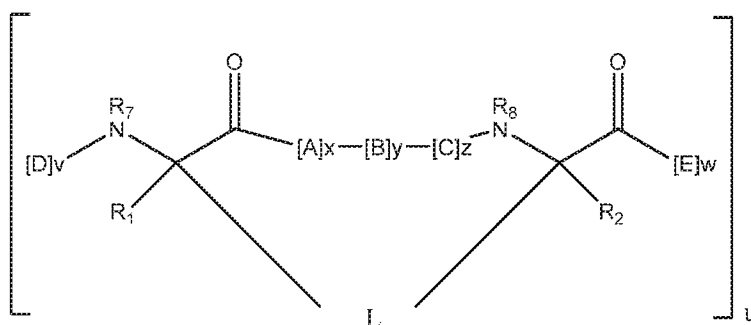
[00195] Briefly, young adult male or female ferrets (five ferrets for each treatment group) that are serologically negative by hemagglutination inhibition assay for currently circulating human influenza A or B viruses are quarantined at least 4 days prior to infection in a BSL-3+ animal holding area, where they are housed in cages contained in bioclean portable laminar flow clean room enclosures (Lab Products, Seaford, Del.). Prior to infection, baseline temperatures are measured twice daily for at least 3 days. Ferrets are anesthetized with ketamine (25 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg) by the intramuscular route and infected intranasally (i.n.) with virus/mL in phosphate-buffered saline (PBS) delivered to the nostrils. Control animals are mock-infected with an equivalent dilution (1:30) of noninfectious allantoic fluid. Stapled Peptides are administered i.v. or i.p. one hour after virus infection. Temperatures are measured twice daily using either a rectal thermometer or a subcutaneous implantable temperature transponder (BioMedic Data Systems, Inc., Seaford, Del.) with pre-infection values averaged to obtain a baseline temperature for each ferret. The change in temperature (in degrees Celsius) is calculated at each time point for each animal. Clinical signs of sneezing (before anesthesia), inappetence, dyspnea, and level of activity are assessed. A scoring system is also used to assess the activity level, and based on the daily scores for each animal in a group a relative inactivity index will be calculated. Rectal temperature and activity scores are used to assess the severity of influenza infection and the ability of Stapled Peptides to prevent flu symptoms

Assaying inhibition of viral polymerase complex assembly and activity.

[00196] The technique of Bimolecular Fluorescence Complementation (“BiFC”) may be used to assay the compounds. In this technique, N- and C-terminal fragments of fluorescent proteins (*e.g.* GFP or its derivatives) are fused to interacting proteins. The two non-functional halves of the fluorophore, following the expression in cells, are brought into close proximity as a result of the specific protein interactions, which initiates folding of the fragments into an active protein and results in a detectable fluorescent signal at the site of the protein-protein complex. Thus, through BiFC, the specific interaction between PB1 and PA subunits can be visualized, quantified and localized within live cells. By disrupting PB1-PA interaction with a compound, the BiFC signal will be reduced, indicative of the presence of potential inhibitors targeting the assembly of PB1-PA complex. See Hemerka et. al., *J. Virol.* 2009, 3944-3955.

Peptidomimetic Macrocycles

[00197] In some embodiments, a peptidomimetic macrocycle has the Formula (I):

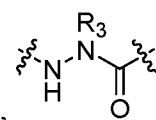


Formula I

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;

each B is independently a natural or non-natural amino acid, amino acid analog,



[-NH-L₃-CO-], [-NH-L₃-SO₂-], or [-NH-L₃-];

each R₁ and R₂ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-;

each R₃ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R₅;

each L is independently a macrocycle-forming linker;

each L₃ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or [-R₄-K-R₄]-_n, each being optionally substituted with R₅;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR₃;

each n is independently an integer from 1-5;

each R_5 is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

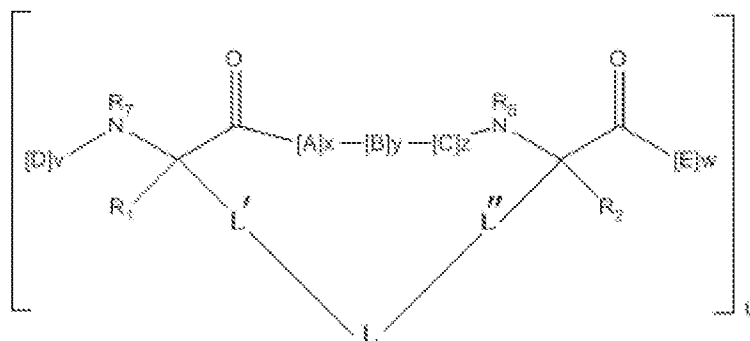
each R_7 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

each R_8 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

each v and w is independently integers from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-40, 1-25, 1-20, 1 to 15, or 1 to 10; and

each u , x , y and z is independently integers from 0-10.

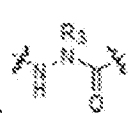
[00198] In some embodiments, a peptidomimetic macrocycle of Formula (I) has Formula (Ic):



Formula (Ic)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;

each B is independently a natural or non-natural amino acid, amino acid analog, , [-NH-L₃-CO-], [-NH-L₃-SO₂-], or [-NH-L₃-];

each L is independently a macrocycle-forming linker;

each L' is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R₅, or a bond, or together with R₁ and the atom to which both R₁ and L' are bound forms a ring;

each L'' is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R₅, or a bond, or together with R₂ and the atom to which both R₂ and L'' are bound forms a ring;

each R₁ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or together with L' and the atom to which both R₁ and L' are bound forms a ring;

each R₂ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or together with L'' and the atom to which both R₂ and L'' are bound forms a ring;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R₅;

each L₃ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or [-R₄-K-R₄]_n, each being optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

n is an integer from 1-5;

each R₅ is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

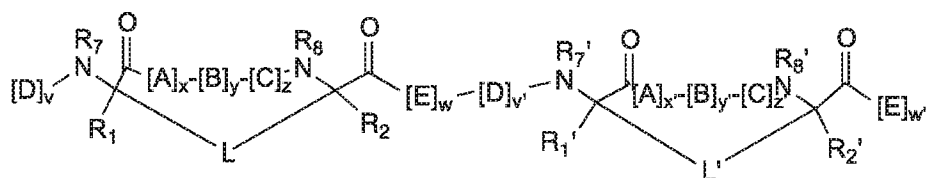
each R₇ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

each R₈ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

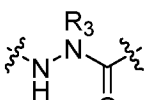
each v and w is independently an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-40, 1-25, 1-20, 1-15, or 1-10; and

each u, x, y and z is independently an integer from 0-10.

[00199] In some embodiments, the peptidomimetic macrocycle of Formula (I) has the Formula:



wherein each A, C, D, and E is independently an amino acid;

each B is independently an amino acid, , $[-NH-L_3-CO-]$, $[-NH-L_3-SO_2-]$, or $[-NH-L_3-]$;

L' is a macrocycle-forming linker of the formula $-L_1'-L_2'-$;

and wherein A, B, C, D, and E, taken together with the crosslinked amino acids connected by the macrocycle-forming linkers L and L' , form the amino acid sequence of the peptidomimetic macrocycle;

R_1' and R_2' are independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-;

L_1' and L_2' and L_3 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R_4-K-R_4-]_n$, each being optionally substituted with R_5 ;

each K is independently O, S, SO, SO_2 , CO, CO_2 , or $CONR_3$;

R_7' is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

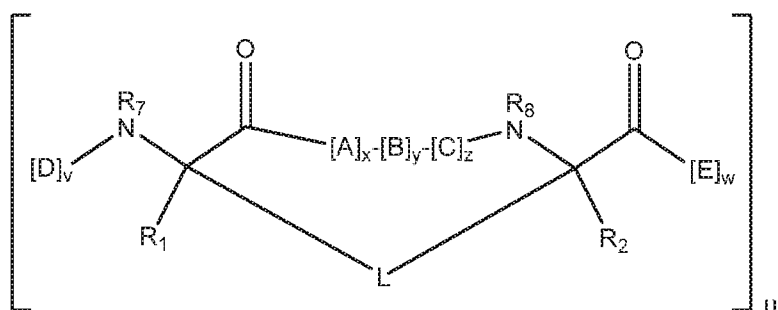
R_8' is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

v' and w' are independently integers from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-40, 1-25, 1-20, 1 to 15, or 1 to 10;

x' , y' and z' are independently integers from 0-10; and

n is an integer from 1-5. In some embodiments, the sum of $x'+y'+z'$ is 2, 3 or 6, for example 3 or 6.

[00200] In some embodiments, the peptidomimetic macrocycles have the Formula (I):

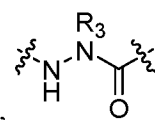


Formula (I)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;

each B is independently a natural or non-natural amino acid, amino acid analog,

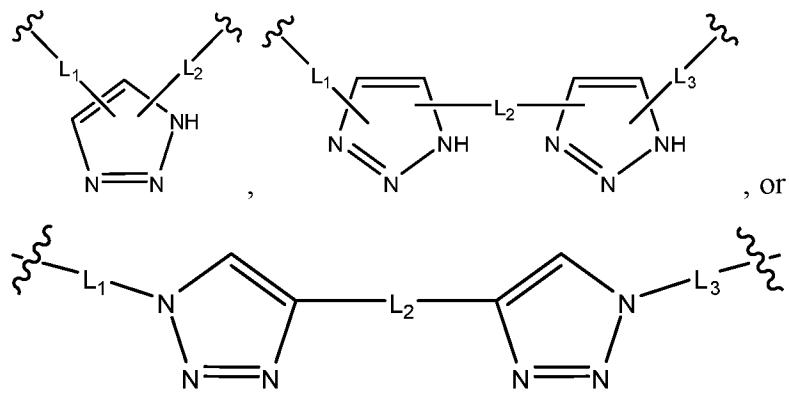


[-NH-L₃-CO-], [-NH-L₃-SO₂-], or [-NH-L₃-];

each R₁ and R₂ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-;

each R₃ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R₅;

each L is independently macrocycle-forming linker of the formula



each L₁, L₂ and L₃ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or [-R₄-K-R₄]_n, each being optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_7 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

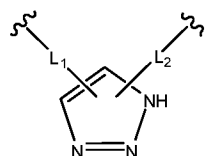
each R_8 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

each v and w is independently an integer from 1-1000;

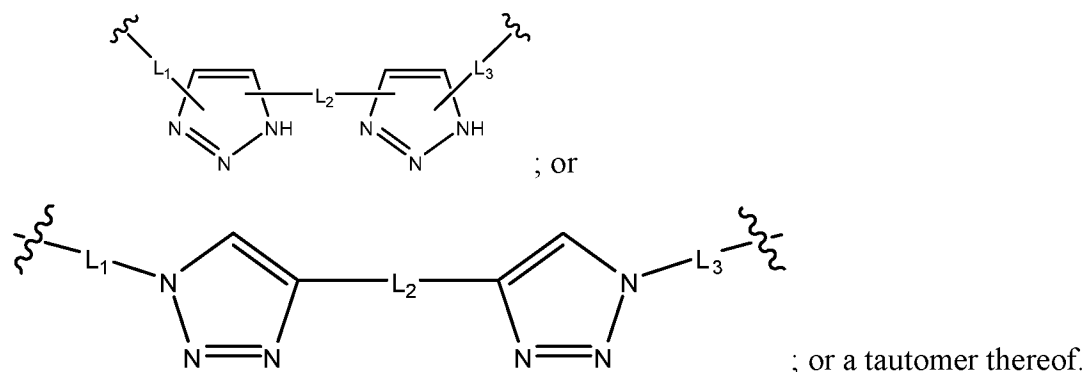
each u , x , y and z is independently integers from 0-10; and

n is an integer from 1-5.

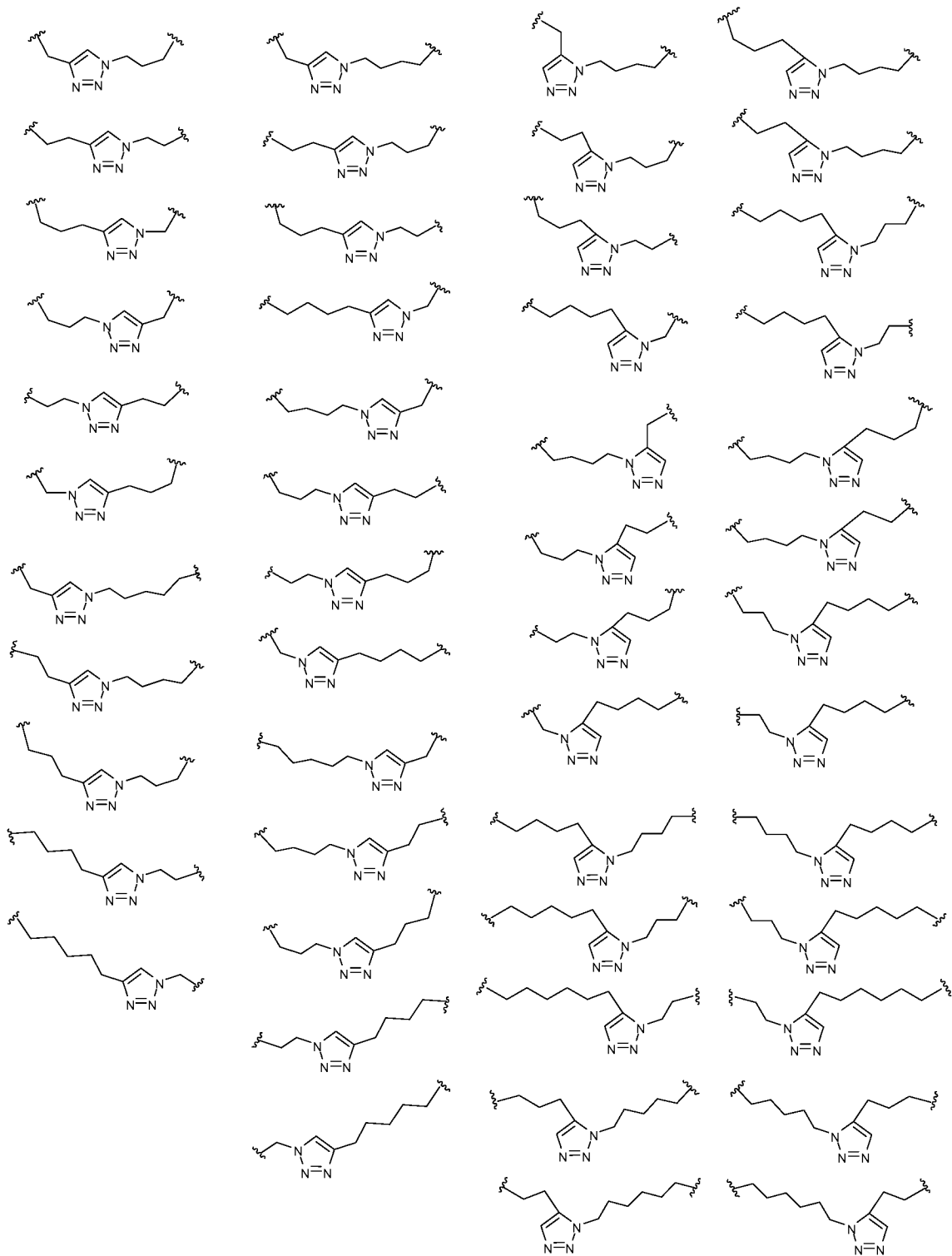
[00201] In some embodiments, L is a macrocycle-forming linker of the formula

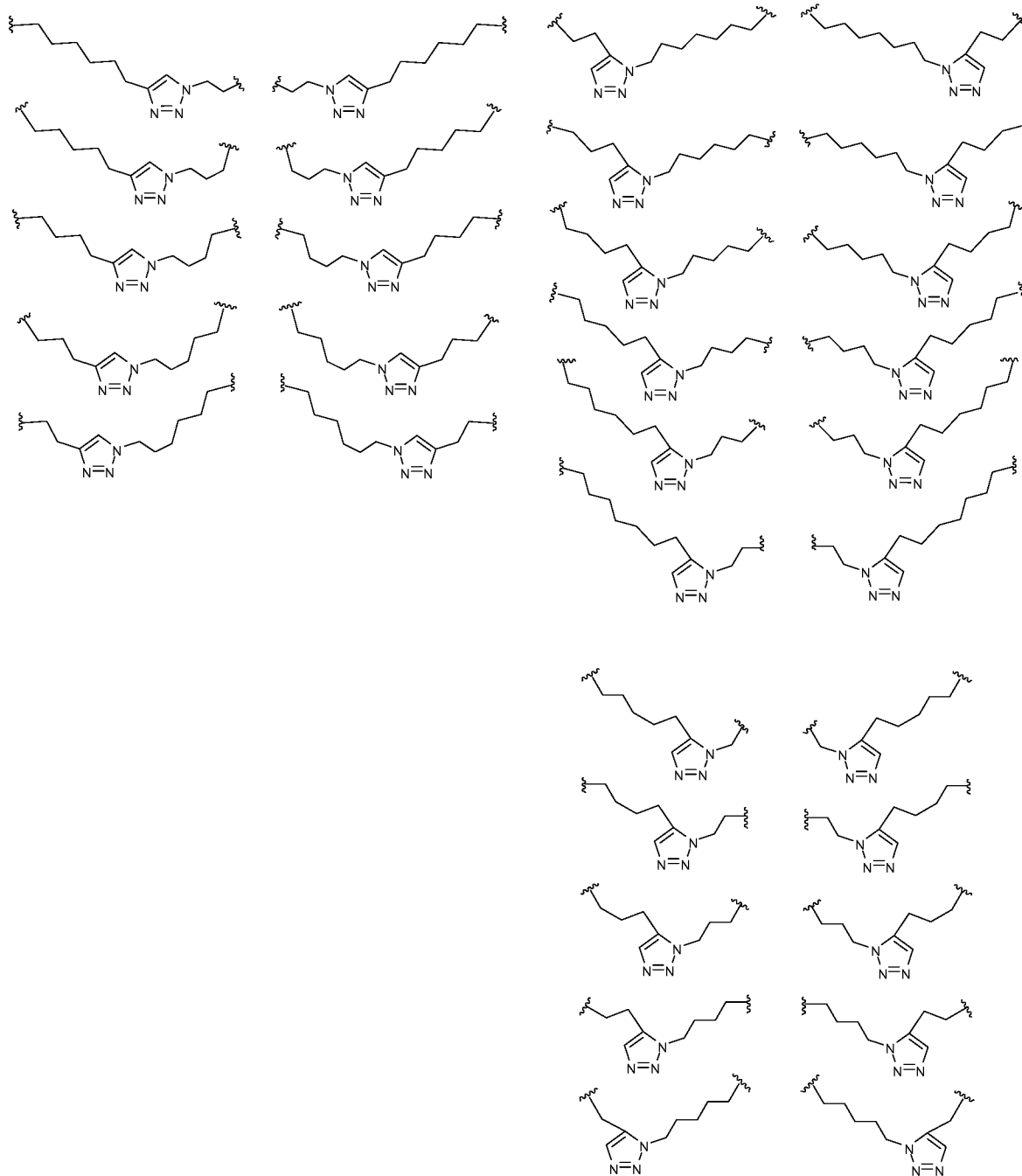


[00202] In some embodiments, L is a macrocycle-forming linker of the formula



[00203] Exemplary embodiments of the macrocycle-forming linker L are shown below.



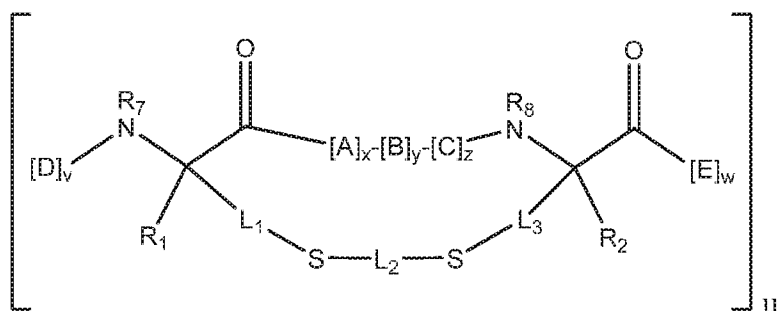


[00204] Amino acids which are used in the formation of triazole crosslinkers are represented according to the legend indicated below. Stereochemistry at the α -position of each amino acid is S unless otherwise indicated. For azide amino acids, the number of carbon atoms indicated refers to the number of methylene units between the α -carbon and the terminal azide. For alkyne amino acids, the number of carbon atoms indicated is the number of methylene units between the α -position and the triazole moiety plus the two carbon atoms within the triazole group derived from the alkyne.

\$5a5	α -Me alkyne 1,5 triazole (5 carbon)
\$5n3	α -Me azide 1,5 triazole (3 carbon)
\$4rn6	α -Me R-azide 1,4 triazole (6 carbon)

\$4a5 α -Me alkyne 1,4 triazole (5 carbon)

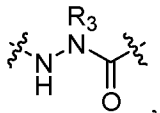
[00205] In other embodiments, the invention provides peptidomimetic macrocycles of Formula (III):



Formula (III)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;

each B is independently a natural or non-natural amino acid, amino acid analog, ,
 [-NH-L4-CO-], [-NH-L4-SO2-], or [-NH-L4-];

each R₁ and R₂ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-;

each R₃ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, unsubstituted or substituted with R₅;

each L₁, L₂, L₃ and L₄ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene or [-R₄-K-R₄]-_n, each being unsubstituted or substituted with R₅;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₄ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each R₅ is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₇ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, unsubstituted or substituted with R₅, or part of a cyclic structure with a D residue;

each R_8 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, unsubstituted or substituted with R_5 , or part of a cyclic structure with an E residue;

each v and w is independently an integer from 1-1000;

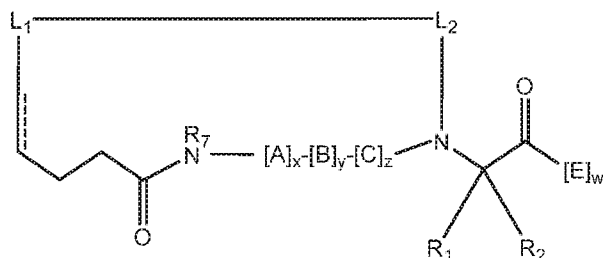
each x , y and z is independently an integer from 0-10;

u is an integer from 0-10; and

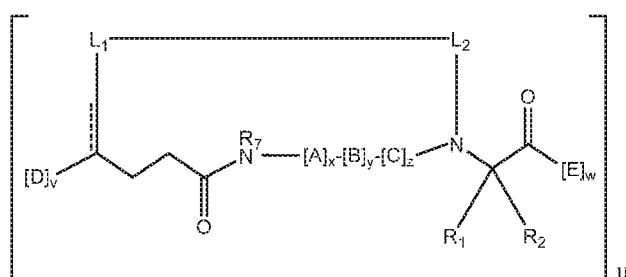
n is an integer from 1-5.

[00206] In some embodiments, the length of the macrocycle-forming linker $[-L_1-S-L_2-S-L_3-]$ as measured from a first α -carbon to a second α -carbon is selected to stabilize a desired secondary peptide structure, such as a helix (including, but not limited to a 3_{10} helix or an α -helix) formed by residues of the peptidomimetic macrocycle including, but not necessarily limited to, those between the first α -carbon to a second α -carbon. In some embodiments, the thiol moieties are the side chains of the amino acid residues L-cysteine, D-cysteine, α -methyl-L cysteine, α -methyl-D-cysteine, L-homocysteine, D-homocysteine, α -methyl-L-homocysteine or α -methyl-D-homocysteine. A bis-alkylating reagent is of the general formula $X-L_2-Y$ wherein L_2 is a linker moiety and X and Y are leaving groups that are displaced by $-SH$ moieties to form bonds with L_2 . In some embodiments, X and Y are halogens such as I, Br, or Cl.

[00207] In other embodiments, the invention provides peptidomimetic macrocycles of Formula (IV) or (IVa):



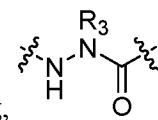
Formula (IV)



Formula (IVa)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;



each B is independently a natural or non-natural amino acid, amino acid analog,

$[-NH-L_3-CO-]$, $[-NH-L_3-SO_2-]$, or $[-NH-L_3-]$;

each R_1 and R_2 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or part of a cyclic structure with an E residue;

each R_3 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R_5 ;

each L is independently a macrocycle-forming linker of the formula $-L_1-L_2-$;

each L_1 and L_2 and L_3 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R_4-K-R_4-]_n$, each being optionally substituted with R_5 ;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO_2 , CO, CO_2 , or $CONR_3$;

each R_5 is independently halogen, alkyl, $-OR_6$, $-N(R_6)_2$, $-SR_6$, $-SOR_6$, $-SO_2R_6$, $-CO_2R_6$, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_7 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 ;

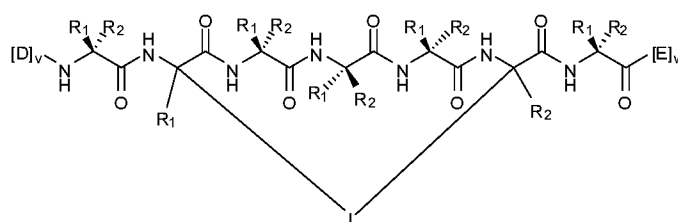
each v and w is independently integers from 1-1000;

each x, y and z is independently an integer from 0-10;

u is an integer from 0-10; and

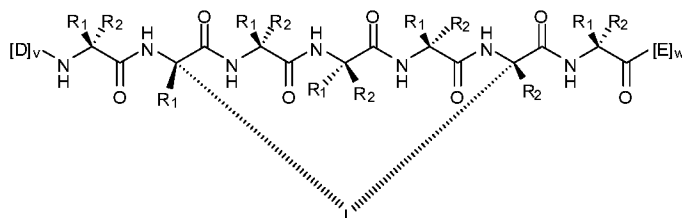
each n is independently an integer from 1-5.

[00208] In one embodiment, the peptidomimetic macrocycle of Formula (I) is:

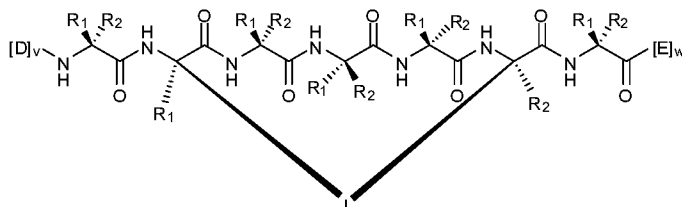


wherein each R_1 and R_2 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-.

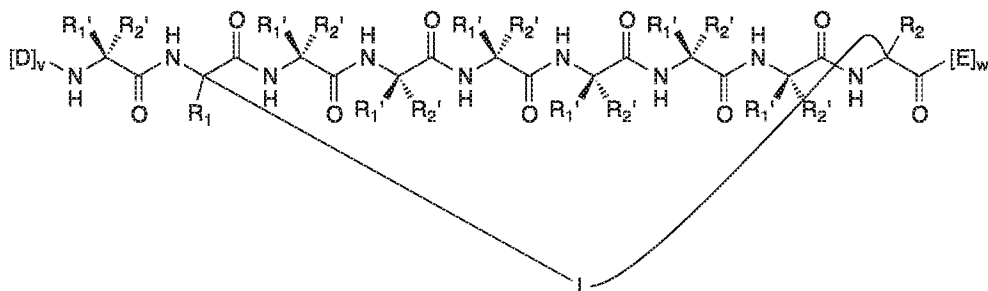
[00209] In related embodiments, the peptidomimetic macrocycle comprises a structure of Formula (I) which is:



or

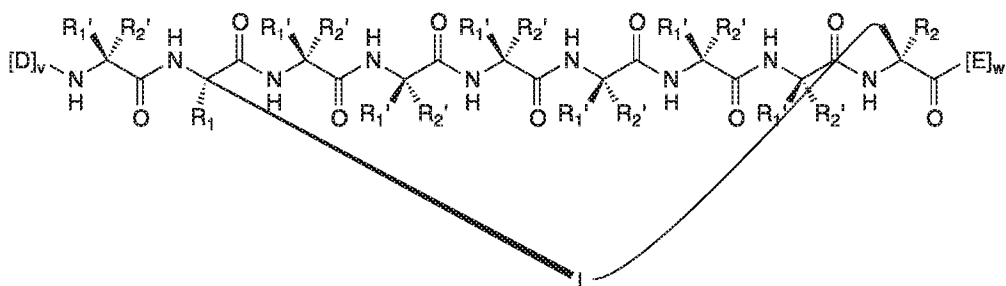


[00210] In some embodiments, the peptidomimetic macrocycle of Formula (I) is:



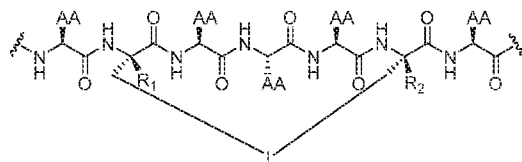
wherein each R₁ and R₂ is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–.

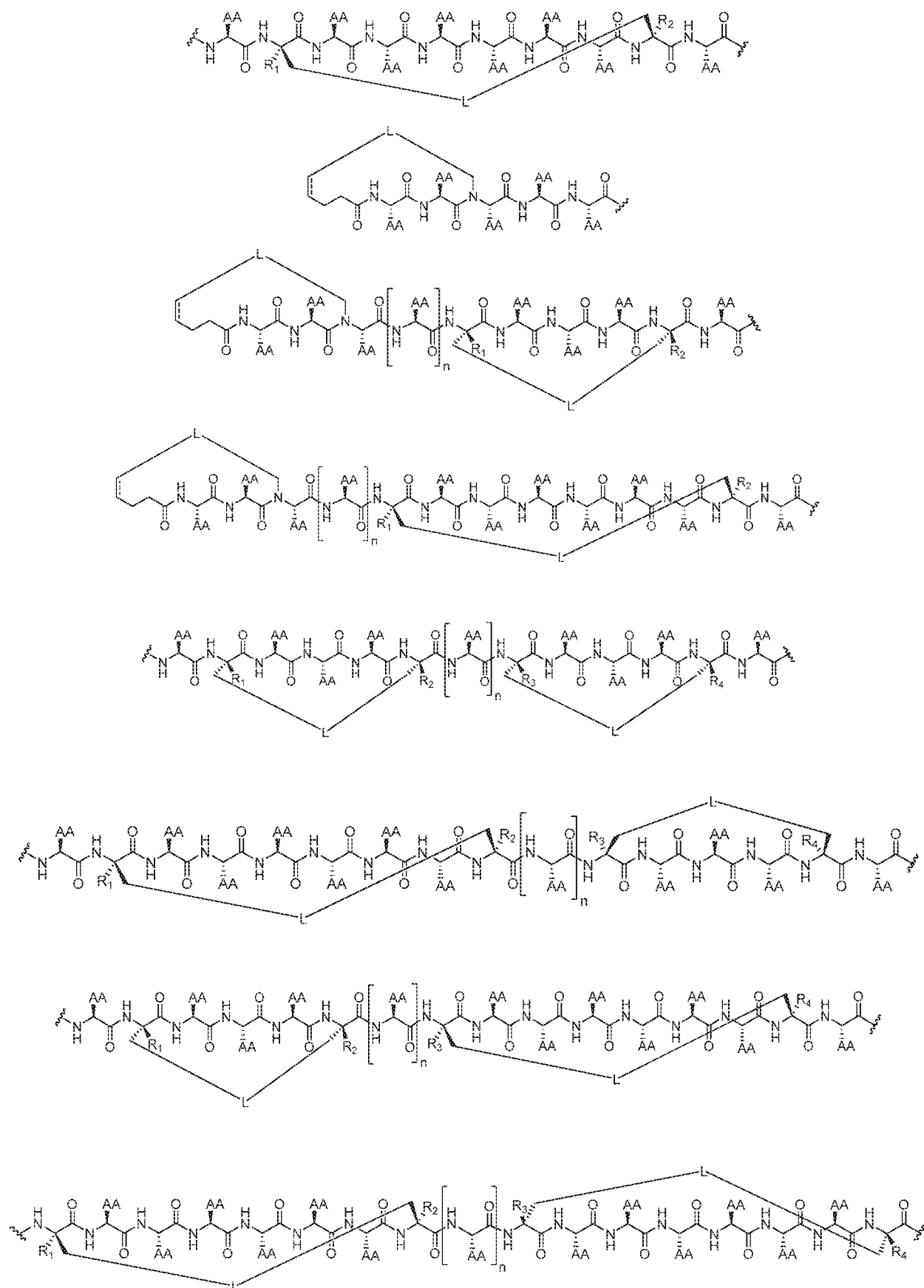
[00211] In related embodiments, the peptidomimetic macrocycle of Formula (I) is:

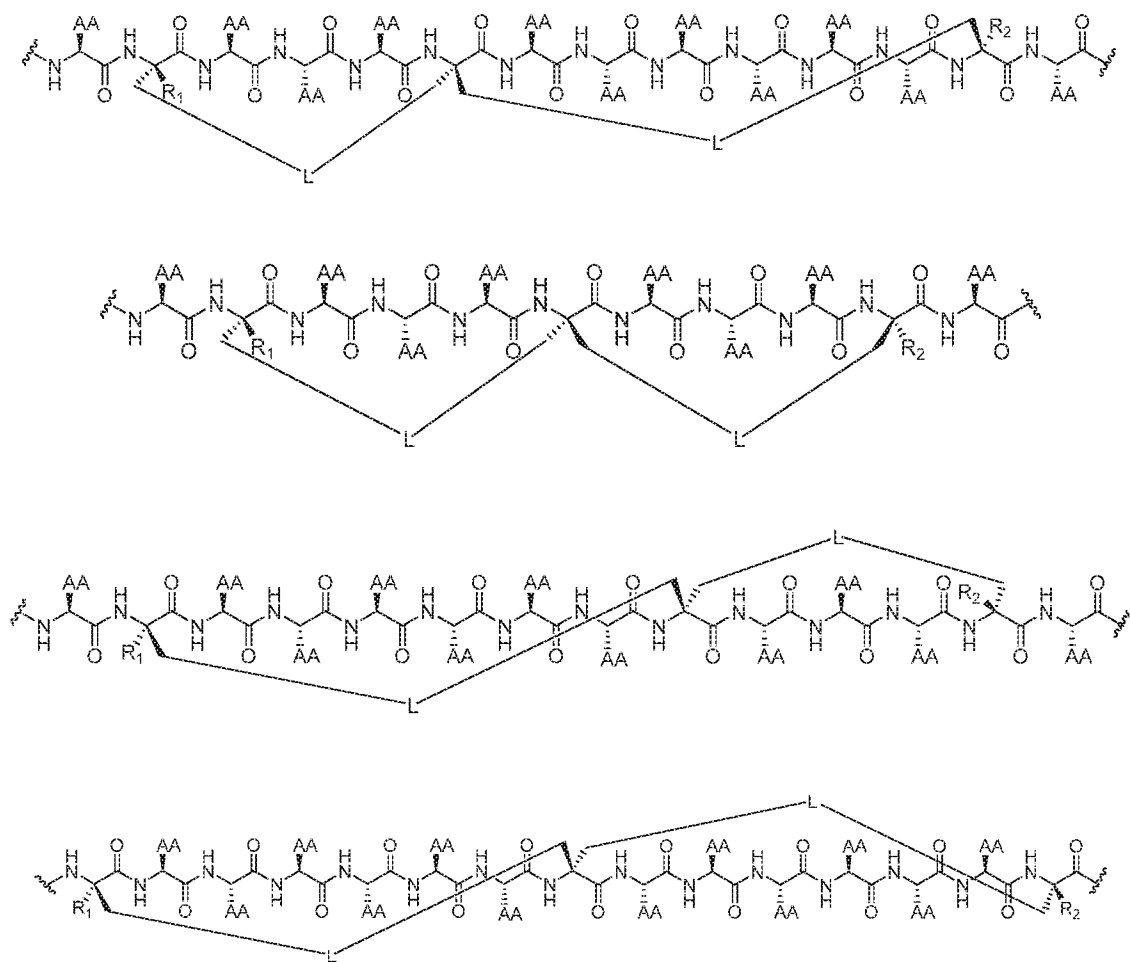


wherein each R₁' and R₂' is independently an amino acid.

[00212] In other embodiments, the peptidomimetic macrocycle of Formula (I) is a compound of any of the formulas shown below:

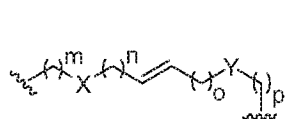




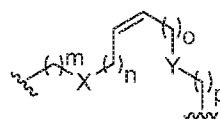


wherein “AA” represents any natural or non-natural amino acid side chain and “ $\sim$ ” is $[D]_v$, $[E]_w$ as defined above, and n is an integer from 0 to 20, 50, 100, 200, 300, 400 or 500. In some embodiments, the substituent “ n ” shown in the preceding paragraph is 0. In other embodiments, the substituent “ n ” shown in the preceding paragraph is less than 50, 40, 30, 20, 10, or 5.

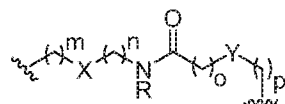
[00213] Exemplary embodiments of the macrocycle-forming linker L are shown below.



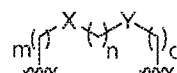
where X, Y = $-\text{CH}_2-$, O, S, or NH
 $m, n, o, p = 0-10$



where X, Y = $-\text{CH}_2-$, O, S, or NH
 $m, n, o, p = 0-10$



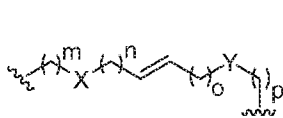
where X, Y = $-\text{CH}_2-$, O, S, or NH
 $m, n, o, p = 0-10$
 $R = \text{H, alkyl, other substituent}$



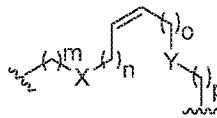
where X, Y = $-\text{CH}_2-$, O, S, or NH
 $m, n, o = 0-10$

[00214] In some embodiments, L is a macrocycle-forming linker of the formula $-L_1-L_2-$. In some embodiments, L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R_4-K-R_4-]_n$, each being optionally substituted with R_5 ; each R_4 is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene; each K is O, S, SO, SO₂, CO, CO₂, or CONR₃; and n is an integer from 1-5.

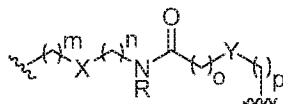
[00215] Exemplary embodiments of the macrocycle-forming linker $-L_1-L_2-$ are shown below.



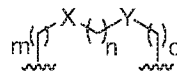
where X, Y = -CH₂-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH₂-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH₂-, O, S, or NH
m, n, o, p = 0-10
R = H, alkyl, other substituent



where X, Y = -CH₂-, O, S, or NH
m, n, o = 0-10

[00216] In an embodiment of any of the Formulas described herein, L_1 and L_2 , either alone or in combination, form a triazole or a thioether.

[00217] In an embodiment of any of the Formulas described herein, L_1 and L_2 , either alone or in combination, do not form a triazole or a thioether.

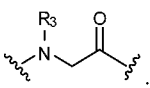
[00218] In other embodiments, the length of the macrocycle-forming linker L as measured from a first α -carbon to a second α -carbon is selected to stabilize a desired secondary peptide structure, such as a helix formed by residues of the peptidomimetic macrocycle including, but not necessarily limited to, those between the first α -carbon to a second α -carbon.

[00219] In one example, at least one of R_1 and R_2 is alkyl, unsubstituted or substituted with halo-. In another example, both R_1 and R_2 are independently alkyl, unsubstituted or substituted with halo-. In some embodiments, at least one of R_1 and R_2 is methyl. In other embodiments, R_1 and R_2 are methyl.

[00220] In some embodiments, $x+y+z$ is at least 2. In other embodiments, $x+y+z$ is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Each occurrence of A, B, C, D or E in a macrocycle or macrocycle precursor is independently selected. For example, a sequence represented by the formula $[A]_x$, when x is 3, encompasses embodiments where the amino acids are not identical, *e.g.* Gln-Asp-Ala as well as embodiments where the amino acids are identical, *e.g.* Gln-Gln-Gln. This applies for any value of x,

y, or z in the indicated ranges. Similarly, when u is greater than 1, each compound may encompass peptidomimetic macrocycles which are the same or different. For example, a compound may comprise peptidomimetic macrocycles comprising different linker lengths or chemical compositions.

[00221] In some embodiments, the peptidomimetic macrocycle comprises a secondary structure which is a helix and R_8 is $-H$, allowing intrahelical hydrogen bonding. In some embodiments, at least one of A, B, C, D or E is an α,α -disubstituted amino acid. In one example, B is an α,α -disubstituted amino acid. For instance, at least one of A, B, C, D or E is 2-aminoisobutyric acid. In other

embodiments, at least one of A, B, C, D or E is .

[00222] In some embodiments, w is from 1 to 1000. For example, the first amino acid represented by E comprises a small hydrophobic side chain. In some embodiments, w is from 2 to 1000. For example, the second amino acid represented by E comprises a small hydrophobic side chain. In some embodiments, w is from 3 to 1000. For example, the third amino acid represented by E can comprise a small hydrophobic side chain. For example, the third amino acid represented by E can comprise a small hydrophobic side chain. In some embodiments, w is from 4 and 1000. In some embodiments, w is from 5 and 1000. In some embodiments, w is from 6 and 1000. In some embodiments, w is from 7 and 1000. In some embodiments, w is from 8 and 1000. In some embodiments, w is an integer from 3-10, for example 3-6, 3-8, 6-8, or 6-10. In some embodiments, w is 3. In other embodiments, w is 6. In some embodiments, v is an integer from 1-10, for example 2-5. In some embodiments, v is 2. In some embodiments, v is 3.

[00223] In some embodiments, each of the first two amino acid represented by E comprises an uncharged side chain or a negatively charged side chain. In some embodiments, each of the first three amino acid represented by E comprises an uncharged side chain or a negatively charged side chain. In some embodiments, each of the first four amino acid represented by E comprises an uncharged side chain or a negatively charged side chain.

[00224] In some embodiments, the first C-terminal amino acid and/or the second C-terminal amino acid represented by E comprise a hydrophobic side chain. For example, the first C-terminal amino acid and/or the second C-terminal amino acid represented by E comprises a hydrophobic side chain, for example a small hydrophobic side chain. In some embodiments, the first C-terminal amino acid, the second C-terminal amino acid, and/or the third C-terminal amino acid represented by E comprise a hydrophobic side chain. For example, the first C-terminal amino acid, the second C-terminal amino acid, and/or the third C-terminal amino acid represented by E comprises a hydrophobic side chain, for example a small hydrophobic side chain.

[00225] In some embodiments, one or more or each of the amino acid that is $i+1$, $i+2$, $i+3$, $i+4$, $i+5$, and/or $i+6$ with respect to a first E comprises an uncharged side chain or a negatively charged side chain. In some embodiments, each E is independently an amino acid selected from the group consisting of Ala (alanine), D-Ala (D-alanine), Aib (α -aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine).

[00226] In other embodiments, D and/or E in the compound of Formula I, II or III are further modified in order to facilitate cellular uptake. In some embodiments, lipidating or PEGylating a peptidomimetic macrocycle facilitates cellular uptake, increases bioavailability, increases blood circulation, alters pharmacokinetics, decreases immunogenicity and/or decreases the needed frequency of administration.

[00227] In other embodiments, at least one of [D] and [E] in the compound of Formula I, II or III represents a moiety comprising an additional macrocycle-forming linker such that the peptidomimetic macrocycle comprises at least two macrocycle-forming linkers. In a specific embodiment, a peptidomimetic macrocycle comprises two macrocycle-forming linkers. In an embodiment, u is 2.

[00228] Unless otherwise stated, any compounds (including peptidomimetic macrocycles, macrocycle precursors, and other compositions) are also meant to encompass compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the described structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C - or ^{14}C -enriched carbon are within the scope of this disclosure.

[00229] In some embodiments, the compounds disclosed herein can contain unnatural proportions of atomic isotopes at one or more of atoms that constitute such compounds. For example, the compounds can be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). In other embodiments, one or more carbon atoms are replaced with a silicon atom. All isotopic variations of the compounds disclosed herein, whether radioactive or not, are contemplated herein.

[00230] A peptidomimetic macrocycle described herein can be at least 1% pure, at least 2% pure, at least 3% pure, at least 4% pure, at least 5% pure, at least 6% pure, at least 7% pure, at least 8% pure, at least 9% pure, at least 10% pure, at least 11% pure, at least 12% pure, at least 13% pure, at least 14% pure, at least 15% pure, at least 16% pure, at least 17% pure, at least 18% pure, at least 19% pure, at least 20% pure, at least 21% pure, at least 22% pure, at least 23% pure, at least 24% pure, at least 25% pure, at least 26% pure, at least 27% pure, at least 28% pure, at least 29% pure, at least 30% pure, at least 31% pure, at least 32% pure, at least 33% pure, at least 34% pure, at least 35% pure, at least 36% pure, at least 37% pure, at least 38% pure, at least 39% pure, at least 40% pure, at

least 41% pure, at least 42% pure, at least 43% pure, at least 44% pure, at least 45% pure, at least 46% pure, at least 47% pure, at least 48% pure, at least 49% pure, at least 50% pure, at least 51% pure, at least 52% pure, at least 53% pure, at least 54% pure, at least 55% pure, at least 56% pure, at least 57% pure, at least 58% pure, at least 59% pure, at least 60% pure, at least 61% pure, at least 62% pure, at least 63% pure, at least 64% pure, at least 65% pure, at least 66% pure, at least 67% pure, at least 68% pure, at least 69% pure, at least 70% pure, at least 71% pure, at least 72% pure, at least 73% pure, at least 74% pure, at least 75% pure, at least 76% pure, at least 77% pure, at least 78% pure, at least 79% pure, at least 80% pure, at least 81% pure, at least 82% pure, at least 83% pure, at least 84% pure, at least 85% pure, at least 86% pure, at least 87% pure, at least 88% pure, at least 89% pure, at least 90% pure, at least 91% pure, at least 92% pure, at least 93% pure, at least 94% pure, at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, at least 99% pure, at least 99.1% pure, at least 99.2% pure, at least 99.3% pure, at least 99.4% pure, at least 99.5% pure, at least 99.6% pure, at least 99.7% pure, at least 99.8% pure, or at least 99.9% pure on a chemical, optical, isomeric, enantiomeric, or diastereomeric basis. Purity can be assessed, for example, by HPLC, MS, LC/MS, melting point, or NMR.

[00231] Two or more peptides can share a degree of homology. A pair of peptides can have, for example, up to about 20% pairwise homology, up to about 25% pairwise homology, up to about 30% pairwise homology, up to about 35% pairwise homology, up to about 40% pairwise homology, up to about 45% pairwise homology, up to about 50% pairwise homology, up to about 55% pairwise homology, up to about 60% pairwise homology, up to about 65% pairwise homology, up to about 70% pairwise homology, up to about 75% pairwise homology, up to about 80% pairwise homology, up to about 85% pairwise homology, up to about 90% pairwise homology, up to about 95% pairwise homology, up to about 96% pairwise homology, up to about 97% pairwise homology, up to about 98% pairwise homology, up to about 99% pairwise homology, up to about 99.5% pairwise homology, or up to about 99.9% pairwise homology. A pair of peptides can have, for example, at least about 20% pairwise homology, at least about 25% pairwise homology, at least about 30% pairwise homology, at least about 35% pairwise homology, at least about 40% pairwise homology, at least about 45% pairwise homology, at least about 50% pairwise homology, at least about 55% pairwise homology, at least about 60% pairwise homology, at least about 65% pairwise homology, at least about 70% pairwise homology, at least about 75% pairwise homology, at least about 80% pairwise homology, at least about 85% pairwise homology, at least about 90% pairwise homology, at least about 95% pairwise homology, at least about 96% pairwise homology, at least about 97% pairwise homology, at least about 98% pairwise homology, at least about 99% pairwise homology, at least about 99.5% pairwise homology, at least about 99.9% pairwise homology.

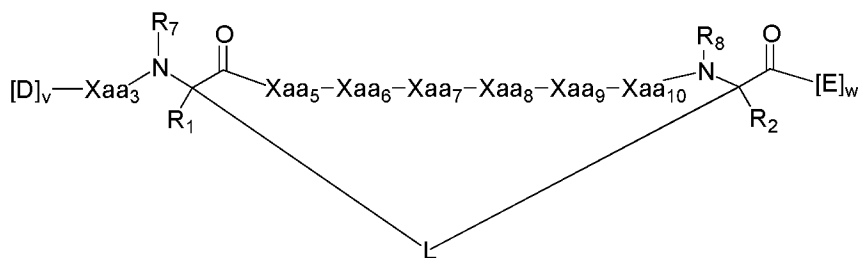
[00232] Various methods and software programs can be used to determine the homology between two or more peptides, such as NCBI BLAST, Clustal W, MAFFT, Clustal Omega, AlignMe, Praline, or another suitable method or algorithm.

[00233] In some embodiments, the peptidomimetic macrocycle comprises at least one helical motif, such as a 3_{10} or an α -helix motif. For example, A, B and/or C in the compound of Formula I, II or III include one or more helices. As a general matter, helices include from 3 to 4 amino acid residues per turn. In some embodiments, the helix of the peptidomimetic macrocycle includes 1 to 5 turns and, therefore, 3 to 20 amino acid residues. In specific embodiments, the helix includes 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns. In some embodiments, the macrocycle-forming linker stabilizes a helix motif included within the peptidomimetic macrocycle. Thus, in some embodiments, the length of the macrocycle-forming linker L from a first α -carbon to a second α -carbon is selected to increase the stability of a helix. In some embodiments, the macrocycle-forming linker spans from 1 turn to 5 turns of the helix. In some embodiments, the macrocycle-forming linker spans approximately 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns of the helix. In some embodiments, the length of the macrocycle-forming linker is approximately 5 Å to 9 Å per turn of the helix, or approximately 6 Å to 8 Å per turn of the helix. Where the macrocycle-forming linker spans approximately 1 turn of a helix, the length is equal to approximately 5 carbon-carbon bonds to 13 carbon-carbon bonds, approximately 7 carbon-carbon bonds to 11 carbon-carbon bonds, or approximately 9 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 2 turns of a helix, the length is equal to approximately 8 carbon-carbon bonds to 16 carbon-carbon bonds, approximately 10 carbon-carbon bonds to 14 carbon-carbon bonds, or approximately 12 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 3 turns of a helix, the length is equal to approximately 14 carbon-carbon bonds to 22 carbon-carbon bonds, approximately 16 carbon-carbon bonds to 20 carbon-carbon bonds, or approximately 18 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 4 turns of a helix, the length is equal to approximately 20 carbon-carbon bonds to 28 carbon-carbon bonds, approximately 22 carbon-carbon bonds to 26 carbon-carbon bonds, or approximately 24 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 5 turns of a helix, the length is equal to approximately 26 carbon-carbon bonds to 34 carbon-carbon bonds, approximately 28 carbon-carbon bonds to 32 carbon-carbon bonds, or approximately 30 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 1 turn of a helix, the linkage contains approximately 4 atoms to 12 atoms, approximately 6 atoms to 10 atoms, or approximately 8 atoms. Where the macrocycle-forming linker spans approximately 2 turns of the helix, the linkage contains approximately 7 atoms to 15 atoms, approximately 9 atoms to 13 atoms, or approximately 11 atoms. Where the macrocycle-forming linker spans approximately 3

turns of the helix, the linkage contains approximately 13 atoms to 21 atoms, approximately 15 atoms to 19 atoms, or approximately 17 atoms. Where the macrocycle-forming linker spans approximately 4 turns of the helix, the linkage contains approximately 19 atoms to 27 atoms, approximately 21 atoms to 25 atoms, or approximately 23 atoms. Where the macrocycle-forming linker spans approximately 5 turns of the helix, the linkage contains approximately 25 atoms to 33 atoms, approximately 27 atoms to 31 atoms, or approximately 29 atoms. Where the macrocycle-forming linker spans approximately 1 turn of the helix, the resulting macrocycle forms a ring containing approximately 17 members to 25 members, approximately 19 members to 23 members, or approximately 21 members. Where the macrocycle-forming linker spans approximately 2 turns of the helix, the resulting macrocycle forms a ring containing approximately 29 members to 37 members, approximately 31 members to 35 members, or approximately 33 members. Where the macrocycle-forming linker spans approximately 3 turns of the helix, the resulting macrocycle forms a ring containing approximately 44 members to 52 members, approximately 46 members to 50 members, or approximately 48 members. Where the macrocycle-forming linker spans approximately 4 turns of the helix, the resulting macrocycle forms a ring containing approximately 59 members to 67 members, approximately 61 members to 65 members, or approximately 63 members. Where the macrocycle-forming linker spans approximately 5 turns of the helix, the resulting macrocycle forms a ring containing approximately 74 members to 82 members, approximately 76 members to 80 members, or approximately 78 members.

[00234] In some embodiments, each v and w is independently an integer from 1-30. In some embodiments, w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10. In some embodiments, the sum of x+y+z is 3 or 6. In some embodiments, the sum of x+y+z is 3. In other embodiments, the sum of x+y+z is 6.

[00235] In some embodiments, peptidomimetic macrocycles are also provided of the formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂, where each X is an amino acid;

each D and E is independently an amino acid;

each R₁ and R₂ is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the α-position of one of the D or E amino acids;

each L or L' is independently a macrocycle-forming linker;

each R₅ is independently halogen, alkyl, –OR₆, –N(R₆)₂, –SR₆, –SOR₆, –SO₂R₆, –CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₇ is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

each R₈ is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

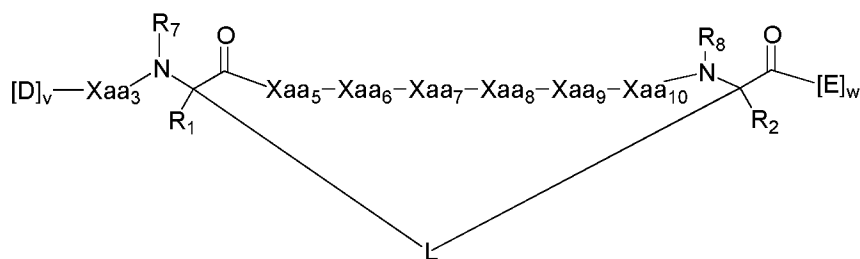
v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20 or 1-10; and

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10.

[00236] In some embodiments, each v and w is independently an integer from 1-30. In some embodiments, w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10. In some embodiments, the sum of x+y+z is 3 or 6. In some embodiments, the sum of x+y+z is 3. In other embodiments, the sum of x+y+z is 6.

[00237] In some embodiments of any of the Formulas described herein, at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂. In other embodiments, at least four of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂. In other embodiments, at least five of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂. In other embodiments, at least six of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂. In other embodiments, at least seven of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂.

[00238] In some embodiments, a peptidomimetic macrocycle has the Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂, where each X is an amino acid;

each D is independently an amino acid;

each E is independently an amino acid, for example an amino acid selected from Ala (alanine), D-Ala (D-alanine), Aib (α -aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine);

each R₁ and R₂ are independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the α -position of one of the D or E amino acids;

each L or L' is independently a macrocycle-forming linker;

each R₅ is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₇ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

each R₈ is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

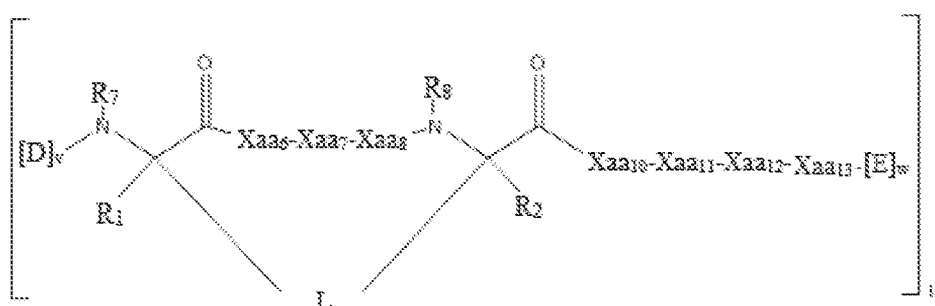
v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10; and

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10.

[00239] In some embodiments of the above Formula, at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂. In other embodiments of the above Formula, at least four of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-

Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂. In other embodiments of the above Formula, at least five of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂. In other embodiments of the above Formula, at least six of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂. In other embodiments of the above Formula, at least seven of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂.

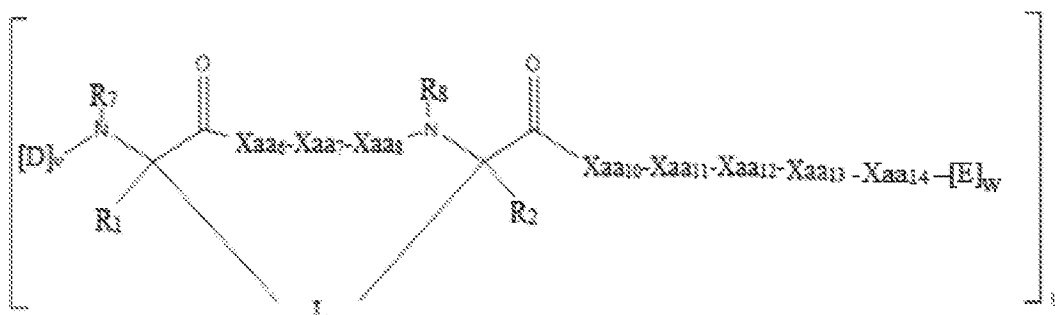
[00240] In one embodiment, the peptidomimetic macrocycle of Formula (I) is Formula (Ia):



Formula (Ia)

or a pharmaceutically-acceptable salt thereof wherein: each of Xaa₆, Xaa₇, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, and Xaa₁₃ is independently an amino acid, wherein at least three, four, five, or each of Xaa₆, Xaa₇, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, are the same amino acid as the amino acid at the corresponding position of the sequence X₅-Thr₆-Leu₇-Leu₈-X₉-Leu₁₀-Lys₁₁/Ala₁₁-Val₁₂/Ala₁₂, where each of X₅ and X₉ is independently an amino acid.

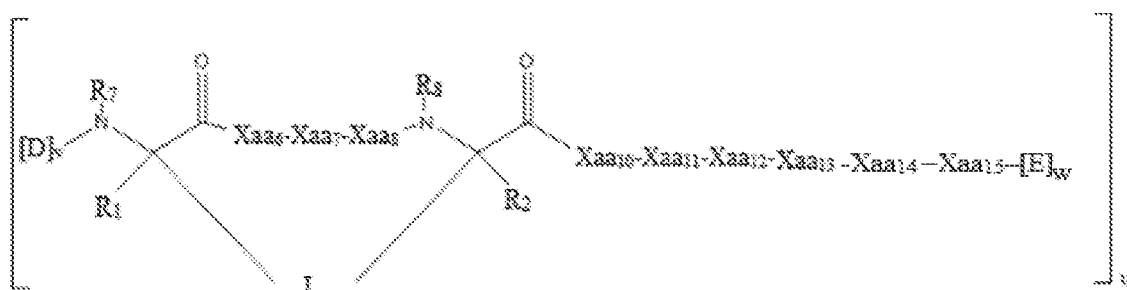
[00241] In some embodiments, the peptidomimetic macrocycle of Formula (Ia) is Formula (Ia-1):



Formula (Ia-1)

or a pharmaceutically-acceptable salt thereof, wherein each Xaa₁₄ is independently an amino acid.

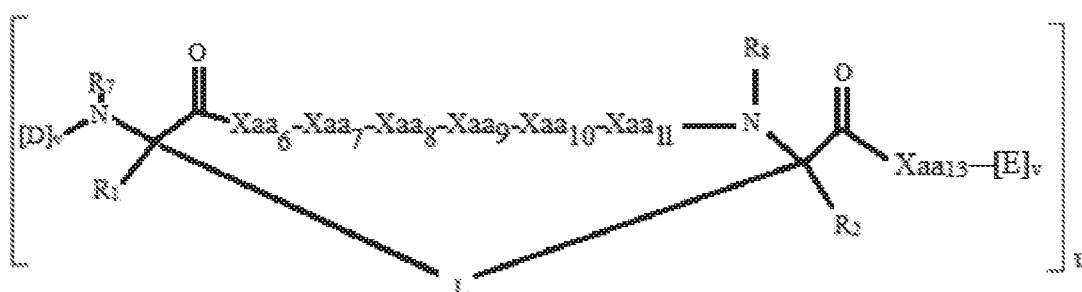
[00242] In some embodiments, the peptidomimetic macrocycle of Formula (Ia) is Formula (Ia-2):



Formula (Ia-2)

or a pharmaceutically-acceptable salt thereof, wherein each Xaa₁₄ and Xaa₁₅ is independently an amino acid.

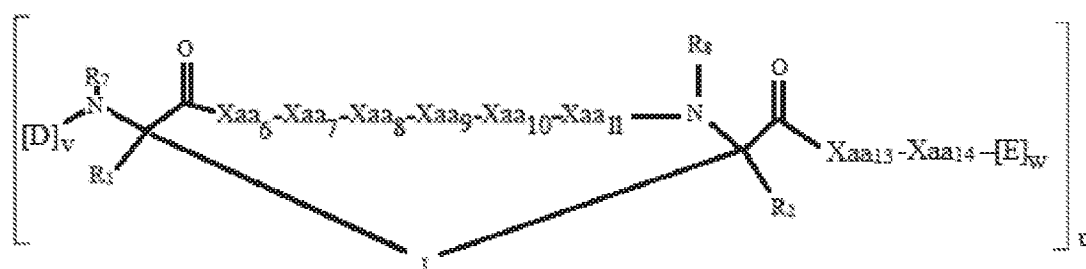
[00243] In one embodiment, the peptidomimetic macrocycle of Formula (I) is Formula (Ib):



Formula (Ib)

or a pharmaceutically-acceptable salt thereof, wherein: each of Xaa₆, Xaa₇, Xaa₈, Xaa₉, Xaa₁₀, Xaa₁₁ and Xaa₁₃ is independently an amino acid, wherein at least three, four, five, or each of Xaa₆, Xaa₇, Xaa₈, Xaa₉, Xaa₁₀, and Xaa₁₁ are the same amino acid as the amino acid at the corresponding position of the sequence X₅-Thr₆-Leu₇-Leu₈-Phe₉-Leu₁₀-Lys₁₁/Ala₁₁-X₁₂, where each of X₅ and X₁₂ is independently an amino acid.

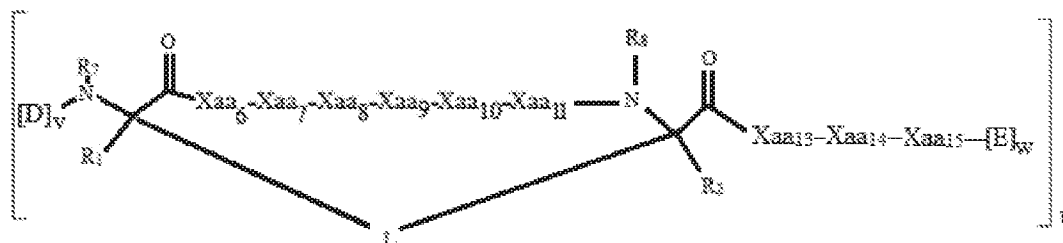
[00244] In some embodiments, the peptidomimetic macrocycle of Formula (Ib) is Formula (Ib-1):



Formula (Ib-1)

or a pharmaceutically-acceptable salt thereof, wherein each Xaa₁₄ is independently an amino acid..

[00245] In some embodiments, the peptidomimetic macrocycle of Formula (Ib) is Formula (Ib-2):



Formula (Ib-2)

or a pharmaceutically-acceptable salt thereof, wherein each Xaa₁₄ and Xaa₁₅ is independently an amino acid.

[00246] In some embodiments, the invention provides a peptidomimetic macrocycle that comprises an amino acid sequence that has at least 60%, 70%, 80%, 90%, 95%, 97%, or 100% identity to any one of the amino acid sequences in Table 3a or Table 3b.

[00247] Myeloid cell leukemia 1 (MCL-1) is a protein that inhibits cell death through the binding and inhibition of pro-death factors such as BCL-2 interacting mediator (BIM). When MCL-1 is over-expressed, the rate of cell death in a cell or tissue is reduced. In some embodiments, the peptide sequences are derived from BIM.

[00248] In some embodiments, a peptidomimetic macrocycle peptide derived from a human BIM peptide can be a peptide comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 amino acids from a BIM peptide sequence.

[00249] In some embodiments, a peptidomimetic macrocycle peptide derived from a human BIM peptide sequence can be a peptide comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 amino acids that are different from the selected sequences from which the peptide is derived. In some embodiments, a peptidomimetic macrocycle peptide derived from a human BIM peptide sequence can be a peptide comprising a mutation at amino acid position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22. In some embodiments, mutations are mutations of non-essential amino acids. In some embodiments, mutations are mutations of essential amino acids. In some embodiments, mutations are mutations of hydrophobic amino acids. In some embodiments, mutations are mutations of naturally occurring amino acids. In some embodiments, mutations are mutations to a conservative amino acid. In some embodiments, a peptidomimetic macrocycle peptide derived from a human BIM peptide sequence can be a peptide comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 amino acid analogues. In some embodiments, a peptidomimetic macrocycle peptide derived from a human BIM peptide sequence can be a peptide comprising 1 or 2 capping groups.

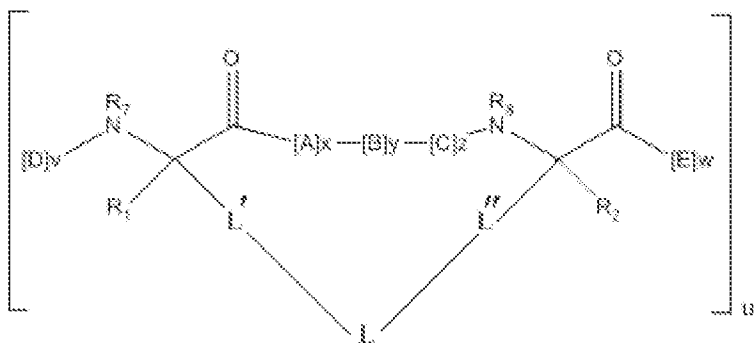
[00250] In some embodiments, the peptidomimetic macrocycle comprises a C-terminal truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acids from an amino acid sequence in Table 1. In some embodiments, the peptidomimetic macrocycle comprises a N-terminal truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 amino acids from the sequence of BIM.

[00251] A non-limiting list of suitable BIM macrocycles for use in the present disclosure are given in Tables 4a and 4b. In Tables 4a and 4b, at the C-terminus, some peptides possess a carboxamide terminus (shown as $-\text{NH}_2$); some peptides possess a hydroxyl terminus (shown as $-\text{OH}$); some peptides possess a 5-carboxyfluorescein terminus (shown as $-\text{5-FAM}$); some peptides possess a isobutylamide terminus (shown as $-\text{NH}i\text{Bu}$); some peptides possess a cyclohexylamide terminus (shown as $-\text{NHChx}$); some peptides possess a cyclohexylmethylamide terminus (shown as $-\text{NHMeChx}$); some peptides possess a phenethylamide terminus (shown as $-\text{NHPE}$); some peptides possess a n-butylamide terminus (shown as $-\text{NHBu}$); some peptides possess a *sec*-butylamide terminus (shown as $-\text{NHsBu}$); and some peptides possess an uncapped terminus (shown as no terminal modification).

[00252] In Tables 4a and 4b, at the N-terminus, some peptides possess an acetyl terminus (shown as $\text{Ac}-$); some peptides possess a fluorescein isothiocyanate terminus (shown as $\text{FITC}-$); some peptides possess a single-unit polyethylene glycol terminus (shown as $\text{dPEG1}-$); some peptides possess a five-unit polyethylene glycol terminus (shown as $\text{dPEG5}-$); some peptides possess an eleven-unit polyethylene glycol terminus (shown as $\text{dPEG11}-$); some peptides possess a propyl terminus (shown as $\text{Pr}-$); some peptides possess a biotin terminus (shown as $\text{Biotin}-$); some peptides possess a KLH terminus (shown as $\text{KLH}-$); some peptides possess an ovalbumin terminus (shown as $\text{OVA}-$); some peptides possess an uncapped terminus (shown as $\text{H}-$); some peptides possess a isobutyl terminus (shown as $i\text{Bu}-$); some peptides possess a decanoyl terminus (shown as $\text{Decac}-$); some peptides possess a benzyl terminus (shown as $\text{Bz}-$); some peptides possess a cyclohexyl terminus (shown as $\text{Chx}-$); some peptides possess a benzyl terminus (shown as $\text{Bz}-$); some peptides possess a Vrl terminus (shown as $\text{Vrl}-$); some peptides possess a HBS terminus (shown as $\text{HBS}-$); some peptides possess a MeIm terminus (shown as $\text{MeImC}-$); some peptides possess a *tert*-butyl terminus (shown as $t\text{-Bu-U}-$); some peptides possess a nonanoyl terminus (shown as $\text{non-U}-$); some peptides possess an ethyl terminus (shown as $\text{Et-U}-$); some peptides possess a cyclohexyl terminus (shown as $\text{Chx-U}-$); some peptides possess an isopropyl terminus (shown as $i\text{Pr-U}-$); some peptides possess a phenyl terminus (shown as $\text{Ph-U}-$); some peptides possess a uric terminus (shown as $\text{NH}_2\text{CO}-$); some peptides possess a palmitoyl terminus (shown as $\text{Pam}-$); some peptides possess a heptenoic terminus

(shown as Hep-); and some peptides possess a 5-carboxytetramethylrhodamine terminus (shown as 5-TAMRA-).

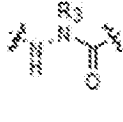
[00253] In some embodiments, the invention provides a peptidomimetic macrocycle of Formula (IX):



Formula (IX)

wherein the peptidomimetic macrocycle binds MCL-1 selectively over another protein that has a BH3 domain, wherein:

each A, C, D, and E is independently a natural or non-natural amino acid;

each B is independently a natural or non-natural amino acid, amino acid analog, , [-NH-L3-CO-], [-NH-L3-SO2-], or [-NH-L3-];

each L is independently a macrocycle-forming linker;

each L' is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R5, or a bond, or together with R1 and the atom to which both R1 and L' are bound forms a ring;

each L'' is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R5, or a bond, or together with R2 and the atom to which both R2 and L'' are bound forms a ring;

each R1 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or together with L' and the atom to which both R1 and L' are bound forms a ring;

each R2 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or together with L'' and the atom to which both R2 and L'' are bound forms a ring;

each R3 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R5;

each L_3 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R_4-K-R_4-]_n$, each being optionally substituted with R_5 ;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR₃;

each n is independently an integer from 1-5;

each R_5 is independently halogen, alkyl, -OR₆, -N(R₆)₂, -SR₆, -SOR₆, -SO₂R₆, -CO₂R₆, a fluorescent moiety, a radioisotope, or a therapeutic agent;

each R_6 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope, or a therapeutic agent;

each R_7 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

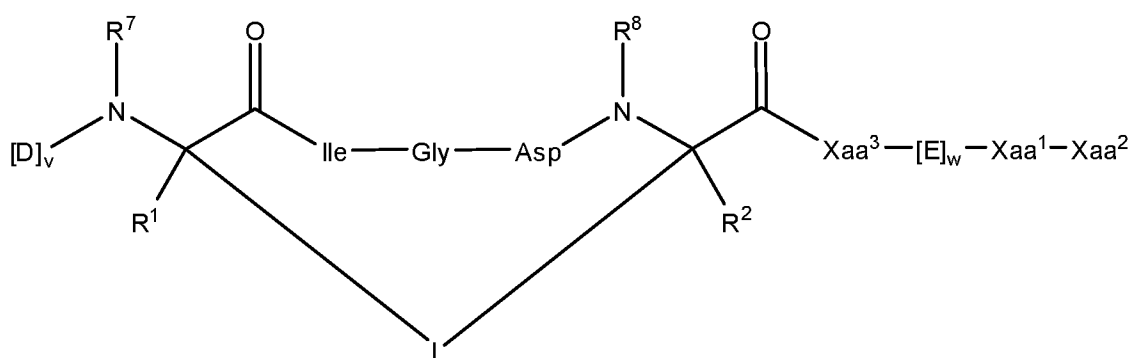
each R_8 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

each v and w is independently an integer from 1-1000;

u is an integer from 1-10; and

each x , y and z is independently an integer from 0-10, or a pharmaceutically-acceptable salt thereof.

[00254] In some embodiments, the invention provides a peptidomimetic macrocycle having the formula:



wherein:

each D and E is independently an amino acid residue;

R^1 and R^2 are independently alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl,

heteroalkyl, or heterocycloalkyl, each being optionally substituted with halo-, -H, or at least one of

R^1 and R^2 forms a macrocycle-forming linker L' connected to the alpha position of one of the D or E amino acid residues;

L is a macrocycle-forming linker of the formula $-L^1-L^2-$ or $-L^1-L^2-L^3-$;

L^1 , L^2 , and L^3 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R^4-K-R^4-]_n$, each being optionally substituted with R^5 ;

each R^3 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 ;

each R^4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R^5 ;

each K is independently O , S , SO , SO_2 , CO , CO_2 , or $CONR^3$;

each R^5 is independently halogen, alkyl, $-OR^6$, $-N(R^6)_2$, $-SR^6$, $-SOR^6$, $-SO_2R^6$, $-CO_2R^6$, a fluorescent moiety, a radioisotope, or a therapeutic agent;

each R^6 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope, or a therapeutic agent;

R^7 is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 , or part of a cyclic structure with a D residue;

R^8 is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 , or part of a cyclic structure with an E residue;

each of Xaa^1 and Xaa^2 is independently an amino acid residue or absent;

Xaa^3 is Ala, Aib, Asp, Asn, Cys, Glu, Gln, His, Ile, Lys, Leu, Met, Arg, Ser, Thr, Val, Trp, Tyr, or an analog of any of the foregoing;

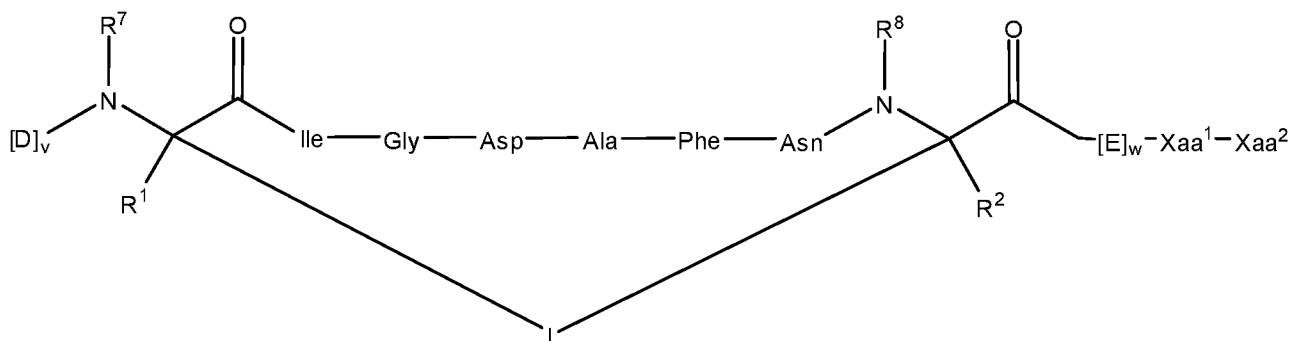
v is an integer from 1-1000;

w is an integer from 0-1000; and

n is an integer from 1-5, or

a pharmaceutically-acceptable salt thereof.

[00255] In some embodiments, the invention provides a peptidomimetic macrocycle of the formula:



wherein:

each D and E is independently an amino acid residue;

R^1 and R^2 are independently alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, each being optionally substituted with halo-, -H, or at least one of R^1 and R^2 forms a macrocycle-forming linker L' connected to the alpha position of one of the D or E amino acid residues;

each L or L' is independently a macrocycle-forming linker of the formula $-L^1-L^2-$ or $-L^1-L^2-L^3-$; L^1 , L^2 , and L^3 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R^4-K-R^4-]_n$, each being optionally substituted with R^5 ;

each R^3 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 ;

each R^4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, each being optionally substituted with R^5 ;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR³;

each R^5 is independently halogen, alkyl, -OR⁶, -N(R⁶)₂, -SR⁶, -SOR⁶, -SO₂R⁶, -CO₂R⁶, a fluorescent moiety, a radioisotope, or a therapeutic agent;

each R^6 is independently -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope, or a therapeutic agent;

R^7 is -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 , or part of a cyclic structure with a D residue;

R^8 is -H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, each being optionally substituted with R^5 , or part of a cyclic structure with an E residue;

each of Xaa¹ and Xaa² is independently an amino acid residue or absent;

v is an integer from 1-1000;

w is an integer from 0-1000; and
n is an integer from 1-5, or
a pharmaceutically-acceptable salt thereof.

[00256] In some embodiments, the invention provides a peptidomimetic macrocycle comprising an amino acid sequence of formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21

wherein:

X1 is Ile, Arg, Ala, Lys, Pro, Leu, Asp, Glu, His, Ser, Gln, Phe, an analog thereof, or absent;

X2 is Trp, Arg, Ala, Asn, Phe, Pro, Leu, Ser, Lys, Tyr, His, Cou, Cou2, Cou4, Cou7, an analog thereof, a crosslinked amino acid, or absent;

X3 is Ile, Ala, Leu, Phe, Tyr, Val, Asp, Trp, Pro, Gln, Chg, Ac5c, Ac6c, Tba, Bip, Cha, Adm, hCha, an analog thereof, or absent;

X4 is Ala, Gln, Asp, Val, Gly, Ser, Leu, Phe, Cha, A4, an analog thereof, a crosslinked amino acid, or absent;

X5 is Gln, Ala, Leu, Phe, Tyr, Gly, Ile, Val, Arg, Glu, Pro, Asp, MO, MO2, an analog thereof, a crosslinked amino acid, or absent;

X6 is Glu, Gln, His, Ala, Ser, Arg, Ile, Leu, Thr, Phe, Val, Tyr, Gly, Nle, St, an analog thereof, or absent;

X7 is Ala, Leu, Phe, Ile, 2Nal, 1Nal, 3cf, Chg, Cha, Adm, hCha, Igl, Bip, an analog thereof, or absent;

X8 is Arg, Ala, Asp, Glu, Thr, His, Gln, Gly, Asn, Phe, Cit, St, an analog thereof, a crosslinked amino acid, or absent;

X9 is Arg, Ala, Asp, Lys, Asn, Gly, Ser, Gln, Cys, Nle, St, an analog thereof, or a crosslinked amino acid;

X10 is Ile, Val, Ala, Asp, Asn, Phe, Tba, hL, hhL, Nle, Chg, Cha, an analog thereof, or a crosslinked amino acid;

X11 is Gly, Val, Ala, Leu, Ile, Asp, Glu, Cha, Aib, Abu, an analog thereof, or a crosslinked amino acid;

X12 is Asp, Ala, Asn, Gly, Arg, Glu, Lys, Leu, Nle, an analog thereof, or a crosslinked amino acid;

X13 is Ala, Glu, Gln, Leu, Lys, Asp, Tyr, Ile, Ser, Cys, St, Sta5, Aib, Nle, an analog thereof, or a crosslinked amino acid;

X14 is Phe, Ala, Leu, Val, Tyr, Glu, His, Ile, Nle, 1Nal, 2Nal, Chg, Cha, BiP, an analog thereof, or a crosslinked amino acid;

X15 is Asn, Gln, Ser, His, Glu, Asp, Ala, Leu, Ile, St, Nle, Aib, an analog thereof, a crosslinked amino acid, or absent;

X16 is Ala, Glu, Asp, Arg, Lys, Phe, Gly, Gln, Aib, Cha, St, an analog thereof, a crosslinked amino acid, or absent;

X17 is Phe, Tyr, Ala, Leu, Asn, Ser, Gln, Arg, His, Thr, Cou2, Cou3, Cou7, Dpr, Amf, Damf, Amye, an analog thereof, a crosslinked amino acid, or absent;

X18 is Tyr, Ala, Ile, Phe, His, Arg, Lys, Trp, Orn, Amf, Amye, Cha, 2Nal, an analog thereof, or absent;

X19 is Ala, Lys, Arg, His, Ser, Gln, Glu, Asp, Thr, Aib, Cha, an analog thereof, a crosslinked amino acid, or absent; and

X20 is Arg, His, Ala, Thr, Lys, Amr, an analog thereof, a crosslinked amino acid, or absent; and

X21 is Arg, His, Ala, Amr, an analog thereof, or absent, or a pharmaceutically-acceptable salt thereof,

wherein at least two of the amino acids of the amino acid sequence are a crosslinked amino acid.

[00257] In some embodiments, the invention provides a peptidomimetic macrocycle comprising an amino acid sequence with C-terminal amino acid residues that are –His-His, wherein the peptidomimetic macrocycle comprises a crosslink connecting at least two amino acid residues, or a pharmaceutically-acceptable salt thereof.

[00258] In some embodiments, the invention provides a peptidomimetic macrocycle that comprises an amino acid sequence that has at least 60%, 70%, 80%, 90%, 95%, 97%, or 100% identity to any one of the amino acid sequences in Table 4a or Table 4b.

Preparation of Peptidomimetic Macrocycles

[00259] Peptidomimetic macrocycles may be prepared by any of a variety of methods known in the art. For example, any of the residues indicated by “\$” or “\$r8” in Tables 1-4 may be substituted with a residue capable of forming a crosslinker with a second residue in the same molecule or a precursor of such a residue.

[00260] Various methods to effect formation of peptidomimetic macrocycles are known in the art. For example, the preparation of peptidomimetic macrocycles of Formula I is described in Schafmeister et al., J. Am. Chem. Soc. 122:5891-5892 (2000); Schafmeister & Verdine, J. Am. Chem. Soc. 122:5891 (2005); Walensky et al., Science 305:1466-1470 (2004); and US Patent No. 7,192,713; and PCT application WO 2008/121767. The α,α -disubstituted amino acids and amino acid precursors disclosed in the cited references may be employed in synthesis of the peptidomimetic macrocycle precursor polypeptides. For example, the “S5-olefin amino acid” is (S)- α -(2'-pentenyl) alanine and the “R8 olefin amino acid” is (R)- α -(2'-octenyl) alanine. Following incorporation of such amino

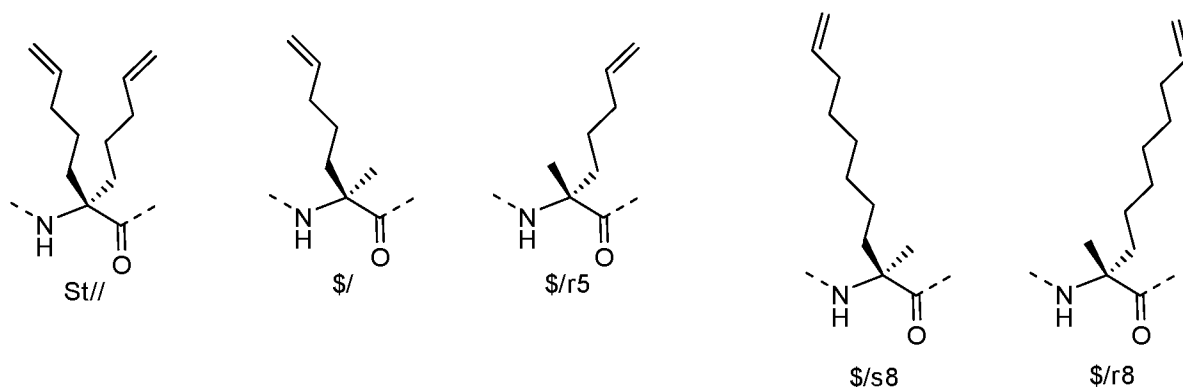
acids into precursor polypeptides, the terminal olefins are reacted with a metathesis catalyst, leading to the formation of the peptidomimetic macrocycle.

[00261] In other embodiments, the peptidomimetic macrocycles are of Formula IV or IVa. Methods for the preparation of such macrocycles are described, for example, in US Patent No. 7,202,332 and PCT application WO 2008/121767.

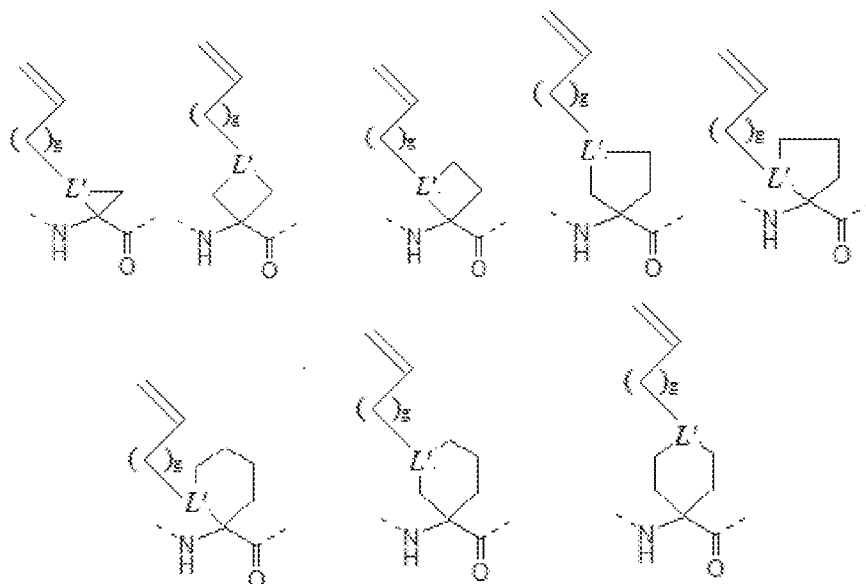
[00262] In some embodiments, the synthesis of these peptidomimetic macrocycles involves a multi-step process that features the synthesis of a peptidomimetic precursor containing an azide moiety and an alkyne moiety; followed by contacting the peptidomimetic precursor with a macrocyclization catalyst to generate a triazole-linked peptidomimetic macrocycle. Such a process is described, for example, in US Application 12/037,041, filed on February 25, 2008. Macrocycles or macrocycle precursors are synthesized, for example, by solution phase or solid-phase methods, and can contain both naturally-occurring and non-naturally-occurring amino acids. See, for example, Hunt, "The Non-Protein Amino Acids" in *Chemistry and Biochemistry of the Amino Acids*, edited by G.C. Barrett, Chapman and Hall, 1985.

[00263] In some embodiments, an azide is linked to the α -carbon of a residue and an alkyne is attached to the α -carbon of another residue. In some embodiments, the azide moieties are azido-analogs of amino acids L-lysine, D-lysine, α -methyl-L-lysine, α -methyl-D-lysine, L-ornithine, D-ornithine, α -methyl-L-ornithine or α -methyl-D-ornithine. In another embodiment, the alkyne moiety is L-propargylglycine. In yet other embodiments, the alkyne moiety is an amino acid selected from the group consisting of L-propargylglycine, D-propargylglycine, (S)-2-amino-2-methyl-4-pentynoic acid, (R)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-2-methyl-5-hexynoic acid, (R)-2-amino-2-methyl-5-hexynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, (R)-2-amino-2-methyl-6-heptynoic acid, (S)-2-amino-2-methyl-7-octynoic acid, (R)-2-amino-2-methyl-7-octynoic acid, (S)-2-amino-2-methyl-8-nonynoic acid and (R)-2-amino-2-methyl-8-nonynoic acid.

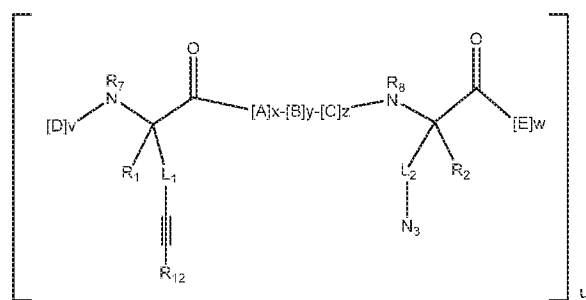
[00264] In various embodiments, the following amino acids may be employed in the synthesis of the peptidomimetic macrocycle:



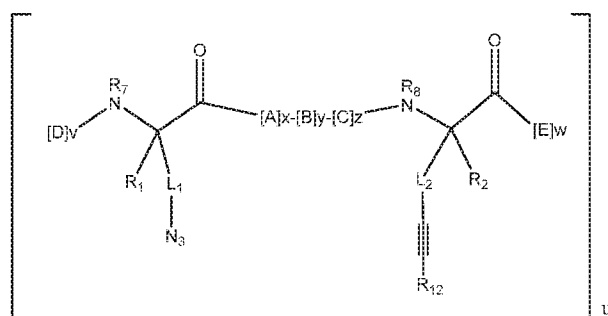
[00265] In various embodiments, the following amino acids may be employed in the synthesis of the peptidomimetic macrocycle, wherein L' is an atom (for example, C, O, N, or S); and g is an integer from 1-20, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20:



[00266] In some embodiments, the invention provides a method for synthesizing a peptidomimetic macrocycle, the method comprising the steps of contacting a peptidomimetic precursor of Formula V or Formula VI:



(Formula V)



(Formula VI)

with a macrocyclization catalyst;

wherein v, w, x, y, z, A, B, C, D, E, R₁, R₂, R₇, R₈, L₁ and L₂ are as defined for Formula (II);

R₁₂ is -H when the macrocyclization catalyst is a Cu catalyst and R₁₂ is -H or alkyl when the

macrocyclization catalyst is a Ru catalyst, and further wherein the contacting step results in a covalent linkage being formed between the alkyne and azide moiety in Formula III or Formula IV. For example, R₁₂ may be methyl when the macrocyclization catalyst t is a Ru catalyst.

[00267] In the peptidomimetic macrocycles, at least one of R₁ and R₂ is alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-. In some embodiments, both R₁ and R₂ are independently alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-. In some embodiments, at least one of A, B, C, D or E is an α,α -disubstituted amino acid. In one example, B is an α,α -disubstituted amino acid. For instance, at least one of A, B, C, D or E is 2-aminoisobutyric acid.

[00268] For example, at least one of R₁ and R₂ is alkyl, unsubstituted or substituted with halo-. In another example, both R₁ and R₂ are independently alkyl, unsubstituted or substituted with halo-. In some embodiments, at least one of R₁ and R₂ is methyl. In other embodiments, R₁ and R₂ are methyl. The macrocyclization catalyst may be a Cu catalyst or a Ru catalyst.

[00269] In some embodiments, the peptidomimetic precursor is purified prior to the contacting step. In other embodiments, the peptidomimetic macrocycle is purified after the contacting step. In still other embodiments, the peptidomimetic macrocycle is refolded after the contacting step. The method may be performed in solution, or, alternatively, the method may be performed on a solid support.

[00270] Also envisioned herein is performing the method in the presence of a target macromolecule that binds to the peptidomimetic precursor or peptidomimetic macrocycle under conditions that favor the binding. In some embodiments, the method is performed in the presence of a target macromolecule that binds preferentially to the peptidomimetic precursor or peptidomimetic macrocycle under conditions that favor the binding. The method may also be applied to synthesize a library of peptidomimetic macrocycles.

[00271] In some embodiments, the alkyne moiety of the peptidomimetic precursor of Formula V or Formula VI is a sidechain of an amino acid selected from the group consisting of L-propargylglycine, D-propargylglycine, (S)-2-amino-2-methyl-4-pentynoic acid, (R)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-2-methyl-5-hexynoic acid, (R)-2-amino-2-methyl-5-hexynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, (R)-2-amino-2-methyl-6-heptynoic acid, (S)-2-amino-2-methyl-7-octynoic acid, (R)-2-amino-2-methyl-7-octynoic acid, (S)-2-amino-2-methyl-8-nonynoic acid, and (R)-2-amino-2-methyl-8-nonynoic acid. In other embodiments, the azide moiety of the peptidomimetic precursor of Formula V or Formula VI is a sidechain of an amino acid selected from the group consisting of ϵ -azido-L-lysine, ϵ -azido-D-lysine, ϵ -azido- α -methyl-L-lysine, ϵ -azido- α -methyl-D-lysine, δ -azido- α -methyl-L-ornithine, and δ -azido- α -methyl-D-ornithine.

[00272] In some embodiments, $x+y+z$ is 2, and A, B and C are independently natural or non-natural amino acids. In other embodiments, $x+y+z$ is 3 or 6, and A, B and C are independently natural or non-natural amino acids.

[00273] In some embodiments, the contacting step is performed in a solvent selected from the group consisting of protic solvent, aqueous solvent, organic solvent, and mixtures thereof. For example, the solvent may be chosen from the group consisting of H₂O, THF, THF/H₂O, tBuOH/H₂O, DMF, DIPEA, CH₃CN or CH₂Cl₂, ClCH₂CH₂Cl or a mixture thereof. The solvent may be a solvent which favors helix formation.

[00274] Alternative but equivalent protecting groups, leaving groups or reagents are substituted, and certain of the synthetic steps are performed in alternative sequences or orders to produce the desired compounds. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein include, for example, those such as described in Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); Greene and Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed. , John Wiley and Sons (1991); Fieser and Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and subsequent editions thereof.

[00275] The peptidomimetic macrocycles disclosed herein are made, for example, by chemical synthesis methods, such as described in Fields *et al.*, Chapter 3 in *Synthetic Peptides: A User's Guide*, ed. Grant, W. H. Freeman & Co., New York, N. Y., 1992, p. 77. Hence, for example, peptides are synthesized using the automated Merrifield techniques of solid phase synthesis with the amine protected by either tBoc or Fmoc chemistry using side chain protected amino acids on, for example, an automated peptide synthesizer (*e.g.*, Applied Biosystems (Foster City, CA), Model 430A, 431, or 433).

[00276] One manner of producing the peptidomimetic precursors and peptidomimetic macrocycles described herein uses solid phase peptide synthesis (SPPS). The C-terminal amino acid is attached to a cross-linked polystyrene resin *via* an acid labile bond with a linker molecule. This resin is insoluble in the solvents used for synthesis, making it relatively simple and fast to wash away excess reagents and by-products. The N-terminus is protected with the Fmoc group, which is stable in acid, but removable by base. Side chain functional groups are protected as necessary with base stable, acid labile groups.

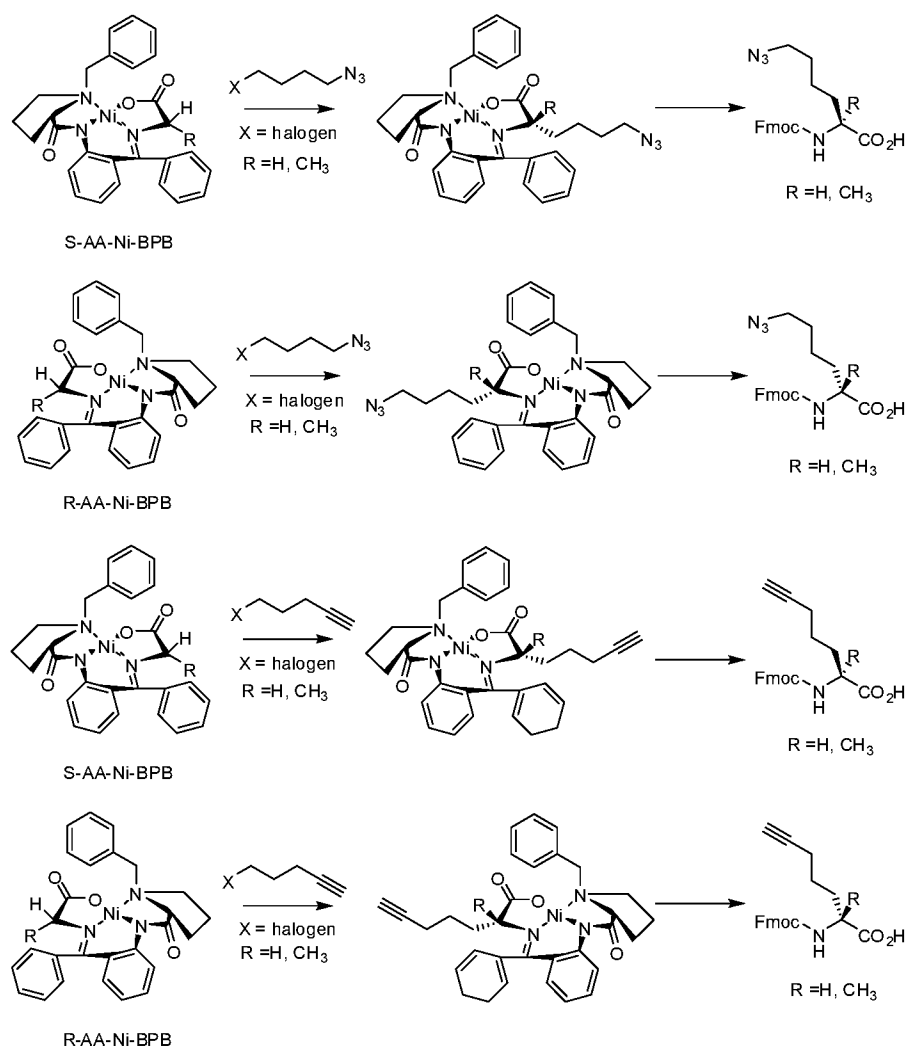
[00277] Longer peptidomimetic precursors are produced, for example, by conjoining individual synthetic peptides using native chemical ligation. Alternatively, the longer synthetic peptides are biosynthesized by well-known recombinant DNA and protein expression techniques. Such

techniques are provided in well-known standard manuals with detailed protocols. To construct a gene encoding a peptidomimetic precursor of this invention, the amino acid sequence is reverse translated to obtain a nucleic acid sequence encoding the amino acid sequence, preferably with codons that are optimum for the organism in which the gene is to be expressed. Next, a synthetic gene is made, typically by synthesizing oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and transfected into a host cell. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

[00278] The peptidomimetic precursors are made, for example, in a high-throughput, combinatorial fashion using, for example, a high-throughput polychannel combinatorial synthesizer (*e.g.*, Thuramed TETRAS multichannel peptide synthesizer from CreoSalus, Louisville, KY or Model Apex 396 multichannel peptide synthesizer from AAPPTec, Inc., Louisville, KY).

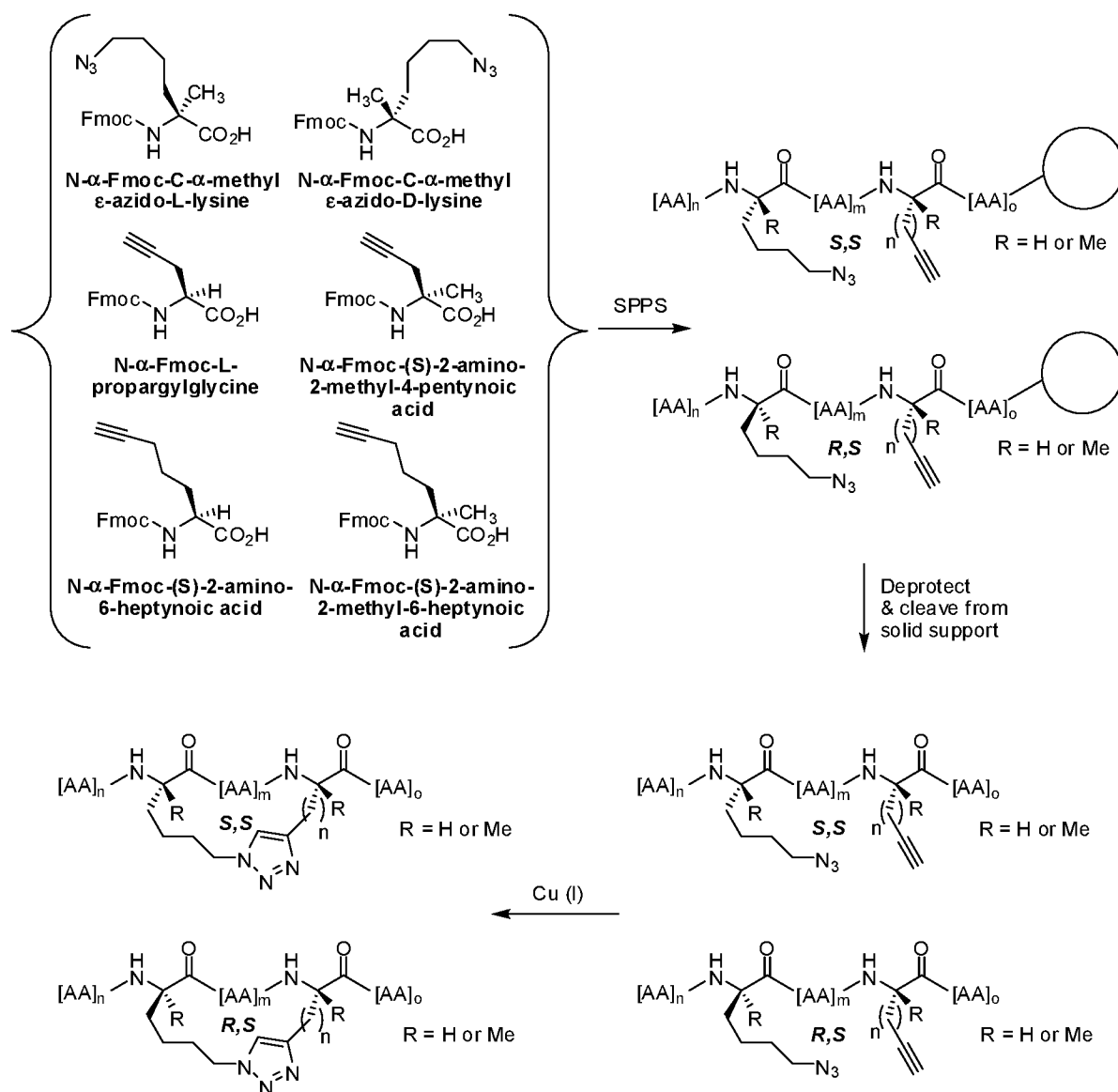
[00279] The following synthetic schemes are provided solely to illustrate the present invention and are not intended to limit the scope of the invention, as described herein. To simplify the drawings, the illustrative schemes depict azido amino acid analogs ϵ -azido- α -methyl-L-lysine and ϵ -azido- α -methyl-D-lysine, and alkyne amino acid analogs L-propargylglycine, (S)-2-amino-2-methyl-4-pentynoic acid, and (S)-2-amino-2-methyl-6-heptynoic acid. Thus, in the following synthetic schemes, each R_1 , R_2 , R_7 and R_8 is -H; each L_1 is $-(CH_2)_4-$; and each L_2 is $-(CH_2)-$. However, as noted throughout the detailed description above, many other amino acid analogs can be employed in which R_1 , R_2 , R_7 , R_8 , L_1 and L_2 can be independently selected from the various structures disclosed herein.

[00280] Synthetic Scheme 1:



[00281] Synthetic Scheme 1 describes the preparation of several compounds of the invention. Ni(II) complexes of Schiff bases derived from the chiral auxiliary (S)-2-[N-(N'-benzylpropyl)amino]benzophenone (BPB) and amino acids such as glycine or alanine are prepared as described in Belokon *et al.* (1998), *Tetrahedron Asymm.* 9:4249-4252. The resulting complexes are subsequently reacted with alkylating reagents comprising an azido or alkynyl moiety to yield enantiomerically enriched compounds. If desired, the resulting compounds can be protected for use in peptide synthesis.

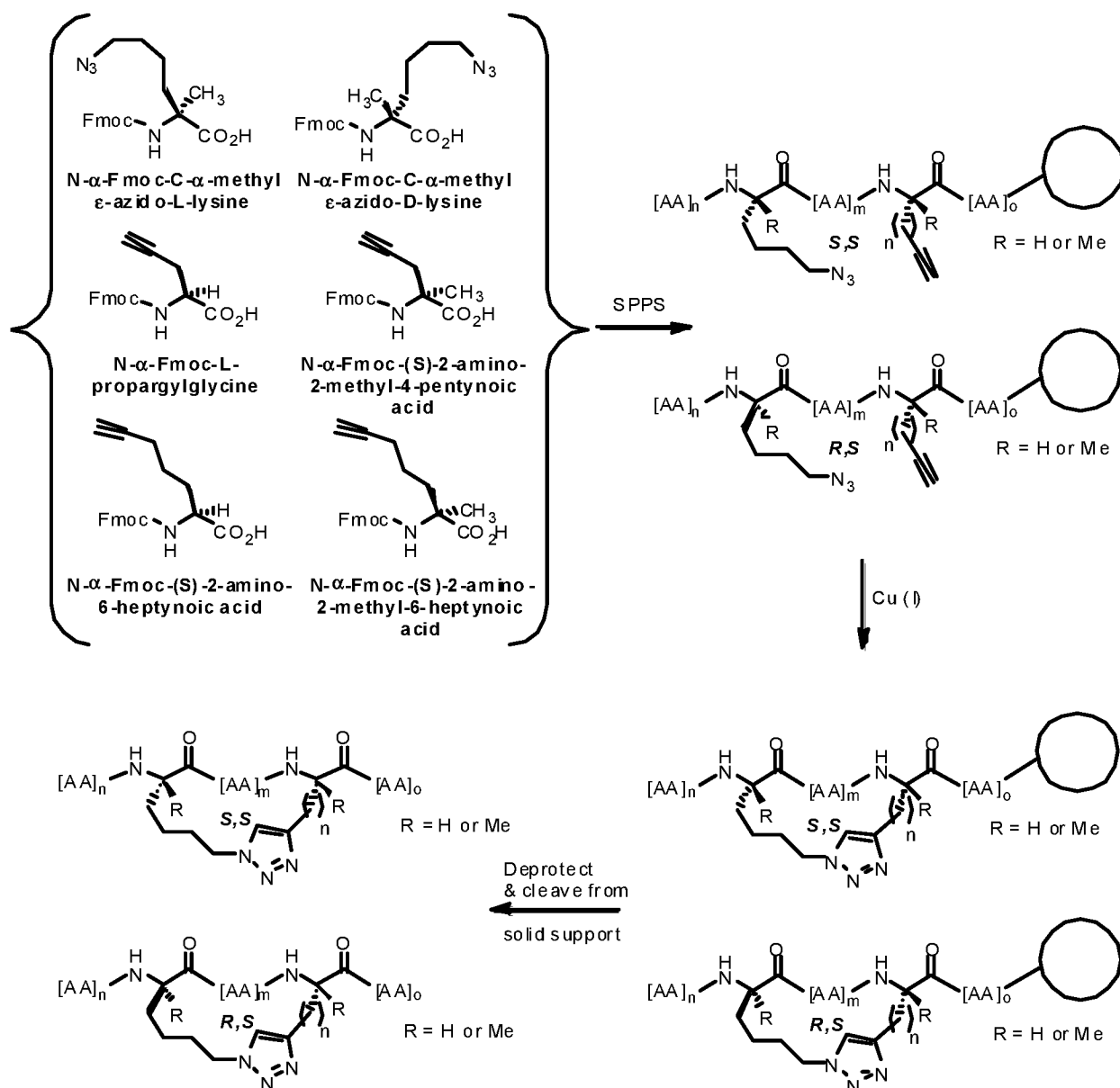
[00282] Synthetic Scheme 2:



[00283] In the general method for the synthesis of peptidomimetic macrocycles shown in Synthetic Scheme 2, the peptidomimetic precursor contains an azide moiety and an alkyne moiety and is synthesized by solution-phase or solid-phase peptide synthesis (SPPS) using the commercially available amino acid N- α -Fmoc-L-propargylglycine and the N- α -Fmoc-protected forms of the amino acids (S)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-6-heptynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, N-methyl- ϵ -azido-L-lysine, and N-methyl- ϵ -azido-D-lysine. The peptidomimetic precursor is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA). The peptidomimetic precursor is reacted as a crude mixture or is purified prior to reaction with a macrocyclization catalyst such as a Cu(I) in organic or aqueous solutions (Rostovtsev *et al.* (2002), *Angew. Chem. Int. Ed.* 41:2596-2599; Tornøe *et al.* (2002), *J. Org. Chem.* 67:3057-3064; Deiters *et al.* (2003), *J. Am. Chem. Soc.* 125:11782-11783; Punna *et al.* (2005), *Angew. Chem. Int. Ed.* 44:2215-2220). In one embodiment, the triazole forming reaction is

performed under conditions that favor helix formation. In one embodiment, the macrocyclization step is performed in a solvent chosen from the group consisting of H₂O, THF, CH₃CN, DMF, DIPEA, tBuOH or a mixture thereof. In another embodiment, the macrocyclization step is performed in DMF. In some embodiments, the macrocyclization step is performed in a buffered aqueous or partially aqueous solvent.

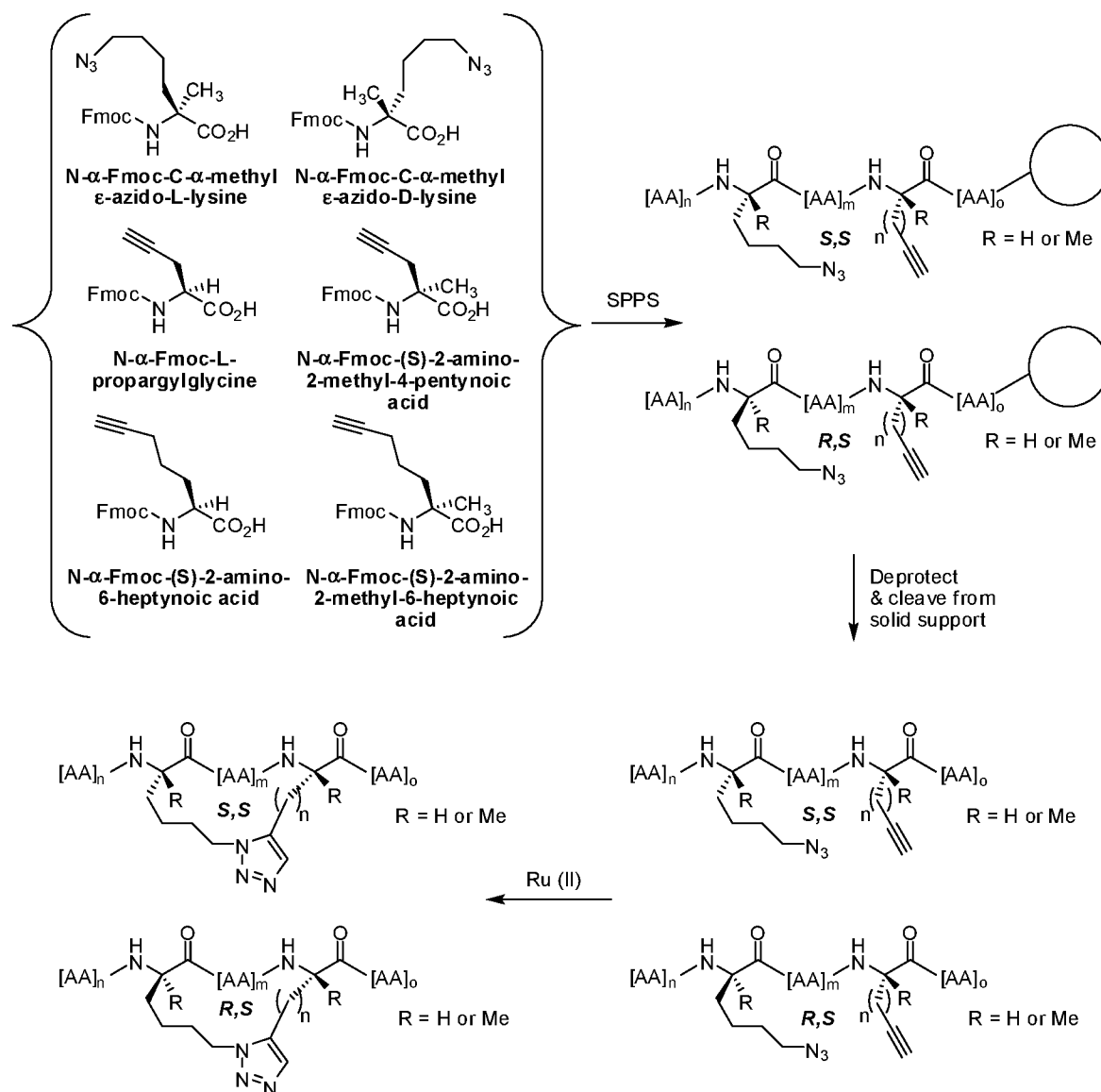
[00284] Synthetic Scheme 3:



[00285] In the general method for the synthesis of peptidomimetic macrocycles shown in Synthetic Scheme 3, the peptidomimetic precursor contains an azide moiety and an alkyne moiety and is synthesized by solid-phase peptide synthesis (SPPS) using the commercially available amino acid N- α -Fmoc-L-propargylglycine and the N- α -Fmoc-protected forms of the amino acids (S)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-6-heptynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, N-

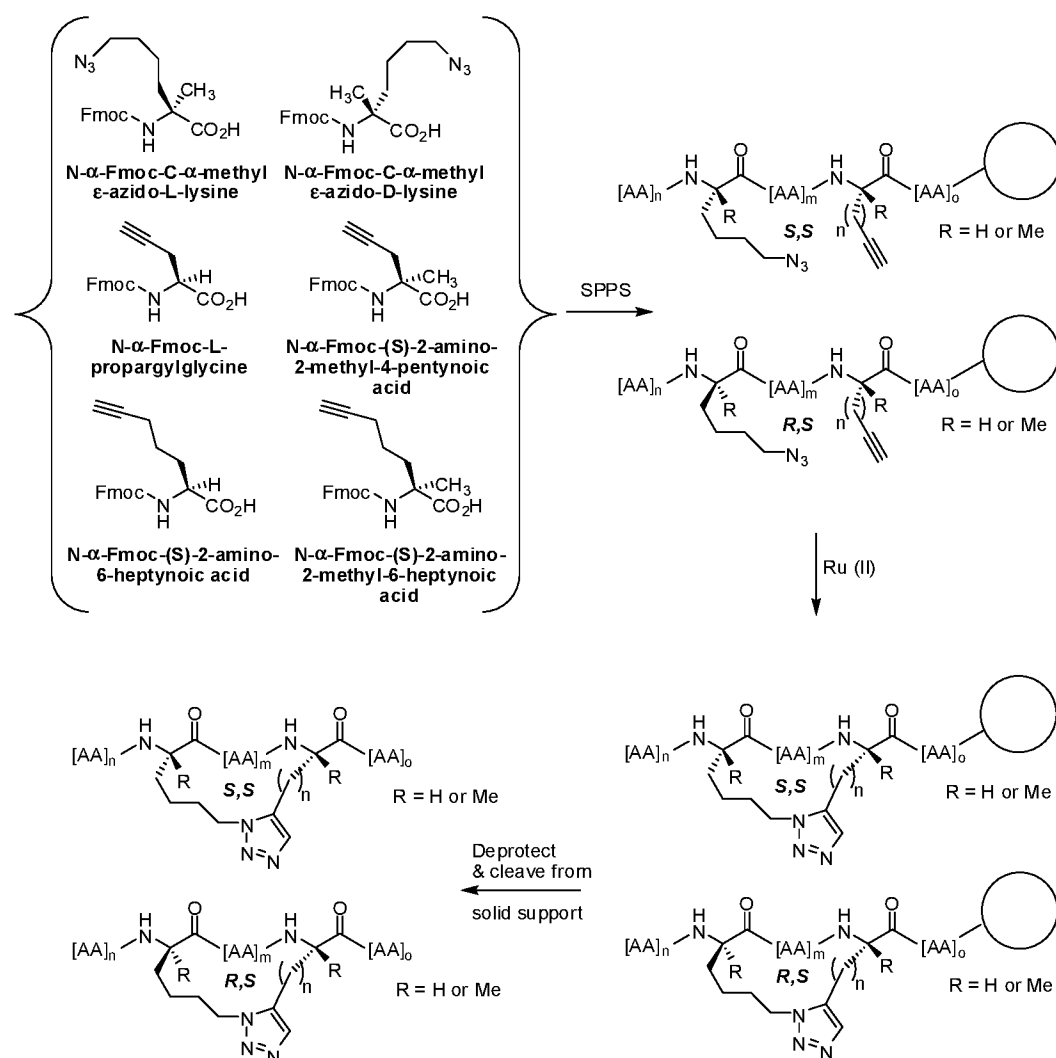
methyl- ϵ -azido-L-lysine, and N-methyl- ϵ -azido-D-lysine. The peptidomimetic precursor is reacted with a macrocyclization catalyst such as a Cu(I) catalyst on the resin as a crude mixture (Rostovtsev *et al.* (2002), *Angew. Chem. Int. Ed.* 41:2596-2599; Tornøe *et al.* (2002), *J. Org. Chem.* 67:3057-3064; Deiters *et al.* (2003), *J. Am. Chem. Soc.* 125:11782-11783; Punna *et al.* (2005), *Angew. Chem. Int. Ed.* 44:2215-2220). The resultant triazole-containing peptidomimetic macrocycle is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA). In some embodiments, the macrocyclization step is performed in a solvent chosen from the group consisting of CH_2Cl_2 , $\text{ClCH}_2\text{CH}_2\text{Cl}$, DMF, THF, NMP, DIPEA, 2,6-lutidine, pyridine, DMSO, H_2O or a mixture thereof. In some embodiments, the macrocyclization step is performed in a buffered aqueous or partially aqueous solvent.

[00286] Synthetic Scheme 4:



[00287] In the general method for the synthesis of peptidomimetic macrocycles shown in Synthetic Scheme 4, the peptidomimetic precursor contains an azide moiety and an alkyne moiety and is synthesized by solution-phase or solid-phase peptide synthesis (SPPS) using the commercially available amino acid N- α -Fmoc-L-propargylglycine and the N- α -Fmoc-protected forms of the amino acids (S)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-6-heptynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, N-methyl- ϵ -azido-L-lysine, and N-methyl- ϵ -azido-D-lysine. The peptidomimetic precursor is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA). The peptidomimetic precursor is reacted as a crude mixture or is purified prior to reaction with a macrocyclization catalyst such as a Ru(II) catalyst, for example Cp*RuCl(PPh₃)₂ or [Cp*RuCl]₄ (Rasmussen *et al.* (2007), *Org. Lett.* 9:5337-5339; Zhang *et al.* (2005), *J. Am. Chem. Soc.* 127:15998-15999). In some embodiments, the macrocyclization step is performed in a solvent chosen from the group consisting of DMF, CH₃CN and THF.

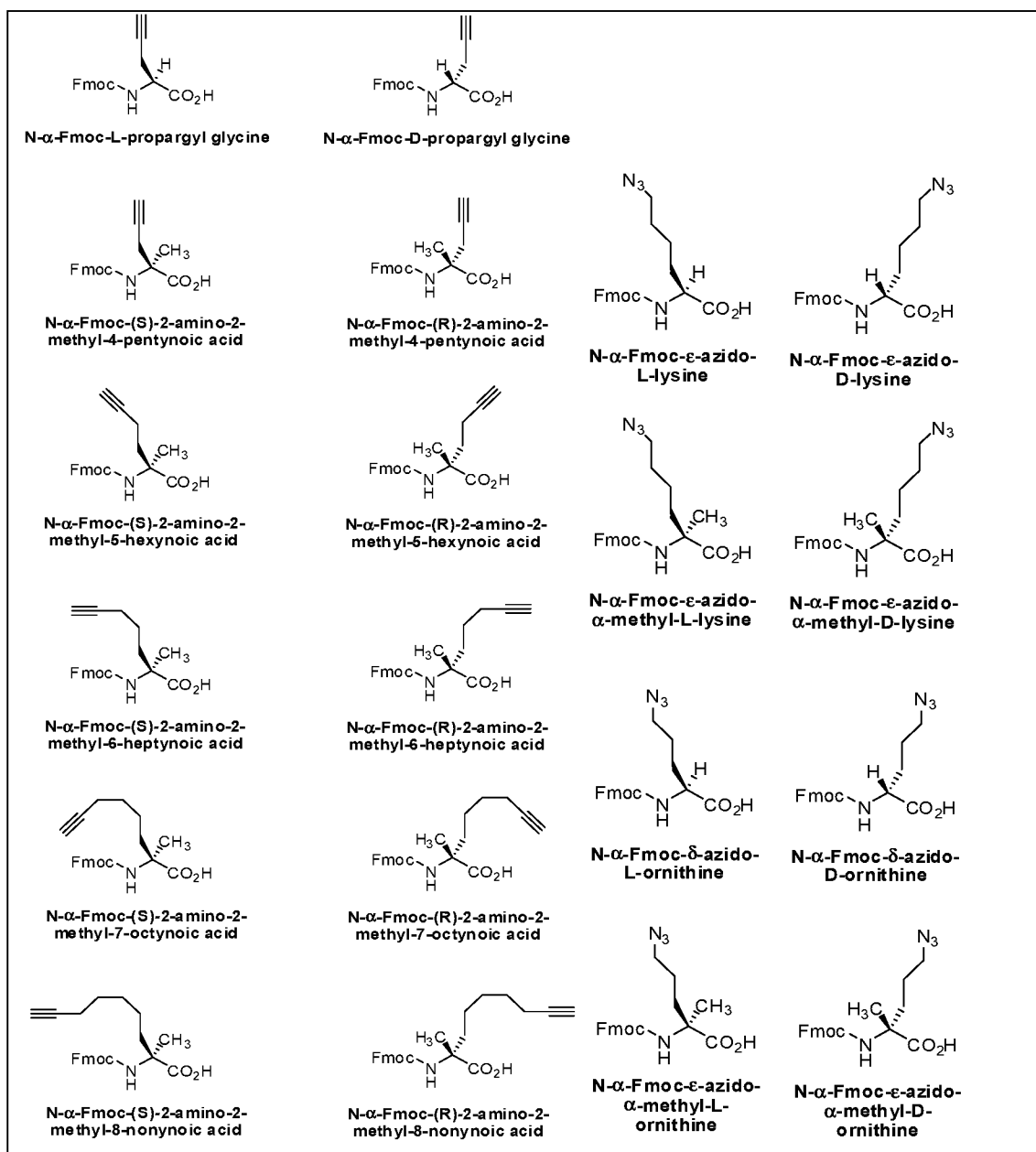
[00288] Synthetic Scheme 5:



[00289] In the general method for the synthesis of peptidomimetic macrocycles shown in Synthetic Scheme 5, the peptidomimetic precursor contains an azide moiety and an alkyne moiety and is synthesized by solid-phase peptide synthesis (SPPS) using the commercially available amino acid N- α -Fmoc-L-propargylglycine and the N- α -Fmoc-protected forms of the amino acids (S)-2-amino-2-methyl-4-pentynoic acid, (S)-2-amino-6-heptynoic acid, (S)-2-amino-2-methyl-6-heptynoic acid, N-methyl- ϵ -azido-L-lysine, and N-methyl- ϵ -azido-D-lysine. The peptidomimetic precursor is reacted with a macrocyclization catalyst such as a Ru(II) catalyst on the resin as a crude mixture. For example, the catalyst can be Cp*RuCl(PPh₃)₂ or [Cp*RuCl]₄ (Rasmussen *et al.* (2007), *Org. Lett.* 9:5337-5339; Zhang *et al.* (2005), *J. Am. Chem. Soc.* 127:15998-15999). In some embodiments, the macrocyclization step is performed in a solvent chosen from the group consisting of CH₂Cl₂, ClCH₂CH₂Cl, CH₃CN, DMF, and THF.

[00290] The present invention contemplates the use of non-naturally-occurring amino acids and amino acid analogs in the synthesis of the peptidomimetic macrocycles described herein. Any amino acid or amino acid analog amenable to the synthetic methods employed for the synthesis of stable triazole containing peptidomimetic macrocycles can be used. For example, L-propargylglycine is contemplated as a useful amino acid. However, other alkyne-containing amino acids that contain a different amino acid side chain are also useful in the invention. For example, L-propargylglycine contains one methylene unit between the α -carbon of the amino acid and the alkyne of the amino acid side chain. The invention also contemplates the use of amino acids with multiple methylene units between the α -carbon and the alkyne. Also, the azido-analogs of amino acids L-lysine, D-lysine, α -methyl-L-lysine, and α -methyl-D-lysine are contemplated as useful amino acids. However, other terminal azide amino acids that contain a different amino acid side chain are also useful in the invention. For example, the azido-analog of L-lysine contains four methylene units between the α -carbon of the amino acid and the terminal azide of the amino acid side chain. The invention also contemplates the use of amino acids with fewer than or greater than four methylene units between the α -carbon and the terminal azide. Table 5 shows some amino acids useful in the preparation of peptidomimetic macrocycles.

Table 5 - Exemplary amino acids useful in the preparation of peptidomimetic macrocycles.

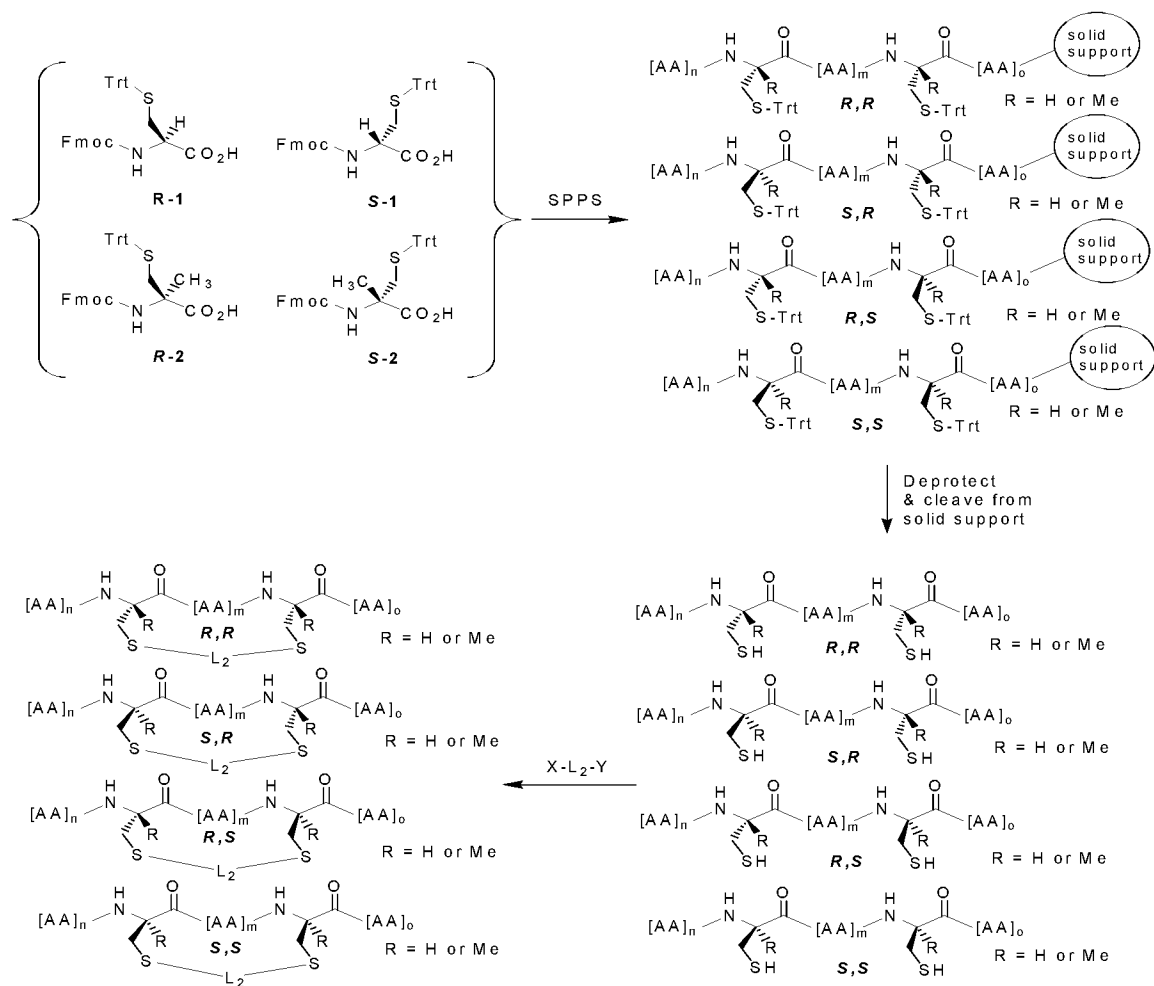


[00291] In some embodiments the amino acids and amino acid analogs are of the D-configuration. In other embodiments they are of the L-configuration. In some embodiments, some of the amino acids and amino acid analogs contained in the peptidomimetic are of the D-configuration while some of the amino acids and amino acid analogs are of the L-configuration. In some embodiments the amino acid analogs are α,α -disubstituted, such as α -methyl-L-propargylglycine, α -methyl-D-propargylglycine, ϵ -azido- α -methyl-L-lysine, and ϵ -azido- α -methyl-D-lysine. In some embodiments the amino acid analogs are N-alkylated, *e.g.*, N-methyl-L-propargylglycine, N-methyl-D-propargylglycine, N-methyl- ϵ -azido-L-lysine, and N-methyl- ϵ -azido-D-lysine.

[00292] In some embodiments, the $-NH$ moiety of the amino acid is protected using a protecting group, including without limitation $-Fmoc$ and $-Boc$. In other embodiments, the amino acid is not protected prior to synthesis of the peptidomimetic macrocycle.

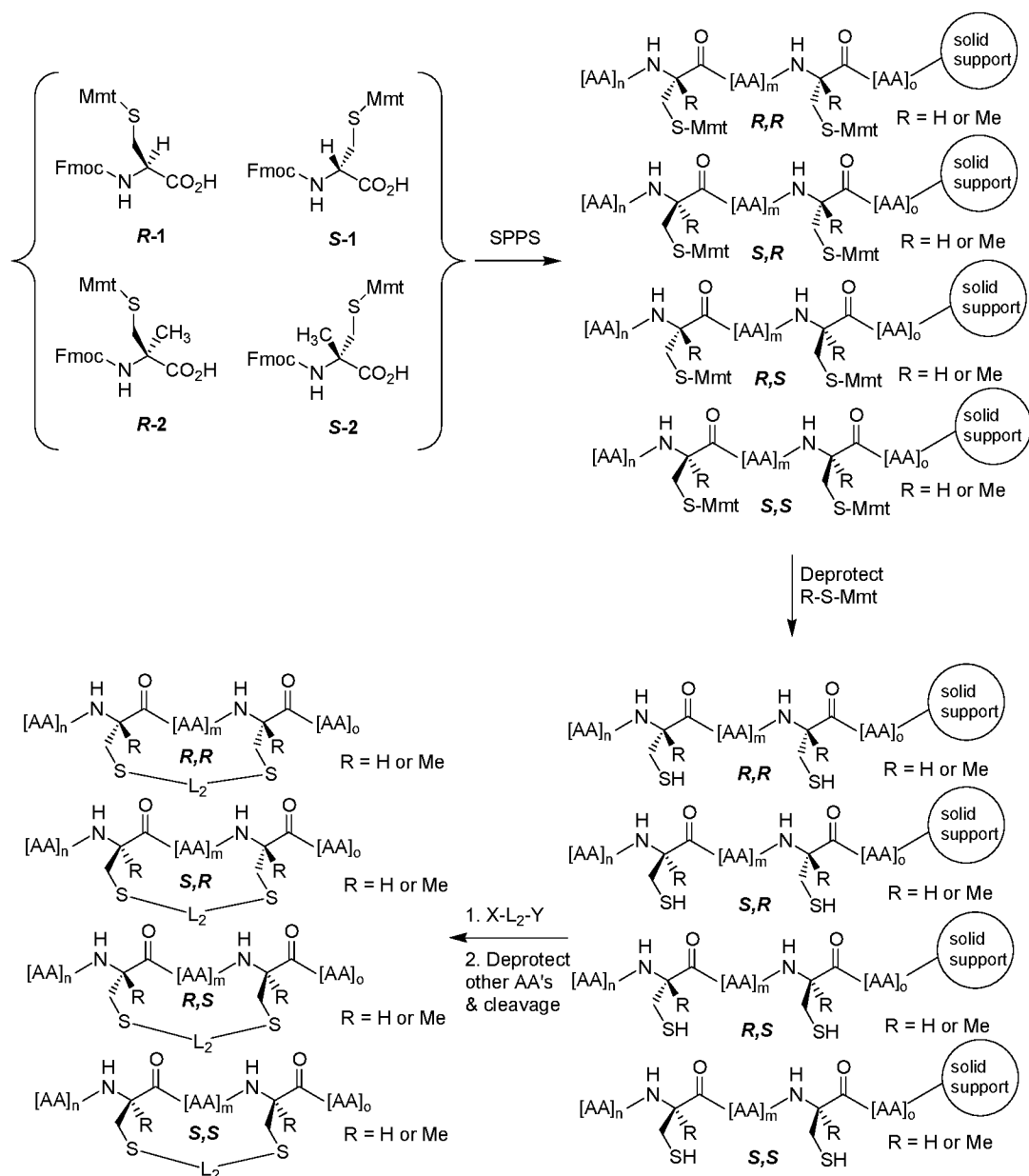
[00293] In other embodiments, peptidomimetic macrocycles of Formula III are synthesized. The preparation of such macrocycles is described, for example, in US Application 11/957,325, filed on December 17, 2007. The following synthetic schemes describe the preparation of such compounds. To simplify the drawings, the illustrative schemes depict amino acid analogs derived from L- or D-cysteine, in which L_1 and L_3 are both $-(CH_2)-$. However, as noted throughout the detailed description above, many other amino acid analogs can be employed in which L_1 and L_3 can be independently selected from the various structures disclosed herein. The symbols “[AA]_m”, “[AA]_n”, “[AA]_o” represent a sequence of amide bond-linked moieties such as natural or unnatural amino acids. As described previously, each occurrence of “AA” is independent of any other occurrence of “AA”, and a formula such as “[AA]_m” encompasses, for example, sequences of non-identical amino acids as well as sequences of identical amino acids.

[00294] Synthetic Scheme 6:



[00295] In Scheme 6, the peptidomimetic precursor contains two -SH moieties and is synthesized by solid-phase peptide synthesis (SPPS) using commercially available N- α -Fmoc amino acids such as N- α -Fmoc-S-trityl-L-cysteine or N- α -Fmoc-S-trityl-D-cysteine. A-methylated versions of D-cysteine or L-cysteine are generated by known methods (Seebach *et al.* (1996), *Angew. Chem. Int. Ed. Engl.* 35:2708-2748, and references therein) and then converted to the appropriately protected N- α -Fmoc-S-trityl monomers by known methods (*Bioorganic Chemistry: Peptides and Proteins*, Oxford University Press, New York: 1998, the entire contents of which are incorporated herein by reference). The precursor peptidomimetic is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA). The precursor peptidomimetic is reacted as a crude mixture or is purified prior to reaction with X-L₂-Y in organic or aqueous solutions. In some embodiments the alkylation reaction is performed under dilute conditions (*i.e.* 0.15 mmol/L) to favor macrocyclization and to avoid polymerization. In some embodiments, the alkylation reaction is performed in organic solutions such as liquid NH₃ (Mosberg *et al.* (1985), *J. Am. Chem. Soc.* 107:2986-2987; Szewczuk *et al.* (1992), *Int. J. Peptide Protein Res.* 40 :233-242), NH₃/MeOH, or NH₃/DMF (Or *et al.* (1991), *J. Org. Chem.* 56:3146-3149). In other embodiments, the alkylation is performed in an aqueous solution such as 6M guanidinium HCL, pH 8 (Brunel *et al.* (2005), *Chem. Commun.* (20):2552-2554). In other embodiments, the solvent used for the alkylation reaction is DMF or dichloroethane.

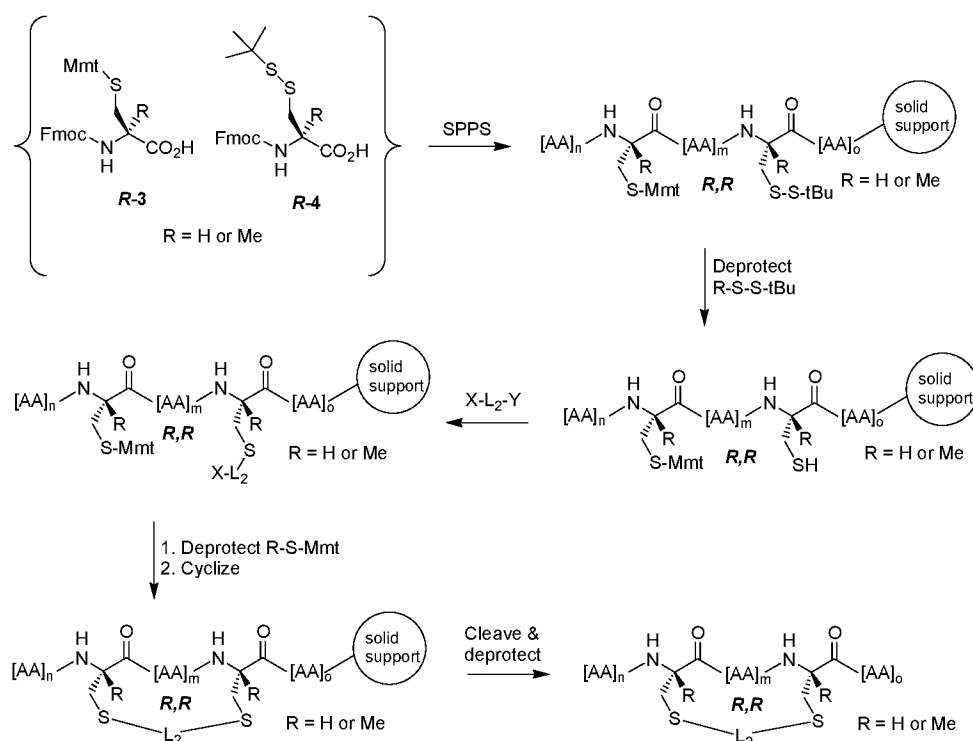
[00296] Synthetic Scheme 7:



[00297] In Scheme 7, the precursor peptidomimetic contains two or more $-SH$ moieties, of which two are specially protected to allow their selective deprotection and subsequent alkylation for macrocycle formation. The precursor peptidomimetic is synthesized by solid-phase peptide synthesis (SPPS) using commercially available N- α -Fmoc amino acids such as N- α -Fmoc-S-*p*-methoxytrityl-L-cysteine or N- α -Fmoc-S-*p*-methoxytrityl-D-cysteine. A-methylated versions of D-cysteine or L-cysteine are generated by known methods (Seebach *et al.* (1996), *Angew. Chem. Int. Ed. Engl.* 35:2708-2748, and references therein) and then converted to the appropriately protected N- α -Fmoc-S-*p*-methoxytrityl monomers by known methods (*Bioorganic Chemistry: Peptides and Proteins*, Oxford University Press, New York: 1998, the entire contents of which are incorporated herein by reference). The Mmt protecting groups of the peptidomimetic precursor are then selectively cleaved by standard conditions (*e.g.*, mild acid such as 1% TFA in DCM). The precursor peptidomimetic is

then reacted on the resin with $X-L_2-Y$ in an organic solution. For example, the reaction takes place in the presence of a hindered base such as diisopropylethylamine. In some embodiments, the alkylation reaction is performed in organic solutions such as liquid NH_3 (Mosberg *et al.* (1985), *J. Am. Chem. Soc.* 107:2986-2987; Szewczuk *et al.* (1992), *Int. J. Peptide Protein Res.* 40 :233-242), $NH_3/MeOH$ or NH_3/DMF (Or *et al.* (1991), *J. Org. Chem.* 56:3146-3149). In other embodiments, the alkylation reaction is performed in DMF or dichloroethane. The peptidomimetic macrocycle is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA).

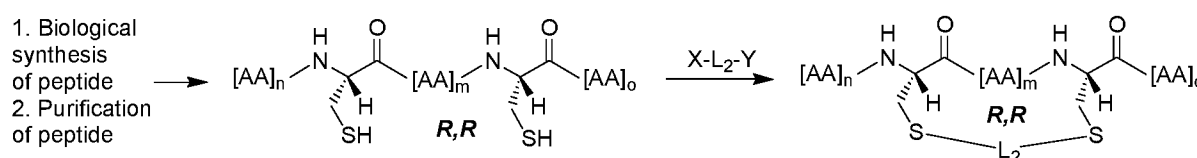
[00298] Synthetic Scheme 8:



[00299] In Scheme 8, the peptidomimetic precursor contains two or more -SH moieties, of which two are specially protected to allow their selective deprotection and subsequent alkylation for macrocycle formation. The peptidomimetic precursor is synthesized by solid-phase peptide synthesis (SPPS) using commercially available N- α -Fmoc amino acids such as N- α -Fmoc-S-*p*-methoxytrityl-L-cysteine, N- α -Fmoc-S-*p*-methoxytrityl-D-cysteine, N- α -Fmoc-S-S-*t*-butyl-L-cysteine, and N- α -Fmoc-S-S-*t*-butyl-D-cysteine. A-methylated versions of D-cysteine or L-cysteine are generated by known methods (Seebach *et al.* (1996), *Angew. Chem. Int. Ed. Engl.* 35:2708-2748, and references therein) and then converted to the appropriately protected N- α -Fmoc-S-*p*-methoxytrityl or N- α -Fmoc-S-S-*t*-butyl monomers by known methods (Bioorganic Chemistry: Peptides and Proteins, Oxford University Press, New York: 1998, the entire contents of which are incorporated herein by reference). The S-S-*t*Butyl protecting group of the peptidomimetic precursor is selectively cleaved by known conditions (*e.g.*, 20% 2-mercaptoethanol in DMF, reference: Galande *et al.* (2005), *J.*

Comb. Chem. 7:174-177). The precursor peptidomimetic is then reacted on the resin with a molar excess of X-L₂-Y in an organic solution. For example, the reaction takes place in the presence of a hindered base such as diisopropylethylamine. The Mmt protecting group of the peptidomimetic precursor is then selectively cleaved by standard conditions (*e.g.*, mild acid such as 1% TFA in DCM). The peptidomimetic precursor is then cyclized on the resin by treatment with a hindered base in organic solutions. In some embodiments, the alkylation reaction is performed in organic solutions such as NH₃/MeOH or NH₃/DMF (Or *et al.* (1991), *J. Org. Chem.* 56:3146-3149). The peptidomimetic macrocycle is then deprotected and cleaved from the solid-phase resin by standard conditions (*e.g.*, strong acid such as 95% TFA).

[00300] Synthetic Scheme 9:



[00301] In Scheme 9, the peptidomimetic precursor contains two L-cysteine moieties. The peptidomimetic precursor is synthesized by known biological expression systems in living cells or by known *in vitro*, cell-free, expression methods. The precursor peptidomimetic is reacted as a crude mixture or is purified prior to reaction with X-L₂-Y in organic or aqueous solutions. In some embodiments the alkylation reaction is performed under dilute conditions (*i.e.* 0.15 mmol/L) to favor macrocyclization and to avoid polymerization. In some embodiments, the alkylation reaction is performed in organic solutions such as liquid NH₃ (Mosberg *et al.* (1985), *J. Am. Chem. Soc.* 107:2986-2987; Szewczuk *et al.* (1992), *Int. J. Peptide Protein Res.* 40 :233-242), NH₃/MeOH, or NH₃/DMF (Or *et al.* (1991), *J. Org. Chem.* 56:3146-3149). In other embodiments, the alkylation is performed in an aqueous solution such as 6M guanidinium HCL, pH 8 (Brunel *et al.* (2005), *Chem. Commun.* (20):2552-2554). In other embodiments, the alkylation is performed in DMF or dichloroethane. In another embodiment, the alkylation is performed in non-denaturing aqueous solutions, and in yet another embodiment the alkylation is performed under conditions that favor helical structure formation. In yet another embodiment, the alkylation is performed under conditions that favor the binding of the precursor peptidomimetic to another protein, so as to induce the formation of the bound helical conformation during the alkylation.

[00302] Various embodiments for X and Y are envisioned which are suitable for reacting with thiol groups. In general, each X or Y is independently selected from the general category shown in Table 6. For example, X and Y are halides such as -Cl, -Br or -I. Any of the macrocycle-forming

linkers described herein may be used in any combination with any of the sequences shown in Tables 1-4 and also with any of the R- substituents indicated herein.

Table 6 - Examples of Reactive Groups Capable of Reacting with Thiol Groups and Resulting Linkages

X or Y	Resulting Covalent Linkage
acrylamide	Thioether
halide (<i>e.g.</i> alkyl or aryl halide)	Thioether
sulfonate	Thioether
aziridine	Thioether
epoxide	Thioether
haloacetamide	Thioether
maleimide	Thioether
sulfonate ester	Thioether

[00303] The present invention contemplates the use of both naturally-occurring and non-naturally-occurring amino acids and amino acid analogs in the synthesis of the peptidomimetic macrocycles of Formula (III). Any amino acid or amino acid analog amenable to the synthetic methods employed for the synthesis of stable bis-sulfhydryl containing peptidomimetic macrocycles can be used. For example, cysteine is contemplated as a useful amino acid. However, sulfur containing amino acids other than cysteine that contain a different amino acid side chain are also useful. For example, cysteine contains one methylene unit between the α -carbon of the amino acid and the terminal -SH of the amino acid side chain. The invention also contemplates the use of amino acids with multiple methylene units between the α -carbon and the terminal -SH. Non-limiting examples include α -methyl-L-homocysteine and α -methyl-D-homocysteine. In some embodiments the amino acids and amino acid analogs are of the D- configuration. In other embodiments they are of the L- configuration. In some embodiments, some of the amino acids and amino acid analogs contained in the peptidomimetic are of the D- configuration while some of the amino acids and amino acid analogs are of the L- configuration. In some embodiments the amino acid analogs are α,α -disubstituted, such as α -methyl-L-cysteine and α -methyl-D-cysteine.

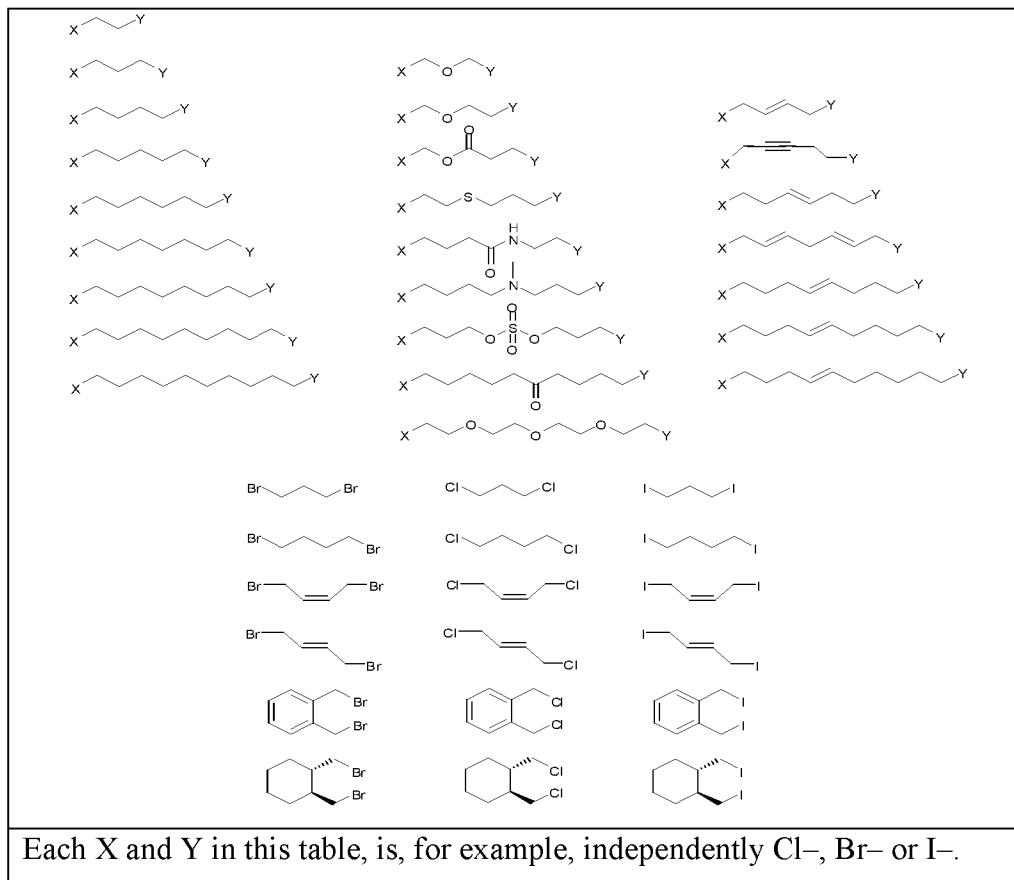
[00304] The invention includes macrocycles in which macrocycle-forming linkers are used to link two or more -SH moieties in the peptidomimetic precursors to form the peptidomimetic macrocycles. As described above, the macrocycle-forming linkers impart conformational rigidity, increased metabolic stability and/or increased cell penetrability. Furthermore, in some embodiments, the macrocycle-forming linkages stabilize a helical secondary structure of the peptidomimetic macrocycles. The macrocycle-forming linkers are of the formula $X-L_2-Y$, wherein both X and Y are the same or different moieties, as defined above. Both X and Y have the chemical characteristics that allow one macrocycle-forming linker $-L_2-$ to bis alkylate the bis-sulfhydryl containing peptidomimetic precursor. As defined above, the linker $-L_2-$ includes alkylene, alkenylene,

alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene, or $-R_4-$ $K-R_4-$, all of which can be optionally substituted with an R_5 group, as defined above. Furthermore, one to three carbon atoms within the macrocycle-forming linkers $-L_2-$, other than the carbons attached to the $-SH$ of the sulfhydryl containing amino acid, are optionally substituted with a heteroatom such as N, S or O.

[00305] The L₂ component of the macrocycle-forming linker X-L₂-Y may be varied in length depending on, among other things, the distance between the positions of the two amino acid analogs used to form the peptidomimetic macrocycle. Furthermore, as the lengths of L₁ and/or L₃ components of the macrocycle-forming linker are varied, the length of L₂ can also be varied in order to create a linker of appropriate overall length for forming a stable peptidomimetic macrocycle. For example, if the amino acid analogs used are varied by adding an additional methylene unit to each of L₁ and L₃, the length of L₂ are decreased in length by the equivalent of approximately two methylene units to compensate for the increased lengths of L₁ and L₃.

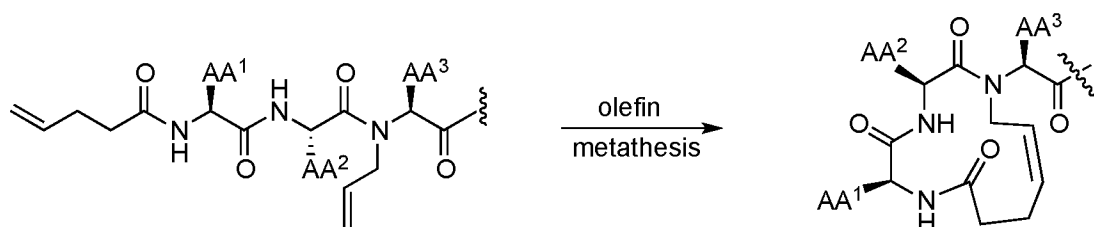
[00306] In some embodiments, L₂ is an alkylene group of the formula $-(CH_2)_n-$, where n is an integer from about 1 to about 15. For example, n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In other embodiments, L₂ is an alkenylene group. In still other embodiments, L₂ is an aryl group.

[00307] Table 7 - Additional embodiments of X-L₂-Y groups.



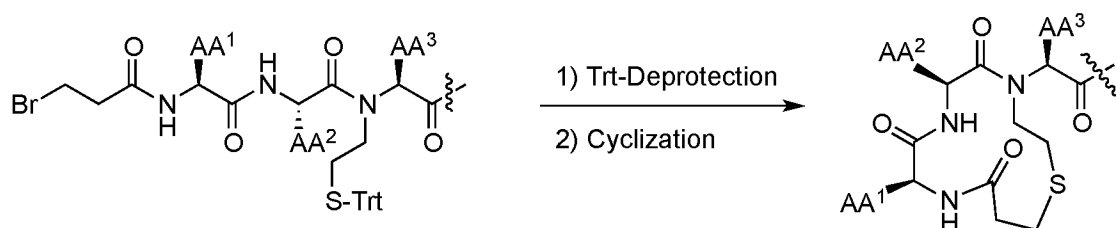
[00308] Additional methods of forming peptidomimetic macrocycles which are envisioned as suitable to perform the present invention include those disclosed by Mustapa, M. Firouz Mohd et al., J. Org. Chem (2003), 68, pp. 8193-8198; Yang, Bin et al. Bioorg Med. Chem. Lett. (2004), 14, pp. 1403-1406; U.S. Patent No. 5,364,851; U.S. Patent No. 5,446,128; U.S. Patent No. 5,824,483; U.S. Patent No. 6,713,280; and U.S. Patent No. 7,202,332. In such embodiments, amino acid precursors are used containing an additional substituent R- at the α -position. Such amino acids are incorporated into the macrocycle precursor at the desired positions, which may be at the positions where the crosslinker is substituted or, alternatively, elsewhere in the sequence of the macrocycle precursor. Cyclization of the precursor is then effected according to the indicated method.

[00309] For example, a peptidomimetic macrocycle of Formula (II) is prepared as indicated:



wherein each AA₁, AA₂, AA₃ is independently an amino acid side chain.

[00310] In other embodiments, a peptidomimetic macrocycle of Formula (II) is prepared as indicated:



wherein each AA₁, AA₂, AA₃ is independently an amino acid side chain.

[00311] In some embodiments, a peptidomimetic macrocycle is obtained in more than one isomer, for example due to the configuration of a double bond within the structure of the crosslinker (*E* vs *Z*). Such isomers can or cannot be separable by conventional chromatographic methods. In some embodiments, one isomer has improved biological properties relative to the other isomer. In one embodiment, an *E* crosslinker olefin isomer of a peptidomimetic macrocycle has better solubility, better target affinity, better *in vivo* or *in vitro* efficacy, higher helicity, or improved cell permeability relative to its *Z* counterpart. In another embodiment, a *Z* crosslinker olefin isomer of a peptidomimetic macrocycle has better solubility, better target affinity, better *in vivo* or *in vitro* efficacy, higher helicity, or improved cell permeability relative to its *E* counterpart.

[00312] In some embodiments, it is desirable to modify the configuration of the resulting peptidomimetic macrocycle. For instance, when a 3_{10} helical configuration is more desirable,

additional substitutions or modifications to the macrocycle can be made to induce or bias such conformations, such as substituting 2-aminoisobutyric acid (Aib) for one or more amino acids in the sequence. See, for example, Boal et. al., *J. Am. Chem. Soc.* 2007, 129, 6986-6987. In one embodiment, the helical macrocycle comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more Aib substitutions

Pharmaceutical Compositions and Routes of Administration

[00313] The peptidomimetic macrocycles also include pharmaceutically acceptable derivatives or prodrugs thereof. A “pharmaceutically acceptable derivative” means any pharmaceutically acceptable salt, ester, salt of an ester, pro-drug or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention. Particularly favored pharmaceutically acceptable derivatives are those that increase the bioavailability of the compounds when administered to a mammal (*e.g.*, by increasing absorption into the blood of an orally administered compound) or which increases delivery of the active compound to a biological compartment (*e.g.*, the brain or lymphatic system) relative to the parent species. Some pharmaceutically acceptable derivatives include a chemical group which increases aqueous solubility or active transport across the gastrointestinal mucosa.

[00314] In some embodiments, the peptidomimetic macrocycles are modified by covalently or non-covalently joining appropriate functional groups to enhance selective biological properties. Such modifications include those which increase biological penetration into a given biological compartment (*e.g.*, blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism, and alter rate of excretion.

[00315] Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, benzoate, benzenesulfonate, butyrate, citrate, digluconate, dodecylsulfate, formate, fumarate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, tosylate and undecanoate. Salts derived from appropriate bases include alkali metal (*e.g.*, sodium), alkaline earth metal (*e.g.*, magnesium), ammonium and N-(alkyl)₄⁺ salts.

[00316] For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers include either solid or liquid carriers. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances, which also acts as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. Details on techniques for

formulation and administration are well described in the scientific and patent literature, see, *e.g.*, the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co, Easton PA.

[00317] In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[00318] Suitable solid excipients are carbohydrate or protein fillers include, but are not limited to sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; as well as proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents are added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

[00319] Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

[00320] The pharmaceutical preparation can be in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

[00321] When the compositions of this invention comprise a combination of a peptidomimetic macrocycle and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of from about 1 to 100%, and more preferably from about 5 to 95% of the dosage normally administered in a monotherapy regimen. In some embodiments, the additional agents are administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents are part of a single dosage form, mixed together with the compounds of this invention in a single composition.

[00322] In some embodiments, the compositions are present as unit dosage forms that can deliver, for example, from about 0.0001 mg to about 1,000 mg of the peptidomimetic macrocycles, salts thereof, prodrugs thereof, derivatives thereof, or any combination of these. Thus, the unit dosage forms can deliver, for example, in some embodiments, from about 1 mg to about 900 mg, from about 1 mg to about 800 mg, from about 1 mg to about 700 mg, from about 1 mg to about 600 mg, from about 1 mg to about 500 mg, from about 1 mg to about 400 mg, from about 1 mg to about 300 mg, from about 1 mg to about 200 mg, from about 1 mg to about 100 mg, from about 1 mg to about 10 mg,

from about 1 mg to about 5 mg, from about 0.1 mg to about 10 mg, from about 0.1 mg to about 5 mg, from about 10 mg to about 1,000 mg, from about 50 mg to about 1,000 mg, from about 100 mg to about 1,000 mg, from about 200 mg to about 1,000 mg, from about 300 mg to about 1,000 mg, from about 400 mg to about 1,000 mg, from about 500 mg to about 1,000 mg, from about 600 mg to about 1,000 mg, from about 700 mg to about 1,000 mg, from about 800 mg to about 1,000 mg, from about 900 mg to about 1,000 mg, from about 10 mg to about 900 mg, from about 100 mg to about 800 mg, from about 200 mg to about 700 mg, or from about 300 mg to about 600 mg of the peptidomimetic macrocycles, salts thereof, prodrugs thereof, derivatives thereof, or any combination of these.

[00323] In some embodiments, the compositions are present as unit dosage forms that can deliver, for example, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, or about 800 mg of peptidomimetic macrocycles, salts thereof, prodrugs thereof, derivatives thereof, or any combination of these.

[00324] Suitable routes of administration include, but are not limited to, oral, intravenous, rectal, aerosol, parenteral, ophthalmic, pulmonary, transmucosal, transdermal, vaginal, otic, nasal, and topical administration. In addition, by way of example only, parenteral delivery includes intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intralymphatic, and intranasal injections.

[00325] In certain embodiments, a composition as described herein is administered in a local rather than systemic manner, for example, via injection of the compound directly into an organ. In specific embodiments, long acting formulations are administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Furthermore, in other embodiments, the drug is delivered in a targeted drug delivery system, for example, in a liposome coated with organ-specific antibody. In such embodiments, the liposomes are targeted to and taken up selectively by the organ. In yet other embodiments, the compound as described herein is provided in the form of a rapid release formulation, in the form of an extended release formulation, or in the form of an intermediate release formulation. In yet other embodiments, the compound described herein is administered topically.

[00326] In another embodiment, compositions described herein are formulated for oral administration. Compositions described herein are formulated by combining a peptidomimetic macrocycle with, *e.g.*, pharmaceutically acceptable carriers or excipients. In various embodiments, the compounds

described herein are formulated in oral dosage forms that include, by way of example only, tablets, powders, pills, dragees, capsules, liquids, gels, syrups, elixirs, slurries, suspensions and the like.

[00327] In certain embodiments, pharmaceutical preparations for oral use are obtained by mixing one or more solid excipient with one or more of the peptidomimetic macrocycles described herein, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as: for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. In specific embodiments, disintegrating agents are optionally added. Disintegrating agents include, by way of example only, cross-linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00328] In one embodiment, dosage forms, such as dragee cores and tablets, are provided with one or more suitable coating. In specific embodiments, concentrated sugar solutions are used for coating the dosage form. The sugar solutions, optionally contain additional components, such as by way of example only, gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs and/or pigments are also optionally added to the coatings for identification purposes. Additionally, the dyestuffs and/or pigments are optionally utilized to characterize different combinations of active compound doses.

[00329] In certain embodiments, therapeutically effective amounts of at least one of the peptidomimetic macrocycles described herein are formulated into other oral dosage forms. Oral dosage forms include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In specific embodiments, push-fit capsules contain the active ingredients in admixture with one or more filler. Fillers include, by way of example only, lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In other embodiments, soft capsules, contain one or more active compound that is dissolved or suspended in a suitable liquid. Suitable liquids include, by way of example only, one or more fatty oil, liquid paraffin, or liquid polyethylene glycol. In addition, stabilizers are optionally added.

[00330] In other embodiments, therapeutically effective amounts of at least one of the peptidomimetic macrocycles described herein are formulated for buccal or sublingual administration. Formulations suitable for buccal or sublingual administration include, by way of example only, tablets, lozenges,

or gels. In still other embodiments, the peptidomimetic macrocycles described herein are formulated for parenteral injection, including formulations suitable for bolus injection or continuous infusion. In specific embodiments, formulations for injection are presented in unit dosage form (*e.g.*, in ampoules) or in multi-dose containers. Preservatives are, optionally, added to the injection formulations. In still other embodiments, pharmaceutical compositions are formulated in a form suitable for parenteral injection as a sterile suspensions, solutions or emulsions in oily or aqueous vehicles. Parenteral injection formulations optionally contain formulatory agents such as suspending, stabilizing and/or dispersing agents. In specific embodiments, pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. In additional embodiments, suspensions of the active compounds are prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles for use in the pharmaceutical compositions described herein include, by way of example only, fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. In certain specific embodiments, aqueous injection suspensions contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension contains suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, in other embodiments, the active ingredient is in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00331] Pharmaceutical compositions herein can be administered, for example, once or twice or three or four or five or six times per day, or once or twice or three or four or five or six times per week, and can be administered, for example, for a day, a week, a month, 3 months, six months, a year, five years, or for example ten years.

Methods of Use

[00332] Cell permeable peptides and peptidomimetics as described herein can be used to regulate expression and/or function of any intracellular protein.

[00333] For example, intracellular proteins which have a mode of action mediated by interactions between one or more α -helix can be targeted by peptidomimetic macrocycles. Such intracellular proteins include, but are not limited to, p53, MDM2, MDMX, Myc, Max, IKK β , NEMO, Hif-1a, CBP/p300, MAML/Notch, influenza RNA-dependent RNA polymerases (*e.g.* PB1 helix of PA protein), β -catenin, Tcf4, BCL-9, HIV gp41, 4ICD, BCL-2, eIF4G, eIF4E, or 4E-BP.

[00334] In one aspect, provided herein are novel peptidomimetic macrocycles that are useful in competitive binding assays to identify agents which bind to the natural ligand(s) of the proteins or

peptides upon which the peptidomimetic macrocycles are modeled. Such binding studies can be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners.

[00335] The invention further provides for the generation of antibodies against the peptidomimetic macrocycles. In some embodiments, these antibodies specifically bind both the peptidomimetic macrocycle and the precursor peptides to which the peptidomimetic macrocycles are related. Such antibodies, for example, disrupt the native protein-protein interaction.

[00336] In other aspects, provided herein are both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant (*e.g.*, insufficient or excessive) expression or activity of the molecules.

[00337] As used herein, the term “treatment” is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

[00338] In some embodiments, a disorder is treated which is caused, at least in part, by an abnormal level of p53 or MDM2 or MDMX, (*e.g.*, over or under expression), or by the presence of p53 or MDM2 or MDMX exhibiting abnormal activity. As such, the reduction in the level and/or activity of p53 or MDM2 or MDMX, or the enhancement of the level and/or activity of p53 or MDM2 or MDMX, by peptidomimetic macrocycles derived from p53, is used, for example, to ameliorate or reduce the adverse symptoms of the disorder.

[00339] For example, in the p53/MDMX system, labeled peptidomimetic macrocycles based on p53 can be used in a MDMX binding assay along with small molecules that competitively bind to MDMX. Competitive binding studies allow for rapid *in vitro* evaluation and determination of drug candidates specific for the p53/MDMX system. Antibodies may also be developed which disrupt the binding between p53 and MDMX. Such binding studies may be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners

[00340] In another aspect, provided herein are methods for treating or preventing a disease including hyperproliferative disease and inflammatory disorder by interfering with the interaction or binding between binding partners, for example, between p53 and MDM2 or p53 and MDMX. In some embodiments, the administration of the compounds of the present invention induces cell growth arrest or apoptosis. In other aspects, the present invention provides for both prophylactic and therapeutic methods of treating a subject infected with, at risk of, or susceptible to an influenza virus. In some embodiments, the administration of the compounds of the present invention prevents the

proliferation or transmission of an influenza virus. These methods comprise administering an effective amount of a compound to a warm blooded animal, including a human.

[00341] In some embodiments, the peptidomimetics macrocycles can be used to treat, prevent, and/or diagnose cancers and neoplastic conditions. As used herein, the terms “cancer”, “hyperproliferative” and “neoplastic” refer to cells having the capacity for autonomous growth, *i.e.*, an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states can be categorized as pathologic, *i.e.*, characterizing or constituting a disease state, or can be categorized as non-pathologic, *i.e.*, a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of breast, lung, liver, colon and ovarian origin. “Pathologic hyperproliferative” cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair. Examples of cellular proliferative and/or differentiative disorders include cancer, *e.g.*, carcinoma, sarcoma, or metastatic disorders. In some embodiments, the peptidomimetics macrocycles are novel therapeutic agents for controlling breast cancer, ovarian cancer, colon cancer, lung cancer, metastasis of such cancers and the like.

[00342] Examples of cancers or neoplastic conditions include, but are not limited to, a fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangi endotheliosarcoma, synovioma, mesothelioma, Ewing’s tumor, leiomyosarcoma, rhabdomyosarcoma, gastric cancer, esophageal cancer, rectal cancer, pancreatic cancer, ovarian cancer, prostate cancer, uterine cancer, cancer of the head and neck, skin cancer, brain cancer, squamous cell carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm’s tumor, cervical cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, or Kaposi sarcoma.

[00343] Examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, *e.g.*, arising from myeloid, lymphoid or

erythroid lineages, or precursor cells thereof. The diseases can arise from poorly differentiated acute leukemias, *e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus (1991), *Crit Rev. Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Stenberg disease.

[00344] Examples of cellular proliferative and/or differentiative disorders of the breast include, but are not limited to, proliferative breast disease including, *e.g.*, epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors, *e.g.*, stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, invasive lobular carcinoma, medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms. Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

[00345] Examples of cellular proliferative and/or differentiative disorders of the skin include, but are not limited to proliferative skin disease such as melanomas, including mucosal melanoma, superficial spreading melanoma, nodular melanoma, lentigo (*e.g.* lentigo maligna, lentigo maligna melanoma, or acral lentiginous melanoma), amelanotic melanoma, desmoplastic melanoma, melanoma with features of a Spitz nevus, melanoma with small nevus-like cells, polypoid melanoma, and soft-tissue melanoma; basal cell carcinomas including micronodular basal cell carcinoma, superficial basal cell carcinoma, nodular basal cell carcinoma (rodent ulcer), cystic basal cell carcinoma, cicatricial basal cell carcinoma, pigmented basal cell carcinoma, aberrant basal cell carcinoma, infiltrative basal cell carcinoma, nevoid basal cell carcinoma syndrome, polypoid basal cell carcinoma, pore-like basal cell carcinoma, and fibroepithelioma of Pinkus; squamous cell carcinomas including acanthoma (large cell acanthoma), adenoid squamous cell carcinoma, basaloid squamous cell carcinoma, clear cell squamous cell carcinoma, signet-ring cell squamous cell carcinoma, spindle cell squamous cell

carcinoma, Marjolin's ulcer, erythroplasia of Queyrat, and Bowen's disease; or other skin or subcutaneous tumors.

[00346] Examples of cellular proliferative and/or differentiative disorders of the lung include, but are not limited to, bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

[00347] Examples of cellular proliferative and/or differentiative disorders of the colon include, but are not limited to, non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

[00348] Examples of cellular proliferative and/or differentiative disorders of the liver include, but are not limited to, nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

[00349] Examples of cellular proliferative and/or differentiative disorders of the ovary include, but are not limited to, ovarian tumors such as, tumors of coelomic epithelium, serous tumors, mucinous tumors, endometrioid tumors, clear cell adenocarcinoma, cystadenofibroma, Brenner tumor, surface epithelial tumors; germ cell tumors such as mature (benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stromal tumors such as, granulosa-theca cell tumors, thecomafibromas, androblastomas, hill cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

[00350] In one aspect, the present invention provides novel peptidomimetic macrocycles that are useful in competitive binding assays to identify agents which bind to the natural ligand(s) of the proteins or peptides upon which the peptidomimetic macrocycles are modeled. For example, in the BH3/BCL-X_L anti-apoptotic system labeled peptidomimetic macrocycles based on BH3 can be used in a BCL-X_L binding assay along with small molecules that competitively bind to BCL-X_L.

Competitive binding studies allow for rapid *in vitro* evaluation and determination of drug candidates specific for the BH3/BCL-X_L system. The invention further provides for the generation of antibodies against the peptidomimetic macrocycles. In some embodiments, these antibodies specifically bind both the peptidomimetic macrocycle and the BH3 peptidomimetic precursors upon which the peptidomimetic macrocycles are derived. Such antibodies, for example, disrupt the BH3/BCL-X_L systems, respectively.

[00351] In other aspects, the present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with

aberrant (*e.g.*, insufficient or excessive) BCL-2 family member expression or activity (*e.g.*, extrinsic or intrinsic apoptotic pathway abnormalities). It is believed that some BCL-2 type disorders are caused, at least in part, by an abnormal level of one or more BCL-2 family members (*e.g.*, over or under expression), or by the presence of one or more BCL-2 family members exhibiting abnormal activity. As such, the reduction in the level and/or activity of the BCL-2 family member or the enhancement of the level and/or activity of the BCL-2 family member, is used, for example, to ameliorate or reduce the adverse symptoms of the disorder.

[00352] In one embodiment, the compounds of the invention are used to treat disorders associated with expression or overexpression of Mcl-1. Mcl-1 has been shown to be expressed in many tissues and neoplastic cell lines and is thought to participate in the development of malignancies (Thallinger et al. (2004) Clin. Cancer Res. 10:4185-4191). The peptidomimetic macrocycles of the invention may be used for the treatment of such malignancies.

[00353] In one embodiment, the disorder being treated (*e.g.* cancer) is differentially responsive to the peptidomimetic macrocycles of the invention. In some embodiments, the cancer is treated with a BIM peptidomimetic macrocycle and is at least 2-fold less sensitive to treatment using a BID polypeptide (such as a BID peptidomimetic macrocycle or uncrosslinked polypeptide) as measured in an *in vitro* cell viability assay. In other embodiments, the cancer is at least 5-fold less sensitive to treatment using a BID polypeptide as measured in an *in vitro* cell viability assay. In yet other embodiments, the cancer is at least 8-fold less sensitive to treatment using a BID polypeptide as measured in an *in vitro* cell viability assay. In other embodiments, the cancer is treated with a BID peptidomimetic macrocycle and is at least 2-fold less sensitive to treatment using a BIM polypeptide (such as a BIM peptidomimetic macrocycle or uncrosslinked polypeptide) as measured in an *in vitro* cell viability assay. In other embodiments, the cancer is at least 5-fold less sensitive to treatment using a BIM polypeptide as measured in an *in vitro* cell viability assay. In yet other embodiments, the cancer is at least 8-fold less sensitive to treatment using a BIM polypeptide as measured in an *in vitro* cell viability assay.

[00354] In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of a BCL-family protein and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of the BCL-family protein is detected. BCL-family proteins include, for example, BCL-2, BCL-X_L, MCL-1, Bfl1/A1, BOO/DIVA, NRH/NR13, BAX, BAD, BAK, BOK, BIK, PUMA, BIM, BMF, BLK, BNIP3, HRK, NIX, SPIKE, and Noxa. In one embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BCL-2 in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BCL-2 is

detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BCL-X_L in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BCL-X_L is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of MCL-1 in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of MCL-1 is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BAX in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BAX is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BAD in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BAD is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BAK in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BAK is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of PUMA in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of PUMA is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of Noxa in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of Noxa is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of Noxa in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of Noxa is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of Noxa in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of Noxa is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of Bfl1/A1 in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of Bfl1/A1 is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BOO/DIVA in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BOO/DIVA is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of NRH/NR13 in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of NRH/NR13 is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to

evaluate the levels of BOK in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BOK is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BIK in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BIK is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BMF in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BMF is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BLK in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BLK is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of BNIP3 in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of BNIP3 is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of HRK in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of HRK is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of Nix in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of Nix is detected. In another embodiment, a method of treating a human patient is provided comprising performing an assay to evaluate the levels of SPIKE in the patient and administering to the patient a peptidomimetic macrocycle if an aberrant or irregular level of expression of SPIKE is detected.

Breast Cancer

[00355] In one aspect, the invention provides methods of treating breast cancer by administering the peptidomimetic macrocycles of the invention. Breast cancer includes invasive breast carcinomas, such as invasive ductal carcinoma, invasive lobular carcinoma, tubular carcinoma, invasive cribriform carcinoma, medullary carcinoma, mucinous carcinoma and other tumours with abundant mucin, cystadenocarcinoma, columnar cell mucinous carcinoma, signet ring cell carcinoma, neuroendocrine tumours (including solid neuroendocrine carcinoma, atypical carcinoid tumour, small cell/oat cell carcinoma, or large cell neuroendocrine carcinoma), invasive papillary carcinoma, invasive micropapillary carcinoma, apocrine carcinoma, metaplastic carcinomas, pure epithelial metaplastic carcinomas, mixed epithelial/mesenchymal metaplastic carcinomas, lipid-rich carcinoma, secretory carcinoma, oncocytic carcinoma, adenoid cystic carcinoma, acinic cell carcinoma,

glycogen-rich clear cell carcinoma, sebaceous carcinoma, inflammatory carcinoma or bilateral breast carcinoma; mesenchymal tumors such as haemangioma, angiomas, haemangiopericytoma, pseudoangiomatous stromal hyperplasia, myofibroblastoma, fibromatosis (aggressive), inflammatory myofibroblastic tumour, lipoma, angiolipoma, granular cell tumour, neurofibroma, schwannoma, angiosarcoma, liposarcoma, rhabdomyosarcoma, osteosarcoma, leiomyoma, or leiomyosarcoma; myoepithelial lesions such as myoepitheliosis, adenomyoepithelial adenosis, adenomyoepithelioma, or malignant myoepithelioma; fibroepithelial tumours such as fibroadenoma, phyllodes tumour, low grade periductal stromal sarcoma, or mammary hamartoma; and tumours of the nipple such as nipple adenoma, syringomatous adenoma, or Paget's disease of the nipple.

[00356] Treatment of breast cancer may be effected in conjunction with any additional therapy, such as a therapy that is part of the standard of care. A surgical technique such as lumpectomy or mastectomy may be performed prior to, during, or following treatment with the peptidomimetic macrocycles of the invention. Alternatively, radiation therapy may be used for the treatment of breast cancer in conjunction with the peptidomimetic macrocycles of the invention. In other cases, the peptidomimetic macrocycles of the invention are administered in combination with a second therapeutic agent. Such an agent may be a chemotherapeutic agent such as an individual drug or combination of drugs and therapies. For example, the chemotherapeutic agent can be an adjuvant chemotherapeutic treatment such as CMF (cyclophosphamide, methotrexate, and 5-fluorouracil); FAC or CAF (5-fluorouracil, doxorubicin, cyclophosphamide); AC or CA (doxorubicin and cyclophosphamide); AC-Taxol (AC followed by paclitaxel); TAC (docetaxel, doxorubicin, and cyclophosphamide); FEC (5-fluorouracil, epirubicin and cyclophosphamide); FECD (FEC followed by docetaxel); TC (docetaxel and cyclophosphamide). In addition to chemotherapy, trastuzumab may also be added to the regimen depending on the tumor characteristics (i.e. HER2/neu status) and risk of relapse. Hormonal therapy may also be appropriate before, during or following chemotherapeutic treatment. For example, tamoxifen may be administered or a compound in the category of aromatase inhibitors including, but not limited to aminoglutethimide, anastrozole, exemestane, formestane, letrozole, or vorozole. In other embodiments, an antiangiogenic agent may be used in combination therapy for the treatment of breast cancer. The antiangiogenic agent may be an anti-VEGF agent including, but not limited to bevacizumab.

Ovarian Cancer

[00357] In another aspect, the peptidomimetic macrocycles of the invention may be used to treat ovarian cancer. Ovarian cancers include ovarian tumors such as, tumors of coelomic epithelium, serous tumors, mucinous tumors, endometrioid tumors, clear cell adenocarcinoma, cystadenofibroma, Brenner tumor, surface epithelial tumors; germ cell tumors such as mature

(benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stromal tumors such as, granulosa-theca cell tumors, thecomafibromas, androblastomas, hilum cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

[00358] The peptidomimetic macrocycles of the invention may be administered in conjunction with a second therapy such as a therapy that is part of the standard of care. Surgery, immunotherapy, chemotherapy, hormone therapy, radiation therapy, or a combination thereof, are some possible treatments available for ovarian cancer. Some possible surgical procedures include debulking, and a unilateral or bilateral oophorectomy and/or a unilateral or bilateral salpingectomy.

[00359] Anti-cancer drugs that may be used include cyclophosphamide, etoposide, altretamine, and ifosfamide. Hormone therapy with the drug tamoxifen may be used to shrink ovarian tumors. Radiation therapy may be external beam radiation therapy and/or brachytherapy.

Prostate Cancer

[00360] In another aspect, the peptidomimetic macrocycles of the invention may be used to treat prostate cancer. Prostate cancers include adenocarcinomas and metastasized adenocarcinomas. The peptidomimetic macrocycles of the invention may be administered in conjunction with a second therapy such as a therapy that is part of the standard of care. Treatment for prostate cancer may involve surgery, radiation therapy, High Intensity Focused Ultrasound (HIFU), chemotherapy, cryosurgery, hormonal therapy, or any combination thereof. Surgery may involve prostatectomy, radical perineal prostatectomy, laparoscopic radical prostatectomy, transurethral resection of the prostate or orchiectomy. Radiation therapy may include external beam radiation therapy and/or brachytherapy. Hormonal therapy may include orchiectomy; administration of antiandrogens such as flutamide, bicalutamide, nilutamide, or cyproterone acetate; medications which inhibit the production of adrenal androgens such as DHEA, such as ketoconazole and aminoglutethimide; and GnRH antagonists or agonists such as Abarelix (Plenaxis®), Cetrorelix (Cetrotide®), Ganirelix (Antagon®), leuprolide, goserelin, triptorelin, or buserelin. Treatment with an anti-androgen agent, which blocks androgen activity in the body, is another available therapy. Such agents include flutamide, bicalutamide, and nilutamide. This therapy is typically combined with LHRH analog administration or an orchiectomy, which is termed a combined androgen blockade (CAB). Chemotherapy includes, but is not limited to, administration of docetaxel, for example with a corticosteroid such as prednisone. Anti-cancer drugs such as doxorubicin, estramustine, etoposide, mitoxantrone, vinblastine, paclitaxel, carboplatin may also be administered to slow the growth of prostate cancer, reduce symptoms and improve the quality of life. Additional compounds such as bisphosphonate drugs may also be administered.

Renal Cancer

[00361] In another aspect, the peptidomimetic macrocycles of the invention may be used to treat renal cancer. Renal cancers include, but are not limited to, renal cell carcinomas, metastases from extra-renal primary neoplasms, renal lymphomas, squamous cell carcinomas, juxtaglomerular tumors (reninomas), transitional cell carcinomas, angiomyolipomas, oncocytomas and Wilm's tumors. The peptidomimetic macrocycles of the invention may be administered in conjunction with a second therapy such as a therapy that is part of the standard of care. Treatment for renal cancer may involve surgery, percutaneous therapies, radiation therapies, chemotherapy, vaccines, or other medication. Surgical techniques useful for treatment of renal cancer in combination with the peptidomimetic macrocycles of the invention include nephrectomy, which may include removal of the adrenal gland, retroperitoneal lymph nodes, and any other surrounding tissues affected by the invasion of the tumor. Percutaneous therapies include, for example, image-guided therapies which may involve imaging of a tumor followed by its targeted destruction by radiofrequency ablation or cryotherapy. In some cases, other chemotherapeutic or other medications useful in treating renal cancer may be α -interferon, interleukin-2, bevacizumab, sorafenib, sunitib, temsirolimus or other kinase inhibitors.

Pancreatic Cancer

[00362] In other aspects, the invention provides methods of treating pancreatic cancer by administering peptidomimetic macrocycles of the invention, such as a pancreatic cancer selected from the following: an epithelioid carcinoma in the pancreatic duct tissue and an adenocarcinoma in a pancreatic duct. The most common type of pancreatic cancer is an adenocarcinoma, which occurs in the lining of the pancreatic duct. Possible treatments available for pancreatic cancer include surgery, immunotherapy, radiation therapy, and chemotherapy. Possible surgical treatment options include a distal or total pancreatectomy and a pancreaticoduodenectomy (Whipple procedure). Radiation therapy may be an option for pancreatic cancer patients, specifically external beam radiation where radiation is focused on the tumor by a machine outside the body. Another option is intraoperative electron beam radiation administered during an operation. Chemotherapy may also be used to treat pancreatic cancer patients. Suitable anti-cancer drugs include, but are not limited to, 5-fluorouracil (5-FU), mitomycin, ifosfamide, doxorubicin, streptozocin, chlorozotocin, and combinations thereof. The methods provided by the invention can provide a beneficial effect for pancreatic cancer patients, by administration of a polypeptide of the invention or a combination of administration of a peptidomimetic macrocycle and surgery, radiation therapy, or chemotherapy.

Colon Cancer

[00363] In one aspect, peptidomimetic macrocycles of the invention may be used for the treatment of colon cancer, including but not limited to non-neoplastic polyps, adenomas, familial syndromes,

colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors. Possible treatments available for colon cancer that may be used in conjunction with the peptidomimetic macrocycles of the invention include surgery, chemotherapy, radiation therapy or targeted drug therapy.

[00364] Radiation therapy may include external beam radiation therapy and/or brachytherapy.

Chemotherapy may be used to reduce the likelihood of metastasis developing, shrink tumor size, or slow tumor growth. Chemotherapy is often applied after surgery (adjuvant), before surgery (neo-adjuvant), or as the primary therapy if surgery is not indicated (palliative). For example, exemplary regimens for adjuvant chemotherapy involve the combination of infusional 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX). First line chemotherapy regimens may involve the combination of infusional 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) with a targeted drug such as bevacizumab, cetuximab or panitumumab or infusional 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) with targeted drug such as bevacizumab, cetuximab or panitumumab. Other chemotherapeutic agents that may be useful in the treatment or prevention of colon cancer in combination with the peptidomimetic macrocycles of the invention are Bortezomib (Velcade®), Oblimersen (Genasense®, G3139), Gefitinib and Erlotinib (Tarceva®) and Topotecan (Hycamtin®).

Lung Cancer

[00365] Some embodiments provide methods for the treatment of lung cancer using the peptidomimetic macrocycles of the invention. Examples of cellular proliferative and/or differentiative disorders of the lung include, but are not limited to, bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

[00366] The most common type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for approximately 80-85% of lung cancers and is divided into squamous cell carcinomas, adenocarcinomas, and large cell undifferentiated carcinomas. Small cell lung cancer, *e.g.* small cell lung carcinomas, accounts for 15-20% of lung cancers. Treatment options for lung cancer include surgery, immunotherapy, radiation therapy, chemotherapy, photodynamic therapy, or a combination thereof. Some possible surgical options for treatment of lung cancer are a segmental or wedge resection, a lobectomy, or a pneumonectomy. Radiation therapy may be external beam radiation therapy or brachytherapy. Some anti-cancer drugs that may be used in chemotherapy to treat lung cancer in combination with the peptidomimetic macrocycles of the invention include cisplatin, carboplatin, paclitaxel, docetaxel, gemcitabine, vinorelbine, irinotecan, etoposide, vinblastine, gefitinib, ifosfamide, methotrexate, or a combination thereof. Photodynamic therapy (PDT) may be

used to treat lung cancer patients. The methods described herein can provide a beneficial effect for lung cancer patients, by administration of a peptidomimetic macrocycle or a combination of administration of a peptidomimetic macrocycle and surgery, radiation therapy, chemotherapy, photodynamic therapy, or a combination thereof.

[00367] Examples of cellular proliferative and/or differentiative disorders of the liver include, but are not limited to, nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

Immunoproliferative Disorders

[00368] Immunoproliferative disorders (also known as “immunoproliferative diseases” or “immunoproliferative neoplasms”) are disorders of the immune system that are characterized by the abnormal proliferation of the primary cells of the immune system, which includes B cells, T cells and Natural Killer (NK) cells, or by the excessive production of immunoglobulins (also known as antibodies). Such disorders include the general categories of lymphoproliferative disorders, hypergammaglobulinemias, and paraproteinemias. Examples of such disorders include, but are not limited to, X-linked lymphoproliferative disorder, autosomal lymphoproliferative disorder, Hyper-IgM syndrome, heavy chain disease, and cryoglobulinemia. Other immunoproliferative disorders can be graft versus host disease (GVHD); psoriasis; immune disorders associated with graft transplantation rejection; T cell lymphoma; T cell acute lymphoblastic leukemia; testicular angiocentric T cell lymphoma; benign lymphocytic angiitis; and autoimmune diseases such as lupus erythematosus, Hashimoto’s thyroiditis, primary myxedema, Graves’ disease, pernicious anemia, autoimmune atrophic gastritis, Addison’s disease, insulin dependent diabetes mellitus, good pasture’s syndrome, myasthenia gravis, pemphigus, Crohn’s disease, sympathetic ophthalmia, autoimmune uveitis, multiple sclerosis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, primary biliary cirrhosis, chronic action hepatitis, ulceratis colitis, Sjogren’s syndrome, rheumatoid arthritis, polymyositis, scleroderma, and mixed connective tissue disease.

Combination Treatments

[00369] In one embodiment, peptidomimetic macrocycles of the invention may be used for the treatment of cancer in conjunction with alkylating and alkylating-like agents. Such agents include, for example, nitrogen mustards such as chlorambucil, chlormethine, cyclophosphamide, ifosfamide, and melphalan; nitrosoureas such as carmustine, fotemustine, lomustine, and streptozocin; platinum therapeutic agents such as carboplatin, cisplatin, oxaliplatin, BBR3464, and satraplatin; or other agents, including but not limited to busulfan, dacarbazine, procarbazine, temozolomide, thiotepea, treosulfan, or uramustine.

[00370] In another embodiment, peptidomimetic macrocycles of the invention may be used in conjunction with an antineoplastic agent which is an antimetabolite. For example, such an antineoplastic agent may be a folic acid such as aminopterin, methotrexate, pemetrexed, or raltitrexed. Alternatively, the antineoplastic agent may be a purine, including but not limited to cladribine, clofarabine, fludarabine, mercaptopurine, pentostatin, thioguanine. In further embodiments, the antineoplastic agent may be a pyrimidine such as capecitabine, cytarabine, fluorouracil, floxuridine, and gemcitabine.

[00371] In still other embodiments, peptidomimetic macrocycles of the invention may be used in conjunction with an antineoplastic agent which is a spindle poison/mitotic inhibitor. Agents in this category include taxanes, for example docetaxel and paclitaxel; and vinca alkaloids such as vinblastine, vincristine, vindesine, and vinorelbine. In yet other embodiments, peptidomimetic macrocycles of the invention may be used in combination with an antineoplastic agent which is a cytotoxic/antitumor antibiotic from the anthracycline family such as daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, pixantrone, or valrubicin; an antibiotic from the streptomyces family such as actinomycin, bleomycin, mitomycin, or plicamycin; or hydroxyurea. Alternatively, agents used for combination therapy may be topoisomerase inhibitors including, but not limited to camptothecin, topotecan, irinotecan, etoposide, or teniposide.

[00372] Alternatively, the antineoplastic agent may be an antibody or antibody-derived agent. For example, a receptor tyrosine kinase-targeted antibody such as cetuximab, panitumumab, or trastuzumab may be used. Alternatively, the antibody may be an anti-CD20 antibody such as rituximab or tositumomab, or any other suitable antibody including but not limited to alemtuzumab, bevacizumab, and gemtuzumab. In other embodiments, the antineoplastic agent is a photosensitizer such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, or verteporfin. In still other embodiments, the antineoplastic agent is a tyrosine kinase inhibitor such as dediranib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib, or vandetanib. Other neoplastic agents suitable in the use of the invention include, for example, alitretinoin, tretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase (pegaspargase), bexarotene, bortezomib, denileukin diftitox, estramustine, ixabepilone, masoprocol, or mitotane.

[00373] In other or further embodiments, the peptidomimetics macrocycles described herein are used to treat, prevent or diagnose conditions characterized by overactive cell death or cellular death due to physiologic insult, etc. Some examples of conditions characterized by premature or unwanted cell death are or alternatively unwanted or excessive cellular proliferation include, but are not limited to hypocellular/hypoplastic, acellular/aplastic, or hypercellular/hyperplastic conditions. Some examples

include hematologic disorders including but not limited to fanconi anemia, aplastic anemia, thalassemia, congenital neutropenia, myelodysplasia

[00374] In other or further embodiments, the peptidomimetics macrocycles of the invention that act to decrease apoptosis are used to treat disorders associated with an undesirable level of cell death. Thus, in some embodiments, the anti-apoptotic peptidomimetics macrocycles of the invention are used to treat disorders such as those that lead to cell death associated with viral infection, *e.g.*, infection associated with infection with human immunodeficiency virus (HIV). A wide variety of neurological diseases are characterized by the gradual loss of specific sets of neurons, and the anti-apoptotic peptidomimetics macrocycles of the invention are used, in some embodiments, in the treatment of these disorders. Such disorders include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) retinitis pigmentosa, spinal muscular atrophy, and various forms of cerebellar degeneration. The cell loss in these diseases does not induce an inflammatory response, and apoptosis appears to be the mechanism of cell death. In addition, a number of hematologic diseases are associated with a decreased production of blood cells. These disorders include anemia associated with chronic disease, aplastic anemia, chronic neutropenia, and the myelodysplastic syndromes. Disorders of blood cell production, such as myelodysplastic syndrome and some forms of aplastic anemia, are associated with increased apoptotic cell death within the bone marrow. These disorders could result from the activation of genes that promote apoptosis, acquired deficiencies in stromal cells or hematopoietic survival factors, or the direct effects of toxins and mediators of immune responses. Two common disorders associated with cell death are myocardial infarctions and stroke. In both disorders, cells within the central area of ischemia, which is produced in the event of acute loss of blood flow, appear to die rapidly as a result of necrosis. However, outside the central ischemic zone, cells die over a more protracted time period and morphologically appear to die by apoptosis.

[00375] In other or further embodiments, the anti-apoptotic peptidomimetics macrocycles of the invention are used to treat all such disorders associated with undesirable cell death.

[00376] Some examples of immunologic disorders that are treated with the peptidomimetics macrocycles described herein include but are not limited to organ transplant rejection, arthritis, lupus, IBD, Crohn's disease, asthma, multiple sclerosis, diabetes, etc.

[00377] Some examples of neurologic disorders that are treated with the peptidomimetics macrocycles described herein include but are not limited to Alzheimer's Disease, Down's Syndrome, Dutch Type Hereditary Cerebral Hemorrhage Amyloidosis, Reactive Amyloidosis, Familial Amyloid Nephropathy with Urticaria and Deafness, Muckle-Wells Syndrome, Idiopathic Myeloma; Macroglobulinemia-Associated Myeloma, Familial Amyloid Polyneuropathy, Familial Amyloid

Cardiomyopathy, Isolated Cardiac Amyloid, Systemic Senile Amyloidosis, Adult Onset Diabetes, Insulinoma, Isolated Atrial Amyloid, Medullary Carcinoma of the Thyroid, Familial Amyloidosis, Hereditary Cerebral Hemorrhage With Amyloidosis, Familial Amyloidotic Polyneuropathy, Scrapie, Creutzfeldt-Jacob Disease, Gerstmann Straussler-Scheinker Syndrome, Bovine Spongiform Encephalitis, a prion-mediated disease, and Huntington's Disease.

[00378] Some examples of endocrinologic disorders that are treated with the peptidomimetics macrocycles described herein include but are not limited to diabetes, hypothyroidism, hypopituitarism, hypoparathyroidism, hypogonadism, etc.

[00379] Examples of cardiovascular disorders (*e.g.*, inflammatory disorders) that are treated or prevented with the peptidomimetics macrocycles of the invention include, but are not limited to, atherosclerosis, myocardial infarction, stroke, thrombosis, aneurism, heart failure, ischemic heart disease, angina pectoris, sudden cardiac death, hypertensive heart disease; non-coronary vessel disease, such as arteriolosclerosis, small vessel disease, nephropathy, hypertriglyceridemia, hypercholesterolemia, hyperlipidemia, xanthomatosis, asthma, hypertension, emphysema and chronic pulmonary disease; or a cardiovascular condition associated with interventional procedures ("procedural vascular trauma"), such as restenosis following angioplasty, placement of a shunt, stent, synthetic or natural excision grafts, indwelling catheter, valve or other implantable devices. Preferred cardiovascular disorders include atherosclerosis, myocardial infarction, aneurism, and stroke.

[00380] In some embodiments, the peptidomimetic macrocycles are useful in the treatment of viral disorders. For example, in the PB1/PA system, labeled peptidomimetic macrocycles based on PB1 can be used in a PA binding assay along with small molecules that competitively bind to PA. Competitive binding studies allow for rapid *in vitro* evaluation and determination of drug candidates specific for the PB1/PA system. Such binding studies may be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners. Antibodies may also be developed which disrupt the binding between PA and PD1.

[00381] For example, peptidomimetic macrocycles derived from the PB1 helix sequence, or peptidomimetic macrocycles that bind selectively to the PB1 peptide binding site of the PA protein, may selectively inhibit influenza RNA-dependent RNA polymerases. Peptidomimetic macrocycles derived from the PB2 helix sequence, or peptidomimetic macrocycles that bind selectively to the PB2 peptide binding site of the PB1 protein, may selectively inhibit influenza RNA-dependent RNA polymerases. When administered within a therapeutic window after infection, such peptidomimetic macrocycles may reduce the severity or duration of an influenza infection. When administered prophylactically, such peptidomimetic macrocycles may prevent infection by influenza viruses and thereby decrease the spread of influenza and reduce large-scale epidemics.

[00382] In one aspect, the present invention provides novel peptidomimetic macrocycles that are useful in competitive binding assays to identify agents which bind to the natural ligand(s) of the proteins or peptides upon which the peptidomimetic macrocycles are modeled. For example, in the PB1/PA system, labeled peptidomimetic macrocycles based on PB1 can be used in a PA binding assay along with small molecules that competitively bind to PA. Competitive binding studies allow for rapid *in vitro* evaluation and determination of drug candidates specific for the PB1/PA system. Such binding studies may be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners.

[00383] In other aspects, the present invention provides for both prophylactic and therapeutic methods of treating a subject infected with, at risk of, or susceptible to an influenza virus. These methods comprise administering an effective amount of a compound to a warm blooded animal, including a human. In some embodiments, the administration of the compounds of the present invention prevents the proliferation or transmission of an influenza virus.

[00384] In some embodiments, peptidomimetic macrocycles are used to treat diseases induced by influenza viruses. Like other viruses, the replication of influenza virus involves six phases; transmission, entry, replication, biosynthesis, assembly, and exit. Entry occurs by endocytosis, replication and vRNP assembly takes place in the nucleus, and the virus buds from the plasma membrane. In the infected patient, the virus targets airway epithelial cells.

[00385] The methods described herein are also useful for development and/or identification of agents for the treatment of infections caused by viruses such as Abelson leukemia virus, Abelson murine leukemia virus, Abelson's virus, Acute laryngotracheobronchitis virus, Adelaide River virus, Adeno associated virus group, Adenovirus, African horse sickness virus, African swine fever virus, AIDS virus, Aleutian mink disease parvovirus, Alpharetrovirus, Alphavirus, ALV related virus, Amapari virus, Aphthovirus, Aquareovirus, Arbovirus, Arbovirus C, arbovirus group A, arbovirus group B, Arenavirus group, Argentine hemorrhagic fever virus, Argentine hemorrhagic fever virus, Arterivirus, Astrovirus, Ateline herpesvirus group, Aujeszky's disease virus, Aura virus, Ausduk disease virus, Australian bat lyssavirus, Aviadenovirus, avian erythroblastosis virus, avian infectious bronchitis virus, avian leukemia virus, avian leukosis virus, avian lymphomatosis virus, avian myeloblastosis virus, avian paramyxovirus, avian pneumoencephalitis virus, avian reticuloendotheliosis virus, avian sarcoma virus, avian type C retrovirus group, Avihepadnavirus, Avipoxvirus, B virus, B19 virus, Babanki virus, baboon herpesvirus, baculovirus, Barmah Forest virus, Bebaru virus, Berrimah virus, Betaretrovirus, Birnavirus, Bittner virus, BK virus, Black Creek Canal virus, bluetongue virus, Bolivian hemorrhagic fever virus, Borna disease virus, border disease of sheep virus, borna virus, bovine alphaherpesvirus 1, bovine alphaherpesvirus 2, bovine

coronavirus, bovine ephemeral fever virus, bovine immunodeficiency virus, bovine leukemia virus, bovine leukosis virus, bovine mammillitis virus, bovine papillomavirus, bovine papular stomatitis virus, bovine parvovirus, bovine syncytial virus, bovine type C oncovirus, bovine viral diarrhea virus, Buggy Creek virus, bullet shaped virus group, Bunyamwera virus supergroup, Bunyavirus, Burkitt's lymphoma virus, Bwamba Fever, CA virus, Calicivirus, California encephalitis virus, camelpox virus, canarypox virus, canid herpesvirus, canine coronavirus, canine distemper virus, canine herpesvirus, canine minute virus, canine parvovirus, Cano Delgadito virus, caprine arthritis virus, caprine encephalitis virus, Caprine Herpes Virus, Capripox virus, Cardiovirus, caviid herpesvirus 1, Cercopithecoid herpesvirus 1, cercopithecine herpesvirus 1, Cercopithecine herpesvirus 2, Chandipura virus, Changuinola virus, channel catfish virus, Charleville virus, chickenpox virus, Chikungunya virus, chimpanzee herpesvirus, chub reovirus, chum salmon virus, Cocal virus, Coho salmon reovirus, coital exanthema virus, Colorado tick fever virus, Coltivirus, Columbia SK virus, common cold virus, contagious ecthyma virus, contagious pustular dermatitis virus, Coronavirus, Corripata virus, coryza virus, cowpox virus, coxsackie virus, CPV (cytoplasmic polyhedrosis virus), cricket paralysis virus, Crimean-Congo hemorrhagic fever virus, croup associated virus, Cryptovirus, Cypovirus, Cytomegalovirus, cytomegalovirus group, cytoplasmic polyhedrosis virus, deer papillomavirus, deltaretrovirus, dengue virus, Densovirus, Dependovirus, Dhori virus, diploma virus, Drosophila C virus, duck hepatitis B virus, duck hepatitis virus 1, duck hepatitis virus 2, duovirus, Duvenhage virus, Deformed wing virus DWV, eastern equine encephalitis virus, eastern equine encephalomyelitis virus, EB virus, Ebola virus, Ebola-like virus, echo virus, echovirus, echovirus 10, echovirus 28, echovirus 9, ectromelia virus, EEE virus, EIA virus, EIA virus, encephalitis virus, encephalomyocarditis group virus, encephalomyocarditis virus, Enterovirus, enzyme elevating virus, enzyme elevating virus (LDH), epidemic hemorrhagic fever virus, epizootic hemorrhagic disease virus, Epstein-Barr virus, equid alphaherpesvirus 1, equid alphaherpesvirus 4, equid herpesvirus 2, equine abortion virus, equine arteritis virus, equine encephalosis virus, equine infectious anemia virus, equine morbillivirus, equine rhinopneumonitis virus, equine rhinovirus, Eubenangu virus, European elk papillomavirus, European swine fever virus, Everglades virus, Eyach virus, felid herpesvirus 1, feline calicivirus, feline fibrosarcoma virus, feline herpesvirus, feline immunodeficiency virus, feline infectious peritonitis virus, feline leukemia /sarcoma virus, feline leukemia virus, feline panleukopenia virus, feline parvovirus, feline sarcoma virus, feline syncytial virus, Filovirus, Flanders virus, Flavivirus, foot and mouth disease virus, Fort Morgan virus, Four Corners hantavirus, fowl adenovirus 1, fowlpox virus, Friend virus, Gammaretrovirus, GB hepatitis virus, GB virus, German measles virus, Getah virus, gibbon ape leukemia virus, glandular fever virus, goatpox virus, golden shinner virus, Gonometa virus, goose parvovirus, granulosis virus,

Gross' virus, ground squirrel hepatitis B virus, group A arbovirus, Guanarito virus, guinea pig cytomegalovirus, guinea pig type C virus, Hantaan virus, Hantavirus, hard clam reovirus, hare fibroma virus, HCMV (human cytomegalovirus), hemadsorption virus 2, hemagglutinating virus of Japan, hemorrhagic fever virus, hendra virus, Henipaviruses, Hepadnavirus, hepatitis A virus, hepatitis B virus group, hepatitis C virus, hepatitis D virus, hepatitis delta virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, hepatitis nonA nonB virus, hepatitis virus, hepatitis virus (nonhuman), hepatoencephalomyelitis reovirus 3, Hepatovirus, heron hepatitis B virus, herpes B virus, herpes simplex virus, herpes simplex virus 1, herpes simplex virus 2, herpesvirus, herpesvirus 7, Herpesvirus ateles, Herpesvirus hominis, Herpesvirus infection, Herpesvirus saimiri, Herpesvirus suis, Herpesvirus varicellae, Highlands J virus, Hirame rhabdovirus, hog cholera virus, human adenovirus 2, human alphaherpesvirus 1, human alphaherpesvirus 2, human alphaherpesvirus 3, human B lymphotropic virus, human betaherpesvirus 5, human coronavirus, human cytomegalovirus group, human foamy virus, human gammaherpesvirus 4, human gammaherpesvirus 6, human hepatitis A virus, human herpesvirus 1 group, human herpesvirus 2 group, human herpesvirus 3 group, human herpesvirus 4 group, human herpesvirus 6, human herpesvirus 8, human immunodeficiency virus, human immunodeficiency virus 1, human immunodeficiency virus 2, human papillomavirus, human T cell leukemia virus, human T cell leukemia virus I, human T cell leukemia virus II, human T cell leukemia virus III, human T cell lymphoma virus I, human T cell lymphoma virus II, human T cell lymphotropic virus type 1, human T cell lymphotropic virus type 2, human T lymphotropic virus I, human T lymphotropic virus II, human T lymphotropic virus III, Ichnovirus, infantile gastroenteritis virus, infectious bovine rhinotracheitis virus, infectious haematopoietic necrosis virus, infectious pancreatic necrosis virus, influenza virus A, influenza virus B, influenza virus C, influenza virus D, influenza virus pr8, insect iridescent virus, insect virus, iridovirus, Japanese B virus, Japanese encephalitis virus, JC virus, Junin virus, Kaposi's sarcoma-associated herpesvirus, Kemerovo virus, Kilham's rat virus, Klamath virus, Kolongo virus, Korean hemorrhagic fever virus, kumba virus, Kysanur forest disease virus, Kyzylgach virus, La Crosse virus, lactic dehydrogenase elevating virus, lactic dehydrogenase virus, Lagos bat virus, Langur virus, lapine parvovirus, Lassa fever virus, Lassa virus, latent rat virus, LCM virus, Leaky virus, Lentivirus, Leporipoxvirus, leukemia virus, leukovirus, lumpy skin disease virus, lymphadenopathy associated virus, Lymphocryptovirus, lymphocytic choriomeningitis virus, lymphoproliferative virus group, Machupo virus, mad itch virus, mammalian type B oncovirus group, mammalian type B retroviruses, mammalian type C retrovirus group, mammalian type D retroviruses, mammary tumor virus, Mapuera virus, Marburg virus, Marburg-like virus, Mason Pfizer monkey virus, Mastadenovirus, Mayaro virus, ME virus, measles virus, Menangle virus, Mengo virus, Mengovirus,

Middelburg virus, milkers nodule virus, mink enteritis virus, minute virus of mice, MLV related virus, MM virus, Mokola virus, Molluscipoxvirus, Molluscum contagiosum virus, monkey B virus, monkeypox virus, Mononegavirales, Morbillivirus, Mount Elgon bat virus, mouse cytomegalovirus, mouse encephalomyelitis virus, mouse hepatitis virus, mouse K virus, mouse leukemia virus, mouse mammary tumor virus, mouse minute virus, mouse pneumonia virus, mouse poliomyelitis virus, mouse polyomavirus, mouse sarcoma virus, mousepox virus, Mozambique virus, Mucambo virus, mucosal disease virus, mumps virus, murid betaherpesvirus 1, murid cytomegalovirus 2, murine cytomegalovirus group, murine encephalomyelitis virus, murine hepatitis virus, murine leukemia virus, murine nodule inducing virus, murine polyomavirus, murine sarcoma virus, Muromegalovirus, Murray Valley encephalitis virus, myxoma virus, Myxovirus, Myxovirus multiforme, Myxovirus parotitidis, Nairobi sheep disease virus, Nairovirus, Nanirnavirus, Nariva virus, Ndumo virus, Neethling virus, Nelson Bay virus, neurotropic virus, New World Arenavirus, newborn pneumonitis virus, Newcastle disease virus, Nipah virus, noncytopathogenic virus, Norwalk virus, nuclear polyhedrosis virus (NPV), nipple neck virus, O'nyong'nyong virus, Ockelbo virus, oncogenic virus, oncogenic viruslike particle, oncornavirus, Orbivirus, Orf virus, Oropouche virus, Orthohepadnavirus, Orthomyxovirus, Orthopoxvirus, Orthoreovirus, Orungo, ovine papillomavirus, ovine catarrhal fever virus, owl monkey herpesvirus, Palyam virus, Papillomavirus, Papillomavirus sylvilagi, Papovavirus, parainfluenza virus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3, parainfluenza virus type 4, Paramyxovirus, Parapoxvirus, paravaccinia virus, Parvovirus, Parvovirus B19, parvovirus group, Pestivirus, Phlebovirus, phocine distemper virus, Picodnavirus, Picornavirus, pig cytomegalovirus, pigeonpox virus, Piry virus, Pixuna virus, pneumonia virus of mice, Pneumovirus, poliomyelitis virus, poliovirus, Polydnavirus, polyhedral virus, polyoma virus, Polyomavirus, Polyomavirus bovis, Polyomavirus cercopithecii, Polyomavirus hominis 2, Polyomavirus maccacae 1, Polyomavirus muris 1, Polyomavirus muris 2, Polyomavirus papionis 1, Polyomavirus papionis 2, Polyomavirus sylvilagi, Pongine herpesvirus 1, porcine epidemic diarrhea virus, porcine hemagglutinating encephalomyelitis virus, porcine parvovirus, porcine transmissible gastroenteritis virus, porcine type C virus, pox virus, poxvirus, poxvirus variolae, Prospect Hill virus, Provirus, pseudocowpox virus, pseudorabies virus, psittacinepox virus, quailpox virus, rabbit fibroma virus, rabbit kidney vacuolating virus, rabbit papillomavirus, rabies virus, raccoon parvovirus, raccoonpox virus, Ranikhet virus, rat cytomegalovirus, rat parvovirus, rat virus, Rauscher's virus, recombinant vaccinia virus, recombinant virus, reovirus, reovirus 1, reovirus 2, reovirus 3, reptilian type C virus, respiratory infection virus, respiratory syncytial virus, respiratory virus, reticuloendotheliosis virus, Rhabdovirus, Rhabdovirus carpia, Rhadinovirus, Rhinovirus, Rhizidiovirus, Rift Valley fever virus, Riley's virus, rinderpest virus, RNA tumor virus,

Ross River virus, Rotavirus, rougeole virus, Rous sarcoma virus, rubella virus, rubeola virus, Rubivirus, Russian autumn encephalitis virus, SA 11 simian virus, SA2 virus, Sabia virus, Sagiyama virus, Saimirine herpesvirus 1, salivary gland virus, sandfly fever virus group, Sandjimba virus, SARS virus, SDAV (sialodacryoadenitis virus), sealpox virus, Semliki Forest Virus, Seoul virus, sheeppox virus, Shope fibroma virus, Shope papilloma virus, simian foamy virus, simian hepatitis A virus, simian human immunodeficiency virus, simian immunodeficiency virus, simian parainfluenza virus, simian T cell lymphotropic virus, simian virus, simian virus 40, Simplexvirus, Sin Nombre virus, Sindbis virus, smallpox virus, South American hemorrhagic fever viruses, sparrowpox virus, Spumavirus, squirrel fibroma virus, squirrel monkey retrovirus, SSV 1 virus group, STLV (simian T lymphotropic virus) type I, STLV (simian T lymphotropic virus) type II, STLV (simian T lymphotropic virus) type III, stomatitis papulosa virus, submaxillary virus, suid alphaherpesvirus 1, suid herpesvirus 2, Suipoxvirus, swamp fever virus, swinepox virus, Swiss mouse leukemia virus, TAC virus, Tacaribe complex virus, Tacaribe virus, Tanapox virus, Taterapox virus, Tench reovirus, Theiler's encephalomyelitis virus, Theiler's virus, Thogoto virus, Thottapalayam virus, Tick borne encephalitis virus, Tioman virus, Togavirus, Torovirus, tumor virus, Tupaia virus, turkey rhinotracheitis virus, turkeypox virus, type C retroviruses, type D oncovirus, type D retrovirus group, ulcerative disease rhabdovirus, Una virus, Uukuniemi virus group, vaccinia virus, vacuolating virus, varicella zoster virus, Varicellovirus, Varicola virus, variola major virus, variola virus, Vasin Gishu disease virus, VEE virus, Venezuelan equine encephalitis virus, Venezuelan equine encephalomyelitis virus, Venezuelan hemorrhagic fever virus, vesicular stomatitis virus, Vesiculovirus, Vilyuisk virus, viper retrovirus, viral haemorrhagic septicemia virus, Visna Maedi virus, Visna virus, volepox virus, VSV (vesicular stomatitis virus), Wallal virus, Warrego virus, wart virus, WEE virus, West Nile virus, western equine encephalitis virus, western equine encephalomyelitis virus, Whataroa virus, Winter Vomiting Virus, woodchuck hepatitis B virus, woolly monkey sarcoma virus, wound tumor virus, WRSV virus, Yaba monkey tumor virus, Yaba virus, Yatapoxvirus, yellow fever virus, and the Yug Bogdanovac virus. In one embodiment an infectome will be produced for each virus that includes an inventory of the host cellular genes involved in virus infection during a specific phase of viral infection, such cellular entry or the replication cycle.

[00386] For some viruses a great deal of progress has been made in the elucidation of the steps involved during infection of host cells, and any of these steps may be targeted using peptidomimetic macrocycles. For example, experiments initiated in the early 1980s showed that influenza virus follows a stepwise, endocytic entry program with elements shared with other viruses such as alpha- and rhabdoviruses (Marsh and Helenius 1989; Whittaker 2006). The steps include: 1) Initial

attachment to sialic acid containing glycoconjugates receptors on the cell surface; 2) signaling induced by the virus particle; 3) endocytosis by clathrin-dependent and clathrin-independent cellular mechanism; 4) acid-induced, hemagglutinin (HA)-mediated penetration from late endosomes; 5) acid-activated, M2 and matrix protein (M1) dependent uncoating of the capsid; and, 6) intra-cytosolic transport and nuclear import of vRNPs. These steps depend on assistance from the host cell in the form of sorting receptors, vesicle formation machinery, kinase-mediated regulation, organelle acidification, and, most likely, activities of the cytoskeleton.

[00387] Influenza attachment to the cells surface occurs via binding of the HA1 subunit to cell surface glycoproteins and glycolipids that carry oligosaccharide moieties with terminal sialic acid residues (Skehel and Wiley 2000). The linkage by which the sialic acid is connected to the next saccharide contributes to species specificity. Avian strains including H5N1 prefer an α -(2,3)-link and human strains α -(2,6)-link (Matrosovich 2006). In epithelial cells, binding occurs preferentially to microvilli on the apical surface, and endocytosis occurs at base of these extensions (Matlin 1982). Whether receptor binding induces signals that prepare the cell for the invasion is not yet known, but it is likely because activation of protein kinase C and synthesis of phosphatidylinositol-3-phosphate (PI3P) are required for efficient entry (Sieczkarski et al. 2003; Whittaker 2006).

[00388] Endocytic internalization occurs within a few minutes after binding (Matlin 1982; Yoshimura and Ohnishi 1984). In tissue culture cells influenza virus makes use of three different types of cellular processes; 1) preexisting clathrin coated pits, 2) virus-induced clathrin coated pits, and 3) endocytosis in vesicles without visible coat (Matlin 1982; Sieczkarski and Whittaker 2002; Rust et al. 2004). Video microscopy using fluorescent viruses showed the virus particles undergoing actin-mediated rapid motion in the cell periphery followed by minus end-directed, microtubule-mediated transport to the perinuclear area of the cell. Live cell imaging indicated that the virus particles first entered a subpopulation of mobile, peripheral early endosomes that carry them deeper into the cytoplasm before penetration takes place (Lakadamyali et al. 2003; Rust et al. 2004). The endocytotic process is regulated by protein and lipid kinases, the proteasome, as well as by Rabs and ubiquitin-dependent sorting factors (Khor et al. 2003; Whittaker 2006).

[00389] The membrane penetration step is mediated by low pH-mediated activation of the trimeric, metastable HA, and the conversion of this Type I viral fusion protein to a membrane fusion competent conformation (Maeda et al. 1981; White et al. 1982). This occurs about 16 min after internalization, and the pH threshold varies between strains in the 5.0-5.6 range. The target membrane is the limiting membrane of intermediate or late endosomes. The mechanism of fusion has been extensively studied (Kielian and Rey 2006). Further it was observed that fusion itself does not seem to require any host cell components except a lipid bilayer membrane and a functional

acidification system (Maeda et al. 1981; White et al. 1982). The penetration step is inhibited by agents such as lysosomotropic weak bases, carboxylic ionophores, and proton pump inhibitors (Matlin 1982; Whittaker 2006).

[00390] To allow nuclear import of the incoming vRNPs, the capsid has to be disassembled. This step involves acidification of the viral interior through the amantadine-sensitive M2-channels causes dissociation of M1 from the vRNPs (Bukrinskaya et al. 1982; Martin and Helenius 1991; Pinto et al. 1992). Transport of the individual vRNPs to the nuclear pore complexes and transfer into the nucleus depends on cellular nuclear transport receptors (O'Neill et al. 1995; Cros et al. 2005). Replication of the viral RNAs (synthesis of positive and negative strands), and transcription occurs in complexes tightly associated with the chromatin in the nucleus. It is evident that, although many of the steps are catalyzed by the viral polymerase, cellular factors are involved including RNA polymerase activating factors, a chaperone HSP90, hCLE, and a human splicing factor UAP56. Viral gene expression is subject to complex cellular control at the transcriptional level, a control system dependent on cellular kinases (Whittaker 2006).

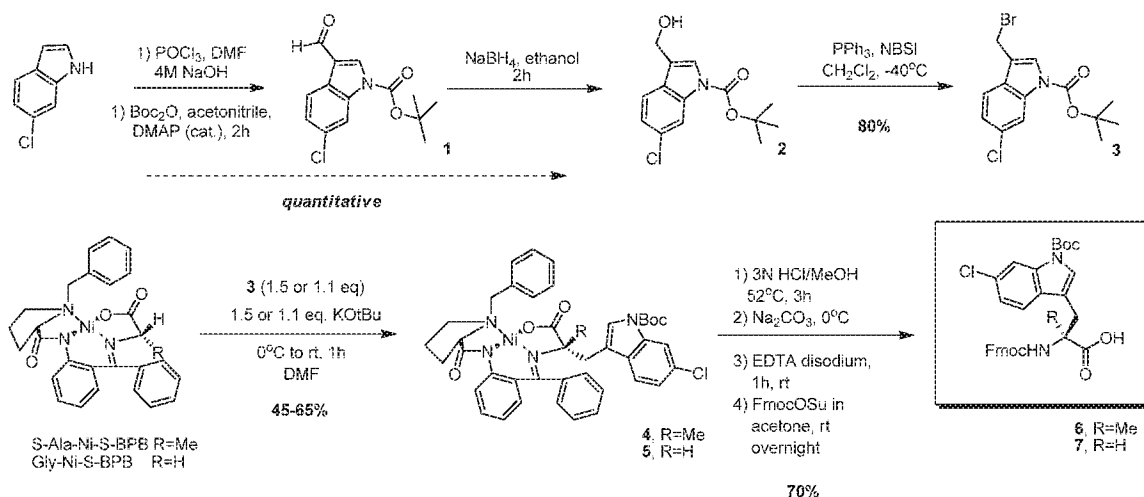
[00391] The final assembly of an influenza particle occurs during a budding process at the plasma membrane. In epithelial cells, budding occurs at the apical membrane domain only (Rodriguez-Boulan 1983). First, the progeny vRNPs are transported within the nucleoplasm to the nuclear envelope, then from the nucleus to the cytoplasm, and finally they accumulate in the cell periphery. Exit from the nucleus is dependent on viral protein NEP and M1, and a variety of cellular proteins including CRM1 (a nuclear export receptor), caspases, and possibly some nuclear protein chaperones. Phosphorylation plays a role in nuclear export by regulating M1 and NEP synthesis, and also through the MAPK/ERK system (Bui et al. 1996; Ludwig 2006). G protein and protein kinase signaling is involved in influenza virus budding from infected host cells (Hui E. and Nayak D, 2002).

[00392] The three membrane proteins of the virus are synthesized, folded and assembled into oligomers in the ER (Doms et al. 1993). They pass through the Golgi complex; undergo maturation through modification of their carbohydrate moieties and proteolytic cleavage. After reaching the plasma membrane they associate with M1 and the vRNPs in a budding process that result in the inclusion of all eight vRNPs and exclusion of most host cell components except lipids.

[00393] Influenza infection is associated with activation of several signaling cascades including the MAPK pathway (ERK, JNK, p38 and BMK-1/ERK5), the κ B/NF- κ B signaling module, the Raf/MEK/ERK cascade, and programmed cell death (Ludwig 2006). These result in a variety of effects that limit the progress of infection such as transcriptional activation of IFN- β , apoptotic cell death, and a block in virus escape of from late endosomes (Ludwig 2006).

Examples

Example 1: Synthesis of 6-chlorotryptophan Fmoc amino acids



[00394] Tert-butyl 6-chloro-3-formyl-1H-indole-1-carboxylate, 1. To a stirred solution of dry DMF (12 mL) was added dropwise POCl_3 (3.92 mL, 43 mmol, 1.3 equiv) at 0°C under Argon. The solution was stirred at the same temperature for 20 min before a solution of 6-chloroindole (5.0 g, 33 mmol, 1 eq.) in dry DMF (30 mL) was added dropwise. The resulting mixture was allowed to warm to room temperature and stirred for an additional 2.5h. Water (50 mL) was added and the solution was neutralized with 4M aqueous NaOH (pH ~ 8). The resulting solid was filtered off, washed with water and dried under vacuum. This material was directly used in the next step without additional purification. To a stirred solution of the crude formyl indole (33 mmol, 1 eq.) in THF (150 mL) was added successively Boc_2O (7.91 g, 36.3 mmol, 1.1 equiv) and DMAP (0.4 g, 3.3 mmol, 0.1 equiv) at room temperature under N_2 . The resulting mixture was stirred at room temperature for 1.5h and the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed with 1N HCl, dried and concentrated to give the formyl indole 1 (9 g, 98 % over 2 steps) as a white solid. ^1H NMR (CDCl_3) δ : 1.70 (s, Boc, 9H); 7.35 (dd, 1H); 8.21 (m, 3H); 10.07 (s, 1H).

[00395] Tert-butyl 6-chloro-3-(hydroxymethyl)-1H-indole-1-carboxylate, 2. To a solution of compound 1 (8.86g, 32 mmol, 1 eq.) in ethanol (150 mL) was added NaBH_4 (2.4g, 63 mmol, 2 eq.). The reaction was stirred for 3 h at room temperature. The reaction mixture was concentrated and the residue was poured into diethyl ether and water. The organic layer was separated, dried over magnesium sulfate and concentrated to give a white solid (8.7g, 98%). This material was directly used in the next step without additional purification. ^1H NMR (CDCl_3) δ : 1.65 (s, Boc, 9H); 4.80 (s, 2H, CH_2); 7.21 (dd, 1H); 7.53 (m, 2H); 8.16 (bs, 1H).

[00396] Tert-butyl 3-(bromomethyl)-6-chloro-1H-indole-1-carboxylate, **3**. To a solution of compound **2** (4.1g, 14.6 mmol, 1 eq.) in dichloromethane (50 mL) under argon was added a solution of triphenylphosphine (4.59g, 17.5 mmol, 1.2 eq.) in dichloromethane (50 mL) at -40 °C. The reaction solution was stirred an additional 30 min at 40 °C. Then NBS (3.38g, 19 mmol, 1.3 eq.) was added. The resulting mixture was allowed to warm to room temperature and stirred overnight. Dichloromethane was evaporated, Carbon Tetrachloride (100 mL) was added and the mixture was stirred for 1h and filtrated. The filtrate was concentrated, loaded in a silica plug and quickly eluted with 25% EtOAc in Hexanes. The solution was concentrated to give a white foam (3.84g, 77%). ¹H NMR (CDCl₃) δ: 1.66 (s, Boc, 9H); 4.63 (s, 2H, CH₂); 7.28 (dd, 1H); 7.57 (d, 1H); 7.64 (bs, 1H); 8.18 (bs, 1H).

[00397] αMe-6Cl-Trp(Boc)-Ni-S-BPB, **4**. To S-Ala-Ni-S-BPB (2.66g, 5.2 mmol, 1 eq.) and KO-*t*Bu (0.87g, 7.8 mmol, 1.5 eq.) was added 50 mL of DMF under argon. The bromide derivative compound **3** (2.68g, 7.8 mmol, 1.5 eq.) in solution of DMF (5.0 mL) was added via syringe. The reaction mixture was stirred at ambient temperature for 1h. The solution was then quenched with 5 % aqueous acetic acid and diluted with water. The desired product was extracted in dichloromethane, dried and concentrated. The oily product **4** was purified by flash chromatography (solid loading) on normal phase using EtOAc and Hexanes as eluents to give a red solid (1.78g, 45% yield). αMe-6Cl-Trp(Boc)-Ni-S-BPB, **4**: M+H calc. 775.21, M+H obs. 775.26; ¹H NMR (CDCl₃) δ: 1.23 (s, 3H, αMe); 1.56 (m, 11H, Boc + CH₂); 1.82-2.20 (m, 4H, 2CH₂); 3.03 (m, 1H, CHα); 3.24 (m, 2H, CH₂); 3.57 and 4.29 (AB system, 2H, CH₂ (benzyl), J= 12.8Hz); 6.62 (d, 2H); 6.98 (d, 1H); 7.14 (m, 2H); 7.23 (m, 1H); 7.32-7.36 (m, 5H); 7.50 (m, 2H); 7.67 (bs, 1H); 7.98 (d, 2H); 8.27 (m, 2H).

[00398] Fmoc-αMe-6Cl-Trp(Boc)-OH, **6**. To a solution of 3N HCl/MeOH (1/3, 15 mL) at 50°C was added a solution of compound **4** (1.75g, 2.3 mmol, 1 eq.) in MeOH (5 mL) dropwise. The starting material disappeared within 3-4 h. The acidic solution was then cooled to 0°C with an ice bath and quenched with an aqueous solution of Na₂CO₃ (1.21g, 11.5 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na₂CO₃ (1.95g, 18.4 mmol) were added to the suspension. The Nickel scavenging EDTA disodium salt dihydrate (1.68g, 4.5 mmol, 2 eq.) was then added and the suspension was stirred for 2h. A solution of Fmoc-OSu (0.84g, 2.5 mmol, 1.1 eq.) in acetone (50 mL) was added and the reaction was stirred overnight. Afterwards, the reaction was diluted with diethyl ether and 1N HCl. The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The desired product **6** was purified on normal phase using acetone and dichloromethane as eluents to give a white foam (0.9g, 70% yield). Fmoc-αMe-6Cl-Trp(Boc)-OH, **6**: M+H calc. 575.19, M+H obs. 575.37; ¹H NMR (CDCl₃) 1.59 (s, 9H, Boc); 1.68 (s, 3H, Me); 3.48 (bs, 2H, CH₂); 4.22

(m, 1H, CH); 4.39 (bs, 2H, CH₂); 5.47 (s, 1H, NH); 7.10 (m, 1H); 7.18 (m, 2H); 7.27 (m, 2H); 7.39 (m, 2H); 7.50 (m, 2H); 7.75 (d, 2H); 8.12 (bs, 1H).

[00399] 6Cl-Trp(Boc)-Ni-S-BPB, 5. To Gly-Ni-S-BPB (4.6g, 9.2 mmol, 1 eq.) and KO-*t*Bu (1.14g, 10.1 mmol, 1.1 eq.) was added 95 mL of DMF under argon. The bromide derivative compound 3 (3.5g, 4.6 mmol, 1.1 eq.) in solution of DMF (10 mL) was added via syringe. The reaction mixture was stirred at ambient temperature for 1h. The solution was then quenched with 5 % aqueous acetic acid and diluted with water. The desired product was extracted in dichloromethane, dried and concentrated. The oily product 5 was purified by flash chromatography (solid loading) on normal phase using EtOAc and Hexanes as eluents to give a red solid (5g, 71% yield). 6Cl-Trp(Boc)-Ni-S-BPB, 5: M+H calc. 761.20, M+H obs. 761.34; ¹H NMR (CDCl₃) δ: 1.58 (m, 11H, Boc + CH₂); 1.84 (m, 1H); 1.96 (m, 1H); 2.24 (m, 2H, CH₂); 3.00 (m, 1H, CH_α); 3.22 (m, 2H, CH₂); 3.45 and 4.25 (AB system, 2H, CH₂ (benzyl), J= 12.8Hz); 4.27 (m, 1H, CH_α); 6.65 (d, 2H); 6.88 (d, 1H); 7.07 (m, 2H); 7.14 (m, 2H); 7.28 (m, 3H); 7.35-7.39 (m, 2H); 7.52 (m, 2H); 7.96 (d, 2H); 8.28 (m, 2H).

[00400] Fmoc-6Cl-Trp(Boc)-OH, 7. To a solution of 3N HCl/MeOH (1/3, 44 mL) at 50°C was added a solution of compound 5 (5g, 6.6 mmol, 1 eq.) in MeOH (10 mL) dropwise. The starting material disappeared within 3-4 h. The acidic solution was then cooled to 0°C with an ice bath and quenched with an aqueous solution of Na₂CO₃ (3.48g, 33 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na₂CO₃ (5.57g, 52 mmol) were added to the suspension. The Nickel scavenging EDTA disodium salt dihydrate (4.89g, 13.1 mmol, 2 eq.) and the suspension was stirred for 2h. A solution of Fmoc-OSu (2.21g, 6.55 mmol, 1.1 eq.) in acetone (100 mL) was added and the reaction was stirred overnight. Afterwards, the reaction was diluted with diethyl ether and 1N HCl. The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The desired product 7 was purified on normal phase using acetone and dichloromethane as eluents to give a white foam (2.6g, 69% yield). Fmoc-6Cl-Trp(Boc)-OH, 7: M+H calc. 561.17, M+H obs. 561.37; ¹H NMR (CDCl₃) 1.63 (s, 9H, Boc); 3.26 (m, 2H, CH₂); 4.19 (m, 1H, CH); 4.39 (m, 2H, CH₂); 4.76 (m, 1H); 5.35 (d, 1H, NH); 7.18 (m, 2H); 7.28 (m, 2H); 7.39 (m, 3H); 7.50 (m, 2H); 7.75 (d, 2H); 8.14 (bs, 1H).

Example 2: Peptidomimetic macrocycles

[00401] Peptidomimetic macrocycles were synthesized, purified and analyzed as previously described and as described below (Schafmeister et al., J. Am. Chem. Soc. 122:5891-5892 (2000); Schafmeister & Verdine, J. Am. Chem. Soc. 122:5891 (2005); Walensky et al., Science 305:1466-1470 (2004); and US Patent No. 7,192,713). Peptidomimetic macrocycles were designed by replacing two or more naturally occurring amino acids with the corresponding synthetic amino acids. Substitutions were made at i and i+4, and i and i+7 positions. Peptide synthesis was performed manually or on an

automated peptide synthesizer (Applied Biosystems, model 433A), using solid phase conditions, rink amide AM resin (Novabiochem), and Fmoc main-chain protecting group chemistry. For the coupling of natural Fmoc-protected amino acids (Novabiochem), 10 equivalents of amino acid and a 1:1:2 molar ratio of coupling reagents HBTU/HOBt (Novabiochem)/DIEA were employed. Non-natural amino acids (4 equiv) were coupled with a 1:1:2 molar ratio of HATU (Applied Biosystems)/HOBt/DIEA. The N-termini of the synthetic peptides were acetylated, while the C-termini were amidated.

[00402] Purification of cross-linked compounds was achieved by high performance liquid chromatography (HPLC) (Varian ProStar) on a reverse phase C18 column (Varian) to yield the pure compounds. Chemical composition of the pure products was confirmed by LC/MS mass spectrometry (Micromass LCT interfaced with Agilent 1100 HPLC system) and amino acid analysis (Applied Biosystems, model 420A).

Example 3: Circular Dichroism (CD) analysis of α -helicity

[00403] Peptide solutions were analyzed by CD spectroscopy using a Jasco J-815 spectropolarimeter (Jasco Inc., Easton, MD) with the Jasco Spectra Manager Ver.2 system software. A Peltier temperature controller was used to maintain temperature control of the optical cell. Results are expressed as mean molar ellipticity $[\theta]$ ($\text{deg cm}^2 \text{ dmol}^{-1}$) as calculated from the equation $[\theta] = \theta_{\text{obs}} \cdot \text{MRW} / 10 \cdot l \cdot c$ where θ_{obs} is the observed ellipticity in millidegrees, MRW is the mean residue weight of the peptide (peptide molecular weight/number of residues), l is the optical path length of the cell in centimeters, and c is the peptide concentration in mg/mL. Peptide concentrations were determined by amino acid analysis. Stock solutions of peptides were prepared in benign CD buffer (20 mM phosphoric acid, pH 2). The stocks were used to prepare peptide solutions of 0.05 mg/mL in either benign CD buffer or CD buffer with 50% trifluoroethanol (TFE) for analyses in a 10 mm pathlength cell. Variable wavelength measurements of peptide solutions were scanned at 4 °C from 195 to 250 nm, in 0.2 nm increments, and a scan rate 50 nm per min. The average of six scans was reported.

Example 4: Competitive Fluorescence polarization assay for MDM2

[00404] The assay was performed according to the following general protocol:

1. Dilute MDM2 (In-house, 41kD) into FP buffer (High salt buffer-200 mM NaCl, 5 mM CHAPS, pH 7.5) to make 84 nM (2X) working stock solution.
2. Add 20 μL of 84 nM (2X) of protein stock solution into each well of 96-well black HE microplate (Molecular Devices)
3. Dilute 1mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μM (dilution 1:10). Then, dilute from 100 μM to 10 μM with water (dilution 1:10) and then dilute with FP buffer

from 10 μ M to 40 nM (dilution 1:250). This is the working solution which will be a 10 nM concentration in well (dilution 1:4). Keep the diluted FAM labeled peptide in the dark until use.

4. Make unlabeled peptide dose plate with FP buffer starting with 1 μ M (final) of peptide and making 5 fold serial dilutions for 6 points using following dilution scheme.

Dilute 10mM (in 100% DMSO) with DMSO to 5mM (dilution 1: 2). Then, dilute from 5mM to 500 μ M with H₂O (dilution 1:10) and then dilute with FP buffer from 500 μ M to 20 μ M (dilution 1:25).

Making 5 fold serial dilutions from 4 μ M (4X) for 6 points.

5. Transfer 10 μ l of serial diluted unlabeled peptides to each well which is filled with 20 μ l of 84 nM of protein.

6. Add 10 μ l of 10nM (4X) of FAM labeled peptide into each well and incubate for 3hr to read.

Example 5: Competitive Fluorescence polarization assay for MDMX

[00405] The assay was performed according to the following general protocol:

1. Dilute MDMX (In-house, 40kD) into FP buffer (High salt buffer-200mM NaCl, 5mM CHAPS, pH 7.5.) to make 300nM (2X) working stock solution.

2. Add 20 μ l of 300 nM (2X) of protein stock solution into each well of 96-well black HE microplate (Molecular Devices)

3. Dilute 1mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μ M (dilution 1: 10). Then, dilute from 100 μ M to 10 μ M with water (dilution 1:10) and then dilute with FP buffer from 10 μ M to 40nM (dilution 1:250). This is the working solution which will be a 10nM concentration in well (dilution 1:4). Keep the diluted FAM labeled peptide in the dark until use.

4. Make unlabeled peptide dose plate with FP buffer starting with 5 μ M (final) of peptide and making 5 fold serial dilutions for 6 points using following dilution scheme.

5. Dilute 10mM (in 100% DMSO) with DMSO to 5mM (dilution 1: 2). Then, dilute from 5mM to 500 μ M with H₂O (dilution 1:10) and then dilute with FP buffer from 500 μ M to 20 μ M (dilution 1:25). Making 5 fold serial dilutions from 20 μ M (4X) for 6 points.

6. Transfer 10 μ l of serial diluted unlabeled peptides to each well which is filled with 20 μ l of 300 nM of protein.

7. Add 10 μ l of 10nM (4X) of FAM labeled peptide into each well and incubate for 3hr to read.

Example 6: Cell Viability assay

[00406] The assay was performed according to the following general protocol:

Cell Plating: Trypsinize, count and seed cells at the pre-determined densities in 96-well plates a day prior to assay. Following cell densities are used for exemplary cell lines used:

- SJSA-1: 7500 cells/ well

- RKO: 5000 cells/well
- RKO-E6: 5000 cells/well
- HCT-116: 5000 cells/well
- SW-480: 2000 cells/well
- MCF-7: 5000 cells/well

[00407] On the day of study, replace media with fresh media with 11% FBS (assay media) at room temperature. Add 180µL of the assay media per well. Control wells with no cells, receive 200 µl media.

[00408] Peptide dilution: all dilutions are made at room temperature and added to cells at room temperature.

- Prepare 10 mM stocks of the peptides in DMSO. Serially dilute the stock using 1:3 dilution scheme to get 10, 3.3, 1.1, 0.33, 0.11, 0.03, 0.01mM solutions using DMSO as diluents. Dilute the serially DMSO-diluted peptides 33.3 times using sterile water. This gives range of 10X working stocks. Also prepare DMSO/sterile water (3% DMSO) mix for control wells.
- Thus the working stocks concentration range µM will be 300, 100, 30, 10, 3, 1, 0.3 and 0 µM. Mix well at each dilution step using multichannel.
- Row H has controls. H1-H3 will receive 20 µl of assay media. H4-H9 will receive 20 µl of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.
- Positive control: MDM2 small molecule inhibitor, Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

[00409] Addition of working stocks to cells:

- Add 20 µl of 10X desired concentration to appropriate well to achieve the final concentrations in total 200 µl volume in well. (20 µl of 300 µM peptide + 180 µl of cells in media = 30 µM final concentration in 200 µl volume in wells). Mix gently a few times using pipette. Thus final concentration range used will be 30, 10, 3, 1, 0.3, 0.1, 0.03 & 0 µM (for potent peptides further dilutions are included).
- Controls include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.
- Incubate for 72 hr t 37 °C in humidified 5% CO₂ atmosphere.
- The viability of cells is determined using MTT reagent from Promega. Viability of SJSA-1, RKO, RKO-E6, HCT-116 cells is determined on day 3, MCF-7 cells on day 5 and SW-480 cells on day 6. At the end of designated incubation time, allow the plates to come to

room temperature. Remove 80 μ l of assay media from each well. Add 15 μ l of thawed MTT reagent to each well.

- Allow plate to incubate for 2h at 37 °C in humidified 5% CO₂ atmosphere and add 100 μ l solubilization reagent as per manufacturer's protocol. Incubate with agitation for 1h at room temperature and read on Synergy Biotek multiplate reader for absorbance at 570 nM.
- Analyze the cell viability against the DMSO controls using GraphPad PRISM analysis tools.

[00410] Reagents:

- Invitrogen cell culture Media
 - i. Falcon 96-well clear cell culture treated plates (Nunc 353072)
- DMSO (Sigma D 2650)
- RPMI 1640 (Invitrogen 72400)
- MTT (Promega G4000)

[00411] Instruments: Multiplate Reader for Absorbance readout (Synergy 2).

Example 7. Cell Lysis by Peptidomimetic Macrocycles

[00412] SJSA-1 cells were plated out one day in advance in clear flat-bottom plates (Costar, catalog number 353072) at 7500 cells/well with 100 μ l/well of growth media, leaving row H columns 10-12 empty for media alone. On the day of the assay, media was exchanged with RPMI 1% FBS media, 90 μ L of media per well.

[00413] 10 mM stock solutions of the peptidomimetic macrocycles were prepared in 100% DMSO. Peptidomimetic macrocycles were then diluted serially in 100% DMSO, and then further diluted 20-fold in sterile water to prepare working stock solutions in 5% DMSO/water of each peptidomimetic macrocycle at concentrations ranging from 500 μ M to 62.5 μ M.

[00414] 10 μ L of each compound was added to the 90 μ L of SJSA-1 cells to yield final concentrations of 50 μ M to 6.25 μ M in 0.5% DMSO-containing media. The negative control (non-lytic) sample was 0.5% DMSO alone and positive control (lytic) samples include 10 μ M Melittin and 1% Triton X-100.

[00415] Cell plates were incubated for 1 hr at 37 °C. After the 1 hr incubation, the morphology of the cells is examined by microscope and then the plates were centrifuged at 1200rpm for 5 min at room temperature. 40 μ L of supernatant for each peptidomimetic macrocycle and control sample is transferred to clear assay plates.

[00416] Cell lysis was evaluated by quantitatively measuring release of LDH, a stable cytosolic enzyme released early in necrosis and only in late stage apoptosis, using a CytoTox96 nonradioactive cytotoxicity assay. Cells were grown for 24 h, treated with peptides at indicated concentrations or

0.04% DMSO for selected time periods. LDH released into the surrounding medium was determined according to the manufacturer's instructions (Promega). 25 mM Paclitaxel for 24 h was used as a positive control for necrosis and 50 nM paclitaxel for 24 h was used as a negative control. The method of measuring LDH was described in part in Do et al. (2003), *Oncogene* 22: 1431–1444, incorporated herein by reference in their entirety.

Example 8: Solubility Determination for Peptidomimetic Macrocycles

[00417] Peptidomimetic macrocycles were first dissolved in neat N, N-dimethylacetamide (DMA, Sigma-Aldrich, 38840-1L-F) to make 20X stock solutions over a concentration range of 20-140 mg/mL. The DMA stock solutions were diluted 20-fold in an aqueous vehicle containing 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol to obtain final concentrations of 1-7 mg/mL of the peptidomimetic macrocycles in 5% DMA, 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol. The final solutions were mixed gently by repeat pipetting or light vortexing, and then the final solutions were sonicated for 10 min at room temperature in an ultrasonic water bath. Careful visual observation is then performed under hood light using a 7x visual amplifier to determine if precipitate exists on the bottom or as a suspension. Additional concentration ranges were tested as needed to determine the maximum solubility limit for each peptidomimetic macrocycle.

Example 9: Solubility Determination for Peptidomimetic Macrocycles by Measuring OD₆₀₀

[00418] Peptidomimetic macrocycles are first dissolved in neat N, N-dimethylacetamide (DMA, Sigma-Aldrich, 38840-1L-F) to make 20X stock solutions over a concentration range of 20-140 mg/mL. The DMA stock solutions are diluted 20-fold in an aqueous vehicle containing 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol to obtain final concentrations of 1-7 mg/mL of the peptidomimetic macrocycles in 5% DMA, 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol. The final solutions are mixed gently by repeat pipetting or light vortexing, and then the final solutions are sonicated for 10 min at room temperature in an ultrasonic water bath.

[00419] Samples of the final solutions are transferred to cuvettes. Turbidity of the solutions is determined by measuring light absorbance of the solutions at 600 nm (i.e., OD₆₀₀) using a spectrophotometer (e.g., Ultrospec™ 10 Cell Density Meter, GE Healthcare). Solvent solution without the peptidomimetic macrocycles is used as a blank reference. Additional concentration ranges are tested as needed to determine the maximum solubility limit for each peptidomimetic macrocycle.

Example 10: Solubility Determination for Peptidomimetic Macrocycles by Nephelometry

[00420] A row of peptidomimetic macrocycles suspensions with different total concentrations (i.e., sum of dissolved and undissolved substance) are prepared, covering the range from 5×10^{-7} to 5×10^{-4} mol/L. This set of suspensions is prepared directly in the 96-well plate used in the nephelometric

assay. The higher concentrations of the dilution row are obtained by mixing different volumes of buffer and 10 mM DMSO stock solution. For concentrations lower than 1×10^{-4} mol/L, the suspensions are prepared by sequential dilution taking an aliquot from the well with the next higher concentration prepared in the previous step. The final volume of all suspensions was set to 300 μ L.

[00421] Nephelometric values of each sample are determined by a laser nephelometer (*e.g.*, NEPHELOstar, BMG LabTechnologies). In a clear solution, a laser beam is passed through without being scattered or changed. In a turbid solution, scattered light is detected by a photodetector at right angles to the incident laser beam. The energy of the scattered light is directly proportional to the particle concentration in the suspension for up to 3 orders of magnitude. Thus, solubility of the peptidomimetic macrocycles can be determined by measuring the energy of the scattered light.

Example 11: Reverse-Phase HPLC retention time determination

[00422] Peptides were analyzed by reverse-phase HPLC on a 100 x 2.1 mm Phenomenex 2.6 micron, 100 Angstrom C18 column using the following mobile phase gradient at room temperature:

Time (min)	Flow rate (mL/min)	% A (0.1% TFA in water)	% B (0.1% TFA in acetonitrile)
0	0.6	80	20
20.0	0.6	20	80
20.1	0.6	5	95
21.0	0.6	5	95
21.1	0.6	80	20
21.2	0.6	5	95
21.5	0.6	5	95
21.8	0.6	80	20
23.5	0.6	80	20

[00423] In some embodiments, the retention time (RT) was then normalized to a 0-100 scale by the following equation: $RT = [RT_raw \text{ (from above)} * 3.317 - 0.534] * 3.3333$. (*e.g.*, see Tables 1b, 8, and 9. In some embodiments, the retention times were not normalized as described above (*e.g.*, see Tables 2b, 3b, 4b, and 10).

[00424] Retention times for exemplary p53 peptidomimetic macrocycles of the invention are shown below in **Table 8** (along with their associated cell penetration efficiencies), and the same RT vs cell permeability data is plotted in **FIG. 2**. Retention times for exemplary BIM peptidomimetic macrocycles of the invention are shown below in **Table 9** (along with their associated cell penetration efficiencies), and the same RT vs cell permeability data for exemplary BIM peptidomimetic macrocycles of the invention are shown in **FIG. 5**.

Example 12: von Heijne (VH) value calculation

[00425] von Heijne values were calculated using a method adapted from Hessa et al., Recognition of transmembrane helices by the endoplasmic reticulum translocon, *Nature*: 433, 377-381 (2005).

Briefly, each amino acid is assigned a fixed value, regardless of location along the polypeptide chain, according to the table below:

Amino Acid	von Heijne Score
I	-0.6
L	-0.5
Nle	-0.5
\$	-0.5
St	-0.5
\$e	-0.5
\$r8	-0.5
\$r5	-0.5
\$s8	-0.5
\$s5	-0.5
\$er8	-0.5
F	-0.3
V	-0.3
Aib	-0.1
M	-0.1
C	-0.1
Abu	-0.1
Ac-	0
-NH ₂	0
A	0.1
a	0.1
W	0.3
T	0.5
Y	0.6
G	0.6
S	0.8
N	2
H	2
P	2.2
Q	2.2
E	2.5
R	2.5
K	2.5
D	3.5

[00426] The von Heijne value (VH) for the polypeptide is then calculated as the sum total of values for all amino acids in the polypeptide. For example, a pentapeptide of the sequence Ac-AAAAA-NH₂ would have a VH score of $5 \times (0.1) = 0.5$.

[00427] Von Heijne (VH) values for exemplary p53 peptidomimetic macrocycles of the invention are shown below in **Table 8** (along with their associated cell penetration efficiencies). Von Heijne (VH)

values for exemplary BIM peptidomimetic macrocycles of the invention are shown below in **Table 9** (along with their associated cell penetration efficiencies), and the VH vs cell permeability data is plotted in **FIG. 6**.

Table 8

SP#	MDM2 IC ₅₀ (nM)	SJSA-1 EC ₅₀ (μM)**	Penetration Efficiency (EC ₅₀ /IC ₅₀ *1000)	RT*	VH
1	140.7	6	42.64	74.2	
2	21.00	28.35	1350.00	58.60	0.20
3	9.50	100.00	10526.32	62.87	
4	19.50	100.00	5128.21	59.70	
5	19.00	100.00	5263.16	47.43	
6	23.00	4.70	204.35	78.27	1.30
7	28.00	1.50	53.57	73.40	1.20
8	10.00	5.49	548.75	51.84	8.00
9	10.00	8.60	860.00	63.56	4.90
10	13.00	100.00	7692.31	62.50	1.50
11	10.00	25.00	2500.00	69.31	4.40
12	1.00	23.07	23070.00	60.87	2.10
13	25.79	1.12	43.29	91.54	1.30
14	19.50	19.98	1024.62	62.27	1.90
15	10.00	1.10	110.00	69.83	
16	10.00	6.91	691.25	67.30	
17	10.00	13.40	1340.00	70.80	
18	5.65	0.86	151.74	77.22	
19	2.50	5.68	2272.00	65.63	3.00
20	9.50	100.00	10526.32	52.40	4.00
21	1.00	100.00	100000.00	53.70	2.60
22	10.00	0.65	65.00	79.60	
23	3.50	100.00	28571.43	53.43	1.90
24	3.00	22.39	7463.33	60.47	4.20
25	5.55	18.61	3354.52	72.63	
26	28.00	100.00	3571.43	54.23	4.00
27	20.00	15.47	773.50	56.27	3.60
28	21.00	28.00	1333.33	50.63	5.40
29	23.00	13.95	606.38	57.93	5.30
30	10.00	2.42	242.25	77.50	
31	27.60	3.65	132.25	108.79	
32	17.00	14.95	879.41	61.13	4.70
33	10.00	1.29	128.75	75.03	
34	16.00	11.31	706.88	70.10	4.70
35	28.00	21.02	750.71	66.63	4.00
36	11.50	100.00	8695.65	52.33	4.20
37	10.00	5.35	535.00	74.63	2.40

SP#	MDM2 IC ₅₀ (nM)	SJSA-1 EC ₅₀ (μM)**	Penetration Efficiency (EC ₅₀ /IC ₅₀ *1000)	RT*	VH
38	4.50	100.00	22222.22	52.07	4.70
39	10.00	4.25	425.00	80.83	1.70
40	4.50	100.00	22222.22	52.13	
41	11.50	100.00	8695.65	52.67	4.70
42	10.00	2.40	240.00	73.40	2.80
43	18.00	100.00	5555.56	44.07	6.70
44	15.00	27.12	1808.00	50.90	5.60
45	15.00	3.65	243.33	76.67	2.70
46	23.00	28.06	1220.00	52.07	5.70
47	10.00	1.85	185.00	76.13	1.90
48	10.00	8.77	876.67	70.75	4.60
49	15.59	2.70	173.19	56.90	
50	15.00	1.85	123.33	82.17	-0.90
51	10.00	1.25	125.00	58.87	7.90
52	26.86	6.90	256.89	71.30	2.30
53	10.00	2.40	240.00	74.07	3.90
54	10.00	1.30	130.00	71.70	3.90
55	11.00	2.25	204.55	82.14	
56	10.00	2.60	260.00	74.97	
57	10.00	5.20	520.00	79.04	4.10
58	10.00	2.65	265.00	68.20	
59	12.00	23.81	1983.80	80.43	1.70
60	16.00	100.00	6250.00	60.03	
61	11.00	3.10	281.82	76.50	1.30
62	10.00	4.50	450.00	60.69	1.80
63	10.00	4.25	425.00	64.56	2.60
64	1.00	100.00	100000.00	63.70	4.40
65	22.50	100.00	4444.44	71.20	
66	10.00	1.75	175.00	70.31	1.90
67	10.00	100.00	10000.00	54.40	
68	10.00	6.60	660.00	58.92	4.50
69	17.00	100.00	5882.35	58.63	
70	17.50	100.00	5714.29	61.97	
71	10.00	6.70	670.00	62.79	5.10
72	11.00	26.85	2440.91	62.57	6.90
73	19.00	4.65	244.74	97.84	0.30
74	10.00	2.10	210.00	76.80	2.10
75	17.00	8.70	511.76	82.40	5.30
76	7.63	11.00	1441.05	70.86	
77	21.00	19.40	923.81	80.15	4.80
78	12.00	28.39	2365.83	61.83	
79	10.00	0.57	57.33	84.46	2.00
80	19.00	12.72	669.47	63.13	

SP#	MDM2 IC ₅₀ (nM)	SJSA-1 EC ₅₀ (μM)**	Penetration Efficiency (EC ₅₀ /IC ₅₀ *1000)	RT*	VH
81	16.50	100.00	6060.61	60.80	
82	20.00	18.87	943.50	65.93	
83	28.50	100.00	3508.77	61.67	
84	13.00	5.60	430.77	76.06	3.10
85	23.00	2.90	126.09	84.90	2.40
86	19.67	2.80	142.37	94.96	
87	7.53	5.70	756.64	79.82	
88	20.00	14.52	726.00	56.27	3.60
89	19.00	100.00	5263.16	74.90	2.60
90	20.00	17.02	851.00	69.63	4.00
91	10.00	5.05	505.00	78.30	1.50
92	10.00	2.25	225.00	74.60	3.60
93	27.50	100.00	3636.36	40.50	
94	10.00	4.35	435.00	74.07	1.50
95	8.67	4.23	488.08	55.05	
96	14.00	100.00	7142.86	62.50	
97	15.50	23.30	1503.23	62.50	4.40
98	1.00	21.83	21830.00	61.53	
99	26.50	19.84	748.68	61.60	
100	11.00	100.00	9090.91	55.52	
101	21.00	100.00	4761.90	49.50	
102	6.50	26.37	4056.92	59.27	
103	7.00	27.17	3881.43	59.57	
104	9.50	25.79	2714.74	59.23	
105	7.50	28.08	3744.00	59.33	
106	10.00	3.05	305.00	64.60	4.50
107	10.00	9.25	925.00	65.00	5.30
108	3.00	23.18	7726.67	59.40	
109	12.93	1.05	81.24	79.60	
110	10.00	1.45	145.00	66.07	2.80
111	10.00	3.80	380.00	68.03	2.60
112	14.50	100.00	6896.55	48.80	
113	10.00	1.10	110.00	65.10	3.20
114	10.00	1.30	130.00	59.47	5.10
115	10.00	10.05	1005.00	58.33	5.10
116	10.00	1.95	195.00	61.07	4.70
117	19.27	2.20	114.19	101.05	
118	17.90	0.70	39.11	104.36	
119	10.00	2.37	236.75	61.33	
120	12.53	0.40	31.91	104.81	
121	19.87	1.65	83.05	89.88	
122	10.00	9.40	940.00	79.70	2.10
123	22.50	23.49	1044.00	77.87	

SP#	MDM2 IC ₅₀ (nM)	SJSA-1 EC ₅₀ (μM)**	Penetration Efficiency (EC ₅₀ /IC ₅₀ *1000)	RT*	VH
124	13.00	100.00	7692.31	41.77	
125	10.98	2.68	244.38	85.79	1.20
126	10.00	3.95	395.00	55.60	5.40
127	10.00	15.50	1550.00	55.47	8.10
128	27.45	1.20	43.72	87.56	
129	10.00	22.95	2295.00	57.40	7.00
130	10.00	2.82	282.00	78.93	1.90
131	10.00	8.95	895.00	77.80	4.00
132	10.00	10.15	1015.00	59.92	5.80
133	10.00	8.00	800.00	81.27	2.80
134	24.00	100.00	4166.67	48.63	3.50
135	17.00	100.00	5882.35	56.57	1.60
136	14.50	100.00	6896.55	47.50	1.90
137	10.00	1.10	110.00	78.93	
138	10.00	3.91	391.00		1.20
139	10.00	3.35	335.00		1.70
140	10.00	3.70	370.00		4.00
141	10.09	0.50	49.55		1.50
142	20.39	0.18	8.81		2.10
143	27.67	21.51	777.61		6.90
144	22.42	21.70	967.89		3.90
145	16.21	0.20	12.34		7.50
146	10.04	0.70	69.76		4.60
147	7.69	0.79	102.04		4.50
148	10.00	0.50	50.00		2.00
149	10.00	3.60	360.00		3.70
150	10.00	3.59	359.00		2.10
151	10.00	0.10	10.00		2.20
152	10.00	4.70	470.00		2.50

* Normalized and calculated according to Example 11 (see table and equation).

**10% serum, 72 hr

VH= von Heijne; RT= retention time

Table 9

SP#	VH	Ch	RT*	EC ₅₀ (μM)**	Ki MCL-1	Ki BCL-X _L	Ki BCL-2
203	16.5	1	45.41		10.6	3.9	12.9
204	18.9	0	39.84			9.2	23.5
205	9.7	0	63.97			212.6	423.8
206	7.5	0	60.88			373.6	877.5
207	8.9	0	58.1			>1000	>1000
208	9.1	0		6.6	8.4	22.4	84.8

SP#	VH	Ch	RT*	EC ₅₀ (μM)**	Ki MCL-1	Ki BCL-X _L	Ki BCL-2
209	9.7	0					410.2
210	9.4	0				308.6	519.2
211	7.3	0				255.9	318.7
212	7	-1				>1000	>1000
213	8.9	0				243.1	272.5
214	9.8	0				>1000	>1000
215	8.9	0				471.5	803.9
216	10.1	-1		>40	19.5	11.6	8.7
217		0	66.45				
218	9.6	-1	58.41		7.0	15.4	21.9
219	11.6	0	46.65				
220		0	64.59		ND	ND	ND
221		0	62.63		ND	ND	ND
222		-1	52.37		ND	ND	ND
223	8.9	0			19.7	22.3	37.7
224	11.5	-1		>40	1.8	1.4	3.2
225	11.2	0			5.2	12.0	67.0
226	9.4	-1			1.0	1.0	6.0
227	9.1	0			6.0	4.0	22.0
228	7	0			6.7	25.6	65.4
229	7.2	0			7.4	55.7	114.6
230		-1		9.1	7.5	109.0	211.6
231		-1			1.7	28.8	88.2
232	8.6	-1			>1000	>1000	>1000
233	10.7	-1			>1000	>1000	>1000
234	8.8	-1			76.2	325.4	364.7
235	10.9	-1			14.8	6.3	27.5
236	9.5	0			7.5	401.7	139.7
237	8.8	0			3.4	14.1	113.0
238	10.9	0					
239	10.1	0			177.0	154.0	502.0
240	8.6	0			96.3	7.7	84.0
241	10.7	0			116.2	7.7	25.6
242	9.8	0			182.7	18.1	59.6
243	11.9	0			122.1	1.9	4.8
244	9.4	0			27.8	15.8	68.5
245	11.5	0			74.1	25.6	66.1
246	9.4	0			22.0	28.0	179.4
247	11.5	0			29.3	25.9	204.6
248	6.7	-1			73.8	386.4	>1000
249	7.6	1			194.7	416.0	404.9
250	9.7	0			>1000	>1000	>1000
251	9.3	0			22.8	53.5	84.9

SP#	VH	Ch	RT*	EC ₅₀ (μM)**	Ki MCL-1	Ki BCL-X _L	Ki BCL-2
252	9.2	0	72.33	3.9	43.8	14.4	104.9
253		0	69.23	5.7	21.2	11.9	160.7
254		0	74.49		21.6	7.3	59.0
255		0	74.18		22.2	13.1	182.3
256		0	64.59	7.2	53.1	69.7	221.2
257		0	66.14	5.9	12.8	145.5	246.4
258		0	66.14		76.7	9.1	237.0
259		0	71.09		150.8	16.9	>1000
260	9.2	0	66.14	4.8	163.2	151.1	264.6
261		0	66.14	6.4	11.0	3.0	>1000
262	9.1	0	71.71		4.2	81.1	>1000
263		0	71.4		3.1	135.9	231.4
264		0	72.33	0.5	40.2	109.5	>1000
265		0	74.8		3.8	>1000	>1000
266		0	76.97		16.6	>1000	240.3
267		0	66.14		7.5	>1000	>1000
268		0	68.62		47.7	146.7	>1000
269		0	68.31		188.1	10.8	>1000
270		0	71.09		169.0	12.7	288.0
271	9.1	0	66.45		119.7	17.3	234.4
272		0	68.62		83.7	8.0	280.1
273	9.2	0	65.52		26.6	27.5	89.0
274	9.2	0	67.69		13.0	6.0	12.7
275	9.2	0	71.09		15.9	7.1	109.1
276		0	67.69		13.7	35.4	227.1
277		0	69.85		34.6	4.0	23.1
278		0	66.14		9.9	17.4	70.6
279	8.7	0	65.21		42.5	83.5	97.9
280		0	69.85		5.2	8.4	48.3
281		0	66.76		19.8	7.2	24.8
282		1	62.12		>1000	>1000	>1000
283	6.8	0	73.57		5.5	59.0	120.1
284		1	58.41	>40	>1000	15.5	>1000
285		0	75.42	2.1	4.8	9.5	91.8
289		0	72.02		10.9	17.2	36.6
290	9.2	0	71.4		3.7	17.0	36.5
291	9.2	0	73.57		4.6	20.9	38.9
292		0	68.92		10.4	7.7	25.7
293		0	65.83		8.9	8.4	68.2
294	10.1	-1	71.4		2.2	72.0	117.8
295	10.1	1	61.5		4.5	32.8	47.8
296	10.1	1	60.26		3.9	27.2	49.7
297	9.6	0	59.95		3.6	25.2	52.0

SP#	VH	Ch	RT*	EC ₅₀ (μM)**	Ki MCL-1	Ki BCL-X _L	Ki BCL-2
298	8.4	0	70.16		3.9	33.4	53.2
299	9.8	0	68.92		4.8	35.9	64.9
300	7.7	0	71.71		3.8	32.6	63.9
301	7.5	0	75.73		4.0	20.3	56.0
302	7.3	0	77.28		6.2	33.9	76.7
303	10.2	-1	59.33		3.0	36.3	41.1
304	9.2	1	46.65		9.8	20.5	39.1
305	8.7	0	47.89		6.6	19.5	43.0
306	7.5	0	60.57		15.3	56.2	89.5
307	8.7	0	57.17		11.2	37.3	62.5
308	6.2	0	85.63		10.2	71.8	125.6
309	6.4	0	80.68		10.2	45.3	95.9
310	6.2	0	84.08		13.7	55.3	144.3
311	7.7	0	73.57		3.6	67.8	117.2
312	6.4	0	80.99		19.7	96.2	170.5
313	6.6	0	79.44		17.2	109.9	125.0
314	6.4	0	83.16		11.6	37.9	75.8
315	7	0	76.04		23.2	11.7	25.6
316	6.3	0	79.44		106.2	112.2	130.6
317	6.1	0	79.44		104.3	139.5	119.8
318	6	0	75.42		63.6	135.4	141.9
319	7.9	0	61.5		29.7	171.4	145.1
320	9.7	0	64.59		2.3	14.5	17.7
321		0	72.33		2.7	16.6	38.9
322		0	73.87		8.2	107.4	103.8
323		0	78.21		21.2	154.1	158.3
324	8.9	0	73.26		4.4	19.1	35.1
325		0	74.8		6.5	100.2	113.5
326	8.5	-1	76.66		1.5	25.9	35.3
327	7.5	-1	77.9		1.8	17.9	30.7
328	7.2	0	76.66		4.9	36.5	71.6
329	5.8	0			8.0	44.1	67.5
330	7	0			8.0	36.3	57.4
331	5.9	0			17.6	69.9	124.9
332	8.6	0			20.3	51.8	102.0
333	8	0			23.4	9.0	18.9
334	8	0			24.1	8.6	28.9
335		0			8.0	12.1	30.7
336	8.8	0			3.9	15.4	23.5
337	8	0			8.0	26.1	65.2
338	10.4	-1			2.2	116.4	137.9
339	9.4	-1			1.4	45.4	56.4
340	9.1	0			4.6	41.1	64.7

SP#	VH	Ch	RT*	EC ₅₀ (μM)**	Ki MCL-1	Ki BCL-X _L	Ki BCL-2
341	7.7	0			4.7	36.0	62.4
342	8.9	0			10.6	73.8	98.8
343	9.4	1			18.5	185.9	141.8
344	9.4	1			6.6	66.3	43.4
345		0		0.98	86.6	>1000	>1000
346		1		15.2	>1000	205.5	>1000
347		0		1.4	14.9	26.0	199.8
348	8.9	0		4.6	29.0	>1000	218.1
349		-1		15.3	>1000	85.0	>1000
350		0		5.0	>1000	>1000	>1000
351		-1		19.3	39.5	>1000	>1000
352		-1		5.8	0.8	>1000	>1000
353	8.6	0		4.0	5.3	12.6	111.6
354		0		1.0	58.0	43.0	
355		0		0.7	27.0	13.0	
356		0		0.7	127.0	121.0	
357		0		0.6	52.0	37.0	
358		0		0.9	23.0	9.0	
359		0		0.5	>1000	>1000	
286	10.7	-1	66.14	>40	1.1	1.1	22.0
287	8.8	-1	62.12	>40	1.6	1.6	19.2
288	11.1	-1	57.48	>40	11.6	24.2	57.7

* Normalized and calculated according to Example 11 (see table and equation).

**Raji Cell Viability, 48 h, 5% serum

Ch = net charge; VH= von Heijne; RT= retention time

Example 13: Synthesis of Peptidomimetic Macrocycles

[00428] Peptidomimetic macrocycles were synthesized, purified and analyzed as previously described (Walensky et al (2004) Science 305:1466-70; Walensky et al (2006) Mol Cell 24:199-210; Bernal et al (2007) J. Am Chem Soc. 9129, 2456-2457) and as indicated below. The macrocycles used in this study are shown in Table 1. The corresponding uncrosslinked polypeptides represent the natural counterparts of the peptidomimetic macrocycles.

[00429] α,α-disubstituted non-natural amino acids containing olefinic side chains were synthesized according to Williams et al. (1991) J. Am. Chem. Soc. 113:9276; Schafmeister et al. (2000) J. Am. Chem Soc. 122:5891 and Verdine et al PCT WO 2008/121767. Peptidomimetic macrocycles were designed by replacing two or more naturally occurring amino acids with the corresponding synthetic amino acids. Substitutions were made at the i and i+3, i and i+4, i and i+6, and i and i+7 positions. Macrocycles were generated by solid phase peptide synthesis followed by olefin metathesis-based crosslinking of the synthetic amino acids via their olefin-containing side chains.

[00430] The non-natural amino acids (R and S enantiomers of the 5-carbon olefinic amino acid and the S enantiomer of the 8-carbon olefinic amino acid) were characterized by nuclear magnetic resonance (NMR) spectroscopy (Varian Mercury 400) and mass spectrometry (Micromass LCT). Peptide synthesis was performed manually or on an automated peptide synthesizer (Applied Biosystems, model 433A), using solid phase conditions, rink amide AM resin (Novabiochem), and Fmoc main-chain protecting group chemistry. For the coupling of natural Fmoc-protected amino acids (Novabiochem), 10 equivalents of amino acid and a 1:1:2 molar ratio of coupling reagents HBTU/HOBt (Novabiochem)/DIEA were employed. Non-natural amino acids (4 equiv) were coupled with a 1:1:2 molar ratio of HATU (Applied Biosystems)/HOBt/DIEA. Olefin metathesis was performed in the solid phase using 10 mM Grubbs catalyst (Blackwell et al. 1994 supra) (Strem Chemicals) dissolved in degassed dichloromethane and reacted for 2 hr at room temperature. Isolation of metathesized compounds was achieved by trifluoroacetic acid-mediated deprotection and cleavage, ether precipitation to yield the crude product, and high performance liquid chromatography (HPLC) (Varian ProStar) on a reverse phase C18 column (Varian) to yield the pure compounds. Chemical composition of the pure products was confirmed by LC/MS mass spectrometry (Micromass LCT interfaced with Agilent 1100 HPLC system) and amino acid analysis (Applied Biosystems, model 420A).

[00431] The synthesized peptides include a norleucine replacement for methionine to avoid issues with unwanted thioether oxidation. In several peptides, the proline residue is replaced with a 2-aminoisobutyric acid residue (Aib) to increase helicity, and the effect of Glu-to-Arg substitution on cell penetrability was also explored. The N-termini of the exemplary synthetic peptides were acetylated, while the C-termini were amidated. Table 1 shows non-limiting list of peptidomimetic macrocycles prepared.

Example 14: Manufacture of peptidomimetic macrocycle

Synthesis of Peptide on Resin

[00432] The initial amino acid of the C-terminus was attached to a polyethylene glycol-grafted polystyrene-based Fmoc-Rink resin (Amphispheres 40 RAM), which was prepared for coupling with the first amino acid by treatment with piperidine in DMF. The subsequent amino acids were coupled individually until the full linear sequence was elaborated. The α -amino group of each amino acid was protected by a 9H-fluoren-9-ylmethoxycarbonyl group (Fmoc) during the coupling of the carboxylic acid of the amino acid with the free amino terminus of the peptide attached to the resin. To avoid any side reactions during the coupling steps performed in DMF, the reactive side-chains of amino acids also carry acid-labile protecting groups that effectively mask the reactive groups until treatment of the resin with acid during the cleavage of the peptide from the solid support. After completion of

each coupling step the Fmoc group of the just-attached amino acid was removed with piperidine and the resin was thoroughly washed to prepare for the coupling of the subsequent Fmoc-amino acid derivative. Diisopropylcarbodiimide (DIC) with 1-hydroxybenzotriazole (HOBt) or (7-azabenzotriazol-1-yloxy) tripyrrolidino-phosphonium hexafluorophosphate (PyAOP) were used as coupling agents to create the amide bond between the free amino terminus of the resin-bound protected peptide and the carboxylic acid of the Fmoc-amino acid.

[00433] The presence or absence of available amino groups was tested with the Kaiser Ninhydrin Test. After each piperidine treatment a small sample of the resin-bound peptide was removed to verify the availability of free amino groups. Additionally, a small sample of the resin-bound peptide was also removed after each Fmoc-amino acid coupling step to verify that no amino groups were still available to react with the next amino acid. In case of a positive test result after coupling (presence of free amino groups), the coupling reaction was repeated. If there were still available amino groups after repeat coupling, the resin-bound peptide was treated with acetic anhydride in the presence of DIPEA in DMF to block the remaining free amine from further reaction with subsequent Fmoc-amino acids. After release from the resin, the shorter blocked peptides were more readily removed from the complete peptide than a peptide containing a deletion.

Cyclization (Metathesis)

[00434] Cyclization was carried out in Toluene with the peptide on the resin resulting in a cross-link of terminal olefin moieties with the application of Hoveyda-Grubbs' 2nd generation catalyst. This cyclization was performed at room temperature with stepwise addition of catalyst (5-10% total amount) over period of 5-7 days. Progress of the cyclization was monitored by TFA cleavage of small sample of peptide resin and LC-MS analysis of cleaved peptide. After cyclization was completed resin was washed extensively and treated with DMSO/DCM mixture (1:1, v/v) for 16 hr. Following extensive washes resin was dried under vacuum before TFA cleavage. Treatment of the resin with trifluoroacetic acid (TFA) removes side chain protecting groups and releases the peptide from the resin as the C-terminal primary amide. Water and triisopropylsilane were added to the TFA cleavage solution to quench reactive side products that result from the removal of side chain protecting groups.

Purification

[00435] Purification was performed by preparative reversed-phase high performance liquid chromatography (RP-HPLC) in three sequential steps on phenyl-hexyl-derivatized silica as purification media. In the first step, the peptide was eluted from the column using a gradient of acetonitrile in 0.1 % TFA in water; in the second step, an acetonitrile gradient of acetonitrile in 0.1 M triethylamine/phosphoric acid in water was used.

[00436] Fractions from each step were tested for peptide purity using an in-process analytical HPLC method. Pooled fractions were lyophilized and stored until the entire batch has been purified.

Desalting, Reconstitution and Lyophilization

[00437] The purified product from the second reverse-phase HPLC purification step was converted to the free carboxylic acid (side chain of Glu) on phenyl-hexyl-derivatized silica and eluted using an acetonitrile gradient in 0.1 % aqueous TFA. The eluate was clarified by filtration through a 0.2 μ m membrane filter and then lyophilized to homogeneity.

Packaging

[00438] Following lyophilization final peptide was packaged into Type III amber glass bottles in a HEPA-filtered environment. The bottles were sealed with Teflon[®]-lined polypropylene screw caps. To ensure that no moisture can enter the containers, the screw cap bottle interface was additionally wrapped with PTFE tape.

Example 15: Pd-catalyzed cross coupling of terminal alkynes to diynes

[00439] The fully protected resin-bound peptides were synthesized on a PEG-PS resin (loading 0.4 mmol/g) on a 0.2 mmol scale. Deprotection of the temporary Fmoc group was achieved by 3 $\times$ 10 min treatments of the resin bound peptide with 20% (v/v) piperidine in DMF. After washing with NMP (3x), dichloromethane (3x) and NMP (3x), coupling of each successive amino acid was achieved with 1 $\times$ 120 min incubation with the appropriate preactivated Fmoc-amino acid derivative. All protected amino acids (0.8 mmol) were dissolved in NMP and activated with HCTU (0.8 mmol) and DIEA (1.6 mmol) prior to transfer of the coupling solution to the deprotected resin-bound peptide. α -methylated amino acids and amino acids immediately followed α -methylated ones were coupled in the similar way with the use of PyAOP instead HCTU as a coupling agent. After all couplings were completed, the resin was washed in preparation for the next deprotection/coupling cycle. Acetylation of the amino terminus was carried out in the presence of acetic anhydride/DIEA in NMP. The LC-MS analysis of a cleaved and deprotected sample obtained from an aliquot of the fully assembled resin-bound peptide was accomplished in order to verifying the completion of each coupling.

[00440] In a typical example, 1,2-dichloroethane (6 mL) and triethylamine (3 mL) were added to the peptide resin (0.2 mmol) in a 40 mL glass vial and shaken for 60 min. Copper iodide (0.008 g, 0.04 mmol) was added to the resin slurry followed by 10 min later with Pd(PPh₃)₂Cl₂ (0.028 g, 0.04 mmol) and triphenylphosphine (0.031 g, 0.12 mmol). The resulting reaction mixture was mechanically shaken 16 hr at 50 °C while open to atmosphere.

[00441] The diyne-cyclized resin-bound peptides were deprotected and cleaved from the solid support by treatment with TFA/H₂O/TIS (95/5/5 v/v) for 2.5 hr at room temperature. After filtration of the

resin the TFA solution was precipitated in cold diethyl ether and centrifuged to yield the desired product as a solid. The crude product was purified by preparative HPLC.

Example 16: Stability

[00442] The ex vivo serum stability of several peptidomimetic macrocycles will be tested by incubating them at 5000 ng/mL (2 μ M at MW=2500) with fresh human serum at 37 °C and taking samples at 0, 0.5, 1, 2, 4, 6 and 24 hr. At each time point the samples will be flash-frozen until analysis in duplicate, then extracted by transferring 100 μ L of sera to 2 mL centrifuge tubes followed by the addition of 10 μ L of 50 % formic acid and 500 μ L acetonitrile and centrifugation at 14,000 RPM for 10 min at 4 $\pm$ 2°C. After protein precipitation, the supernatants will be transferred to fresh 2 mL tubes and evaporated on Turbovap under N₂ at < 10 psi, 37°C. The samples will be reconstituted in 100 μ L of 50:50 acetonitrile/water and quantified by LC-MS/MS analysis. The response for each compound will be normalized to estimate a percent decrease in concentration versus time.

Example 17: PK Properties

[00443] Several peptidomimetic macrocycles will be tested for PK properties in single IV dose in rats. The in-life portion of the study will be conducted at ViviSource Laboratories (Waltham, MA). A single intravenous dose of 3 mg/kg Stapled Peptide formulated in water containing 5% PEG-400 and 2% Dextrose will be administered to a pair of jugular vein-cannulated male Sprague-Dawley rats. The IV dose that is mostly well-tolerated and whether animals appear healthy within the study duration will be determined. Blood samples will be collected over thirteen sampling times up to 24 hr and the plasma samples will be shipped on dry ice to Tandem Bioanalytical Facilities, Inc. (Woburn, MA) for the analytical phase of the study.

[00444] Quantification in plasma samples will be preceded by the preparation of sample extracts by combining 50 μ L of ammonium hydroxide (14.5 M ammonia), 1 mL of a 1:1 acetonitrile/methanol solution, and 50 μ L of internal standard with 50 μ L of each plasma sample. The mixtures will be centrifuged to separate liquid supernatant from solid precipitate and supernatants will be dried at 40 °C under flowing nitrogen gas. The dried sample extracts will be reconstituted in 50 μ L of a 1:1 water/methanol solution that contained 0.1% (v/v) trifluoroacetic acid. Plasma sample extracts will be analyzed by a liquid chromatography-mass spectrometry method that utilized an API 5000 (Applied Biosystems) instrument operated in positive ionization mode at a temperature of 500°C using a multiple reaction monitoring mode of detection (MRM). The analytical column for liquid chromatography will be a Varian Metasil C18, 50 mm x 2 mm and mobile phases A (0.1 % formic acid in water) and B (0.1 % formic acid in acetonitrile) will be pumped at a flow rate of 0.5 mL/min. Quantification in plasma extracts will be made by linear regression analysis employing a pure reference standard Stapled Peptide diluted in normal rat plasma to prepare eight calibration standards

over the working concentration range of 20 – 10,000 ng/mL. The calibration standards will be extracted in identical fashion as sample extracts and analyzed before and after the sample extracts. [00445] Pharmacokinetic parameters will be calculated using a non-compartmental model using the PK Functions add-in for Microsoft Excel. The terminal elimination half-life will be calculated as $\ln(2)/(\lambda_z)$, where the rate constant (λ_z) will be calculated as -1 times the estimated slope of the log-concentration versus time data over 2-12 hr. AUC values (hr*ng/mL) will be calculated by statistical moment and linear trapezoidal approximation methods over time points of 0 – 24 hr and 24 hr concentration values will be divided by (λ_z) was added in order to extrapolate AUMC and AUC values to infinite time. Total body clearance (per kg body weight) will be calculated as dose divided by AUC. The volume of distribution at steady state (V_{ss}) will be calculated as the product of clearance and mean residence time ($MRT = AUC / AUMC$).

[00446] An experiment will be also performed to compare different modes of administration. Subcutaneous injection of peptidomimetic macrocycle will be performed and compared to intravenous administration. Two groups of two animals each will be injected subcutaneously with a 3 mg/kg dose. Plasma will be collected at regular time points (*e.g.*, 5, 20 min; 1, 2, 4, 8 12, and 48 hr) and the samples will be analyzed as indicated above.

Example 18: *In vitro* Testing for Inhibition of Influenza Replication

[00447] This influenza antiviral evaluation assay will examine the effects of peptidomimetic macrocycles at designated dose-response concentrations. See also Noah, J.W., W. Severson, D.L. Noah, L. Rasmussen, E.L. White, and C.B. Jonsson, *Antiviral Res*, 2007. **73**(1): p. 50-9. Madin Darby canine kidney (MDCK) cells will be used in the assay to test the efficacy of the compounds in preventing the cytopathic effect (CPE) induced by influenza infection. Either Ribavirin or Tamiflu will be included in each run as a positive control compound. Subconfluent cultures of MDCK cells will be plated into 96-well plates for the analysis of cell viability (cell cytotoxicity (CC)) and antiviral activity (CPE). Drugs will be added to the cells 24 hr later. At a designated time, the CPE wells will also receive 100 tissue culture infectious doses (100 TCID₅₀s) of titered influenza virus. 72 hr later the cell viability will be determined. The effective compound concentrations which reduced viral-induced CPE by 25% (IC₂₅), 50% (IC₅₀), and 90% (IC₉₀) will be calculated by regression analysis with semi-log curve fitting. Cell viability will be assessed using CellTiter-Glo (Promega). The toxic concentration of drug that reduces cell numbers by 50% and 90% (TC₅₀ and TC₉₀, respectively) will be calculated as well. Selectivity (therapeutic) indices (SI = TC/IC) will also be calculated. Cells will also be used in a virus yield reduction (VYR) assay to determine antiviral efficacy of the compounds in reducing viral titers. Cells will be plated and infected as described above and then incubated. Supernatants will then be harvested and viral titers will be determined by

plaque assay, TCID, or immunocytochemistry. Antiviral efficacy will be calculated based on viral titer reduction compared to an untreated virus control and used to calculate IC₅₀. Exemplary results are shown in Table 10 below.

Table 10

SP#	Crosslink	Ch	L	VH	RT (min)*	Ala (%)	CPE, EC ₅₀ (μM)
174	i, i+4	0	15	7.1	9.46	7	
175	i, i+4	0	15	7.5	8.16	13	36
176	i, i+4	0	15	5	10.03	13	51
177	i, i+4	0	15	9.2	6.86	0	100
178	i, i+4	0	15	7.3	9.59	7	100
179	i, i+4	-1	16		11.23	13	34
180	i, i+4	-1	15	4.7	10.68	13	23
181	i, i+4	-1	17	4.9	11.55	24	11
182	i, i+4	-1	17	5.1	10.35	29	19
183	i, i+4	-1	17	3	12.00	35	22
184	i, i+4	-1	17	5.8	8.92	24	35
185	i, i+4	-1	17	7.2	8.02	24	4.1
186	i, i+4	-2	17	7.5	8.71	24	8.4
187	i, i+4	-1	17	3.7	10.50	29	31
188	i, i+4	-1	17	5.1	9.54	29	6.1
189	i, i+4	-2	17	5.4	10.33	29	28
190	i, i+4	-1	17	4.9	10.65	18	19
191	i, i+4	-2	17	4	10.89	18	30
192	i, i+7	-1	15	4.9	10.18	20	4.5
193	i, i+4	-1	17	7		18	
194	i, i+4	-1	17	7	8.47	18	
195	i, i+4	-1	17	4.9	10.01	24	
196	i, i+4	-1	17	4.9	10.18	24	
197	i, i+4	-1	17	4.9	10.26	4	
198	i, i+7	-1	17	5.1	10.92	29	
199	i, i+7	-1	17	3	13.03	35	
200	i, i+7	-1	17	7.2	8.86	24	
201	i, i+7	-1	17	5.1	10.83	29	

* See Example 11 table

Ch = net charge; L = length in amino acids; VH= von Heijne; RT= retention time; Ala= alanine content

Example 19:

[00448] Cells were thawed from a liquid nitrogen preserved state. Once cells were expanded and divide at their expected doubling times, screening began. Cells were seeded in growth media in black 384-well tissue culture treated plates at 500 cells/well. Cells were equilibrated in assay plates via centrifugation and placed in incubators attached to the Dosing Modules at 37 °C for 24 hr before treatment, resulting in cell densities of approximately 500 cells/plate. At the time of treatment, a set of assay plates (which did not receive treatment) were collected and ATP levels were measured by

adding ATPLite (Perkin Elmer). These T-zero (T_0) plates were read using ultra-sensitive luminescence on Envision Plate Readers. Using an automated acoustic dispensing system, assay plates were treated with compound or peptide from a 1000x DMSO stock, achieving a 1:1000 working dilution. Final treatment concentrations in the plates were 0 (vehicle), 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 μ M. The assay plates (4 replicates per treatment) were incubated with compound or peptide for 72 hr. After 72 hr, plates were developed for endpoint analysis using ATPLite. All data points were collected via automated processes; quality controlled; and analyzed using Zalicus proprietary software. Assay plates were accepted if they passed the following quality control standards: relative luciferase values were consistent throughout the entire experiment, Z-factor scores were greater than 0.6, and untreated/vehicle controls behaved consistently on the plate.

[00449] Growth Inhibition (GI), used as a measure of cell viability, was measured at the time of dosing (T_0) and after 72 hr (T_{72}). A GI reading of 0% represents no growth inhibition; a GI 100% represents complete growth inhibition, a cytostatic effect. A GI 200% represents complete death of all cells in the culture well. Compounds reaching an activity plateau of GI 200% were considered cytotoxic. GI was calculated by the following test and equation:

$$\text{If } T < V_0 : 100 * (1 - \frac{T-V_0}{V_0})$$

$$\text{If } T \geq V_0 : 100 * (1 - \frac{T-V_0}{V-V_0})$$

[00450] Where T is the signal measure for a test article, V is the vehicle-treated control measure, and V_0 is the vehicle control measure at time zero. This formula was derived from the Growth Inhibition calculation used in the National Cancer Institute's NCI-60 high throughput screen

[00451] Cell lines were assigned as p53 wild-type, mutant, or null from information available from the Cancer Cell Line Encyclopedia. Results for exemplary p53 peptidomimetic macrocycles are shown in the table below

Cell Viability in p53^{WT} / p53^{MUT} cancer cell lines

Cell lines	Cancer Type	p53 ^{WT} / p53 ^{MUT}	SP-18	SP-172
			EC ₅₀ (μ M)	EC ₅₀ (μ M)
A2058	skin	p53 ^{MUT}	18.6	30
AsPC-1	Pancreas	p53 ^{MUT}	30	30
Caov-3	ovary	p53 ^{MUT}	12.9	30
Caov-4	ovary	p53 ^{MUT}	30	30
COLO-679	skin	p53 ^{MUT}	13.5	30
COLO-684	endometrium	p53 ^{MUT}	10.1	30
COLO-741	skin	p53 ^{MUT}	18.5	30
EBC-1	Lung-NSCLC	p53 ^{MUT}	30	30
ECC10	gastric	p53 ^{MUT}	15.2	30

Cell lines	Cancer Type	p53 ^{WT} / p53 ^{MUT}	SP-18	SP-172
KG-1	erythroleukemia	p53 ^{MUT}	30	30
KM12	colorectal	p53 ^{MUT}	30	30
LS-123	colorectal	p53 ^{MUT}	30	30
MIA PaCa-2	pancreas	p53 ^{MUT}	30	30
NCI-H508	colorectal	p53 ^{MUT}	30	30
OVCAR-3	ovary	p53 ^{MUT}	30	30
RPMI-7951	skin	p53 ^{MUT}	10.8	30
SF126	brain	p53 ^{MUT}	11	30
SK-OV-3	ovary	p53 ^{MUT}	14.3	30
SW480	colorectal	p53 ^{MUT}	30	30
HCT-116	colorectal	p53 ^{WT}	0.464	1.02
HEC-151	endometrium	p53 ^{WT}	1.19	30
HEC-265	endometrium	p53 ^{WT}	1.78	30
HUH-6-clone5	liver	p53 ^{WT}	0.865	0.791
IST-MES1	lung	p53 ^{WT}	1.43	30
KP-N-RT-BM-1	nerve	p53 ^{WT}	0.285	0.255
KP-N-S19s	nerve	p53 ^{WT}	0.176	0.0168
LoVo	colorectal	p53 ^{WT}	0.431	0.134
LS-174T	colorectal	p53 ^{WT}	0.402	0.205
MSTO-211H	lung	p53 ^{WT}	0.288	0.209
MV-4-11	AML	p53 ^{WT}	0.159	0.307
NCI-H929	multiple myeloma	p53 ^{WT}	0.858	0.24
PA-1	ovary	p53 ^{WT}	0.339	0.592
WM-115	skin	p53 ^{WT}	0.425	0.429
COLO-205	colorectal	p53 ^{WT}	0.646	0.429
COLO-849	skin	p53 ^{WT}	1.66	0.501
NCI-H28	lung	p53 ^{WT}	3.42	30

CLAIMS

WHAT IS CLAIMED IS:

1. A method of selecting a peptidomimetic macrocycle with enhanced cell permeability, the method comprising:
 - a) synthesizing a peptidomimetic macrocycle comprising a polypeptide with a crosslinker connecting a first amino acid and a second amino acid;
 - b) determining if the peptidomimetic macrocycle fulfills two or more conditions selected from a group consisting of:
 - a length of the peptidomimetic macrocycle ranges from 10-24 amino acids,
 - a molecular weight of the peptidomimetic macrocycle ranges from about 500 to about 1500 Da,
 - a percent polar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a percent nonpolar amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a percent hydrophobic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a percent β -branched amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a percent aromatic amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a von Heijne value of the peptidomimetic macrocycle ranges from 2 to 9,
 - a net charge carried by the peptidomimetic macrocycle ranges from -4 to +2,
 - a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the C-terminus of the peptidomimetic macrocycle ranges from -4 to +2,
 - a net charge carried by the 1, 2, 3, 4, 5, or 6 amino acids at the N-terminus of the peptidomimetic macrocycle ranges from -4 to +2,
 - a ratio of charged amino acids over uncharged amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10,
 - a positively charged amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a negatively charged amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 - a side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,

a charged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 an uncharged side chain hydrogen bonding amino acids content of the peptidomimetic macrocycle ranges from 15% to 80%,
 a ratio of charged side chain hydrogen bonding amino acids over uncharged side chain hydrogen bonding amino acids of the peptidomimetic macrocycle ranges from 0.1 to 10,
 a tyrosine content of the peptidomimetic macrocycle ranges from 5% to 50%,
 an alanine content of the peptidomimetic macrocycle ranges from 5% to 50%,
 a tryptophan content of the peptidomimetic macrocycle ranges from 5% to 50%,
 a non-natural amino acid content of the peptidomimetic macrocycle ranges from 5% to 50%,
 a number of halo-substituents of the peptidomimetic macrocycle ranges from 1 to 20,
 a number of *ortho*-substituted, *meta*-substituted, and/or *para*-substituted phenylalanine of the peptidomimetic macrocycle ranges from 1 to 20,
 a ratio of fluorine atoms to chlorine atoms of the peptidomimetic macrocycle ranges from 0.1 to 10,
 a presence of a carboxylic acid with a substituent in the capping groups of the peptidomimetic macrocycle,
 a presence of an amine with a substituent in the capping groups of the peptidomimetic macrocycle, and
 a partition coefficient ($\log P_{\text{octanol/water}}$ at 37°C) of the peptidomimetic macrocycle ranges from -5.0 to 5.0; and

c) selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the peptidomimetic macrocycle fulfills two or more conditions in (b).

2. The method of claim 1, further comprising determining an aqueous solubility of the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the aqueous solubility indicates a lack of precipitated polypeptide.
3. The method of claim 1 or 2, wherein the aqueous solubility of the peptidomimetic macrocycle is determined by evaluating the turbidity of a solution comprising the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic macrocycle as an enhanced peptidomimetic macrocycle if the turbidity of the aqueous solubility indicates a lack of precipitated polypeptide.
4. The method of any of claims 1-3, further comprising calculating an amphipathicity value of the peptidomimetic macrocycle, wherein the selecting comprises selecting the peptidomimetic

macrocycle as an enhanced peptidomimetic macrocycle if the amphipathicity of the peptidomimetic macrocycle falls in a range that is optimal for cell permeability.

5. The method of any of claims 1-4, wherein the selected peptidomimetic macrocycle is soluble, does not have off-target effects, or a combination thereof.
6. The method of any of claims 1-5, wherein the selected peptidomimetic macrocycle comprises a first C-terminal amino acid that is hydrophobic.
7. The method of any of claims 1-6, wherein the selected peptidomimetic macrocycle comprises a second C-terminal amino acid that is hydrophobic.
8. The method of any of claims 1-7, wherein the selected peptidomimetic macrocycle comprises a third C-terminal amino acid that is hydrophobic.
9. The method of any of claims 1-8, wherein the selected peptidomimetic macrocycle comprises a fourth C-terminal amino acid that is hydrophobic.
10. The method of any of claims 1-9, wherein the selected peptidomimetic macrocycle comprises a fifth C-terminal amino acid that is hydrophobic.
11. The method of any of claims 1-10, wherein the selected peptidomimetic macrocycle comprises a sixth C-terminal amino acid that is hydrophobic.
12. The method of any of claims 1-11, wherein the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, 3, 4, 5, 6, 7, or 8 amino acids that are C-terminal to the second amino acid connected to the crosslinker.
13. The method of any of claims 1-12, wherein the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, 3, 4, 5, or 6 hydrophobic amino acids that are C-terminal to the second amino acid connected to the crosslinker.
14. The method of any of claims 1-13, wherein the first amino acid connected to the crosslinker is N-terminal to the second amino acid connected to the crosslinker, and wherein the selected peptidomimetic macrocycle comprises 1, 2, or 3 glutamines that are C-terminal to the second amino acid connected to the crosslinker.
15. The method of any of claims 1-14, wherein the amino acid that is hydrophobic is a small hydrophobic amino acid.
16. The method of any of claims 1-15, wherein the amino acid that is hydrophobic is an alanine, a D-alanine, or an Aib.
17. The method of any of claims 1-16, wherein the selected peptidomimetic macrocycle is a helical polypeptide.

18. The method of any of claims 1-17, wherein the selected peptidomimetic macrocycle comprises an α -helix.
19. The method of any of claims 1-18, wherein the selected peptidomimetic macrocycle comprises an amphipathic α -helix.
20. The method of any of claims 1-19, wherein the first amino acid connected to the crosslinker or the second amino acid connected to the crosslinker is an α,α -disubstituted amino acid.
21. The method of any of claims 1-20, wherein the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are α,α -disubstituted amino acids.
22. The method of any of claims 1-21, wherein the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by two amino acids.
23. The method of any of claims 1-21, wherein the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by three amino acids.
24. The method of any of claims 1-21, wherein the first amino acid connected to the crosslinker and the second amino acid connected to the crosslinker are separated by six amino acids.
25. The method of any of claims 1-24, wherein the crosslinker spans 1 turn of an α -helix of the peptidomimetic macrocycle.
26. The method of any of claims 1-24, wherein the crosslinker spans 2 turns of an α -helix of the peptidomimetic macrocycle.
27. The method of any of claims 1-26, wherein the length of the crosslinker is from about 5 Å to about 9 Å per turn of an α -helix of the peptidomimetic macrocycle.
28. The method of any of claims 1-27, wherein the selected peptidomimetic macrocycle provides a therapeutic effect.
29. The method of any of claims 1-28, wherein an ability of the selected peptidomimetic macrocycle to penetrate cell membranes by an energy-dependent process is improved relative to a corresponding uncrosslinked peptidomimetic macrocycle.
30. The method of any of claims 1-29, wherein an ability of the selected peptidomimetic macrocycle to penetrate cell membranes by an energy-independent process is improved relative to a corresponding uncrosslinked peptidomimetic macrocycle.
31. The method of claim 29, wherein the energy-dependent process is primary active transport, secondary transport, endocytosis, or a combination thereof.
32. The method of claim 31, wherein the energy-dependent process is active transport.
33. The method of claim 30, wherein the energy-independent process is passive diffusion, passive transport, facilitated diffusion, filtration, or a combination thereof.
34. The method of claim 33, wherein the energy-independent process is passive transport.

35. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to MDM2 with a K_D value of 1×10^{-7} M or less.
36. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to MDM2 or MDMX with a K_D value of 1×10^{-7} M or less.
37. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to MDMX with a K_D value of 1×10^{-7} M or less.
38. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to a PB1 peptide binding site of a PA protein with a K_D value of 1×10^{-7} M or less.
39. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to a PB2 peptide binding site of a PB1 protein with a K_D value of 1×10^{-7} M or less.
40. The method of any of claims 1-34, wherein the selected peptidomimetic macrocycle binds to viral polymerase, for example, a RNA-dependent RNA polymerase with a K_D value of 1×10^{-7} M or less.
41. The method of any of claim 38-40, wherein the selected peptidomimetic macrocycle inhibits an influenza RNA-dependent RNA polymerase.
42. The peptidomimetic macrocycle of claim 40 or 41, wherein the virus is influenza virus.
43. The peptidomimetic macrocycle of any one of claims 40-42, wherein, wherein the peptidomimetic macrocycle is capable of competing with the binding of a peptide of the sequence MDVNPTLLFLKVPAQ or MERIKELRNLM to the viral RNA-dependent RNA polymerase.
44. The method of any of claims 1-43, wherein the selected peptidomimetic macrocycle binds to MCL-1, BCL-X_L, BCL-2, or a combination thereof with a K_D value of 1×10^{-7} M or less.
45. The method of any of claims 1-43, wherein the selected peptidomimetic macrocycle binds to MCL-1 with a K_D value of 1×10^{-7} M or less.
46. The method of any of claims 1-43, wherein the selected peptidomimetic macrocycle binds to BCL-X_L with a K_D value of 1×10^{-7} M or less.
47. The method of any of claims 1-43, wherein the selected peptidomimetic macrocycle binds to BCL-2 with a K_D value of 1×10^{-7} M or less.
48. The method of any of claims 1-47, wherein the selected peptidomimetic macrocycle has an IC₅₀ value of 100 nM or less to a target protein.
49. The method of any of claims 1-48, wherein the selected peptidomimetic macrocycle has an EC₅₀ value of 100 μ M or less.
50. The method of any of claims 1-49, wherein the selected peptidomimetic macrocycle has an IC₅₀ value of 10 nM or less to a target protein.

51. The method of any of claims 1-50, wherein the selected peptidomimetic macrocycle has an EC₅₀ value of 10 μ M or less.
52. The method of any of claims 1-51, wherein the selected peptidomimetic macrocycle has an IC₅₀ value of 1 nM or less to a target protein.
53. The method of any of claims 1-51, wherein the selected peptidomimetic macrocycle has an EC₅₀ value of 1 μ M or less.
54. The method of any of claims 1-53, wherein the selected peptidomimetic macrocycle has a penetration efficiency value of 100 or less.
55. The method of any of claims 1-54, wherein the selected peptidomimetic macrocycle has a penetration efficiency value of 10 or less.
56. The method of any of claims 1-55, wherein the selected peptidomimetic macrocycle has a penetration efficiency value of 1 or less.
57. A peptidomimetic macrocycle selected according to the method of any of claims 1-56, wherein the peptidomimetic macrocycle penetrates cell membranes by an energy-dependent process and binds to a target protein with a K_D value of 1×10^{-7} M or less, wherein the target protein is not MDM2 or MDMX.
58. The peptidomimetic macrocycle of claim 58, wherein the energy-dependent process comprises primary active transport, secondary transport, or endocytosis.
59. The peptidomimetic macrocycle of claim 57 or 58, wherein the energy-dependent process comprises active transport.
60. A peptidomimetic macrocycle selected according to the method of any of claims 1-56, wherein the peptidomimetic macrocycle penetrates cell membranes by an energy-independent process and binds to a target protein with a K_D value of 1×10^{-7} M or less, wherein the target protein is not MDM2 or MDMX.
61. The peptidomimetic macrocycle of claim 60, wherein the energy-independent process comprises passive diffusion, passive transport, facilitated diffusion, or filtration.
62. The peptidomimetic macrocycle of claim 60 or 61, wherein the energy-independent process comprises passive transport.
63. A method of treating or controlling a disease or condition, comprising administering an effective amount of a peptidomimetic macrocycle selected according to the method of claims 1-56, to a subject in need thereof.
64. A peptidomimetic macrocycle selected according to the method of any of claims 1-56, comprising two non-natural amino acids connected by a macrocycle-forming crosslinker and an

amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence of Table 3a or 3b.

65. A peptidomimetic macrocycle comprising two non-natural amino acids connected by a macrocycle-forming crosslinker and an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence of Table 4a or 4b.
66. The peptidomimetic macrocycle of any of claims 57-62, wherein the target protein is an intracellular protein.
67. The peptidomimetic macrocycle of claim 66, wherein the target protein is a viral polymerase RNA-dependent RNA polymerase.
68. The peptidomimetic macrocycle of claim 66 or 67, wherein the target protein is MCL-1, BCL-X_L, BCL-2, or a combination thereof.

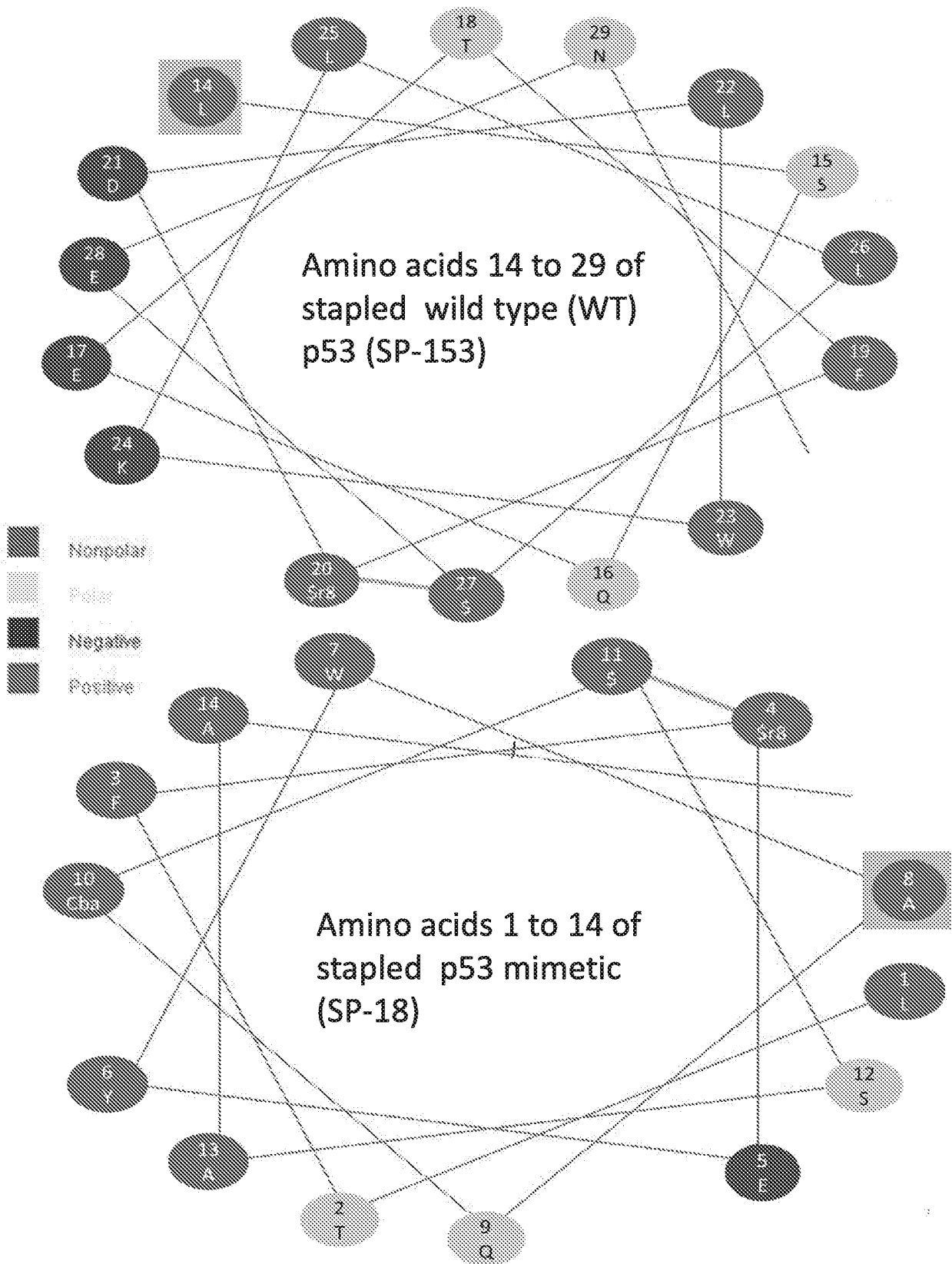


FIG. 1
1/9

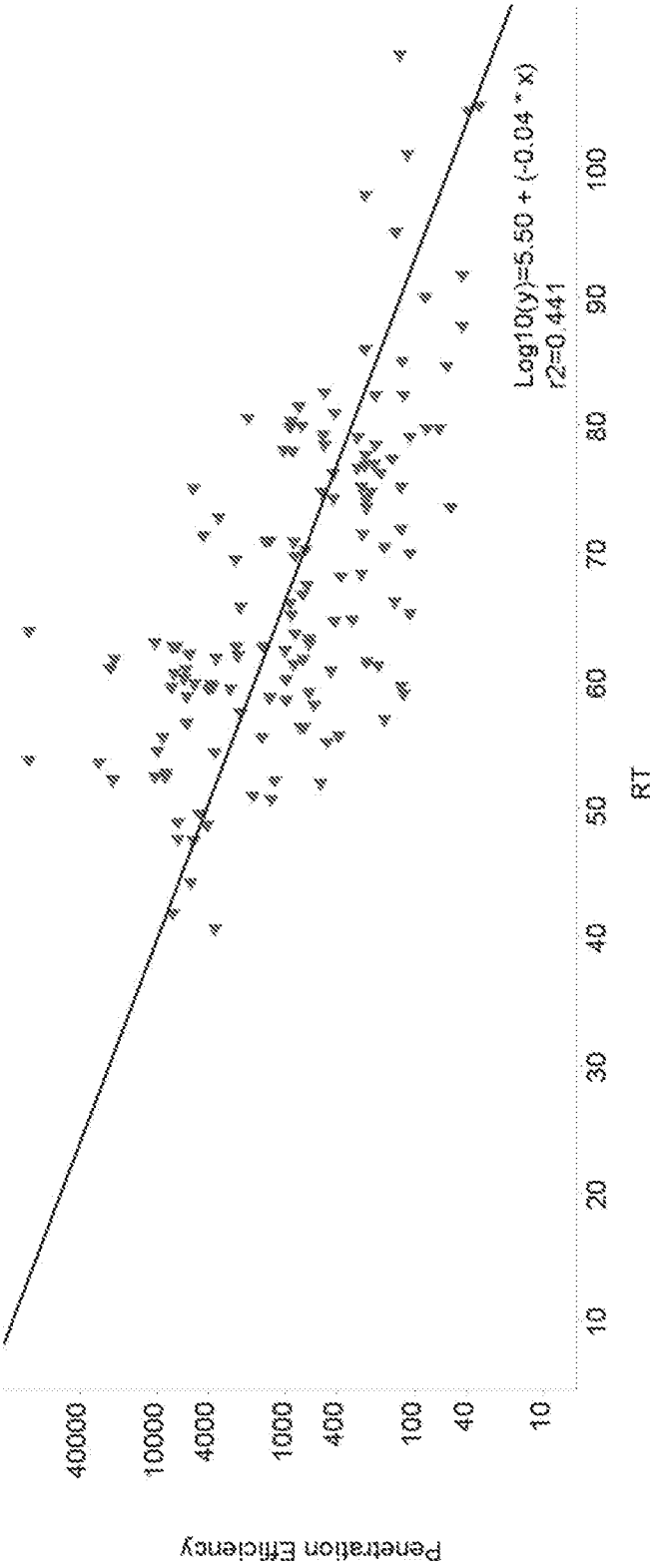


FIG. 2

SJSA-1 cell Viability (72h) 10%

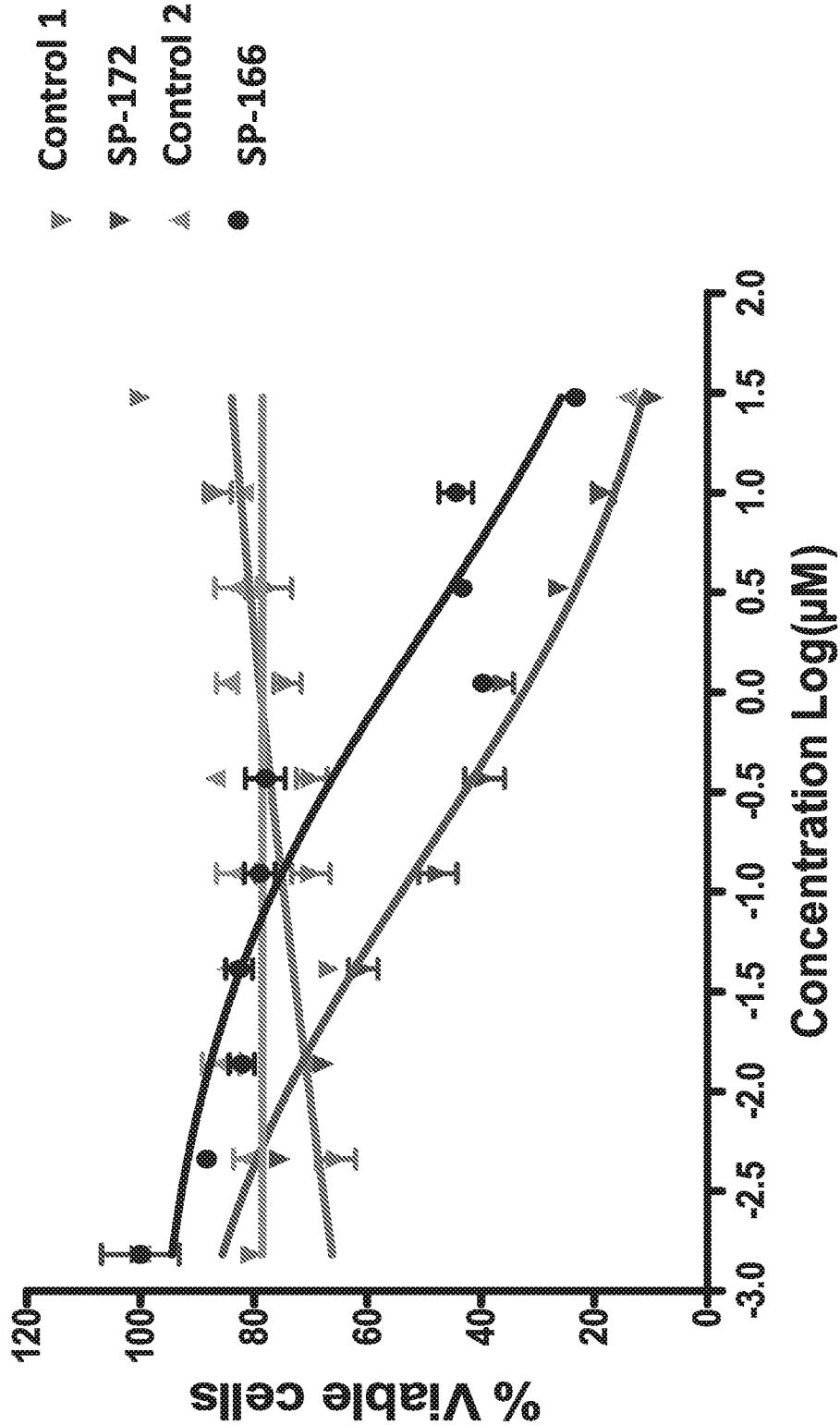


FIG. 3

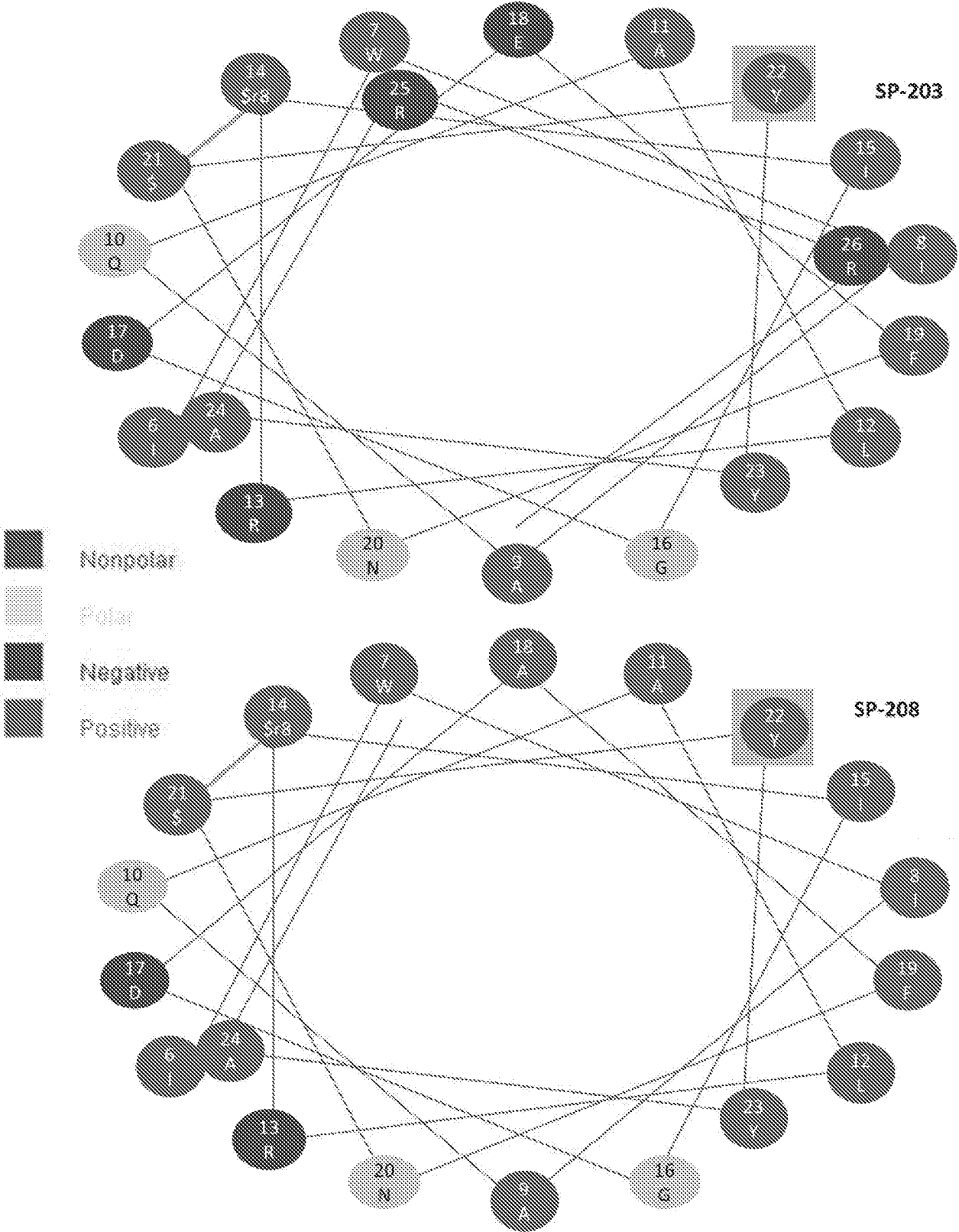


FIG. 4

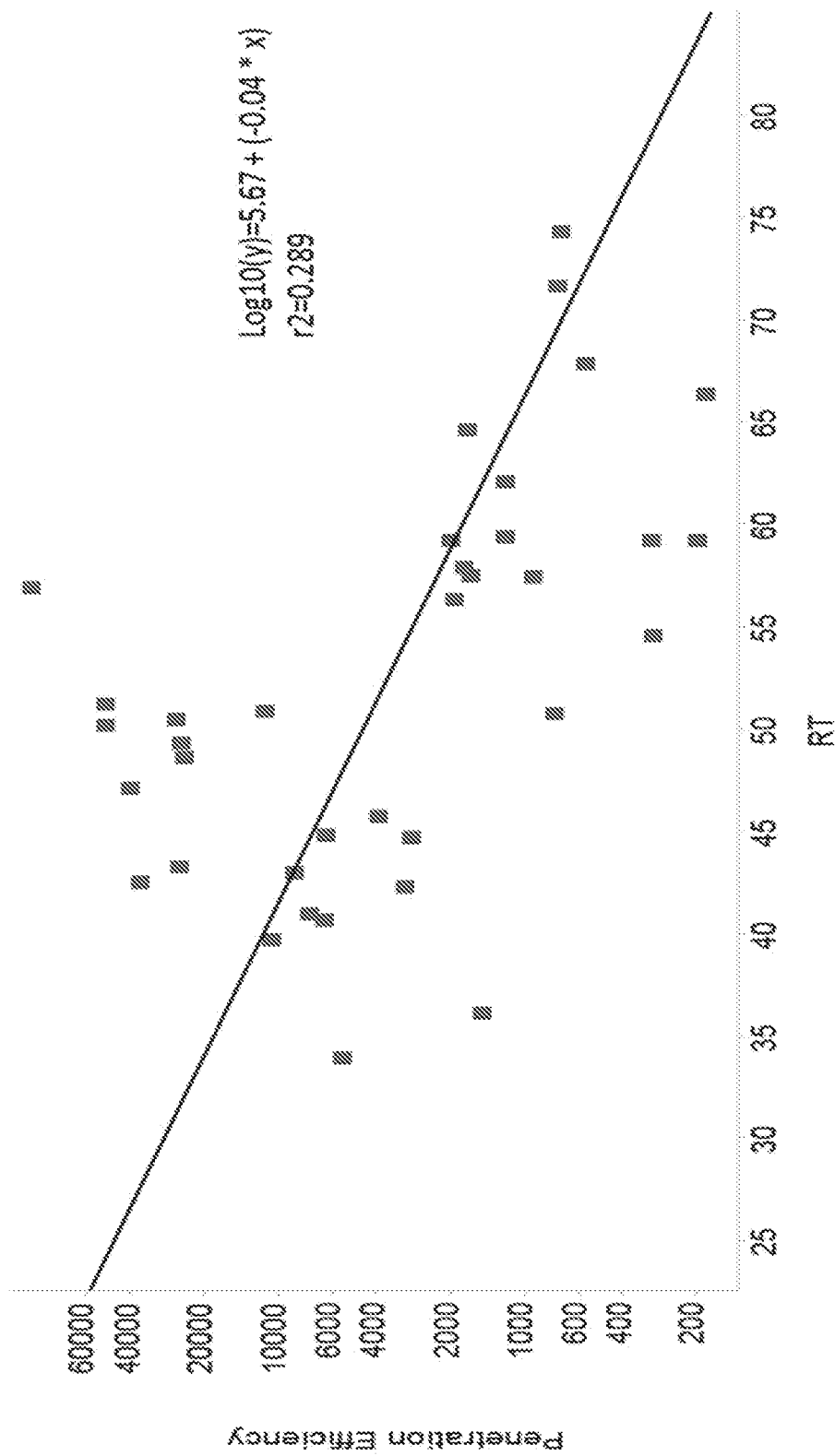


FIG. 5

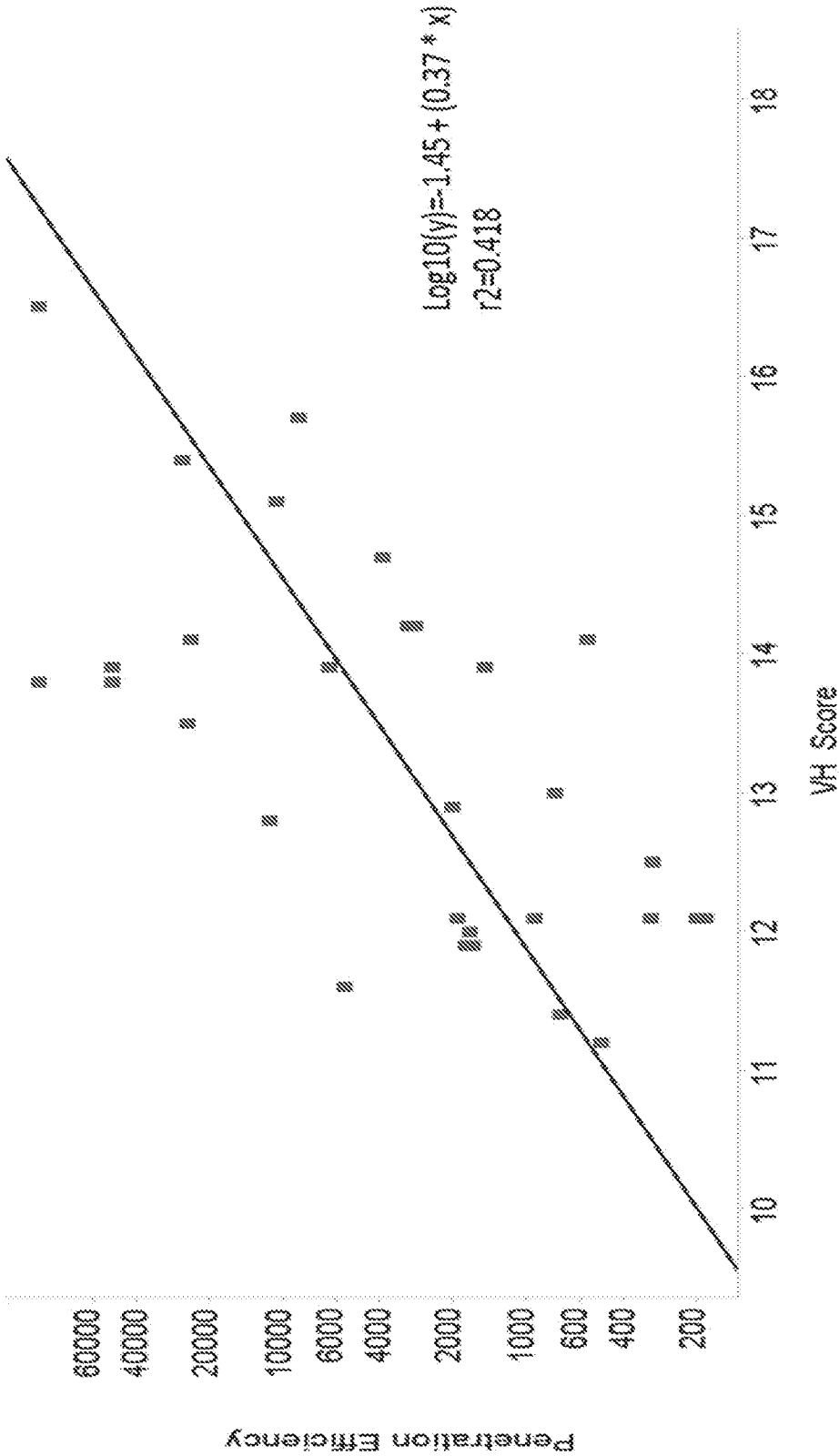


FIG. 6

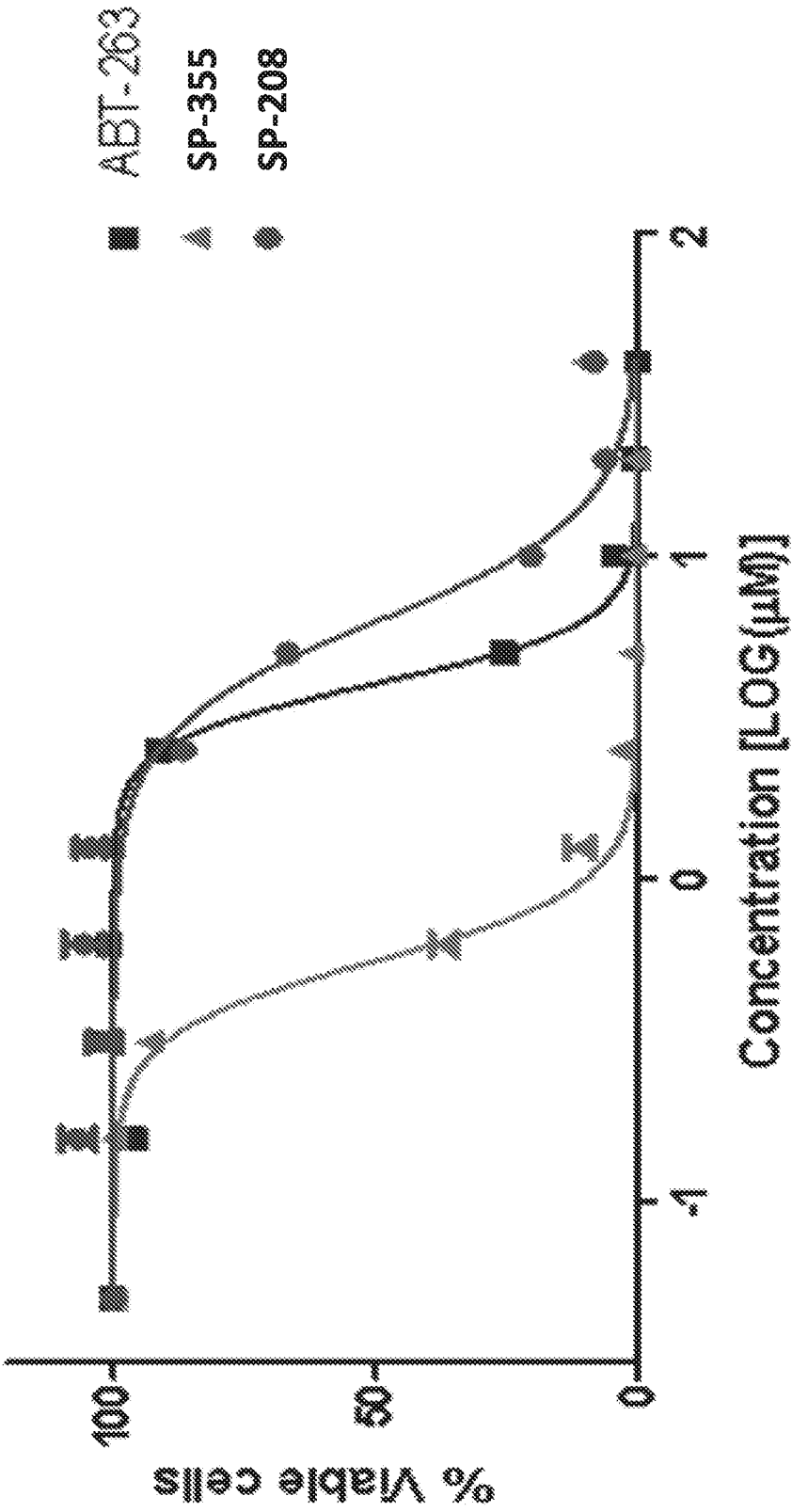


FIG. 7

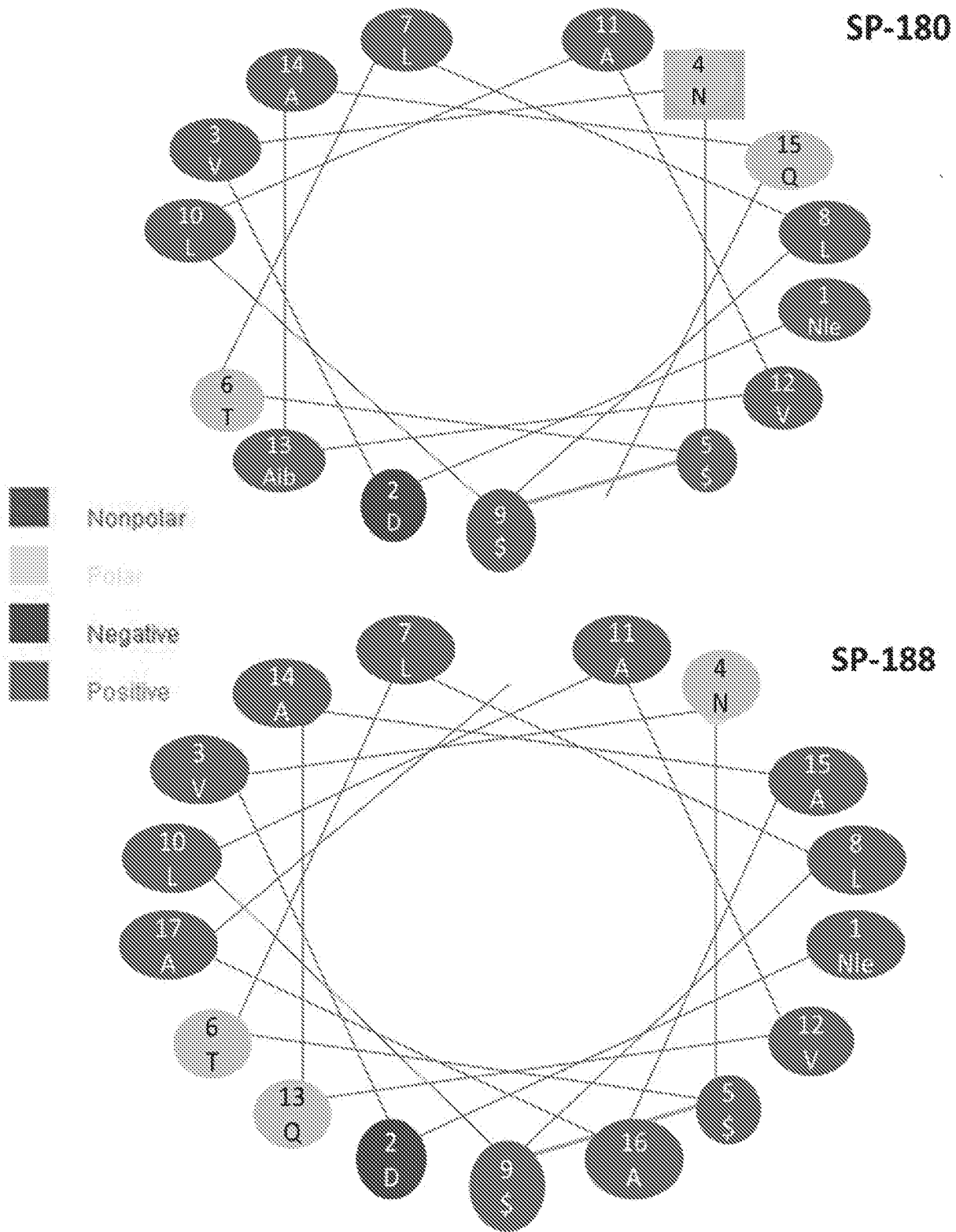


FIG. 8

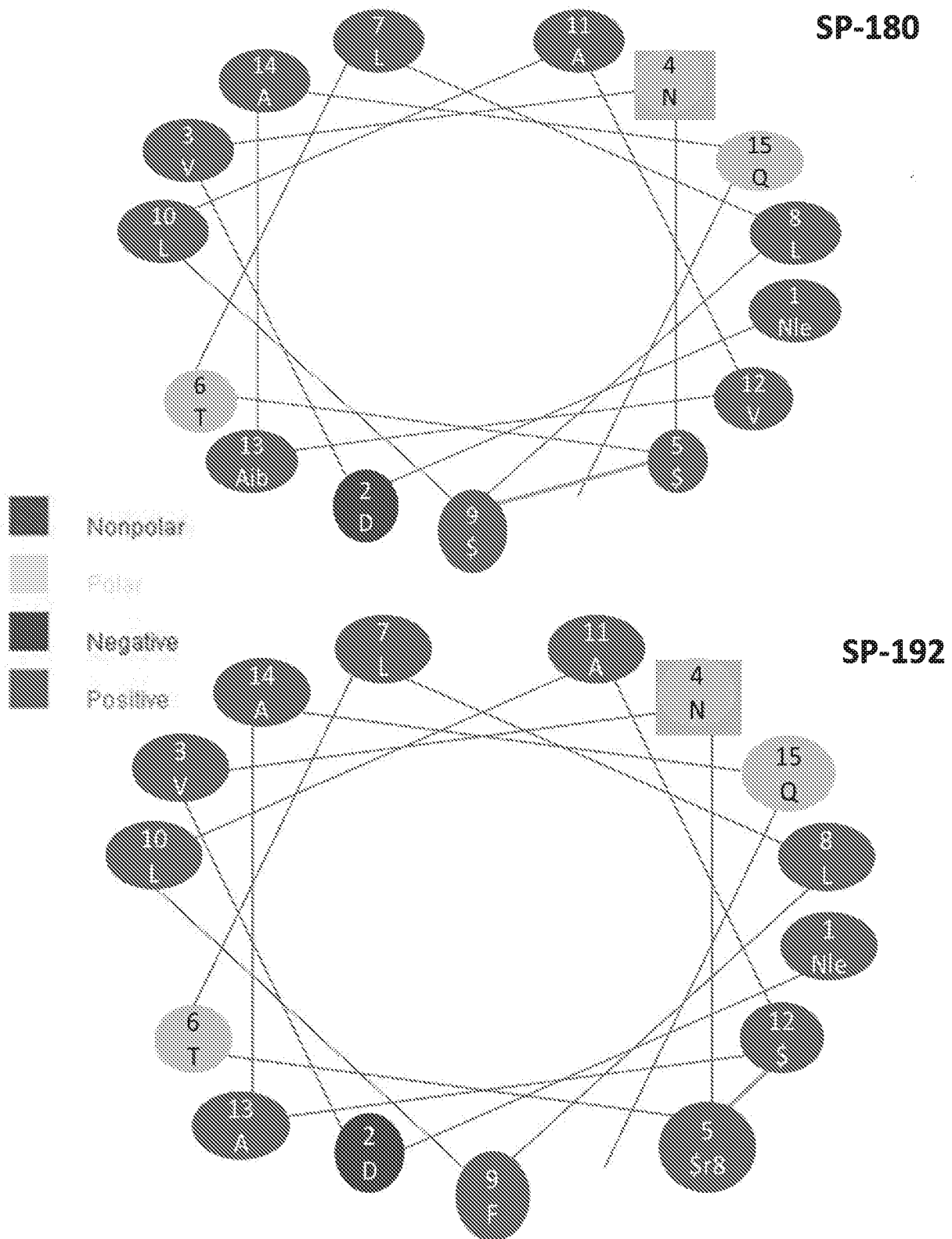


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/034752

A. CLASSIFICATION OF SUBJECT MATTER

C07K 7/50 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

\$PATEN (fulltext databases) via EPOQUE; CAPLUS, MEDLINE, BIOSIS, EMBASE via STN; Google: aileron therapeutics, influenza PB1, stapled, peptidomimetic, stability, permeability and like terms. Consensus sequence of Table 3b searched in STN Registry.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 11 August 2017		Date of mailing of the international search report 11 August 2017	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au		Authorised officer Felix White AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832565	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2017/034752
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/049355 A1 (AILERON THERAPEUTICS, INC.) 31 March 2016 Table 3, p. 138-9	64 (in part)
X	US 2012/0040889 A1 (NASH et al) 16 February 2012 Table 1a, Table 5, compounds SP-48, SP-43	64 (in part)
A	WUNDERLICH, K. et al "Identification of High-Affinity PB1-Derived Peptides with Enhanced Affinity to the PA Protein of Influenza A Virus Polymerase" Antimicrobial Agents and Chemotherapy, 2011, vol. 55, pps. 696-702 Whole document	64 (in part)
A	MATUSEVICH, O.V. et al "Synthesis and antiviral activity of PB1 component of the influenza A RNA polymerase peptide fragments" Antiviral Research, 2015, vol. 113, pps. 4-10 Whole document	64 (in part)
A	WALENSKY, L.D. et al "Hydrocarbon-Stapled Peptides: Principles, Practice, and Progress" Journal of Medicinal Chemistry, 2014, vol. 57, pps. 6275-6288 Whole document	64 (in part)
A	WO 2013/116829 A1 (THE TRUSTEES OF PRINCETON UNIVERSITY) 08 August 2013 Whole document	64 (in part)

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
64 (in part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box

Continuation of: Box III

The present application does not comply with the requirement for unity of invention, and includes a large number of inventive concepts, as set forth in the analysis below.

A preliminary communication (PCT/ISA/224) was issued by the ISA on 30 June 2017. The applicant responded on 25 July 2017 that they wished the peptides of Table 3b (claim 64 in part) to be searched and did not wish to pay additional fees.

Therefore this search and opinion has been limited to claim 64 in part with respect to the peptides listed in Table 3b.

Claims 1-63 and 66-68 are drawn to methods of selecting whether a crosslinked ("stapled") peptidomimetic peptide is a candidate for improved cell permeability, based on various combinations of two or more of the twenty-eight (28) criteria in item b) of claim 1, and peptides selected by said combinations.

These criteria are distinct from each other and do not appear to share any common features a priori.

Net charge has already been disclosed to be a relevant factor for influencing cellular uptake of stapled peptides (see D5: Walensky & Bird, J Med Chem 57, 6275 (Feb 2014) at Fig 5 and p. 6283 last paragraph). Therefore, these criteria also appear to lack unity a posteriori.

There are four hundred and six (406) different combinations of pairs of these criteria and **each of these pairs could be considered to be a distinct inventive concept.**

Claims 64 and 65 are directed to structurally defined stapled peptides. Claim 64 (the peptides of Tables 3a and 3b) are peptides based on the PA-binding helix of pB1, and claim 65 (the peptides of Tables 4a and 4b) are peptides based on the MCL-1 binding helix of BIM.

However, to the extent that these peptides could be said to share a common activity and a common backbone structure, those backbones are not novel (D2: US2012/0040889 discloses stapled peptides from the same pB1 helix and D6: WO2013/116829 discloses stapled peptides from the same BIM helix).

Therefore, formally speaking, the peptides recited in tables 3-4 do not satisfy the definition of a Markush grouping and **each peptide would be considered a separate invention.**

However this Authority considered that for the fee already paid, all of the peptides of Table 3b could be searched.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2017/034752	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2016/049355 A1	31 March 2016	WO 2016049355 A1	31 Mar 2016
		AU 2015320545 A1	06 Apr 2017
		CA 2961029 A1	31 Mar 2016
		EP 3197477 A1	02 Aug 2017
		KR 20170063798 A	08 Jun 2017
		SG 11201702175Y A	27 Apr 2017
		US 2016101145 A1	14 Apr 2016
US 2012/0040889 A1	16 February 2012	US 2012040889 A1	16 Feb 2012
		US 9175047 B2	03 Nov 2015
		AU 2010204648 A1	22 Jul 2010
		AU 2010204648 B2	01 Sep 2016
		BR PI1006139 A2	30 May 2017
		CA 2744088 A1	22 Jul 2010
		CN 102256615 A	23 Nov 2011
		EP 2376100 A2	19 Oct 2011
		JP 2012515172 A	05 Jul 2012
		US 2016068573 A1	10 Mar 2016
		US 2016250278 A1	01 Sep 2016
		US 2016251399 A1	01 Sep 2016
		WO 2010083347 A2	22 Jul 2010
WO 2013/116829 A1	08 August 2013	WO 2013116829 A1	08 Aug 2013
		US 2015045310 A1	12 Feb 2015
		US 9464125 B2	11 Oct 2016
		US 2016376336 A1	29 Dec 2016
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			