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(54) **Titre : IMMUNOMODULATEURS**
(54) **Title: IMMUNOMODULATORS**

(57) **Abrégé/Abstract:**

The present disclosure provides novel macrocyclic peptides which inhibit the PD-1/PD-L1 and PD-L1/CD80 protein/protein interaction, and thus are useful for the amelioration of various diseases, including cancer and infectious diseases.



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(54) **Title:** IMMUNOMODULATORS

(57) **Abstract:** The present disclosure provides novel macrocyclic peptides which inhibit the PD-1/PD-L1 and PD-L1/CD80 protein/protein interaction, and thus are useful for the amelioration of various diseases, including cancer and infectious diseases.

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IMMUNOMODULATORS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to Provisional Patent Application USSN 62/134,686 filed March 18, 2015, hereby incorporated by reference in its entirety.

 The present disclosure provides novel macrocyclic peptides which inhibit the PD-1/PD-L1 and CD80/PD-L1 protein/protein interaction, and are thus useful for the amelioration of various diseases, including cancer and infectious diseases.

10 The protein Programmed Death 1 (PD-1) is an inhibitory member of the CD28 family of receptors, that also includes CD28, CTLA-4, ICOS and BTLA. PD-1 is expressed on activated B cells, T cells, and myeloid cells (Agata et al., *supra*; Okazaki et al., *Curr. Opin. Immunol.*, 14:779-782 (2002); Bennett et al., *J. Immunol.*, 170:711-718 (2003)).

15 The PD-1 protein is a 55 kDa type I transmembrane protein that is part of the Ig gene superfamily (Agata et al., *Int. Immunol.*, 8:765-772 (1996)). PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M.L., *J. Exp. Med.*, 181:1953-1956 (1995); Vivier, E. et al., *Immunol. Today*, 18:286-291 (1997)).

20 Although structurally similar to CTLA-4, PD-1 lacks the MYPPY motif that is critical for CD80 CD86 (B7-2) binding. Two ligands for PD-1 have been identified, PD-L1 (B7-H1) and PD-L2 (b7-DC). The activation of T cells expressing PD-1 has been shown to be downregulated upon interaction with cells expressing PD-L1 or PD-L2 (Freeman et al., *J. Exp. Med.*, 192:1027-1034 (2000); Latchman et al., *Nat. Immunol.*, 2:261-268 (2001); Carter et al., *Eur. J. Immunol.*, 32:634-643 (2002)).

25 Both PD-L1 and PD-L2 are B7 protein family members that bind to PD-1, but do not bind to other CD28 family members. The PD-L1 ligand is abundant in a variety of human cancers (Dong et al., *Nat. Med.*, 8:787-789 (2002)). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in
30 T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al., *J. Mol. Med.*, 81:281-287 (2003); Blank et al., *Cancer Immunol. Immunother.*, 54:307-314 (2005); Konishi et al., *Clin. Cancer Res.*, 10:5094-5100

- (2004)). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai et al., *Proc. Natl. Acad. Sci. USA*, 99:12293-12297 (2002); Brown et al., *J. Immunol.*, 170:1257-1266 (2003)).
- 5 PD-L1 has also been shown to interact with CD80 (Butte MJ et al, *Immunity*;27:111-122 (2007)). The interaction PD-L1/CD80 on expressing immune cells has been shown to be an inhibitory one. Blockade of this interaction has been shown to abrogate this inhibitory interaction (Paterson AM, et al., *J Immunol.*, 187:1097-1105 (2011); Yang J, et al. *J Immunol.* Aug 1;187(3):1113-9 (2011)).
- 10 When PD-1 expressing T cells contact cells expressing its ligands, functional activities in response to antigenic stimuli, including proliferation, cytokine secretion, and cytotoxicity, are reduced. PD-1/PD-L1 or PD-L2 interactions down regulate immune responses during resolution of an infection or tumor, or during the development of self tolerance (Keir, M.E. et al., *Annu. Rev. Immunol.*, 26:Epub
- 15 (2008)). Chronic antigen stimulation, such as that which occurs during tumor disease or chronic infections, results in T cells that express elevated levels of PD-1 and are dysfunctional with respect to activity towards the chronic antigen (reviewed in Kim et al., *Curr. Opin. Imm.* (2010)). This is termed "T cell exhaustion". B cells also display PD-1/PD-ligand suppression and "exhaustion".
- 20 Blockade of PD-1/PD-L1 ligation using antibodies to PD-L1 has been shown to restore and augment T cell activation in many systems. Patients with advanced cancer benefit from therapy with a monoclonal antibody to PD-L1 (Brahmer et al., *New Engl. J. Med.* (2012)). Preclinical animal models of tumors and chronic infections have shown that blockade of the PD-1/PD-L1 pathway by monoclonal
- 25 antibodies can enhance the immune response and result in tumor rejection or control of infection. Antitumor immunotherapy via PD-1/PD-L1 blockade may augment therapeutic immune response to a number of histologically distinct tumors (Dong, H. et al., "B7-H1 pathway and its role in the evasion of tumor immunity", *J. Mol. Med.*, 81(5):281-287 (2003); Dong, H. et al., "Tumor-associated B7-H1 promotes T-cell
- 30 apoptosis: a potential mechanism of immune evasion", *Nat. Med.*, 8(8):793-800 (2002)).

Interference with the PD-1/PD-L1 interaction causes enhanced T cell activity in systems with chronic infection. Blockade of PD-L1 caused improved viral clearance and restored immunity in mice with chronic lymphocytic chorio meningitis virus infection (Barber, D.L. et al., "Restoring function in exhausted CD8 T cells during chronic viral infection", *Nature*, 439(7077):682-687 (2006)).

Humanized mice infected with HIV-1 show enhanced protection against viremia and viral depletion of CD4⁺ T cells (Palmer et al., *J. Immunol.* (2013)). Blockade of PD-1/PD-L1 through monoclonal antibodies to PD-L1 can restore *in vitro* antigen-specific functionality to T cells from HIV patients (Day, *Nature* (2006); Petrovas, *J. Exp. Med.* (2006); Trautman, *Nature Med.* (2006); D'Souza, *J. Immunol.* (2007); Zhang, *Blood* (2007); Kaufmann, *Nature Imm.* (2007); Kasu, *J. Immunol.* (2010); Porichis, *Blood* (2011)), HCV patients (Golden-Mason, *J. Virol.* (2007); Jeung, *J. Leuk. Biol.* (2007); Urbani, *J. Hepatol.* (2008); Nakamoto, *PLoS Path.* (2009); Nakamoto, *Gastroenterology* (2008)) and HBV patients (Boni, *J. Virol.* (2007); Fisicaro, *Gastro.* (2010); Fisicaro et al., *Gastroenterology* (2012); Boni et al., *Gastro.* (2012); Penna et al., *J. Hep.* (2012); Raziorrough, *Hepatology* (2009); Liang, *World J. Gastro.* (2010); Zhang, *Gastro.* (2008)).

Blockade of the PD-L1/CD80 interaction has also been shown to stimulate immunity (Yang J., et al., *J Immunol.* Aug 1;187(3):1113-9 (2011)). Immune stimulation resulting from blockade of the PD-L1/CD80 interaction has been shown to be enhanced through combination with blockade of further PD-1/PD-L1 or PD-1/PD-L2 interactions.

Alterations in immune cell phenotypes are hypothesized to be an important factor in septic shock (Hotchkiss, et al., *Nat Rev Immunol* (2013)). These include increased levels of PD-1 and PD-L1 (Guignant, et al, *Crit. Care* (2011)), Cells from septic shock patients with increased levels of PD-1 and PD-L1 exhibit an increased level of T cell apoptosis. Antibodies directed to PD-L1, can reduce the level of Immune cell apoptosis (Zhang et al, *Crit. Care* (2011)). Furthermore, mice lacking PD-1 expression are more resistant to septic shock symptoms than wildtype mice.

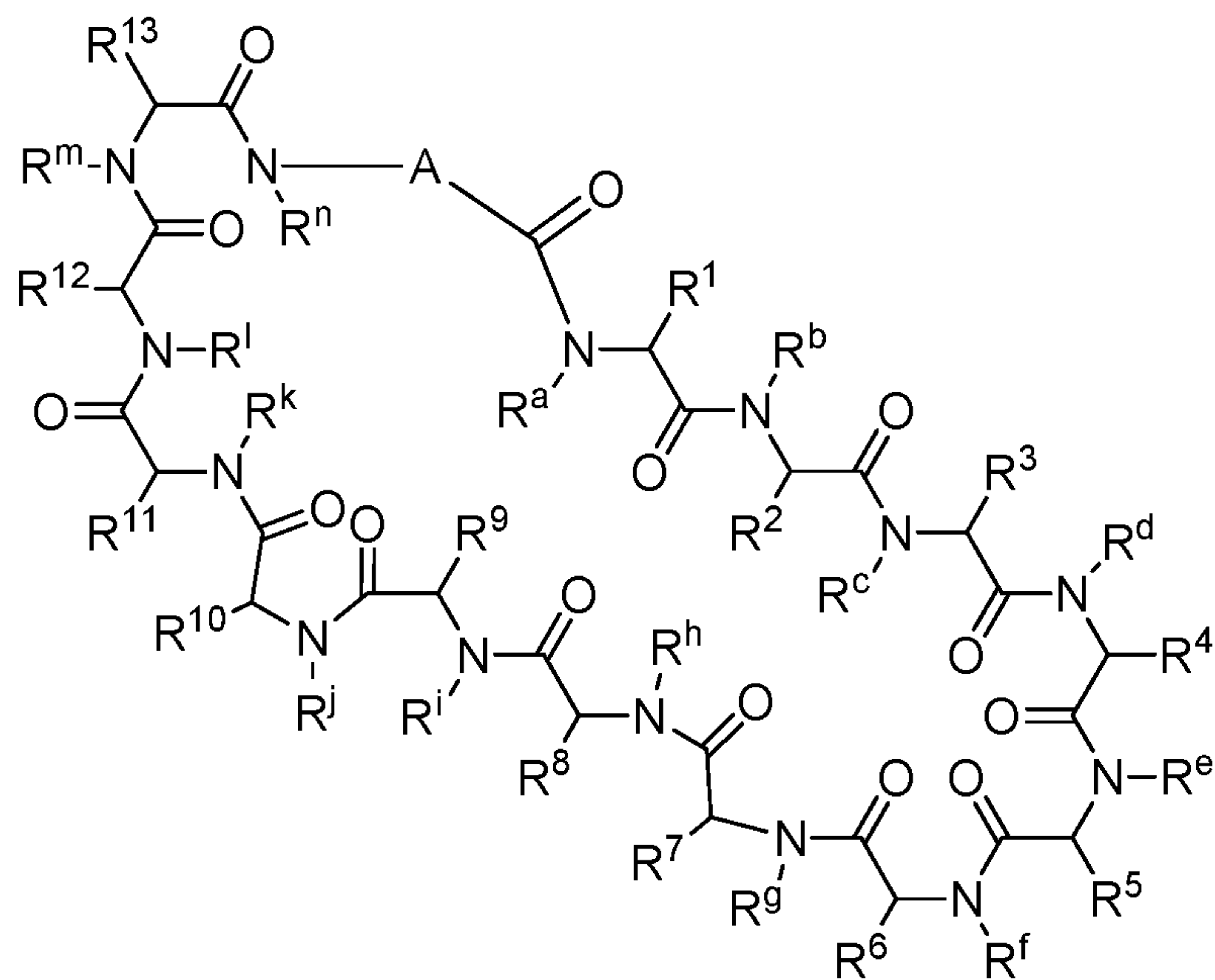
Yang J., et al.. *J Immunol.* Aug 1;187(3):1113-9 (2011)). Studies have revealed that blockade of the interactions of PD-L1 using antibodies can suppress inappropriate immune responses and ameliorate disease signs.

In addition to enhancing immunologic responses to chronic antigens, blockade of the PD-1/PD-L1 pathway has also been shown to enhance responses to vaccination, including therapeutic vaccination in the context of chronic infection (Ha, S.J. et al., "Enhancing therapeutic vaccination by blocking PD-1-mediated inhibitory
5 signals during chronic infection", *J. Exp. Med.*, 205(3):543-555 (2008); Finnefrock, A.C. et al., "PD-1 blockade in rhesus macaques: impact on chronic infection and prophylactic vaccination", *J. Immunol.*, 182(2):980-987 (2009); Song, M.-Y. et al., "Enhancement of vaccine-induced primary and memory CD8+ t-cell responses by soluble PD-1", *J. Immunother.*, 34(3):297-306 (2011)).

10 The molecules described herein demonstrate the ability to block the interaction of PD-L1 with PD-1, in both biochemical and cell-based experimental systems. These results are consistent with a potential for therapeutic administration to enhance immunity in cancer or chronic infection, including therapeutic vaccine.

The macrocyclic peptides described herein are capable of inhibiting the
15 interaction of PD-L1 with PD-1 and with CD80. These compounds have demonstrated highly efficacious binding to PD-L1, blockade of the interaction of PD-L1 with either PD-1 or CD80, and are capable of promoting enhanced T cell functional activity, thus making them candidates for parenteral, oral, pulmonary, nasal, buccal and sustained release formulations.

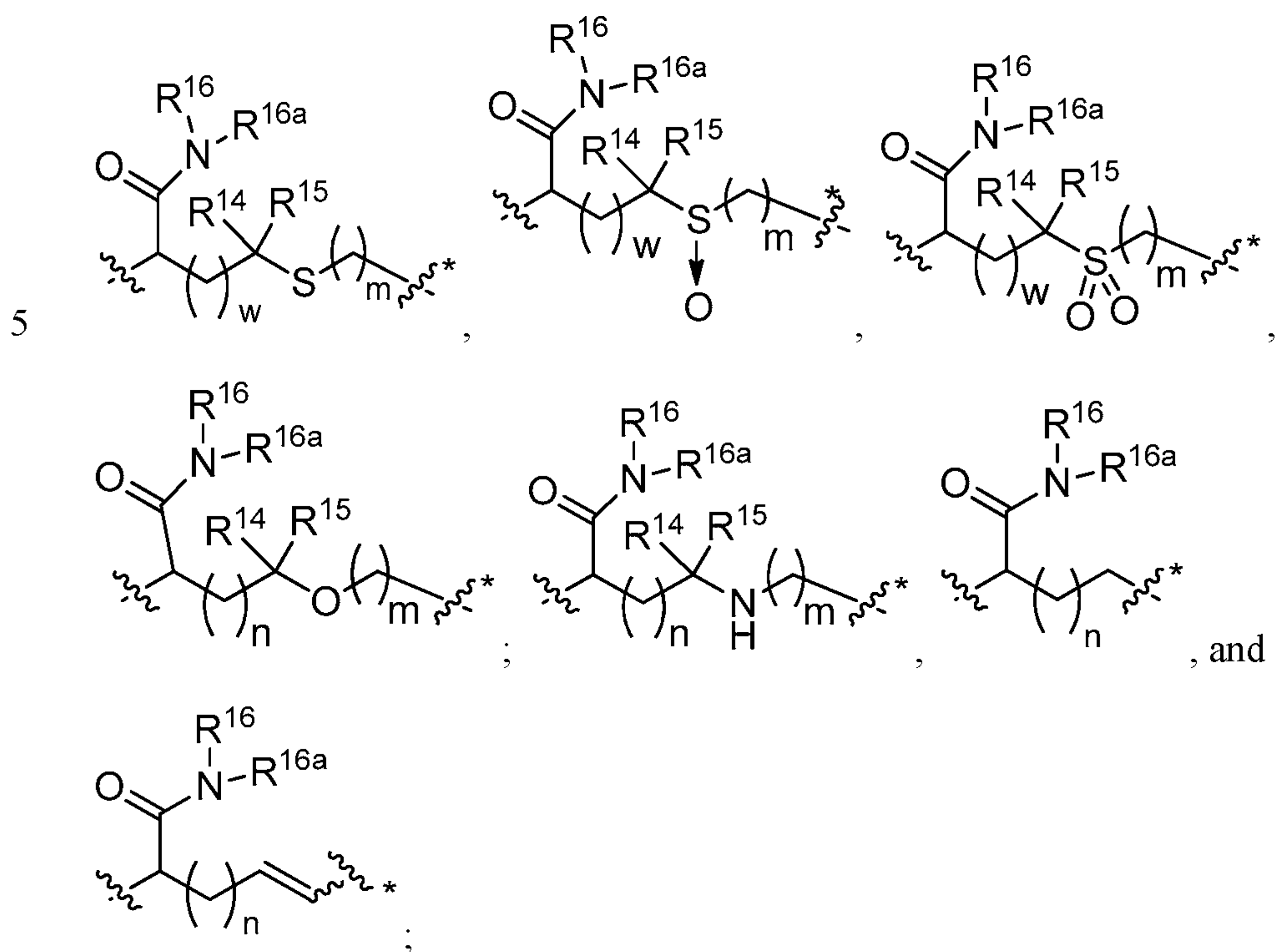
20 In one aspect the present disclosure provides a compound of formula (I)



(I),

or a pharmaceutically acceptable salt thereof, wherein:

A is selected from



wherein:

 denotes the point of attachment to the carbonyl group and  denotes the point of attachment to the nitrogen atom;

n is 0 or 1;

m is 1 or 2;

5 w is 0, 1, or 2;

R¹⁴ and R¹⁵ are independently selected from hydrogen and methyl;

R^{16a} is selected from hydrogen and C₁-C₆ alkyl;

R¹⁶ is selected from

-(C(R^{17a})₂)-C(O)-NR⁵⁰R⁵¹;

10 wherein:

each R^{17a} is independently selected from hydrogen and C₁-C₆alkyl;

one of R⁵⁰ and R⁵¹ is selected from hydrogen and C₁-C₆alkyl and the other is selected from

15 -(CH₂)_{n'}X, C₁-C₆alkyl, C₃-C₇cycloalkyl, heterocyclyl, and phenyl, wherein the cycloalkyl is optionally substituted with one, two, or three groups independently selected from C₁-C₃alkoxy, C₁-C₃alkyl, amino, cyano, and hydroxy, or;

R⁵⁰ and R⁵¹, together with the nitrogen atom to which they are attached, form a four-, five- six- or seven-membered saturated or unsaturated ring optionally containing one or two additional heteroatoms independently selected from nitrogen, oxygen, and sulfur; wherein said ring is optionally substituted with one, two, or three groups selected from C₁-C₆alkoxy, C₁-C₃alkyl, cyano, halo, haloC₁-C₃alkyl, hydroxy, hydroxy(C₁-C₃alkyl), -NR⁷⁰R⁷¹, oxo, and phenyl; wherein the phenyl is further optionally substituted with one, two, or three groups independently selected from C₁-C₃alkoxy, cyano, and halo;

25 n' is 1-5;

X is selected from $\text{---C}\equiv\text{CH}$, $\text{---}\overset{\text{H}}{\underset{\text{H}}{\text{C}}}=\text{CH}_2$, C₂-C₆alkoxymethyl, C₁-C₆alkoxycarbonylmethyl, C₁-C₆alkylsulfanylmethyl, C₁-C₆alkylsulfonylmethyl, azidomethyl, *tert*-butoxymethyl, C₃-C₇cycloalkyl, haloalkoxymethyl, halomethyl, heterocyclyl, hydroxymethyl, isopropoxymethyl, (NR⁷⁰R⁷¹)methyl, phenyl, phenoxyethyl, phenylsulfanylmethyl,

30

one of R^{70} and R^{71} is selected from hydrogen, C_1 - C_6 alkyl, and hydroxy C_2 - C_6 alkyl and the other is selected from C_1 - C_6 alkoxycarbonyl, C_1 - C_6 alkylcarbonyl, C_1 - C_6 alkylsulfonyl, and hydroxy C_2 - C_6 alkyl;

 denotes the point of attachment to the carbonyl group and  denotes the point of attachment to the nitrogen atom;

R^c , R^f , R^h , R^i , R^m , and R^n are hydrogen;

R^a , R^e , R^j , and R^k , are each independently selected from hydrogen and methyl;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , and R^{13} are independently selected from a natural amino acid side chain and an unnatural amino acid side chain or form a ring with the corresponding vicinal R group as described below;

R^e and R^k can each form a ring with the corresponding vicinal R group and the atoms to which they are attached selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, and hydroxy;

R^b is methyl or, R^b and R^2 , together with the atoms to which they are attached, form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, and hydroxy;

R^d is hydrogen or methyl, or, R^d and R^4 , together with the atoms to which they are attached, can form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, hydroxy, and phenyl;

R^g is hydrogen or methyl or R^g and R^7 , together with the atoms to which they are attached, can form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, benzyl optionally substituted with a halo group, benzyloxy, cyano, cyclohexyl, methyl, halo,

hydroxy, isoquinolinylloxy optionally substituted with a methoxy group, quinolinylloxy optionally substituted with a halo group, and tetrazolyl; and wherein the pyrrolidine and the piperidine ring are optionally fused to a cyclohexyl, phenyl, or indole group; and

5 R¹ is methyl or, R¹ and R¹², together with the atoms to which they are attached, form a ring selected from azetidine and pyrrolidine, wherein each ring is optionally substituted with one to four independently selected from amino, cyano, methyl, halo, and hydroxy.

 In a first embodiment of the first aspect the present disclosure provides a
10 compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein:

 R^d and R⁴, together with the atoms to which they are attached, form a pyrrolidine ring;

 R^g and R⁷, together with the atoms to which they are attached, form a pyrrolidine ring, wherein said ring is optionally substituted with one hydroxy group;
15 and

 R^k is methyl.

 In a second embodiment of the first aspect the present disclosure provides a compound of formula (I), or a therapeutically acceptable salt thereof, wherein:

 R^d and R⁴, together with the atoms to which they are attached, form a
20 pyrrolidine ring;

 R^g and R⁷, together with the atoms to which they are attached, form a pyrrolidine ring, wherein said ring is optionally substituted with one hydroxy group;

 R^k is methyl;

 R^a, R^e, and R^j hydrogen;

25 R^b, R^k, and R^l are methyl;

 Rⁿ is hydrogen;

 R¹ is phenylmethyl wherein the phenyl is substituted with hydroxy;

 R² is methyl;

 R³ is selected from -CH₂C(O)NH₂ and -CH₂CO₂H;

30 R⁵ is selected from -CH₂NH₂, -CH₂OH, and -CH₂C(O)NH₂;

 R⁶ is selected from -CH₂CH(CH₃)₂, -(CH₂)₂CO₂H, and (CH₂)₂C(O)NH₂;

R⁸ and R¹⁰ are -CH₂(indolyl), wherein the indolyl is optionally substituted with -CH₂CO₂H;

R⁹ is selected from hydrogen, -(CH₂)₂NH₂, CH₂OH, and -CH₂C(O)NH₂;

R¹¹ and R¹² are -(CH₂)₃CH₃; and

5 R¹³ is selected from methyl, -CH₂CH(CH₃)₂, and -(CH₂)₂CO₂H.

In another embodiment the present disclosure provides a method of enhancing, stimulating, and/or increasing the immune response in a subject in need thereof, said method comprising administering to the subject a therapeutically effective amount of a compound of formula (I) or a therapeutically acceptable salt thereof. In another embodiment the method further comprises administering an additional agent prior to, after, or simultaneously with the compound of formula (I) or a therapeutically acceptable salt thereof. In another embodiment the additional agent is an antimicrobial agent, an antiviral agent, a cytotoxic agent, and/or an immune response modifier. In another embodiment the additional agent is an HDAC
10 inhibitor. In another embodiment the additional agent is a TLR7 and/or TLR8 agonist.
15

In another embodiment the present disclosure provides a method of inhibiting growth, proliferation, or metastasis of cancer cells in a subject in need thereof, said method comprising administering to the subject a therapeutically effective amount of a compound of formula (I) or a therapeutically acceptable salt thereof. It should be understood that said inhibition can be direct or indirect. In another embodiment the cancer is selected from melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.
20
25

In another embodiment the present disclosure provides a method of treating an infectious disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of formula (I) or a therapeutically acceptable salt thereof. In another embodiment the infectious disease is caused by a virus. In another embodiment the virus is selected from HIV, Hepatitis A, Hepatitis B, Hepatitis C, herpes virus, and influenza.
30

In another embodiment the present disclosure provides a method of treating septic shock in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of one or more macrocyclic peptides described herein.

5 In another embodiment the present disclosure provides a method blocking the interaction of PD-L1 with PD-1 and/or CD80 in a subject, said method comprising administering to the subject a therapeutically effective amount of at least one macrocyclic peptide described herein.

In compounds of formula (I) where the R side chains are part of a ring that is
10 substituted with methyl, it is understood that the methyl group may be on any substitutable carbon atom in the ring, including the carbon that is part of the macrocyclic parent structure.

In compounds of formula (I), preferred R¹ side chains are: phenylalanine, tyrosine, 3-thien-2-yl, 4-methylphenylalanine, 4-chlorophenylalanine, 3-
15 methoxyphenylalanine, isotryptophan, 3-methylphenylalanine, 1-naphthylalanine, 3,4-difluorophenylalanine, 4-fluorophenylalanine, 3,4-dimethoxyphenylalanine, 3,4-dichlorophenylalanine, 4-difluoromethylphenylalanine, 2-methylphenylalanine, 2-naphthylalanine, tryptophan, 4-pyridinyl, 4-bromophenylalanine, 3-pyridinyl, 4-trifluoromethylphenylalanine, 4-carboxyphenylalanine, 4-methoxyphenylalanine,
20 biphenylalanine, and 3-chlorophenylalanine; and 2,4-diaminobutane.

In compounds of formula (I) where R² is not part of a ring, preferred R² side chains are: alanine, serine, and glycine.

In compounds of formula (I), preferred R³ side chains are: asparagine, aspartic acid, glutamic acid, glutamine, serine, ornithine, lysine, histidine, threonine,
25 leucine, alanine, 2,3-diaminopropane, and 2,4-diaminobutane.

In compounds of formula (I) where R⁴ is not part of a ring, preferred R⁴ side chains are: valine, alanine, isoleucine, and glycine.

In compounds of formula (I), preferred R⁵ side chains are: aminomethane, histidine, asparagine, 2,3-diaminopropane, serine, glycine, 2,4-diaminobutane,
30 threonine, alanine, lysine, aspartic acid, alanine, and 3-thiazolylalanine.

In compounds of formula (I), preferred R⁶ side chains are: leucine, aspartic acid, asparagine, glutamic acid, glutamine, serine, lysine, 3-cyclohexane, threonine, ornithine, 2,4-diaminobutane, alanine, arginine, and ornithine (COCH₃).

In compounds of formula (I) where R⁷ is not part of a ring, preferred R⁷ side chains are: glycine, 2,4-diaminobutane, serine, lysine, arginine, ornithine, histidine, asparagine, glutamine, alanine, and 2,4-diaminobutane (C(O)cyclobutane).

In compounds of formula (I) preferred R⁸ side chains are tryptophan and 1,2-benzisothiazolinyllalanine.

In compounds of formula (I) preferred R⁹ side chains are: serine, histidine, lysine, ornithine, 2,4-dibutylamine, threonine, lysine, glycine, glutamic acid, valine, 2,3-diaminopropane, arginine, aspartic acid, and tyrosine.

In compounds of formula (I) preferred R¹⁰ side chains are: optionally substituted tryptophan, benzisothiazolylalanine, 1-naphthylalanine, and methionine.

In compounds of formula (I) preferred R¹¹ side chains are: norleucine, leucine, asparagine, phenylalanine, methionine, ethoxymethane, alanine, tryptophan, isoleucine, phenylpropane, glutamic acid, hexane, and heptane.

In compounds of formula (I) where R¹² is not part of a ring, preferred R¹² side chains are: norleucine, alanine, ethoxymethane, methionine, serine, phenylalanine, methoxyethane, leucine, tryptophan, isoleucine, glutamic acid, hexane, heptane, and glycine.

In compounds of formula (I) preferred R¹³ side chains : arginine, ornithine, alanine, 2,4-diaminobutane, 2,3-diaminopropane, leucine, aspartic acid, glutamic acid, serine, lysine, threonine, cyclopropylmethane, glycine, valine, isoleucine, histidine, and 2-aminobutane.

In accordance with the present disclosure, we have discovered peptides that specifically bind to PD-L1 and are capable of inhibiting the interaction of PD-L1 with PD-1 and CD80. These macrocyclic peptides exhibit *in vitro* immunomodulatory efficacy thus making them therapeutic candidates for the treatment of various diseases including cancer and infectious diseases.

The terms "specific binding" or "specifically bind" refer to the interaction between a protein and a binding molecule, such as a compound or ligand. The interaction is dependent upon the presence of a particular structure (*i.e.*, an enzyme

binding site, an antigenic determinant or epitope) of the protein that is recognized by the binding molecule. For example, if a compound has specific binding for protein binding site "A", the presence of the compound in a reaction containing a protein including binding site A, and a labeled peptide that specifically binds to protein binding site A will reduce the amount of labeled peptide bound to the protein. In contrast, nonspecific binding of a compound to the protein does not result in a concentration-dependent displacement of the labeled peptide from the protein.

The present disclosure is intended to include all isotopes of atoms occurring in the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include deuterium and tritium. Isotopes of carbon include ^{13}C and ^{14}C . Isotopically-labeled compounds of the invention can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein, using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed. Such compounds may have a variety of potential uses, for example as standards and reagents in determining biological activity. In the case of stable isotopes, such compounds may have the potential to favorably modify biological, pharmacological, or pharmacokinetic properties.

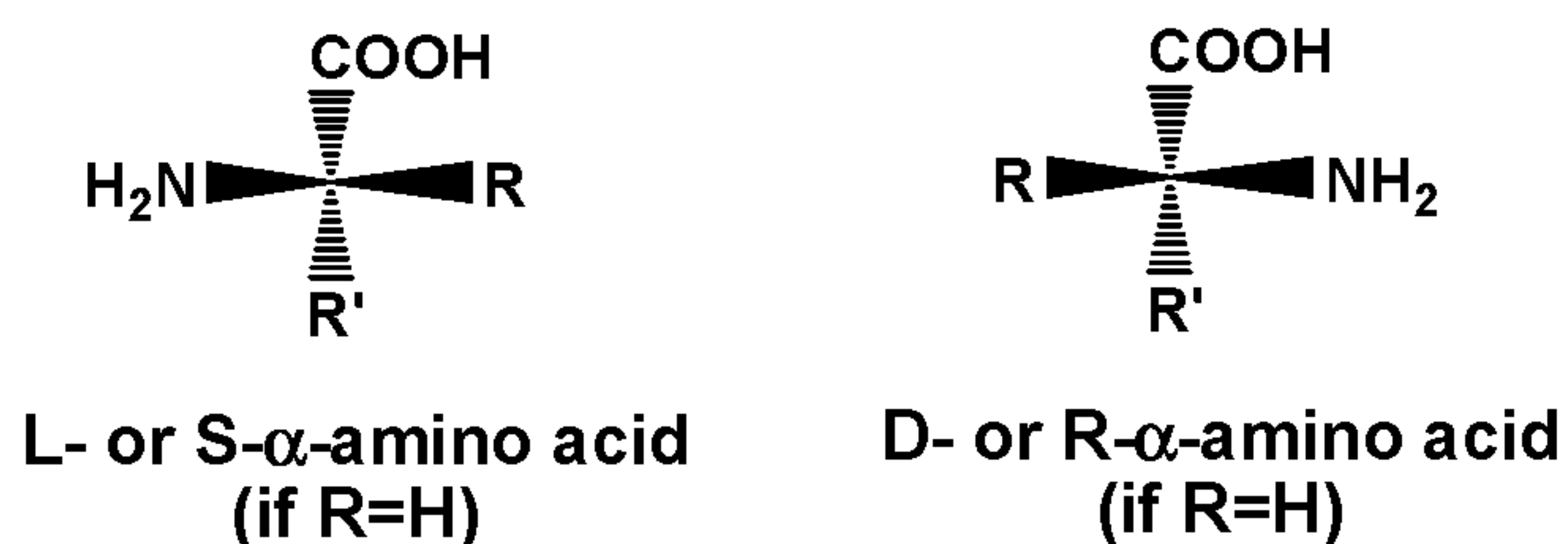
An additional aspect of the subject matter described herein is the use of the disclosed peptides as radiolabeled ligands for development of ligand binding assays or for monitoring of *in vivo* adsorption, metabolism, distribution, receptor binding or occupancy, or compound disposition. For example, a macrocyclic peptide described herein may be prepared using the radioactive isotope ^{125}I and the resulting radiolabeled peptide may be used to develop a binding assay or for metabolism studies. Alternatively, and for the same purpose, a macrocyclic peptide described herein may be converted to a radiolabeled form by catalytic tritiation using methods known to those skilled in the art.

The macrocyclic peptides of the present disclosure can also be used as PET imaging agents by adding a radioactive tracer using methods known to those skilled in the art.

Preferred peptides include at least one of the macrocyclic peptides provided herein and these peptides may be included in pharmaceutical compositions and combinations.

The definitions provided herein apply, without limitation, to the terms as used throughout this specification, unless otherwise limited in specific instances.

Those of ordinary skill in the art of amino acid and peptide chemistry are aware that an amino acid includes a compound represented by the general structure:



where R and R' are as discussed herein.

Unless otherwise indicated, the term "amino acid" as employed herein, alone or as part of another group, includes, without limitation, an amino group and a carboxyl group linked to the same carbon, referred to as " α " carbon, where R and/or R' can be a natural or an un-natural side chain, including hydrogen. The absolute "S" configuration at the " α " carbon is commonly referred to as the "L" or "natural" configuration. In the case where both the "R" and the "R'"(prime) substituents equal hydrogen, the amino acid is glycine and is not chiral.

The terms "natural amino acid side chain" and "naturally occurring amino acid side chain," as used herein, refer to side chain of any of the naturally occurring amino acids (i.e., alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, -histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) usually in the S-configuration (i.e., the L-amino acid).

The terms "unnatural amino acid side chain" and "non-naturally occurring amino acid side chain," as used herein, refer to a side chain of any naturally occurring amino acid usually in the R-configuration (i.e., the D-amino acid) or to a group other than a naturally occurring amino acid side chain in R- or S-configuration (i.e., the D- or L-amino acid, respectively) selected from:

C₂-C₇alkenyl, C₁-C₃alkoxyC₁-C₃alkyl, C₁-C₆alkoxycarbonylC₁-C₃alkyl, C₁-C₇alkyl, C₁-C₃alkylsulfanylC₁-C₃alkyl, amidoC₁-C₃alkyl, aminoC₁-C₃alkyl, azaindolyC₁-C₃alkyl, benzothiazolyC₁-C₃alkyl, benzothienylC₁-C₃alkyl, benzyloxyC₁-C₃alkyl, carboxyC₁-C₃alkyl, C₃-C₁₄cycloalkylC₁-C₃alkyl, C₃-C₆cycloalkylC₁-C₃alkyl, diphenylmethyl, furanylC₁-C₃alkyl, imidazolylC₁-C₃alkyl, naphthylC₁-C₃alkyl, pyridinylC₁-C₃alkyl, thiazolylC₁-C₃alkyl, thienylC₁-C₃alkyl;

biphenylC₁-C₃alkyl wherein the biphenyl is optionally substituted with a methyl group;

heterorocyclyl optionally substituted with one, two, three, four, or five groups independently selected from C₁-C₄alkoxy, C₁-C₄alkyl, C₁-C₃alkylsulfonylamino, amido, amino, aminoC₁-C₃alkyl, aminosulfonyl, carboxy, cyano, halo, haloC₁-C₃alkyl, hydroxy, -NC(NH₂)₂, nitro, and -OP(O)(OH)₂;

indolylC₁-C₃alkyl, wherein the indolyl part is optionally substituted with one group selected from C₁-C₃alkyl, carboxyC₁-C₃alkyl, halo, hydroxy, and phenyl, wherein the phenyl is further optionally substituted by one, two, or three groups independently selected from C₁-C₃alkoxy, C₁-C₃alkyl, and halo;

phenyl optionally substituted with one, two, three, four, or five groups independently selected from C₁-C₄alkoxy, C₁-C₄alkyl, C₁-C₃alkylsulfonylamino, amido, amino, aminoC₁-C₃alkyl, aminosulfonyl, carboxy, cyano, halo, haloC₁-C₃alkyl, hydroxy, -NC(NH₂)₂, nitro, and -OP(O)(OH)₂;

NR^aR^b(C₁-C₇alkyl), wherein R^a and R^b are independently selected from hydrogen, C₂-C₄alkenyloxy, carbonyl, C₁-C₃alkyl, C₁-C₃alkylcarbonyl, C₃-C₆cycloalkylcarbonyl, furanylcarbonyl, and phenylcarbonyl. When the alkyl linker contains more than one carbon an additional NR^aR^b group can be on the chain.

NR^cR^dcarbonylC₁-C₃alkyl, wherein R^c and R^d are independently selected from hydrogen, C₁-C₃alkyl, and triphenylmethyl;

phenylC₁-C₃alkyl wherein the phenyl part is optionally substituted with one, two, three, four, or five groups independently selected from C₁-C₄alkoxy, C₁-C₄alkyl, C₁-C₃alkylsulfonylamino, amido, amino, aminoC₁-C₃alkyl, aminosulfonyl, carboxy, cyano, halo, haloC₁-C₃alkyl, hydroxy, -NC(NH₂)₂, nitro, and -OP(O)(OH)₂; and

phenoxyC₁-C₃alkyl wherein the phenyl is optionally substituted with a C₁-C₃alkyl group.

The term “C₂-C₄alkenyl,” as used herein, refers to a straight or branched chain group of two to four carbon atoms containing at least one carbon-carbon double bond.

5 The term “C₂-C₇alkenyl,” as used herein, refers to a straight or branched chain group of two to seven carbon atoms containing at least one carbon-carbon double bond.

The term “C₂-C₄alkenyloxy,” as used herein, refers to a C₂-C₄alkenyl group attached to the parent molecular moiety through an oxygen atom.

10 The term “C₁-C₃alkoxy,” as used herein, refers to a C₁-C₃alkyl group attached to the parent molecular moiety through an oxygen atom.

The term “C₁-C₄alkoxy,” as used herein, refers to a C₁-C₄alkyl group attached to the parent molecular moiety through an oxygen atom.

The term “C₁-C₆alkoxy,” as used herein, refers to a C₁-C₆alkyl group attached to the parent molecular moiety through an oxygen atom.

15 The term “C₁-C₃alkoxyC₁-C₃alkyl,” as used herein, refers to a C₁-C₃alkoxy group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “C₁-C₆alkoxycarbonyl,” as used herein, refers to a C₁-C₆alkoxy group attached to the parent molecular moiety through a carbonyl group.

20 The term “C₁-C₆alkoxycarbonylC₁-C₃alkyl,” as used herein, refers to a C₁-C₆alkoxycarbonyl group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “C₁-C₃alkyl,” as used herein, refers to a group derived from a straight or branched chain saturated hydrocarbon containing from one to three carbon atoms.

25 The term “C₁-C₄alkyl,” as used herein, refers to a group derived from a straight or branched chain saturated hydrocarbon containing from one to four carbon atoms.

30 The term “C₁-C₆alkyl,” as used herein, refers to a group derived from a straight or branched chain saturated hydrocarbon containing from one to six carbon atoms.

The term “C₁-C₃alkylcarbonyl,” as used herein, refers to a C₁-C₃alkyl group attached to the parent molecular moiety through a carbonyl group.

The term “C₁-C₃alkylsulfanyl,” as used herein, refers to a C₁-C₃alkyl group attached to the parent molecular moiety through a sulfur atom.

5 The term “C₁-C₃alkylsulfanylC₁-C₃alkyl,” as used herein, refers to a C₁-C₃alkylsulfanyl group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “C₁-C₃alkylsulfonyl,” as used herein, refers to a C₁-C₃alkyl group attached to the parent molecular moiety through a sulfonyl group.

10 The term “C₁-C₃alkylsulfonylamino,” as used herein, refers to a C₁-C₃alkylsulfonyl group attached to the parent molecular moiety through an amino group.

The term “amido,” as used herein, refers to –C(O)NH₂.

The term “amidoC₁-C₃alkyl,” as used herein, refers to an amido group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “amino,” as used herein, refers to –NH₂.

15 The term “aminoC₁-C₃alkyl,” as used herein, refers to an amino group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “aminosulfonyl,” as used herein, refers to an amino group attached to the parent molecular moiety through a sulfonyl group.

20 The term “azaindolylC₁-C₃alkyl,” as used herein, refers to an azaindolyl group attached to the parent molecular through a C₁-C₃alkyl group. The azaindolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

25 The term “benzothiazolylC₁-C₃alkyl,” as used herein, refers to an benzothiazolyl group attached to the parent molecular through a C₁-C₃alkyl group. The benzothiazolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

30 The term “benzothienylC₁-C₃alkyl,” as used herein, refers to a benzothienyl group attached to the parent molecular through a C₁-C₃alkyl group. The benzothienyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “benzyloxy,” as used herein, refers to a benzyl group attached to the parent molecular moiety through an oxygen atom.

The term “benzyloxyC₁-C₃alkyl,” as used herein, refers to a benzyloxy group attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “biphenylC₁-C₃alkyl,” as used herein, refers to a biphenyl group attached to the parent molecular moiety through a C₁-C₃alkyl group. The biphenyl
5 group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “carbonyl,” as used herein, refers to -C(O)-.

The term “carboxy,” as used herein, refers to -CO₂H.

The term “carboxyC₁-C₃alkyl,” as used herein, refers to a carboxy group
10 attached to the parent molecular moiety through a C₁-C₃alkyl group.

The term “cyano,” as used herein, refers to -CN.

The term “C₃-C₁₄cycloalkyl,” as used herein, refers to a saturated monocyclic, bicyclic, or tricyclic hydrocarbon ring system having three to fourteen carbon atoms and zero heteroatoms. The bicyclic and tricyclic rings may be fused, spirocyclic, or
15 bridged. Representative examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclopentyl, bicyclo[3.1.1]heptyl, and adamantyl.

The term “C₃-C₁₄cycloalkylC₁-C₃alkyl,” as used herein, refers to a C₃-C₁₄cycloalkyl group attached to the parent molecular moiety through a C₁-C₃alkyl group.

20 The term “C₃-C₁₄cycloalkylcarbonyl,” as used herein, refers to a C₃-C₁₄cycloalkyl group attached to the parent molecular moiety through a carbonyl group.

The term “C₃-C₆cycloalkyl,” as used herein, refers to a saturated monocyclic, hydrocarbon ring system having three to six carbon atoms and zero heteroatoms.

The term “C₃-C₆cycloalkylC₁-C₃alkyl,” as used herein, refers to a C₃-C₆cycloalkyl group attached to the parent molecular moiety through a C₁-C₃alkyl
25 group.

The term “C₃-C₆cycloalkylcarbonyl,” as used herein, refers to a C₃-C₆cycloalkyl group attached to the parent molecular moiety through a carbonyl group.

The term “furanylC₁-C₃alkyl,” as used herein, refers to a furanyl group
30 attached to the parent molecular moiety through a C₁-C₃alkyl group. The furanyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “furanylcarbonyl,” as used herein, refers to a furanyl group attached to the parent molecular moiety through a carbonyl group.

The terms “halo” and “halogen,” as used herein, refer to F, Cl, Br, or I.

The term “haloC₁-C₃alkyl,” as used herein, refers to a C₁-C₃alkyl group
5 substituted with one, two, or three halogen atoms.

The term “halomethyl,” as used herein, refers to a methyl group substituted with one, two, or three halogen atoms.

The term “heterocyclyl,” as used herein, refers to a five-, six-, or seven-membered ring containing one, two, or three heteroatoms independently selected
10 from nitrogen, oxygen, and sulfur. The five-membered ring has zero to two double bonds and the six- and seven-membered rings have zero to three double bonds. The term “heterocyclyl” also includes bicyclic groups in which the heterocyclyl ring is fused to a four- to six-membered aromatic or non-aromatic carbocyclic ring or another monocyclic heterocyclyl group. The heterocyclyl groups of the present
15 disclosure are attached to the parent molecular moiety through a carbon atom in the group. Examples of heterocyclyl groups include, but are not limited to, benzothienyl, furyl, imidazolyl, indolinyl, indolyl, isothiazolyl, isoxazolyl, morpholinyl, oxazolyl, piperazinyl, piperidinyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrrolopyridinyl, pyrrolyl, thiazolyl, thienyl, and thiomorpholinyl.

20 The term “hydroxy,” as used herein, refers to –OH.

The term “imidazolylC₁-C₃alkyl,” as used herein, refers to an imidazolyl group attached to the parent molecular moiety through a C₁-C₃alkyl group. The imidazolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

25 The term “indolylC₁-C₃alkyl,” as used herein, refers to an indolyl group attached to the parent molecular moiety through a C₁-C₃alkyl group. The indolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “naphthylC₁-C₃alkyl,” as used herein, refers to a naphthyl group
30 attached to the parent molecular moiety through a C₁-C₃alkyl group. The naphthyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “nitro,” as used herein, refers to $-\text{NO}_2$.

The term “ NR^aR^b ,” as used herein, refers to two groups, R^a and R^b , which are attached to the parent molecular moiety through a nitrogen atom. R^a and R^b are independently selected from hydrogen, C_2 - C_4 alkenyloxy carbonyl, C_1 -
 5 C_3 alkyl carbonyl, C_3 - C_6 cycloalkyl carbonyl, furanyl carbonyl, and phenyl carbonyl.

The term “ $\text{NR}^a\text{R}^b(\text{C}_1\text{-C}_3)\text{alkyl}$,” as used herein, refers to an NR^aR^b group attached to the parent molecular moiety through a C_1 - C_3 alkyl group.

The term “ NR^cR^d ,” as used herein, refers to two groups, R^c and R^d , which are attached to the parent molecular moiety through a nitrogen atom. R^c and R^d are
 10 independently selected from hydrogen, C_1 - C_3 alkyl, and triphenylmethyl.

The term “ $\text{NR}^c\text{R}^d\text{carbonyl}$,” as used herein, refers to an NR^cR^d group attached to the parent molecular moiety through a carbonyl group.

The term “ $\text{NR}^c\text{R}^d\text{carbonylC}_1\text{-C}_3\text{alkyl}$,” as used herein, refers to an $\text{NR}^c\text{R}^d\text{carbonyl}$ group attached to the parent molecular moiety through a C_1 - C_3 alkyl
 15 group.

The term “phenoxy,” as used herein, refers to a phenyl group attached to the parent molecular moiety through an oxygen atom.

The term “phenoxy $\text{C}_1\text{-C}_3\text{alkyl}$,” as used herein, refers to a phenoxy group attached to the parent molecular moiety through a C_1 - C_3 alkyl group.

20 The term “phenyl $\text{C}_1\text{-C}_3\text{alkyl}$,” as used herein, refers to a phenyl group attached to the parent molecular moiety through a C_1 - C_3 alkyl group.

The term “phenyl carbonyl,” as used herein, refers to a phenyl group attached to the parent molecular moiety through a carbonyl group.

The term “pyridinyl $\text{C}_1\text{-C}_3\text{alkyl}$,” as used herein, refers to a pyridinyl group
 25 attached to the parent molecular moiety through a C_1 - C_3 alkyl group. The pyridinyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term “sulfanyl,” as used herein, refers to $-\text{S}-$.

The term “sulfonyl,” as used herein, refers to $-\text{SO}_2-$.

30 The term “thiazolyl $\text{C}_1\text{-C}_3\text{alkyl}$,” as used herein, refers to a thiazolyl group attached to the parent molecular moiety through a C_1 - C_3 alkyl group. The thiazolyl

group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "thienylC₁-C₃alkyl," as used herein, refers to a thienyl group attached to the parent molecular moiety through a C₁-C₃alkyl group. The thienyl
5 group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "treating" refers to: (i) preventing a disease, disorder, or condition from occurring in a patient that may be predisposed to the disease, disorder, and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease,
10 disorder, or condition, i.e., arresting its development; and (iii) relieving the disease, disorder, or condition, i.e., causing regression of the disease, disorder, and/or condition and/or symptoms associated with the disease, disorder, and/or condition.

Binding of the macrocyclic peptides to PD-L1 can be measured, for example, by methods such as homogeneous time-resolved fluorescence (HTRF), Surface
15 Plasmon Resonance (SPR), isothermal titration calorimetry (ITC), nuclear magnetic resonance spectroscopy (NMR), and the like. Further, binding of the macrocyclic peptides to PD-L1 expressed on the surface of cells can be measured as described herein in cellular binding assays.

Administration of a therapeutic agent described herein includes, without
20 limitation, administration of a therapeutically effective amount of therapeutic agent. The term "therapeutically effective amount" as used herein refers, without limitation, to an amount of a therapeutic agent to treat or prevent a condition treatable by administration of a composition of the PD-1/PD-L1 binding inhibitors described herein. That amount is the amount sufficient to exhibit a detectable therapeutic or
25 preventative or ameliorative effect. The effect may include, for example and without limitation, treatment or prevention of the conditions listed herein. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition being treated, recommendations of the treating physician, and therapeutics or combination of therapeutics selected for
30 administration. Thus, it is not useful to specify an exact effective amount in advance.

In another aspect, the disclosure pertains to methods of inhibiting growth of tumor cells in a subject using the macrocyclic peptides of the present disclosure. As

demonstrated herein, the macrocyclic peptides of the present disclosure are capable of binding to PD-L1, disrupting the interaction between PD-L1 and PD-1, competing with the binding of PD-L1 with anti-PD-1 monoclonal antibodies that are known to block the interaction with PD-1, enhancing CMV-specific T cell IFN γ secretion, and
5 enhancement of HIV-specific T cell IFN γ secretion. As a result, the macrocyclic peptides of the present disclosure are useful for modifying an immune response, treating diseases such as cancer or infectious disease, stimulating a protective autoimmune response or to stimulate antigen-specific immune responses (*e.g.*, by coadministration of PD-L1 blocking peptides with an antigen of interest).

10 In order that the present disclosure may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "Programmed Death Ligand 1", "Programmed Cell Death Ligand 1", "Protein PD-L1", "PD-L1", "PDL1", "PDCDL1", "hPD-L1", "hPD-LI", "CD274"
15 and "B7-H1" are used interchangeably, and include variants, isoforms, species homologs of human PD-L1, and analogs having at least one common epitope with PD-L1. The complete PD-L1 sequence can be found under GENBANK® Accession No. NP_054862.

The terms "Programmed Death 1", "Programmed Cell Death 1", "Protein PD-1", "PD-1", "PD1", "PDCD1", "hPD-1" and "hPD-I" are used interchangeably, and
20 include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GENBANK® Accession No. U64863.

The terms "cytotoxic T lymphocyte-associated antigen-4", "CTLA-4",
25 "CTLA4", "CTLA-4 antigen" and "CD152" (see, *e.g.*, Murata, *Am. J. Pathol.*, 155:453-460 (1999)) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (see, *e.g.*, Balzano, *Int. J. Cancer Suppl.*, 7:28-32 (1992)). The complete CTLA-4 nucleic acid sequence can be found under GENBANK®
30 Accession No. L15006.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble

macromolecules produced by the above cells or the liver (including macrocyclic peptides, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

An "adverse event" (AE) as used herein is any unfavorable and generally unintended, even undesirable, sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

As used herein, "hyperproliferative disease" refers to conditions wherein cell growth is increased over normal levels. For example, hyperproliferative diseases or disorders include malignant diseases (*e.g.*, esophageal cancer, colon cancer, biliary cancer) and non-malignant diseases (*e.g.*, atherosclerosis, benign hyperplasia, and benign prostatic hypertrophy).

As used herein, "about" or "comprising essentially of" mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within one or more than one standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value.

As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited

range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

Competition Assays

5 The present disclosure is also directed to macrocyclic peptides that are capable of competing with the binding of a reference anti-PD-L1 antibody (MDX-1105) by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 100%. Such macrocyclic peptides may share structural homology
10 with one or more macrocyclic peptides disclosed herein, including mutant, conservative substitution, functional substitution, and deletion forms, provided they specific bind to PD-L1. For example, if a macrocyclic peptide binds substantially to the same region of PD-L1 as a reference anti-PD-L1 antibody, the macrocyclic peptide should bind to an epitope of PD-L1 that at least overlaps with the PD-L1
15 epitope that the anti-PD-L1 monoclonal antibody binds to. The overlapping region can range from one amino acid residue to several hundred amino acid residues. The macrocyclic peptide should then compete with and/or block the binding of the anti-PD-L1 monoclonal antibody to PD-L1 and thereby decrease the binding of the anti-PD-L1 monoclonal antibody to PD-L1, preferably by at least about 50% in a
20 competition assay.

 Anti-PD-L1 antibodies that may be used as reference antibodies for competition assay purposes are known in the art. For example, the following representative anti-PD-L1 antibodies may be used: MDX-1105 (BMS); L01X-C (Serono), L1X3 (Serono), MSB-0010718C (Serono), and PD-L1 Probody (CytomX),
25 and the PD-L1 antibodies disclosed in co-owned WO 2007/005874.

 Anti-PD-1 antibodies that may be used as reference antibodies for competition assay purposes are known in the art. For example, the following representative anti-PD-1 antibodies may be used: nivolumab (BMS); 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 each disclosed in co-owned U.S. Patent No. 8,008,449
30 (BMS), MK-3475 (Merck, disclosed in U.S. Patent No. 8,168,757), and the antibodies disclosed in U.S. Patent No. 7,488,802.

Pharmaceutical Compositions

In another aspect, the present disclosure provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of macrocyclic peptides of the present disclosure, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) macrocyclic peptides, or immunoconjugates or bispecific molecules of the disclosure. For example, a pharmaceutical composition of the disclosure can comprise a combination of macrocyclic peptides (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

Pharmaceutical compositions of the disclosure also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include a macrocyclic peptide combined with at least one other anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the macrocyclic peptides of the disclosure.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, a macrocyclic peptide, immunoconjugate, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compounds of the disclosure may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" or "therapeutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M. et al., *J. Pharm. Sci.*, 66:1-19 (1977)). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric,

phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from
5 alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of the disclosure also may include a
10 pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol,
15 and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable
20 mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting
25 agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions.
30 In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any
5 conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the disclosure is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a
10 solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the
15 maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example,
20 monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active
25 compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously
30 sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being

treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01
5 percent to about ninety-nine percent of active ingredient, preferably from about 0.1 percent to about 70 percent, most preferably from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several
10 divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be
15 treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the
20 art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the macrocyclic peptide, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg
25 body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per day, twice per day, bi-weekly, tri-weekly, weekly, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for a macrocyclic peptide of the
30 disclosure include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the macrocycle being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every

three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

In some methods, two or more macrocyclic peptides with different binding specificities are administered simultaneously, in which case the dosage of each
5 compound administered falls within the ranges indicated. The compounds are usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of macrocyclic peptide to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma
10 concentration of about 1-1000 $\mu\text{g/ml}$ and in some methods about 25-300 $\mu\text{g/ml}$.

Alternatively, the macrocyclic peptide can be administered as a sustained release formulation, in which case less frequent administration is required. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is
15 administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease.
20 Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to
25 the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in
30 combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "therapeutically effective dosage" of a macrocyclic peptide of the disclosure preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth and/or HIV can be evaluated in an animal model system predictive of efficacy in human tumors or viral efficacy. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, decrease viral load, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

In another aspect, the instant disclosure provides a pharmaceutical kit of parts comprising a macrocyclic peptide and an another immunomodulator, as described herein. The kit may also further comprise instructions for use in the treatment of a hyperproliferative disease (such as cancer as described herein) and/or anti-viral disease.

A composition of the present disclosure can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for macrocyclic peptides of the disclosure include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital,

intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Alternatively, a macrocyclic peptide of the disclosure can be administered via
5 a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including
10 implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, Robinson, J.R., ed., *Sustained and*
15 *Controlled Release Drug Delivery Systems*, Marcel Dekker, Inc., New York (1978).

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the disclosure can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163, 5,383,851, 5,312,335,
20 5,064,413, 4,941,880, 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present disclosure include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medication through the skin; U.S. Patent No. 4,447,233, which
25 discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These
30 patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the macrocyclic peptides of the disclosure can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that therapeutic compounds of the disclosure cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patent Nos. 4,522,811, 5,374,548, and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, Ranade, V.V., *J. Clin. Pharmacol.*, 29:685 (1989)). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent No. 5,416,016 to Low et al.); mannosides (Umezawa et al., *Biochem. Biophys. Res. Commun.*, 153:1038 (1988)); macrocyclic peptides (Bloeman, P.G. et al., *FEBS Lett.*, 357:140 (1995); Owais, M. et al., *Antimicrob. Agents Chemother.*, 39:180 (1995)); surfactant protein A receptor (Briscoe et al., *Am. J. Physiol.*, 1233:134 (1995)); p120 (Schreier et al., *J. Biol. Chem.*, 269:9090 (1994)); see also Keinanen, K. et al., *FEBS Lett.*, 346:123 (1994); Killion, J.J. et al., *Immunomethods* 4:273 (1994).

Uses and Methods of the Disclosure

The macrocyclic peptides, compositions and methods of the present disclosure have numerous *in vitro* and *in vivo* utilities involving, for example, detection of PD-L1 or enhancement of immune response by blockade of PD-L1. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the disclosure provides a method of modifying an immune response in a subject comprising administering to the subject the macrocyclic peptide of the disclosure such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated. In other respects, the macrocyclic peptide may have anti-cyno, anti-mouse, and/or anti-woodchuck binding and therapeutic activity.

As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses,

chickens, woodchuck, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses. Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that
5 can be treated by augmenting the T-cell mediated immune response. In a particular embodiment, the methods are particularly suitable for treatment of cancer cells *in vivo*. To achieve antigen-specific enhancement of immunity, the macrocyclic peptides can be administered together with an antigen of interest. When macrocyclic peptides to PD-L1 are administered together with another agent, the two can be
10 administered in either order or simultaneously.

The disclosure further provides methods for detecting the presence of human, woodchuck, cyno, and/or mouse PD-L1 antigen in a sample, or measuring the amount of human, woodchuck, cyno, and/or mouse PD-L1 antigen, comprising contacting the sample, and a control sample, with a reference macrocyclic peptide
15 which specifically binds to human, woodchuck, cyno, and/or mouse PD-L1, under conditions that allow for formation of a complex between the macrocycle and human, woodchuck, cyno, and/or mouse PD-L1. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human, woodchuck, cyno, and/or
20 mouse PD-L1 antigen in the sample.

Given the specific binding of the macrocyclic peptides of the disclosure for PD-L1, compared to CD28, ICOS and CTLA-4, the macrocyclic peptides of the disclosure can be used to specifically detect PD-L1 expression on the surface of cells and, moreover, can be used to purify PD-L1 via immunoaffinity purification.

25

Cancer

Blockade of PD-1 by macrocyclic peptides can enhance the immune response to cancerous cells in the patient. The ligand for PD-1, PD-L1, is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong et al., *Nat. Med.*, 8:787-789 (2002)). The interaction between PD-1 and PD-L1 results in a
30 decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al., *J. Mol. Med.*,

81:281-287 (2003); Blank et al., *Cancer Immunol. Immunother.*, 54:307-314 (2005); Konishi et al., *Clin. Cancer Res.*, 10:5094-5100 (2004)). Immune suppression can be reversed by inhibiting the local interaction of PD-1 to PD-L1 and the effect is additive when the interaction of PD-1 to PD-L2 is blocked as well (Iwai et al., *Proc. Natl. Acad. Sci.*, 99:12293-12297 (2002); Brown et al., *J. Immunol.*, 170:1257-1266 (2003)). While previous studies have shown that T-cell proliferation can be restored by inhibiting the interaction of PD-1 to PD-L1, there have been no reports of a direct effect on cancer tumor growth *in vivo* by blocking the PD-1/PD-L1 interaction. In one aspect, the present disclosure relates to treatment of a subject *in vivo* using a macrocyclic peptide such that growth of cancerous tumors is inhibited. A macrocyclic peptide may be used alone to inhibit the growth of cancerous tumors. Alternatively, a macrocyclic peptide may be used in conjunction with other immunogenic agents, standard cancer treatments, or other macrocyclic peptides, as described below.

Accordingly, in one embodiment, the disclosure provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of a macrocyclic peptide.

Preferred cancers whose growth may be inhibited using the macrocyclic peptides of the disclosure include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cell carcinoma (*e.g.*, clear cell carcinoma), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma and castration-resistant prostate cancer), breast cancer, colorectal cancer and lung cancer (*e.g.*, squamous and non-squamous non-small cell lung cancer). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the macrocyclic peptides of the disclosure.

Examples of other cancers that may be treated using the methods of the disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, colon cancer, rectal cancer, cancer of the anal region, stomach/gastric cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the

vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present disclosure is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai et al., *Int. Immunol.*, 17:133-144 (2005)).

Optionally, macrocyclic peptides to PD-L1 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al., *J. Immunol.*, 173:4919-4928 (2004)). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

In humans, some tumors have been shown to be immunogenic such as melanomas. It is anticipated that by raising the threshold of T cell activation by PD-L1 blockade, we may expect to activate tumor responses in the host.

PD-L1 blockade is likely to be most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., *Development of Cancer Vaccines*, ASCO Educational Book Spring: 60-62 (2000); Logothetis, C., *ASCO Educational Book Spring: 300-302* (2000); Khayat, D., *ASCO Educational Book Spring: 414-428* (2000); Foon, K., *ASCO Educational Book Spring: 730-738* (2000); see also Restifo, N. et al., *Cancer Vaccines*, Chapter 61, pp. 3023-3043, in DeVita, V. et al., eds.,

Cancer: Principles and Practice of Oncology, Fifth Edition (1997)). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al., *Proc. Natl. Acad. Sci. USA*, 90: 3539-3543 (1993)).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, S.A., *Immunity*, 10:281-287 (1999)). In many cases, these tumor specific antigens are differentiated antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. PD-L1 blockade may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N et al., *Science*, 266:2011-2013 (1994)). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such as Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-L1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R.

et al., *Science*, 269:1585-1588 (1995); Tamura, Y. et al., *Science*, 278:117-120 (1997)).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. et al., *Nat. Med.*, 4:328-332 (1998)). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. et al., *Nat. Med.*, 6:332-336 (2000)). As a method of vaccination, DC immunization may be effectively combined with PD-L1 blockade to activate more potent anti-tumor responses.

PD-L1 blockade may also be combined with standard cancer treatments. PD-L1 blockade may be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. et al., *Cancer Res.*, 58:5301-5304 (1998)). An example of such a combination is a macrocyclic peptide in combination with decarbazine for the treatment of melanoma. Another example of such a combination is a macrocyclic peptide in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-L1 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with PD-L1 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with PD-L1 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

PD-L1 blocking macrocyclic peptides can also be used in combination with bispecific macrocyclic peptides that target Fc alpha or Fc gamma receptor-expressing effectors cells to tumor cells (see, *e.g.*, U.S. Patent Nos. 5,922,845 and 5,837,243). Bispecific macrocyclic peptides can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific macrocyclic peptides have been used to target macrophages to sites of tumor. This targeting may

more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the use of PD-L1 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific macrocyclic peptides which bind to tumor antigen and a dendritic cell specific cell surface marker.

5 Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-beta (Kehrl, J. et al., *J. Exp. Med.*, 163:1037-1050 (1986)), IL-10 (Howard, M. et al., *Immunology Today*, 13:198-200 (1992)), and Fas ligand
10 (Hahne, M. et al., *Science*, 274:1363-1365 (1996)). Macrocyclic peptides to each of these entities may be used in combination with anti-PD-L1 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other macrocyclic peptides which may be used to activate host immune responsiveness can be used in combination with anti-PD-L1. These include
15 molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 macrocyclic peptides are able to substitute effectively for T cell helper activity (Ridge, J. et al., *Nature*, 393:474-478 (1998)) and can be used in conjunction with PD-1 antibodies (Ito, N. et al., *Immunobiology*, 201(5):527-540 (2000)). Activating macrocyclic peptides to T cell costimulatory molecules such as
20 CTLA-4 (e.g., U.S. Patent No. 5,811,097), OX-40 (Weinberg, A. et al., *Immunol.*, 164:2160-2169 (2000)), 4-1BB (Melero, I. et al., *Nat. Med.*, 3:682-685 (1997), and ICOS (Hutloff, A. et al., *Nature*, 397:262-266 (1999)) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of
25 tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. PD-L1 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo*
30 activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. et al., *Science*, 285:546-551 (1999)). These methods may also be used to activate T

cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of macrocyclic peptides may be expected to increase the frequency and activity of the adoptively transferred T cells.

5 Infectious Diseases

Other methods of the disclosure are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the disclosure provides a method of treating an infectious disease in a subject comprising administering to the subject a macrocyclic peptide of the present disclosure such that
10 the subject is treated for the infectious disease.

Similar to its application to tumors as discussed above, PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for
15 which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, and C), Influenza, Herpes, Giardia, Malaria (Butler, N.S. et al., *Nature Immunology* 13, 188-195 (2012); Hafalla, J.C.R., et al. *PLOS Pathogens*; February 2, 2012)), Leishmania, Staphylococcus aureus, Pseudomonas Aeruginosa.
20 PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human PD-L1 administration, thus provoking a strong T cell response that is not dampened by negative signals through PD-L1.

Some examples of pathogenic viruses causing infections treatable by methods of the disclosure include HIV, hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus,
30 HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections treatable by methods of the disclosure include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, 5 tetanus, botulism, anthrax, plague, leptospirosis, and Lyme disease bacteria.

Some examples of pathogenic fungi causing infections treatable by methods of the disclosure include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, 10 Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Some examples of pathogenic parasites causing infections treatable by methods of the disclosure include Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lamblia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, 15 Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

In all of the above methods, PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, interferons, agents targeting VEGF activity or VEGF-receptors, GM-CSF, G-CSF, IL-2), or bispecific 20 antibody therapy, which provides for enhanced presentation of tumor antigens (see, *e.g.*, Holliger, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993); Poljak, *Structure*, 2:1121-1123 (1994)).

Autoimmune Reactions

25 The macrocyclic peptides may provoke and amplify autoimmune responses. Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines reveals that many anti-tumor responses involve anti-self reactivities (depigmentation observed in anti-CTLA-4+GM-CSF-modified B 16 melanoma in van Elsas et al. supra; depigmentation in Trp-2 vaccinated mice (Overwijk, W. et al., *Proc. Natl. Acad. Sci. USA*, 96:2982-2987 (1999)); autoimmune prostatitis evoked by TRAMP 30 tumor cell vaccines (Hurwitz, A., *supra* (2000)), melanoma peptide antigen

vaccination and vitiligo observed in human clinical trials (Rosenberg, S.A. et al., *J. Immunother. Emphasis Tumor Immunol.*, 19(1):81-84 (1996)).

Therefore, it is possible to consider using anti-PD-L1 blockade in conjunction with various self proteins in order to devise vaccination protocols to efficiently
5 generate immune responses against these self proteins for disease treatment. For example, Alzheimer's disease involves inappropriate accumulation of A.beta. peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk et al., *Nature*, 400:173-177 (1999)).

Other self proteins may also be used as targets such as IgE for the treatment
10 of allergy and asthma, and TNF.alpha for rheumatoid arthritis. Finally, antibody responses to various hormones may be induced by the use of the macrocycles disclosed herein. Neutralizing antibody responses to reproductive hormones may be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors may also be
15 considered as possible vaccination targets.

Analogous methods as described above for the use of anti-PD-L1
macrocycles can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including A.beta. in Alzheimer's disease, cytokines such as TNF.alpha., and
20 IgE.

Vaccines

The macrocyclic peptides may be used to stimulate antigen-specific immune responses by coadministration of an anti-PD-1 macrocycle with an antigen of interest
25 (e.g., a vaccine). Accordingly, in another aspect the disclosure provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 macrocycle such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen.
30 Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

Suitable routes of administering the compositions (*e.g.*, macrocyclic peptides, multispecific and bispecific molecules and immunoconjugates) of the disclosure *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the compositions can be administered by injection (*e.g.*,
5 intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the composition.

As previously described the macrocyclic peptides of the disclosure can be co-administered with one or other more therapeutic agents, *e.g.*, a cytotoxic agent, a
10 radiotoxic agent or an immunosuppressive agent. The peptide can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the peptide can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. Such therapeutic agents include, among
15 others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, decarbazine and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once
20 every 21 days. Co-administration of the macrocyclic peptides of the present disclosure with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them
25 unreactive with the peptides.

Also within the scope of the present disclosure are kits comprising the compositions of the disclosure (*e.g.*, macrocyclic peptides, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain at least one additional reagent, or one or more additional macrocyclic
30 peptides of the disclosure (*e.g.*, a human antibody having a complementary activity which binds to an epitope in PD-L1 antigen distinct from the macrocycle). Kits typically include a label indicating the intended use of the contents of the kit. The

term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

Combination Therapy

5 The combination of the macrocyclic peptides of the present disclosure with another PD-L1 antagonist and/or other immunomodulator is useful for enhancement of an immune response against a hyperproliferative disease. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in
10 one aspect, the disclosure provides a method of modifying an immune response in a subject comprising administering to the subject a macrocyclic peptide of the disclosure such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated. In another embodiment, the instant disclosure provides a method of altering adverse events associated with treatment of a
15 hyperproliferative disease with an immunostimulatory therapeutic agent, comprising administering a macrocyclic peptide of the present disclosure and a subtherapeutic dose of another immunomodulator to a subject.

 Blockade of PD-L1 by macrocyclic peptides can enhance the immune response to cancerous cells in the patient. Cancers whose growth may be inhibited
20 using the macrocyclic peptides of the instant disclosure include cancers typically responsive to immunotherapy. Representative examples of cancers for treatment with the combination therapy of the instant disclosure include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer. Examples of other cancers that may be treated using the
25 methods of the instant disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the
30 vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue,

cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present disclosure is also useful for treatment of metastatic cancers.

10 In certain embodiments, the combination of therapeutic agents containing at least one macrocyclic peptide discussed herein may be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions wherein each agent can be administered sequentially. For example, a second immunomodulator and a macrocyclic peptide of the present disclosure can be administered sequentially, such as the second immunomodulator administered first and the macrocyclic peptide second, or the macrocyclic peptide being administered first and the second immunomodulator second. Furthermore, if more than one dose of the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration, sequential administrations may be combined with concurrent administrations, or any combination thereof. For example, the first administration of a second immunomodulator and the macrocyclic peptide may be concurrent, the second administration may be sequential with the second immunomodulator first and the macrocyclic peptide second, and the third administration may be sequential with the macrocyclic peptide first and second immunomodulator second, etc. Another representative dosing scheme may involve a first administration that is sequential with the macrocyclic peptide first and the second immunomodulator second, and subsequent administrations may be concurrent.

30 Optionally, the combination of the macrocyclic peptide and a second immunomodulator can be further combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides,

and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al., *J. Immunol.*, 173:4919-4928 (2004)). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or
5 tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

A combined PD-L1 macrocyclic peptide and a second immunomodulator can be further combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., Development of
10 Cancer Vaccines, ASCO Educational Book Spring: 60-62 (2000); Logothetis, C., ASCO Educational Book Spring: 300-302 (2000); Khayat, D., ASCO Educational Book Spring: 414-428 (2000); Foon, K., ASCO Educational Book Spring: 730-738 (2000); see also Restifo et al., Cancer Vaccines, Chapter 61, pp. 3023-3043 in DeVita et al., eds., *Cancer: Principles and Practice of Oncology*, Fifth Edition
15 (1997)). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al., *Proc. Natl. Acad. Sci. USA*, 90:3539-3543 (1993)).

20 The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, *Immunity*, 10:281-287 (1999)). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens,
25 and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. In certain embodiments, a combined PD-L1 macrocyclic peptide and a second immunomodulator may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally
30 viewed by the immune system as self-antigens and are, therefore, tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of

human cancers and in only a limited number of somatic tissues (Kim et al., *Science*, 266:2011-2013 (1994)). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion
5 proteins between two unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such as Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific
10 antigen which may be used in conjunction with PD-L1 macrocyclic peptide blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot et al., *Science*, 269:1585-1588 (1995); Tamura et al., *Science*, 278:117-120
15 (1997)).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle et al., *Nat. Med.*, 4:328-332 (1998)). DCs may also be transduced by genetic means to express
20 these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler et al., *Nat. Med.*, 6:332-336 (2000)). As a method of vaccination, DC immunization may be effectively further combined with a combined anti-PD-L1 macrocyclic peptide and a second immunomodulator to activate more potent anti-tumor responses.

25 A combined anti-PD-L1 macrocyclic peptide and additional immunomodulator may also be further combined with standard cancer treatments. For example, a combination of a macrocyclic peptide and a second immunomodulator may be effectively combined with chemotherapeutic regimes. In these instances, as is observed with the combination of a macrocyclic peptide and a
30 second immunomodulator, it may be possible to reduce the dose of other chemotherapeutic reagent administered with the combination of the instant disclosure (Mokyr et al., *Cancer Res.*, 58:5301-5304 (1998)). An example of such a

combination is a combination of a macrocyclic peptide and a second immunomodulator further in combination with decarbazine for the treatment of melanoma. Another example is a combination of a macrocyclic peptide and a second immunomodulatory agent further in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-L1 macrocyclic peptide and another immunomodulator with chemotherapy is that cell death, which is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with a combined anti-PD-L1 macrocyclic peptide and additional immunomodulator through cell death include radiation, surgery, or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with a combined PD-L1 and second immunomodulator. Inhibition of angiogenesis leads to tumor cell death, which may also be a source of tumor antigen to be fed into host antigen presentation pathways.

A combination of PD-L1 and another immunomodulator can also be used in combination with bispecific macrocyclic peptides that target Fc.alpha. or Fc.gamma. receptor-expressing effector cells to tumor cells (see, *e.g.*, U.S. Patent Nos. 5,922,845 and 5,837,243). Bispecific macrocyclic peptides can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific macrocyclic peptides have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the use of a combined PD-L1 and a second immunomodulator. Alternatively, antigen may be delivered directly to DCs by the use of bispecific macrocyclic peptides which bind to tumor antigen and a dendritic cell specific cell surface marker.

In another example, a combination of a macrocyclic peptide and a second immunomodulator can be used in conjunction with anti-neoplastic macrocyclic agents, such as RITUXAN® (rituximab), HERCEPTIN® (trastuzumab), BEXXAR® (tositumomab), ZEVALIN® (ibritumomab), CAMPATH® (alemtuzumab), Lymphocide (epratuzumab), AVASTIN® (bevacizumab), and TARCEVA® (erlotinib), and the like. By way of example and not wishing to be bound by theory,

treatment with an anti-cancer antibody or an anti-cancer antibody conjugated to a toxin can lead to cancer cell death (*e.g.*, tumor cells) which would potentiate an immune response mediated by the second immunomodulator target or PD-L1. In an exemplary embodiment, a treatment of a hyperproliferative disease (*e.g.*, a cancer
5 tumor) may include an anti-cancer antibody in combination with a macrocyclic peptide and a second immunomodulator concurrently or sequentially or any combination thereof, which may potentiate an anti-tumor immune responses by the host.

Tumors evade host immune surveillance by a large variety of mechanisms.
10 Many of these mechanisms may be overcome by the inactivation of proteins, which are expressed by the tumors and which are immunosuppressive. These include, among others, TGF- β . (Kehrl, J. et al., *J. Exp. Med.*, 163:1037-1050 (1986)), IL-10 (Howard, M. et al., *Immunology Today*, 13:198-200 (1992)), and Fas ligand (Hahne, M. et al., *Science*, 274:1363-1365 (1996)). In another example, antibodies
15 to each of these entities may be further combined with a macrocyclic peptide and another immunomodulator to counteract the effects of immunosuppressive agents and favor anti-tumor immune responses by the host.

Other agents that may be used to activate host immune responsiveness can be further used in combination with a macrocyclic peptide of the present disclosure.
20 These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 macrocyclic peptides are able to substitute effectively for T cell helper activity (Ridge, J. et al., *Nature*, 393:474-478 (1998)) and can be used in conjunction with the macrocyclic peptides of the present disclosure, either alone or in combination with an anti-CTLA-4 combination (Ito, N.
25 et al., *Immunobiology*, 201(5):527-540 (2000)). Activating macrocyclic peptides to T cell costimulatory molecules, such as OX-40 (Weinberg, A. et al., *Immunol.*, 164:2160-2169 (2000)), 4-1BB (Melero, I. et al., *Nat. Med.*, 3:682-685 (1997), and ICOS (Hutloff, A. et al., *Nature*, 397:262-266 (1999)) may also provide for increased levels of T cell activation.

30 Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses.

A macrocyclic peptide of the present disclosure, either alone or in combination with another immunomodulator, can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. et al., *Science*, 285:546-551 (1999)). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence a macrocyclic peptide of the present disclosure, either alone or in combination with another immunomodulator, may be expected to increase the frequency and activity of the adoptively transferred T cells.

In certain embodiments, the present disclosure provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering a macrocyclic peptide of the present disclosure in combination with a subtherapeutic dose of another immunomodulator to a subject. For example, the methods of the present disclosure provide for a method of reducing the incidence of immunostimulatory therapeutic antibody-induced colitis or diarrhea by administering a non-absorbable steroid to the patient. Because any patient who will receive an immunostimulatory therapeutic antibody is at risk for developing colitis or diarrhea induced by such treatment, this entire patient population is suitable for therapy according to the methods of the present disclosure. Although steroids have been administered to treat inflammatory bowel disease (IBD) and prevent exacerbations of IBD, they have not been used to prevent (decrease the incidence of) IBD in patients who have not been diagnosed with IBD. The significant side effects associated with steroids, even non-absorbable steroids, have discouraged prophylactic use.

In further embodiments, a macrocyclic peptide of the present disclosure, either alone or in combination with another immunomodulator, can be further combined with the use of any non-absorbable steroid. As used herein, a "non-absorbable steroid" is a glucocorticoid that exhibits extensive first pass metabolism such that, following metabolism in the liver, the bioavailability of the steroid is low, *i.e.*, less than about 20%. In one embodiment of the disclosure, the non-absorbable

steroid is budesonide. Budesonide is a locally-acting glucocorticosteroid, which is extensively metabolized, primarily by the liver, following oral administration. ENTOCORT® EC (Astra-Zeneca) is a pH- and time-dependent oral formulation of budesonide developed to optimize drug delivery to the ileum and throughout the colon. ENTOCORT® EC is approved in the U.S. for the treatment of mild to moderate Crohn's disease involving the ileum and/or ascending colon. The usual oral dosage of ENTOCORT® EC for the treatment of Crohn's disease is 6 to 9 mg/day. ENTOCORT® EC is released in the intestines before being absorbed and retained in the gut mucosa. Once it passes through the gut mucosa target tissue, ENTOCORT® EC is extensively metabolized by the cytochrome P450 system in the liver to metabolites with negligible glucocorticoid activity. Therefore, the bioavailability is low (about 10%). The low bioavailability of budesonide results in an improved therapeutic ratio compared to other glucocorticoids with less extensive first-pass metabolism. Budesonide results in fewer adverse effects, including less hypothalamic-pituitary suppression, than systemically-acting corticosteroids. However, chronic administration of ENTOCORT® EC can result in systemic glucocorticoid effects such as hypercorticism and adrenal suppression. See *Physicians' Desk Reference Supplement*, 58th Edition, 608-610 (2004).

In still further embodiments, a combination PD-L1 and another immunomodulator in conjunction with a non-absorbable steroid can be further combined with a salicylate. Salicylates include 5-ASA agents such as, for example: sulfasalazine (AZULFIDINE®, Pharmacia & Upjohn); olsalazine (DIPENTUM®, Pharmacia & Upjohn); balsalazide (COLAZAL®, Salix Pharmaceuticals, Inc.); and mesalamine (ASACOL®, Procter & Gamble Pharmaceuticals; PENTASA®, Shire US; CANASA®, Axcan Scandipharm, Inc.; ROWASA®, Solvay).

Dosage and Formulation

A suitable peptide of Formula I, or more specifically a macrocyclic peptide described herein, can be administered to patients to treat diabetes and other related diseases as the compound alone and or mixed with an acceptable carrier in the form of pharmaceutical formulations. Those skilled in the art of treating diabetes can easily determine the dosage and route of administration of the compound to

mammals, including humans, in need of such treatment. The route of administration may include but is not limited to oral, intraoral, rectal, transdermal, buccal, intranasal, pulmonary, subcutaneous, intramuscular, intradermal, sublingual, intracolonic, intraocular, intravenous, or intestinal administration. The compound is formulated
5 according to the route of administration based on acceptable pharmacy practice (Fingl et al., in *The Pharmacological Basis of Therapeutics*, Chapter 1, p. 1 (1975); *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co., Easton, PA (1990)).

The pharmaceutically acceptable peptide compositions described herein can
10 be administered in multiple dosage forms such as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, in situ gels, microspheres, crystalline complexes, liposomes, micro-emulsions, tinctures, suspensions, syrups, aerosol sprays and emulsions. The compositions described herein can also be administered in oral, intravenous (bolus or
15 infusion), intraperitoneal, subcutaneous, transdermally or intramuscular form, all using dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compositions may be administered alone, but generally will be administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

20 The dosage regimen for the compositions described herein will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of
25 administration, the renal and hepatic function of the patient, and the effect desired. A physician or veterinarian can determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the disease state.

By way of general guidance, the daily oral dosage of the active ingredient, when used for the indicated effects, will range between about 0.001 to 1000 mg/kg of
30 body weight, preferably between about 0.01 to 100 mg/kg of body weight per day, and most preferably between about 0.6 to 20 mg/kg/day. Intravenously, the daily dosage of the active ingredient when used for the indicated effects will range between

0.001ng to 100.0 ng per min/per Kg of body weight during a constant rate infusion. Such constant intravenous infusion can be preferably administered at a rate of 0.01 ng to 50 ng per min per Kg body weight and most preferably at 0.01 ng to 10.0 mg per min per Kg body weight. The compositions described herein may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three, or four times daily. The compositions described herein may also be administered by a depot formulation that will allow sustained release of the drug over a period of days/weeks/months as desired.

The compositions described herein can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using transdermal skin patches. When administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The compositions are typically administered in a mixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as pharmaceutical carriers) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, aerosol sprays generated with or without propellant and syrups, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as but not limited to, lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, and sorbitol; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as, but not limited to, ethanol, glycerol, and water. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include, but not limited to, starch, gelatin, natural sugars such as, but not limited to, glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, and waxes. Lubricants used in these dosage forms include sodium oleate, sodium stearate,

magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride.

Disintegrants include, but are not limited to, starch, methyl cellulose, agar, bentonite, and xanthan gum.

5 The compositions described herein may also be administered in the form of mixed micellar or liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. Permeation enhancers may be added to enhance drug absorption.

10 Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (*i.e.*, solubility, bioavailability, manufacturing, etc.) the compounds described herein may be delivered in prodrug form. Thus, the subject matter described herein is intended to cover prodrugs of the presently claimed compounds, methods of delivering the same, and compositions containing the same.

15 The compositions described herein may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compositions described herein may be
20 combined with a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacylates, and crosslinked or amphipathic block copolymers of hydrogels.

25 Dosage forms (pharmaceutical compositions) suitable for administration may contain from about 0.01 milligram to about 500 milligrams of active ingredient per dosage unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

30 Gelatin capsules may contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivative, magnesium stearate, and stearic acid. Similar diluents can be used to make compressed tablets. Both tablets and capsules

can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

5 Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

 In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solution for parenteral administration
10 preferably contains a water-soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium
15 chloride, methyl- or propyl-paraben, and chlorobutanol.

 Suitable pharmaceutical carriers are described in *Remington: The Science and Practice of Pharmacy*, Nineteenth Edition, Mack Publishing Company (1995), a standard reference text in this field.

 Representative useful pharmaceutical dosage forms for administration of the
20 compounds described herein can be illustrated as follows:

Capsules

 A large number of unit capsules can be prepared by filling standard two-piece hard gelatin capsules with 100 milligrams of powdered active ingredient, 150
25 milligrams of lactose, 50 milligrams of cellulose, and 6 milligrams magnesium stearate.

Soft Gelatin Capsules

 A mixture of active ingredient in a digestible oil such as soybean oil,
30 cottonseed oil or olive oil may be prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 100 milligrams of the active ingredient. The capsules should be washed and dried.

Tablets

Tablets may be prepared by conventional procedures so that the dosage unit, for example is 100 milligrams of active ingredient, 0.2 milligrams of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 milligrams of microcrystalline
5 cellulose, 11 milligrams of starch and 98.8 milligrams of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

Injectable

An injectable formulation of a peptide composition described herein may or
10 may not require the use of excipients such as those that have been approved by regulatory bodies. These excipients include, but are not limited to, solvents and co-solvents, solubilizing, emulsifying or thickening agents, chelating agents, anti-oxidants and reducing agents, antimicrobial preservatives, buffers and pH adjusting agents, bulking agents, protectants and tonicity adjustors and special additives. An
15 injectable formulation has to be sterile, pyrogen free and, in the case of solutions, free of particulate matter.

A parenteral composition suitable for administration by injection may be prepared by stirring for example, 1.5% by weight of active ingredient in a pharmaceutically acceptable buffer that may or may not contain a co-solvent or other
20 excipient. The solution should be made isotonic with sodium chloride and sterilized.

Suspension

An aqueous suspension can be prepared for oral and/or parenteral administration so that, for example, each 5 mL contains 100 mg of finely divided
25 active ingredient, 20 mg of sodium carboxymethyl cellulose, 5 mg of sodium benzoate, 1.0 g of sorbitol solution, U.S.P., and 0.025 mL of vanillin or other palatable flavoring.

Biodegradable Microparticles

30 A sustained-release parenteral composition suitable for administration by injection may be prepared, for example, by dissolving a suitable biodegradable polymer in a solvent, adding to the polymer solution the active agent to be

incorporated, and removing the solvent from the matrix thereby forming the matrix of the polymer with the active agent distributed throughout the matrix.

The abbreviations used in the present application, including particularly in the illustrative examples which follow, are well-known to those skilled in the art. Some of the abbreviations used are as follows: HOBt for hydroxybenzotriazole; HOAt for 1-hydroxy-7-azabenzotriazole; DIC for N,N'-diisopropylcarbodiimide; BOP for benzotriazol-1-yloxy tris(dimethylamino)phosphonium hexafluorophosphate; PyBOP for benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; HCTU for 1H-benzotriazolium 1-[bis(dimethylamino)methylene]-5chloro-,hexafluorophosphate (1-),3-oxide; HATU for 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; TFA for trifluoroacetic acid; TIS for triisopropylsilane; DMSO for dimethylsulfoxide; MeCN or ACN or AcCN for acetonitrile; DMF for N,N-dimethylformamide; DCM for dichloromethane; DIPEA or DIEA for diisopropylethylamine; Et₂O for diethyl ether; MeOH for methanol; rt for room temperature; h for hours; min for minutes; and iPr for isopropyl.

Peptide Synthesis

The description of the present disclosure herein should be construed in congruity with the laws and principals of chemical bonding. It should be understood that the compounds encompassed by the present disclosure are those that are suitably stable for use as pharmaceutical agent. One of skill in the art will know what compounds would and would not be stable based on the general principles of chemical bonding and stability.

Chemical synthesis of a macrocyclic peptide of the present disclosure can be carried out using a variety of art recognized methods, including stepwise solid phase synthesis, semi-synthesis through the conformationally-assisted re-ligation of peptide fragments, enzymatic ligation of cloned or synthetic peptide segments, and chemical ligation. A preferred method to synthesize the macrocyclic peptides and analogs thereof described herein is chemical synthesis using various solid-phase techniques such as those described in Chan, W.C. et al., eds., *Fmoc Solid Phase Synthesis*, Oxford University Press, Oxford (2000); Barany, G. et al., *The Peptides: Analysis, Synthesis, Biology*, Vol. 2: "Special Methods in Peptide Synthesis, Part A", pp. 3-

284, Gross, E. et al., eds., Academic Press, New York (1980); and in Stewart, J.M. et al., *Solid-Phase Peptide Synthesis*, 2nd Edition, Pierce Chemical Co., Rockford, IL (1984). The preferred strategy is based on the Fmoc (9-Fluorenylmethyl methoxycarbonyl) group for temporary protection of the α -amino group, in combination
5 with the *tert*-butyl group for temporary protection of the amino acid side chains (see for example Atherton, E. et al., "The Fluorenylmethoxycarbonyl Amino Protecting Group", in *The Peptides: Analysis, Synthesis, Biology*, Vol. 9: "Special Methods in Peptide Synthesis, Part C", pp. 1-38, Udenfriend, S. et al., eds., Academic Press, San Diego (1987).

10 The peptides can be synthesized in a stepwise manner on an insoluble polymer support (also referred to as "resin") starting from the C-terminus of the peptide. A synthesis is begun by appending the C-terminal amino acid of the peptide to the resin through formation of an amide or ester linkage. This allows the eventual release of the resulting peptide as a C-terminal amide or carboxylic acid, respectively.

15 The C-terminal amino acid and all other amino acids used in the synthesis are required to have their α -amino groups and side chain functionalities (if present) differentially protected such that the α -amino protecting group may be selectively removed during the synthesis. The coupling of an amino acid is performed by activation of its carboxyl group as an active ester and reaction thereof with the
20 unblocked α -amino group of the N-terminal amino acid appended to the resin. The sequence of α -amino group deprotection and coupling is repeated until the entire peptide sequence is assembled. The peptide is then released from the resin with concomitant deprotection of the side chain functionalities, usually in the presence of appropriate scavengers to limit side reactions. The resulting peptide is finally
25 purified by reverse phase HPLC.

The synthesis of the peptidyl-resins required as precursors to the final peptides utilizes commercially available cross-linked polystyrene polymer resins (Novabiochem, San Diego, CA; Applied Biosystems, Foster City, CA). Preferred solid supports are: 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetyl-p-
30 methyl benzhydrylamine resin (Rink amide MBHA resin); 9-Fmoc-amino-xanthen-3-yloxy-Merrifield resin (Sieber amide resin); 4-(9-Fmoc)aminomethyl-3,5-dimethoxyphenoxy)valeryl-aminomethyl-Merrifield resin (PAL resin), for C-terminal

carboxamides. Coupling of first and subsequent amino acids can be accomplished using HOBt, 6-Cl-HOBt or HOAt active esters produced from DIC/HOBt, HBTU/HOBt, BOP, PyBOP, or from DIC/6-Cl-HOBt, HCTU, DIC/HOAt or HATU, respectively. Preferred solid supports are: 2-Chlorotrityl chloride resin and 9-Fmoc-amino-xanthen-3-yloxy-Merrifield resin (Sieber amide resin) for protected peptide
5 fragments. Loading of the first amino acid onto the 2-chlorotrityl chloride resin is best achieved by reacting the Fmoc-protected amino acid with the resin in dichloromethane and DIEA. If necessary, a small amount of DMF may be added to facilitate dissolution of the amino acid.

10 The syntheses of the peptide analogs described herein can be carried out by using a single or multi-channel peptide synthesizer, such as an CEM Liberty Microwave synthesizer, or a Protein Technologies, Inc. Prelude (6 channels) or Symphony (12 channels) synthesizer.

The peptidyl-resin precursors for their respective peptides may be cleaved and
15 deprotected using any standard procedure (see, for example, King, D.S. et al., *Int. J. Peptide Protein Res.*, 36:255-266 (1990)). A desired method is the use of TFA in the presence of water and TIS as scavengers. Typically, the peptidyl-resin is stirred in TFA/water/TIS (94:3:3, v:v:v; 1 mL/100 mg of peptidyl resin) for 2-6 hrs at room temperature. The spent resin is then filtered off and the TFA solution is concentrated
20 or dried under reduced pressure. The resulting crude peptide is either precipitated and washed with Et₂O or is redissolved directly into DMSO or 50% aqueous acetic acid for purification by preparative HPLC.

Peptides with the desired purity can be obtained by purification using preparative HPLC, for example, on a Waters Model 4000 or a Shimadzu Model LC-
25 8A liquid chromatograph. The solution of crude peptide is injected into a YMC S5 ODS (20X 100 mm) column and eluted with a linear gradient of MeCN in water, both buffered with 0.1% TFA, using a flow rate of 14-20 mL/min with effluent monitoring by UV absorbance at 220 nm. The structures of the purified peptides can be confirmed by electro-spray MS analysis.

30

Analytical Data:

Mass Spectrometry: “ESI-MS(+)” signifies electrospray ionization mass spectrometry performed in positive ion mode; “ESI-MS(-)” signifies electrospray ionization mass spectrometry performed in negative ion mode; “ESI-HRMS(+)” signifies high-resolution electrospray ionization mass spectrometry performed in positive ion mode; “ESI-HRMS(-)” signifies high-resolution electrospray ionization mass spectrometry performed in negative ion mode. The detected masses are reported following the “ m/z ” unit designation. Compounds with exact masses greater than 1000 were often detected as double-charged or triple-charged ions.

10

Analysis Condition A:

Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0%B, 0-100% B over 3 minutes, then a 0.5-minute hold at 100% B; Flow: 1 mL/min; Detection: UV at 220 nm.

Analysis Condition B:

Column: Waters BEH C18, 2.0 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 methanol:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0%B, 0-100% B over 3 minutes, then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min; Detection: UV at 220 nm.

Analysis condition C:

Column: Waters BEH C18, 2.1 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 70 °C; Gradient: 0%B, 0-100% B over 3 minutes, then a 2.0-minute hold at 100% B; Flow: 0.75 mL/min; Detection: UV at 220 nm.

30

Analysis condition D:

Column: Waters CSH C18, 2.1 x 50 mm, 1.7- μ m particles; Mobile Phase A: 5:95 acetonitrile:water with trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile:water with trifluoroacetic acid; Temperature: 70 °C; Gradient: 0%B, 0-
 5 100% B over 3 minutes, then a 2.0-minute hold at 100% B; Flow: 0.75 mL/min; Detection: UV at 220 nm.

General Procedures for Examples and Intermediates

All manipulations were performed under automation on a Symphony-X
 10 peptide synthesizer (Protein Technologies). All procedures unless noted were performed in a 10 mL polypropylene tube fitted with a bottom frit. The tube connects to the Prelude peptide synthesizer through both the bottom and the top of the tube. DMF and DCM can be added through the top of the tube, which washes down the sides of the tube equally. The remaining reagents are added through the bottom of the
 15 tube and pass up through the frit to contact the resin. All solutions are removed through the bottom of the tube. "Periodic agitation" describes a brief pulse of N₂ gas through the bottom frit; the pulse lasts approximately 5 seconds and occurs every 30 seconds. Chloroacetyl chloride solutions in DMF were used within 24h of preparation. Amino acid solutions were generally not used beyond three weeks from
 20 preparation. HATU solution was used within 5 days of preparation. DMF = dimethylformamide; HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; DIPEA = diisopropylethylamine; Rink = (2,4-dimethoxyphenyl)(4-alkoxyphenyl)methanamine, where "4-alkoxy" describes the position and type of connectivity to the polystyrene
 25 resin. The resin used is Merrifield polymer (polystyrene) with a Rink linker (Fmoc-protected at nitrogen); 100-200 mesh, 1% DVB, 0.56 mmol/g loading. Common amino acids used are listed below with side-chain protecting groups indicated inside parenthesis.

Fmoc-Ala-OH; Fmoc-Arg(Pbf)-OH; Fmoc-Asn(Trt)-OH; Fmoc-Asp(OtBu)-OH;
 30 Fmoc-Bzt-OH; Fmoc-Cys(Trt)-OH; Fmoc-Dab(Boc)-OH; Fmoc-Dap(Boc)-OH; Fmoc-Gln(Trt)-OH; Fmoc-Gly-OH; Fmoc-His(Trt)-OH; Fmoc-Hyp(tBu)-OH; Fmoc-Ile-OH; Fmoc-Leu-OH; Fmoc-Lys(Boc)-OH; Fmoc-Nle-OH; Fmoc-Met-OH; Fmoc-

[N-Me]Ala-OH; Fmoc-[N-Me]Nle-OH; Fmoc-Phe-OH; Fmoc-Pro-OH; Fmoc-Sar-OH; Fmoc-Ser(tBu)-OH; Fmoc-Thr(tBu)-OH; Fmoc-Trp(Boc)-OH; Fmoc-Tyr(tBu)-OH; Fmoc-Val-OH

For carboxamide products: the procedures describe an experiment performed on a
5 0.100 mmol scale, where the scale is determined by the amount of Rink linker bound to the resin. This scale corresponds to approximately 178 mg of the Rink-Merrifield resin described above. Prior to amino acid coupling, all peptide synthesis sequences began with a resin-swelling procedure, described below as “Resin-swelling procedure”. Coupling of amino acids to a primary amine N-terminus used the
10 “Single-coupling procedure” described below. Coupling of amino acids to a secondary amine N-terminus used the “Double-coupling procedure” described below. Coupling of chloroacetylchloride to the N-terminus of the peptide is described by the “Chloroacetyl chloride coupling procedure” detailed below.

15 *Resin-swelling procedure:*

To a 10 mL polypropylene solid-phase reaction vessel was added Merrifield:Rink resin (178 mg, 0.100 mmol). The resin was washed (swelled) three times as follows: to the reaction vessel was added DMF (2.0 mL), upon which the mixture was periodically agitated for 10 minutes before the solvent was drained
20 through the frit.

Single-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3
25 minutes and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically
30 agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA (0.4M in DMF, 1.0 mL, 4 eq). The

mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the
5 solution was drained through the frit. To the reaction vessel was added acetic anhydride (2.0 mL). The mixture was periodically agitated for 10 minutes, then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds
10 before the solution was drained through the frit. The resulting resin was used directly in the next step.

Single-coupling for-(S)-2-amino-3-(1-(carboxymethyl)-1H-indol-3-yl)propanoic acid:

The coupling was performed as above, only a 30 min agitation time was used.
15

Double-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. To the reaction vessel
20 was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit.
25 To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA (0.4M in DMF, 1.0 mL, 4 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was twice washed as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the
30 resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA

(0.4M in DMF, 1.0 mL, 4 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was twice washed as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds
5 before the solution was drained through the frit. To the reaction vessel was added acetic anhydride (2.0 mL). The mixture was periodically agitated for 10 minutes, then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for
10 90 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Chloroacetyl chloride coupling procedure:

To the reaction vessel containing resin from the previous step was added
15 piperdine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. To the reaction vessel was added piperdine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was
20 added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added DIPEA (0.4M in DMF, 3.0 mL, 24 eq), then chloroacetyl chloride (0.8M in DMF, 1.5 mL, 13.2 eq). The mixture was periodically agitated for 30 minutes, then the solution was drained through the frit. The resin was
25 washed successively three times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, CH₂Cl₂ (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting
30 mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resulting resin was placed under a N₂ stream for 15 minutes upon which the resin became rigid and easily handled.

For carboxylic acid C-terminal products: The procedures describe an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of 2-chlorotrityl linker bound to the resin. Commercial Fmoc-Gly-2-chlorotrityl resin was used, usually as a 0.92 meq/g loading. This scale corresponds to approximately 109
5 mg of the Fmoc-Gly-2-chlorotrityl resin described above. Prior to amino acid coupling, all peptide synthesis sequences began with a resin-swelling procedure, described below as “Resin-swelling procedure”. Coupling of amino acids to a primary amine N-terminus used the “Single-coupling procedure” described below. Coupling of amino acids to a secondary amine N-terminus used the “Double-
10 coupling procedure” described below. Coupling of chloroacetylchloride to the N-terminus of the peptide is described by the “Chloroacetyl chloride coupling procedure” detailed below.

Resin-swelling procedure:

15 To a 10 mL polypropylene solid-phase reaction vessel was added Merrifield:Rink resin (178 mg, 0.100 mmol). The resin was washed (swelled) three times as follows: to the reaction vessel was added DMF (2.0 mL), upon which the mixture was periodically agitated for 10 minutes before the solvent was drained through the frit.

20

Single-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperdine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. To the reaction vessel
25 was added piperdine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit.
30 To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA (0.4M in DMF, 1.0 mL, 4 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution

was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added
5 DIPEA (0.4M in DMF, 1.0 mL, 4 eq), then acetic anhydride (2.0 mL). The mixture was periodically agitated for 10 minutes, then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds before the solution was drained
10 through the frit. The resulting resin was used directly in the next step.

Single-coupling for-(S)-2-amino-3-(1-(carboxymethyl)-1H-indol-3-yl)propanoic acid:

The coupling was performed as above, only a 30 min agitation time was used.

15 *Double-coupling procedure:*

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically
20 agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then
25 HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA (0.4M in DMF, 1.0 mL, 4 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was twice washed as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was
30 drained through the frit. To the reaction vessel was added the amino acid (0.2M in DMF, 1.0 mL, 2 eq), then HATU (0.2M in DMF, 1.0 mL, 2 eq), and finally DIPEA (0.4M in DMF, 1.0 mL, 4 eq). The mixture was periodically agitated for 15 minutes,

then the reaction solution was drained through the frit. The resin was twice washed as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added
5 DIPEA (0.4M in DMF, 1.0 mL, 4 eq), then acetic anhydride (2.0 mL). The mixture was periodically agitated for 10 minutes, then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds before the solution was drained
10 through the frit. The resulting resin was used directly in the next step.

Chloroacetyl chloride coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3
15 minutes and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.0 mL). The mixture was periodically agitated for 3 minutes and then the solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was
20 periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added DIPEA (0.4M in DMF, 3.0 mL, 24 eq), then chloroacetyl chloride (0.8M in DMF, 1.5 mL, 13.2 eq). The mixture was periodically agitated for 30 minutes, then the solution was drained through the frit. The resin was washed successively three times as follows: for each wash, DMF (2.0 mL) was added
25 to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, CH₂Cl₂ (2.0 mL) was added to top of the vessel (not through the bottom frit) and the resulting mixture was periodically agitated for 90 seconds before the solution was drained
30 through the frit. The resulting resin was placed under a N₂ stream for 15 minutes upon which the resin became rigid and easily handled.

Global deprotection procedure:

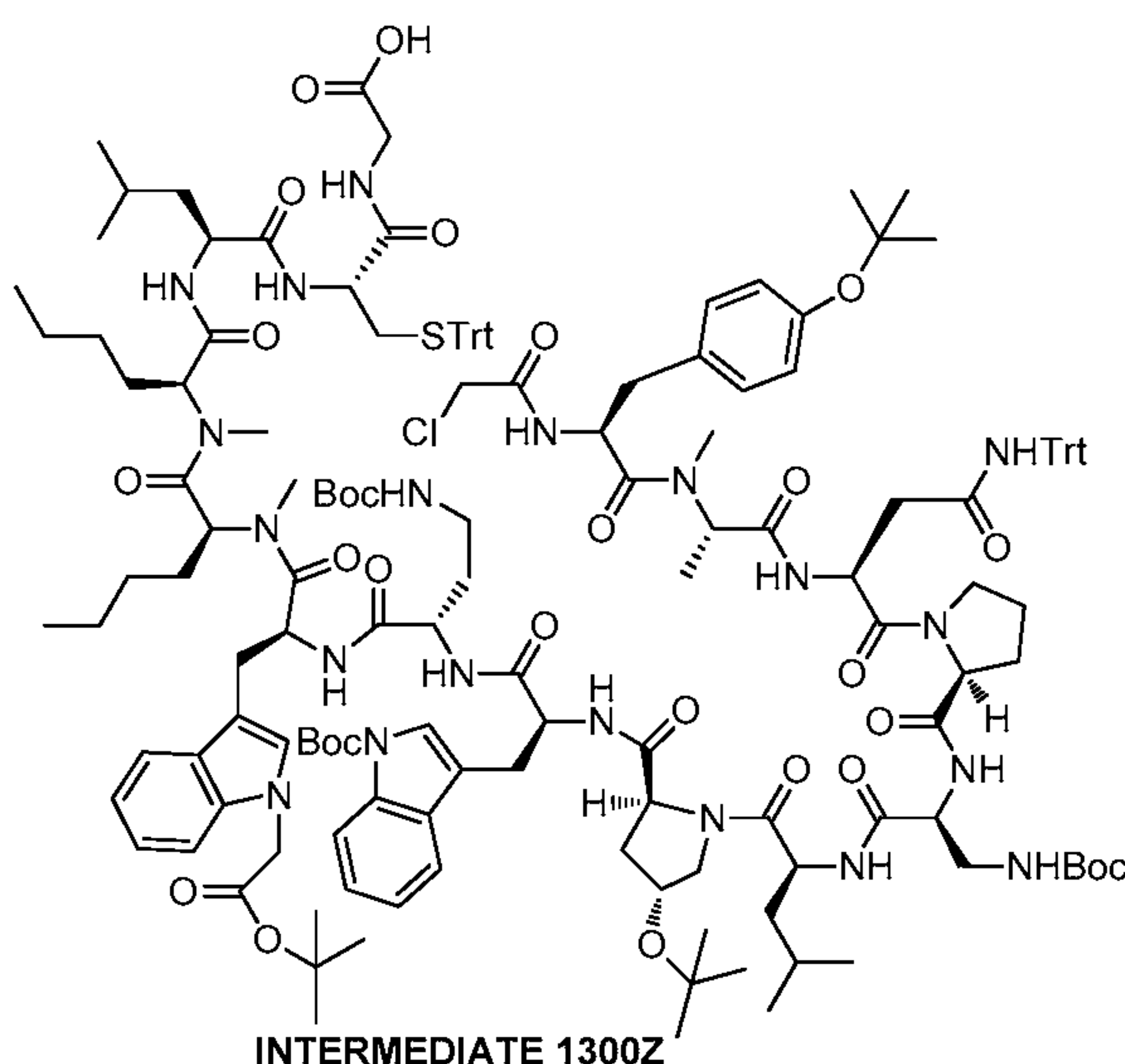
A “deprotection solution” was prepared by combining in a 40 mL glass vial trifluoroacetic acid (22 mL), phenol (1.325 g), water (1.25 mL) and triisopropylsilane (0.5 mL). The resin was removed from the reaction vessel and transferred to a 4 mL glass vial. To the vial was added the “deprotection solution” (2.0 mL). The mixture was vigorously mixed in a shaker (1000 RPM for 1 minute, then 500 RPM for 1.5h). The mixture was filtered through a 0.2 micron syringe filter into a 18X150 mm test tube, and the solids were extracted with a second portion of the “deprotection solution” (1.0 mL). The combined filtrates, in the 18X150 mm test tube, were diluted with Et₂O (15 mL) upon which a significant amount of a white solid precipitated. The mixture was centrifuged for 2 minutes, then the solution was decanted. The solids were suspended in Et₂O (20 mL); the mixture was centrifuged for 5 minutes; and the solution was decanted. For a final time, the solids were suspended in Et₂O (20 mL); the mixture was centrifuged for 5 minutes; and the solution was decanted.

15

Cyclization procedure:

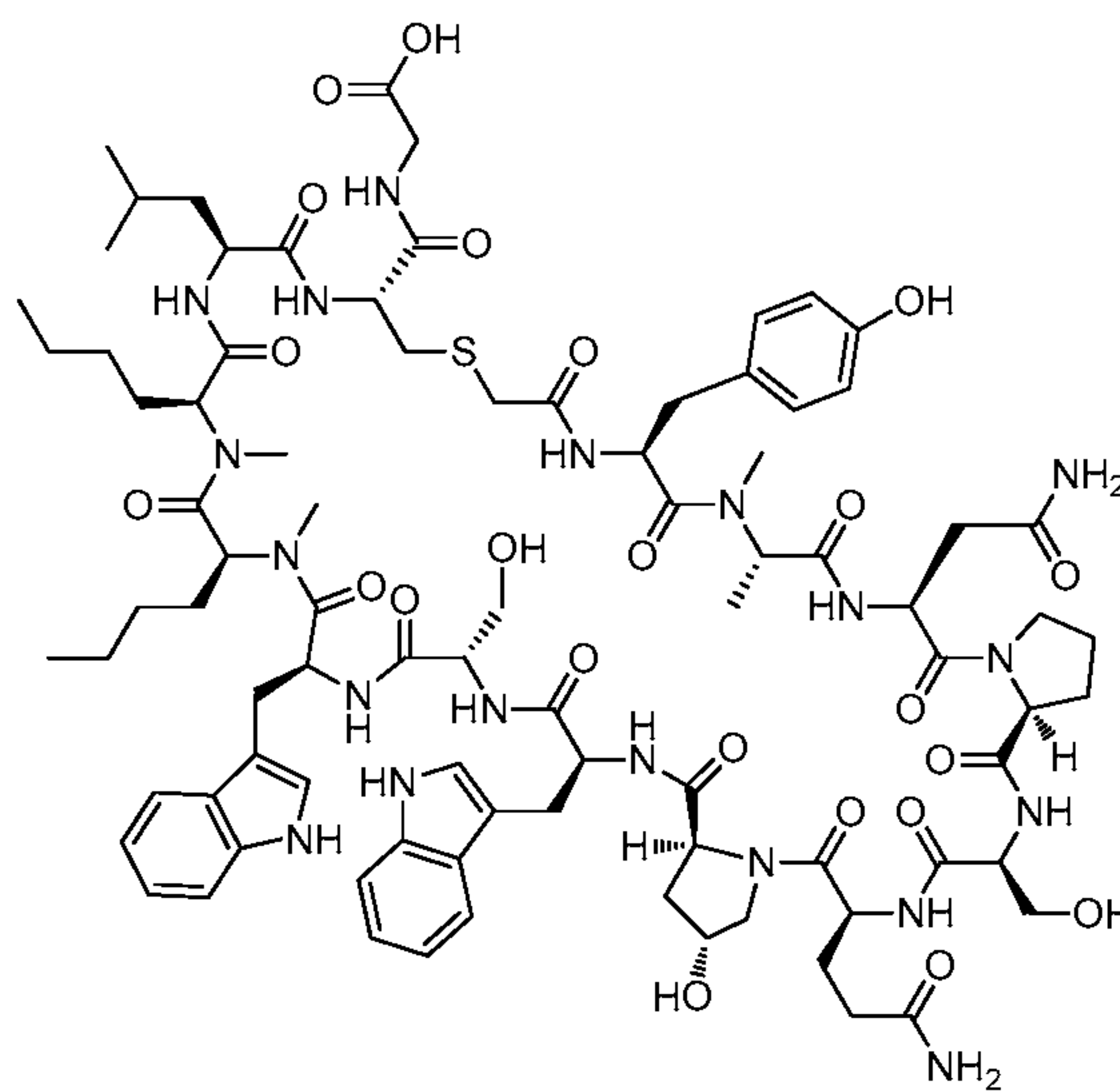
The solids were dissolved in 20 mL MeOH, and the solution was adjusted to pH = 11 using Hunig’s base. The solution was then allowed to stand (stirring not necessary) overnight (app. 18h). Removal of the solvent under rotary evaporation provided the crude product.

20

Preparation of INT-1300Z

The following peptide was synthesized on a .4 mmol scale according to the procedures above. The underlined steps employed the double-coupling procedure, and italicized residues were coupled with a 30 min single coupling. ClAc-**Tyr**-[N-Me]Ala-**Asn**-Pro-Dap-**Leu**-Hyp-Trp-Dab-[(*S*)-2-amino-3-(1-(carboxymethyl)-1*H*-*indol*-3-yl)propanoic acid]-[**N-Me**]**Nle**-[N-Me]Nle-Leu-Cys-Gly; where the Gly was incorporated onto 2-chlorotrityl resin. Cleavage from the resin was accomplished by shaking the resin for 5 min in 20% hexafluoroisopropanol/DCM, followed by filtration. The filtrate was concentrated in vacuo to afford the desired product. HPLC RT = 2.21 min, Column: Waters Aquity UPLC BEH C18 2.1 X 50 mm 1.7-
 10 μ m particles; Mobile Phase A: acetonitrile with 0.05% TFA; Mobile Phase B: water with 0.05% TFA; Gradient: 2-98% B over 1.5 minutes, then a 1.55-minute hold at 98% B; Flow: 0.8 mL/min.

Preparation of INT-130AA



15

The following peptide was synthesized on a .8 mmol scale according to the procedures above. The underlined steps employed the double-coupling procedure. ClAc-**Tyr**-[N-Me]Ala-**Asn**-Pro-Ser-**Gln**-Hyp-Trp-Ser-Trp-[**N-Me**]**Nle**-[N-Me]Nle-Leu-Cys-Gly; where the Gly was incorporated onto 2-chlorotrityl resin. After
 20 deprotection and cyclization according to the procedures above, the compound was purified as follows: The crude material was purified via preparative LC/MS with the following conditions: Column: waters CSH c-18, 19 x 200 mm, 5- μ m particles;

Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-80% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation.

5 The yield of the product was 29.3 mg, and its estimated purity by LCMS analysis was 97%.

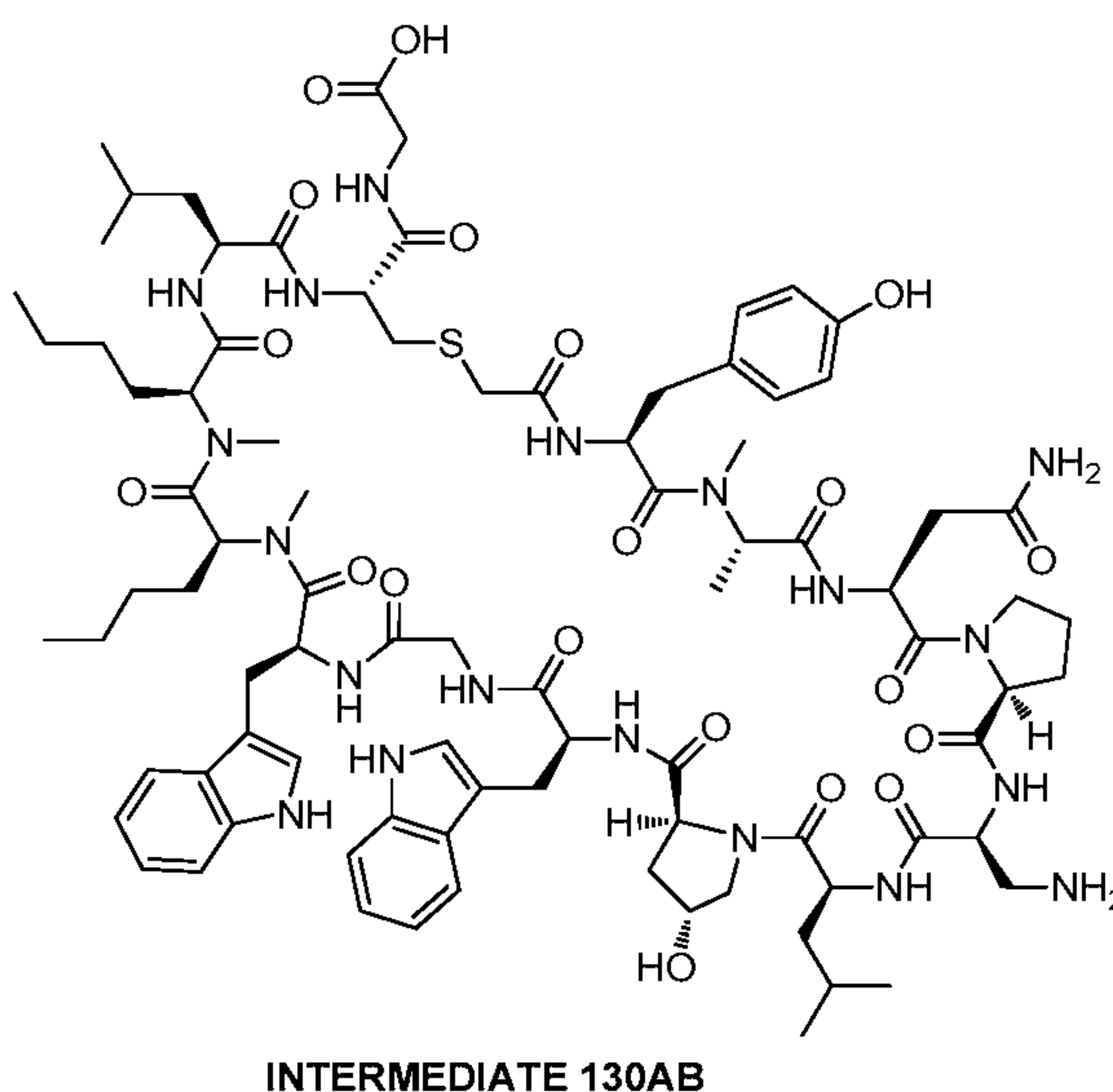
Analysis condition C: Retention time = 1.59 min; ESI-MS(+) m/z 917.4 (M + 2H)

Analysis condition D: Retention time = 1.84 min; ESI-MS(+) m/z 917.4 (M + 2H)

ESI-HRMS(+) m/z : Calculated: 916.9374 (M+2H) Found: 916.9371

10 (M+2H).

Preparation of INT-130AB

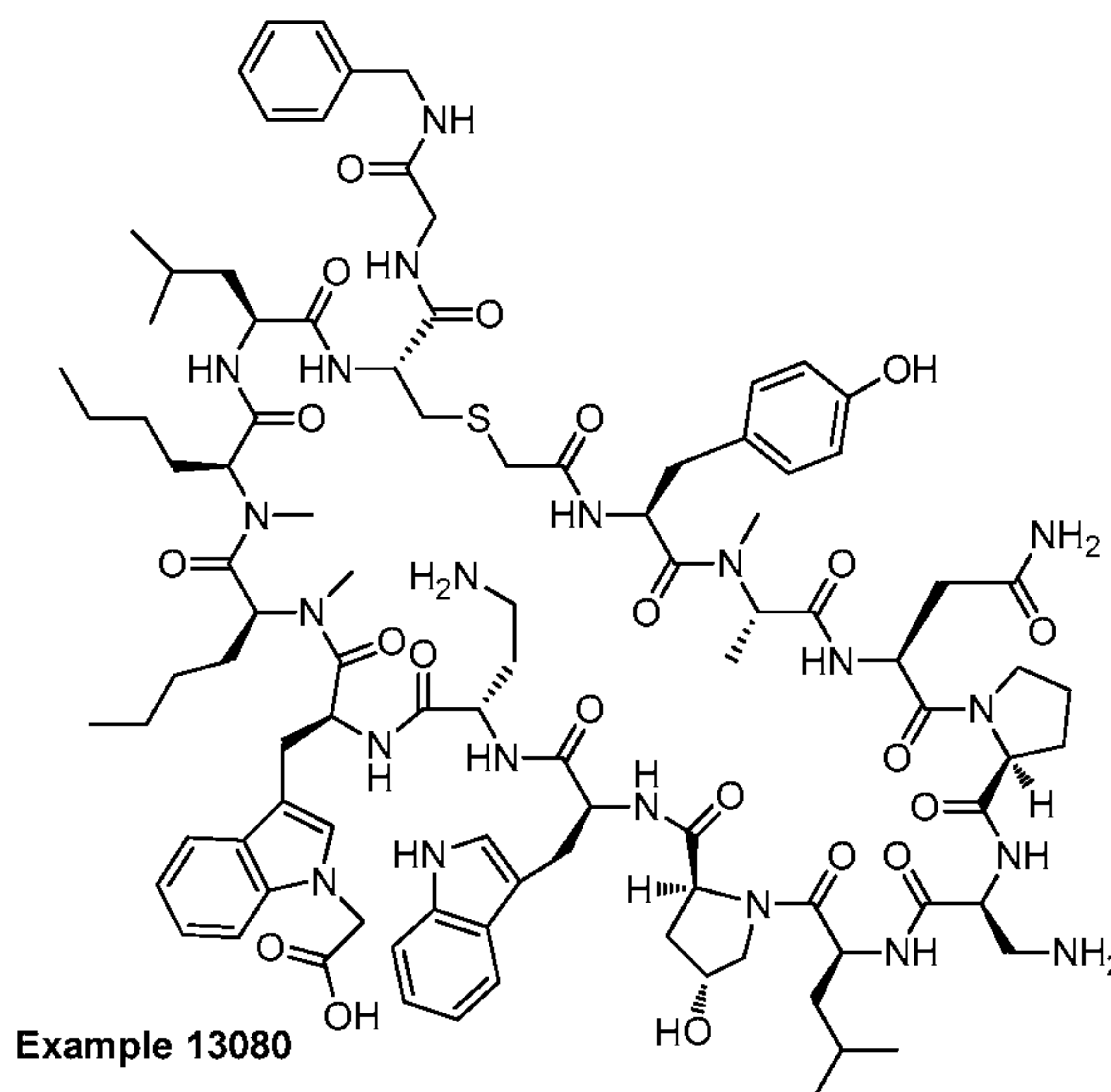


The synthesis was conducted by the attached general method on a 1.400 mmol scale (.1 mmol). Double-coupling (procedure B) was used when a secondary amine at the N-terminus was encountered (denoted with an underlined residue). The sequence was:

ClAc-**Tyr**-mAla-**Asn**-Pro-Dap-**Leu**-Hyp-Trp-Gly-Trp-m**Nle**-mNle-Leu-Cys-[Gly],
The peptide was cleaved from the resin following the TFA/phenol/water/iPr₃SiH cocktail and precipitation/washing in ether procedures described above. The
20 resulting material was cyclized by taking the material up in MeOH (~20 mL) and adding 4-6 drops of Hunig's base (pH ~11). After sitting at rt overnight, the crude

material was obtained by removal of the solvent via rotary evaporation. The material was used without purification for Examples 13082-13089 and 13091-13117.

Preparation of Example 13080



5

Step 1: To a solution of Intermediate 1300Z (61.8 mg, 0.021 mmol) in DMF (537 μ l) was added HATU (12.25 mg, 0.032 mmol), followed by Hunig'sBase (11.25 μ l, 0.064 mmol). Finally, BENZYLAMINE (3.52 μ l, 0.032 mmol) was added, and the resulting solution allowed to stir at rt. Within 3 h, the product had formed as shown by LC/MS. Water was added, and the resulting mixture was filtered to afford the solid tert-butyl 3-((7R,10S,13S,16S,19S,22S,25S)-25-((2S,4R)-4-(tert-butoxy)-1-((S)-2-((S)-2-((S)-1-((S)-2-((S)-2-((S)-3-(4-(tert-butoxy)phenyl)-2-(2-chloroacetamido)-N-methylpropanamido)propanamido)-4-oxo-4-(tritylamino)butanoyl)pyrrolidine-2-carboxamido)-3-((tert-butoxycarbonyl)amino)propanamido)-4-methylpentanoyl)pyrrolidine-2-carboxamido)-19-((1-(2-(tert-butoxy)-2-oxoethyl)-1H-indol-3-yl)methyl)-22-(2-((tert-butoxycarbonyl)amino)ethyl)-13,16-dibutyl-10-isobutyl-14,17-dimethyl-3,6,9,12,15,18,21,24-octaoxo-1-phenyl-7-((tritylthio)methyl)-2,5,8,11,14,17,20,23-octaazahexacosan-26-yl)-1H-indole-1-carboxylate (38.1 mg, 0.013 mmol, 59.8 % yield). The material was taken on as-is.

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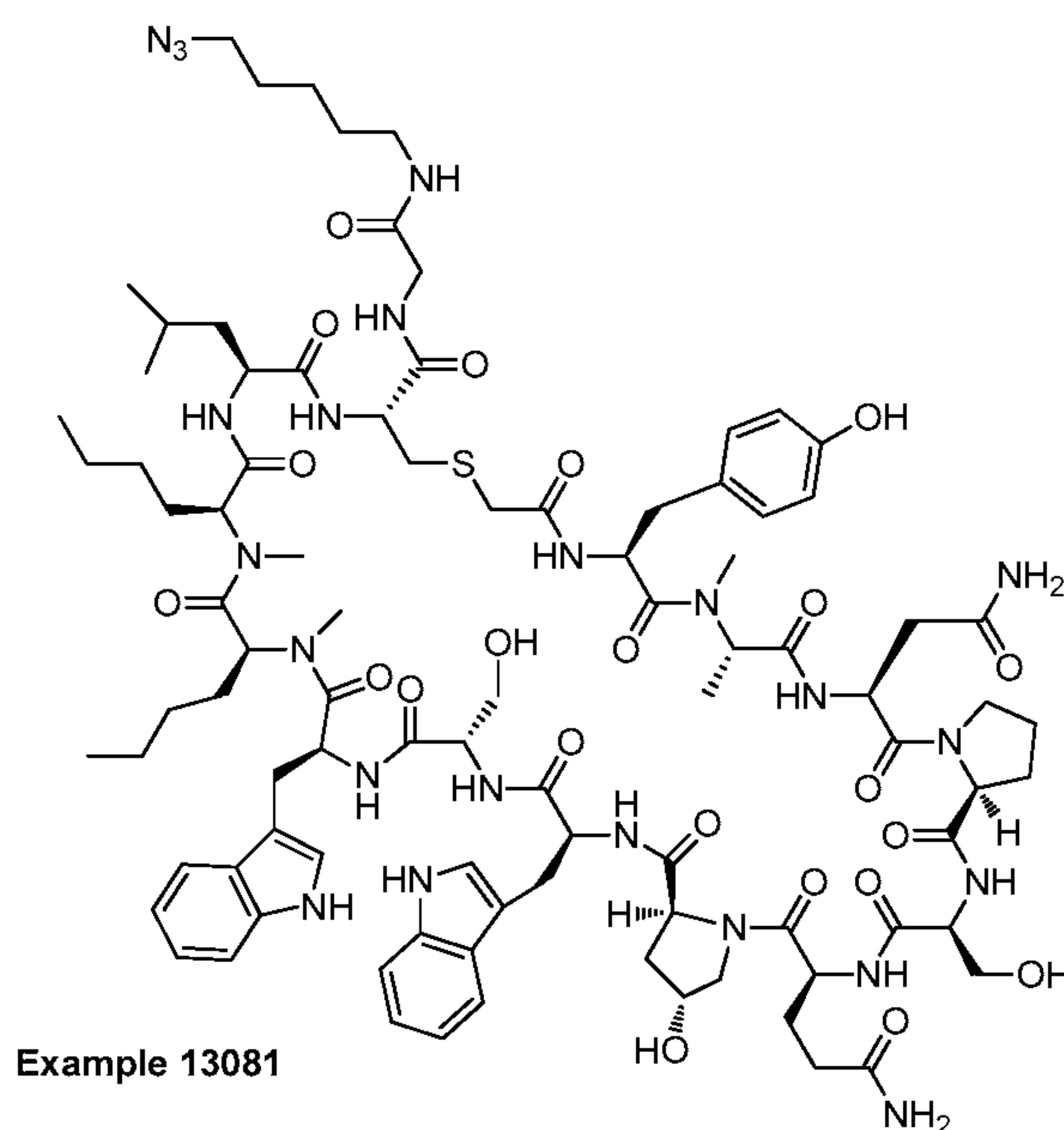
Step 2: To solid tert-butyl 3-((7R,10S,13S,16S,19S,22S,25S)-25-((2S,4R)-4-(tert-butoxy)-1-((S)-2-((S)-2-((S)-1-((S)-2-((S)-2-((S)-3-(4-(tert-butoxy)phenyl)-2-(2-

chloroacetamido)-N-methylpropanamido)propanamido)-4-oxo-4-(tritylamino)butanoyl)pyrrolidine-2-carboxamido)-3-((tert-butoxycarbonyl)amino)propanamido)-4-methylpentanoyl)pyrrolidine-2-carboxamido)-19-((1-(2-(tert-butoxy)-2-oxoethyl)-1H-indol-3-yl)methyl)-22-(2-((tert-butoxycarbonyl)amino)ethyl)-13,16-dibutyl-10-isobutyl-14,17-dimethyl-3,6,9,12,15,18,21,24-octaoxo-1-phenyl-7-((tritylthio)methyl)-2,5,8,11,14,17,20,23-octaazahexacosan-26-yl)-1H-indole-1-carboxylate (38.1 mg, 0.013 mmol) was added a cleavage cocktail (2 mL TFA, 120.4 mg phenol, 45.4 μ L of Et₃SiH and 113.6 μ L of water). The resulting yellow solution was held at rt for 45 min, then diluted with ~20 mL ether. The resulting solid was spun into a pellet on a centrifuge, and the ether was removed by decanting. After an additional ether wash cycle, the solid was dissolved in 1:1 AcCN: ammonium acetate (20 mL), and the pH was adjusted to ~9 with 1M NaOH. The solution was left to stand at rt overnight. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 6.6 mg, and its estimated purity by LCMS analysis was 100%.

Analysis condition C: Retention time = 1.88 min; ESI-MS(+) *m/z* 989.4 (M + 2H)
 Analysis condition D: Retention time = 1.64 min; ESI-MS(+) *m/z* 989.7 (M + 2H)
 ESI-HRMS(+) *m/z*: Calculated: 989.0082 (M+2H) Found: 989.0073 (M+2H).

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Preparation of Example 13081

To a solution of 2-

5 ((6S,9S,12S,18R,21S,24S,27S,30S,33S,36S,38aS,40R,44S,47S,49aS)-30,36-bis((1H-indol-3-yl)methyl)-6-(2-amino-2-oxoethyl)-44-(3-amino-3-oxopropyl)-24,27-dibutyl-40-hydroxy-12-(4-hydroxybenzyl)-33,47-bis(hydroxymethyl)-21-isobutyl-9,10,25,28-tetramethyl-5,8,11,14,20,23,26,29,32,35,38,43,46,49-tetradecaooxooctatetracontahydrodipyrrolo[2,1-g₁:2',1'-x][1,4,7,10,13,16,19,22,25,28,31,34,37,40,43]thiatetradecaazacyclopentatetracontine-10 18-carboxamido)acetic acid (21.2 mg, 0.012 mmol) in DMF (289 μ l) was added HATU (5.72 mg, 0.015 mmol), then a mixture of Hunig'sBase (8.08 μ l, 0.046 mmol) and 5-azidopentan-1-amine (14.82 mg, 0.116 mmol) in DMF. The mixture was stirred at rt. After ~1 h, the product was seen in small amount, with the vast majority of SM remaining. After an additional hour, LC/MS did not change much. More 15 HATU and amine mixture was sequentially added, and the yellow solution stirred at rt overnight. LC/MS showed the consumption of starting material and the formation of the desired material. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 20 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 60-100% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing

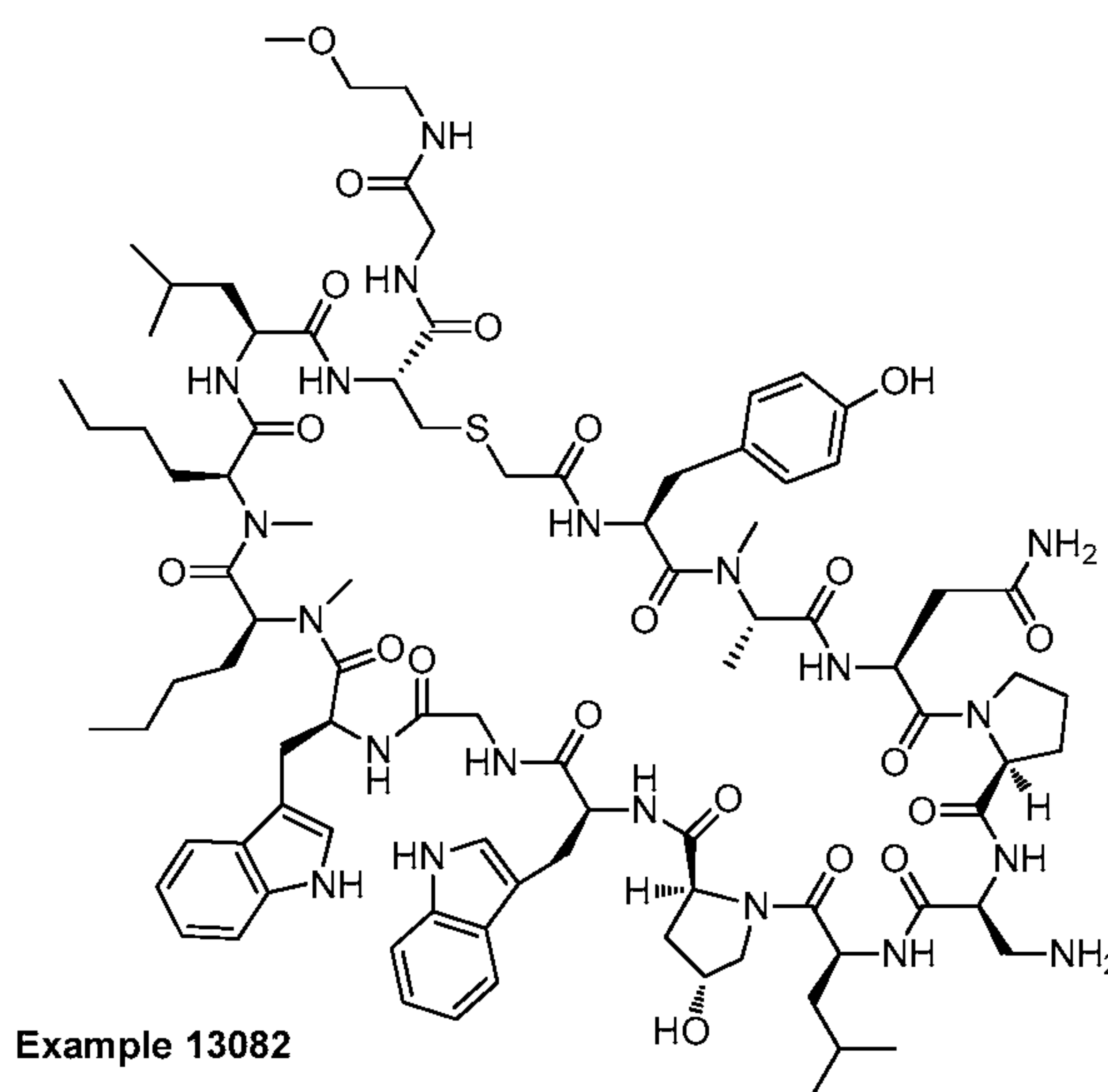
the desired product were combined and dried via centrifugal evaporation. The yield of the product was 8.1 mg, and its estimated purity by LCMS analysis was 99%.

Analysis condition C: Retention time = 2.01 min;

Analysis condition D: Retention time = 2.02 min;

5 ESI-HRMS(+) m/z : Calculated: 971.9853 (M+2H) Found: 971.9856 (M+2H).

Preparation of Example 13082



10 To a solution of crude Intermediate 130AB (16.0 mg, 8.95 μ mol) in DMF (298 μ l) was sequentially added Hunig's Base (9.38 μ l, 0.054 mmol) and 2-methoxyethanamine (15.42 μ l, 0.179 mmol). HATU (10.21 mg, 0.027 mmol) was then added. The mixture was stirred at rt. LC/MS indicated a mixture of SM and product which did not grow further over time. More HATU was added and the

15 mixture stirred overnight, after which LC/MS indicated the consumption of starting material. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a

20 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 3.4 mg, and its estimated purity by LCMS analysis was 96%.

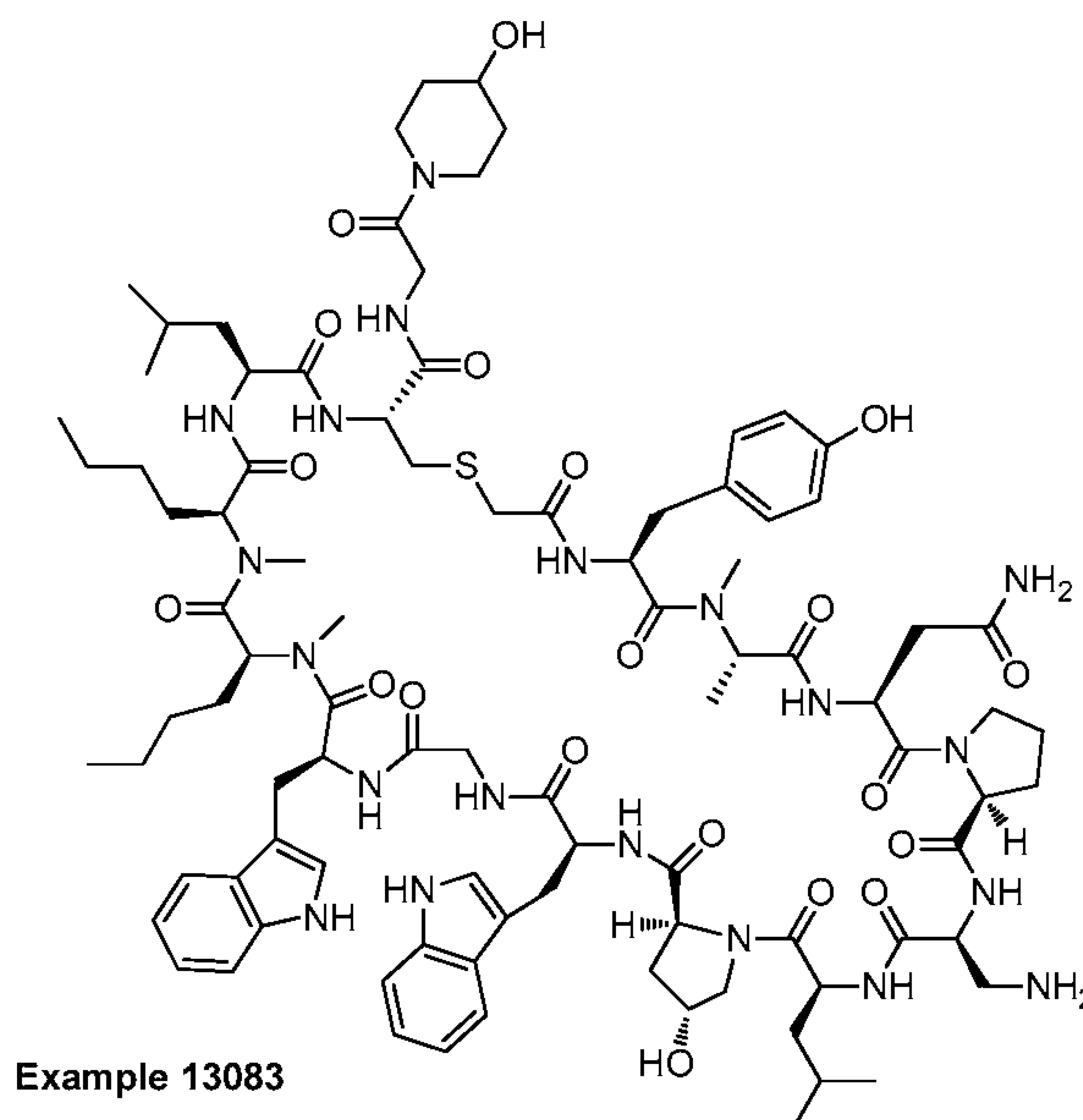
Analysis condition C: Retention time = 1.92 min; ESI-MS(+) m/z 922.8 (M + 2H)

Analysis condition D: Retention time = 1.76 min; ESI-MS(+) m/z 922.9 (M + 2H)

ESI-HRMS(+) m/z : Calculated: 922.4818 (M+2H) Found: 922.4807 (M+2H).

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Preparation of Example 13083



Example 13083

To a solution of crude Intermediate 130AB (49 mg, 0.027 mmol) in DMF (548 μ l) was sequentially added Hunig'sBase (28.7 μ l, 0.165 mmol) and piperidin-4-ol (55.5 mg, 0.548 mmol). HATU (11.47 mg, 0.030 mmol) was then added. The mixture was stirred at rt. LC/MS showed overlapping SM and product, with the major mass peaks in ~1:1 ratio. An additional 0.5 eq HATU was added, and the reaction was allowed to proceed an additional hour. The desired mass was then the major component, but some SM as well as the guanidine adduct peaks was visible. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.6 mg, and its estimated purity by LCMS analysis was 95%.

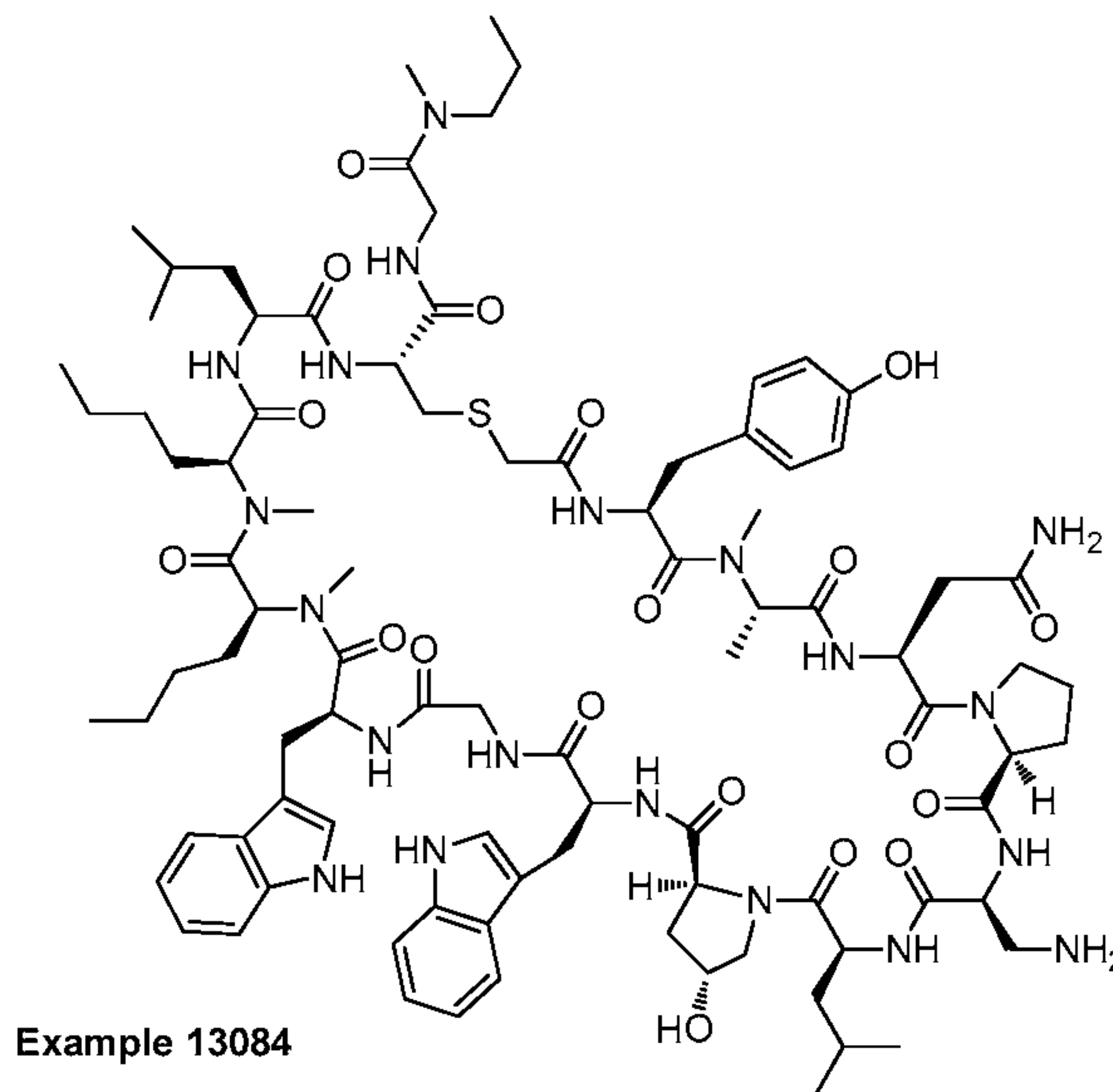
Analysis condition C: Retention time = 1.85 min;

Analysis condition D: Retention time = 1.76 min; ESI-MS(+) m/z 936.2 (M + 2H)

ESI-HRMS(+) m/z : Calculated: 935.4896 (M+2H) Found: 935.4882 (M+2H).

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Preparation of Example 13084

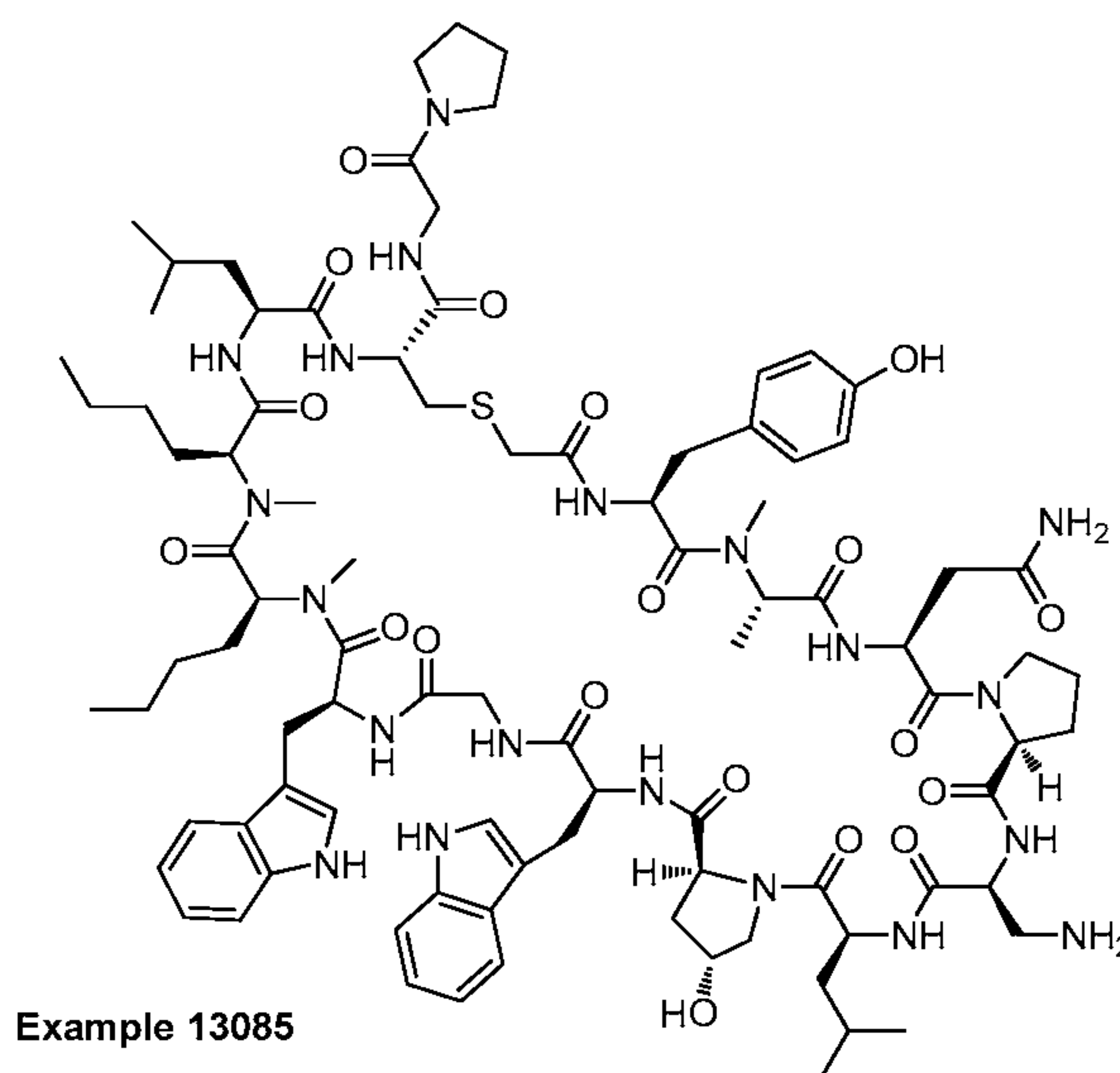


To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and N-methylpropan-1-amine (40.9 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis condition B: Retention time = 2.89 min; ESI-MS(+) m/z 921.8 (M + 2H)

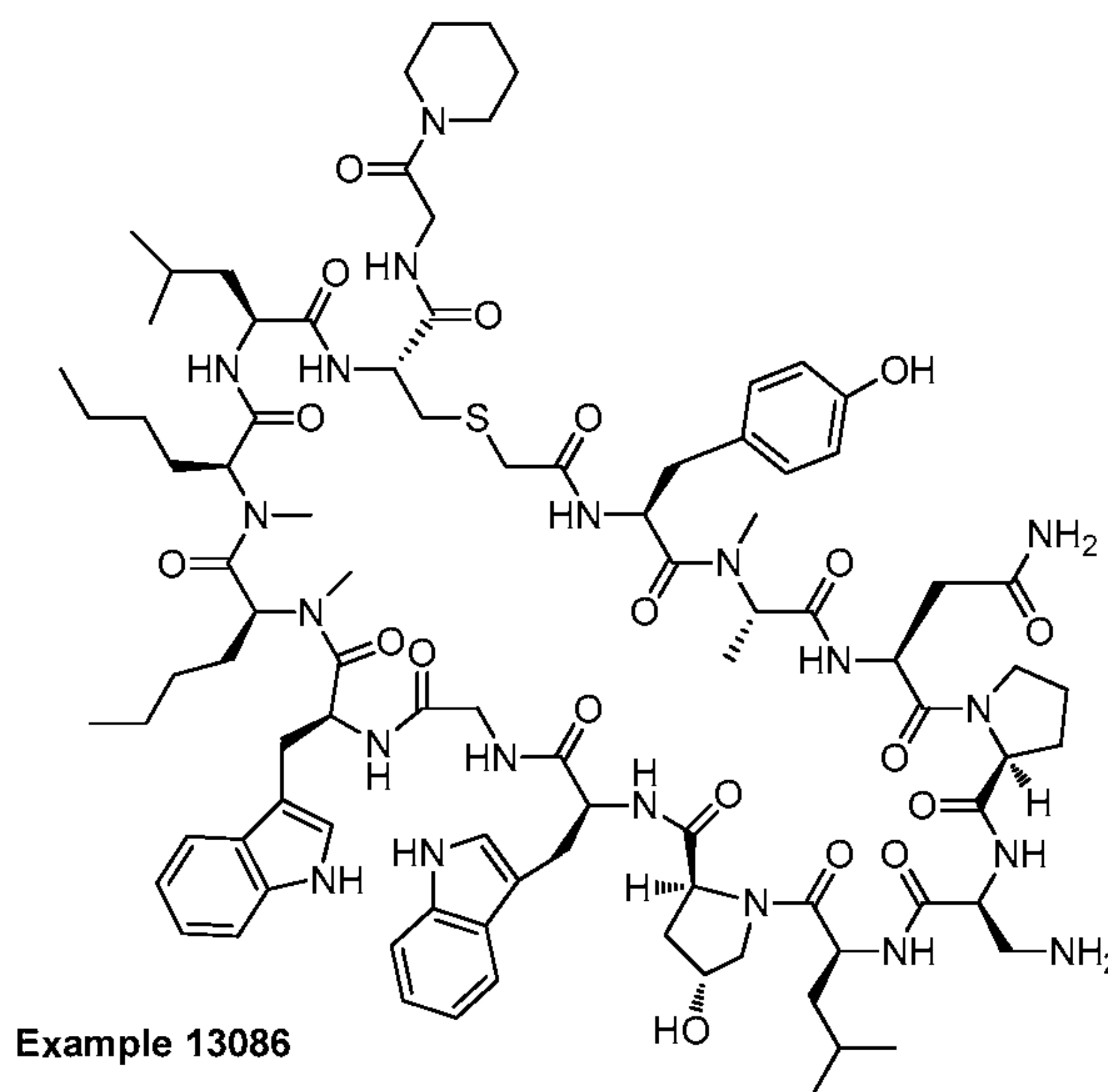
Analysis condition C: Retention time = 1.90 min; ESI-MS(+) m/z 922.0 (M + 2H)

ESI-HRMS(+) m/z : Calculated: 921.4922 (M+2H) Found: 921.4910 (M+2H).

Preparation of Example 13085

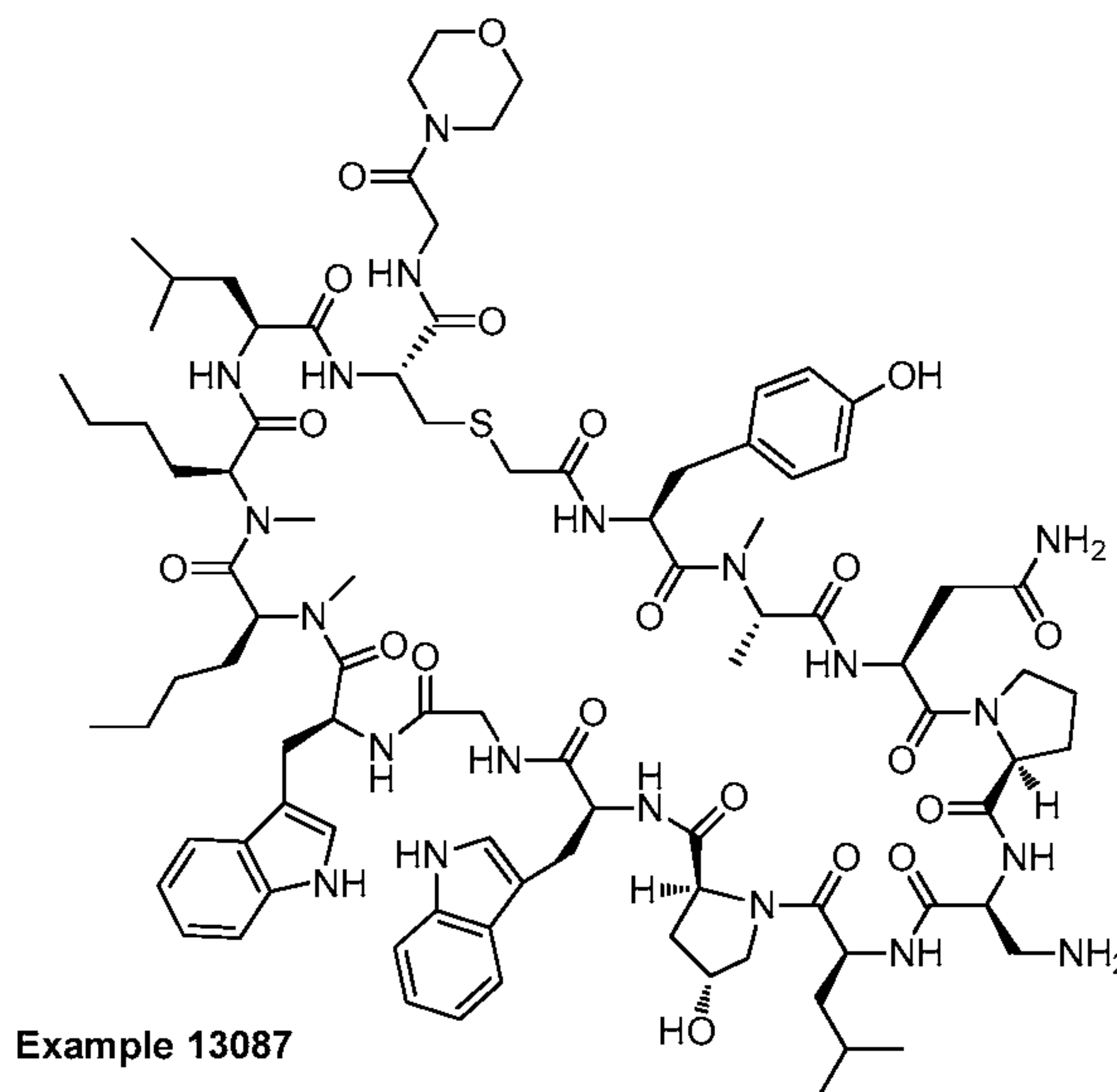
To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and pyrrolidine (39.8 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.2 mg, and its estimated purity by LCMS analysis was 96%.

Analysis condition B: Retention time = 2.81 min; ESI-MS(+) m/z 921.1 (M + 2H)
 Analysis condition C: Retention time = 1.76 min; ESI-MS(+) m/z 920.8 (M + 2H)
 ESI-HRMS(+) m/z : Calculated: 920.4844 (M+2H) Found: 920.4833 (M+2H).

Preparation of Example 13086

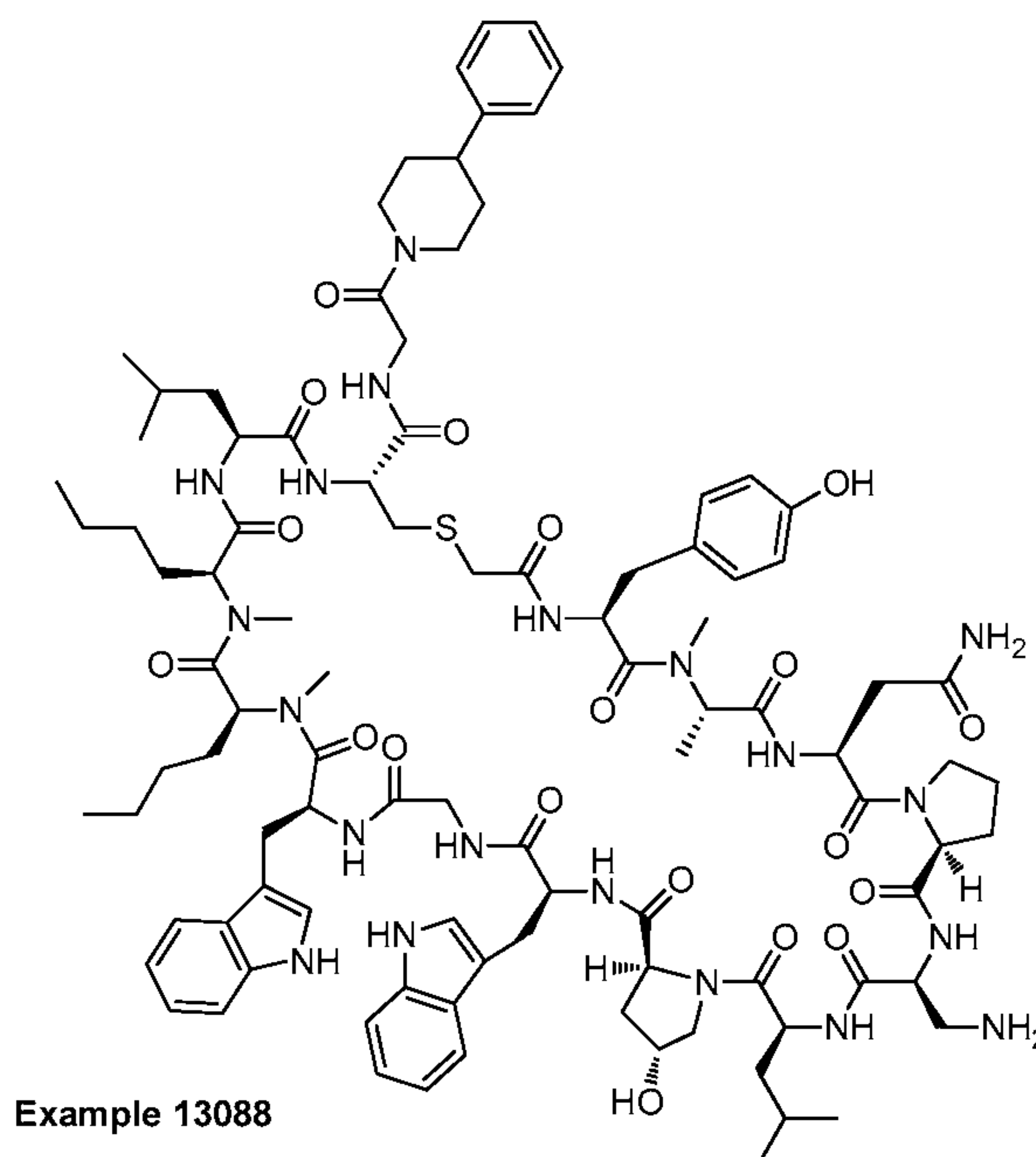
To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and piperidine (47.6 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis condition C: Retention time = 2.01 min; ESI-MS(+) m/z 928.0 (M + 2H)
 Analysis condition D: Retention time = 1.72 min; ESI-MS(+) m/z 927.9 (M + 2H)
 ESI-HRMS(+) m/z : Calculated: 927.4922 (M+2H) Found: 927.4905 (M+2H).

Preparation of Example 13087

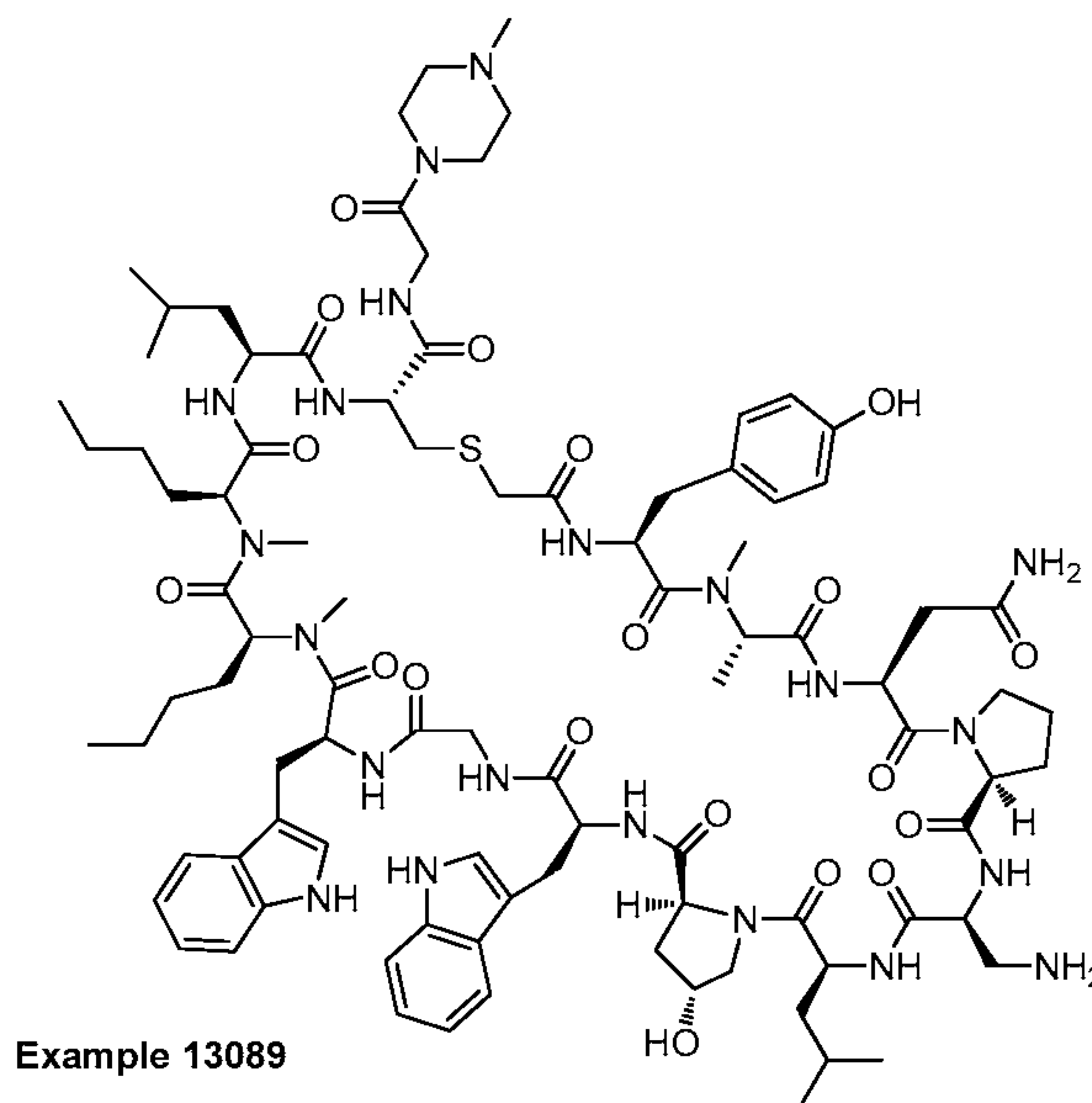
To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and morpholine (48.7 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 5.3 mg, and its estimated purity by LCMS analysis was 95%.

Analysis condition C: Retention time = 1.81 min; ESI-MS(+) m/z 929.3 (M + 2H)
 Analysis condition D: Retention time = 1.66 min; ESI-MS(+) m/z 929.3 (M + 2H)
 ESI-HRMS(+) m/z : Calculated: 928.4818 (M+2H) Found: 928.4807 (M+2H).

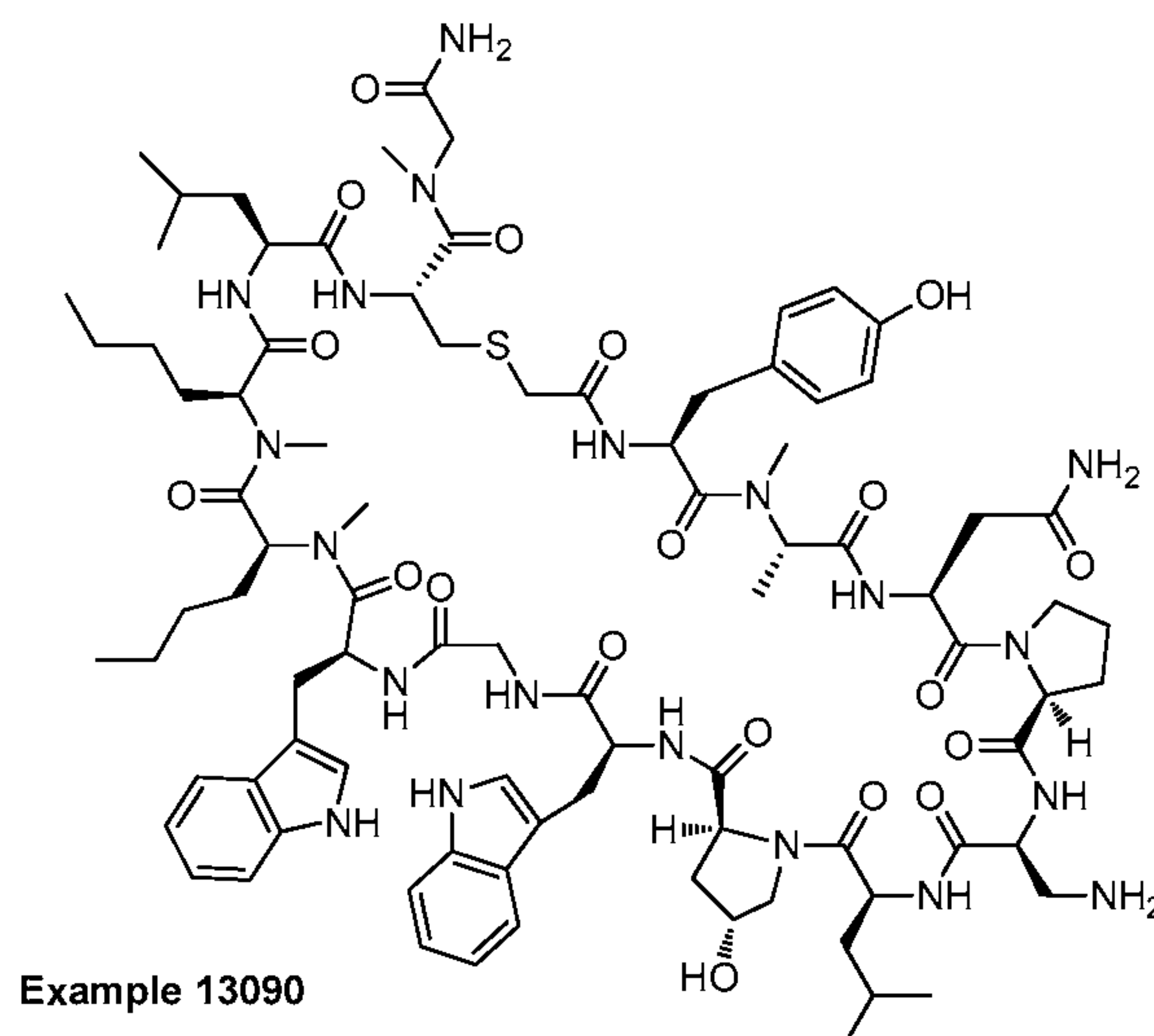
Preparation of Example 13088

To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and 4-phenylpiperidine (90 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 55-95% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 3.1 mg, and its estimated purity by LCMS analysis was 100%.

Analysis condition C: Retention time = 2.14 min; ESI-MS(+) m/z 966.1 (M + 2H)
 Analysis condition D: Retention time = 1.89 min; ESI-MS(+) m/z 966.0 (M + 2H)
 ESI-HRMS(+) m/z : Calculated: 965.5078 (M+2H) Found: 965.5070 (M+2H).

Preparation of Example 13089

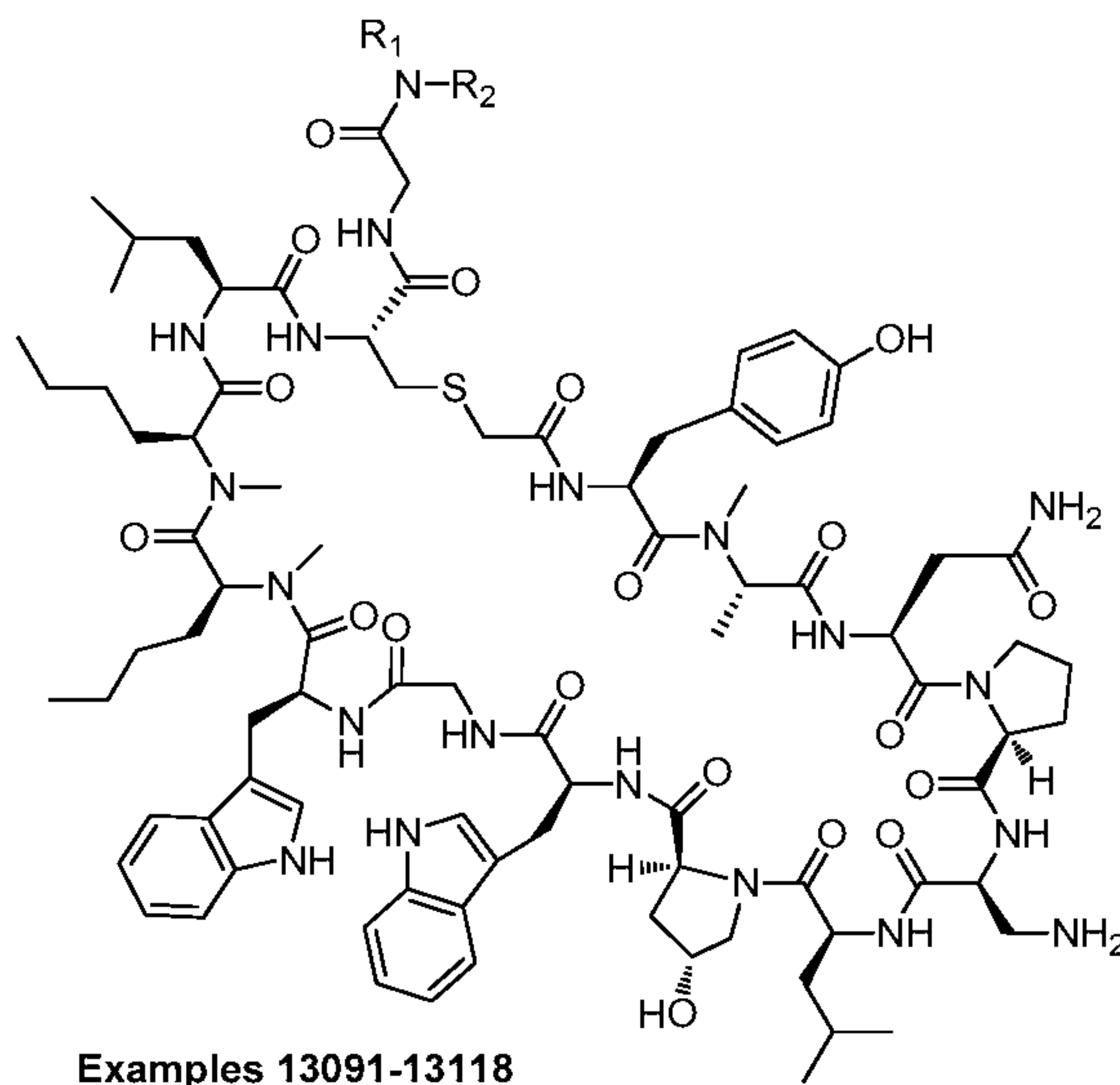
- To a solution of crude Intermediate 130AB (50 mg, 0.028 mmol) in DMF
- 5 (933 μ l) was sequentially added Hunig'sBase (29.3 μ l, 0.168 mmol) and methylpiperazine (56 mg, 0.560 mmol). HATU (11.70 mg, 0.031 mmol) was then added. The mixture was stirred at rt. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate;
- 10 Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 4.9 mg, and its estimated purity by LCMS analysis was 96%.
- 15 Analysis condition C: Retention time = 1.79 min; ESI-MS(+) m/z 935.6 (M + 2H)
 Analysis condition D: Retention time = 1.38 min; ESI-MS(+) m/z 935.6 (M + 2H)
 ESI-HRMS(+) m/z : Calculated: 934.9976 (M+2H) Found: 934.9962 (M+2H).

Preparation of Example 13090

The synthesis was conducted by the attached general method on a 0.20 mmol scale (2 X 0.1 mmol). Double-coupling (procedure B) was used when a secondary amine at the N-terminus was encountered (denoted with an underlined residue). The sequence was: ClAc-**Tyr**-mAla-**Asn**-Pro-Dap-**Leu**-Hyp-Trp-Gly-Trp-**mNle**-mNle-Leu-**Cys**-Sarc. The peptide was cleaved from the Rink resin following the TFA/phenol/water/iPr₃SiH cocktail and precipitation/washing in ether procedures described above. The resulting material was cyclized by taking the material up in MeOH (~20 mL) and adding 4-6 drops of Hunig's base (pH ~11). After sitting at rt overnight, the crude material was obtained by removal of the solvent via rotary evaporation. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 36.4 mg, and its estimated purity by LCMS analysis was 95%.

Analysis condition C: Retention time = 1.69 min; ESI-MS(+) *m/z* 900.6 (M + 2H)

Analysis condition D: Retention time = 1.54 min; ESI-MS(+) *m/z* 900.9 (M + 2H).

Preparation of Examples 13091-13118

A solution of Intermediate 130AB (1.1 gm, 592 μmol) and DIPEA (629 μL , 3.6 mmol) in DMF (9.25) mL was allowed to shake at room temp for 15 minutes. A solution of HATU (248 mg, 651 μmol) in DMF (9.25 mL) was also prepared. To each of the amines (15 eq) weighed into 16x48 mm threaded vials was added 250 μL of the Intermediate 130AB / DIPEA solution and 250 μL of the HATU solution. The vials were capped and allowed to shake at room temp. After overnight, LC/MS indicated an incomplete reaction, and another 250 μL aliquot of HATU solution was added. After an additional day, a final aliquot of HATU solution was added.

Purification of Example 13091:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.7 mg, and its estimated purity by LCMS analysis was 96%.

Purification of Example 13092:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μm particles; Mobile Phase A:

5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.7 mg, and its estimated purity by LCMS analysis was 95%.

Purification of Example 13093:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.2 mg, and its estimated purity by LCMS analysis was 94%.

Purification of Example 13094:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.8 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13095:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 30

minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.5 mg, and its estimated purity by LCMS analysis was 91%.

Purification of Example 13096:

5 The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 3-minute hold at 100% B; Flow: 20 mL/min. Fractions
10 containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.1 mg, and its estimated purity by LCMS analysis was 96%.

Purification of Example 13097:

 The crude material was purified via preparative LC/MS with the following
15 conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 3-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield
20 of the product was 2.3 mg, and its estimated purity by LCMS analysis was 96%.

Purification of Example 13098:

 The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5
25 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.7 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13099:

30 The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5

acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 3-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.4 mg, and its estimated purity by LCMS analysis was 94%.

5 *Purification of Example 13100:*

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30
10 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.1 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13101:

The crude material was purified via preparative LC/MS with the following
15 conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield
20 of the product was 2.0 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13102:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5
25 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95
30 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product

were combined and dried via centrifugal evaporation. The yield of the product was 0.1 mg, and its estimated purity by LCMS analysis was 94%.

Purification of Example 13103:

The crude material was purified via preparative LC/MS with the following
5 conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The
10 material was further purified via preparative LC/MS with the following conditions: Column: Waters CSH C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the
15 desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.1 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13104:

The crude material was purified via preparative LC/MS with the following
conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A:
20 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.4 mg, and its estimated purity by LCMS analysis was 100%.

25 *Purification of Example 13105:*

The crude material was purified via preparative LC/MS with the following
conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A:
5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5
methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30
30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.9 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13106:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-100% B over 30 minutes, then a 3-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.7 mg, and its estimated purity by LCMS analysis was 90%.

Purification of Example 13107:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 30 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.6 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13108:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 25-65% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.9 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13109:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 25-65% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.0 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13110:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.7 mg, and its estimated purity by LCMS analysis was 90%.

Purification of Example 13111:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.6 mg, and its estimated purity by LCMS analysis was 96%.

Purification of Example 13112:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 4-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.6 mg, and its estimated purity by LCMS analysis was 100%.

Purification of Example 13113:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 25-65% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.8 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13114:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.8 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13115:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 25-65% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 0.5 mg, and its estimated purity by LCMS analysis was 96%.

Purification of Example 13116:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.6 mg, and its estimated purity by LCMS analysis was 92%.

Purification of Example 13117:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 2.0 mg, and its estimated purity by LCMS analysis was 96%.

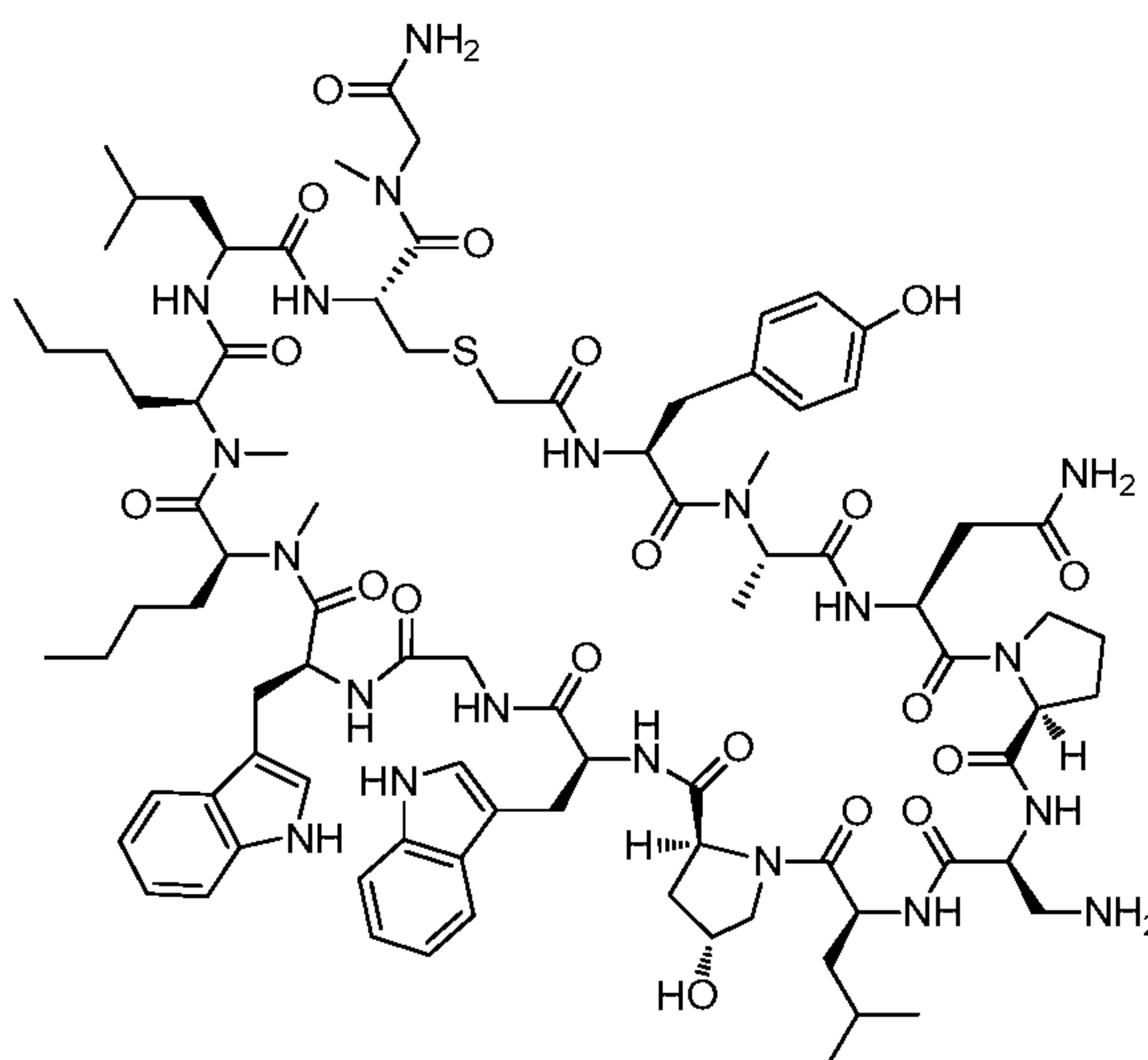
Purification of Example 13118:

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5- μ m particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5
5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 4.3 mg, and its estimated purity by LCMS analysis was 100%.

Analysis conditions, retention times, and ESI-MS(+) m/z (M + 2H) for
10 Examples 13091-13118:

Example Number	Amine used	analysis C rt (min)	m/z observed	analysis D rt (min)	m/z observed
13091	CYCLOPROPYLAMINE	1.82	914.1	1.59	914.0
13092	CYCLOBUTYLAMINE	1.90	921.2	1.66	921.1
13093	N-(3-AMINOPROPYL)MORPHOLINE	1.69	957.2	1.34	957.1
13094	3-(AMINOMETHYL)PYRIDINE	1.78	939.1	1.34	940.0
13095	ETHANOLAMINE	1.72	916.0		
13096	PROPARGYLAMINE	1.81	913.0	1.58	912.9
13097	ALLYLAMINE	1.83	913.9	1.61	914.1
13098	N,N-DIMETHYL-1,3-PROPANEDIAMINE	1.64	938.1	1.32	937.0
13099	1-(3-AMINOPROPYL)IMIDAZOLE	1.73	947.9	1.33	947.9
13100	BUTYLAMINE	1.86	922.2	Condition B rt = 2.91 min	922.0
13101	2-(PHENYLTHIO)ETHANAMINE	2.02	962.1	1.77	961.7
13102	N,N,N'-TRIMETHYL-1,3-PROPANEDIAMINE	1.65	943.3	1.36	943.7
13103	4-(DIMETHYLAMINO)PIPERIDINE	1.65	949.1	1.36	949.3
13104	CYCLOPROPYLMETHYLAMINE	1.86	920.9	1.69	920.7
13105	METHYL 3-AMINOPROPIONATE HYDROCHLORIDE	1.79	936.7	1.59	937.2
13106	4-AMINOCYCLOHEXANOL	1.72	942.6	1.53	942.9

Example Number	Amine used	analysis C rt (min)	m/z observed	analysis D rt (min)	m/z observed
13107	N-(3-AMINOPROPYL)DIETHANOLAMINE	1.64	966.1	1.33	966.5
13108	2-PHENOXYETHYLAMINE	1.96	954.2	1.73	954.0
13109	TERT-BUTYL 3-AMINOPROPANOATE HYDROCHLORIDE	1.96	958.8	1.73	958.3
13110	3-AMINOPROPANAMIDE HYDROCHLORIDE	1.68	929.9	1.50	929.6
13111	4-PIPERIDINEMETHANOL	1.76	943.0	1.57	943.2
13112	N-BOC-1,5-DIAMINOPENTANE	2.00	986.3	1.76	986.3
13113	N-(2-AMINOETHYL)-N-METHYL CARBAMIC ACID TERT-BUTYL ESTER	1.97	972.2	1.74	972.9
13114	PIPERAZIN-2-ONE	1.69	935.2	1.51	935.2
13115	4-(4-CHLOROPHENYL)PIPERIDINE	2.20	982.7	1.93	982.9
13116	2-AMINOETHYLMETHYLSULFONE HYDROCHLORIDE	1.72	947.1	1.53	947.4
13117	PHENYLPIPERAZINE	2.01	966.2	1.69	966.2
13118	BETA-ALANINE ETHYL ESTER	1.83	944.1	1.62	944.1

Preparation of Example 13119**Example 13119**

The synthesis was conducted by the attached general method using .2 mmol Rink resin. Double-coupling (procedure B) was used when a secondary amine at the N-terminus was encountered (denoted with an underlined residue). The sequence was: ClAc-**Tyr**-mAla-**Asn**-Pro-Dap-**Leu**-Hyp-Trp-Gly-Trp-m**Nle**-mNle-Leu-Cys-Sar. The peptide was cleaved from the resin following the TFA/phenol/water/iPr₃SiH cocktail and precipitation/washing in ether procedures described above. The resulting material was cyclized by taking the material up in MeOH (~20 mL) and adding 4-6 drops of Hunig's base (pH ~11). After sitting at rt overnight, the crude material was obtained by removal of the solvent via rotary evaporation. The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 36.4 mg, and its estimated purity by LCMS analysis was 95%. Analysis condition C: Retention time = 1.69 min; ESI-MS(+) *m/z* 900.6 (M + 2H) Analysis condition D: Retention time = 1.54 min; ESI-MS(+) *m/z* 900.9 (M + 2H) ESI-HRMS(+) *m/z*: Calculated: 900.4687 (M+2H) Found: 900.4671 (M+2H).

METHODS FOR TESTING THE ABILITY OF MACROCYCLIC PEPTIDES TO COMPETE FOR THE BINDING OF PD-1 TO PD-L1 USING HOMOGENOUS TIME-RESOLVED FLUORESCENCE (HTRF) BINDING ASSAYS

5 The ability of the macrocyclic peptides of the present disclosure to bind to PD-L1 was investigated using a PD-1/PD-L1 Homogenous Time-Resolved Fluorescence (HTRF) binding assay.

Methods

 Homogenous Time-Resolved Fluorescence (HTRF) Assays of Binding of
 10 Soluble PD-1 to Soluble PD-L1. Soluble PD-1 and soluble PD-L1 refers to proteins with carboxyl-end truncations that remove the transmembrane-spanning regions and are fused to heterologous sequences, specifically the Fc portion of the human immunoglobulin G sequence (Ig) or the hexahistidine epitope tag (His). All binding studies were performed in an HTRF assay buffer consisting of dPBS supplemented
 15 with 0.1% (w/v) bovine serum albumin and 0.05% (v/v) Tween-20. For the PD-1-Ig/PD-L1-His binding assay, inhibitors were pre-incubated with PD-L1-His (10 nM final) for 15m in 4 µl of assay buffer, followed by addition of PD-1-Ig (20 nM final) in 1 µl of assay buffer and further incubation for 15m. PD-L1 fusion proteins from either human, cynomolgous macaques, mouse, or other species were used. HTRF
 20 detection was achieved using europium cryptate-labeled anti-Ig monoclonal antibody (1 nM final) and allophycocyanin (APC) labeled anti-His monoclonal antibody (20 nM final). Antibodies were diluted in HTRF detection buffer and 5 µl was dispensed on top of binding reaction. The reaction was allowed to equilibrate for 30 minutes and signal (665nm/620nm ratio) was obtained using an EnVision fluorometer.

25 Additional binding assays were established between PD-1-Ig/PD-L2-His (20 and 5 nM, respectively), CD80-His/PD-L1-Ig (100 and 10 nM, respectively) and CD80-His/CTLA4-Ig (10 and 5 nM, respectively). Binding/competition studies between biotinylated Compound No. 71 and human PD-L1-His were performed as follows. Macrocyclic peptide inhibitors were pre-incubated with PD-L1-His (10 nM final) for
 30 60 minutes in 4 µl of assay buffer followed by addition of biotinylated Compound No. 71 (0.5 nM final) in 1 µl of assay buffer. Binding was allowed to equilibrate for 30 minutes followed by addition of europium cryptate labeled Streptavidin (2.5 pM

final) and APC-labeled anti-His (20 nM final) in 5 µl of HTRF buffer. The reaction was allowed to equilibrate for 30m and signal (665nm/620nm ratio) was obtained using an EnVision fluorometer.

Recombinant Proteins. Carboxyl-truncated human PD-1 (amino acids 25-167) with a C-terminal human Ig epitope tag [hPD-1 (25-167)-3S-IG] and human PD-L1 (amino acids 18-239) with a C-terminal His epitope tag [hPD-L1(19-239)-tobacco vein mottling virus protease cleavage site (TVMV)-His] were expressed in HEK293T cells and purified sequentially by recombinant Protein A affinity chromatography and size exclusion chromatography. Human PD-L2-His (Sino Biologicals), CD80-His (Sino Biologicals), CTLA4-Ig (RnD Systems) were all obtained through commercial sources.

Sequence of Recombinant Human PD-1-Ig

hPD1(25-167)-3S-IG					
1	LDSPDRFWNF	PTPSPALLVV	TEGDNATFTC	SPENTSESFV	LNWYRMSPSN
51	QTDKLAAPFE	DRSQFGQDCR	FRVTQLPNGR	DFHMSVVRAR	RNDGGTYLGG
101	AISLAPKAQT	KESLRAELEV	TERRAEVPTA	HPSPSPRPAG	QFCGSPGGGG
151	GEEPKSSDKT	HTSPFPSPAF	LLOGSSVFLF	PPKPKDTLMI	SRTPEVTCVV
201	VDVSHEDPEV	KFNWYVDGVE	VHNKTKPRE	EQYNSTYEVV	SVLTVLHQDW
251	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SEDELTKNQV
301	SLTCLVKGPY	PSDIAVENES	NGQPENNYKT	TPPVLDSDGS	PFLYSKLTVD
351	KSRWQQGNVF	SCSVMEALH	NHYTQKSLSL	SPGK	

(SEQ ID NO:1)

15

Sequence of Recombinant Human PD-L1-TVMV-His (PD-L1-His)

hPD1(19-239)-TVMV-His					
1	FTVTVPKDLY	VVEYGSNMTI	ECKFPVEKQL	DLAALIVYWE	MEDKNIIQFV
51	HGEEDLKVQH	SSYRQARLL	KDQLSLGNAA	LOITDVKLQD	AGVYRCMISY
101	GGADYKRITV	KVNAPYNKIN	QRILVVDPVT	SEHELTCQAE	GYPKAEVIWT
151	SSDHQVLGSK	TTTTNSKEEE	KLPNVTSTLE	INTTTNEIFY	CTFRRLDPFE
201	NHTAELVIPE	LPLAHPPNER	TGSSETVRPQ	GHHHHHH	

(SEQ ID NO:2)

The results are shown in Table 1. As shown, the macrocyclic peptides of the present disclosure demonstrated potent inhibition of PD-1-Ig binding activity to PD-L1-TVMV-His (PD-L1-His). Ranges are as follows: A = 0.01-0.06 µM; B = 0.004 – 0.0099 µM.

Table 1

<i>Example Number</i>	<i>HTRF IC₅₀ (μM)</i>
Example 13080	B
Example 13081	A
Example 13082	A
Example 13083	---
Example 13084	A
Example 13085	A
Example 13086	B
Example 13087	A
Example 13088	0.05
Example 13089	A
Example 13090	B
Example 13091	B
Example 13092	A
Example 13093	8.17E-03
Example 13094	A
Example 13095	A
Example 13096	A
Example 13097	A
Example 13098	A
Example 13099	A
Example 13100	A
Example 13101	A
Example 13102	---
Example 13103	A
Example 13104	A
Example 13105	0.01
Example 13006	A
Example 13007	A
Example 13008	A

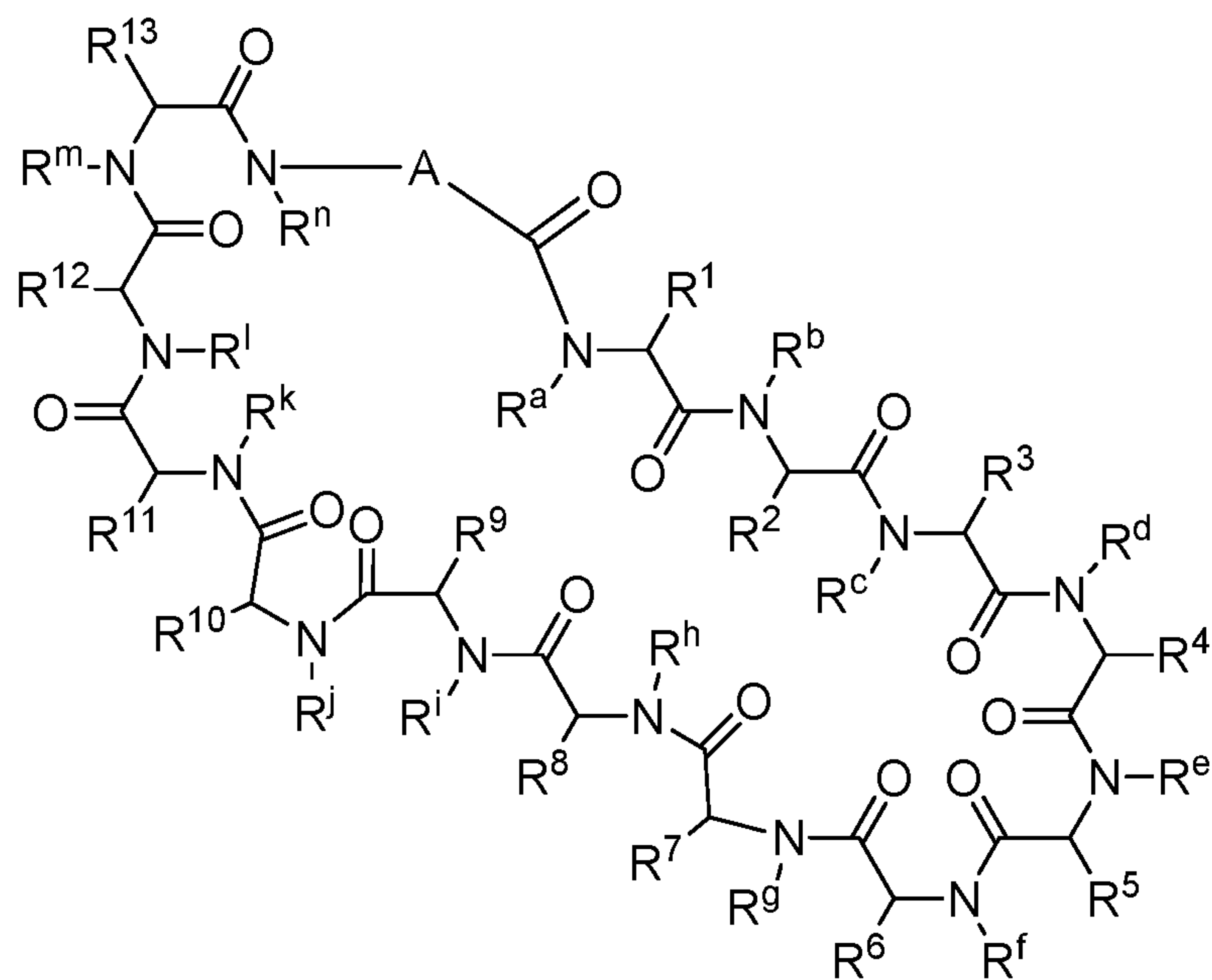
Example 13009	A
Example 13110	B
Example 13111	A
Example 13112	A
Example 13113	A
Example 13114	B
Example 13115	A
Example 13116	A
Example 13117	B
Example 13118	---
Example 13119	A

It will be evident to one skilled in the art that the present disclosure is not limited to the foregoing illustrative examples, and that it can be embodied in other specific forms without departing from the essential attributes thereof. It is therefore
5 desired that the examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing examples, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

CLAIMS

WHAT IS CLAIMED IS:

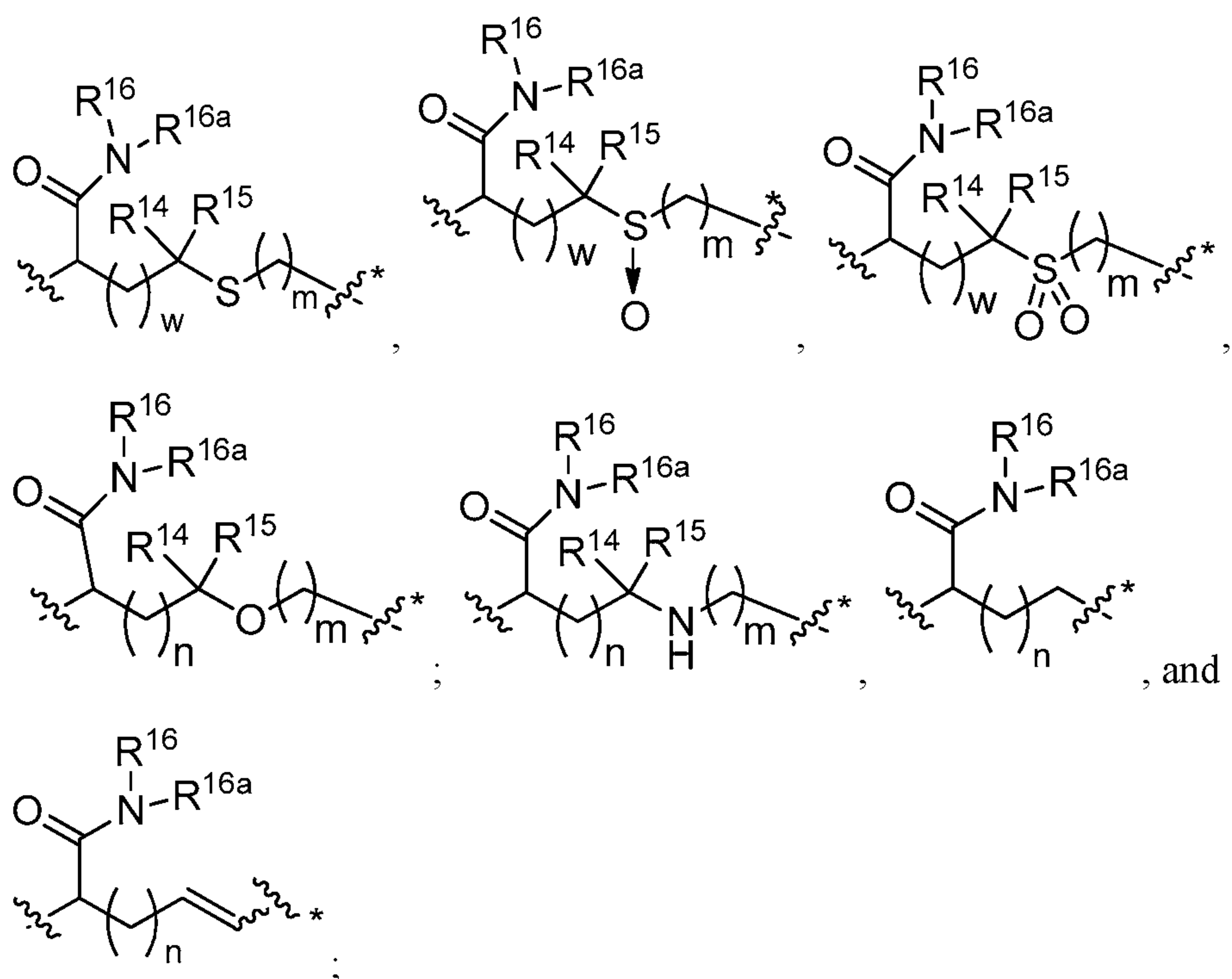
1. A compound of formula (I)



(I),

or a pharmaceutically acceptable salt thereof, wherein:

A is selected from



wherein:

* denotes the point of attachment to the carbonyl group and * denotes the point of attachment to the nitrogen atom;

n is 0 or 1;

m is 1 or 2;

w is 0, 1, or 2;

R^{14} and R^{15} are independently selected from hydrogen and methyl;

R^{16a} is selected from hydrogen and C_1 - C_6 alkyl;

R^{16} is selected from

$-(C(R^{17a})_2)-C(O)-NR^{50}R^{51}$;

wherein:

each R^{17a} is independently selected from hydrogen and C_1 - C_6 alkyl;

one of R^{50} and R^{51} is selected from hydrogen and C_1 - C_6 alkyl and the other is selected from $-(CH_2)_nX$, C_1 - C_6 alkyl, C_3 - C_7 cycloalkyl, heterocyclyl, and phenyl, wherein the cycloalkyl is optionally substituted with one, two, or three groups independently selected from C_1 - C_3 alkoxy, C_1 - C_3 alkyl, amino, cyano, and hydroxy, or;

R^{50} and R^{51} , together with the nitrogen atom to which they are attached, form a four-, five-, six- or seven-membered saturated or unsaturated ring optionally containing one or two additional heteroatoms independently selected from nitrogen, oxygen, and sulfur; wherein said ring is optionally substituted with one, two, or three groups selected from C_1 - C_6 alkoxy, C_1 - C_3 alkyl, cyano, halo, halo C_1 - C_3 alkyl, hydroxy, hydroxy(C_1 - C_3 alkyl), $-NR^{70}R^{71}$, oxo, and phenyl; wherein the phenyl is further optionally substituted with one, two, or three groups independently selected from C_1 - C_3 alkoxy, cyano, and halo;

n' is 1-5;

X is selected from $-\text{C}\equiv\text{CH}$, $-\text{C}(\text{H})=\text{CH}_2$, C_2 - C_6 alkoxymethyl, C_1 - C_6 alkoxycarbonylmethyl, C_1 - C_6 alkylsulfanylmethyl, C_1 - C_6 alkylsulfonylmethyl, azidomethyl, *tert*-butoxymethyl, C_3 - C_7 cycloalkyl, haloalkoxymethyl, halomethyl, heterocyclyl, hydroxymethyl, isopropoxymethyl, $(NR^{70}R^{71})$ methyl, phenyl, phenoxymethyl, phenylsulfanylmethyl,

one of R^{70} and R^{71} is selected from hydrogen, C_1 - C_6 alkyl, and hydroxy C_2 - C_6 alkyl and the other is selected from C_1 - C_6 alkoxycarbonyl, C_1 - C_6 alkylcarbonyl, C_1 - C_6 alkylsulfonyl, and hydroxy C_2 - C_6 alkyl;

 denotes the point of attachment to the carbonyl group and  denotes the point of attachment to the nitrogen atom;

R^c , R^f , R^h , R^i , R^m , and R^n are hydrogen;

R^a , R^e , R^j , and R^k , are each independently selected from hydrogen and methyl;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , and R^{13} are independently selected from a natural amino acid side chain and an unnatural amino acid side chain or form a ring with the corresponding vicinal R group as described below;

R^e and R^k can each form a ring with the corresponding vicinal R group and the atoms to which they are attached selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, and hydroxy;

R^b is methyl or, R^b and R², together with the atoms to which they are attached, form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, and hydroxy;

R^d is hydrogen or methyl, or, R^d and R⁴, together with the atoms to which they are attached, can form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, hydroxy, and phenyl;

R^g is hydrogen or methyl or R^g and R⁷, together with the atoms to which they are attached, can form a ring selected from azetidine, pyrrolidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, benzyl optionally substituted with a halo group, benzyloxy, cyano, cyclohexyl, methyl, halo, hydroxy, isoquinolinyloxy optionally substituted with a methoxy group, quinolinyloxy optionally substituted with a halo group, and tetrazolyl; and wherein the pyrrolidine and the piperidine ring are optionally fused to a cyclohexyl, phenyl, or indole group; and

R^l is methyl or, R^l and R¹², together with the atoms to which they are attached, form a ring selected from azetidine and pyrrolidine, wherein each ring is optionally substituted with one to four independently selected from amino, cyano, methyl, halo, and hydroxy.

2. A compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein

R^d and R⁴, together with the atoms to which they are attached, form a pyrrolidine ring;

R^g and R⁷, together with the atoms to which they are attached, form a pyrrolidine ring, wherein said ring is optionally substituted with one hydroxy group; and

R^k is methyl.

3. A compound of claim 2, or a therapeutically acceptable salt thereof, wherein:
 - R^a , R^e , and R^j hydrogen;
 - R^b , R^k , and R^l are methyl;
 - R^n is hydrogen;
 - R^1 is phenylmethyl wherein the phenyl is substituted with hydroxy;
 - R^2 is methyl;
 - R^3 is selected from $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$ and $-\text{CH}_2\text{CO}_2\text{H}$;
 - R^5 is selected from $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{OH}$, and $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$;
 - R^6 is selected from $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-(\text{CH}_2)_2\text{CO}_2\text{H}$, and $(\text{CH}_2)_2\text{C}(\text{O})\text{NH}_2$;
 - R^8 and R^{10} are $-\text{CH}_2(\text{indolyl})$, wherein the indolyl is optionally substituted with $-\text{CH}_2\text{CO}_2\text{H}$;
 - R^9 is selected from hydrogen, $-(\text{CH}_2)_2\text{NH}_2$, CH_2OH , and $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$;
 - R^{11} and R^{12} are $-(\text{CH}_2)_3\text{CH}_3$; and
 - R^{13} is selected from methyl, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, and $-(\text{CH}_2)_2\text{CO}_2\text{H}$.

4. A compound selected from:

Example 13080, Example 13081, Example 13082, Example 13083, Example 13084, Example 13085, Example 13086, Example 13087, Example 13088, Example 13089, Example 13090, Example 13091, Example 13092, Example 13093, Example 13094, Example 13095, Example 13096, Example 13097, Example 13098, Example 13099, Example 13100, Example 13101, Example 13102, Example 13103, Example 13104, Example 13105, Example 13106, Example 13107, Example 13108, Example 13109, Example 13110, Example 13111, Example 13112, Example 13113, Example 13114, Example 13115, Example 13116, Example 13117, Example 13118, and Example 13119; or a pharmaceutically acceptable salt thereof.

5. A method of enhancing, stimulating, and/or increasing the immune response in a subject in need thereof, said method comprising administering to the subject a therapeutically effective amount of a compound of claim 1 or a therapeutically acceptable salt thereof.

6. The method of claim 5 further comprising administering an additional agent prior to, after, or simultaneously with the compound of claim 1 or a therapeutically acceptable salt thereof.
7. The method of claim 6 wherein the additional agent is an antimicrobial agent, an antiviral agent, a cytotoxic agent, and/or an immune response modifier.
8. The method of claim 6 wherein the additional agent is an HDAC inhibitor.
9. The method of claim 6 wherein the additional agent is a TLR7 and/or TLR8 agonist
10. A method of inhibiting growth, proliferation, or metastasis of cancer cells in a subject in need thereof, said method comprising administering to the subject a therapeutically effective amount a compound of claim 1 or a therapeutically acceptable salt thereof.
11. The method of claim 10 wherein the cancer is selected from melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and hematological malignancies.
12. A method of treating an infectious disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of claim 1 or a therapeutically acceptable salt thereof.
13. The method of claim 11 wherein the infectious disease is caused by a virus.
14. The method of claim 13 wherein the virus is selected from HIV, Hepatitis A, Hepatitis B, Hepatitis C, herpes viruses, and influenza.

15. A method of treating septic shock in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of claim 1 or a therapeutically acceptable salt thereof.
16. A method blocking the interaction of PD-L1 with PD-1 and/or CD80 in a subject, said method comprising administering to the subject a therapeutically effective amount of a compound of claim 1 or a therapeutically acceptable salt thereof.