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(54) **Title**: PEPTIDOMIMETIC MACROCYCLES

(57) **Abstract**:

PEPTIDOMIMETIC MACROCYCLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial Nos. 61/373,701 filed August 13, 2010, 61/373,638 filed August 13, 2010, and 61/374,163 filed August 16, 2010, which are hereby incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] The human transcription factor protein p53 induces cell cycle arrest and apoptosis in response to DNA damage and cellular stress, and thereby plays a critical role in protecting cells from malignant transformation. The E3 ubiquitin ligase HDM2 negatively regulates p53 function through a direct binding interaction that neutralizes the p53 transactivation activity, leads to export from the nucleus of p53 protein, and targets p53 for degradation via the ubiquitylation-proteasomal pathway. Loss of p53 activity, either by deletion, mutation, or HDM2 overexpression, is the most common defect in human cancers. Tumors that express wild type p53 are vulnerable to pharmacologic agents that stabilize or increase the concentration of active p53. In this context, inhibition of the activities of HDM2 has emerged as a validated approach to restore p53 activity and resensitize cancer cells to apoptosis *in vitro* and *in vivo*. HDMX (HDM4) has more recently been identified as a similar negative regulator of p53, and studies have revealed significant structural homology between the p53 binding interfaces of HDM2 and HDMX.

[0003] The p53-HDM2 and p53-HDMX protein-protein interactions are mediated by the same 15-residue alpha-helical transactivation domain of p53, which inserts into hydrophobic clefts on the surface of HDM2 and HDMX. Three residues within this domain of p53 (F19, W23, and L26) are essential for binding to HDM2 and HDMX. The present invention provides p53-based peptidomimetic macrocycles that modulate the activities of p53 by inhibiting the interactions between p53 and HDM2, p53 and HDMX, or p53 and both HDM2 and HDMX proteins, and that may be used for treating diseases including but not limited to cancer and other hyperproliferative diseases.

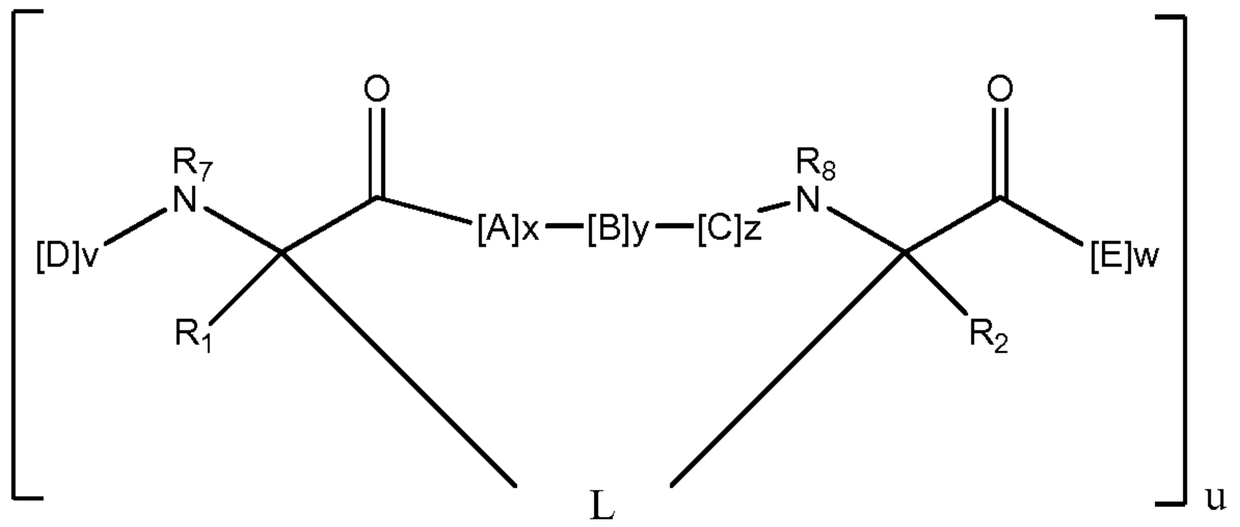
SUMMARY OF THE INVENTION

[0004] Described below are stably cross-linked peptides related to a portion of human 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 alpha-helical secondary structure of a portion of p53 that is thought to be important for binding of p53 to HDM2 and for binding of p53 to HDMX. 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 HDM2 and/or of p53 to HDMX, 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 HDM2 or HDMX. The p53 peptidomimetic macrocycles may 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 immunedeficiencies. In some instances, the p53 peptidomimetic macrocycles bind to HDM2 (e.g., GenBank® Accession No.: 228952; GI:228952) and/or HDMX (also referred to as HDM4; GenBank® AccessionNo.: 88702791; GI:88702791).

[0005] In one aspect, the present invention provides a peptidomimetic macrocycle comprising an amino acid sequence which is at least about 60%, 80%, 90%, or 95% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4. Alternatively, an amino acid sequence of said peptidomimetic macrocycle is chosen from the group consisting of the amino acid sequences in Table 1. Alternatively, an amino acid sequence of said peptidomimetic macrocycle is chosen as above, and further wherein the macrocycle does not include a thioether or a triazole. In some embodiments, the peptidomimetic macrocycle comprises a helix, such as an α-helix. In other embodiments, the peptidomimetic macrocycle comprises an α,α-disubstituted amino acid. A peptidomimetic macrocycle of the invention may comprise a crosslinker linking the α-positions of at least two amino acids. At least one of said two amino acids may be an α,α-disubstituted amino acid.

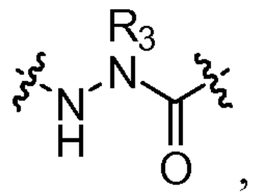
[0006] In some embodiments, the peptidomimetic macrocycle has the formula:



Formula I

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid, and the terminal D and E independently optionally include a capping group;

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

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

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

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

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

v and w are independently integers from 1-1000;

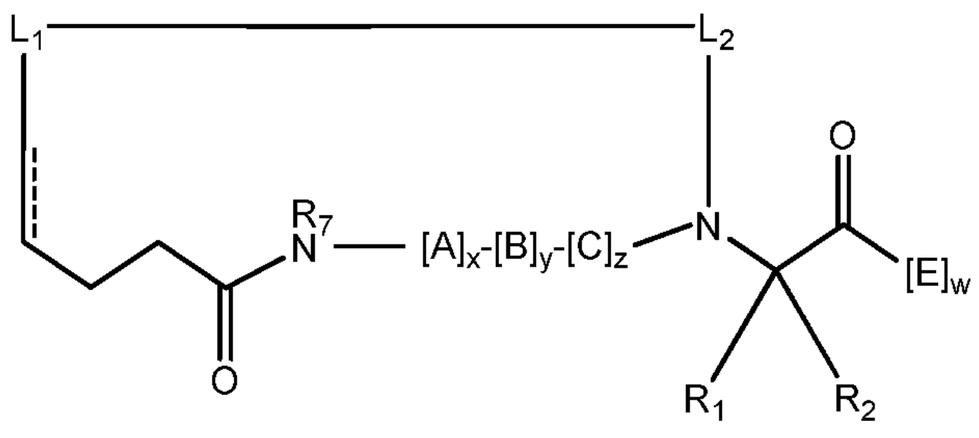
u is an integer from 1-10;

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

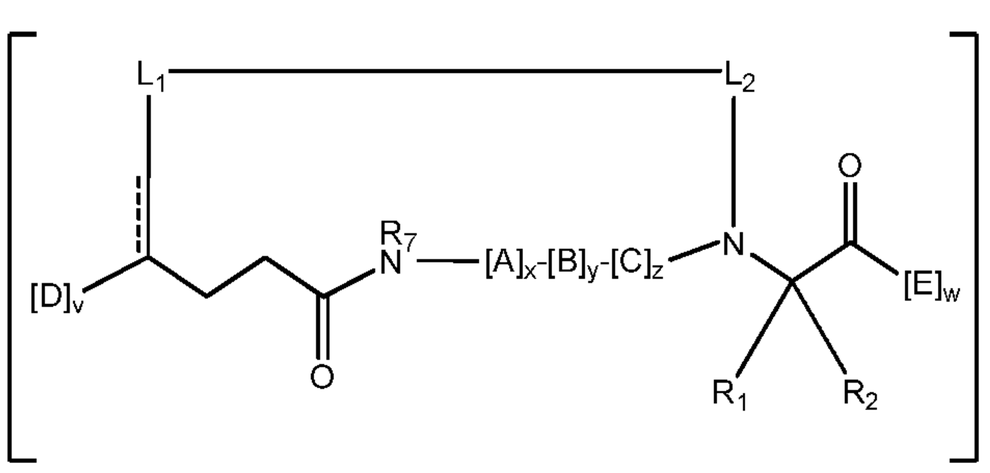
n is an integer from 1-5.

[0007] In various embodiments, the peptidomimetic macrocycle includes L_1 and L_2 wherein L_1 and L_2 either alone or in combination do not include a thioether or a triazole.

[0008] In other embodiments, the peptidomimetic macrocycle may comprise a crosslinker linking a backbone amino group of a first amino acid to a second amino acid within the peptidomimetic macrocycle. For example, the invention provides peptidomimetic macrocycles of the formula (IV) or (IVa):



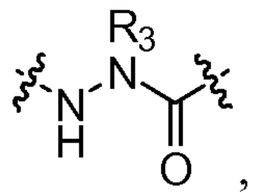
Formula (IV)



Formula (IVa)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid, and the terminal D and E independently optionally include a capping group;

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

R_1 and R_2 are 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;

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

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

n is an integer from 1-5.

[0009] Additionally, the invention provides a method of treating cancer in a subject comprising administering to the subject a peptidomimetic macrocycle of the invention. Also provided is a method of modulating the activity of p53 or HDM2 or HDMX in a subject comprising administering to the subject a peptidomimetic macrocycle of the invention, or a method of antagonizing the interaction between p53 and HDM2 and/or HDMX proteins in a subject comprising administering to the subject such a peptidomimetic macrocycle.

INCORPORATION BY REFERENCE

[0010] 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

[0011] 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:

[0012] **FIGURE 1** describes the synthesis of Fmoc-Me-6-Chloro-Tryptophan & Fmoc-6-Chloro-Tryptophan.

[0013] **FIGURE 2** shows an LC-MS trace of Me-6-Chloro-(Boc)Tryptophan-Ni-S-BPB.

[0014] **FIGURE 3** shows a 1H-NMR spectrum of Me-6-Chloro-(Boc)Tryptophan-Ni-S-BPB.

[0015] **FIGURE 4** shows an LC-MS trace of Fmoc-Me-6-Chloro-(Boc)Tryptophan.

[0016] **FIGURE 5** shows a 1H-NMR spectrum of Fmoc-Me-6-Chloro-(Boc)Tryptophan.

[0017] **FIGURES 6a-f** describe the results of a cell viability assay, a competition ELISA assay, GRIP assay, Kd data, p21 activation assay, fluorescence polarization competition binding and circular helicity data for exemplary peptidomimetic macrocycles of the invention.

[0018] **FIGURES 7A-D** provide data from a variety of macrocycles.

[0019] **FIGURES 8A-B** provide data from a variety of macrocycles.

DETAILED DESCRIPTION OF THE INVENTION

[0020] 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.

[0021] 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. 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.

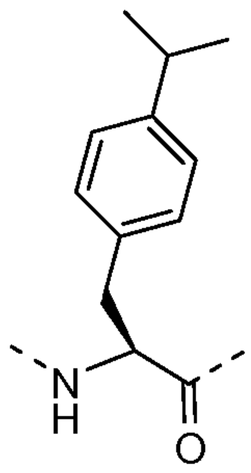
[0022] As used herein, the term "stability" refers to the maintenance of a defined secondary structure in solution by a peptidomimetic macrocycle of the invention as measured by circular dichroism, NMR or another biophysical measure, or resistance to proteolytic degradation *in vitro* or *in vivo*. Non-limiting examples of secondary structures contemplated in this invention are α-helices, β-turns, and β-pleated sheets.

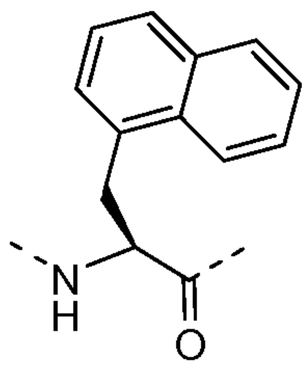
[0023] As used herein, the term "helical stability" refers to the maintenance of α helical structure by a peptidomimetic macrocycle of the invention as measured by circular dichroism or NMR. For example, in some embodiments, the peptidomimetic macrocycles of the invention exhibit at least a 1.25, 1.5, 1.75 or 2-fold increase in α-helicity as determined by circular dichroism compared to a corresponding uncrosslinked macrocycle.

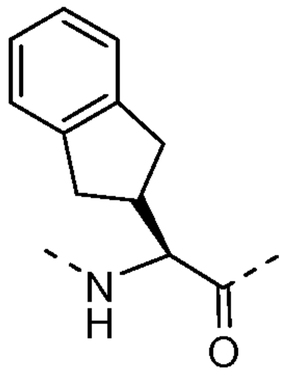
[0024] The term "α-amino acid" or simply "amino acid" refers to a molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the α-carbon. Suitable amino acids include, without limitation, both the D-and L-isomers of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. Unless the context specifically indicates otherwise, the term amino acid, as used herein, is intended to include amino acid analogs.

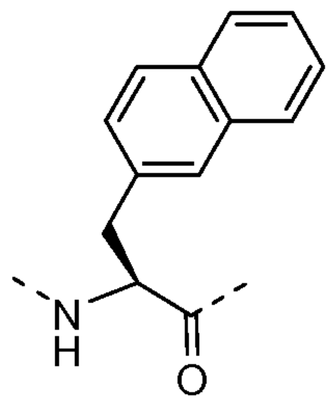
[0025] The term "naturally occurring amino acid" refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

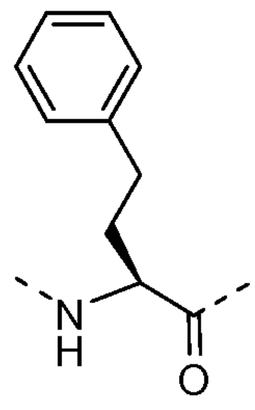
[0026] The term "amino acid analog" or "non-natural amino acid" refers to a molecule which is structurally similar to an amino acid and which can be substituted for an amino acid in the formation of a peptidomimetic macrocycle. Amino acid analogs include, without limitation, compounds which are structurally identical to an amino acid, as defined herein, except for the inclusion of one or more additional methylene groups between the amino and carboxyl group (*e.g.*, α-amino β-carboxy acids), or for the substitution of the amino or carboxy group 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). Non-natural amino acids include structures according to the following:


F4tBu


1-Naphtalanine (1Nal)


Indanyl glycine (Igl)


2-Naphtalanine (2Nal)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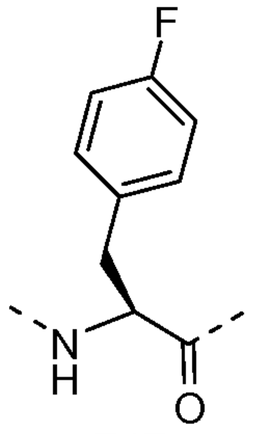
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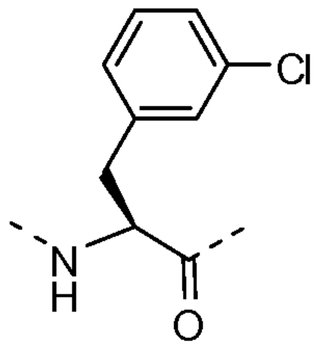
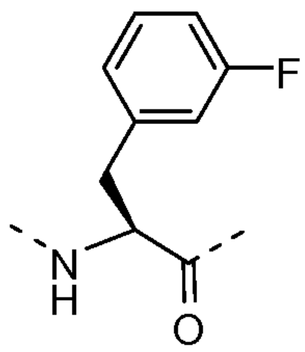

F3Cl=3cf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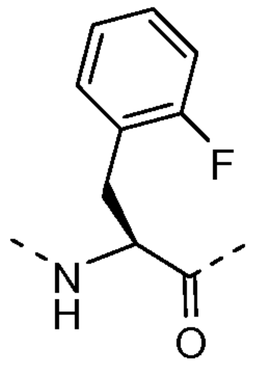
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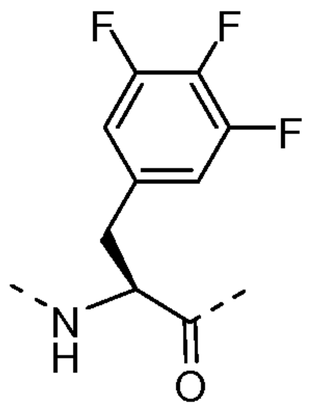
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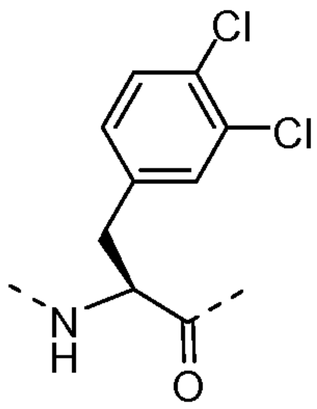
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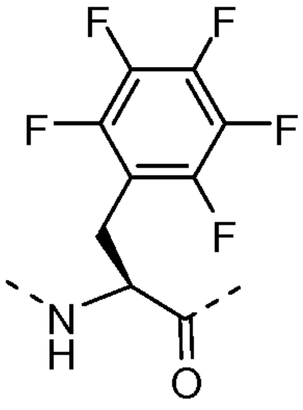
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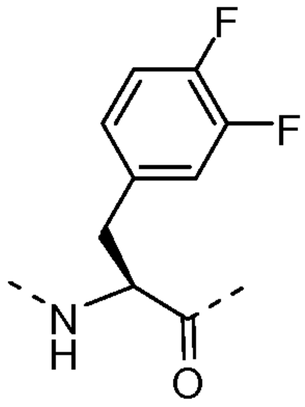
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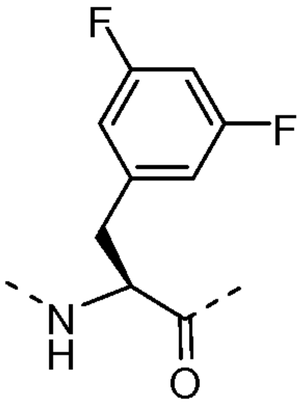
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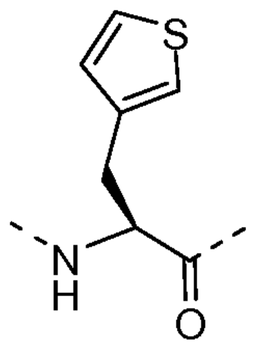
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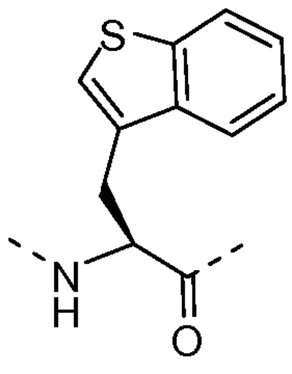
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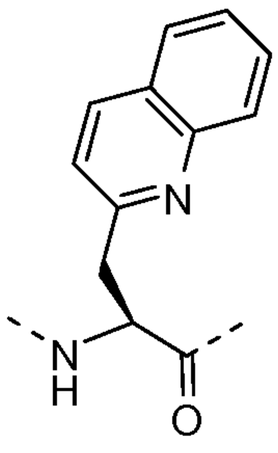
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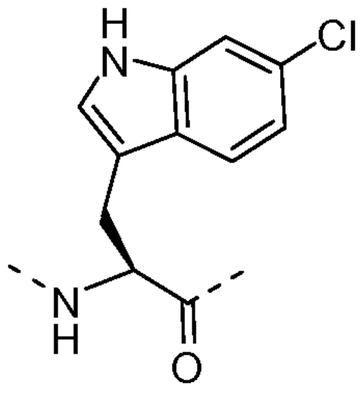
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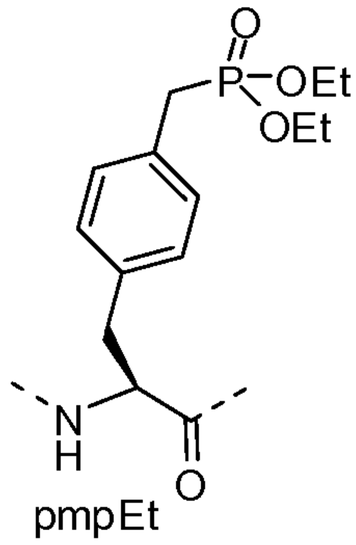
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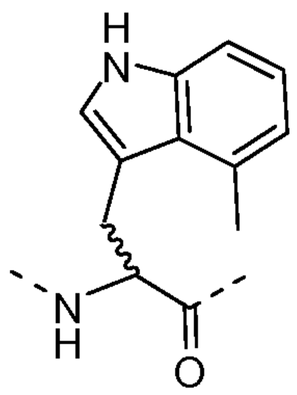

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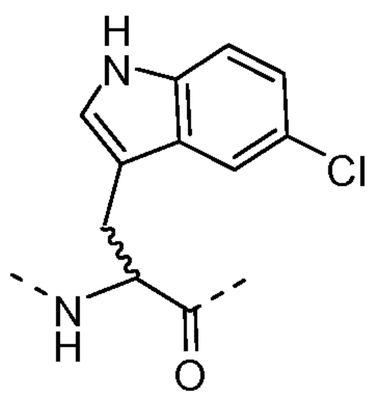

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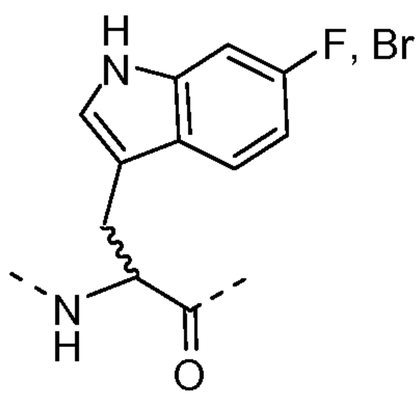
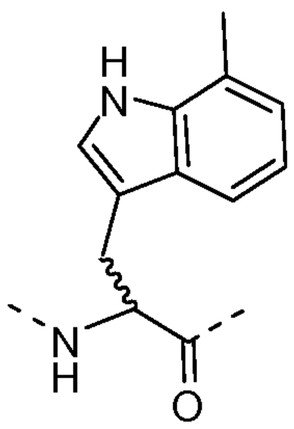


dl6fW & dl6brW



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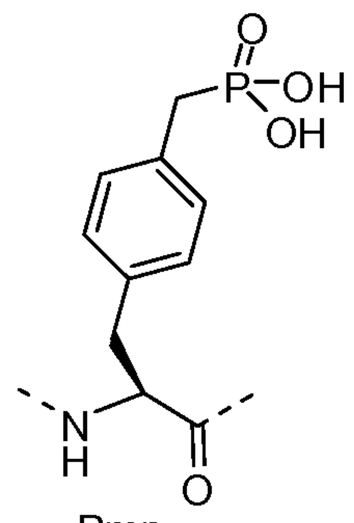

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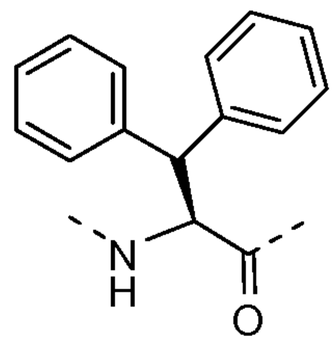
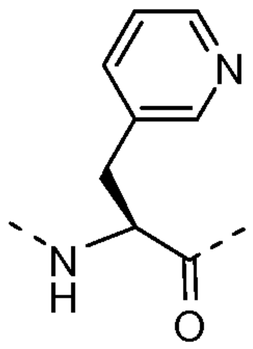
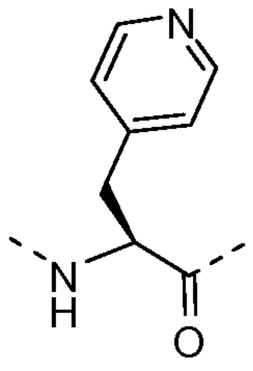


3,3-diphenyl-alanine
(Dip)



3-pyridyl-alanine
(3Pal)



4-pyridyl-alanine
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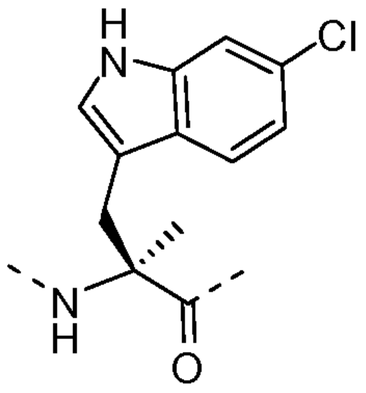
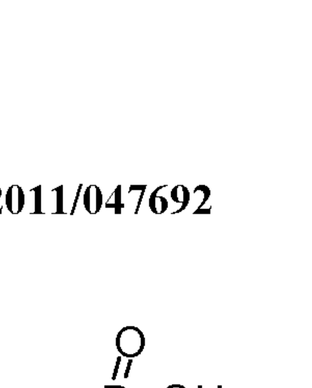
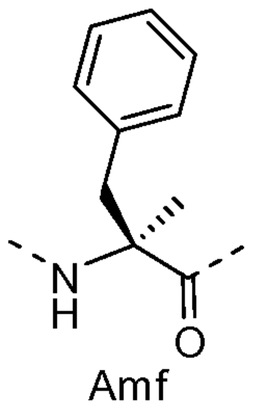


Me6clW



homotyrosine
(hY)



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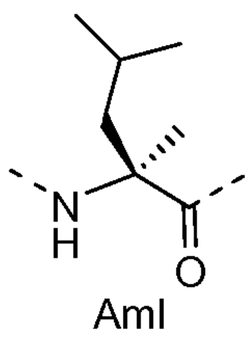

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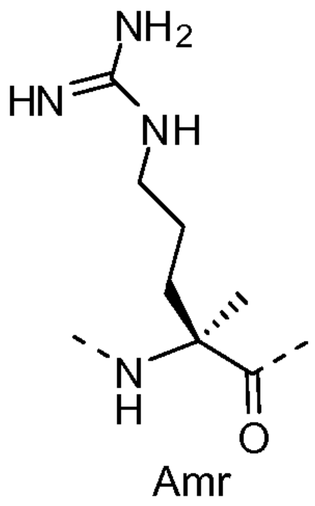

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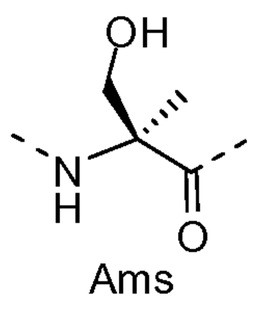
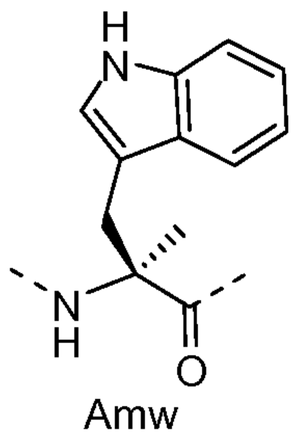


Ams



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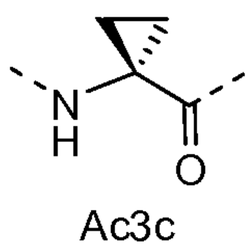

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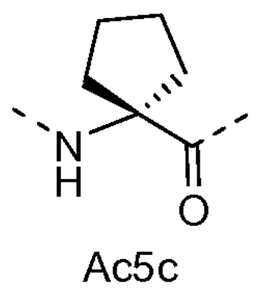

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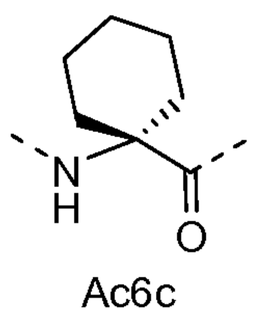

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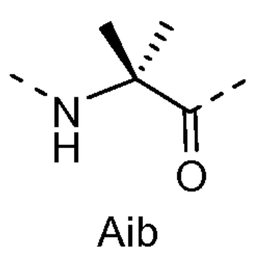

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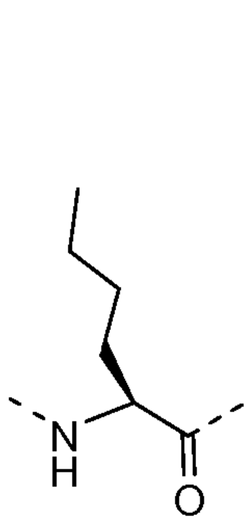
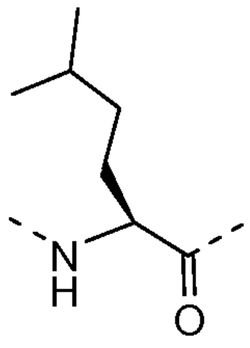
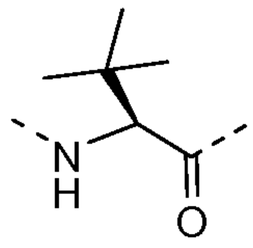


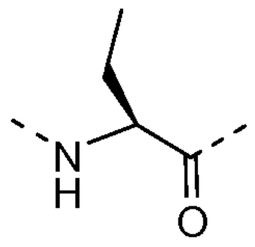
Norleucine (Nle)



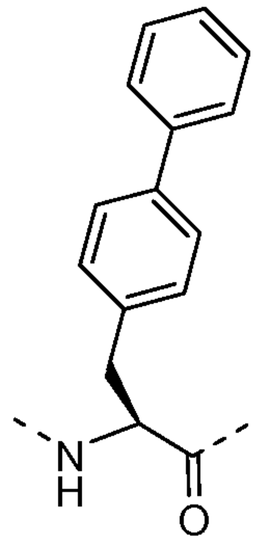
Homoleucine (hL)



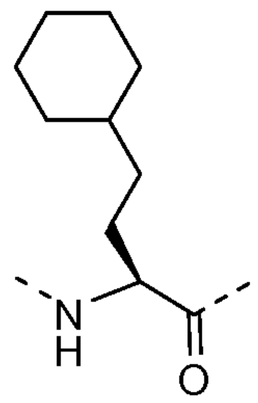
tert-Butyl glycine (Tle)



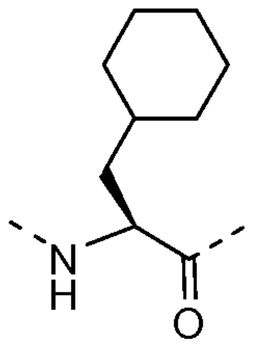
Abu



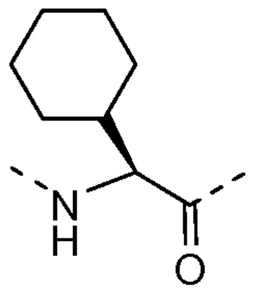
Bip



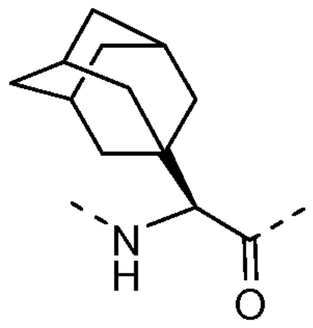
homocyclohexyl alanine (hCha)



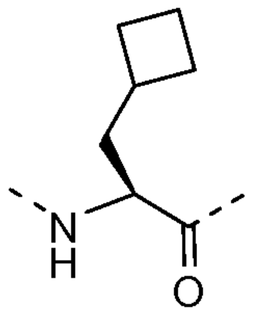
cyclohexyl alanine (Cha)



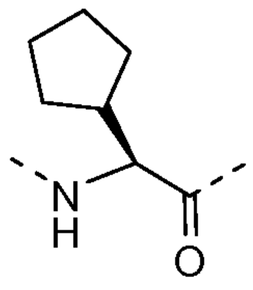
cyclohexyl glycine (Chg)



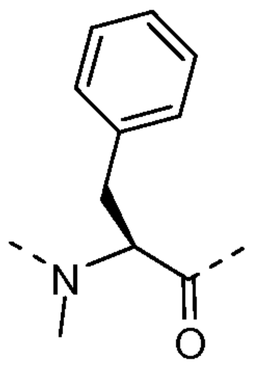
Adamantyl glycine (Adm)



cyclobutyl alanine (Cba)



cyclopentyl glycine (Cpg)



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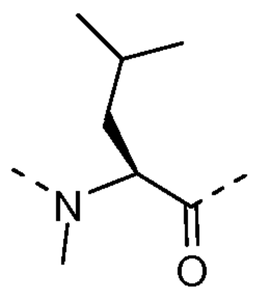

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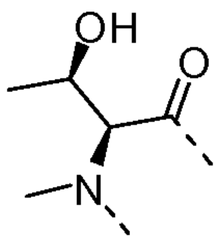

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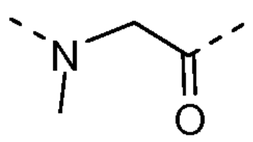

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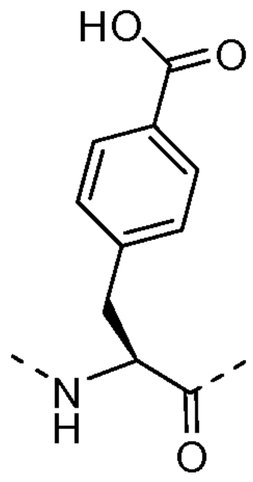
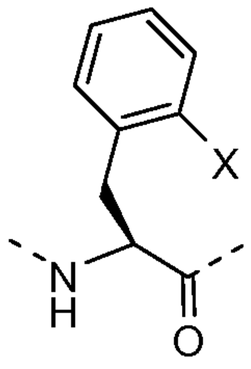
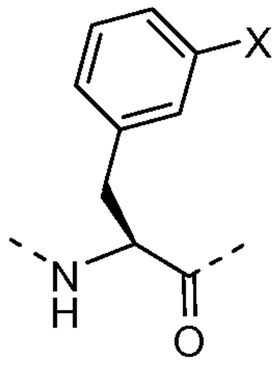


F4cooh



F2X
X=Cl, Br, CF3, CN, Me, NO2



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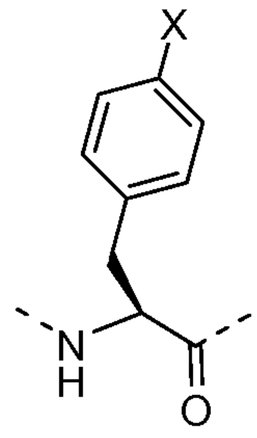
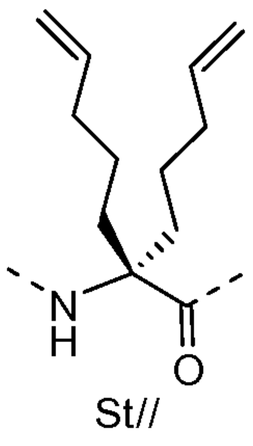


F4X
X=Cl, Br, CF3, CN, Me, NO2, I



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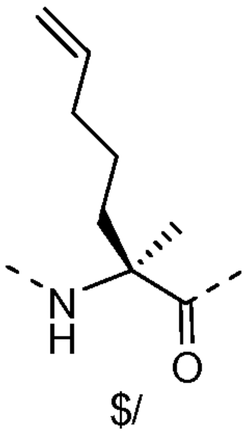

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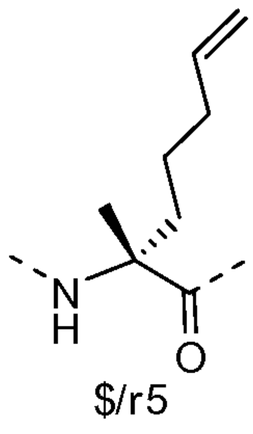

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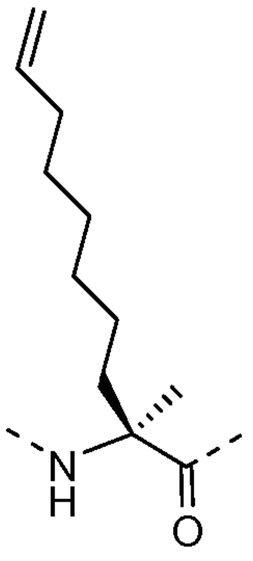
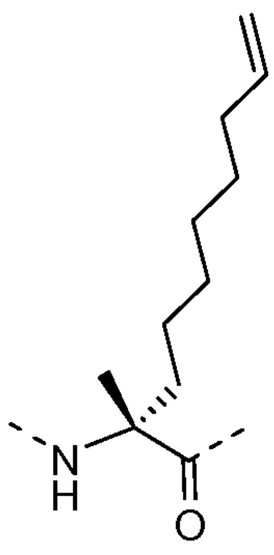


$/s8

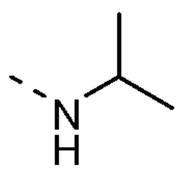


$/r8

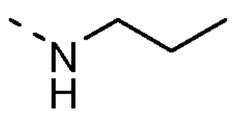
[0027] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of a polypeptide without abolishing or substantially altering its essential biological or biochemical activity (*e.g.*, receptor binding or activation). An "essential" amino acid residue is a residue 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.

[0028] 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), beta-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, for example, is preferably 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).

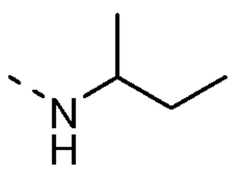
[0029] The term "capping group" refers to the chemical moiety occurring at either the carboxy or amino terminus of the polypeptide chain of the subject peptidomimetic macrocycle. The capping group of a carboxy terminus includes an unmodified carboxylic acid (ie –COOH) or a carboxylic acid with a substituent. For example, the carboxy terminus may be substituted with an amino group to yield a carboxamide at the C-terminus. Various substituents include but are not limited to primary and secondary amines, including pegylated secondary amines. Representative secondary amine capping groups for the C-terminus include:



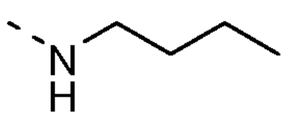
isopropylamide
(-NHPr)



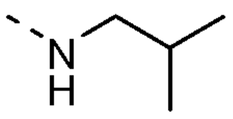
propylamide
(-NHnPr)



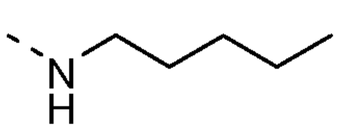
sec-butylamide
(-NHsBu)



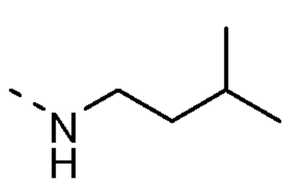
butylamide
(-NHnBu)



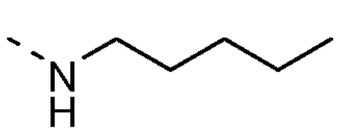
isobutylamide
(-NHiBu)



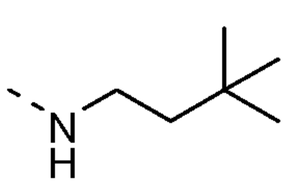
amylamide
(-NHAm)



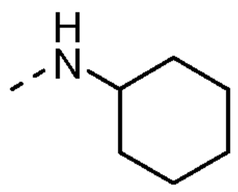
isoamylamide
(-NHiAm)



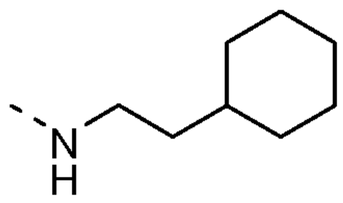
hexylamide
(-NHHex)



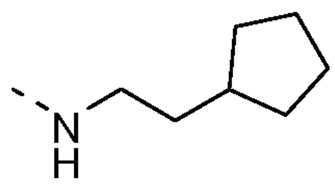
3,3-dimethylbutylamide
(-NHnBu3,3Me)



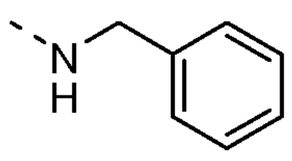
cyclohexylamide
(-NHChx)



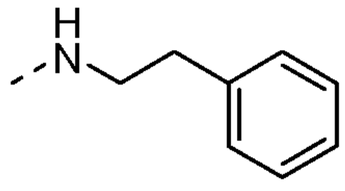
2-cyclohexylethylamide
(-NHnEt2Ch)



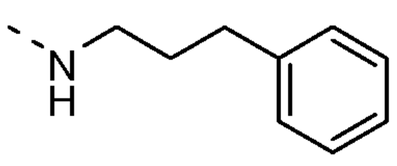
2-cyclopentylethylamide
(-NHnEt2Cp)



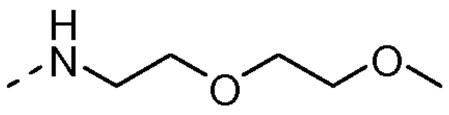
benzylamide
(-NHBn)



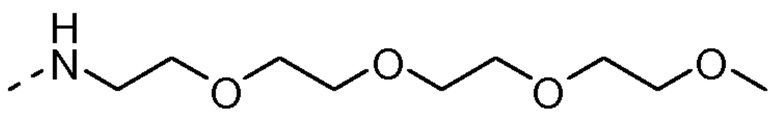
phenetylamide
(-NHPe)



3-phenyl-1-propylamide
(-NHnPr3Ph)

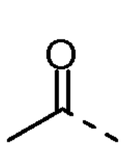


n-diPeg2-amide
(-NHmdPeg2)

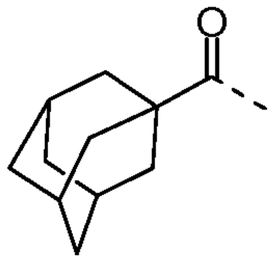


n-diPeg4-amide
(-NHmdPeg4)

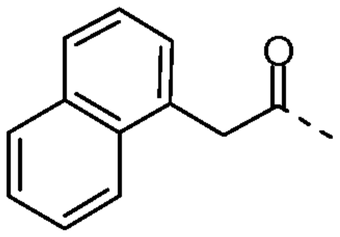
The capping group of an amino terminus includes an unmodified amine (ie $-NH_2$) or an amine with a substituent. For example, the amino terminus may be substituted with an acyl group to yield a carboxamide at the N-terminus. Various substituents include but are not limited to substituted acyl groups, including C_1-C_6 carbonyls, C_7-C_{30} carbonyls, and pegylated carbamates. Representative capping groups for the N-terminus include:



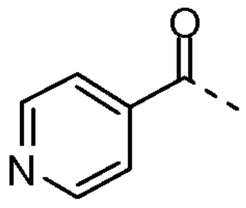
Ac-



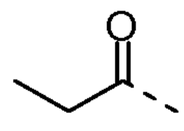
Adamantylcarbonyl
(Admac)



1-Naphthyl
(Napac)

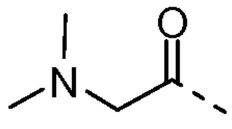


Isonicotinyl
(Isoac)

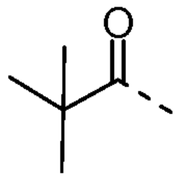


Pr-

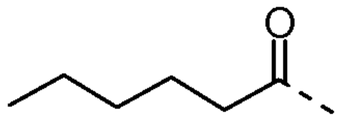
H-
(uncapped)



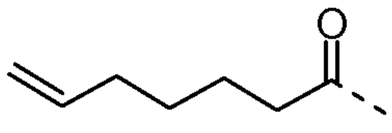
N,N-Dimethylaminoacetyl
(Dmaac)



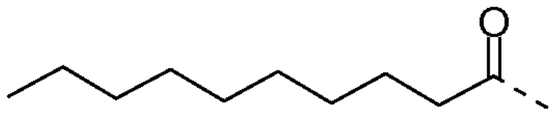
Trimethylacetyl
(Tmac)



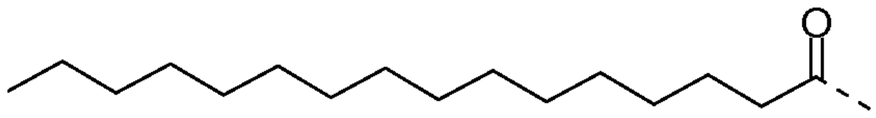
Hexanoyl
(Hexac)



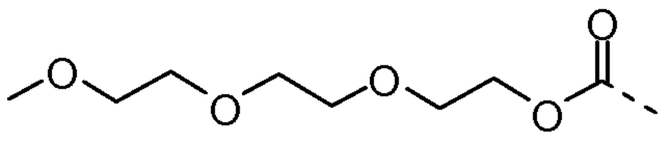
Hep/



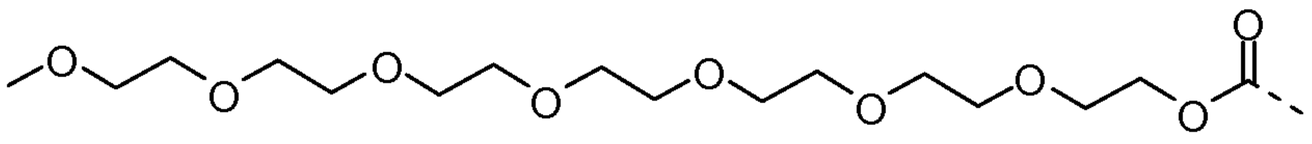
Decanoyl
(Decac)



Palmityl
(Pam)



mdPEG3



mdPEG7

[0030] 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 or fluoro substituents or methyl side chains do not participate in forming the macrocycle.

[0031] The symbol " " when used as part of a molecular structure refers to a single bond or a *trans* or *cis* double bond.

[0032] The term "amino acid side chain" refers to a moiety attached to the α-carbon 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, for example, 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).

[0033] 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.

[0034] 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).

[0035] The term "macrocyclization reagent" or "macrocycle-forming reagent" as used herein refers to any reagent which may be used to prepare a peptidomimetic macrocycle of the invention by mediating the reaction between two reactive groups. Reactive groups may be, for example, an azide and alkyne, in which case macrocyclization reagents include, without limitation, Cu reagents such as reagents which provide a reactive Cu(I) species, such as CuBr, CuI or CuOTf, as well as Cu(II) salts such as $Cu(CO_2CH_3)_2$, $CuSO_4$, and $CuCl_2$ that can be converted in situ to an active Cu(I) reagent by the addition of a reducing agent such as ascorbic acid or sodium ascorbate. Macrocyclization reagents may additionally include, for example, Ru reagents known in the art such as $Cp^*RuCl(PPh_3)_2$, $[Cp^*RuCl]_4$ or other Ru reagents which may provide a reactive Ru(II) species. In other cases, the reactive groups are terminal olefins. In such embodiments, the macrocyclization reagents or macrocycle-forming reagents 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. Additional catalysts are disclosed in Grubbs et al., "Ring Closing Metathesis and Related Processes in Organic Synthesis" Acc. Chem. Res. 1995, 28, 446-452, and U.S. Pat. No. 5,811,515. In yet other cases, the reactive groups are thiol groups. In such embodiments, the

macrocyclization reagent is, for example, a linker functionalized with two thiol-reactive groups such as halogen groups.

[0036] The term "halo" or "halogen" refers to fluorine, chlorine, bromine or iodine or a radical thereof.

[0037] 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, C_1-C_{10} indicates that the group has from 1 to 10 (inclusive) carbon atoms in it. In the absence of any numerical designation, "alkyl" is a chain (straight or branched) having 1 to 20 (inclusive) carbon atoms in it.

[0038] The term "alkylene" refers to a divalent alkyl (*i.e.*, -R-).

[0039] 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, C_2-C_{10} indicates that the group has from 2 to 10 (inclusive) carbon atoms in it. The term "lower alkenyl" refers to a C_2-C_6 alkenyl chain. In the absence of any numerical designation, "alkenyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

[0040] 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, C_2-C_{10} indicates that the group has from 2 to 10 (inclusive) carbon atoms in it. The term "lower alkynyl" refers to a C_2-C_6 alkynyl chain. In the absence of any numerical designation, "alkynyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

[0041] The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring are substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "arylalkyl" or the term "aralkyl" refers to alkyl substituted with an aryl. The term "arylalkoxy" refers to an alkoxy substituted with aryl.

[0042] "Arylalkyl" refers to an aryl group, as defined above, wherein one of the aryl group's hydrogen atoms has been replaced with a C_1-C_5 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.

[0043] "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 -$C(O)NH_2$ groups. Representative examples of an arylamido group include 2-C(O)NH2-phenyl, 3-$C(O)NH_2$-phenyl, 4-$C(O)NH_2$-phenyl, 2-$C(O)NH_2$-pyridyl, 3-$C(O)NH_2$-pyridyl, and 4-$C(O)NH_2$-pyridyl,

[0044] "Alkylheterocycle" refers to a C_1-C_5 alkyl group, as defined above, wherein one of the C_1-C_5 alkyl group's hydrogen atoms has been replaced with a heterocycle. Representative examples of an alkylheterocycle group include, but are not limited to, -CH_2CH_2-morpholine, -CH_2CH_2-piperidine, -$CH_2CH_2CH_2$-morpholine, and -$CH_2CH_2CH_2$-imidazole.

[0045] "Alkylamido" refers to a C_1-C_5 alkyl group, as defined above, wherein one of the C_1-C_5 alkyl group's hydrogen atoms has been replaced with a -$C(O)NH_2$ group. Representative examples of an alkylamido group include, but are not limited to, -CH_2-$C(O)NH_2$, -CH_2CH_2-$C(O)NH_2$, -$CH_2CH_2CH_2C(O)NH_2$, -$CH_2CH_2CH_2CH_2C(O)NH_2$, -$CH_2CH_2CH_2CH_2CH_2C(O)NH_2$, -$CH_2CH(C(O)NH_2)CH_3$, -$CH_2CH(C(O)NH_2)CH_2CH_3$, -$CH(C(O)NH_2)CH_2CH_3$, -$C(CH_3)_2CH_2C(O)NH_2$, –$CH_2$-$CH_2$–NH-C(O)-$CH_3$, –$CH_2$-$CH_2$–NH-C(O)-$CH_3$-CH3, and –$CH_2$-$CH_2$–NH-C(O)-CH=$CH_2$.

[0046] "Alkanol" refers to a C_1-C_5 alkyl group, as defined above, wherein one of the C_1-C_5 alkyl group's hydrogen atoms has been replaced with a hydroxyl group. Representative examples of an alkanol group include, but are not limited to, -CH_2OH, -CH_2CH_2OH, -$CH_2CH_2CH_2OH$, -$CH_2CH_2CH_2CH_2OH$, -$CH_2CH_2CH_2$ CH_2CH_2OH, -$CH_2CH(OH)CH_3$, -$CH_2CH(OH)CH_2CH_3$, -$CH(OH)CH_3$ and -$C(CH_3)_2CH_2OH$.

[0047] "Alkylcarboxy" refers to a C_1-C_5 alkyl group, as defined above, wherein one of the C_1-C_5 alkyl group's hydrogen atoms has been replaced with a --COOH group. Representative examples of an alkylcarboxy group include, but are not limited to, -CH_2COOH, -CH_2CH_2COOH, -$CH_2CH_2CH_2COOH$, -$CH_2CH_2CH_2CH_2COOH$, -$CH_2CH(COOH)CH_3$, -$CH_2CH_2CH_2CH_2CH_2COOH$, -$CH_2CH(COOH)CH_2CH_3$, -$CH(COOH)CH_2CH_3$ and -$C(CH_3)_2CH_2COOH$.

[0048] The term "cycloalkyl" as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, preferably 3 to 8 carbons, and more preferably 3 to 6 carbons, wherein the cycloalkyl group additionally is optionally substituted. Some cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0049] The term "heteroaryl" refers to 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, said 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, or 4 atoms of each ring are substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like.

[0050] 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.

[0051] 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.

[0052] 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, said 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.

[0053] 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.

[0054] 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 in the present invention 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 in the present invention unless expressly provided otherwise. All crystal forms of the compounds described herein are included in the present invention unless expressly provided otherwise.

[0055] 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%.

[0056] As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable 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 between 0 and 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.

[0057] 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."

[0058] The term "on average" represents the mean value derived from performing at least three independent replicates for each data point.

[0059] The term "biological activity" encompasses structural and functional properties of a macrocycle of the invention. Biological activity is, for example, structural stability, alpha-helicity, affinity for a target, resistance to proteolytic degradation, cell penetrability, intracellular stability, *in vivo* stability, or any combination thereof.

[0060] 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.

[0061] In some embodiments, the peptide sequences are derived from the p53 protein.

[0062] A non-limiting exemplary list of suitable p53 peptides for use in the present invention is given below.

TABLE 1

Sequence (bold = critical residue; X = cross-linked amino acid)																		Design Notes
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	Asn	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	linear
Ac-	X	Gln	Ser	Gln	X	Thr	**Phe**	Ser	Asn	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #1
Ac-		X	Ser	Gln	Gln	X	**Phe**	Ser	Asn	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #2
Ac-		Gln	Ser	X	Gln	Thr	**Phe**	X	Asn	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #3
Ac-		Gln	Ser	Gln	X	Thr	**Phe**	Ser	X	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #4
Ac-		Gln	Ser	Gln	Gln	X	**Phe**	Ser	Asn	X	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #5
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	X	Asn	Leu	**Trp**	X	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #6
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	X	Leu	**Trp**	Arg	X	**Leu**	Pro	Gln	Asn	-NH2	i--> i+4 x-link #7
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	Asn	Leu	**Trp**	X	Leu	**Leu**	Pro	X	Asn	-NH2	i--> i+4 x-link #8
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	Asn	Leu	**Trp**	Arg	X	**Leu**	Pro	Gln	X	-NH2	i--> i+4 x-link #9
Ac-	X	Gln	Ser	Gln	Gln	Thr	**Phe**	X	Asn	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+7 x-link #1
Ac-		X	Ser	Gln	Gln	Thr	**Phe**	Ser	X	Leu	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+7 x-link #2
Ac-		Gln	X	Gln	Gln	Thr	**Phe**	Ser	Asn	X	**Trp**	Arg	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+7 x-link #3
Ac-		Gln	Ser	Gln	X	Thr	**Phe**	Ser	Asn	Leu	**Trp**	X	Leu	**Leu**	Pro	Gln	Asn	-NH2	i--> i+7 x-link #4
Ac-		Gln	Ser	Gln	Gln	X	**Phe**	Ser	Asn	Leu	**Trp**	Arg	X	**Leu**	Pro	Gln	Asn	-NH2	i--> i+7 x-link #5
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	X	Asn	Leu	**Trp**	Arg	Leu	**Leu**	X	Gln	Asn	-NH2	i--> i+7 x-link #6
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	X	Leu	**Trp**	Arg	Leu	**Leu**	Pro	X	Asn	-NH2	i--> i+7 x-link #7
Ac-		Gln	Ser	Gln	Gln	Thr	**Phe**	Ser	Asn	X	**Trp**	Arg	Leu	**Leu**	Pro	Gln	X	-NH2	i--> i+7 x-link #8

TABLE 2

Sequence (bold = critical residue; X = cross-linked amino acid)																Design Notes
Ac-		Leu	Thr	**Phe**	Glu	His	Tyr	**Trp**	Ala	Gln	**Leu**	Thr	Ser		-NH2	linear
Ac-	**X**	Leu	Thr	**Phe**	**X**	His	Tyr	**Trp**	Ala	Gln	**Leu**	Thr	Ser		-NH2	i--> i+4 x-link #1
Ac-		**X**	Thr	**Phe**	Glu	**X**	Tyr	**Trp**	Ala	Gln	**Leu**	Thr	Ser		-NH2	i--> i+4 x-link #2
Ac-		Leu	**X**	**Phe**	Glu	His	**X**	**Trp**	Ala	Gln	**Leu**	Thr	Ser		-NH2	i--> i+4 x-link #3
Ac-		Leu	Thr	**Phe**	**X**	His	Tyr	**Trp**	**X**	Gln	**Leu**	Thr	Ser		-NH2	i--> i+4 x-link #4
Ac-		Leu	Thr	**Phe**	Glu	**X**	Tyr	**Trp**	Ala	**X**	**Leu**	Thr	Ser		-NH2	i--> i+4 x-link #5
Ac-		Leu	Thr	**Phe**	Glu	His	Tyr	**Trp**	**X**	Gln	**Leu**	Thr	**X**		-NH2	i--> i+4 x-link #6
Ac-		Leu	Thr	**Phe**	Glu	His	Tyr	**Trp**	Ala	**X**	**Leu**	Thr	Ser	**X**	-NH2	i--> i+4 x-link #7
Ac-		**X**	Thr	**Phe**	Glu	His	Tyr	**Trp**	**X**	Gln	**Leu**	Thr	Ser		-NH2	i--> i+7 x-link #1
Ac-		Gln	**X**	**Phe**	Glu	His	Tyr	**Trp**	Ala	**X**	**Leu**	Thr	Ser		-NH2	i--> i+7 x-link #2
Ac-		Gln	Thr	**Phe**	**X**	His	Tyr	**Trp**	Ala	Gln	**Leu**	**X**	Ser		-NH2	i--> i+7 x-link #3
Ac-		Gln	Thr	**Phe**	Glu	**X**	Tyr	**Trp**	Ala	Gln	**Leu**	Thr	**X**		-NH2	i--> i+7 x-link #4
Ac-		Gln	Thr	**Phe**	Glu	His	**X**	**Trp**	Ala	Gln	**Leu**	Thr	Ser	**X**	-NH2	i--> i+7 x-link #5

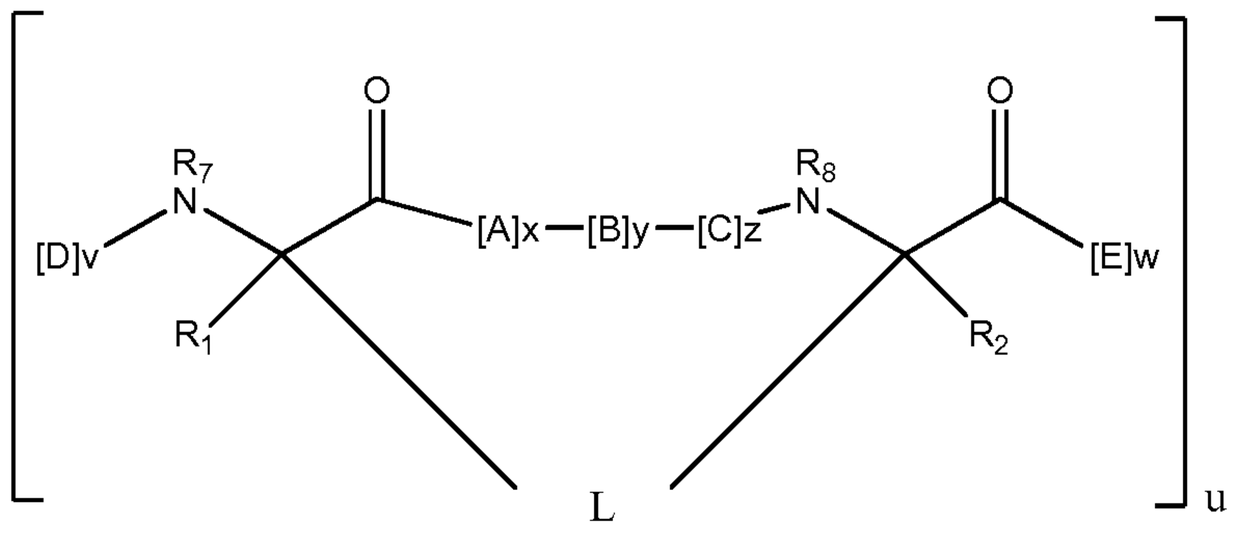
TABLE 3

Sequence (bold = critical residue; X = cross-linked amino acid)														Design Notes
Ac-		**Phe**	Met	Aib/His/Asn	Tyr	**6-ClTrp**	Glu	Ac3c/Gln/Leu	**Leu**				-NH2	linear
Ac-	**X**	**Phe**	Met	Aib/His/Asn	**X**	**6-ClTrp**	Glu	Ac3c/Gln/Leu	**Leu**				-NH2	i--> i+4 x-link #1
Ac-		**Phe**	**X**	Aib/His/Asn	Tyr	**6-ClTrp**	**X**	Ac3c/Gln/Leu	**Leu**				-NH2	i--> i+4 x-link #2
Ac-		**Phe**	Met	**X**	Tyr	**6-ClTrp**	Glu	**X**	**Leu**				-NH2	i--> i+4 x-link #3
Ac-	**X**	**Phe**	Met	Aib/His/Asn	Tyr	**6-ClTrp**	Glu	**X**	**Leu**				-NH2	i--> i+7 x-link #1
Ac-		**Phe**	**X**	Aib/His/Asn	Tyr	**6-ClTrp**	Glu	Ac3c/Gln/Leu	**Leu**	**X**			-NH2	i--> i+7 x-link #2

In Table 3 and elsewhere, "Aib" represents a 2-aminoisobutyric acid residue, while "Ac3c" represents a aminocyclopropane carboxylic acid residue.

Peptidomimetic Macrocycles

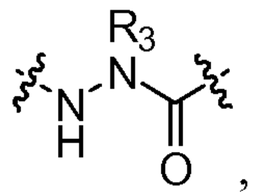
[0063] In some embodiments, a peptidomimetic macrocycle of the invention has the Formula (I):



Formula I

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid, and the terminal D and E independently optionally include a capping group;

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

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

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

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

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

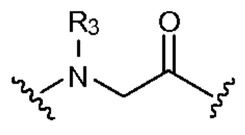
x, y and z are independently integers from 0-10; and

n is an integer from 1-5.

[0064] In one embodiment, L_1 and L_2, either alone or in combination, do not form a triazole or a thioether.

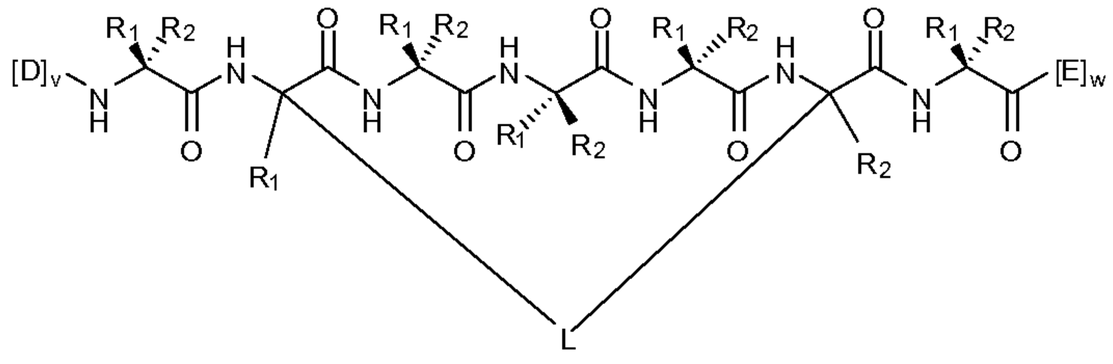
[0065] In one example, at least one of R_1 and R_2 is alkyl, unsubstituted or substituted with halo–. In another example, both R_1 and R_2 are independently alkyl, unsubstituted or substituted with halo–. In some embodiments, at least one of R_1 and R_2 is methyl. In other embodiments, R_1 and R_2 are methyl.

[0066] In some embodiments of the invention, x+y+z is at least 3. In other embodiments of the invention, 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 of the invention 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 of the invention may encompass peptidomimetic macrocycles which are the same or different. For example, a compound of the invention may comprise peptidomimetic macrocycles comprising different linker lengths or chemical compositions.

[0067] In some embodiments, the peptidomimetic macrocycle of the invention comprises a secondary structure which is an α-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  .

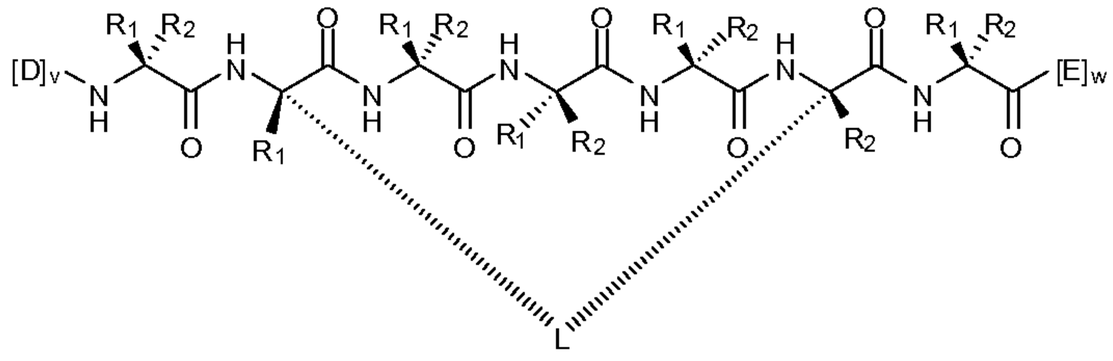
[0068] In other embodiments, the length of the macrocycle-forming linker L as measured from a first Cα to a second Cα is selected to stabilize a desired secondary peptide structure, such as an α-helix formed by residues of the peptidomimetic macrocycle including, but not necessarily limited to, those between the first Cα to a second Cα.

[0069] In one embodiment, the peptidomimetic macrocycle of Formula (I) is:

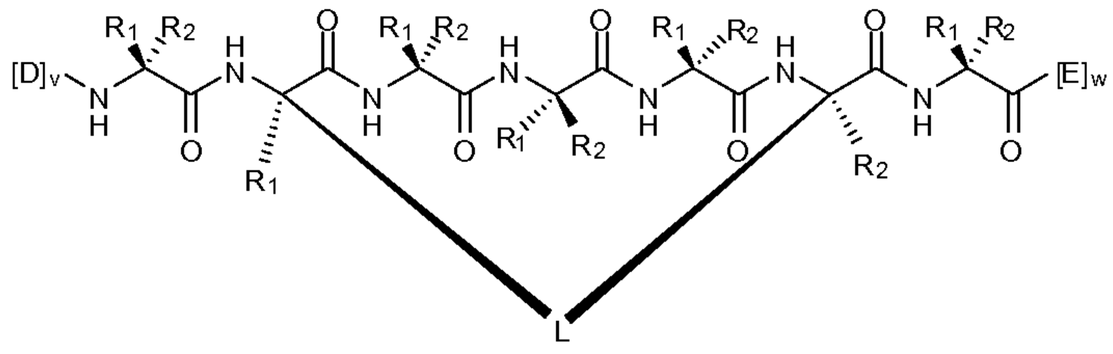


wherein each R_1 and R_2 is independently independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–.

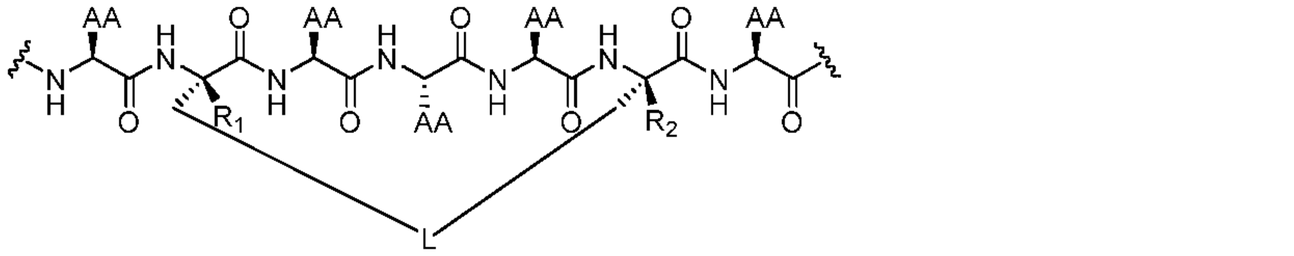
[0070] In related embodiments, the peptidomimetic macrocycle of Formula (I) is:

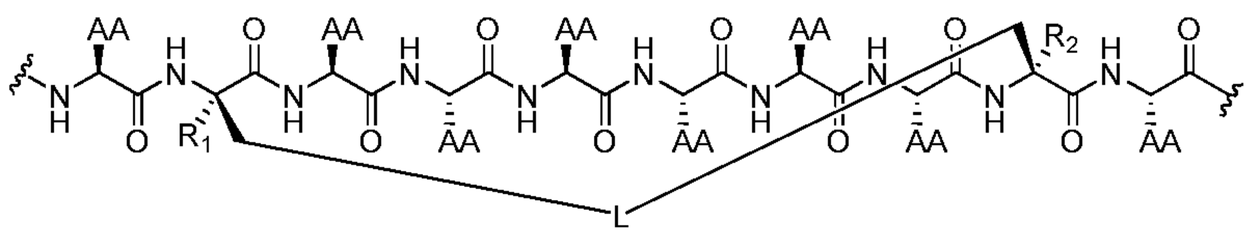


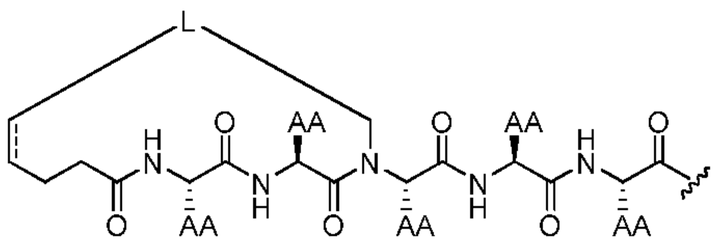
or

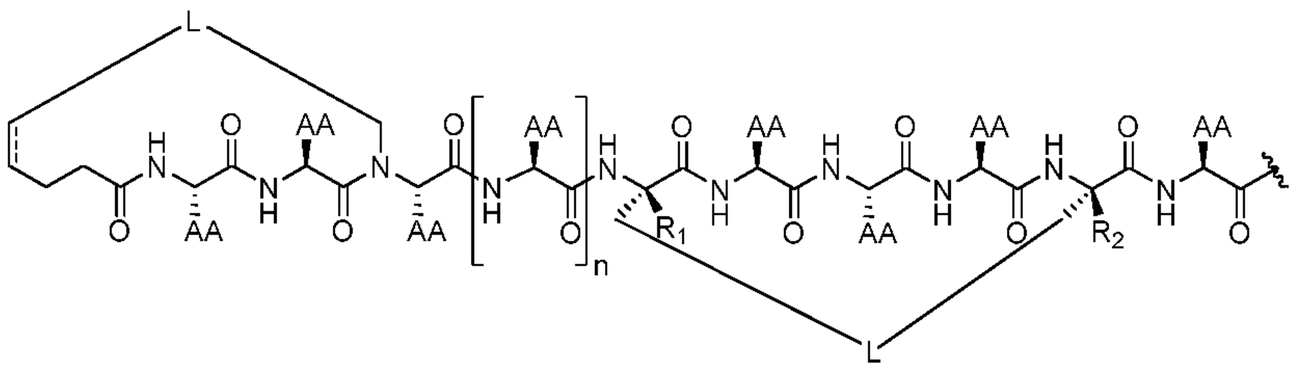


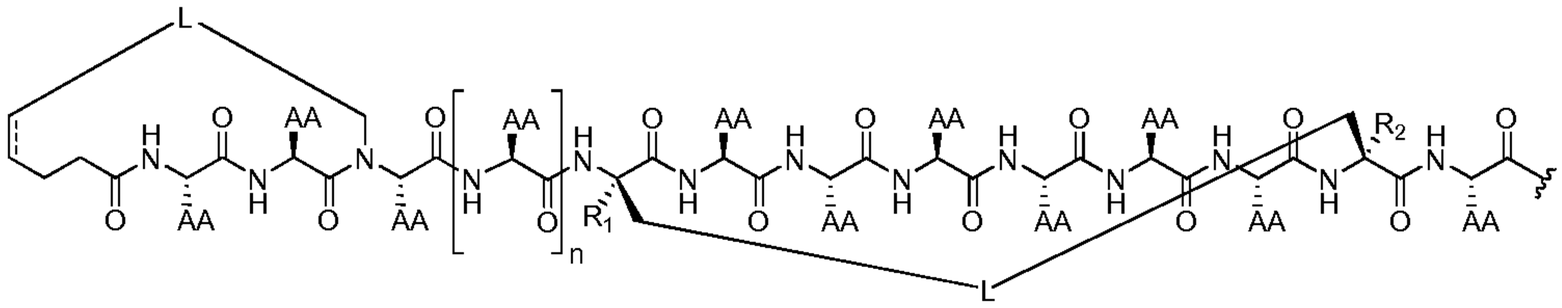
[0071] In other embodiments, the peptidomimetic macrocycle of Formula (I) is a compound of any of the formulas shown below:

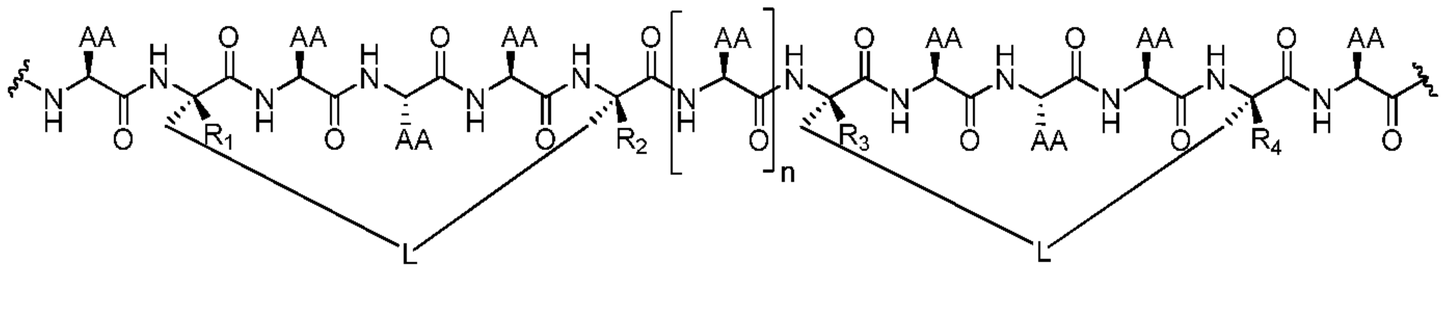


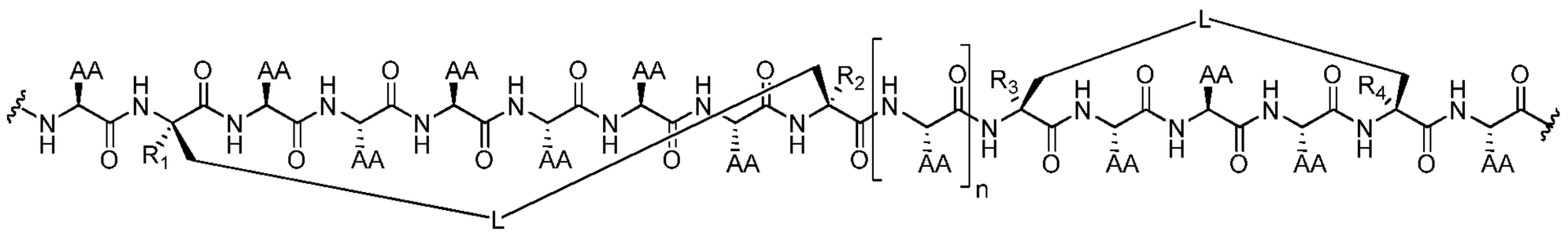


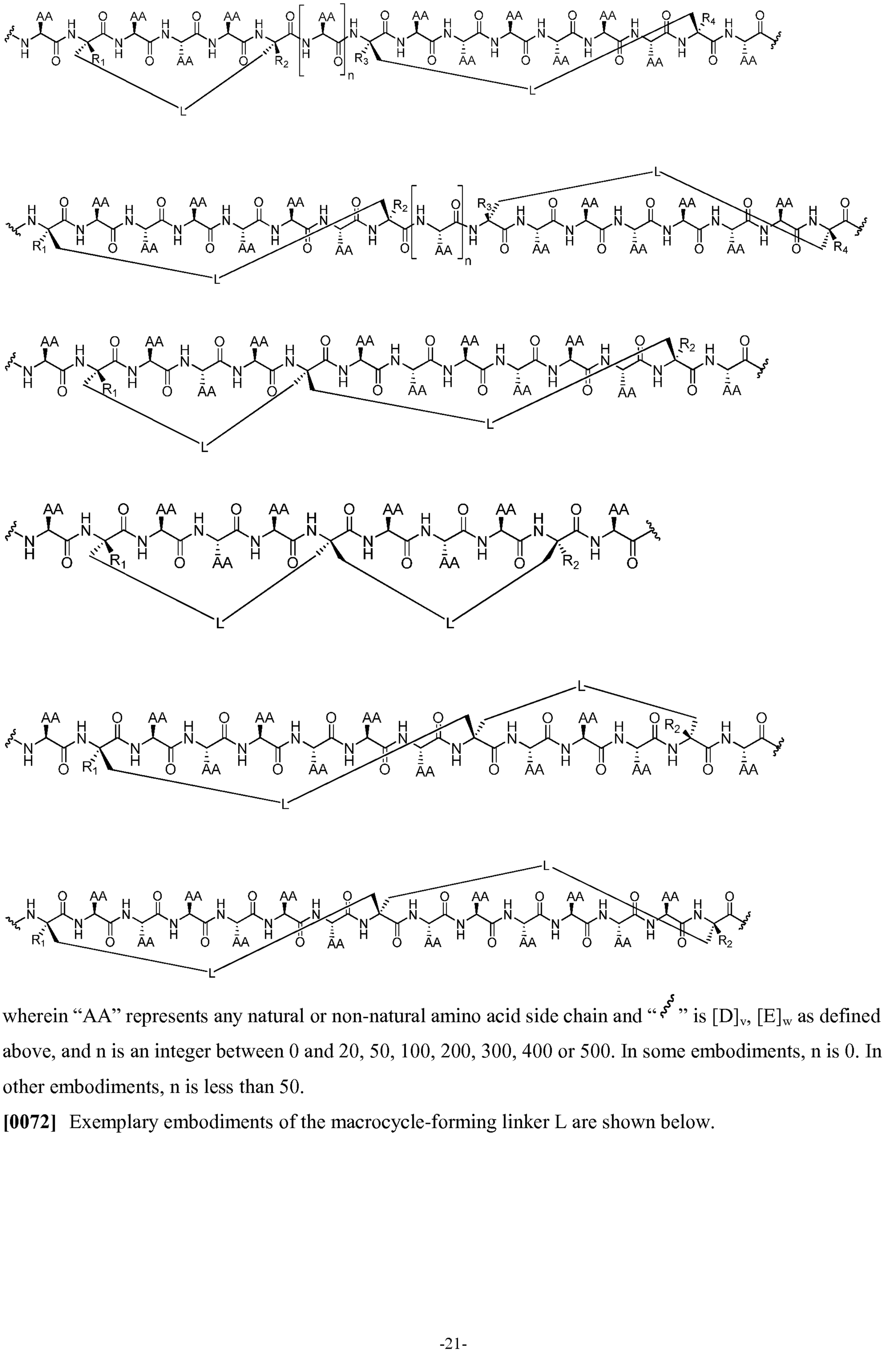






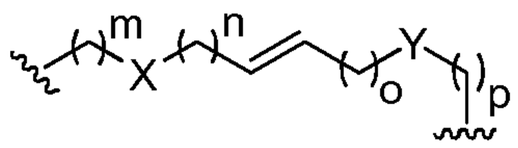




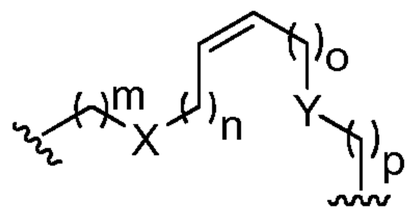


wherein "AA" represents any natural or non-natural amino acid side chain and "〰" is $[D]_v$, $[E]_w$ as defined above, and n is an integer between 0 and 20, 50, 100, 200, 300, 400 or 500. In some embodiments, n is 0. In other embodiments, n is less than 50.

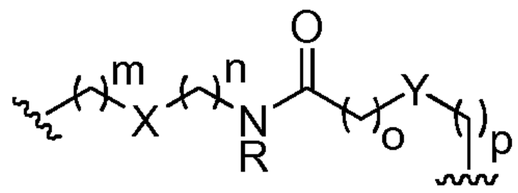
[0072] Exemplary embodiments of the macrocycle-forming linker L are shown below.



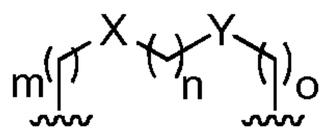
where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10
R = H, alkyl, other substituent



where X, Y = -CH_2-, O, S, or NH
m, n, o = 0-10

[0073] In other embodiments, D and/or E in the compound of Formula I 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.

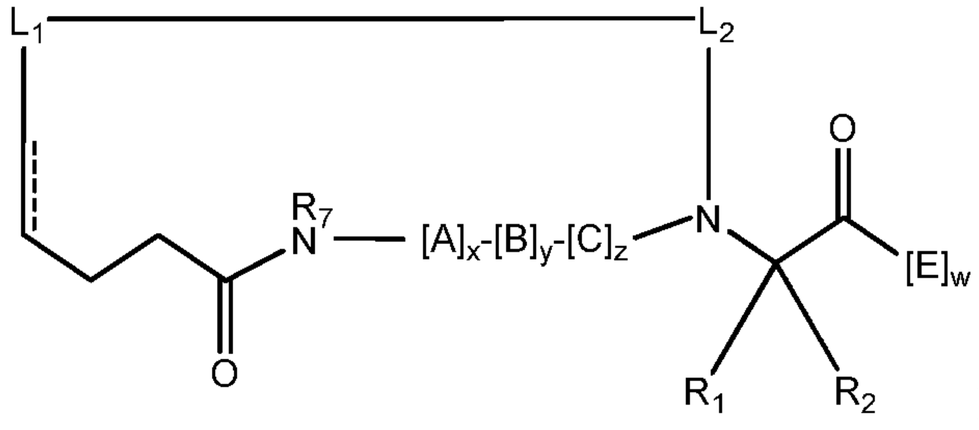
[0074] In other embodiments, at least one of [D] and [E] in the compound of Formula I represents a moiety comprising an additional macrocycle-forming linker such that the peptidomimetic macrocycle comprises at least two macrocycle-forming linkers. In a specific embodiment, a peptidomimetic macrocycle comprises two macrocycle-forming linkers. In an embodiment, u is 2.

[0075] In the peptidomimetic macrocycles of the invention, 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.

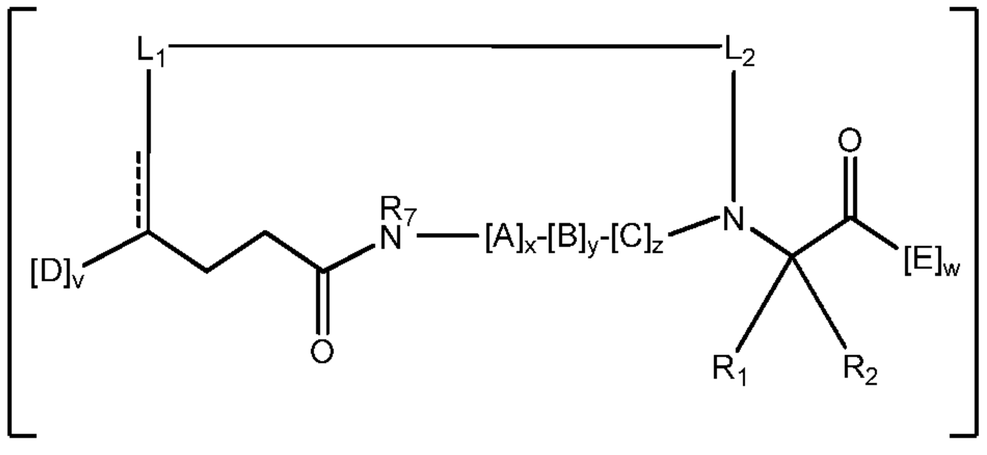
[0076] In some embodiments, the peptidomimetic macrocycle comprises at least one α-helix motif. For example, A, B and/or C in the compound of Formula I include one or more α-helices. As a general matter, α-helices include between 3 and 4 amino acid residues per turn. In some embodiments, the α-helix of the peptidomimetic macrocycle includes 1 to 5 turns and, therefore, 3 to 20 amino acid residues. In specific embodiments, the α-helix includes 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns. In some embodiments, the macrocycle-forming linker stabilizes an α-helix motif included within the peptidomimetic macrocycle. Thus, in some embodiments, the length of the macrocycle-forming linker L from a first Cα to a second Cα is selected to increase the stability of an α-helix. In some embodiments, the macrocycle-forming linker spans from 1 turn to 5 turns of the α-helix. In some embodiments, the macrocycle-forming linker spans approximately 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns of the α-helix. In some embodiments, the length of the macrocycle-forming linker is approximately 5 Å to 9 Å per turn of the α-helix, or approximately 6 Å to 8 Å per turn of the α-helix. Where the macrocycle-forming linker spans approximately 1 turn of an α-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 an α-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 an α-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 an α-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 an α-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 an α-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 α-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 α-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 α-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 α-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 α-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 α-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 α-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 α-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 α-helix, the resulting macrocycle forms a ring containing approximately 74 members to 82 members, approximately 76 members to 80 members, or approximately 78 members.

[0077] In other embodiments, the invention provides peptidomimetic macrocycles of Formula (IV) or (IVa):



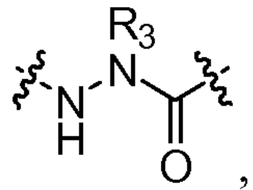
Formula (IV)



Formula (IVa)

wherein:

each A, C, D, and E is independently a natural or non-natural amino acid, and the terminal D and E independently optionally include a capping group;

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

R_1 and R_2 are 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;

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

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

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

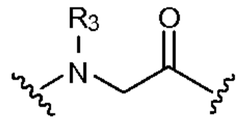
x, y and z are independently integers from 0-10; and

n is an integer from 1-5.

[0078] In one example, L_1 and L_2, either alone or in combination, do not form a triazole or a thioether.

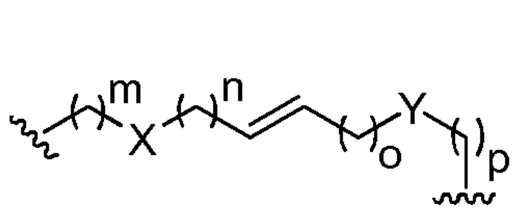
[0079] In one example, at least one of R_1 and R_2 is alkyl, unsubstituted or substituted with halo–. In another example, both R_1 and R_2 are independently alkyl, unsubstituted or substituted with halo–. In some embodiments, at least one of R_1 and R_2 is methyl. In other embodiments, R_1 and R_2 are methyl.

[0080] In some embodiments of the invention, x+y+z is at least 1. In other embodiments of the invention, x+y+z is at least 2. In other embodiments of the invention, 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 of the invention 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.

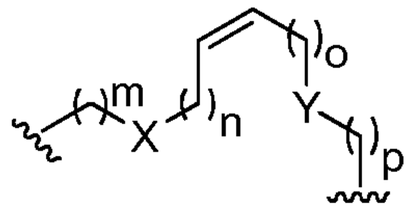
[0081] In some embodiments, the peptidomimetic macrocycle of the invention comprises a secondary structure which is an α-helix and R_8 is –H, allowing intrahelical hydrogen bonding. In some embodiments, at least one of A, B, C, D or E is an α,α-disubstituted amino acid. In one example, B is an α,α-disubstituted 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  .

[0082] In other embodiments, the length of the macrocycle-forming linker L as measured from a first Cα to a second Cα is selected to stabilize a desired secondary peptide structure, such as an α-helix formed by residues of the peptidomimetic macrocycle including, but not necessarily limited to, those between the first Cα to a second Cα.

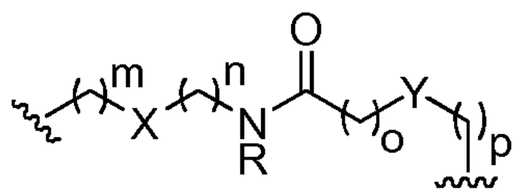
[0083] Exemplary embodiments of the macrocycle-forming linker -L_1-L_2- are shown below.



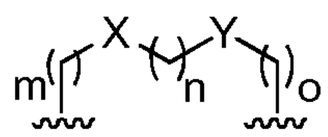
where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10



where X, Y = -CH_2-, O, S, or NH
m, n, o, p = 0-10
R = H, alkyl, other substituent

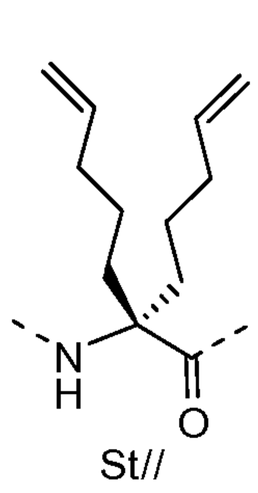


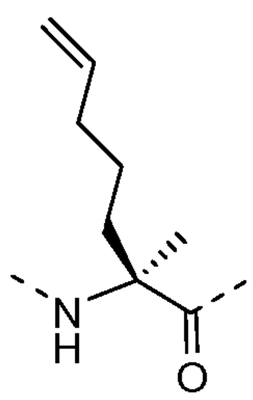
where X, Y = -CH_2-, O, S, or NH
m, n, o = 0-10

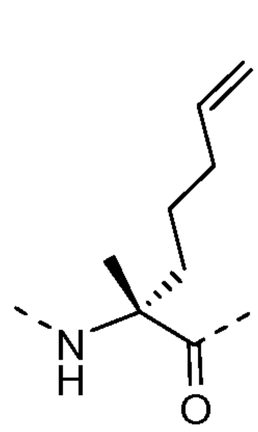
Preparation of Peptidomimetic Macrocycles

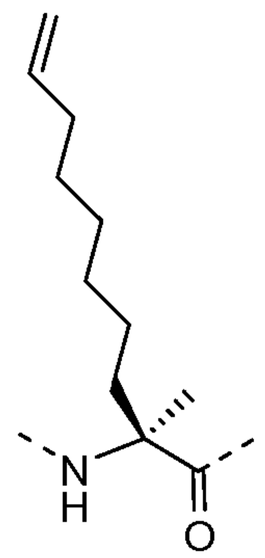
[0084] Peptidomimetic macrocycles of the invention may be prepared by any of a variety of methods known in the art. For example, any of the residues indicated by "X" in Tables 1, 2, 3, or 4 may be substituted with a residue capable of forming a crosslinker with a second residue in the same molecule or a precursor of such a residue.

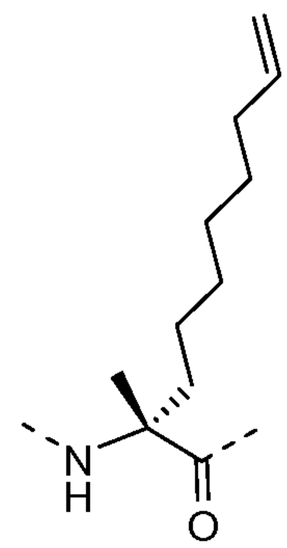
[0085] Various methods to effect formation of peptidomimetic macrocycles are known in the art. For example, the preparation of peptidomimetic macrocycles of Formula I is described in Schafmeister et al., J. Am. Chem. Soc. 122:5891-5892 (2000); Schafmeister & Verdine, J. Am. Chem. Soc. 122:5891 (2005); Walensky et al., Science 305:1466-1470 (2004); 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. In various embodiments, the following amino acids may be employed in the synthesis of the peptidomimetic macrocycle:











[0086] In other embodiments, the peptidomimetic macrocyles of the invention are of Formula IV or IVa. Methods for the preparation of such macrocycles are described, for example, in US Patent No. 7,202,332.

[0087] 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, aminoacid precursors are used containing an additional substituent R- at the alpha position. Such aminoacids 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.

Assays

[0088] The properties of the peptidomimetic macrocycles of the invention are assayed, for example, by using the methods described below. In some embodiments, a peptidomimetic macrocycle of the invention has improved biological properties relative to a corresponding polypeptide lacking the substituents described herein.

Assay to Determine α-helicity.

[0089] In solution, the secondary structure of polypeptides with α-helical domains will reach a dynamic equilibrium between random coil structures and α-helical structures, often expressed as a "percent helicity". Thus, for example, alpha-helical domains are predominantly random coils in solution, with α-helical content usually under 25%. Peptidomimetic macrocycles with optimized linkers, on the other hand, possess, for example, an alpha-helicity that is at least two-fold greater than that of a corresponding uncrosslinked polypeptide. In some embodiments, macrocycles of the invention will possess an alpha-helicity of greater than 50%. To assay the helicity of peptidomimetic macrocyles of the invention, the compounds are dissolved in an aqueous solution (e.g. 50 mM potassium phosphate solution at pH 7, or distilled H_2O, to concentrations of 25-50 μM). 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. [Φ]222obs) by the reported value for a model helical decapeptide (Yang *et al*. (1986), *Methods Enzymol*. 130:208)).

Assay to Determine Melting Temperature (Tm).

[0090] A peptidomimetic macrocycle of the invention comprising a secondary structure such as an α-helix exhibits, for example, a higher melting temperature than a corresponding uncrosslinked polypeptide. Typically peptidomimetic macrocycles of the invention exhibit Tm of > 60°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 H_2O (e.g. at a final concentration of 50 μM) and the Tm is determined by measuring the change in ellipticity over a temperature range (e.g. 4 to 95 °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°C/min; path length, 0.1 cm).

Protease Resistance Assay.

[0091] 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 may shield it from proteolytic cleavage. The peptidomimetic macrocycles of the present invention may 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 minutes. 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=-1Xslope).

Ex Vivo Stability Assay.

[0092] 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 hours or more. For *ex vivo* serum stability studies, a variety of assays may 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 hours. To determine the level of intact compound, the following procedure may be used: The samples are extracted by transferring 100 µl of sera to 2 ml centrifuge tubes followed by the addition of 10 µL of 50 % formic acid and 500µL acetonitrile and centrifugation at 14,000 RPM 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 100µL of 50:50 acetonitrile:water and submitted to LC-MS/MS analysis.

In vitro Binding Assays.

[0093] To assess the binding and affinity of peptidomimetic macrocycles and peptidomimetic precursors to acceptor proteins, a fluorescence polarization assay (FPA) isused, 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).

[0094] For example, fluoresceinated peptidomimetic macrocycles (25 nM) are incubated with the acceptor protein (25- 1000nM) in binding buffer (140mM NaCl, 50 mM Tris-HCL, pH 7.4) for 30 minutes at room temperature. Binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (e.g. Perkin-Elmer LS50B). Kd values may be determined by nonlinear regression analysis using, for example, Graphpad Prism software (GraphPad Software, Inc., San Diego, CA). A peptidomimetic macrocycle of the invention shows, in some instances, similar or lower Kd than a corresponding uncrosslinked polypeptide.

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

[0095] 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.

[0096] 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 (140mM NaCl, 50 mM Tris-HCL, pH 7.4) for 30 minutes at room temperature. Antagonist binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (e.g. Perkin-Elmer LS50B). Kd values may be determined by nonlinear regression analysis using, for example, Graphpad Prism software (GraphPad Software, Inc., San Diego, CA).

[0097] 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

[0098] 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 μM peptidomimetic macrocycle plus 5 μM hMDM2. A 1 μL DMSO aliquot of a 40 μM stock solution of peptidomimetic macrocycle is dissolved in 19 μL 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 μL aliquot of the resulting supernatant is added 4 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 plus 1 μM 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 Kd Titration Experiments.

[0099] To assess the binding and affinity of test compounds for proteins, a protein-ligand Kd 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 °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

[0100] To determine the ability of test compounds to bind competitively to proteins, an affiinity 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 °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.

[0101] 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 hrs in the absence of serum, followed by serum replacement and further incubation that ranges from 4-18 hrs. 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 minutes at 4°C. Extracts are centrifuged at 14,000 rpm for 15 minutes and supernatants collected and incubated with 10 µl goat anti-FITC antibody for 2 hrs, rotating at 4°C followed by further 2 hrs incubation at 4°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.

[0102] 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 fluoresceinated peptidomimetic macrocycles or corresponding uncrosslinked macrocycle (10 µM) for 4 hrs 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.

[0103] 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 hrs of incubation with peptidomimetic macrocycles (0.5 to 50 μM) to identify those that kill at EC50<10 μM. 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.

[0104] 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 hrs, 8 hrs and 24 hours post-injection. Levels of intact compound in 25 μL of fresh serum are then measured by LC-MS/MS as above.

In vivo Efficacy in Animal Models.

[0105] To determine the anti-oncogenic activity of peptidomimetic macrocycles of the invention *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 x 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 hrs 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.

[0106] To determine the suitability of the peptidomimetic macrocycles of the invention for treatment of humans, clinical trials are performed. For example, patients diagnosed with cancer and in need of treatment are selected and separated in treatment and one or more control groups, wherein the treatment group is administered a peptidomimetic macrocycle of the invention, while the control groups receive a placebo or a known anti-cancer drug. The treatment safety and efficacy of the peptidomimetic macrocycles of the invention 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 macrocyle show improved long-term survival compared to a patient control group treated with a placebo.

Pharmaceutical Compositions and Routes of Administration

[0107] The peptidomimetic macrocycles of the invention 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 of the invention 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.

[0108] In some embodiments, the peptidomimetic macrocycles of the invention 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] The pharmaceutical preparation is preferably 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.

[0115] 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 between about 1 to 100%, and more preferably between 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.

Methods of Use

[0116] 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 p53/HDMX system, labeled peptidomimetic macrocycles based on p53 can be used in a HDMX binding assay along with small molecules that competitively bind to HDMX. Competitive binding studies allow for rapid *in vitro* evaluation and determination of drug candidates specific for the p53/HDMX system.

Such binding studies may be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners.

[0117] 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, such as p53, to which the peptidomimetic macrocycles are related. Such antibodies, for example, disrupt the native protein-protein interaction, for example, binding between p53 and HDMX.

[0118] 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) expression or activity of the molecules including p53, HDM2 or HDMX.

[0119] In another embodiment, a disorder is caused, at least in part, by an abnormal level of p53 or HDM2 or HDMX, (*e.g.*, over or under expression), or by the presence of p53 or HDM2 or HDMX exhibiting abnormal activity. As such, the reduction in the level and/or activity of p53 or HDM2 or HDMX, or the enhancement of the level and/or activity of p53 or HDM2 or HDMX, by peptidomimetic macrocycles derived from p53, is used, for example, to ameliorate or reduce the adverse symptoms of the disorder.

[0120] In another aspect, the present invention provides 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 HDM2 or p53 and HDMX. These methods comprise administering an effective amount of a compound of the invention to a warm blooded animal, including a human. In some embodiments, the administration of the compounds of the present invention induces cell growth arrest or apoptosis.

[0121] 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.

[0122] In some embodiments, the peptidomimetics macrocycles of the invention is 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 may be categorized as pathologic, *i.e.*, characterizing or constituting a disease state, or may 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.

[0123] Examples of cancers or neoplastic conditions include, but are not limited to, a fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, 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.

[0124] 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. Preferably, the diseases 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./Hemotol.* 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-Stemberg disease.

[0125] 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.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] 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-stomal tumors such as, granulosa-theca cell tumors, thecomafibromas, androblastomas, hill cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

[0130] 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, thalaessemia, congenital neutropenia, and myelodysplasia.

[0131] 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. One example is Alzheimer's disease (AD). Alzheimer's disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions. Both

amyloid plaques and neurofibrillary tangles are visible in brains of those afflicted by AD. Alzheimer's disease has been identified as a protein misfolding disease, due to the accumulation of abnormally folded A-beta and tau proteins in the brain. Plaques are made up of β-amyloid. β-amyloid is a fragment from a larger protein called amyloid precursor protein (APP). APP is critical to neuron growth, survival and post-injury repair. In AD, an unknown process causes APP to be cleaved into smaller fragments by enzymes through proteolysis. One of these fragments is fibrils of β-amyloid, which form clumps that deposit outside neurons in dense formations known as senile plaques. Plaques continue to grow into insoluble twisted fibers within the nerve cell, often called tangles. Disruption of the interaction between β-amyloid and its native receptor is therefore important in the treatment of AD. The anti-apoptotic peptidomimetics macrocycles of the invention are used, in some embodiments, in the treatment of AD and other neurological disorders associated with cell apoptosis. Such neurological 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.

[0132] 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. 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.

[0133] 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.

[0134] In another embodiment, the peptidomimetics macrocycles described herein are used to treat, prevent or diagnose inflammatory disorders. Numerous types of inflammatory disorders exist. Certain inflammatory diseases are associated with the immune system, for example, autoimmune diseases. Autoimmune diseases arise from an overactive immune response of the body against substances and tissues normally present in the body, i.e. self antigens. In other words, the immune system attacks its own cells. Autoimmune diseases are a major cause of immune-mediated diseases. Rheumatoid arthritis is an example of an autoimmune disease, in which the immune system attacks the joints, where it causes inflammation (i.e. arthritis) and destruction. It can also damage some organs, such as the lungs and skin. Rheumatoid arthritis can lead to substantial loss of functioning and mobility. Rheumatoid arthritis is diagnosed with blood tests especially the rheumatoid factor test. Some examples of autoimmune diseases that are treated with the peptidomimetics macrocycles described herein include, but are not limited to, acute disseminated encephalomyelitis (ADEM), Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome (APS), autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, Bechet's disease, bullous pemphigoid, coeliac disease, Chagas disease, Churg-Strauss syndrome, chronic obstructive pulmonary disease (COPD), Crohn's disease, dermatomyositis, diabetes mellitus type 1, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's disease, Hidradenitis suppurativa, idiopathic thrombocytopenic purpura, inflammatory bowl disease (IBD), interstitial cystitis, lupus erythematosus, morphea, multiple sclerosis, myasthenia gravis, narcolepsy, neuromyotonia, pemphigus vulgaris, pernicious anaemia, Polymyositis, polymyalgia rheumatica, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, schizophrenia, scleroderma, Sjögren's syndrome, temporal arteritis (also known as "giant cell arteritis"), Takayasu's arteritis, Vasculitis, Vitiligo, and Wegener's granulomatosis.

[0135] Some examples of other types of inflammatory disorders that are treated with the peptidomimetics macrocycles described herein include, but are not limited to, allergy including allergic rhinitis/sinusitis, skin allergies (urticaria/hives, angioedema, atopic dermatitis), food allergies, drug allergies, insect allergies, and rare allergic disorders such as mastocytosis, asthma, arthritis including osteoarthritis, rheumatoid arthritis, and spondyloarthropathies, primary angitis of the CNS, sarcoidosis, organ transplant rejection, fibromyalgia, fibrosis, pancreatitis, and pelvic inflammatory disease.

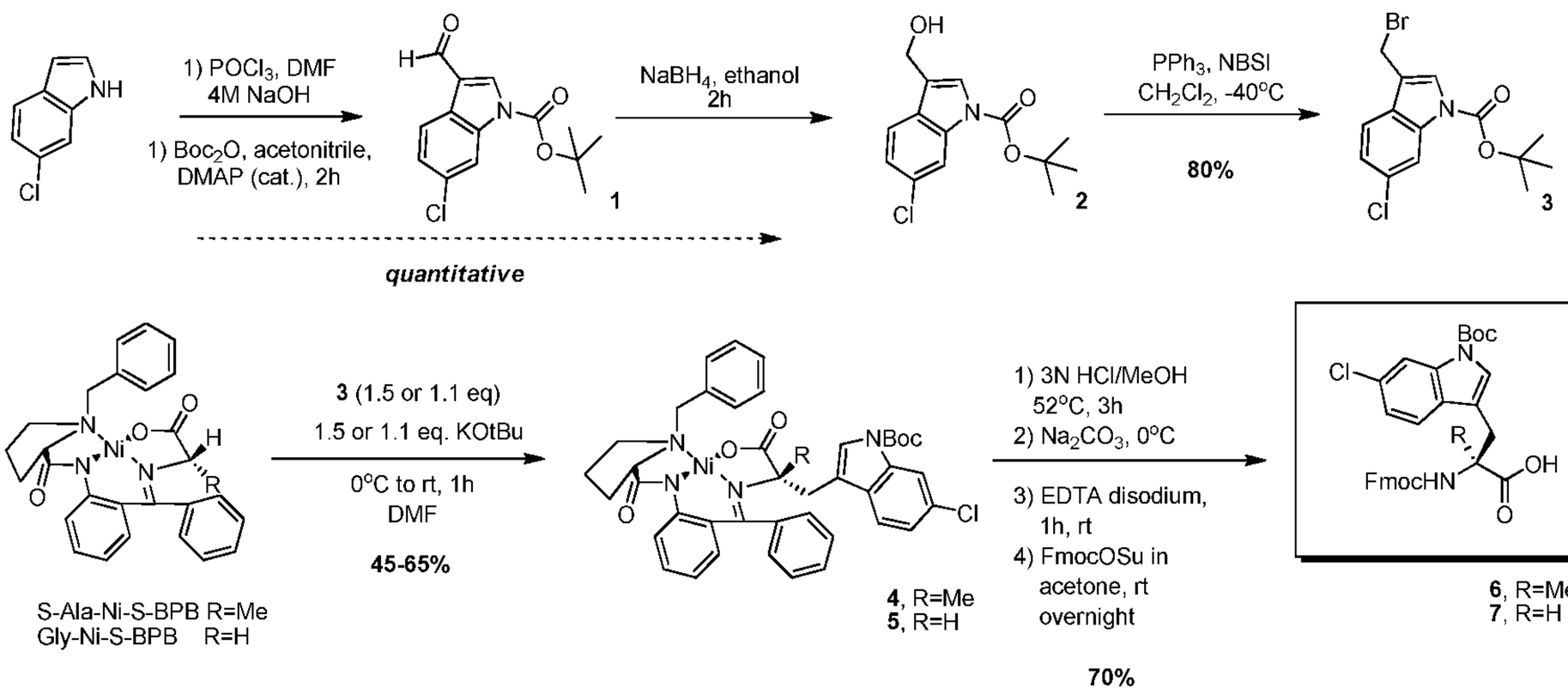
[0136] Examples of cardiovascular disorders (*e.g.*, inflammatory disorders) that are treated or prevented with the peptidomimetics macrocycles of the invention include, but are not limited to, aortic valve stenosis, atherosclerosis, myocardial infarction, stroke, thrombosis, aneurism, heart failure, 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.

[0137] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Examples

Example 1: Synthesis of 6-chlorotryptophan Fmoc amino acids



[0138] 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).

[0139] 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. ^{1}H 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).

[0140] 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%). ^{1}H NMR ($CDCl_3$) δ: 1.66 (s, Boc, 9H); 4.63 (s, 2H, CH_2); 7.28 (dd, 1H); 7.57 (d, 1H); 7.64 (bs, 1H); 8.18 (bs, 1H).

[0141] 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; ^{1}H NMR ($CDCl_3$) δ: 1.23 (s, 3H, αMe); 1.56 (m, 11H, Boc + CH_2); 1.82-2.20 (m, 4H, $2CH_2$); 3.03 (m, 1H, CH_α); 3.24 (m, 2H, CH_2); 3.57 and 4.29 (AB system, 2H, CH_2 (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).

[0142] 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 Na_2CO_3 (1.21g, 11.5 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na_2CO_3 (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; ^{1}H NMR ($CDCl_3$) 1.59 (s, 9H, Boc); 1.68 (s, 3H, Me); 3.48 (bs, 2H, CH_2); 4.22 (m, 1H, CH); 4.39 (bs, 2H, CH_2); 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).

[0143] 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; ^{1}H NMR ($CDCl_3$) δ: 1.58 (m, 11H, Boc + CH_2); 1.84 (m, 1H); 1.96 (m, 1H); 2.24 (m, 2H, CH_2); 3.00 (m, 1H, CH_α); 3.22 (m, 2H, CH_2); 3.45 and 4.25 (AB system, 2H, CH_2 (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).

[0144] 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 Na_2CO_3 (3.48g, 33 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na_2CO_3 (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; ^{1}H NMR ($CDCl_3$) 1.63 (s, 9H, Boc); 3.26 (m, 2H, CH_2); 4.19 (m, 1H, CH); 4.39 (m, 2H, CH_2); 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 of the invention

[0145] 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 either 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.

[0146] 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).

[0147] Table 4 shows a list of peptidomimetic macrocycles of the invention prepared.

Table 4

SP	Seq	Exact Mass	M+2	Observed mass (m/e)
SP-1	Ac-LSQETF$r8DLWKLL$EN-NH2	2068.13	1035.07	1035.36
SP-2	Ac-LSQETF$r8NLWKLL$QN-NH2	2066.16	1034.08	1034.31
SP-3	Ac-LSQQTF$r8NLWRLL$QN-NH2	2093.18	1047.59	1047.73
SP-4	Ac-QSQQTF$r8NLWKLL$QN-NH2	2080.15	1041.08	1041.31
SP-5	Ac-QSQQTF$r8NLWRLL$QN-NH2	2108.15	1055.08	1055.32
SP-6	Ac-QSQQTA$r8NLWRLL$QN-NH2	2032.12	1017.06	1017.24
SP-7	Ac-QAibQQTF$r8NLWRLL$QN-NH2	2106.17	1054.09	1054.34
SP-8	Ac-QSQQTFSNLWRLLPQN-NH2	2000.02	1001.01	1001.26
SP-9	Ac-QSQQTF$/r8NLWRLL$/QN-NH2	2136.18	1069.09	1069.37
SP-10	Ac-QSQAibTF$r8NLWRLL$QN-NH2	2065.15	1033.58	1033.71
SP-11	Ac-QSQQTF$r8NLWRLL$AN-NH2	2051.13	1026.57	1026.70
SP-12	Ac-ASQQTF$r8NLWRLL$QN-NH2	2051.13	1026.57	1026.90
SP-13	Ac-QSQQTF$r8ALWRLL$QN-NH2	2065.15	1033.58	1033.41
SP-14	Ac-QSQETF$r8NLWRLL$QN-NH2	2109.14	1055.57	1055.70
SP-15	Ac-RSQQTF$r8NLWRLL$QN-NH2	2136.20	1069.10	1069.17
SP-16	Ac-RSQQTF$r8NLWRLL$EN-NH2	2137.18	1069.59	1069.75
SP-17	Ac-LSQETFSDLWKLLPEN-NH2	1959.99	981.00	981.24
SP-18	Ac-QSQTFSLWRLLPQN-NH2	2008.09	1005.05	1004.97
SP-19	Ac-QSQQFSNWRLLPQN-NH2	2036.06	1019.03	1018.86
SP-20	Ac-QSQQTSNLRLLPQN-NH2	1917.04	959.52	959.32
SP-21	Ac-QSQQTFNLWLLPQN-NH2	2007.06	1004.53	1004.97
SP-22	Ac-RTQATF$r8NQWAibANle$TNAibTR-NH2	2310.26	1156.13	1156.52
SP-23	Ac-QSQQTF$r8NLWRLL$RN-NH2	2136.20	1069.10	1068.94
SP-24	Ac-QSQRTF$r8NLWRLL$QN-NH2	2136.20	1069.10	1068.94
SP-25	Ac-QSQQTF$r8NNleWRLL$QN-NH2	2108.15	1055.08	1055.44
SP-26	Ac-QSQQTF$r8NLWRNleL$QN-NH2	2108.15	1055.08	1055.84

SP-27	Ac-QSQQTF$r8NLWRLNle$QN-NH2	2108.15	1055.08	1055.12
SP-28	Ac-QSQQTY$r8NLWRLL$QN-NH2	2124.15	1063.08	1062.92
SP-29	Ac-RAibQQTF$r8NLWRLL$QN-NH2	2134.22	1068.11	1068.65
SP-30	Ac-MPRFMDYWEGLN-NH2	1598.70	800.35	800.45
SP-31	Ac-RSQQRF$r8NLWRLL$QN-NH2	2191.25	1096.63	1096.83
SP-32	Ac-QSQQRF$r8NLWRLL$QN-NH2	2163.21	1082.61	1082.87
SP-33	Ac-RAibQQRF$r8NLWRLL$QN-NH2	2189.27	1095.64	1096.37
SP-34	Ac-RSQQRF$r8NFWRLL$QN-NH2	2225.23	1113.62	1114.37
SP-35	Ac-RSQQRF$r8NYWRLL$QN-NH2	2241.23	1121.62	1122.37
SP-36	Ac-RSQQTF$r8NLWQLL$QN-NH2	2108.15	1055.08	1055.29
SP-37	Ac-QSQQTF$r8NLWQAmlL$QN-NH2	2094.13	1048.07	1048.32
SP-38	Ac-QSQQTF$r8NAmlWRLL$QN-NH2	2122.17	1062.09	1062.35
SP-39	Ac-NlePRF$r8DYWEGL$QN-NH2	1869.98	935.99	936.20
SP-40	Ac-NlePRF$r8NYWRLL$QN-NH2	1952.12	977.06	977.35
SP-41	Ac-RF$r8NLWRLL$Q-NH2	1577.96	789.98	790.18
SP-42	Ac-QSQQTF$r8N2ffWRLL$QN-NH2	2160.13	1081.07	1081.40
SP-43	Ac-QSQQTF$r8N3ffWRLL$QN-NH2	2160.13	1081.07	1081.34
SP-44	Ac-QSQQTF#r8NLWRLL#QN-NH2	2080.12	1041.06	1041.34
SP-45	Ac-RSQQTA$r8NLWRLL$QN-NH2	2060.16	1031.08	1031.38
SP-46	Ac-QSQQTF%r8NLWRLL%QN-NH2	2110.17	1056.09	1056.55
SP-47	HepQSQ$TFSNLWRLLPQN-NH2	2051.10	1026.55	1026.82
SP-48	HepQSQTFr8NLWRLL$QN-NH2	2159.23	1080.62	1080.89
SP-49	Ac-QSQQTF$r8NL6clWRLL$QN-NH2	2142.11	1072.06	1072.35
SP-50	Ac-QSQQTF$r8NLMe6clwRLL$QN-NH2	2156.13	1079.07	1079.27
SP-51	Ac-LTFEHYWAQLTS-NH2	1535.74	768.87	768.91
SP-52	Ac-LTFHYWQLTS-NH2	1585.83	793.92	794.17
SP-53	Ac-LTFEYWALTS-NH2	1520.79	761.40	761.67
SP-54	Ac-LTF$zr8HYWAQL$zS-NH2	1597.87	799.94	800.06
SP-55	Ac-LTF$r8HYWRQL$S-NH2	1682.93	842.47	842.72
SP-56	Ac-QS$QTFStNLWRLL$s8QN-NH2	2145.21	1073.61	1073.90
SP-57	Ac-QSQQTASNLWRLLPQN-NH2	1923.99	963.00	963.26
SP-58	Ac-QSQQTA$/r8NLWRLL$/QN-NH2	2060.15	1031.08	1031.24
SP-59	Ac-ASQQTF$/r8NLWRLL$/QN-NH2	2079.16	1040.58	1040.89
SP-60	Ac-SQQFSNLWRLLAibQN-NH2	2009.09	1005.55	1005.86
SP-61	Ac-QSQTFNLWRLLAibQN-NH2	2023.10	1012.55	1012.79
SP-62	Ac-QSQQFSNWRLLAibQN-NH2	2024.06	1013.03	1013.31
SP-63	Ac-QSQQTFNLWLLAibQN-NH2	1995.06	998.53	998.87
SP-64	Ac-QSQQTFSLWRLAibQN-NH2	2011.06	1006.53	1006.83
SP-65	Ac-QSQQTFSNLWLLAN-NH2	1940.02	971.01	971.29
SP-66	Ac-$/SQQ$/FSNLWRLLAibQN-NH2	2037.12	1019.56	1019.78
SP-67	Ac-QS$/QTF$/NLWRLLAibQN-NH2	2051.13	1026.57	1026.90
SP-68	Ac-QSQQ$/FSN$/WRLLAibQN-NH2	2052.09	1027.05	1027.36
SP-69	Ac-QSQQTF$/NLW$/LLAibQN-NH2	2023.09	1012.55	1013.82
SP-70	Ac-QSQTFSLWRLLAibQN-NH2	1996.09	999.05	999.39
SP-71	Ac-QSQ$/TFS$/LWRLLAibQN-NH2	2024.12	1013.06	1013.37
SP-72	Ac-QS$/QTFSt//NLWRLL$/s8QN-NH2	2201.27	1101.64	1102.00
SP-73	Ac-$r8SQQTFS$LWRLLAibQN-NH2	2038.14	1020.07	1020.23
SP-74	Ac-QSQ$r8TFSNLW$LLAibQN-NH2	1996.08	999.04	999.32
SP-75	Ac-QSQQTFS$r8LWRLLA$N-NH2	2024.12	1013.06	1013.37
SP-76	Ac-QS$r5QTFStNLW$LLAibQN-NH2	2032.12	1017.06	1017.39

SP-77	Ac-$/r8SQQTFS$/LWRLLAibQN-NH2	2066.17	1034.09	1034.80
SP-78	Ac-QSQ$/r8TFSNLW$/LLAibQN-NH2	2024.11	1013.06	1014.34
SP-79	Ac-QSQQTFS$/r8LWRLLA$/N-NH2	2052.15	1027.08	1027.16
SP-80	Ac-QS$/r5QTFSt//NLW$/LLAibQN-NH2	2088.18	1045.09	1047.10
SP-81	Ac-QSQQTFSNLWRLLAibQN-NH2	1988.02	995.01	995.31
SP-82	Hep/QSQ$/TF$/r8NLWRLL$/QN-NH2	2215.29	1108.65	1108.93
SP-83	Ac-ASQQTF$r8NLRWLL$QN-NH2	2051.13	1026.57	1026.90
SP-84	Ac-QSQQTF$/r8NLWRLL$/Q-NH2	2022.14	1012.07	1012.66
SP-85	Ac-QSQQTF$r8NLWRLL$Q-NH2	1994.11	998.06	998.42
SP-86	Ac-AAARAA$r8AAARAA$AA-NH2	1515.90	758.95	759.21
SP-87	Ac-LTFEHYWAQLTSA-NH2	1606.78	804.39	804.59
SP-88	Ac-LTF$r8HYWAQL$SA-NH2	1668.90	835.45	835.67
SP-89	Ac-ASQQTFSNLWRLLPQN-NH2	1943.00	972.50	973.27
SP-90	Ac-QS$QTFStNLW$r5LLAibQN-NH2	2032.12	1017.06	1017.30
SP-91	Ac-QSQQTFAibNLWRLLAibQN-NH2	1986.04	994.02	994.19
SP-92	Ac-QSQQTFNleNLWRLLNleQN-NH2	2042.11	1022.06	1022.23
SP-93	Ac-QSQQTF$/r8NLWRLLAibQN-NH2	2082.14	1042.07	1042.23
SP-94	Ac-QSQQTF$/r8NLWRLLNleQN-NH2	2110.17	1056.09	1056.29
SP-95	Ac-QSQQTFAibNLWRLL$/QN-NH2	2040.09	1021.05	1021.25
SP-96	Ac-QSQQTFNleNLWRLL$/QN-NH2	2068.12	1035.06	1035.31
SP-97	Ac-QSQQTF%r8NL6clWRNleL%QN-NH2	2144.13	1073.07	1073.32
SP-98	Ac-QSQQTF%r8NLMe6clWRLL%QN-NH2	2158.15	1080.08	1080.31
SP-101	Ac-FNleYWEL-NH2	1160.63	-	1161.70
SP-102	Ac-F$r8AYWELL$A-NH2	1344.75	-	1345.90
SP-103	Ac-F$r8AYWQLL$A-NH2	1343.76	-	1344.83
SP-104	Ac-NlePRF$r8NYWELL$QN-NH2	1925.06	963.53	963.69
SP-105	Ac-NlePRF$r8DYWRLL$QN-NH2	1953.10	977.55	977.68
SP-106	Ac-NlePRF$r8NYWRLL$Q-NH2	1838.07	920.04	920.18
SP-107	Ac-NlePRF$r8NYWRLL$-NH2	1710.01	856.01	856.13
SP-108	Ac-QSQQTF$r8DLWRLL$QN-NH2	2109.14	1055.57	1055.64
SP-109	Ac-QSQQTF$r8NLWRLL$EN-NH2	2109.14	1055.57	1055.70
SP-110	Ac-QSQQTF$r8NLWRLL$QD-NH2	2109.14	1055.57	1055.64
SP-111	Ac-QSQQTF$r8NLWRLL$S-NH2	1953.08	977.54	977.60
SP-112	Ac-ESQQTF$r8NLWRLL$QN-NH2	2109.14	1055.57	1055.70
SP-113	Ac-LTF$r8NLWRNleL$Q-NH2	1635.99	819.00	819.10
SP-114	Ac-LRF$r8NLWRNleL$Q-NH2	1691.04	846.52	846.68
SP-115	Ac-QSQQTF$r8NWWRNleL$QN-NH2	2181.15	1091.58	1091.64
SP-116	Ac-QSQQTF$r8NLWRNleL$Q-NH2	1994.11	998.06	998.07
SP-117	Ac-QTF$r8NLWRNleL$QN-NH2	1765.00	883.50	883.59
SP-118	Ac-NlePRF$r8NWWRLL$QN-NH2	1975.13	988.57	988.75
SP-119	Ac-NlePRF$r8NWWRLL$A-NH2	1804.07	903.04	903.08
SP-120	Ac-TSFAEYWNLLSP-NH2	1467.70	734.85	734.90
SP-121	Ac-QTF$r8HWWSQL$S-NH2	1651.85	826.93	827.12
SP-122	Ac-FMYWEL-NH2	1178.58	-	1179.64
SP-123	Ac-QTFEHWWSQLLS-NH2	1601.76	801.88	801.94
SP-124	Ac-QSQQTF$r8NLAmwRLNle$QN-NH2	2122.17	1062.09	1062.24
SP-125	Ac-FMAibY6clWEAc3cL-NH2	1130.47	-	1131.53
SP-126	Ac-FNle$Y6clWE$L-NH2	1194.59	-	1195.64
SP-127	Ac-F$zr8AY6clWEAc3cL$z-NH2	1277.63	639.82	1278.71
SP-128	Ac-F$r8AY6clWEAc3cL$A-NH2	1348.66	-	1350.72

SP-129	Ac-NlePRF$r8NY6clWRLL$QN-NH2	1986.08	994.04	994.64
SP-130	Ac-AF$r8AAWALA$A-NH2	1223.71	-	1224.71
SP-131	Ac-TF$r8AAWRLA$Q-NH2	1395.80	698.90	399.04
SP-132	Pr-TF$r8AAWRLA$Q-NH2	1409.82	705.91	706.04
SP-133	Ac-QSQQTF%r8NLWRNleL%QN-NH2	2110.17	1056.09	1056.22
SP-134	Ac-LTF%r8HYWAQL%SA-NH2	1670.92	836.46	836.58
SP-135	Ac-NlePRF%r8NYWRLL%QN-NH2	1954.13	978.07	978.19
SP-136	Ac-NlePRF%r8NY6clWRLL%QN-NH2	1988.09	995.05	995.68
SP-137	Ac-LTF%r8HY6clWAQL%S-NH2	1633.84	817.92	817.93
SP-138	Ac-QS%QTF%StNLWRLL%s8QN-NH2	2149.24	1075.62	1075.65
SP-139	Ac-LTF%r8HY6clWRQL%S-NH2	1718.91	860.46	860.54
SP-140	Ac-QSQQTF%r8NL6clWRLL%QN-NH2	2144.13	1073.07	1073.64
SP-141	Ac-%r8SQQTFS%LWRLLAibQN-NH2	2040.15	1021.08	1021.13
SP-142	Ac-LTF%r8HYWAQL%S-NH2	1599.88	800.94	801.09
SP-143	Ac-TSF%r8QYWNLL%P-NH2	1602.88	802.44	802.58
SP-147	Ac-LTFEHYWAQLTS-NH2	1535.74	768.87	769.5
SP-152	Ac-F$er8AY6clWEAc3cL$e-NH2	1277.63	639.82	1278.71
SP-153	Ac-AF$r8AAWALA$A-NH2	1277.63	639.82	1277.84
SP-154	Ac-TF$r8AAWRLA$Q-NH2	1395.80	698.90	699.04
SP-155	Pr-TF$r8AAWRLA$Q-NH2	1409.82	705.91	706.04
SP-156	Ac-LTF$er8HYWAQL$eS-NH2	1597.87	799.94	800.44
SP-159	Ac-CCPGCCBaQSQQTF$r8NLWRLL$QN-NH2	2745.30	1373.65	1372.99
SP-160	Ac-CCPGCCBaQSQQTA$r8NLWRLL$QN-NH2	2669.27	1335.64	1336.09
SP-161	Ac-CCPGCCBaNlePRF$r8NYWRLL$QN-NH2	2589.26	1295.63	1296.2
SP-162	Ac-LTF$/r8HYWAQL$/S-NH2	1625.90	813.95	814.18
SP-163	Ac-F%r8HY6clWRAc3cL%-NH2	1372.72	687.36	687.59
SP-164	Ac-QTF%r8HWWSQL%S-NH2	1653.87	827.94	827.94
SP-165	Ac-LTA$r8HYWRQL$S-NH2	1606.90	804.45	804.66
SP-166	Ac-Q$r8QQTFSN$WRLLAibQN-NH2	2080.12	1041.06	1041.61
SP-167	Ac-QSQQ$r8FSNLWR$LAibQN-NH2	2066.11	1034.06	1034.58
SP-168	Ac-F$r8AYWEAc3cL$A-NH2	1314.70	658.35	1315.88
SP-169	Ac-F$r8AYWEAc3cL$S-NH2	1330.70	666.35	1331.87
SP-170	Ac-F$r8AYWEAc3cL$Q-NH2	1371.72	686.86	1372.72
SP-171	Ac-F$r8AYWEAibL$S-NH2	1332.71	667.36	1334.83
SP-172	Ac-F$r8AYWEAL$S-NH2	1318.70	660.35	1319.73
SP-173	Ac-F$r8AYWEQL$S-NH2	1375.72	688.86	1377.53
SP-174	Ac-F$r8HYWEQL$S-NH2	1441.74	721.87	1443.48
SP-175	Ac-F$r8HYWAQL$S-NH2	1383.73	692.87	1385.38
SP-176	Ac-F$r8HYWAAc3cL$S-NH2	1338.71	670.36	1340.82
SP-177	Ac-F$r8HYWRAc3cL$S-NH2	1423.78	712.89	713.04
SP-178	Ac-F$r8AYWEAc3cL#A-NH2	1300.69	651.35	1302.78
SP-179	Ac-NlePTF%r8NYWRLL%QN-NH2	1899.08	950.54	950.56
SP-180	Ac-TF$r8AAWRAL$Q-NH2	1395.80	698.90	699.13
SP-181	Ac-TSF%r8HYWAQL%S-NH2	1573.83	787.92	787.98
SP-184	Ac-F%r8AY6clWEAc3cL%A-NH2	1350.68	676.34	676.91
SP-185	Ac-LTF$r8HYWAQI$S-NH2	1597.87	799.94	800.07
SP-186	Ac-LTF$r8HYWAQNle$S-NH2	1597.87	799.94	800.07
SP-187	Ac-LTF$r8HYWAQL$A-NH2	1581.87	791.94	792.45
SP-188	Ac-LTF$r8HYWAQL$Abu-NH2	1595.89	798.95	799.03

SP-189	Ac-LTF$r8HYWAbuQL$S-NH2	1611.88	806.94	807.47
SP-190	Ac-LTF$er8AYWAQL$eS-NH2	1531.84	766.92	766.96
SP-191	Ac-LAF$r8HYWAQL$S-NH2	1567.86	784.93	785.49
SP-192	Ac-LAF$r8AYWAQL$S-NH2	1501.83	751.92	752.01
SP-193	Ac-LTF$er8AYWAQL$eA-NH2	1515.85	758.93	758.97
SP-194	Ac-LAF$r8AYWAQL$A-NH2	1485.84	743.92	744.05
SP-195	Ac-LTF$r8NLWANleL$Q-NH2	1550.92	776.46	776.61
SP-196	Ac-LTF$r8NLWANleL$A-NH2	1493.90	747.95	1495.6
SP-197	Ac-LTF$r8ALWANleL$Q-NH2	1507.92	754.96	755
SP-198	Ac-LAF$r8NLWANleL$Q-NH2	1520.91	761.46	761.96
SP-199	Ac-LAF$r8ALWANleL$A-NH2	1420.89	711.45	1421.74
SP-200	Ac-A$r8AYWEAc3cL$A-NH2	1238.67	620.34	1239.65
SP-201	Ac-F$r8AYWEAc3cL$AA-NH2	1385.74	693.87	1386.64
SP-202	Ac-F$r8AYWEAc3cL$Abu-NH2	1328.72	665.36	1330.17
SP-203	Ac-F$r8AYWEAc3cL$Nle-NH2	1356.75	679.38	1358.22
SP-204	Ac-F$r5AYWEAc3cL$s8A-NH2	1314.70	658.35	1315.51
SP-205	Ac-F$AYWEAc3cL$r8A-NH2	1314.70	658.35	1315.66
SP-206	Ac-F$r8AYWEAc3cI$A-NH2	1314.70	658.35	1316.18
SP-207	Ac-F$r8AYWEAc3cNle$A-NH2	1314.70	658.35	1315.66
SP-208	Ac-F$r8AYWEAmlL$A-NH2	1358.76	680.38	1360.21
SP-209	Ac-F$r8AYWENleL$A-NH2	1344.75	673.38	1345.71
SP-210	Ac-F$r8AYWQAc3cL$A-NH2	1313.72	657.86	1314.7
SP-211	Ac-F$r8AYWAAc3cL$A-NH2	1256.70	629.35	1257.56
SP-212	Ac-F$r8AYWAbuAc3cL$A-NH2	1270.71	636.36	1272.14
SP-213	Ac-F$r8AYWNleAc3cL$A-NH2	1298.74	650.37	1299.67
SP-214	Ac-F$r8AbuYWEAc3cL$A-NH2	1328.72	665.36	1329.65
SP-215	Ac-F$r8NleYWEAc3cL$A-NH2	1356.75	679.38	1358.66
SP-216	5-FAM-BaLTFEHYWAQLTS-NH2	1922.82	962.41	962.87
SP-217	5-FAM-BaLTF%r8HYWAQL%S-NH2	1986.96	994.48	994.97
SP-218	Ac-LTF$r8HYWAQhL$S-NH2	1611.88	806.94	807
SP-219	Ac-LTF$r8HYWAQTle$S-NH2	1597.87	799.94	799.97
SP-220	Ac-LTF$r8HYWAQAdm$S-NH2	1675.91	838.96	839.09
SP-221	Ac-LTF$r8HYWAQhCha$S-NH2	1651.91	826.96	826.98
SP-222	Ac-LTF$r8HYWAQCha$S-NH2	1637.90	819.95	820.02
SP-223	Ac-LTF$r8HYWAc6cQL$S-NH2	1651.91	826.96	826.98
SP-224	Ac-LTF$r8HYWAc5cQL$S-NH2	1637.90	819.95	820.02
SP-225	Ac-LThF$r8HYWAQL$S-NH2	1611.88	806.94	807
SP-226	Ac-LTIgl$r8HYWAQL$S-NH2	1625.90	813.95	812.99
SP-227	Ac-LTF$r8HYWAQChg$S-NH2	1623.88	812.94	812.99
SP-228	Ac-LTF$r8HYWAQF$S-NH2	1631.85	816.93	816.99
SP-229	Ac-LTF$r8HYWAQIgl$S-NH2	1659.88	830.94	829.94
SP-230	Ac-LTF$r8HYWAQCba$S-NH2	1609.87	805.94	805.96
SP-231	Ac-LTF$r8HYWAQCpg$S-NH2	1609.87	805.94	805.96
SP-232	Ac-LTF$r8HhYWAQL$S-NH2	1611.88	806.94	807
SP-233	Ac-F$r8AYWEAc3chL$A-NH2	1328.72	665.36	665.43
SP-234	Ac-F$r8AYWEAc3cTle$A-NH2	1314.70	658.35	1315.62
SP-235	Ac-F$r8AYWEAc3cAdm$A-NH2	1392.75	697.38	697.47
SP-236	Ac-F$r8AYWEAc3chCha$A-NH2	1368.75	685.38	685.34
SP-237	Ac-F$r8AYWEAc3cCha$A-NH2	1354.73	678.37	678.38
SP-238	Ac-F$r8AYWEAc6cL$A-NH2	1356.75	679.38	679.42

SP-239	Ac-F$r8AYWEAc5cL$A-NH2	1342.73	672.37	672.46
SP-240	Ac-hF$r8AYWEAc3cL$A-NH2	1328.72	665.36	665.43
SP-241	Ac-Igl$r8AYWEAc3cL$A-NH2	1342.73	672.37	671.5
SP-243	Ac-F$r8AYWEAc3cF$A-NH2	1348.69	675.35	675.35
SP-244	Ac-F$r8AYWEAc3cIgl$A-NH2	1376.72	689.36	688.37
SP-245	Ac-F$r8AYWEAc3cCba$A-NH2	1326.70	664.35	664.47
SP-246	Ac-F$r8AYWEAc3cCpg$A-NH2	1326.70	664.35	664.39
SP-247	Ac-F$r8AhYWEAc3cL$A-NH2	1328.72	665.36	665.43
SP-248	Ac-F$r8AYWEAc3cL$Q-NH2	1371.72	686.86	1372.87
SP-249	Ac-F$r8AYWEAibL$A-NH2	1316.72	659.36	1318.18
SP-250	Ac-F$r8AYWEAL$A-NH2	1302.70	652.35	1303.75
SP-251	Ac-LAF$r8AYWAAL$A-NH2	1428.82	715.41	715.49
SP-252	Ac-LTF$r8HYWAAc3cL$S-NH2	1552.84	777.42	777.5
SP-253	Ac-NleTF$r8HYWAQL$S-NH2	1597.87	799.94	800.04
SP-254	Ac-VTF$r8HYWAQL$S-NH2	1583.85	792.93	793.04
SP-255	Ac-FTF$r8HYWAQL$S-NH2	1631.85	816.93	817.02
SP-256	Ac-WTF$r8HYWAQL$S-NH2	1670.86	836.43	836.85
SP-257	Ac-RTF$r8HYWAQL$S-NH2	1640.88	821.44	821.9
SP-258	Ac-KTF$r8HYWAQL$S-NH2	1612.88	807.44	807.91
SP-259	Ac-LNleF$r8HYWAQL$S-NH2	1609.90	805.95	806.43
SP-260	Ac-LVF$r8HYWAQL$S-NH2	1595.89	798.95	798.93
SP-261	Ac-LFF$r8HYWAQL$S-NH2	1643.89	822.95	823.38
SP-262	Ac-LWF$r8HYWAQL$S-NH2	1682.90	842.45	842.55
SP-263	Ac-LRF$r8HYWAQL$S-NH2	1652.92	827.46	827.52
SP-264	Ac-LKF$r8HYWAQL$S-NH2	1624.91	813.46	813.51
SP-265	Ac-LTF$r8NleYWAQL$S-NH2	1573.89	787.95	788.05
SP-266	Ac-LTF$r8VYWAQL$S-NH2	1559.88	780.94	780.98
SP-267	Ac-LTF$r8FYWAQL$S-NH2	1607.88	804.94	805.32
SP-268	Ac-LTF$r8WYWAQL$S-NH2	1646.89	824.45	824.86
SP-269	Ac-LTF$r8RYWAQL$S-NH2	1616.91	809.46	809.51
SP-270	Ac-LTF$r8KYWAQL$S-NH2	1588.90	795.45	795.48
SP-271	Ac-LTF$r8HNleWAQL$S-NH2	1547.89	774.95	774.98
SP-272	Ac-LTF$r8HVWAQL$S-NH2	1533.87	767.94	767.95
SP-273	Ac-LTF$r8HFWAQL$S-NH2	1581.87	791.94	792.3
SP-274	Ac-LTF$r8HWWAQL$S-NH2	1620.88	811.44	811.54
SP-275	Ac-LTF$r8HRWAQL$S-NH2	1590.90	796.45	796.52
SP-276	Ac-LTF$r8HKWAQL$S-NH2	1562.90	782.45	782.53
SP-277	Ac-LTF$r8HYWNleQL$S-NH2	1639.91	820.96	820.98
SP-278	Ac-LTF$r8HYWVQL$S-NH2	1625.90	813.95	814.03
SP-279	Ac-LTF$r8HYWFQL$S-NH2	1673.90	837.95	838.03
SP-280	Ac-LTF$r8HYWWQL$S-NH2	1712.91	857.46	857.5
SP-281	Ac-LTF$r8HYWKQL$S-NH2	1654.92	828.46	828.49
SP-282	Ac-LTF$r8HYWANleL$S-NH2	1582.89	792.45	792.52
SP-283	Ac-LTF$r8HYWAVL$S-NH2	1568.88	785.44	785.49
SP-284	Ac-LTF$r8HYWAFL$S-NH2	1616.88	809.44	809.47
SP-285	Ac-LTF$r8HYWAWL$S-NH2	1655.89	828.95	829
SP-286	Ac-LTF$r8HYWARL$S-NH2	1625.91	813.96	813.98
SP-287	Ac-LTF$r8HYWAQL$Nle-NH2	1623.92	812.96	813.39
SP-288	Ac-LTF$r8HYWAQL$V-NH2	1609.90	805.95	805.99
SP-289	Ac-LTF$r8HYWAQL$F-NH2	1657.90	829.95	830.26

SP-290	Ac-LTF$r8HYWAQL$W-NH2	1696.91	849.46	849.5
SP-291	Ac-LTF$r8HYWAQL$R-NH2	1666.94	834.47	834.56
SP-292	Ac-LTF$r8HYWAQL$K-NH2	1638.93	820.47	820.49
SP-293	Ac-Q$r8QQTFSN$WRLLAibQN-NH2	2080.12	1041.06	1041.54
SP-294	Ac-QSQQ$r8FSNLWR$LAibQN-NH2	2066.11	1034.06	1034.58
SP-295	Ac-LT2Pal$r8HYWAQL$S-NH2	1598.86	800.43	800.49
SP-296	Ac-LT3Pal$r8HYWAQL$S-NH2	1598.86	800.43	800.49
SP-297	Ac-LT4Pal$r8HYWAQL$S-NH2	1598.86	800.43	800.49
SP-298	Ac-LTF2CF3$r8HYWAQL$S-NH2	1665.85	833.93	834.01
SP-299	Ac-LTF2CN$r8HYWAQL$S-NH2	1622.86	812.43	812.47
SP-300	Ac-LTF2Me$r8HYWAQL$S-NH2	1611.88	806.94	807
SP-301	Ac-LTF3Cl$r8HYWAQL$S-NH2	1631.83	816.92	816.99
SP-302	Ac-LTF4CF3$r8HYWAQL$S-NH2	1665.85	833.93	833.94
SP-303	Ac-LTF4tBu$r8HYWAQL$S-NH2	1653.93	827.97	828.02
SP-304	Ac-LTF5F$r8HYWAQL$S-NH2	1687.82	844.91	844.96
SP-305	Ac-LTF$r8HY3BthAAQL$S-NH2	1614.83	808.42	808.48
SP-306	Ac-LTF2Br$r8HYWAQL$S-NH2	1675.78	838.89	838.97
SP-307	Ac-LTF4Br$r8HYWAQL$S-NH2	1675.78	838.89	839.86
SP-308	Ac-LTF2Cl$r8HYWAQL$S-NH2	1631.83	816.92	816.99
SP-309	Ac-LTF4Cl$r8HYWAQL$S-NH2	1631.83	816.92	817.36
SP-310	Ac-LTF3CN$r8HYWAQL$S-NH2	1622.86	812.43	812.47
SP-311	Ac-LTF4CN$r8HYWAQL$S-NH2	1622.86	812.43	812.47
SP-312	Ac-LTF34Cl2$r8HYWAQL$S-NH2	1665.79	833.90	833.94
SP-313	Ac-LTF34F2$r8HYWAQL$S-NH2	1633.85	817.93	817.95
SP-314	Ac-LTF35F2$r8HYWAQL$S-NH2	1633.85	817.93	817.95
SP-315	Ac-LTDip$r8HYWAQL$S-NH2	1673.90	837.95	838.01
SP-316	Ac-LTF2F$r8HYWAQL$S-NH2	1615.86	808.93	809
SP-317	Ac-LTF3F$r8HYWAQL$S-NH2	1615.86	808.93	809
SP-318	Ac-LTF4F$r8HYWAQL$S-NH2	1615.86	808.93	809
SP-319	Ac-LTF4I$r8HYWAQL$S-NH2	1723.76	862.88	862.94
SP-320	Ac-LTF3Me$r8HYWAQL$S-NH2	1611.88	806.94	807.07
SP-321	Ac-LTF4Me$r8HYWAQL$S-NH2	1611.88	806.94	807
SP-322	Ac-LT1Nal$r8HYWAQL$S-NH2	1647.88	824.94	824.98
SP-323	Ac-LT2Nal$r8HYWAQL$S-NH2	1647.88	824.94	825.06
SP-324	Ac-LTF3CF3$r8HYWAQL$S-NH2	1665.85	833.93	834.01
SP-325	Ac-LTF4NO2$r8HYWAQL$S-NH2	1642.85	822.43	822.46
SP-326	Ac-LTF3NO2$r8HYWAQL$S-NH2	1642.85	822.43	822.46
SP-327	Ac-LTF$r82ThiYWAQL$S-NH2	1613.83	807.92	807.96
SP-328	Ac-LTF$r8HBipWAQL$S-NH2	1657.90	829.95	830.01
SP-329	Ac-LTF$r8HF4tBuWAQL$S-NH2	1637.93	819.97	820.02
SP-330	Ac-LTF$r8HF4CF3WAQL$S-NH2	1649.86	825.93	826.02
SP-331	Ac-LTF$r8HF4ClWAQL$S-NH2	1615.83	808.92	809.37
SP-332	Ac-LTF$r8HF4MeWAQL$S-NH2	1595.89	798.95	799.01
SP-333	Ac-LTF$r8HF4BrWAQL$S-NH2	1659.78	830.89	830.98
SP-334	Ac-LTF$r8HF4CNWAQL$S-NH2	1606.87	804.44	804.56
SP-335	Ac-LTF$r8HF4NO2WAQL$S-NH2	1626.86	814.43	814.55
SP-336	Ac-LTF$r8H1NalWAQL$S-NH2	1631.89	816.95	817.06
SP-337	Ac-LTF$r8H2NalWAQL$S-NH2	1631.89	816.95	816.99
SP-338	Ac-LTF$r8HWAQL$S-NH2	1434.80	718.40	718.49
SP-339	Ac-LTF$r8HY1NalAQL$S-NH2	1608.87	805.44	805.52

SP-340	Ac-LTF$r8HY2NalAQL$S-NH2	1608.87	805.44	805.52
SP-341	Ac-LTF$r8HYWAQI$S-NH2	1597.87	799.94	800.07
SP-342	Ac-LTF$r8HYWAQNle$S-NH2	1597.87	799.94	800.44
SP-343	Ac-LTF$er8HYWAQL$eA-NH2	1581.87	791.94	791.98
SP-344	Ac-LTF$r8HYWAQL$Abu-NH2	1595.89	798.95	799.03
SP-345	Ac-LTF$r8HYWAbuQL$S-NH2	1611.88	806.94	804.47
SP-346	Ac-LAF$r8HYWAQL$S-NH2	1567.86	784.93	785.49
SP-347	Ac-LTF$r8NLWANleL$Q-NH2	1550.92	776.46	777.5
SP-348	Ac-LTF$r8ALWANleL$Q-NH2	1507.92	754.96	755.52
SP-349	Ac-LAF$r8NLWANleL$Q-NH2	1520.91	761.46	762.48
SP-350	Ac-F$r8AYWAAc3cL$A-NH2	1256.70	629.35	1257.56
SP-351	Ac-LTF$r8AYWAAL$S-NH2	1474.82	738.41	738.55
SP-352	Ac-LVF$r8AYWAQL$S-NH2	1529.87	765.94	766
SP-353	Ac-LTF$r8AYWAbuQL$S-NH2	1545.86	773.93	773.92
SP-354	Ac-LTF$r8AYWNleQL$S-NH2	1573.89	787.95	788.17
SP-355	Ac-LTF$r8AbuYWAQL$S-NH2	1545.86	773.93	773.99
SP-356	Ac-LTF$r8AYWHQL$S-NH2	1597.87	799.94	799.97
SP-357	Ac-LTF$r8AYWKQL$S-NH2	1588.90	795.45	795.53
SP-358	Ac-LTF$r8AYWOQL$S-NH2	1574.89	788.45	788.5
SP-359	Ac-LTF$r8AYWRQL$S-NH2	1616.91	809.46	809.51
SP-360	Ac-LTF$r8AYWSQL$S-NH2	1547.84	774.92	774.96
SP-361	Ac-LTF$r8AYWRAL$S-NH2	1559.89	780.95	780.95
SP-362	Ac-LTF$r8AYWRQL$A-NH2	1600.91	801.46	801.52
SP-363	Ac-LTF$r8AYWRAL$A-NH2	1543.89	772.95	773.03
SP-364	Ac-LTF$r5HYWAQL$s8S-NH2	1597.87	799.94	799.97
SP-365	Ac-LTF$HYWAQL$r8S-NH2	1597.87	799.94	799.97
SP-366	Ac-LTF$r8HYWAAL$S-NH2	1540.84	771.42	771.48
SP-367	Ac-LTF$r8HYWAAbuL$S-NH2	1554.86	778.43	778.51
SP-368	Ac-LTF$r8HYWALL$S-NH2	1582.89	792.45	792.49
SP-369	Ac-F$r8AYWHAL$A-NH2	1310.72	656.36	656.4
SP-370	Ac-F$r8AYWAAL$A-NH2	1244.70	623.35	1245.61
SP-371	Ac-F$r8AYWSAL$A-NH2	1260.69	631.35	1261.6
SP-372	Ac-F$r8AYWRAL$A-NH2	1329.76	665.88	1330.72
SP-373	Ac-F$r8AYWKAL$A-NH2	1301.75	651.88	1302.67
SP-374	Ac-F$r8AYWOAL$A-NH2	1287.74	644.87	1289.13
SP-375	Ac-F$r8VYWEAc3cL$A-NH2	1342.73	672.37	1343.67
SP-376	Ac-F$r8FYWEAc3cL$A-NH2	1390.73	696.37	1392.14
SP-377	Ac-F$r8WYWEAc3cL$A-NH2	1429.74	715.87	1431.44
SP-378	Ac-F$r8RYWEAc3cL$A-NH2	1399.77	700.89	700.95
SP-379	Ac-F$r8KYWEAc3cL$A-NH2	1371.76	686.88	686.97
SP-380	Ac-F$r8ANleWEAc3cL$A-NH2	1264.72	633.36	1265.59
SP-381	Ac-F$r8AVWEAc3cL$A-NH2	1250.71	626.36	1252.2
SP-382	Ac-F$r8AFWEAc3cL$A-NH2	1298.71	650.36	1299.64
SP-383	Ac-F$r8AWWEAc3cL$A-NH2	1337.72	669.86	1338.64
SP-384	Ac-F$r8ARWEAc3cL$A-NH2	1307.74	654.87	655
SP-385	Ac-F$r8AKWEAc3cL$A-NH2	1279.73	640.87	641.01
SP-386	Ac-F$r8AYWVAc3cL$A-NH2	1284.73	643.37	643.38
SP-387	Ac-F$r8AYWFAc3cL$A-NH2	1332.73	667.37	667.43
SP-388	Ac-F$r8AYWWAc3cL$A-NH2	1371.74	686.87	686.97
SP-389	Ac-F$r8AYWRAc3cL$A-NH2	1341.76	671.88	671.94

SP-390	Ac-F$r8AYWKAc3cL$A-NH2	1313.75	657.88	657.88
SP-391	Ac-F$r8AYWEVL$A-NH2	1330.73	666.37	666.47
SP-392	Ac-F$r8AYWEFL$A-NH2	1378.73	690.37	690.44
SP-393	Ac-F$r8AYWEWL$A-NH2	1417.74	709.87	709.91
SP-394	Ac-F$r8AYWERL$A-NH2	1387.77	694.89	1388.66
SP-395	Ac-F$r8AYWEKL$A-NH2	1359.76	680.88	1361.21
SP-396	Ac-F$r8AYWEAc3cL$V-NH2	1342.73	672.37	1343.59
SP-397	Ac-F$r8AYWEAc3cL$F-NH2	1390.73	696.37	1392.58
SP-398	Ac-F$r8AYWEAc3cL$W-NH2	1429.74	715.87	1431.29
SP-399	Ac-F$r8AYWEAc3cL$R-NH2	1399.77	700.89	700.95
SP-400	Ac-F$r8AYWEAc3cL$K-NH2	1371.76	686.88	686.97
SP-401	Ac-F$r8AYWEAc3cL$AV-NH2	1413.77	707.89	707.91
SP-402	Ac-F$r8AYWEAc3cL$AF-NH2	1461.77	731.89	731.96
SP-403	Ac-F$r8AYWEAc3cL$AW-NH2	1500.78	751.39	751.5
SP-404	Ac-F$r8AYWEAc3cL$AR-NH2	1470.80	736.40	736.47
SP-405	Ac-F$r8AYWEAc3cL$AK-NH2	1442.80	722.40	722.41
SP-406	Ac-F$r8AYWEAc3cL$AH-NH2	1451.76	726.88	726.93
SP-407	Ac-LTF2NO2$r8HYWAQL$S-NH2	1642.85	822.43	822.54
SP-408	Ac-LTA$r8HYAAQL$S-NH2	1406.79	704.40	704.5
SP-409	Ac-LTF$r8HYAAQL$S-NH2	1482.82	742.41	742.47
SP-410	Ac-QSQQTF$r8NLWALL$AN-NH2	1966.07	984.04	984.38
SP-411	Ac-QAibQQTF$r8NLWALL$AN-NH2	1964.09	983.05	983.42
SP-412	Ac-QAibQQTF$r8ALWALL$AN-NH2	1921.08	961.54	961.59
SP-413	Ac-AAAATF$r8AAWAAL$AA-NH2	1608.90	805.45	805.52
SP-414	Ac-F$r8AAWRAL$Q-NH2	1294.76	648.38	648.48
SP-415	Ac-TF$r8AAWAAL$Q-NH2	1310.74	656.37	1311.62
SP-416	Ac-TF$r8AAWRAL$A-NH2	1338.78	670.39	670.46
SP-417	Ac-VF$r8AAWRAL$Q-NH2	1393.82	697.91	697.99
SP-418	Ac-AF$r8AAWAAL$A-NH2	1223.71	612.86	1224.67
SP-420	Ac-TF$r8AAWKAL$Q-NH2	1367.80	684.90	684.97
SP-421	Ac-TF$r8AAWOAL$Q-NH2	1353.78	677.89	678.01
SP-422	Ac-TF$r8AAWSAL$Q-NH2	1326.73	664.37	664.47
SP-423	Ac-LTF$r8AAWRAL$Q-NH2	1508.89	755.45	755.49
SP-424	Ac-F$r8AYWAQL$A-NH2	1301.72	651.86	651.96
SP-425	Ac-F$r8AWWAAL$A-NH2	1267.71	634.86	634.87
SP-426	Ac-F$r8AWWAQL$A-NH2	1324.73	663.37	663.43
SP-427	Ac-F$r8AYWEAL$-NH2	1231.66	616.83	1232.93
SP-428	Ac-F$r8AYWAAL$-NH2	1173.66	587.83	1175.09
SP-429	Ac-F$r8AYWKAL$-NH2	1230.72	616.36	616.44
SP-430	Ac-F$r8AYWOAL$-NH2	1216.70	609.35	609.48
SP-431	Ac-F$r8AYWQAL$-NH2	1230.68	616.34	616.44
SP-432	Ac-F$r8AYWAQL$-NH2	1230.68	616.34	616.37
SP-433	Ac-F$r8HYWDQL$S-NH2	1427.72	714.86	714.86
SP-434	Ac-F$r8HFWEQL$S-NH2	1425.74	713.87	713.98
SP-435	Ac-F$r8AYWHQL$S-NH2	1383.73	692.87	692.96
SP-436	Ac-F$r8AYWKQL$S-NH2	1374.77	688.39	688.45
SP-437	Ac-F$r8AYWOQL$S-NH2	1360.75	681.38	681.49
SP-438	Ac-F$r8HYWSQL$S-NH2	1399.73	700.87	700.95
SP-439	Ac-F$r8HWWEQL$S-NH2	1464.76	733.38	733.44
SP-440	Ac-F$r8HWWAQL$S-NH2	1406.75	704.38	704.43

SP-441	Ac-F$r8AWWHQL$S-NH2	1406.75	704.38	704.43
SP-442	Ac-F$r8AWWKQL$S-NH2	1397.79	699.90	699.92
SP-443	Ac-F$r8AWWOQL$S-NH2	1383.77	692.89	692.96
SP-444	Ac-F$r8HWWSQL$S-NH2	1422.75	712.38	712.42
SP-445	Ac-LTF$r8NYWANleL$Q-NH2	1600.90	801.45	801.52
SP-446	Ac-LTF$r8NLWAQL$Q-NH2	1565.90	783.95	784.06
SP-447	Ac-LTF$r8NYWANleL$A-NH2	1543.88	772.94	773.03
SP-448	Ac-LTF$r8NLWAQL$A-NH2	1508.88	755.44	755.49
SP-449	Ac-LTF$r8AYWANleL$Q-NH2	1557.90	779.95	780.06
SP-450	Ac-LTF$r8ALWAQL$Q-NH2	1522.89	762.45	762.45
SP-451	Ac-LAF$r8NYWANleL$Q-NH2	1570.89	786.45	786.5
SP-452	Ac-LAF$r8NLWAQL$Q-NH2	1535.89	768.95	769.03
SP-453	Ac-LAF$r8AYWANleL$A-NH2	1470.86	736.43	736.47
SP-454	Ac-LAF$r8ALWAQL$A-NH2	1435.86	718.93	719.01
SP-455	Ac-LAF$r8AYWAAL$A-NH2	1428.82	715.41	715.41
SP-456	Ac-F$r8AYWEAc3cL$AAib-NH2	1399.75	700.88	700.95
SP-457	Ac-F$r8AYWAQL$AA-NH2	1372.75	687.38	687.78
SP-458	Ac-F$r8AYWAAc3cL$AA-NH2	1327.73	664.87	664.84
SP-459	Ac-F$r8AYWSAc3cL$AA-NH2	1343.73	672.87	672.9
SP-460	Ac-F$r8AYWEAc3cL$AS-NH2	1401.73	701.87	701.84
SP-461	Ac-F$r8AYWEAc3cL$AT-NH2	1415.75	708.88	708.87
SP-462	Ac-F$r8AYWEAc3cL$AL-NH2	1427.79	714.90	714.94
SP-463	Ac-F$r8AYWEAc3cL$AQ-NH2	1442.76	722.38	722.41
SP-464	Ac-F$r8AFWEAc3cL$AA-NH2	1369.74	685.87	685.93
SP-465	Ac-F$r8AWWEAc3cL$AA-NH2	1408.75	705.38	705.39
SP-466	Ac-F$r8AYWEAc3cL$SA-NH2	1401.73	701.87	701.99
SP-467	Ac-F$r8AYWEAL$AA-NH2	1373.74	687.87	687.93
SP-468	Ac-F$r8AYWENleL$AA-NH2	1415.79	708.90	708.94
SP-469	Ac-F$r8AYWEAc3cL$AbuA-NH2	1399.75	700.88	700.95
SP-470	Ac-F$r8AYWEAc3cL$NleA-NH2	1427.79	714.90	714.86
SP-471	Ac-F$r8AYWEAibL$NleA-NH2	1429.80	715.90	715.97
SP-472	Ac-F$r8AYWEAL$NleA-NH2	1415.79	708.90	708.94
SP-473	Ac-F$r8AYWENleL$NleA-NH2	1457.83	729.92	729.96
SP-474	Ac-F$r8AYWEAibL$Abu-NH2	1330.73	666.37	666.39
SP-475	Ac-F$r8AYWENleL$Abu-NH2	1358.76	680.38	680.39
SP-476	Ac-F$r8AYWEAL$Abu-NH2	1316.72	659.36	659.36
SP-477	Ac-LTF$r8AFWAQL$S-NH2	1515.85	758.93	759.12
SP-478	Ac-LTF$r8AWWAQL$S-NH2	1554.86	778.43	778.51
SP-479	Ac-LTF$r8AYWAQI$S-NH2	1531.84	766.92	766.96
SP-480	Ac-LTF$r8AYWAQNle$S-NH2	1531.84	766.92	766.96
SP-481	Ac-LTF$r8AYWAQL$SA-NH2	1602.88	802.44	802.48
SP-482	Ac-LTF$r8AWWAQL$A-NH2	1538.87	770.44	770.89
SP-483	Ac-LTF$r8AYWAQI$A-NH2	1515.85	758.93	759.42
SP-484	Ac-LTF$r8AYWAQNle$A-NH2	1515.85	758.93	759.42
SP-485	Ac-LTF$r8AYWAQL$AA-NH2	1586.89	794.45	794.94
SP-486	Ac-LTF$r8HWWAQL$S-NH2	1620.88	811.44	811.47
SP-487	Ac-LTF$r8HRWAQL$S-NH2	1590.90	796.45	796.52
SP-488	Ac-LTF$r8HKWAQL$S-NH2	1562.90	782.45	782.53
SP-489	Ac-LTF$r8HYWAQL$W-NH2	1696.91	849.46	849.5
SP-491	Ac-F$r8AYWAbuAL$A-NH2	1258.71	630.36	630.5

SP-492	Ac-F$r8AbuYWEAL$A-NH2	1316.72	659.36	659.51
SP-493	Ac-NlePRF%r8NYWRLL%QN-NH2	1954.13	978.07	978.54
SP-494	Ac-TSF%r8HYWAQL%S-NH2	1573.83	787.92	787.98
SP-495	Ac-LTF%r8AYWAQL%S-NH2	1533.86	767.93	768
SP-496	Ac-HTF$r8HYWAQL$S-NH2	1621.84	811.92	811.96
SP-497	Ac-LHF$r8HYWAQL$S-NH2	1633.88	817.94	818.02
SP-498	Ac-LTF$r8HHWAQL$S-NH2	1571.86	786.93	786.94
SP-499	Ac-LTF$r8HYWHQL$S-NH2	1663.89	832.95	832.38
SP-500	Ac-LTF$r8HYWAHL$S-NH2	1606.87	804.44	804.48
SP-501	Ac-LTF$r8HYWAQL$H-NH2	1647.89	824.95	824.98
SP-502	Ac-LTF$r8HYWAQL$S-NHPr	1639.91	820.96	820.98
SP-503	Ac-LTF$r8HYWAQL$S-NHsBu	1653.93	827.97	828.02
SP-504	Ac-LTF$r8HYWAQL$S-NHiBu	1653.93	827.97	828.02
SP-505	Ac-LTF$r8HYWAQL$S-NHBn	1687.91	844.96	844.44
SP-506	Ac-LTF$r8HYWAQL$S-NHPe	1700.92	851.46	851.99
SP-507	Ac-LTF$r8HYWAQL$S-NHChx	1679.94	840.97	841.04
SP-508	Ac-ETF$r8AYWAQL$S-NH2	1547.80	774.90	774.96
SP-509	Ac-STF$r8AYWAQL$S-NH2	1505.79	753.90	753.94
SP-510	Ac-LEF$r8AYWAQL$S-NH2	1559.84	780.92	781.25
SP-511	Ac-LSF$r8AYWAQL$S-NH2	1517.83	759.92	759.93
SP-512	Ac-LTF$r8EYWAQL$S-NH2	1589.85	795.93	795.97
SP-513	Ac-LTF$r8SYWAQL$S-NH2	1547.84	774.92	774.96
SP-514	Ac-LTF$r8AYWEQL$S-NH2	1589.85	795.93	795.9
SP-515	Ac-LTF$r8AYWAEL$S-NH2	1532.83	767.42	766.96
SP-516	Ac-LTF$r8AYWASL$S-NH2	1490.82	746.41	746.46
SP-517	Ac-LTF$r8AYWAQL$E-NH2	1573.85	787.93	787.98
SP-518	Ac-LTF2CN$r8HYWAQL$S-NH2	1622.86	812.43	812.47
SP-519	Ac-LTF3Cl$r8HYWAQL$S-NH2	1631.83	816.92	816.99
SP-520	Ac-LTDip$r8HYWAQL$S-NH2	1673.90	837.95	838.01
SP-521	Ac-LTF$r8HYWAQTle$S-NH2	1597.87	799.94	800.04
SP-522	Ac-F$r8AY6clWEAL$A-NH2	1336.66	669.33	1338.56
SP-523	Ac-F$r8AYdl6brWEAL$A-NH2	1380.61	691.31	692.2
SP-524	Ac-F$r8AYdl6fWEAL$A-NH2	1320.69	661.35	1321.61
SP-525	Ac-F$r8AYdl4mWEAL$A-NH2	1316.72	659.36	659.36
SP-526	Ac-F$r8AYdl5clWEAL$A-NH2	1336.66	669.33	669.35
SP-527	Ac-F$r8AYdl7mWEAL$A-NH2	1316.72	659.36	659.36
SP-528	Ac-LTF%r8HYWAQL%A-NH2	1583.89	792.95	793.01
SP-529	Ac-LTF$r8HCouWAQL$S-NH2	1679.87	840.94	841.38
SP-530	Ac-LTFEHCouWAQLTS-NH2	1617.75	809.88	809.96
SP-531	Ac-LTA$r8HCouWAQL$S-NH2	1603.84	802.92	803.36
SP-532	Ac-F$r8AYWEAL$AbuA-NH2	1387.75	694.88	694.88
SP-533	Ac-F$r8AYWEAI$AA-NH2	1373.74	687.87	687.93
SP-534	Ac-F$r8AYWEANle$AA-NH2	1373.74	687.87	687.93
SP-535	Ac-F$r8AYWEAmlL$AA-NH2	1429.80	715.90	715.97
SP-536	Ac-F$r8AYWQAL$AA-NH2	1372.75	687.38	687.48
SP-537	Ac-F$r8AYWAAL$AA-NH2	1315.73	658.87	658.92
SP-538	Ac-F$r8AYWAbuAL$AA-NH2	1329.75	665.88	665.95
SP-539	Ac-F$r8AYWNleAL$AA-NH2	1357.78	679.89	679.94
SP-540	Ac-F$r8AbuYWEAL$AA-NH2	1387.75	694.88	694.96
SP-541	Ac-F$r8NleYWEAL$AA-NH2	1415.79	708.90	708.94

SP-542	Ac-F$r8FYWEAL$AA-NH2	1449.77	725.89	725.97
SP-543	Ac-LTF$r8HYWAQhL$S-NH2	1611.88	806.94	807
SP-544	Ac-LTF$r8HYWAQAdm$S-NH2	1675.91	838.96	839.04
SP-545	Ac-LTF$r8HYWAQIgl$S-NH2	1659.88	830.94	829.94
SP-546	Ac-F$r8AYWAQL$AA-NH2	1372.75	687.38	687.48
SP-547	Ac-LTF$r8ALWAQL$Q-NH2	1522.89	762.45	762.52
SP-548	Ac-F$r8AYWEAL$AA-NH2	1373.74	687.87	687.93
SP-549	Ac-F$r8AYWENleL$AA-NH2	1415.79	708.90	708.94
SP-550	Ac-F$r8AYWEAibL$Abu-NH2	1330.73	666.37	666.39
SP-551	Ac-F$r8AYWENleL$Abu-NH2	1358.76	680.38	680.38
SP-552	Ac-F$r8AYWEAL$Abu-NH2	1316.72	659.36	659.36
SP-553	Ac-F$r8AYWEAc3cL$AbuA-NH2	1399.75	700.88	700.95
SP-554	Ac-F$r8AYWEAc3cL$NleA-NH2	1427.79	714.90	715.01
SP-555	H-LTF$r8AYWAQL$S-NH2	1489.83	745.92	745.95
SP-556	mdPEG3-LTF$r8AYWAQL$S-NH2	1679.92	840.96	840.97
SP-557	mdPEG7-LTF$r8AYWAQL$S-NH2	1856.02	929.01	929.03
SP-558	Ac-F$r8ApmpEt6clWEAL$A-NH2	1470.71	736.36	788.17
SP-559	Ac-LTF3Cl$r8AYWAQL$S-NH2	1565.81	783.91	809.18
SP-560	Ac-LTF3Cl$r8HYWAQL$A-NH2	1615.83	808.92	875.24
SP-561	Ac-LTF3Cl$r8HYWWQL$S-NH2	1746.87	874.44	841.65
SP-562	Ac-LTF3Cl$r8AYWWQL$S-NH2	1680.85	841.43	824.63
SP-563	Ac-LTF$r8AYWWQL$S-NH2	1646.89	824.45	849.98
SP-564	Ac-LTF$r8HYWWQL$A-NH2	1696.91	849.46	816.67
SP-565	Ac-LTF$r8AYWWQL$A-NH2	1630.89	816.45	776.15
SP-566	Ac-LTF4F$r8AYWAQL$S-NH2	1549.83	775.92	776.15
SP-567	Ac-LTF2F$r8AYWAQL$S-NH2	1549.83	775.92	776.15
SP-568	Ac-LTF3F$r8AYWAQL$S-NH2	1549.83	775.92	785.12
SP-569	Ac-LTF34F2$r8AYWAQL$S-NH2	1567.83	784.92	785.12
SP-570	Ac-LTF35F2$r8AYWAQL$S-NH2	1567.83	784.92	1338.74
SP-571	Ac-F3Cl$r8AYWEAL$A-NH2	1336.66	669.33	705.28
SP-572	Ac-F3Cl$r8AYWEAL$AA-NH2	1407.70	704.85	680.11
SP-573	Ac-F$r8AY6clWEAL$AA-NH2	1407.70	704.85	736.83
SP-574	Ac-F$r8AY6clWEAL$-NH2	1265.63	633.82	784.1
SP-575	Ac-LTF$r8HYWAQLSt/S-NH2	16.03	9.02	826.98
SP-576	Ac-LTF$r8HYWAQL$S-NHsBu	1653.93	827.97	828.02
SP-577	Ac-STF$r8AYWAQL$S-NH2	1505.79	753.90	753.94
SP-578	Ac-LTF$r8AYWAEL$S-NH2	1532.83	767.42	767.41
SP-579	Ac-LTF$r8AYWAQL$E-NH2	1573.85	787.93	787.98
SP-580	mdPEG3-LTF$r8AYWAQL$S-NH2	1679.92	840.96	840.97
SP-581	Ac-LTF$r8AYWAQhL$S-NH2	1545.86	773.93	774.31
SP-583	Ac-LTF$r8AYWAQCha$S-NH2	1571.88	786.94	787.3
SP-584	Ac-LTF$r8AYWAQChg$S-NH2	1557.86	779.93	780.4
SP-585	Ac-LTF$r8AYWAQCba$S-NH2	1543.84	772.92	780.13
SP-586	Ac-LTF$r8AYWAQF$S-NH2	1565.83	783.92	784.2
SP-587	Ac-LTF4F$r8HYWAQhL$S-NH2	1629.87	815.94	815.36
SP-588	Ac-LTF4F$r8HYWAQCha$S-NH2	1655.89	828.95	828.39
SP-589	Ac-LTF4F$r8HYWAQChg$S-NH2	1641.87	821.94	821.35
SP-590	Ac-LTF4F$r8HYWAQCba$S-NH2	1627.86	814.93	814.32
SP-591	Ac-LTF4F$r8AYWAQhL$S-NH2	1563.85	782.93	782.36
SP-592	Ac-LTF4F$r8AYWAQCha$S-NH2	1589.87	795.94	795.38

SP-593	Ac-LTF4F$r8AYWAQChg$S-NH2	1575.85	788.93	788.35
SP-594	Ac-LTF4F$r8AYWAQCba$S-NH2	1561.83	781.92	781.39
SP-595	Ac-LTF3Cl$r8AYWAQhL$S-NH2	1579.82	790.91	790.35
SP-596	Ac-LTF3Cl$r8AYWAQCha$S-NH2	1605.84	803.92	803.67
SP-597	Ac-LTF3Cl$r8AYWAQChg$S-NH2	1591.82	796.91	796.34
SP-598	Ac-LTF3Cl$r8AYWAQCba$S-NH2	1577.81	789.91	789.39
SP-599	Ac-LTF$r8AYWAQhF$S-NH2	1579.84	790.92	791.14
SP-600	Ac-LTF$r8AYWAQF3CF3$S-NH2	1633.82	817.91	818.15
SP-601	Ac-LTF$r8AYWAQF3Me$S-NH2	1581.86	791.93	791.32
SP-602	Ac-LTF$r8AYWAQ1Nal$S-NH2	1615.84	808.92	809.18
SP-603	Ac-LTF$r8AYWAQBip$S-NH2	1641.86	821.93	822.13
SP-604	Ac-LTF$r8FYWAQL$A-NH2	1591.88	796.94	797.33
SP-605	Ac-LTF$r8HYWAQL$S-NHAm	1667.94	834.97	835.92
SP-606	Ac-LTF$r8HYWAQL$S-NHiAm	1667.94	834.97	835.55
SP-607	Ac-LTF$r8HYWAQL$S-NHnPr3Ph	1715.94	858.97	859.79
SP-608	Ac-LTF$r8HYWAQL$S-NHnBu3,3Me	1681.96	841.98	842.49
SP-610	Ac-LTF$r8HYWAQL$S-NHnPr	1639.91	820.96	821.58
SP-611	Ac-LTF$r8HYWAQL$S-NHnEt2Ch	1707.98	854.99	855.35
SP-612	Ac-LTF$r8HYWAQL$S-NHHex	1681.96	841.98	842.4
SP-613	Ac-LTF$r8AYWAQL$S-NHmdPeg2	1633.91	817.96	818.35
SP-614	Ac-LTF$r8AYWAQL$A-NHmdPeg2	1617.92	809.96	810.3
SP-615	Ac-LTF$r8AYWAQL$A-NHmdPeg4	1705.97	853.99	854.33
SP-616	Ac-F$r8AYdl4mWEAL$A-NH2	1316.72	659.36	659.44
SP-617	Ac-F$r8AYdl5clWEAL$A-NH2	1336.66	669.33	669.43
SP-618	Ac-LThF$r8AYWAQL$S-NH2	1545.86	773.93	774.11
SP-619	Ac-LT2Nal$r8AYWAQL$S-NH2	1581.86	791.93	792.43
SP-620	Ac-LTA$r8AYWAQL$S-NH2	1455.81	728.91	729.15
SP-621	Ac-LTF$r8AYWVQL$S-NH2	1559.88	780.94	781.24
SP-622	Ac-LTF$r8HYWAAL$A-NH2	1524.85	763.43	763.86
SP-623	Ac-LTF$r8VYWAQL$A-NH2	1543.88	772.94	773.37
SP-624	Ac-LTF$r8IYWAQL$S-NH2	1573.89	787.95	788.17
SP-625	Ac-FTF$r8VYWSQL$S-NH2	1609.85	805.93	806.22
SP-626	Ac-ITF$r8FYWAQL$S-NH2	1607.88	804.94	805.2
SP-627	Ac-2NalTF$r8VYWSQL$S-NH2	1659.87	830.94	831.2
SP-628	Ac-ITF$r8LYWSQL$S-NH2	1589.89	795.95	796.13
SP-629	Ac-FTF$r8FYWAQL$S-NH2	1641.86	821.93	822.13
SP-630	Ac-WTF$r8VYWAQL$S-NH2	1632.87	817.44	817.69
SP-631	Ac-WTF$r8WYWAQL$S-NH2	1719.88	860.94	861.36
SP-632	Ac-VTF$r8AYWSQL$S-NH2	1533.82	767.91	768.19
SP-633	Ac-WTF$r8FYWSQL$S-NH2	1696.87	849.44	849.7
SP-634	Ac-FTF$r8IYWAQL$S-NH2	1607.88	804.94	805.2
SP-635	Ac-WTF$r8VYWSQL$S-NH2	1648.87	825.44	824.8
SP-636	Ac-FTF$r8LYWSQL$S-NH2	1623.87	812.94	812.8
SP-637	Ac-YTF$r8FYWSQL$S-NH2	1673.85	837.93	837.8
SP-638	Ac-LTF$r8AY6clWEAL$A-NH2	1550.79	776.40	776.14
SP-639	Ac-LTF$r8AY6clWSQL$S-NH2	1581.80	791.90	791.68
SP-640	Ac-F$r8AY6clWSAL$A-NH2	1294.65	648.33	647.67
SP-641	Ac-F$r8AY6clWQAL$AA-NH2	1406.72	704.36	703.84
SP-642	Ac-LHF$r8AYWAQL$S-NH2	1567.86	784.93	785.21
SP-643	Ac-LTF$r8AYWAQL$S-NH2	1531.84	766.92	767.17

SP-644	Ac-LTF$r8AHWAQL$S-NH2	1505.84	753.92	754.13
SP-645	Ac-LTF$r8AYWAHL$S-NH2	1540.84	771.42	771.61
SP-646	Ac-LTF$r8AYWAQL$H-NH2	1581.87	791.94	792.15
SP-647	H-LTF$r8AYWAQL$A-NH2	1473.84	737.92	737.29
SP-648	Ac-HHF$r8AYWAQL$S-NH2	1591.83	796.92	797.35
SP-649	Ac-aAibWTF$r8VYWSQL$S-NH2	1804.96	903.48	903.64
SP-650	Ac-AibWTF$r8HYWAQL$S-NH2	1755.91	878.96	879.4
SP-651	Ac-AibAWTF$r8HYWAQL$S-NH2	1826.95	914.48	914.7
SP-652	Ac-fWTF$r8HYWAQL$S-NH2	1817.93	909.97	910.1
SP-653	Ac-AibWWTF$r8HYWAQL$S-NH2	1941.99	972.00	972.2
SP-654	Ac-WTF$r8LYWSQL$S-NH2	1662.88	832.44	832.8
SP-655	Ac-WTF$r8NleYWSQL$S-NH2	1662.88	832.44	832.6
SP-656	Ac-LTF$r8AYWSQL$a-NH2	1531.84	766.92	767.2
SP-657	Ac-LTF$r8EYWARL$A-NH2	1601.90	801.95	802.1
SP-658	Ac-LTF$r8EYWAHL$A-NH2	1582.86	792.43	792.6
SP-659	Ac-aTF$r8AYWAQL$S-NH2	1489.80	745.90	746.08
SP-660	Ac-AibTF$r8AYWAQL$S-NH2	1503.81	752.91	753.11
SP-661	Ac-AmfTF$r8AYWAQL$S-NH2	1579.84	790.92	791.14
SP-662	Ac-AmwTF$r8AYWAQL$S-NH2	1618.86	810.43	810.66
SP-663	Ac-NmLTF$r8AYWAQL$S-NH2	1545.86	773.93	774.11
SP-664	Ac-LNmTF$r8AYWAQL$S-NH2	1545.86	773.93	774.11
SP-665	Ac-LSarF$r8AYWAQL$S-NH2	1501.83	751.92	752.18
SP-667	Ac-LGF$r8AYWAQL$S-NH2	1487.82	744.91	745.15
SP-668	Ac-LTNmF$r8AYWAQL$S-NH2	1545.86	773.93	774.2
SP-669	Ac-TF$r8AYWAQL$S-NH2	1418.76	710.38	710.64
SP-670	Ac-ETF$r8AYWAQL$A-NH2	1531.81	766.91	767.2
SP-671	Ac-LTF$r8EYWAQL$A-NH2	1573.85	787.93	788.1
SP-672	Ac-LT2Nal$r8AYWSQL$S-NH2	1597.85	799.93	800.4
SP-673	Ac-LTF$r8AYWAAL$S-NH2	1474.82	738.41	738.68
SP-674	Ac-LTF$r8AYWAQhCha$S-NH2	1585.89	793.95	794.19
SP-675	Ac-LTF$r8AYWAQChg$S-NH2	1557.86	779.93	780.97
SP-676	Ac-LTF$r8AYWAQCba$S-NH2	1543.84	772.92	773.19
SP-677	Ac-LTF$r8AYWAQF3CF3$S-NH2	1633.82	817.91	818.15
SP-678	Ac-LTF$r8AYWAQ1Nal$S-NH2	1615.84	808.92	809.18
SP-679	Ac-LTF$r8AYWAQBip$S-NH2	1641.86	821.93	822.32
SP-680	Ac-LT2Nal$r8AYWAQL$S-NH2	1581.86	791.93	792.15
SP-681	Ac-LTF$r8AYWVQL$S-NH2	1559.88	780.94	781.62
SP-682	Ac-LTF$r8AWWAQL$S-NH2	1554.86	778.43	778.65
SP-683	Ac-FTF$r8VYWSQL$S-NH2	1609.85	805.93	806.12
SP-684	Ac-ITF$r8FYWAQL$S-NH2	1607.88	804.94	805.2
SP-685	Ac-ITF$r8LYWSQL$S-NH2	1589.89	795.95	796.22
SP-686	Ac-FTF$r8FYWAQL$S-NH2	1641.86	821.93	822.41
SP-687	Ac-VTF$r8AYWSQL$S-NH2	1533.82	767.91	768.19
SP-688	Ac-LTF$r8AHWAQL$S-NH2	1505.84	753.92	754.31
SP-689	Ac-LTF$r8AYWAQL$H-NH2	1581.87	791.94	791.94
SP-690	Ac-LTF$r8AYWAHL$S-NH2	1540.84	771.42	771.61
SP-691	Ac-aAibWTF$r8VYWSQL$S-NH2	1804.96	903.48	903.9
SP-692	Ac-AibWTF$r8HYWAQL$S-NH2	1755.91	878.96	879.5
SP-693	Ac-AibAWTF$r8HYWAQL$S-NH2	1826.95	914.48	914.7
SP-694	Ac-fWTF$r8HYWAQL$S-NH2	1817.93	909.97	910.2

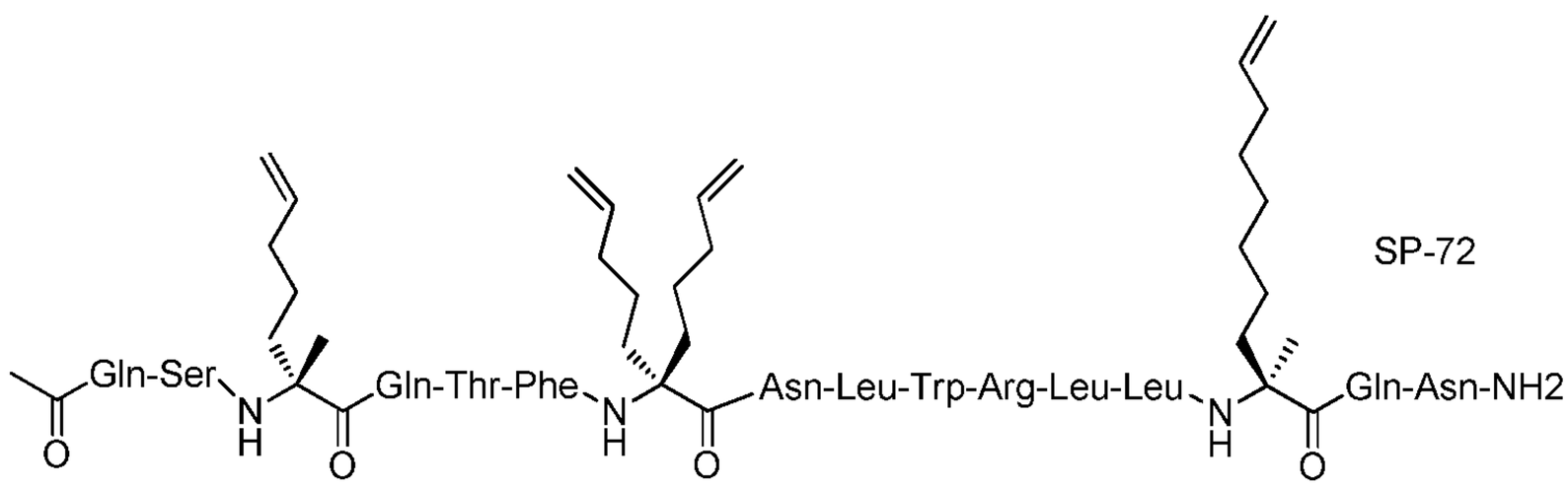
SP-695	Ac-AibWWTF$r8HYWAQL$S-NH2	1941.99	972.00	972.7
SP-696	Ac-WTF$r8LYWSQL$S-NH2	1662.88	832.44	832.7
SP-697	Ac-WTF$r8NleYWSQL$S-NH2	1662.88	832.44	832.7
SP-698	Ac-LTF$r8AYWSQL$a-NH2	1531.84	766.92	767.2
SP-699	Ac-LTF$r8EYWARL$A-NH2	1601.90	801.95	802.2
SP-700	Ac-LTF$r8EYWAHL$A-NH2	1582.86	792.43	792.6
SP-701	Ac-aTF$r8AYWAQL$S-NH2	1489.80	745.90	746.1
SP-702	Ac-AibTF$r8AYWAQL$S-NH2	1503.81	752.91	753.2
SP-703	Ac-AmfTF$r8AYWAQL$S-NH2	1579.84	790.92	791.2
SP-704	Ac-AmwTF$r8AYWAQL$S-NH2	1618.86	810.43	810.7
SP-705	Ac-NmLTF$r8AYWAQL$S-NH2	1545.86	773.93	774.1
SP-706	Ac-LNmTF$r8AYWAQL$S-NH2	1545.86	773.93	774.4
SP-707	Ac-LSarF$r8AYWAQL$S-NH2	1501.83	751.92	752.1
SP-708	Ac-TF$r8AYWAQL$S-NH2	1418.76	710.38	710.8
SP-709	Ac-ETF$r8AYWAQL$A-NH2	1531.81	766.91	767.4
SP-710	Ac-LTF$r8EYWAQL$A-NH2	1573.85	787.93	788.2
SP-711	Ac-WTF$r8VYWSQL$S-NH2	1648.87	825.44	825.2
SP-713	Ac-YTF$r8FYWSQL$S-NH2	1673.85	837.93	837.3
SP-714	Ac-F$r8AY6clWSAL$A-NH2	1294.65	648.33	647.74
SP-715	Ac-ETF$r8EYWVQL$S-NH2	1633.84	817.92	817.36
SP-716	Ac-ETF$r8EHWAQL$A-NH2	1563.81	782.91	782.36
SP-717	Ac-ITF$r8EYWAQL$S-NH2	1589.85	795.93	795.38
SP-718	Ac-ITF$r8EHWVQL$A-NH2	1575.88	788.94	788.42
SP-719	Ac-ITF$r8EHWAQL$S-NH2	1563.85	782.93	782.43
SP-720	Ac-LTF4F$r8AYWAQCba$S-NH2	1561.83	781.92	781.32
SP-721	Ac-LTF3Cl$r8AYWAQhL$S-NH2	1579.82	790.91	790.64
SP-722	Ac-LTF3Cl$r8AYWAQCha$S-NH2	1605.84	803.92	803.37
SP-723	Ac-LTF3Cl$r8AYWAQChg$S-NH2	1591.82	796.91	796.27
SP-724	Ac-LTF3Cl$r8AYWAQCba$S-NH2	1577.81	789.91	789.83
SP-725	Ac-LTF$r8AY6clWSQL$S-NH2	1581.80	791.90	791.75
SP-726	Ac-LTF4F$r8HYWAQhL$S-NH2	1629.87	815.94	815.36
SP-727	Ac-LTF4F$r8HYWAQCba$S-NH2	1627.86	814.93	814.32
SP-728	Ac-LTF4F$r8AYWAQhL$S-NH2	1563.85	782.93	782.36
SP-729	Ac-LTF4F$r8AYWAQChg$S-NH2	1575.85	788.93	788.35
SP-730	Ac-ETF$r8EYWVAL$S-NH2	1576.82	789.41	788.79
SP-731	Ac-ETF$r8EHWAAL$A-NH2	1506.79	754.40	754.8
SP-732	Ac-ITF$r8EYWAAL$S-NH2	1532.83	767.42	767.75
SP-733	Ac-ITF$r8EHWVAL$A-NH2	1518.86	760.43	760.81
SP-734	Ac-ITF$r8EHWAAL$S-NH2	1506.82	754.41	754.8
SP-735	Pam-LTF$r8EYWAQL$S-NH2	1786.07	894.04	894.48
SP-736	Pam-ETF$r8EYWAQL$S-NH2	1802.03	902.02	902.34
SP-737	Ac-LTF$r8AYWLQL$S-NH2	1573.89	787.95	787.39
SP-738	Ac-LTF$r8EYWLQL$S-NH2	1631.90	816.95	817.33
SP-739	Ac-LTF$r8EHWLQL$S-NH2	1605.89	803.95	804.29
SP-740	Ac-LTF$r8VYWAQL$S-NH2	1559.88	780.94	781.34
SP-741	Ac-LTF$r8AYWSQL$S-NH2	1547.84	774.92	775.33
SP-742	Ac-ETF$r8AYWAQL$S-NH2	1547.80	774.90	775.7
SP-743	Ac-LTF$r8EYWAQL$S-NH2	1589.85	795.93	796.33
SP-744	Ac-LTF$r8HYWAQL$S-NHAm	1667.94	834.97	835.37
SP-745	Ac-LTF$r8HYWAQL$S-NHiAm	1667.94	834.97	835.27

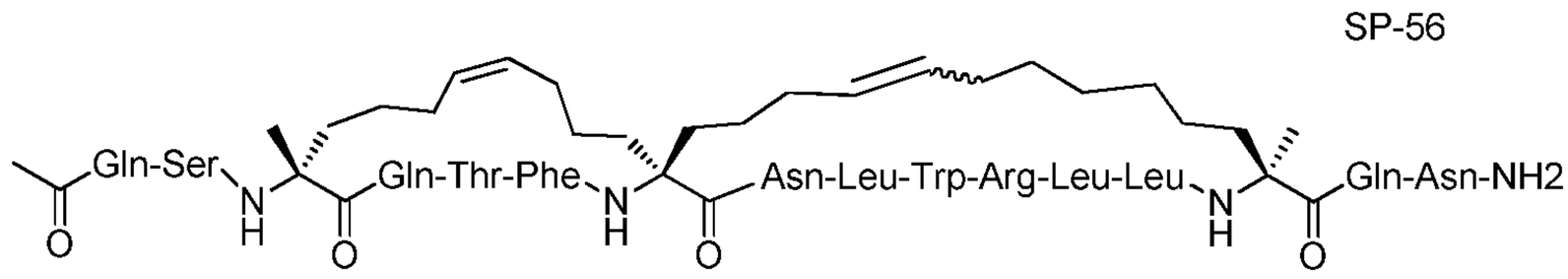
SP-746	Ac-LTF$r8HYWAQL$S-NHnPr3Ph	1715.94	858.97	859.42
SP-747	Ac-LTF$r8HYWAQL$S-NHnBu3,3Me	1681.96	841.98	842.67
SP-748	Ac-LTF$r8HYWAQL$S-NHnBu	1653.93	827.97	828.24
SP-749	Ac-LTF$r8HYWAQL$S-NHnPr	1639.91	820.96	821.31
SP-750	Ac-LTF$r8HYWAQL$S-NHnEt2Ch	1707.98	854.99	855.35
SP-751	Ac-LTF$r8HYWAQL$S-NHHex	1681.96	841.98	842.4
SP-752	Ac-LTF$r8AYWAQL$S-NHmdPeg2	1633.91	817.96	855.35
SP-753	Ac-LTF$r8AYWAQL$A-NHmdPeg2	1617.92	809.96	810.58
SP-754	Ac-LTF$r5AYWAAL$s8S-NH2	1474.82	738.41	738.79
SP-755	Ac-LTF$r8AYWCouQL$S-NH2	1705.88	853.94	854.61
SP-756	Ac-LTF$r8CouYWAQL$S-NH2	1705.88	853.94	854.7
SP-757	Ac-CouTF$r8AYWAQL$S-NH2	1663.83	832.92	833.33
SP-758	H-LTF$r8AYWAQL$A-NH2	1473.84	737.92	737.29
SP-759	Ac-HHF$r8AYWAQL$S-NH2	1591.83	796.92	797.72
SP-760	Ac-LT2Nal$r8AYWSQL$S-NH2	1597.85	799.93	800.68
SP-761	Ac-LTF$r8HCouWAQL$S-NH2	1679.87	840.94	841.38
SP-762	Ac-LTF$r8AYWCou2QL$S-NH2	1789.94	895.97	896.51
SP-763	Ac-LTF$r8Cou2YWAQL$S-NH2	1789.94	895.97	896.5
SP-764	Ac-Cou2TF$r8AYWAQL$S-NH2	1747.90	874.95	875.42
SP-765	Ac-LTF$r8ACou2WAQL$S-NH2	1697.92	849.96	850.82
SP-766	Dmaac-LTF$r8AYWAQL$S-NH2	1574.89	788.45	788.82
SP-767	Hexac-LTF$r8AYWAQL$S-NH2	1587.91	794.96	795.11
SP-768	Napac-LTF$r8AYWAQL$S-NH2	1657.89	829.95	830.36
SP-769	Pam-LTF$r8AYWAQL$S-NH2	1728.06	865.03	865.45
SP-770	Ac-LT2Nal$r8HYAAQL$S-NH2	1532.84	767.42	767.61
SP-771	Ac-LT2Nal$/r8HYWAQL$/S-NH2	1675.91	838.96	839.1
SP-772	Ac-LT2Nal$r8HYFAQL$S-NH2	1608.87	805.44	805.9
SP-773	Ac-LT2Nal$r8HWAAQL$S-NH2	1555.86	778.93	779.08
SP-774	Ac-LT2Nal$r8HYAWQL$S-NH2	1647.88	824.94	825.04
SP-775	Ac-LT2Nal$r8HYAAQW$S-NH2	1605.83	803.92	804.05
SP-776	Ac-LTW$r8HYWAQL$S-NH2	1636.88	819.44	819.95
SP-777	Ac-LT1Nal$r8HYWAQL$S-NH2	1647.88	824.94	825.41

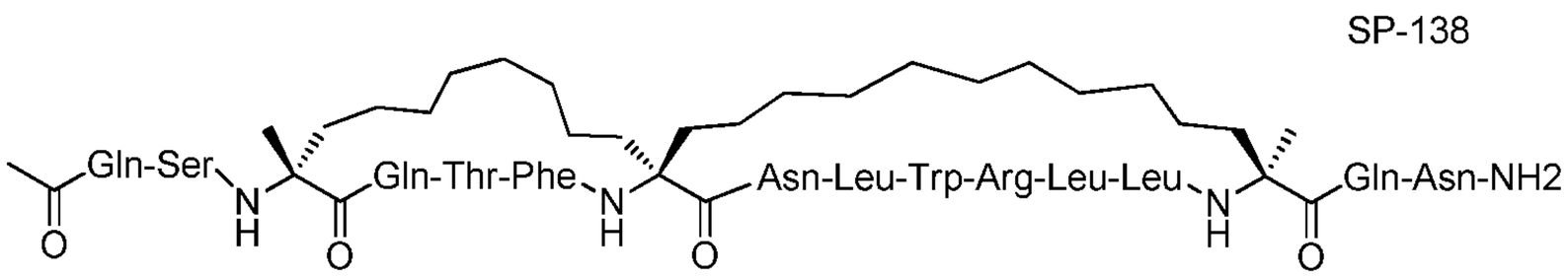
[0148] In the sequences shown above and elsewhere, the following abbreviations are used: “Nle” represents norleucine, “Aib” represents 2-aminoisobutyric acid, “Ac” represents acetyl, and “Pr” represents propionyl. Amino acids represented as “$” are alpha-Me S5-pentenyl-alanine olefin amino acids connected by an all-carbon i to i+4 crosslinker comprising one double bond. Amino acids represented as “$r5” are alpha-Me R5-pentenyl-alanine olefin amino acids connected by an all-carbon i to i+4 crosslinker comprising one double bond. Amino acids represented as “$s8” are alpha-Me S8-octenyl-alanine olefin amino acids connected by an all-carbon i to i+7 crosslinker comprising one double bond. Amino acids represented as “$r8” are alpha-Me R8-octenyl-alanine olefin amino acids connected by an all-carbon i to i+7 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 alpha carbons of each amino acid. Amino acids represented as “$/” are alpha-Me S5-pentenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “$/r5” are alpha-Me R5-pentenyl-alanine olefin amino acids that are not

connected by any crosslinker. Amino acids represented as "$/s8" are alpha-Me S8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as "$/r8" are alpha-Me R8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as "Amw" are alpha-Me tryptophan amino acids. Amino acids represented as "Aml" are alpha-Me leucine 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.

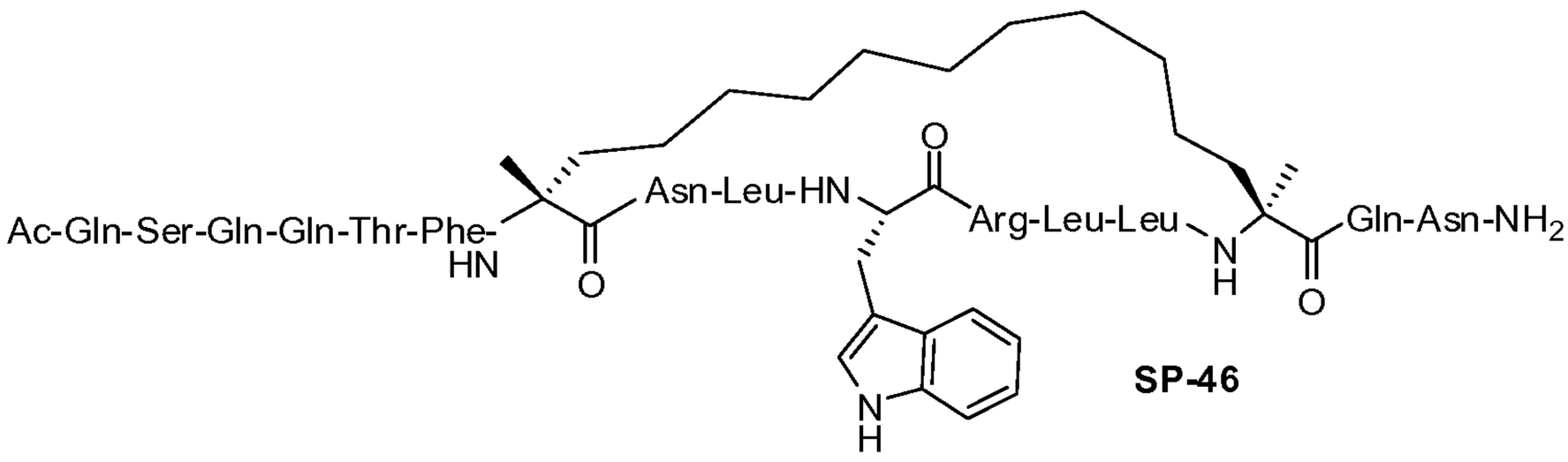
[0149] For example, the compounds represented as SP-72, SP-56 and SP-138 have the following structures:



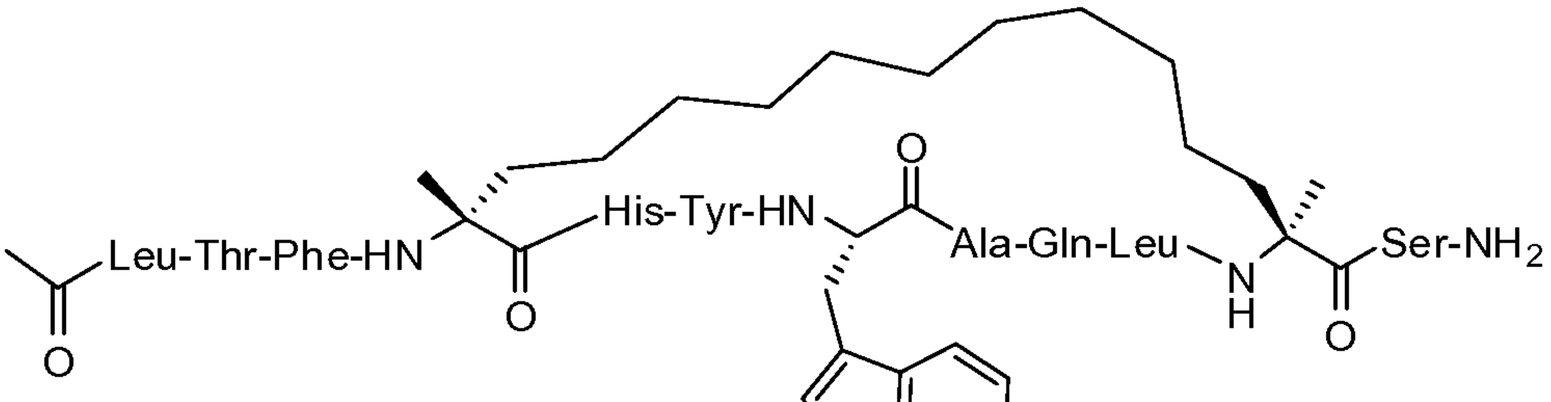




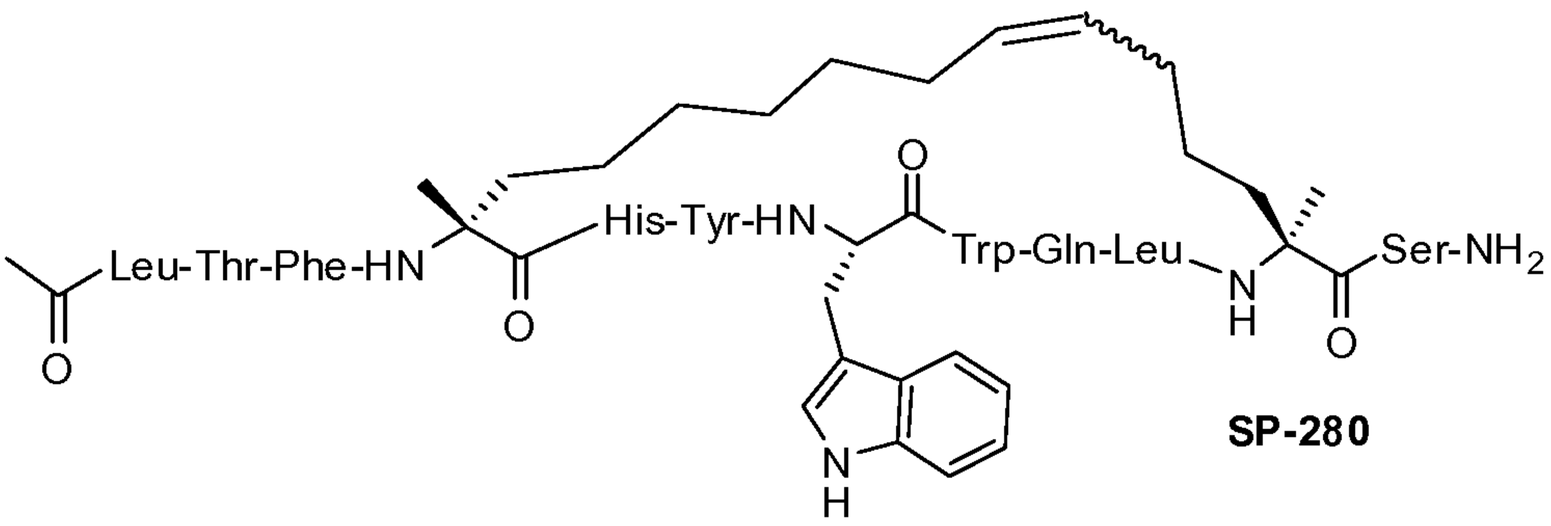
[0150] For example, additional compounds have the following structures:



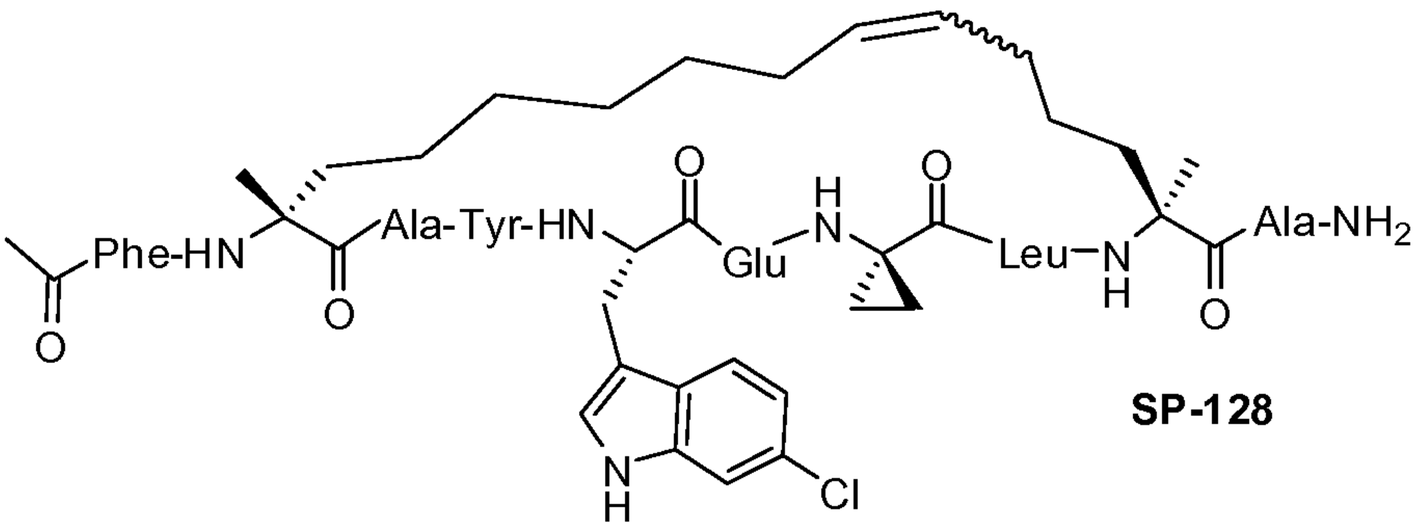
SP-46



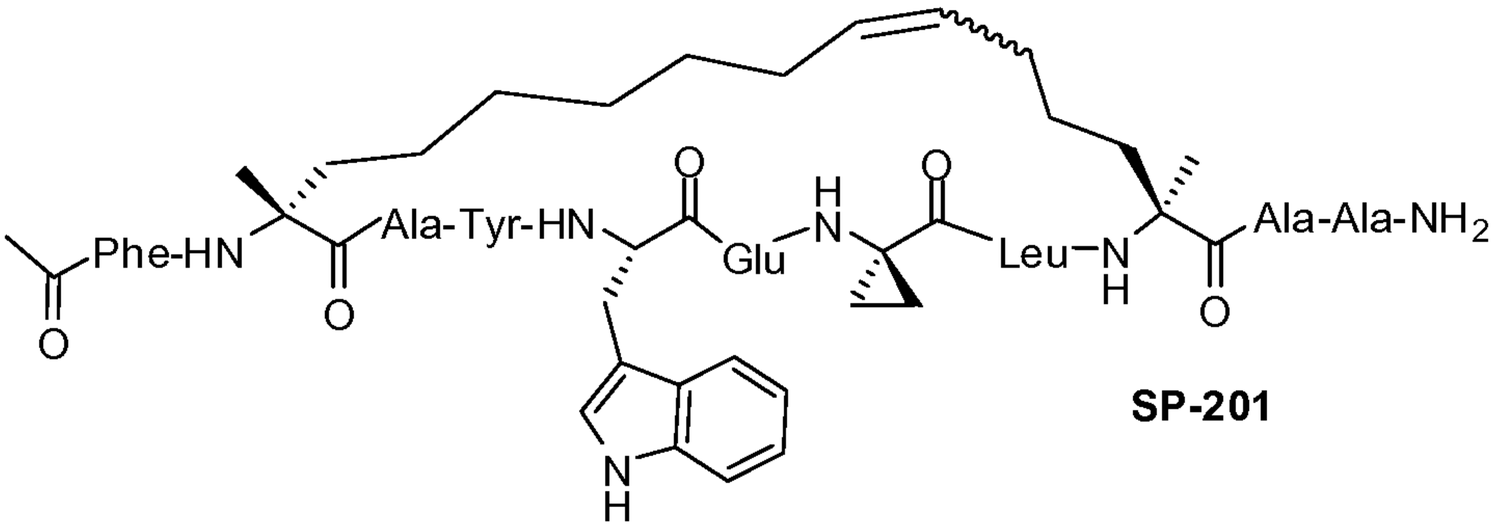
SP-142



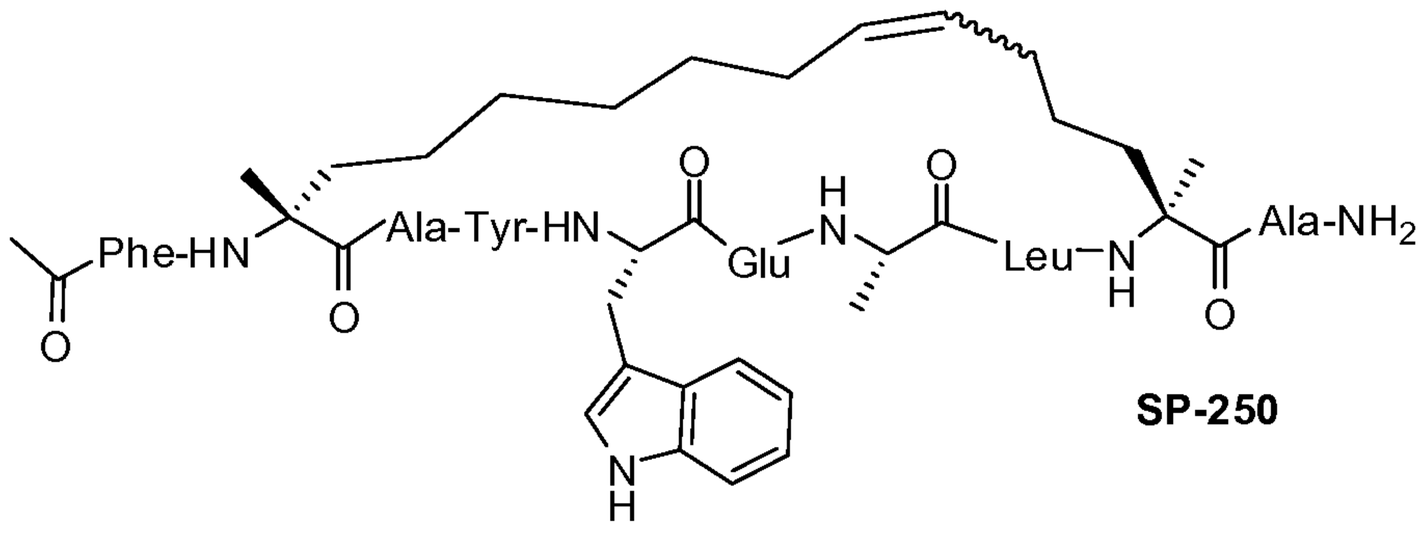
SP-280



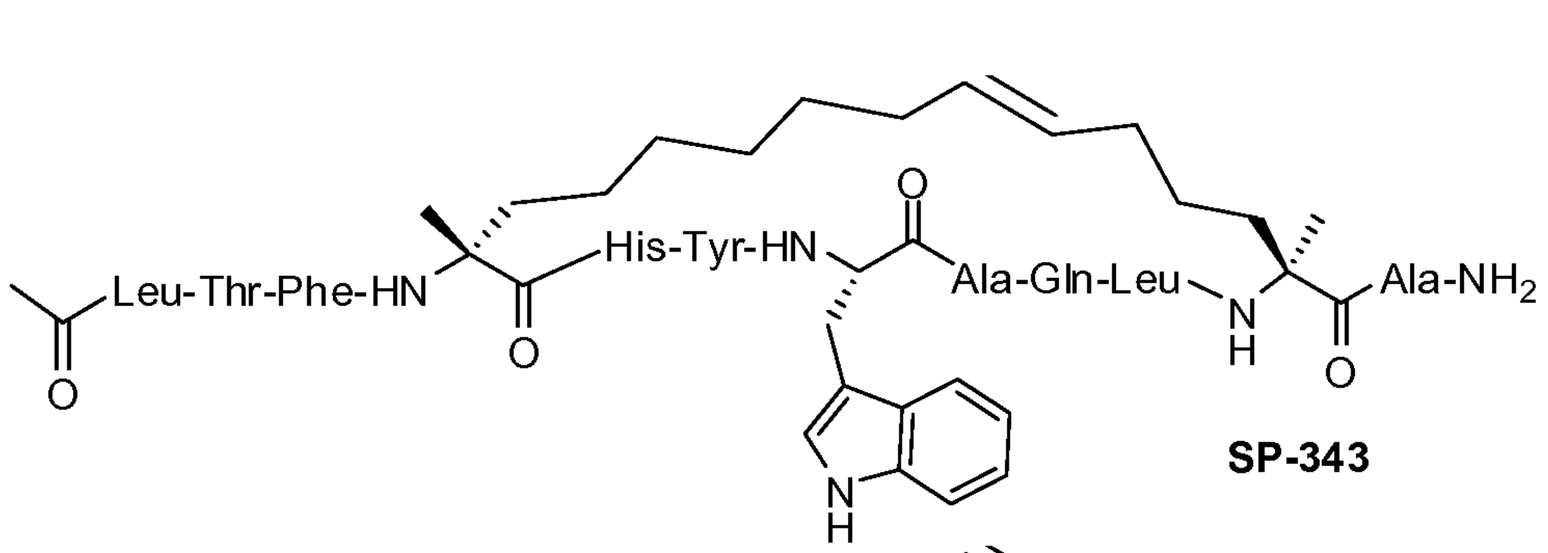
SP-128



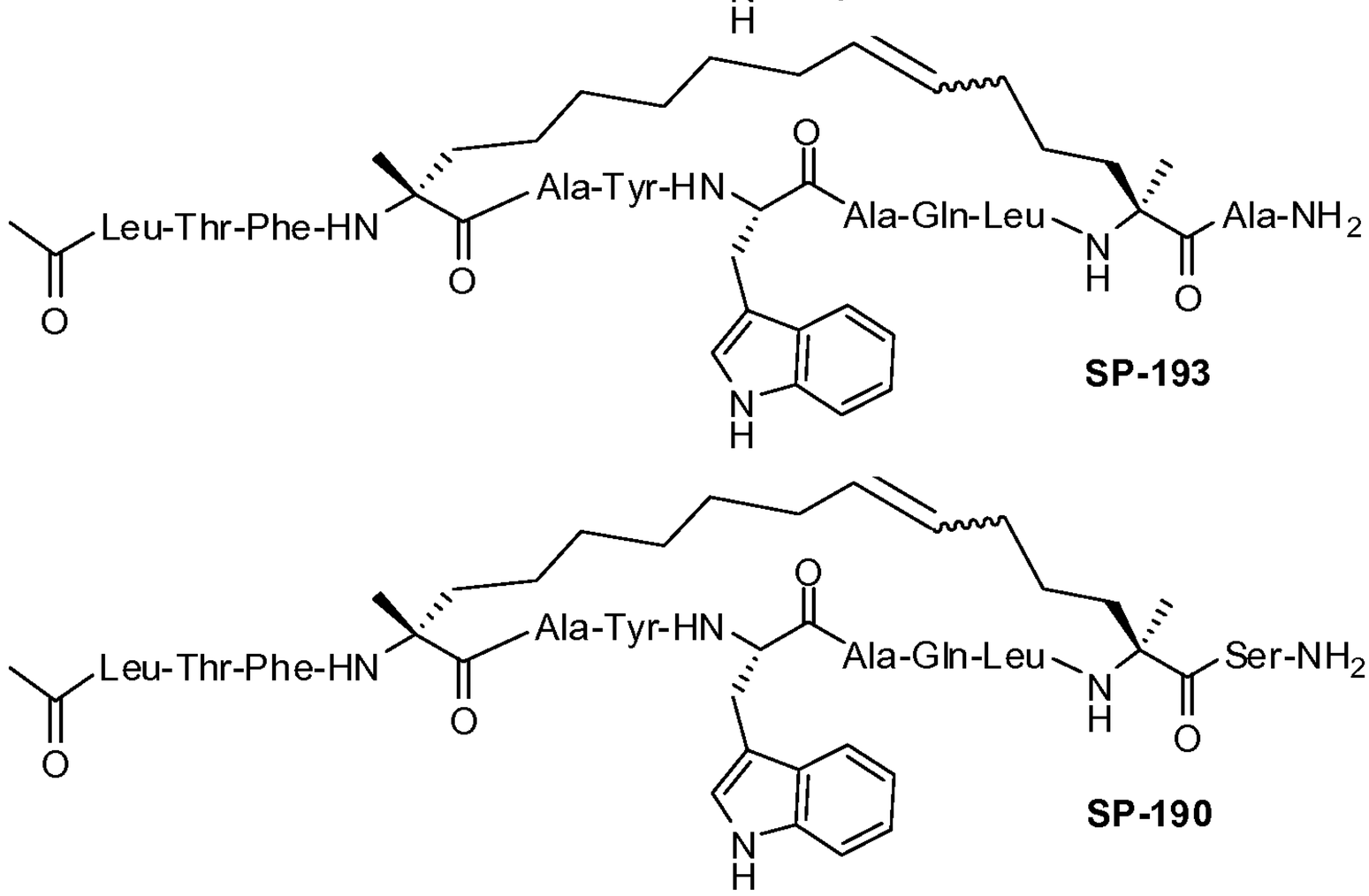
SP-201



SP-250

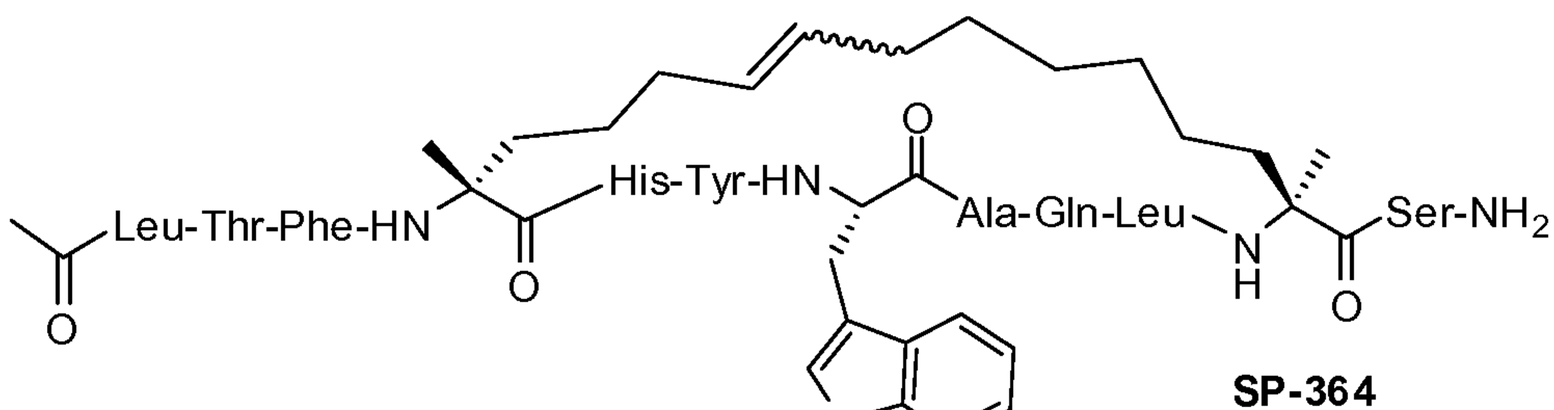


SP-343

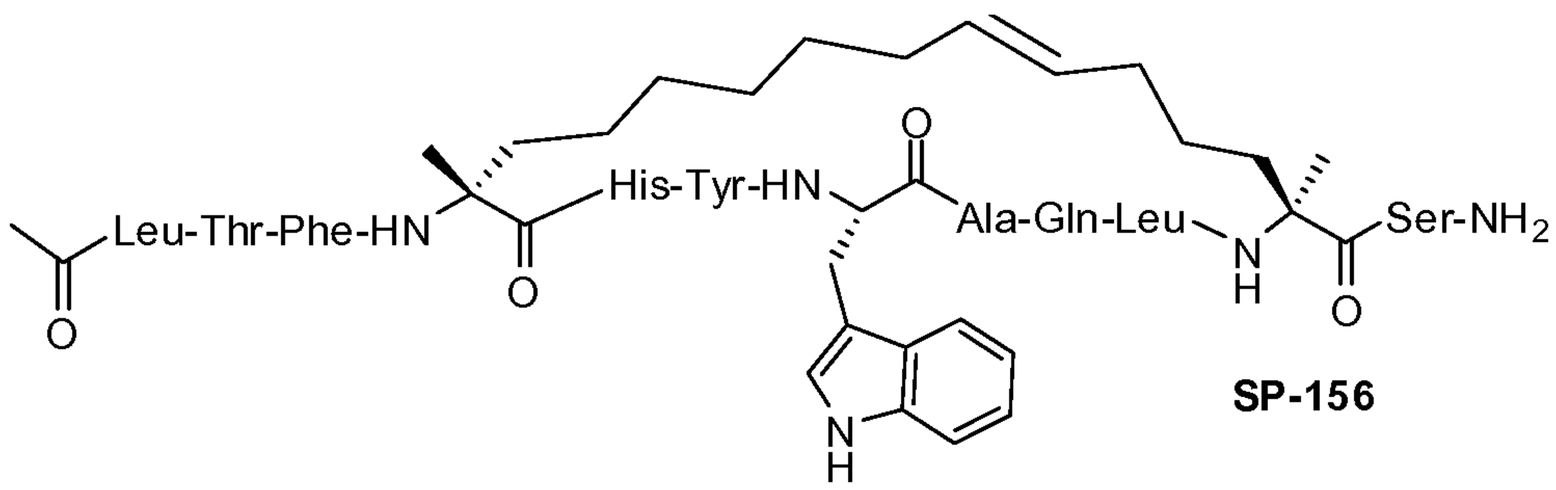


SP-193

SP-190



SP-364



SP-156

Example 3: Competition Binding ELISA (HDM2 & HDMX)

[0151] p53-His6 protein (30 nM/well) is coated overnight at room temperature in the wells of a 96-well Immulon plates. On the day of the experiment, plates are washed with 1X PBS-Tween 20 (0.05%) using an automated ELISA plate washer, blocked with ELISA Micro well Blocking for 30 minutes at room temperature; excess blocking agent is washed off by washing plates with 1X PBS-Tween 20 (0.05%). Peptides are diluted from 10 mM DMSO stocks to 500 µM working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. The peptides are added to wells at 2X desired concentrations in 50 µl volumes, followed by addition of diluted GST-HDM2 or GST-HMDX protein (final concentration: 10nM). Samples are incubated at room temperature for 2h, plates are washed with PBS-Tween 20 (0.05%) prior to adding 100 µl of HRP-conjugated anti-GST antibody [Hypromatrix, INC] diluted to 0.5 µg/ml in HRP-stabilizing buffer. Post 30 min incubation with detection antibody, plates are washed and incubated with 100 µl per well of TMB-E Substrate solution up to 30 minutes; reactions are stopped using 1M HCL and absorbance measured at 450 nm on micro plate reader. Data is analyzed using Graph Pad PRISM software.

Example 4: SJSA-1 Cell Viability assay

[0152] SJSA1 cells are seeded at the density of 5000 cells/ 100 µl/well in 96-well plates a day prior to assay. On the day of study cells are washed once with Opti-MEM Media and 90µL of the Opti-MEM Media is added to cells. Peptides are diluted from 10 mM DMSO stocks to 500 µM working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. The final concentration range µM will be 50, 25, 12.5, 6.25, 3.1, 1.56, 0.8 and 0 µM in 100 µL final volume per well for peptides. Final highest DMSO concentration is 0.5% and will be used as the negative control. Cayman Chemicals Cell-Based Assay (-)-Nutlin-3 (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides 10 µl of 10X desired concentrations is added to the appropriate well to achieve the final desired concentrations. Cells are then incubated with peptides for 20-24h at 37°C in humidified 5% CO2 atmosphere. Post-incubation period, cell viability is measured using Promega Cell Titer-Glo reagents according to manufacturer' instructions.

Example 5: SJSA-1 p21 up-regulation assay

[0153] SJSA1 cells are seeded at the density of 0.8 million cells/ 2 ml/well in 6-well plates a day prior to assay. On the day of study cells are washed once with Opti-MEM Media and 1350µL of the Opti-MEM Media is added to cells.Peptides are diluted from 10 mM DMSO stocks to 500 µM working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. Final highest DMSO concentration is 0.5% and is used as the negative control. Cayman Chemicals Cell-Based Assay (-)-Nutlin-3 (10 mM) is used as positive control. Nutlin is diluted using the same dilution scheme as peptides 150 µl of 10X desired concentrations is added to the appropriate well to achieve the final desired concentrations. Cells are then incubated with peptides for 18-20 h at 37°C in humidified 5% CO2 atmosphere. Post-incubation period, cells are harvested, washed with 1X PBS (without Ca++/Mg++) and lysed in 1X Cell lysis buffer (Cell Signaling technologies 10X buffer diluted to 1X and supplemented with protease inhibitors and Phosphatase inhibitors) on ice for 30 min.Lysates are centrifuged at 13000 rpm speed in a microfuge at 40C for 8 min; clear supernatants are collected and stored at -80 0C till further use. Total protein content of the lysates is measured using BCA protein detection kit and BSA standards from Thermofisher. 25 µg of the total protein is used for p21 detection ELISA assay. Each condition is set in triplicate for ELISA plate. The ELISA assay protocol is followed as per the manufacturer's instructions. 25 µg total protein used for each well, and each well is set up in triplicate. Data is analyzed using Graph Pad PRISM software.

Example 6: p53 GRIP assay

[0154] Thermo Scientific* BioImage p53-Hdm2 Redistribution Assay monitors the protein interaction with Hdm2 and cellular translocation of GFP-tagged p53 in response to drug compounds or other stimuli. Recombinant CHO-hIR cells stably express human p53(1-312) fused to the C-terminus of enhanced green fluorescent protein (EGFP) and PDE4A4-Hdm2(1-124), a fusion protein between PDE4A4 and Hdm2(1-124). They provide a ready-to-use assay system for measuring the effects of experimental conditions on the interaction of p53 and Hdm2. Imaging and analysis is performed with a HCS platform.

[0155] CHO-hIR cells are regularly maintained in Ham's F12 media supplemented with 1% Penicillin-Streptomycin, 0.5 mg/ml Geneticin, 1 mg/ml Zeocin and 10% FBS. Cells seeded into 96-well plates at the density of 7000 cells/ 100 µl per well 18-24 hours prior to running the assay using culture media. The next day, media is refreshed and PD177 is added to cells to the final concentration of 3µM to activate foci formation. Control wells are kept without PD-177 solution. 24h post stimulation with PD177, cells are washed once with Opti-MEM Media and 50 µL of the Opti-MEM Media supplemented with PD-177(6 µM) is added to cells. Peptides are diluted from 10 mM DMSO stocks to 500 µM working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. Final highest DMSO concentration is 0.5% and

is used as the negative control. Cayman Chemicals Cell-Based Assay (-)-Nutlin-3 (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.50 µl of 2X desired concentrations is added to the appropriate well to achieve the final desired concentrations. Cells are then incubated with peptides for 6 h at 37°C in humidified 5% CO2 atmosphere. Post-incubation period, cells are fixed by gently aspirating out the media and adding 150 µl of fixing solution per well for 20 minutes at room temperature. Fixed cells are washed 4 times with 200 µl PBS per well each time. At the end of last wash, 100 µl of 1µM Hoechst staining solution is added. Sealed plates incubated for at least 30 min in dark, washed with PBS to remove excess stain and PBS is added to each well. Plates can be stored at 4°C in dark up to 3 days. The translocation of p53/HDM2 is imaged using Molecular translocation module on Cellomics Arrayscan instrument using 10x objective, XF-100 filter sets for Hoechst and GFP. The output parameters was Mean- CircRINGAveIntenRatio (the ratio of average fluorescence intensities of nucleus and cytoplasm,(well average)). The minimally acceptable number of cells per well used for image analysis was set to 500 cells.

Example 7: Direct binding assay hDM2 with Fluorescence polarization (FP)

[0156] The assay was performed according to the following general protocol:

1. Dilute hDM2 (In-house, 41kD) into FP buffer (High salt buffer-200mM Nacl,5mM CHAPS, pH 7.5) to make 10µM working stock solution.
2. Add 30µl of 10µM of protein stock solution into A1 and B1 well of 96-well black HE microplate (Molecular Devices).
3. Fill in 30µl of FP buffer into column A2 to A12, B2 to B12, C1 to C12, and D1 to D12.
4. 2 or 3 fold series dilution of protein stock from A1, B1 into A2, B2; A2, B2 to A3, B3; … to reach the single digit nM concentration at the last dilution point.
5. Dilute 1mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100µM (dilution 1: 10). Then, dilutefrom 100µM to 10µM with water (dilution 1:10) and then dilute with FP buffer from 10µM 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.
6. Add 10µl of 10nM of FAM labeled peptide into each well and incubate, and read at different time points. Kd with 5-FAM-BaLTFEHYWAQLTS-NH_2 is ~13.38 nM.

Example 8: Competitive Fluorescence polarization assay for hDM2

[0157] The assay was performed according to the following general protocol:

1. Dilute hDM2 (In-house, 41kD) into FP buffer (High salt buffer-200mM Nacl,5mM CHAPS, pH 7.5) to make 84nM (2X) working stock solution.
2. Add 20µl of 84nM (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 100μM (dilution 1: 10). Then, dilute from 100μM to 10μM with water (dilution 1:10) and then dilute with FP buffer from 10μM 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 1μM (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 500μM with H_2O (dilution 1:10) and then dilute with FP buffer from 500μM to 20μM (dilution 1:25). Making 5 fold serial dilutions from 4μM (4X) for 6 points.

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

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

Results of Examples 7 and 8 are provided in HDM2 data in Figures 7A-D.

Example 9: Direct binding assay hDMX with Fluorescence polarization (FP)

[0158] The assay was performed according to the following general protocol:

1. Dilute hDMX (In-house, 40kD) into FP buffer (High salt buffer-200mM Nacl,5mM CHAPS, pH 7.5) to make 10μM working stock solution.

2. Add 30μl of 10μM of protein stock solution into A1 and B1 well of 96-well black HE microplate (Molecular Devices).

3. Fill in 30μl of FP buffer into column A2 to A12, B2 to B12, C1 to C12, and D1 to D12.

4. 2 or 3 fold series dilution of protein stock from A1, B1 into A2, B2; A2, B2 to A3, B3; … to reach the single digit nM concentration at the last dilution point.

5. Dilute 1mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100μM (dilution 1: 10). Then, dilute from 100μM to 10μM with water (dilution 1:10) and then dilute with FP buffer from 10μM 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.

6. Add 10μl of 10nM of FAM labeled peptide into each well and incubate, and read at different time points.

Kd with 5-FAM-BaLTFEHYWAQLTS-NH_2 is ~51 nM.

Example 10: Competitive Fluorescence polarization assay for hDMX

[0159] The assay was performed according to the following general protocol:

1. Dilute hDMX (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 20μl of 300nM (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 100μM (dilution 1: 10). Then, dilute from 100μM to 10μM with water (dilution 1:10) and then dilute with FP buffer from 10μM 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 5μM (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 500μM with H_2O (dilution 1:10) and then dilute with FP buffer from 500μM to 20μM (dilution 1:25). Making 5 fold serial dilutions from 20μM (4X) for 6 points.

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

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

Results of Examples 9 and 10 are provided in HDMX data in Figures 7A-D.

Example 11: Cell Viability assay

[0160] 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 each cell line in use:

- 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

[0161] 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.

[0162] 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 ul of assay media. H4-H9 will receive 20 ul of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.
- Positive control: HDM2 small molecule inhibitor,Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

[0163] 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 hours at 37°C in humidified 5% CO_2 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 µl of assay media from each well. Add 15 µl of thawed MTT reagent to each well.
- Allow plate to incubate for 2h at 37°C in humidified 5% CO_2 atmosphere and add 100 µl 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 570nM.
- Analyze the cell viability against the DMSO controls using GraphPad PRISM analysis tools.

[0164] 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)

[0165] **Instruments:** Multiplate Reader for Absorbance readout (Synergy 2)

Results of Example 11 are provided in SJSA-1 EC50 data in Figures 7A-D.

Example 12. P21 ELISA assay

[0166] The assay was performed according to the following general protocol:

Cell Platimg:

- Trypsinize, count and seed SJSA1 cells at the density of 7500 cells/ 100 µl/well in 96-well plates a day prior to assay.
- On the day of study, replace media with fresh RPMI-11% FBS (assay media). Add 90µL of the assay media per well. Control wells with no cells, receive 100 µl media.

[0167] **Peptide dilution:**

- 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 10 ul of assay media. H4-H9 will receive 10 ul of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.
- Positive control: HDM2 small molecule inhibitor,Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

[0168] Addition of working stocks to cells:

- Add 10 μl of 10X desired concentration to appropriate well to achieve the final concentrations in total 100 μl volume in well. (10 μl of 300 μM peptide + 90 μl of cells in media = 30 μM final concentration in 100 μl volume in wells). Thus final concentration range used will be 30, 10, 3, 1, 0.3& 0 μM.
- Controls will include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.
- 20h-post incubation, aspirate the media; wash cells with 1X PBS (without Ca^{++}/Mg^{++}) and lyse in 60 μl of 1X Cell lysis buffer (Cell Signaling technologies 10X buffer diluted to 1X and supplemented with protease inhibitors and Phosphatase inhibitors) on ice for 30 min.
- Centrifuge plates in at 5000 rpm speed in at 4^0C for 8 min; collect clear supernatants and freeze at -80 ^{0}C till further use.

[0169] Protein Estimation:

- Total protein content of the lysates is measured using BCA protein detection kit and BSA standards from Thermofisher. Typically about 6-7 μg protein is expected per well.
- Use 50 μl of the lysate per well to set up p21 ELISA.

[0170] Human Total p21 ELISA: The ELISA assay protocol is followed as per the manufacturer's instructions. 50 μl lysate is used for each well, and each well is set up in triplicate.

[0171] Reagents:

- -Cell-Based Assay (-)-Nutlin-3 (10 mM): Cayman Chemicals, catalog # 600034
- - OptiMEM, Invitrogen catalog # 51985
- -Cell Signaling Lysis Buffer (10X), Cell signaling technology, Catalog # 9803
- -Protease inhibitor Cocktail tablets(mini), Roche Chemicals, catalog # 04693124001
- -Phosphatase inhibitor Cocktail tablet, Roche Chemicals, catalog # 04906837001
- -Human total p21 ELISA kit, R&D Systems, DYC1047-5
- -STOP Solution (1M HCL), Cell Signaling Technologies, Catalog # 7002

[0172] Instruments: Micro centrifuge- Eppendorf 5415D and Multiplate Reader for Absorbance readout (Synergy 2)

Results of Example 12 are provided in p21 data in Figures 7A-D.

Example 13: Caspase 3 Detection assay:

[0173] The assay was performed according to the following general protocol:

Cell Platimg: Trypsinize, count and seed SJSA1 cells at the density of 7500 cells/ 100 µl/well in 96-well plates a day prior to assay. On the day of study, replace media with fresh RPMI-11% FBS (assay media). Add 180µL of the assay media per well. Control wells with no cells, receive 200 µl media.

[0174] Peptide dilution:

- 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. Add 20 ul of 10X working stocks to appropriate wells.
- Row H has controls. H1- H3 will receive 20 ul of assay media. H4-H9 will receive 20 ul of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.
- Positive control: HDM2 small molecule inhibitor,Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

[0175] Addition of working stocks to cells:

- **Add 10 µl of 10X desired concentration** to appropriate well **to achieve the final concentrations in total 100 µl volume in well**. (10 µl of 300 µM peptide + 90 µl of cells in media = 30 µM final concentration in 100 µl volume in wells). Thus final concentration range used will be 30, 10, 3, 1, 0.3& 0 µM.
- Controls will include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.
- 48 h-post incubation, aspirate 80 µl media from each well; add 100 µl Caspase3/7Glo assay reagent (Promega Caspase 3/7 glo assay system, G8092)per well, incubate with gentle shaking for 1h at room temperature.
- read on Synergy Biotek multiplate reader for luminescence.
- Data is analyzed as Caspase 3 activation over DMSO-treated cells.

Results of Example 13 are provided in p21 data in Figures 7A-D.

CLAIMS

WHAT IS CLAIMED IS:

1. A peptidomimetic macrocycle comprising an amino acid sequence which is at least about 60% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4.

2. The peptidomimetic macrocycle of claim 1, wherein the amino acid sequence of said peptidomimetic macrocycle is at least about 60% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4, and the macrocycle does not contain a triazole or a thioether.

3. The peptidomimetic macrocycle of claim 1, wherein the amino acid sequence of said peptidomimetic macrocycle is at least about 80% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4.

4. The peptidomimetic macrocycle of claim 1, wherein the amino acid sequence of said peptidomimetic macrocycle is at least about 90% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4.

5. The peptidomimetic macrocycle of claim 1, wherein the amino acid sequence of said peptidomimetic macrocycle is chosen from the group consisting of the amino acid sequences in Table 1, 2, 3, or 4.

6. The peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle comprises a helix.

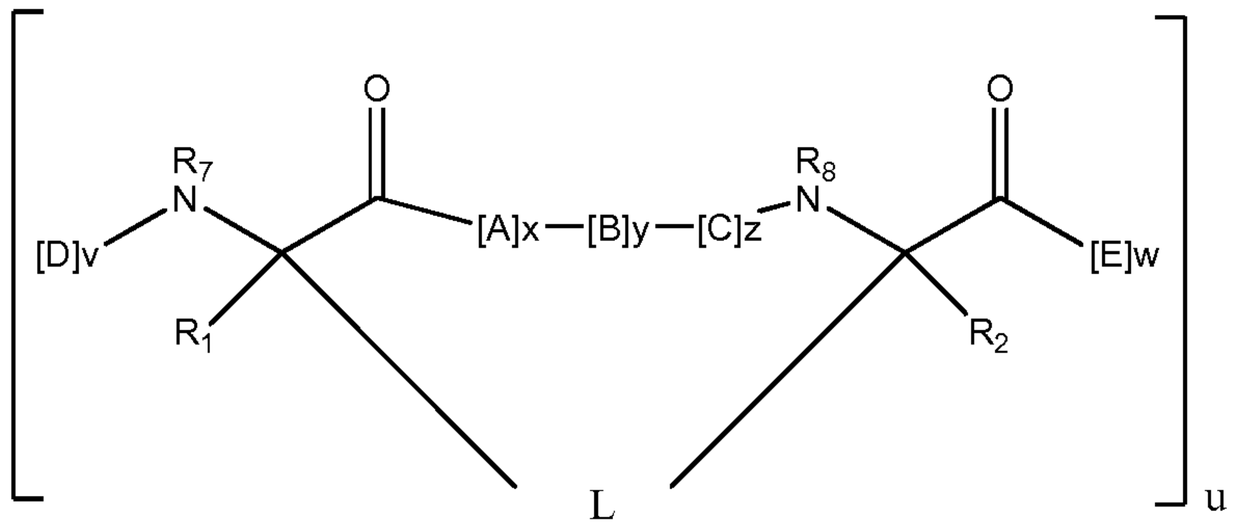
7. The peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle comprises an α-helix.

8. The peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle comprises an α,α-disubstituted amino acid.

9. The peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle comprises a crosslinker linking the α-positions of at least two amino acids.

10. The peptidomimetic macrocycle of claim 9, wherein at least one of said two amino acids is an α,α-disubstituted amino acid.

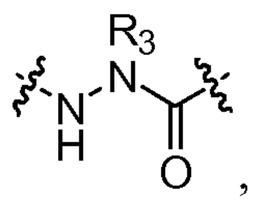
11. The peptidomimetic macrocycle of any one of claims 1-10, wherein the peptidomimetic macrocycle has the formula:



Formula I

wherein:

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

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

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

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

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

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

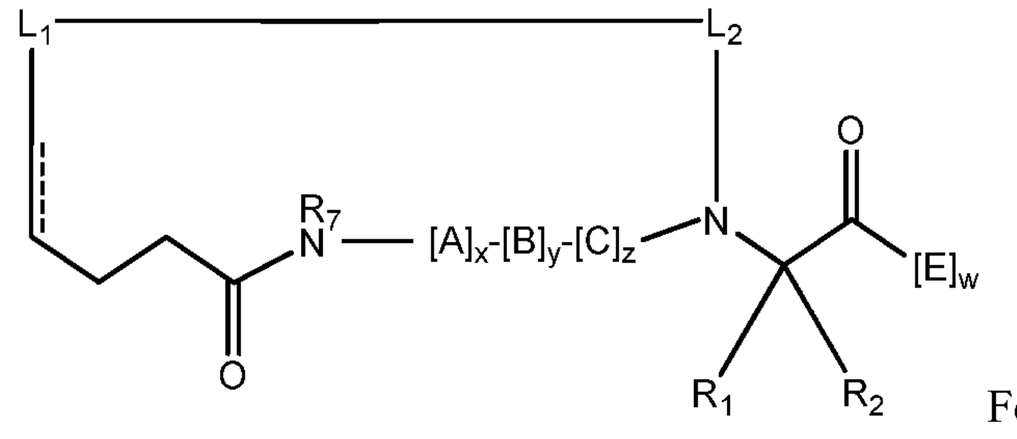
x, y and z are independently integers from 0-10; and

n is an integer from 1-5.

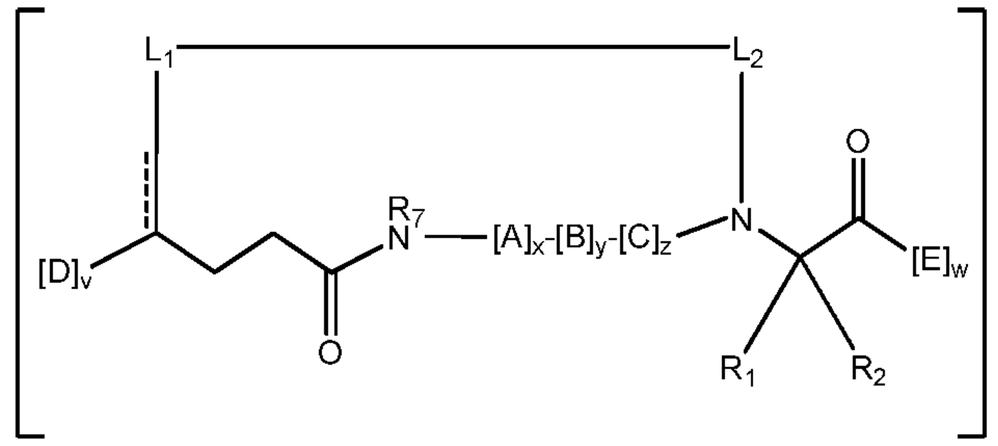
12. The peptidomimetic macrocycle of claim 11, wherein L does not include a thioether or a triazole.

13. The peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle comprises a crosslinker linking a backbone amino group of a first amino acid to a second amino acid within the peptidomimetic macrocycle.

14. The peptidomimetic macrocycle of claim 13, wherein the peptidomimetic macrocycle has the formula (IV) or (IVa):



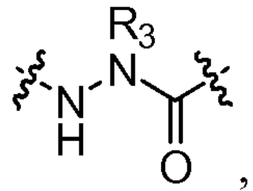
Formula (IV)



Formula (IVa)

wherein:

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

B is a natural or non-natural amino acid, amino acid analog,  , [-NH-L_3-CO-], [-NH-L_3-SO_2-], or [-NH-L_3-];

R_1 and R_2 are 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;

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, 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, 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, cycloaryl, or heterocycloaryl, optionally substituted with R_5;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

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

n is an integer from 1-5.

15. The peptidomimetic macrocycle of claim 14, wherein L_1 and L_2 either alone or in combination do not include a thioether or a triazole.

16. The peptidomimetic macrocycle of claim 11, wherein L_1 and L_2 are independently alkylene, alkenylene or alkynylene.

17. The peptidomimetic macrocycle of claim 11, wherein L_1 and L_2 are independently C_3-C_{10} alkylene or alkenylene

18. The peptidomimetic macrocycle of claim 17, wherein L_1 and L_2 are independently C_3-C_6 alkylene or alkenylene.

19. The peptidomimetic macrocycle of claim 11, wherein R_1 and R_2 are H.

20. The peptidomimetic macrocycle of claim 11, wherein R_1 and R_2 are independently alkyl.

21. The peptidomimetic macrocycle of claim 11, wherein R_1 and R_2 are methyl.

22. A method of treating cancer in a subject comprising administering to the subject a peptidomimetic macrocycle of claim 1.

23. A method of modulating the activity of p53 and/or HDM2 and/or HDMX in a subject comprising administering to the subject a peptidomimetic macrocycle of claim 1.

24. A method of antagonizing the interaction between p53 and HDM2 and/or between p53 and HDMXproteins in a subject comprising administering to the subject a peptidomimetic macrocycle of claim 1.

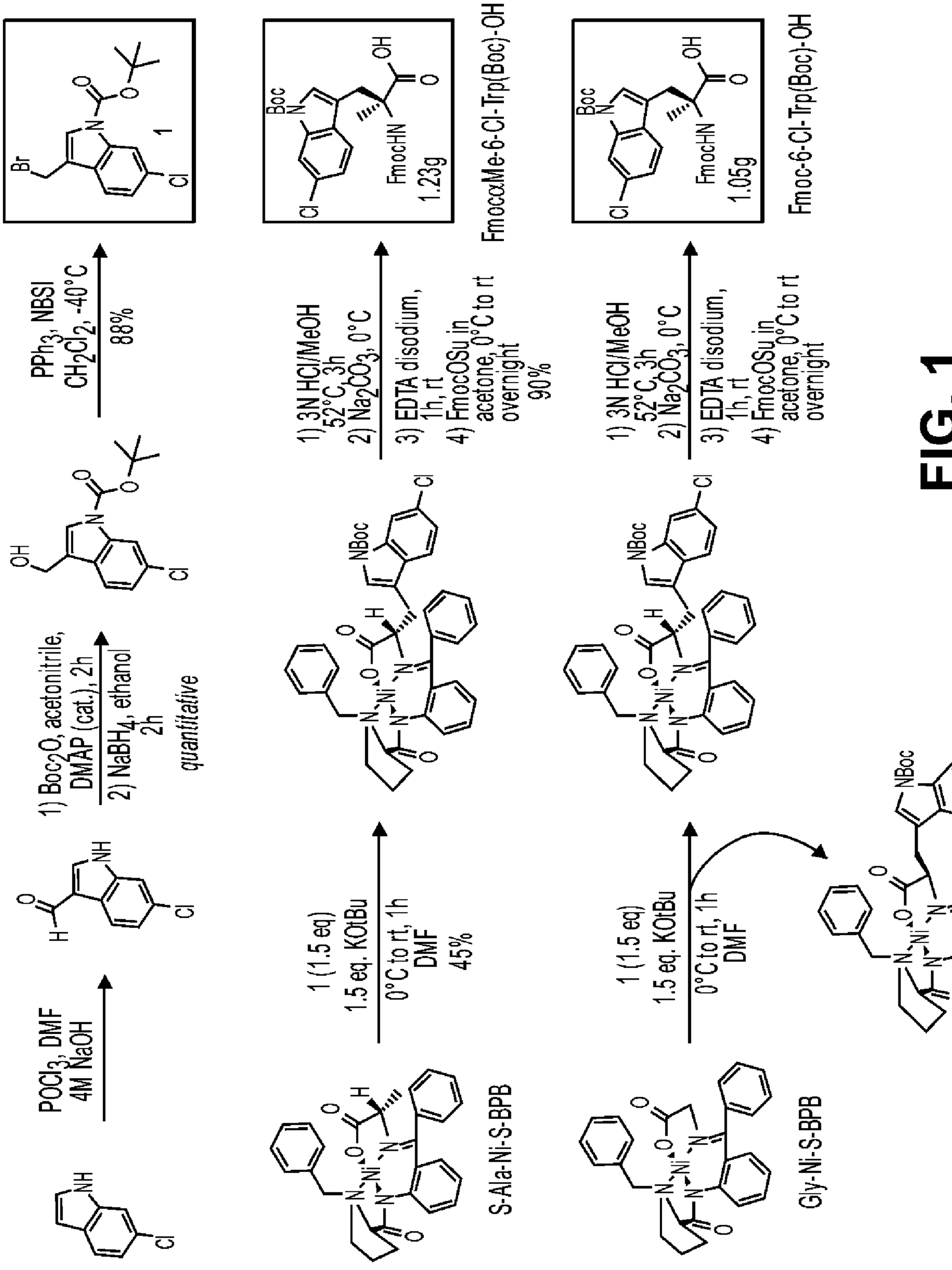

Br
Cl
1
PPh3, NBSI
CH2Cl2, -40°C
88%
OH
Cl
POCl3, DMF
4M NaOH
NH
Cl
1) Boc2O, acetonitrile,
DMAP (cat.), 2h
2) NaBH4, ethanol
2h
quantitative
NBoc
Cl
1 (1.5 eq)
1.5 eq. KOtBu
0°C to rt, 1h
DMF
45%
S-Ala-Ni-S-BPB
1) 3N HCl/MeOH
52°C, 3h
2) Na2CO3, 0°C
3) EDTA disodium,
1h, rt
4) FmocOSu in
acetone, 0°C to rt
overnight
90%
Boc
N
OH
O
FmocHN
1.23g
FmocαMe-6-Cl-Trp(Boc)-OH
1.05g
Fmoc-6-Cl-Trp(Boc)-OH
Gly-Ni-S-BPB

FIG. 1

FIG. 2

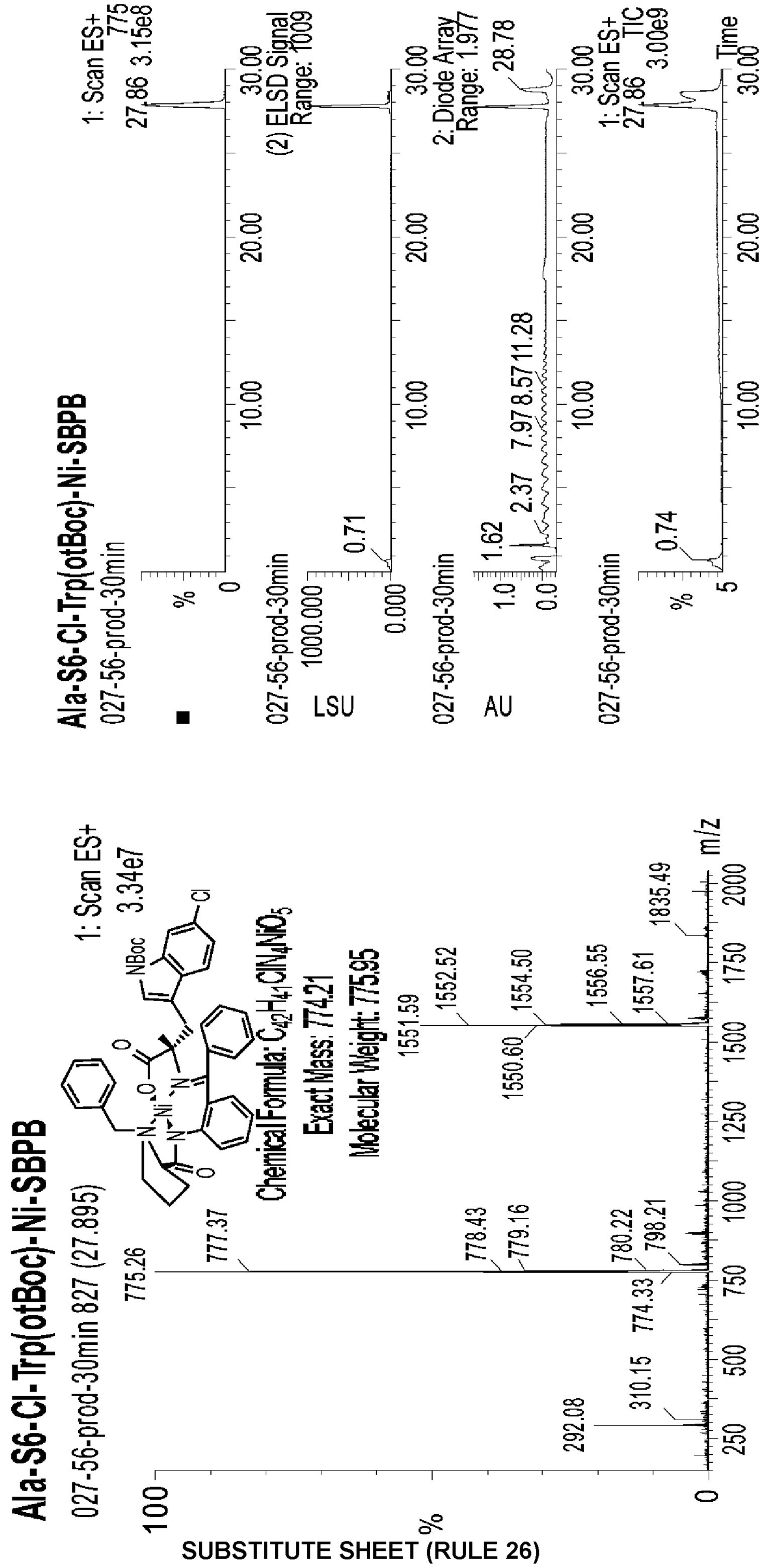

Ala-S6-Cl-Trp(otBoc)-Ni-SBPB
027-56-prod-30min
1: Scan ES+ 775 27.86 3.15e8
(2) ELSD Signal Range: 1009
2: Diode Array Range: 1.977
28.78
1: Scan ES+ TIC 3.00e9
Time
0.71
0.74
1.62
2.37
7.97
8.57
11.28
LSU
AU
%
Ala-S6-Cl-Trp(otBoc)-Ni-SBPB
027-56-prod-30min 827 (27.895)
1: Scan ES+ 3.34e7
NBoc
Cl
Chemical Formula: C42H41ClN4NiO5
Exact Mass: 774.21
Molecular Weight: 775.95
m/z
775.26
777.37
778.43
779.16
780.22
798.21
774.33
310.15
292.08
1551.59
1552.52
1550.60
1554.50
1556.55
1557.61
1835.49

FIG. 3

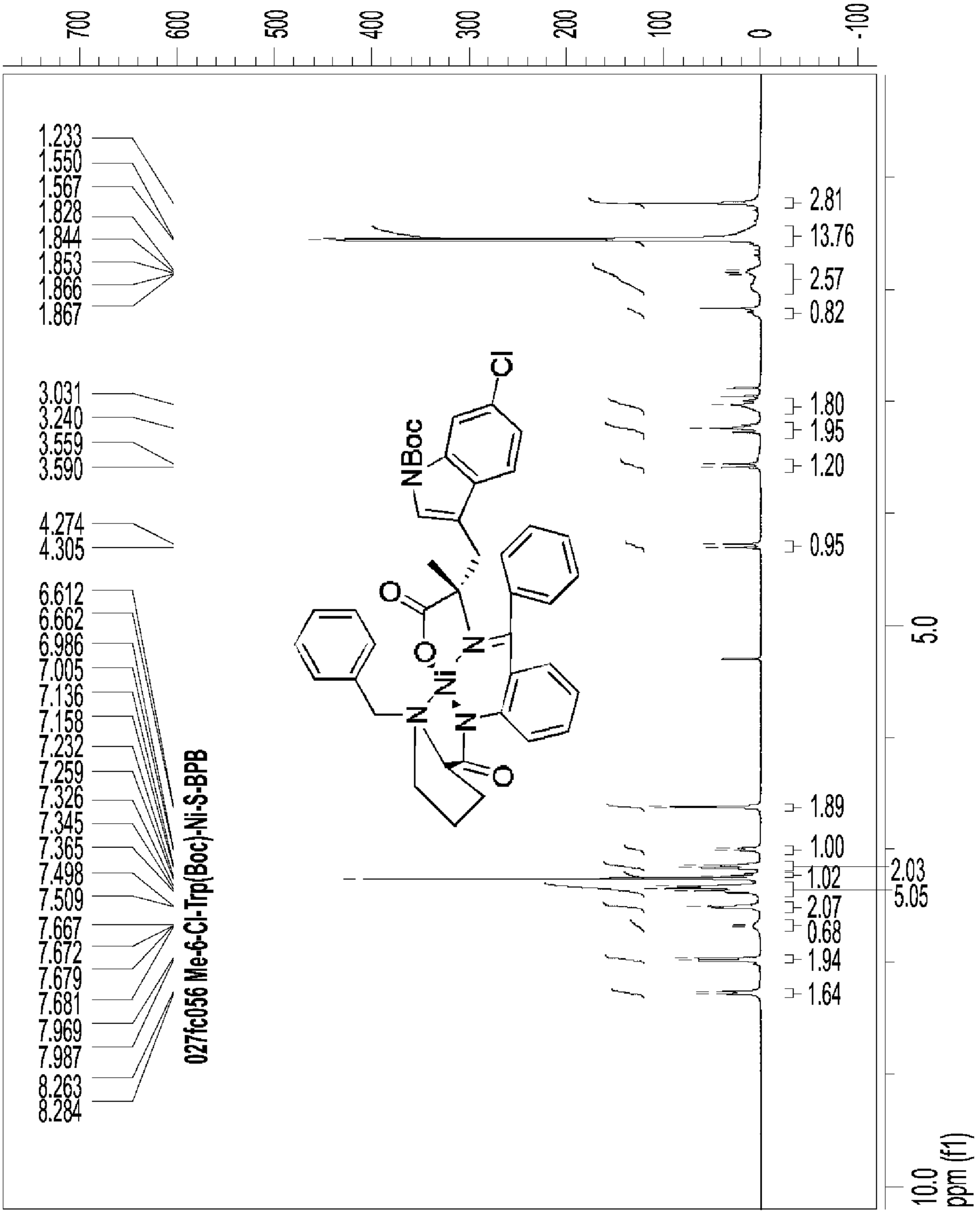

700
600
500
400
300
200
100
0
-100
1.233
1.550
1.567
1.828
1.844
1.853
1.866
1.867
3.031
3.240
3.559
3.590
4.274
4.305
6.612
6.662
6.986
7.005
7.136
7.158
7.232
7.259
7.326
7.345
7.365
7.498
7.509
7.667
7.672
7.679
7.681
7.969
7.987
8.263
8.284
027fc056 Me-6-Cl-Trp(Boc)-Ni-S-BPB
Cl
NBoc
O
O
Ni
N
N
N
N
O
2.81
13.76
2.57
0.82
1.80
1.95
1.20
0.95
1.89
1.00
1.02
2.07
0.68
1.94
1.64
2.03
5.05
5.0
10.0
ppm (f1)

FIG. 4

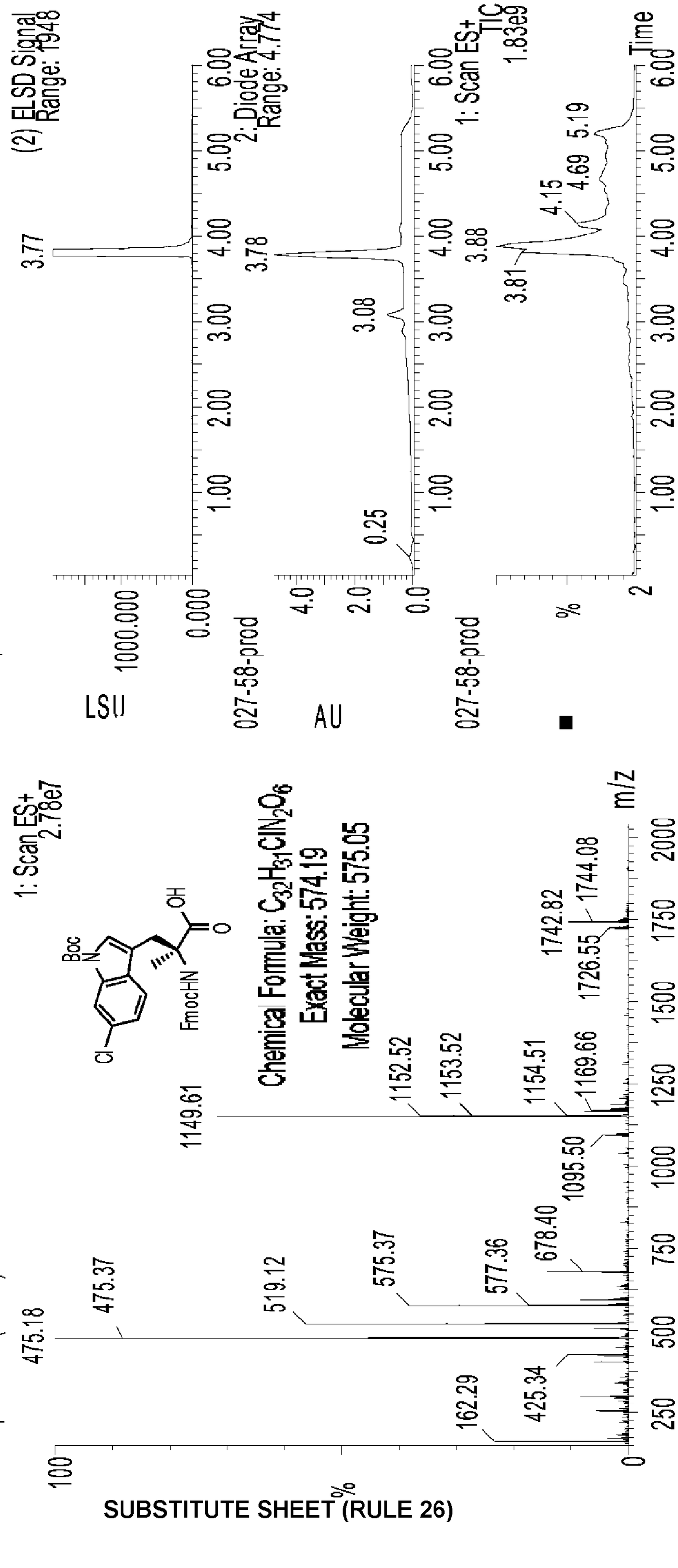

Fmoc-SMeTrp(6-Cl)(NtBoc)-OH
027-58-prod
(2) ELSD Signal
Range: 1948
3.77
LSU
1000.000
0.000
4.00
5.00
6.00
1.00
2.00
3.00
2: Diode Array
Range: 4.774
3.78
AU
4.0
2.0
0.0
0.25
3.08
1: Scan ES+
TIC
1.83e9
3.88
4.15
4.69
5.19
3.81
%
Time
1: Scan ES+
2.78e7
Boc
N
Cl
OH
O
FmocHN
1149.61
Chemical Formula: C32H31ClN2O6
Exact Mass: 574.19
Molecular Weight: 575.05
1152.52
1153.52
1154.51
1742.82
1169.66
1726.55
1744.08
m/z
Fmoc-SMeTrp(6-Cl)(NtBoc)-OH
027-58-prod 115 (3.879)
475.18
475.37
519.12
575.37
577.36
678.40
1095.50
162.29
425.34
100
%
0
250
500
750
1000
1250
1500
1750
2000

FIG. 5

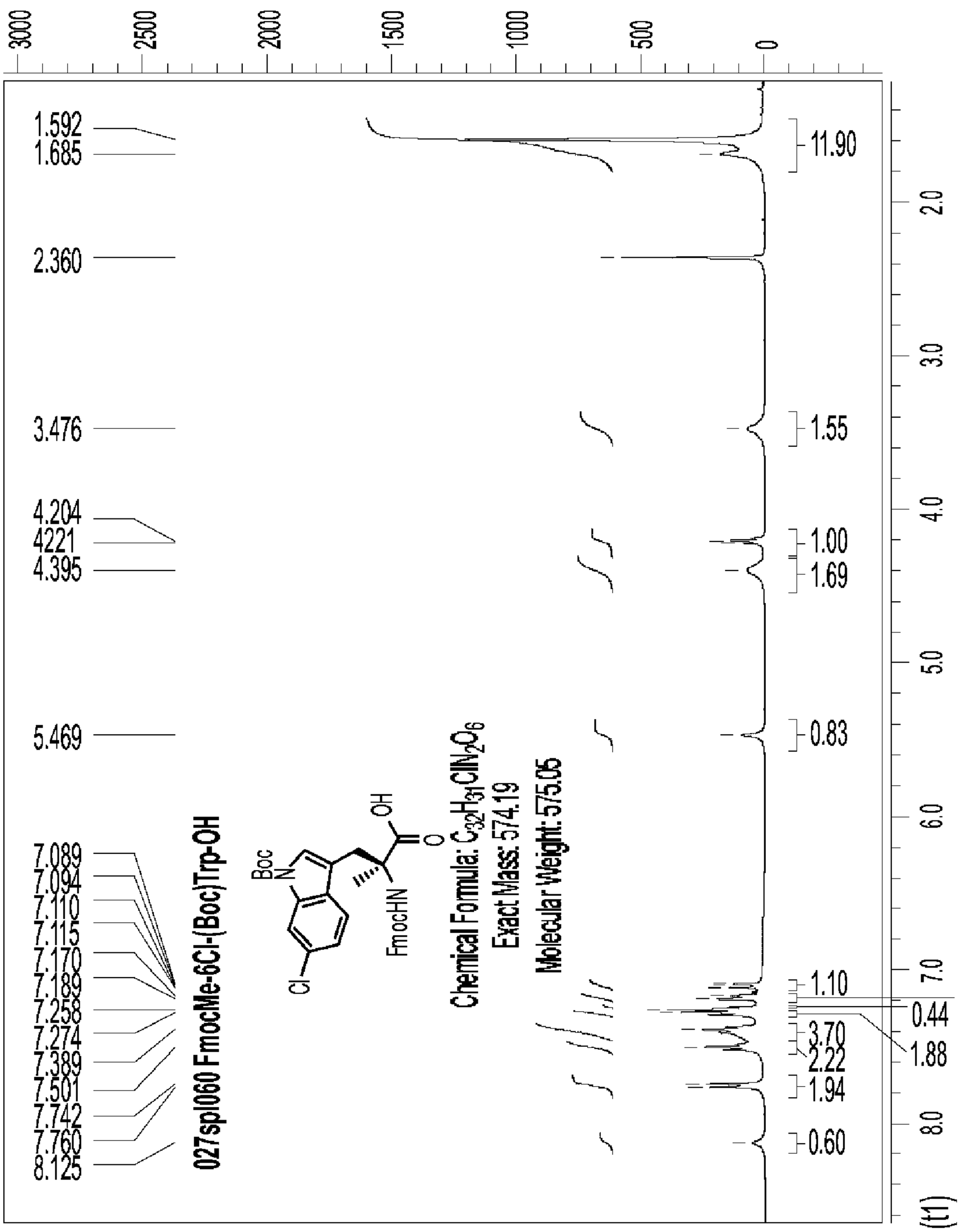

3000
2500
2000
1500
1000
500
0
1.592
1.685
2.360
3.476
4.204
4221
4.395
5.469
7.089
7.094
7.110
7.115
7.170
7.189
7.258
7.274
7.389
7.501
7.742
7.760
8.125
11.90
1.55
1.00
1.69
0.83
1.10
0.44
3.70
2.22
1.94
0.60
1.48
1.88
027spl060 FmocMe-6Cl-(Boc)Trp-OH
Boc
N
Cl
OH
O
FmocHN
Chemical Fomula: C32H31ClN2O6
Exact Mass: 574.19
Molecular Weight: 575.05
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ppm (t1)

FIG. 6A

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD % Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0% TFE	50% TFE
SP-1	2069.40	Ac-LSQETF$r8DLWKLL$EN-NH2	>50													
SP-2	2067.43	Ac-LSQETF$r8NLWKLL$QN-NH2	>50													
SP-3	2094.46	Ac-LSQQTF$r8NLWRLL$QN-NH2	17.6; 10.3		13.4											
SP-4	2081.42	Ac-QSQQTF$r8NLWKLL$QN-NH2	36.8; 21.2													
SP-5	2109.4	Ac-QSQQTF$r8NLWRLL$QN-NH2	22.6, 16.5, 18.4, 18.4, 17.1, 17.9	12.0	2.7; 5.4	67; 56	14.3	25	90	32; 16	1	0.9			74.8, 76.5	100
SP-6	2033.33	Ac-QSQQTA$r8NLWRLL$QN-NH2	>50	>25	>1000	>1000	>50									
SP-7	2107.46	Ac-QAibQQTF$r8NLWRLL$QN-NH2	19.9; 14.3; 10.3													
SP-8	2001.21	Ac-QSQQTFSNLWRLLPQN-NH2	>50	>25											5	34
SP-9	2137.48	Ac-QSQQTF$/r8NLWRLL$/QN-NH2	7.3		>1000	>1000	>50								55	98
SP-10	2066.40	Ac-QSQAibTF$r8NLWRLL$QN-NH2	11.4		26.6											
SP-11	2052.38	Ac-QSQQTF$r8NLWRLL$AN-NH2	13.0		15.9,											
SP-12	2052.38	Ac-ASQQTF$r8NLWRLL$QN-NH2	10.0		17.4											
SP-13	2066.40	Ac-QSQQTF$r8ALWRLL$QN-NH2	13.7													
SP-14	2110.41	Ac-QSQETF$r8NLWRLL$QN-NH2	>50													
SP-15	2137.49	Ac-RSQQTF$r8NLWRLL$QN-NH2	5.6		42.3,	199,	13.45								62.3	94.6
SP-16	2138.47	Ac-RSQQTF$r8NLWRLL$EN-NH2	10.3		13.5											
SP-17	1961.17	Ac-LSQETFSDLWKLLPEN-NH2	>50													
SP-18	2009.31	Ac-QSQTFSLWRLLPQN-NH2	>50													
SP-19	2037.28	Ac-QSQQFSNWRLLPQN-NH2	>50													
SP-20	1918.16	Ac-QSQQTSNLRLLPQN-NH2	>50													
SP-21	2008.28	Ac-QSQQTFNLWLLPQN-NH2	>50													
SP-22	2311.64	Ac-RTQATF$r8NQWAibANle$TNAibTR-NH2	35.2													
SP-23	2137.49	Ac-QSQQTF$r8NLWRLL$RN-NH2	>50													
SP-24	2137.49	Ac-QSQRTF$r8NLWRLL$QN-NH2	>50													

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FIG. 6B

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD %Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0%TFE	50%TFE
SP-25	2109.43	Ac-QSQQTF$r8NNleWRLL$QN-NH2														
SP-26	2109.43	Ac-QSQQTF$r8NLWRNleL$QN-NH2	14.8; 6.2; 11.8; 19.7			64	26.8			62; 6	1.1	0.9				
SP-27	2109.43	Ac-QSQQTF$r8NLWRLNle$QN-NH2	14.8													
SP-28	2125.43	Ac-QSQQTY$r8NLWRLL$QN-NH2	>50													
SP-29	2135.51	Ac-RAibQQTF$r8NLWRLL$QN-NH2	5; 4.9; 11.5; 19.5													
SP-30	1599.83	Ac-MPRFMDYWEGLN-NH2													7.5	58.2
SP-31	2192.57	Ac-RSQQRF$r8NLWRLL$QN-NH2	8.6													
SP-32	2164.51	Ac-QSQQRF$r8NLWRLL$QN-NH2	15.5,16.9													
SP-33	2190.59	Ac-RAibQQRF$r8NLWRLL$QN-NH2	7.6													
SP-34	2226.58	Ac-RSQQRF$r8NFWRLL$QN-NH2	5.2, 4.8													
SP-35	2242.58	Ac-RSQQRF$r8NYWRLL$QN-NH2														
SP-36	2109.43	Ac-RSQQTF$r8NLWQLL$QN-NH2	5.3, 5.7, 11.4													
SP-37	2095.40	Ac-QSQQTF$r8NLWQAmlL$QN-NH2	41.9													
SP-38	2123.46	Ac-QSQQTF$r8NAmlWRLL$QN-NH2	9.1													
SP-39	1871.14	Ac-NlePRF$r8DYWEGL$QN-NH2	>50			3.82				0.8	1.5	1.3				
SP-40	1953.33	Ac-NlePRF$r8NYWRLL$QN-NH2	5.2; 5.9			6.3				3.1	1.7	1			62.6	90.6
SP-41	1578.94	Ac-RF$r8NLWRLL$Q-NH2	>50													
SP-42	2161.44	Ac-QSQQTF$r8N2fiWRLL$QN-NH2	14.5													
SP-43	2161.44	Ac-QSQQTF$r8N3fiWRLL$QN-NH2	18.3													
SP-44	2081.38	Ac-QSQQTF#r8NLWRLL#QN-NH2	14			29.3		25	180						50.5	94.8
SP-45	2061.39	Ac-RSQQTA$r8NLWRLL$QN-NH2	32.85													
SP-46	2111.45	Ac-QSQQTF%r8NLWRLL%QN-NH2	6.8		175	10.1									64.3, 85	88.5, 121
SP-47	2052.33	HepQSQ$TFSNLWRLLPQN-NH2	>50													
SP-48	2160.56	HepQSQTFr8NLWRLL$QN-NH2	>50													

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CA 02807685 2013-02-06

FIG. 6C

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD %Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0%TFE	50%TFE
SP-49	2143.87	Ac-QSQQTF$r8NL6clWRLL$QN-NH2	15.94				11.25									
SP-50	2157.90	Ac-QSQQTF$r8NLMe6clwRLL$QN-NH2					>50								60.9	82.4
SP-51	1536.68	Ac-LTFEHYWAQLTS-NH2	>50												-0.5	61.6
SP-52	1586.83	Ac-LTFHYWQLTS-NH2	>50													
SP-53	1521.75	Ac-LTFEYWALTS-NH2	>50													
SP-54	1598.88	Ac-LTF$r8HYWAQL$S-NH2													85.6	96.6
SP-55	1683.99	Ac-LTF$r8HYWRQL$S-NH2	16.3				19.3			25.2	15.1				59.5	84.2
SP-56	2146.53	Ac-QS$QTFStNLWRLL$s8QN-NH2	12.8			383	>50			1	1				98.9, 109	110, 124
SP-57	1925.11	Ac-QSQQTASNLWRLLPQN-NH2	>50													
SP-58	2061.39	Ac-QSQQTA$/r8NLWRLL$/QN-NH2	>50													
SP-59	2080.43	Ac-ASQQTF$/r8NLWRLL$/QN-NH2														
SP-60	2010.30	Ac-SQQFSNLWRLLAibQN-NH2														
SP-61	2024.32	Ac-QSQTFNLWRLLAibQN-NH2	35.76													
SP-62	2025.27	Ac-QSQQFSNWRLLAibQN-NH2	>50													
SP-63	1996.27	Ac-QSQQTFNLWLLAibQN-NH2	>50													
SP-64	2012.27	Ac-QSQQTFSLWRLAibQN-NH2	>50													
SP-65	1941.19	Ac-QSQQTFSNLWLLAN-NH2	>50													
SP-66	2038.35	Ac-$/SQQ$/FSNLWRLLAibQN-NH2														
SP-67	2052.38	Ac-QS$/QTF$/NLWRLLAibQN-NH2	16.57													
SP-68	2053.32	Ac-QSQQ$/FSN$/WRLLAibQN-NH2	36.94													
SP-69	2024.32	Ac-QSQQTF$/NLW$/LLAibQN-NH2	>50													
SP-70	1997.30	Ac-QSQTFSLWRLLAibQN-NH2	36.06													
SP-71	2025.35	Ac-QSQ$/TFS$/LWRLLAibQN-NH2	30.36													
SP-72	2202.64	Ac-QS$/QTFSt//NLWRLL$/s8QN-NH2	5.1			>1000	10.68								99	108

FIG. 6D

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD %Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0%TFE	50%TFE
SP-73	2039.38	Ac-$r8SQQTFS$LWRLLAibQN-NH2	14.01 and 26.91													
SP-74	1997.30	Ac-QSQ$r8TFSNLW$LLAibQN-NH2	>50 (both)													
SP-75	2025.35	Ac-QSQQTFS$r8LWRLLA$N-NH2	>50													
SP-76	2033.37	Ac-QS$r5QTFStNLW$LLAibQN-NH2	>50												0	58
SP-77	2067.43	Ac-$/r8SQQTFS$/LWRLLAibQN-NH2														
SP-78	2025.35	Ac-QSQ$/r8TFSNLW$/LLAibQN-NH2														
SP-79	2053.41	Ac-QSQQTFS$/r8LWRLLA$/N-NH2														
SP-80	2089.48	Ac-QS$/r5QTFSt//NLW$/LLAibQN-NH2														
SP-81	1989.19	Ac-QSQQTFSNLWRLLAibQN-NH2														
SP-82	2216.67	Hep/QSQ$/TF$/r8NLWRLL$/QN-NH2														
SP-83	2052.38	Ac-ASQQTF$r8NLRWLL$QN-NH2														
SP-84	2023.38	Ac-QSQQTF$/r8NLWRLL$/Q-NH2					>50									
SP-85	1995.33	Ac-QSQQTF$r8NLWRLL$Q-NH2	9.35				20.7								77.8	95.7
SP-86	1516.79	Ac-AAARAA$r8AAARAA$AA-NH2													131	143
SP-87	1607.76	Ac-LTFEHYWAQLTSA-NH2														
SP-88	1669.96	Ac-LTF$r8HYWAQL$SA-NH2					6.5		12.1						80.8	81.8
SP-89	1944.15	Ac-ASQQTFSNLWRLLPQN-NH2														
SP-90	2033.37	Ac-QS$QTFStNLW$r5LLAibQN-NH2														
SP-91	1987.22	Ac-QSQQTFAibNLWRLLAibQN-NH2														
SP-92	2043.33	Ac-QSQQTFNleNLWRLLNleQN-NH2														
SP-93	2083.39	Ac-QSQQTF$/r8NLWRLLAibQN-NH2														
SP-94	2111.45	Ac-QSQQTF$/r8NLWRLLNleQN-NH2														
SP-95	2041.31	Ac-QSQQTFAibNLWRLL$/QN-NH2														
SP-96	2069.37	Ac-QSQQTFNleNLWRLL$/QN-NH2														

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FIG. 6E

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD %Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0%TFE	50%TFE
SP-97	2145.89	Ac-QSQQTF%r8NL6clWRNIeL%QN-NH2														
SP-98	2159.92	Ac-QSQQTF%r8NLMe6clwRLL%QN-NH2													62.4	71.1
SP-101	1161.39	Ac-FNleYWEL-NH2														
SP-102	1345.63	Ac-F$r8AYWELL$A-NH2														
SP-103	1344.64	Ac-F$r8AYWQLL$A-NH2														
SP-104	1926.26	Ac-NlePRF$r8NYWELL$QN-NH2														
SP-105	1954.32	Ac-NlePRF$r8DYWRLL$QN-NH2														
SP-106	1839.23	Ac-NlePRF$r8NYWRLL$Q-NH2														
SP-107	1711.10	Ac-NlePRF$r8NYWRLL$-NH2													54.4	80.5
SP-108	2110.41	Ac-QSQQTF$r8DLWRLL$QN-NH2														
SP-109	2110.41	Ac-QSQQTF$r8NLWRLL$EN-NH2														
SP-110	2110.41	Ac-QSQQTF$r8NLWRLL$QD-NH2														
SP-111	1954.27	Ac-QSQQTF$r8NLWRLL$S-NH2														
SP-112	2110.41	Ac-ESQQTF$r8NLWRLL$QN-NH2														
SP-113	1637.02	Ac-LTF$r8NLWRNIeL$Q-NH2														
SP-114	1692.10	Ac-LRF$r8NLWRNIeL$Q-NH2														
SP-115	2182.48	Ac-QSQQTF$r8NWWRNIeL$QN-NH2														
SP-116	1995.33	Ac-QSQQTF$r8NLWRNIeL$Q-NH2														
SP-117	1766.09	Ac-QTF$r8NLWRNIeL$QN-NH2														
SP-118	1976.37	Ac-NlePRF$r8NWWRLL$QN-NH2														
SP-119	1805.22	Ac-NlePRF$r8NWWRLL$A-NH2														
SP-120	1468.61	Ac-TSFAEYWNLLSP-NH2													6.8	30.6
SP-121	1652.89	Ac-QTF$r8HWWSQL$S-NH2					4.1								21.6	88.2
SP-122	1179.43	Ac-FMYWEL-NH2														
SP-123	1602.75	Ac-QTFEHWWSQLLS-NH2													21.6	87.6

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FIG. 6F

SP#	MW	Sequence	SJSA-1 Cell viability EC50 (μM) serum free		Competition ELISA IC50 (nM)		p53 Nuclear translocation of p53 (GRIP assay)	Affinity selection MS Kd (nM)		p21 activation ELISA			FP Competition Binding IC50 (nM)		CD % Helicity	
			24h	72h	GST-hDM2	GST-hDMX		GST-hDM2	GST-hDMX	20μM	10μM	1μM	HDM2	HDMx	0% TFE	50% TFE
SP-124	2123.46	Ac-QSQQTF$r8NLAmwRLNle$QN-NH2														
SP-125	1131.73	Ac-FMAibY6clWEAc3cL-NH2													-3.6	16.3
SP-126	1195.84	Ac-FNle$Y6clWE$L-NH2														
SP-127	1278.92	Ac-F$r8AY6clWEAc3cL$-NH2													4.6 and 2.3	58.1
SP-128	1350.00	Ac-F$r8AY6clWEAc3cL$A-NH2														
SP-129	1987.78	Ac-NlePRF$r8NY6clWRLL$QN-NH2														
SP-130	1224.49	Ac-AF$r8AAWALA$A-NH2														
SP-131	1396.68	Ac-TF$r8AAWRLA$Q-NH2														
SP-132	1410.70	Pr-TF$r8AAWRLA$Q-NH2														
SP-133	2111.45	Ac-QSQQTF%r8NLWRNIeL%QN-NH2													84.7	110.8
SP-134	1671.98	Ac-LTF%r8HYWAQL%SA-NH2													78.4	82
SP-135	1955.35	Ac-NlePRF%r8NYWRLL%QN-NH2													61.6	84.8
SP-136	1989.80	Ac-NlePRF%r8NY6clWRLL%QN-NH2					2.6									
SP-137	1635.34	Ac-LTF%r8HY6clWAQL%S-NH2					2								69.5	78.6
SP-138	2150.56	Ac-QS%QTF%StNLWRLL%s8QN-NH2													96	101.3
SP-139	1720.45	Ac-LTF%r8HY6clWRQL%S-NH2													74.2	94.2
SP-140	2145.89	Ac-QSQQTF%r8NL6clWRLL%QN-NH2													92.4	120.8
SP-141	2041.40	Ac-%r8SQQTFS%LWRLLAibQN-NH2													66.7	105.6
SP-142	1600.90	Ac-LTF%r8HYWAQL%S-NH2					0.65								45.2	447
SP-143	1603.90	Ac-TSF%r8QYWNLL%P-NH2													2.6	26.5

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FIG. 7A

SP#	SEQUENCE																	
Nutlin 3a																		
SP-5	Ac-	Q	S	Q	Q	T	F	$r8	N	L	W	R	L	L	$	Q	N	-NH2
SP-128						Ac-	F	$r8	A	Y	6clW	E	Ac3c	L	$	A	-NH2	
SP-142				Ac-	L	T	F	$r8	H	Y	W	A	Q	L	$	S	-NH2	
SP-191				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	S	-NH2	
SP-194				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	A	-NH2	
SP-344				Ac-	L	T	F	$r8	H	Y	W	A	Q	L	$	A	A	
SP-266				Ac-	L	T	F	$r8	V	Y	W	A	Q	L	$	S	-NH2	
SP-267				Ac-	L	T	F	$r8	F	Y	W	A	Q	L	$	S	-NH2	
SP-360				Ac-	L	T	F	$r8	A	Y	W	S	Q	L	$	S	-NH2	
SP-512				Ac-	L	T	F	$r8	E	Y	W	A	Q	F	$	S	-NH2	
SP-508				Ac-	E	T	F	$r8	A	Y	W	A	Q		$	S	-NH2	
SP-522						Ac-	F	$r8	A	Y	6clW	E	A	L	$	A	-NH2	
SP-580				mdPEG3-	L	T	F	$r8	A	Y	W	A	Q	L	$	S	-NH2	
SP-523						Ac-	F	$r8	A	Y	dI6brW	E	A	L	$	A	-NH2	
SP-573						Ac-	F	$r8	A	Y	6clW	E	A	L	$	A	-NH2	
SP-256				Ac-	W	T	F	$r8	H	Y	W	A	Q	L	$	S	-NH2	
SP-351				Ac-	L	T	F	$r8	A	Y	W	A	A	L	$	S	-NH2	
SP-641						Ac-	F	$r8	A	Y	6clW	Q	A	L	$	A	-NH2	

FP Competition Blinding Assay				SJSA-1 Cell Viability 72h (10% FBS) EC50 (uM)		p21 levels at 21h (10% FBS) (pg/ml)			
HDM2 IC50 (nM)	HDMx IC50 (nM)	HDM2 Ki (IC50) - FAM-pDi	HDMx Ki (IC50) - FAM-pDi	AVE EC50 (uM)	SD	30uM	10uM	3uM	1uM
442	42500	82.3	6876	1.6	0.55	3290 ± 619	2659 ± 749	915 ±262	BDL
94.1	667	14	91	>30		ND	ND	ND	ND
114.5	967	2.5	85.1	4.5	1.70	2400 ± 584	1535 ± 166	522 ± 82	BDL
88.13	408	4.5	63.9	14.2	5.40	2598	170	BDL	BDL
74.92	97	8.4	N/D	5.7	0.80	1672 ± 267	1068 ± 93	105 ± 79	BDL
140.55	463	9.1	n/D	5.2	1.00	1723 ± 494	937 ± 117	178 ± 88	BDL
59.41	348	5.2	n/D	7.4	1.20	1688 ± 443	1009 ± 668	BDL	BDL
66	301	7	80	4.9	0.50	3751 ± 942	1620 ± 552	102 ± 45	BDL
190.5	318.5	21	85	5.3	1.27	3983 ± 957	1106 ± 519-	43 ± 19	BDL
90	110	<1	27	5.0	2.36	3337 ± 836	1182 ± 509	265 ± 38	BDL
16.2	26.1	1.86	4.76	11.3	3.59	3137 ± 630	879 ± 289	BDL	BDL
22.76	88.82	2.18	15.12	7.1	3.00	2322 ± 572	972 ± 182	451 ± 103	BDL
ND	ND	ND	ND	1.88	0.82	1117 ± 267	1038 ± 184	1023 ± 205	BDL
81	195	10	24	8.6	2.8	505 ± 70	(592, 169)	BDL	BDL
ND	ND	ND	ND	6.14	2.47	1016 ± 280	407 ± 25	41 ± 26	BDL
ND	ND	ND	ND	1.44	0.74	1103 ± 319	1118 ± 298	709 ± 217	BDL
185	204	201	54	6.7	3.3	3625 ± 856	330 ± 112	BDL	BDL
47.2	143	<10	24.1	0.7	0.44	1784	1860	1184 ± 756	132
ND	ND	ND	ND	2.7	0.23	1755	958	497	75

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FIG. 7B

SP#	SEQUENCE																		Fold Caspase Activation relative to DMSO in 10% FBS @ 48h				Cell Viability (10% FBS) EC50 (uM)					% Helicity @ 222 nM vs parent seq	
																			30uM	10uM	3uM	1uM	RKO (72h)	HCT-116, 5k/well,(72h)	MCF-7 (7500 cells/well, 5day)	SW-480, 2k/well, 6d	RKO-E6 (72h)	0% TFE	50% TFE
Nutlin 3a																			45.5	26.5	13.3	1.0	6.7 ± 2.5	8.9 ± 4.9	5 ± 1.9	>30	>30		
SP-5	Ac-	Q	S	Q	Q	T	F	$r8	N	L	W	R	L	L	$	Q	N	-NH2	ND	ND	ND	ND	ND	ND	ND	ND	ND	74%	100%
SP-128						Ac-	F	$r8	A	Y	6clW	E	Ac3c	L	$	A		-NH2	29.6	9.1	2.0	1.0	20.8 ± 10.5	>30	10.7 ± 0.4	>30	>30	10%	188%
SP-142				Ac-	L	T	F	$r8	H	Y	W	A	Q	L	$	S		-NH2	30.0	2.0	1.7	1.0	12.6 ± 1.6	24.1 ± 4.3	ND	26.7	>30	94%	94%
SP-191				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	S		-NH2	34.5	5.1	1.8	1.0	10.6 ± 3.8	11.1 ± 2.4	6.4 + 1.2	>30	>30	4%	151%
SP-194				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	A		-NH2	32.6	5.8	2.2	1.0	ND	11.2 ± 6.5	4 + 0.4	>30	>30	5%	143%
SP-344				Ac-	L	T	F	$r8	H	Y	W	A	Q	L	$	A		A	38.8	5.3	1.6	1.0	15.3 ± 5.1	11.3 ± 3.7	10.2 + 1.6	>30	>30	140%	140%
SP-266				Ac-	L	T	F	$r8	V	Y	W	A	Q	L	$	S		-NH2	30 ± 0	12 ± 11	1 ± 0	1.0	16 ± 2	7.5 ± 0.85	ND	19.2 ± 2.7	>30	2%	142%
SP-267				Ac-	L	T	F	$r8	F	Y	W	A	Q	L	$	S		-NH2	29 ± 4	15 ± 8	1 ± 0	1.0	9.5 ± 2.5	5.5 ± 0.5	ND	17.5 ± 7.8	~29uM	1%	120%
SP-360				Ac-	L	T	F	$r8	A	Y	W	S	Q	L	$	S		-NH2	30 ± 4	11 ± 8	2 ± 1	1.0	5.5 ± 0.7	6.6	ND	>30	>30	8%	119%
SP-512				Ac-	L	T	F	$r8	E	Y	W	A	Q	F	$	S		-NH2	24 ± 6	11 ± 2	3 ± 0	1.0	>30	ND	ND	>30	>30	2%	131%
SP-508				Ac-	E	T	F	$r8	A	Y	W	A	Q		$	S		-NH2	19 ± 6	8 ± 4	4 ± 2	1.0	>30	>30	ND	>30	>30	8%	126%
SP-522						Ac-	F	$r8	A	Y	6clW	E	A	L	$	A		-NH2	27 ± 1.7	17 ± 2.2	5 ± 1	1.0	>30	27.1 ± 5.1	ND	>30	>30	5%	127%
SP-580				mdPEG3-	L	T	F	$r8	A	Y	W	A	Q	L	$	S		-NH2	16 ± 2.4	8 ± 0.1	11 ± 0.5	1.0	11.7 ± 1	13 ± 5.8	ND	>30	>30	5%	134%
SP-523						Ac-	F	$r8	A	Y	dl6brW	E	A	L	$	A		-NH2	19 ± 1	4 ± 0.5	1 ± 0.1	1.0	>30	26.3 ± 1.7	ND	>30	>30	ND	ND
SP-573						Ac-	F	$r8	A	Y	6clW	E	A	L	$	A		-NH2	23 ± 1.2	16 ± 1.8	6 ± 2	1.0	25 ± 8.6	>30	ND	>30	>30	ND	ND
SP-256				Ac-	W	T	F	$r8	H	Y	W	A	Q	L	$	S		-NH2	36 ± 2	4 ± 5	1 ± 0	1.0	2.25uM ± 7.7	16.8uM ± 6.2	ND	26.4uM ± 4.8	>30	ND	ND
SP-351				Ac-	L	T	F	$r8	A	Y	W	A	A	L	$	S		-NH2	31	29	15 ± 5.5	2	1.73	1.11	ND	4.46	9.07	ND	ND
SP-641						Ac-	F	$r8	A	Y	6clW	Q	A	L	$	A		-NH2	20	13	6 ± 1.8	1	25.49	28.25 ± 12.52	ND	>30	>30	ND	ND

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FIG. 7C

SP#	SEQUENCE																		FP Competition Blinding Assay				SJSA-1 Cell Viability 72h (10% FBS) EC50 (uM)		p21 levels at 21h (10% FBS) (pg/ml)			
																			HDM2 IC50 (nM)	HDMx IC50 (nM)	HDM2 Ki (IC50) - FAM-pDi	HDMx Ki (IC50) - FAM-pDi	AVE EC50 (uM)	SD	30uM	10uM	3uM	1uM
SP-684				Ac-	I	T	F	$r8	F	Y	W	A	Q	L	$	S	-NH2		103.6	442	<10	74.6	2.7	0.00	1687	563	51	25
SP-681				Ac-	L	T	F	$r8	A	Y	W	V	Q	L	$	S	-NH2		118	395	<10	66.8	2.8	0.14	1983	368 ± 290	57	76
SP-686				Ac-	F	T	F	$r8	F	Y	W	A	Q	L	$	S	-NH2		102.5	269	<10	45.4	3.6	0.63	1798	196	0	BDL
SP-688				Ac-	L	T	F	$r8	A	H	W	A	Q	L	$	S	-NH2		77.1	293.2	<10	49.5	3.6	2.26	1282	398	6	BDL
SP-671				Ac-	L	T	F	$r8	E	Y	W	A	Q	L	$	A	-NH2		63.6	110	<10	18.6	3.7	0.69	1398	372	86	11
SP-485				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	A	A	-NH2	ND	ND	ND	ND	3.9	0.50	1604	1125	208	88
SP-604				Ac-	L	T	F	$r8	F	Y	W	A	Q	L	$	A	-NH2		115	309.5	<10	52.3	3.9	0.97	2183	254	-6	BDLE
SP-685				Ac-	I	T	F	$r8	L	Y	W	S	Q	L	$	S	-NH2		97.9	491	<10	83	4.0	2.68	1442	225	12	18
SP-698				Ac-	L	T	F	$r8	A	Y	W	S	Q	L	$	a	-NH2		82.2	156.7	<10	26.5	4.4	0.90	1574	530	88	42
SP-586				Ac-	L	T	F	$r8	A	Y	W	A	Q	F	$	S	-NH2		111	673	<10	113.7	4.7	1.53	2259	516	0	BDL
SP-585				Ac-	L	T	F	$r8	A	Y	W	A	Q	Cba	$	S	-NH2		92	282	<10	47.7	5.0	1.63	2112	739 ± 305	32	51
SP-623				Ac-	L	T	F	$r8	V	Y	W	A	Q	L	$	A	-NH2		68.3	251	<10	42.4	5.1	1.23	2343	1701 ± 667	41	0
SP-673				Ac-	L	T	F	$r8	A	Y	W	A	A	L	$	S	-NH2		136.6	717	11	121	6.5	0.59	1693	606	80	0
SP-714						Ac-	F	$r8	A	Y	6clW	S	A	L	$	A	-NH2		ND	ND	ND	ND	5.5	1.44	2064 ± 24	1284 ± 155	249 ± 46	15 ± 21
SP-352				Ac-	L	V	F	$r8	A	Y	W	A	Q	L	$	S	-NH2		80.9	386	<10	65.3	5.8	2.58	1535 ± 143	1028 ± 439	56 ± 37	0 ± 0
SP-709				Ac-	E	T	F	$r8	A	Y	W	A	Q	L	$	A	-NH2		103	235	<10	39.7	5.8	3.05	1574 ± 47	787 ± 149	278 ± 27	56 ± 37
SP-643				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	S	-NH2		ND	ND	ND	ND	6.0	0.20	2137 ± 287	1343 ± 303	51 ± 44	6 ± 8
SP-566				Ac-	L	T	F4F	$r8	A	Y	W	A	Q	L	$	S	-NH2		ND	ND	ND	ND	6.1	1.35	2243 ± 84	1111 ± 29	24 ± 3	4 ± 6
SP-696				Ac-	W	T	F	$r8	L	Y	W	S	Q	L	$	S	-NH2		105	379	<10	64	6.2	0.45	2177 ± 162	247 ± 365	0 ± 0	0 ± 0

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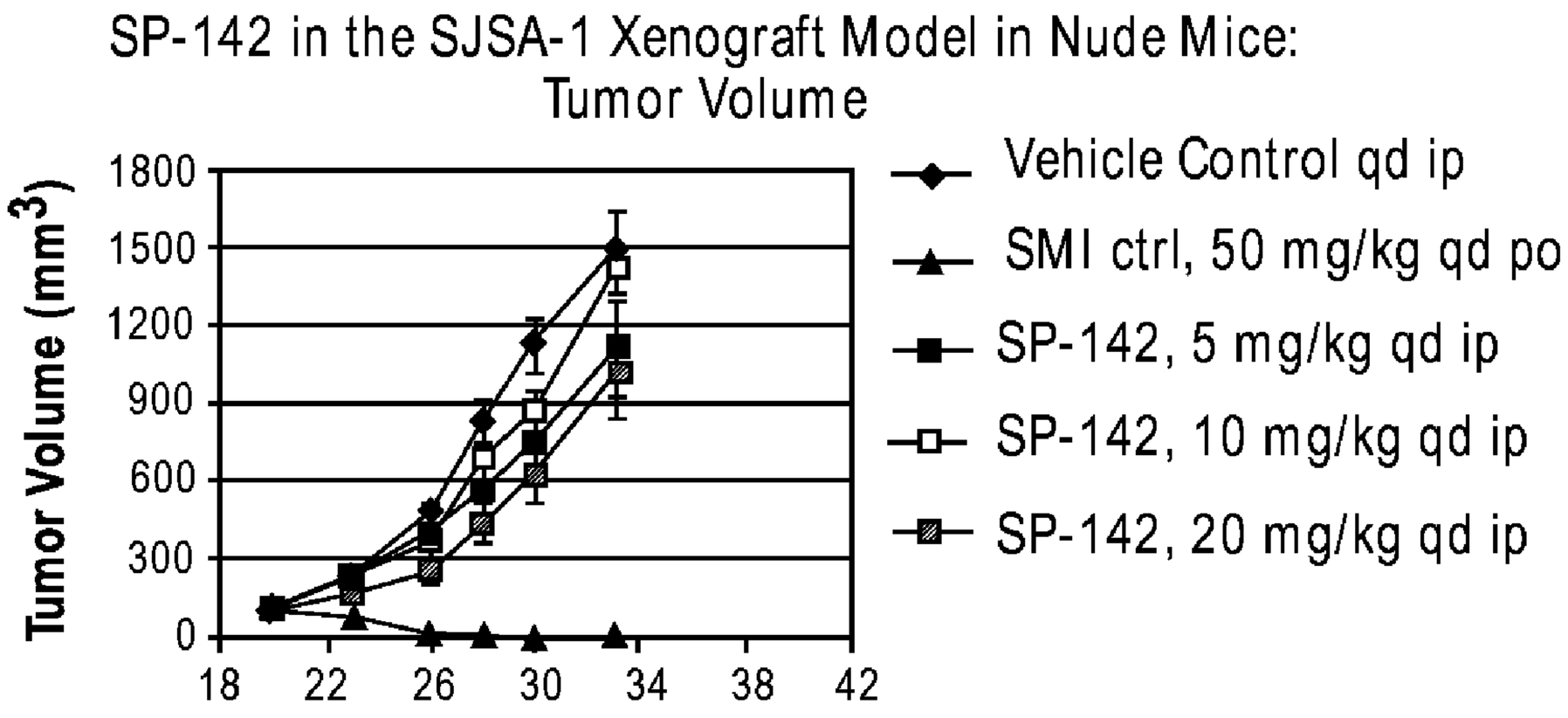
FIG. 7D

SP#	SEQUENCE																		Fold Caspase Activation relative to DMSO in 10% FBS @ 48h				Cell Viability (10% FBS) EC50 (uM)					% Helicity @ 222 nM vs parent seq	
																			30uM	10uM	3uM	1uM	RKO (72h)	HCT-116, 5k/well,(72h)	MCF-7 (7500 cells/well, 5day)	SW-480, 2k/well, 6d	RKO-E6 (72h)	0% TFE	50% TFE
SP-684				Ac-	I	T	F	$r8	F	Y	W	A	Q	L	$	S	-NH2		24	9 + 6.0	1	1	11.26	9.37	ND	12.14 ± 4.56	27.63	ND	ND
SP-681				Ac-	L	T	F	$r8	A	Y	W	V	Q	L	$	S	-NH2		23	5	1	1	12.89	10.83 ± 5.46	ND	9.40 ± 3.10	24.07 ± 8.49	ND	ND
SP-686				Ac-	F	T	F	$r8	F	Y	W	A	Q	L	$	S	-NH2		24	2	1	1	11.2	2.1	ND	11.84 ± 5.19	>30	ND	ND
SP-688				Ac-	L	T	F	$r8	A	H	W	A	Q	L	$	S	-NH2		17	6	1	1	23.86 ± 9.66	14.55	ND	>30	28.12	ND	ND
SP-671				Ac-	L	T	F	$r8	E	Y	W	A	Q	L	$	A	-NH2		19	4	1	1	>30	15.59	ND	>30	>30	ND	ND
SP-485				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	A	A	-NH2	22	19	3	1	5.84	4.78	ND	13.83	29.88	ND	ND
SP-604				Ac-	L	T	F	$r8	F	Y	W	A	Q	L	$	A	-NH2		28	2	1	1	5.3 ± 4.1	5.6 ± 2.6	ND	9.3 ± 4	14.4 ± 8.1	ND	ND
SP-685				Ac-	I	T	F	$r8	L	Y	W	S	Q	L	$	S	-NH2		17	2	1	1	30.24 ± 4.25	7.32 ± 4.75	ND	15.82	>30	ND	ND
SP-698				Ac-	L	T	F	$r8	A	Y	W	S	Q	L	$	a	-NH2		21	8	1	1	12.08 ± 4.57	4.05	ND	>30	>30	ND	ND
SP-586				Ac-	L	T	F	$r8	A	Y	W	A	Q	F	$	S	-NH2		20	5	1	1	25.03 ± 4.46	11.05	ND	5.79	>30	ND	ND
SP-585				Ac-	L	T	F	$r8	A	Y	W	A	Q	Cba	$	S	-NH2		22	7	1	1	9.79 ± 4.47	9.55	ND	17.68	>30	ND	ND
SP-623				Ac-	L	T	F	$r8	V	Y	W	A	Q	L	$	A	-NH2		21	15	1	1	7.37	7.5	ND	10.51	24.2	ND	ND
SP-673				Ac-	L	T	F	$r8	A	Y	W	A	A	L	$	S	-NH2		25	9 + 5.3	1	1	8.03	6.93	ND	16.84 ± 5.95	27.98	ND	ND
SP-714					Ac-	F	$r8	A	Y	6clW	S	A	L	$	A	-NH2			33 ± 1.3	16 ± 1.9	2 ± 0.3	1 ± 0	4.4 ± 1.7	7.6 ± 3.6	ND	13.7 ± 0.9	>30	ND	ND
SP-352				Ac-	L	V	F	$r8	A	Y	W	A	Q	L	$	S	-NH2			13 ± 1.2	11 ± 1.6	1 ± 0.2	1 ± 0	5.6 ± 0.3	5 ± 0.3	ND	4.6 ± 1.6	10.4 ± 1.8	ND	ND
SP-709				Ac-	E	T	F	$r8	A	Y	W	A	Q	L	$	A	-NH2			25 ± 0.1	11 ± 2.3	2 ± 0.4	1 ± 0.1	29.2 ± 3.3	>30	ND	>30	>30	ND	ND
SP-643				Ac-	L	T	F	$r8	A	Y	W	A	Q	L	$	S	-NH2			29 ± 2.2	16 ± 7.2	1 ± 0.1	1 ± 0	8.6 ± 1.8	7.1 ± 0.3	ND	17.1 ± 8.5	>30	ND	ND
SP-566				Ac-	L	T	F4F	$r8	A	Y	W	A	Q	L	$	S	-NH2			28 ± 2.6	17 ± 2.9	1 ± 0.1	1 ± 0.1	5.6 ± 3.6	9.1 ± 0	ND	9.8 ± 3.2	27.3 ± 3.1	ND	ND
SP-696				Ac-	W	T	F	$r8	L	Y	W	S	Q	L	$	S	-NH2			26 ± 0.2	4 ± 4.8	1 ± 0	1 ± 0	9.1 ± 4.5	11.8 ± 4.9	ND	10 ± 4.3	29.8 ± 0.2	ND	ND

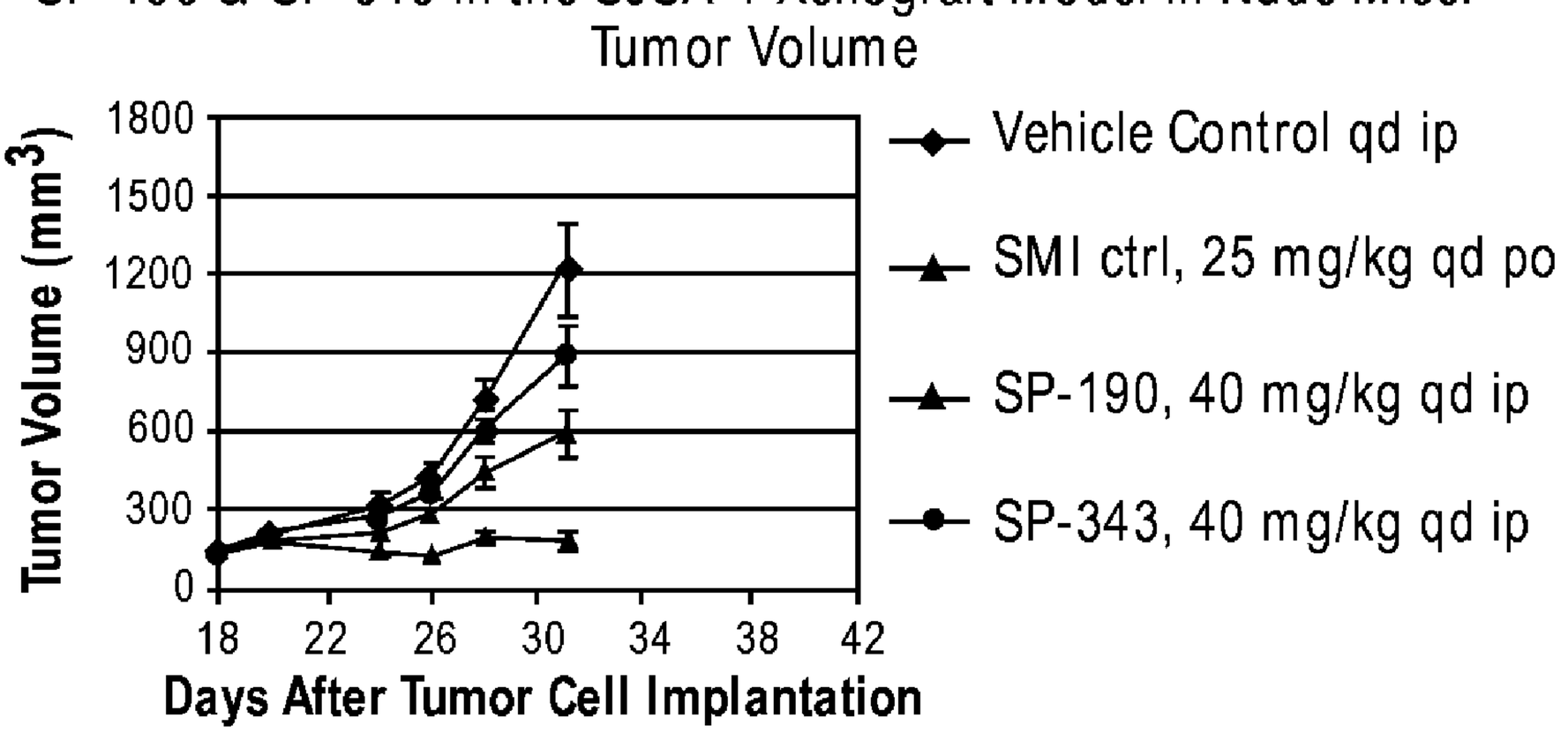
SUBSTITUTE SHEET (RULE 26)

FIG. 8A



Group No.	Treatment	Amount/dose (mg/kg)	Schedule	ROA	TGI (%) after 13 days dosing
1	Vehicle control	-	qd	ip	-
2	Small molecule inhibitor control	50	qd	po	> 100%
3	SP-142	5	qd	ip	27%
4	SP-142	10	qd	ip	4.5%
5	SP-142	20	qd	ip	34%

FIG. 8B



Group No.	Treatment	Amount/dose (mg/kg)	Schedule	ROA	TGI (%) after 13 days dosing
1	Vehicle control	-	qd	ip	-
2	Small molecule inhibitor control	25	qd	po	97%
3	SP-190	40	qd	ip	58%
4	SP-343	40	qd	ip	30%