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(72) **Inventeurs/Inventors:**
MILLER, MICHAEL MATTHEW, US;
MAPELLI, CLAUDIO, US;
ALLEN, MARTIN PATRICK, US;
BOWSHER, MICHAEL S., US;
GILLIS, ERIC P., US;
LANGLEY, DAVID R., US;
...

(73) **Propriétaire/Owner:**
BRISTOL-MYERS SQUIBB COMPANY, US

(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : INHIBITEURS MACROCYCLIQUES DES INTERACTIONS PROTEINE/PROTEINE PD-1/PD-L1 ET CD80(B7-1)/PD-L1**
(54) **Title: MACROCYCLIC INHIBITORS OF THE PD-1/PD-L1 AND CD80(B7-1)/PD-L1 PROTEIN/PROTEIN INTERACTIONS**

(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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(72) **Inventeurs(suite)/Inventors(continued):** MULL, ERIC, US; POIRIER, MAUDE A., US; SANGHVI, NISHITH, US; SUN, LI-QIANG, US; TENNEY, DANIEL J., US; YEUNG, KAP-SUN, US; ZHU, JULIANG, US; GILLMAN, KEVIN W., US; ZHAO, QIAN, US; GRANT-YOUNG, KATHARINE A., US; SCOLA, PAUL MICHAEL, US; CORNELIUS, LYNDON A.M., US

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(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and ProvinceLine Road, Princeton, New Jersey 08543 (US).

(72) Inventors: **MILLER, Michael Matthew**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **MAPELLI, Claudio**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **ALLEN, Martin Patrick**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **BOWSHER, Michael S.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **GILLIS, Eric P.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **LANGLEY, David R.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **MULL, Eric**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **POIRIER, Maude A.**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **SANGHVI, Nishith**; 3 Kershaw Court, Bridgewater, New Jersey 08807 (US). **SUN, Li-Qiang**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **TENNEY, Daniel J.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **YEUNG, Kap-Sun**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **ZHU, Juliang**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **GILLMAN, Kevin W.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **ZHAO, Qian**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **GRANT-YOUNG, Katharine A.**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US). **SCOLA, Paul Michael**; c/o Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, Connecticut 06492 (US).

(74) Agents: **MINGO, Pamela, A.** et al.; Bristol-Myers Squibb Company, Route 206 & Province Line Road, Princeton, New Jersey 08543 (US).

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(54) **Title**: MACROCYCLIC INHIBITORS OF THE PD-1/PD-L1 AND CD80 (B7-1)/PD-LI PROTEIN/PROTEIN INTERACTIONS

(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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JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

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MACROCYCLIC INHIBITORS OF THE PD-1/PD-L1 AND CD80(B7-1)/PD-L1 PROTEIN/PROTEIN INTERACTIONS

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.

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)).

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)). 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)). 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 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)).

PD-L1 has also been shown to interact with CD80 (Butte, M.J. 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, A.M. et al., *J. Immunol.*, 187:1097-1105 (2011); Yang, J. et al., *J. Immunol.*,187(3):1113-1119 (Aug 1 2011)).

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 (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".

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 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 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 chromoic 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.*, 187(3):1113-1119 (Aug 1 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.*, 187(3):1113-1119 (Aug 1 2011)). Studies have revealed that blockade of the interactions of PD-L1 using antibodies can suppress inappropriate immune responses and ameliorate disease signs.

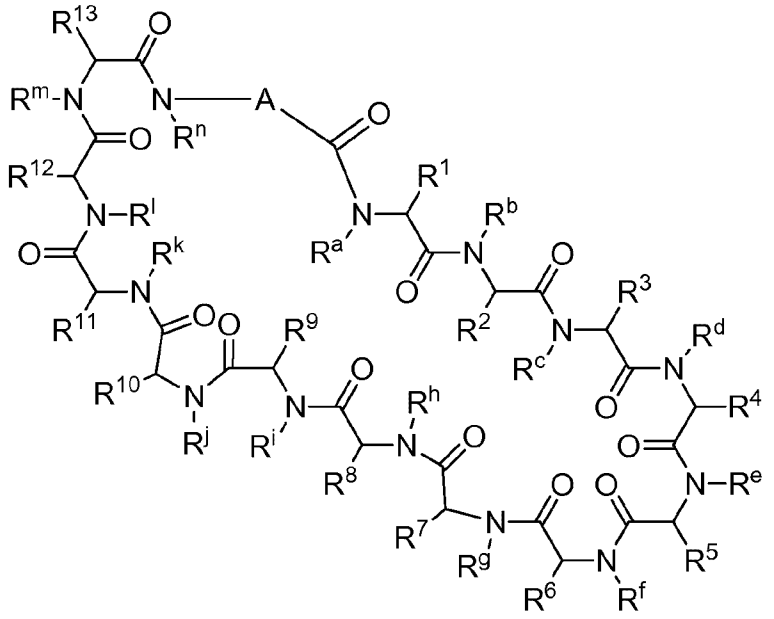
In addition to enhancing immunologic responses to chronic antigens, blockade of the PD-1/PD-L1 pathway has also been shown to enhance responses to vaccination, including therapeutic vaccination in the context of chronic infection (Ha, S.J. et al., "Enhancing therapeutic vaccination by blocking PD-1-mediated inhibitory 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)).

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 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.

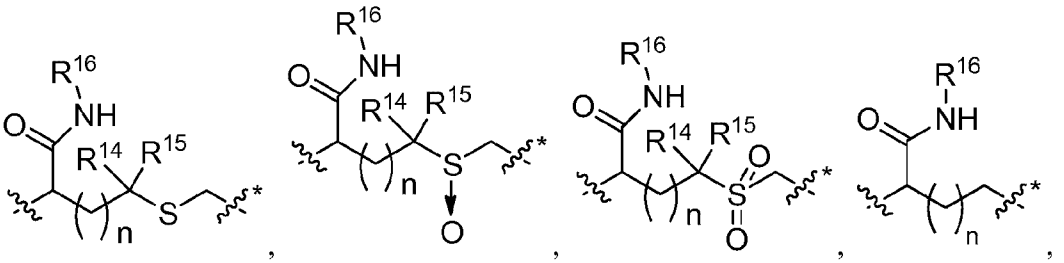
In one embodiment the present disclosure provides a compound of formula (I)

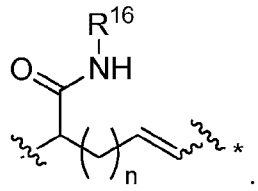


(I),

or a pharmaceutically acceptable salt thereof, wherein:

A is selected from a bond,



and  ;

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;

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

R^{16} is selected from hydrogen, $-CHR^{17}C(O)NH_2$, $-CHR^{17}C(O)NHCHR^{18}C(O)NH_2$, and $-CHR^{17}C(O)NHCHR^{18}C(O)NHCH_2C(O)NH_2$; wherein R^{17} is selected from hydrogen and $-CH_2OH$ and wherein R^{18} is selected from hydrogen and methyl;

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, pyrollidine, 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, pyrollidine, morpholine, piperidine, piperazine, and

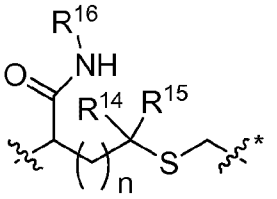
tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, and hydroxy;

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, pyrollidine, 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;

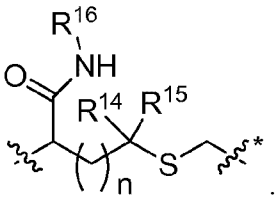
R^g is hydrogen or methyl or R^g and R^7, together with the atoms to which they are attached, can form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from 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^1 is methyl or, R^1 and R^{12}, together with the atoms to which they are attached, form a ring selected from azetidine and pyrollidine, wherein each ring is optionally substituted with one to four independently selected from amino, cyano, methyl, halo, and hydroxy.

In another embodiment the present disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein A is



In another embodiment the present disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein A is



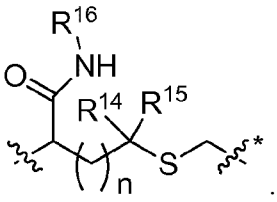
;

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

R^g is methyl or, R^g and R^7, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two 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^k is methyl or, R^k and R^{11}, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, and hydroxy.

In another embodiment the present disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein A is



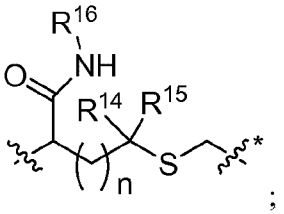
;

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

R^g and R^7, together with the atoms to which they are attached, form a pyrollidine ring, wherein said ring is optionally substituted with one or two 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^k is methyl.

In another embodiment the present disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein A is



;

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

R^g and R^7, together with the atoms to which they are attached, form a pyrollidine ring, wherein said ring is optionally substituted with one or two 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;

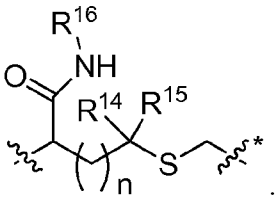
R^k is methyl; and

R^8 is selected from:

azaindolylC_1-C_3alkyl, benzothiazolylC_1-C_3alkyl, benzothienylC_1-C_3alkyl, benzyloxyC_1-C_3alkyl, diphenylmethyl, furanylC_1-C_3alkyl, imidazolylC_1-C_3alkyl, naphthylC_1-C_3alkyl, pyridinylC_1-C_3alkyl, thiazolylC_1-C_3alkyl, thienylC_1-C_3alkyl; and

indolylC_1-C_3alkyl, wherein the indolyl part is optionally substituted with one group selected from C_1-C_3alkyl, cyano, halo, and hydroxy.

In another embodiment the present disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, wherein A is



;

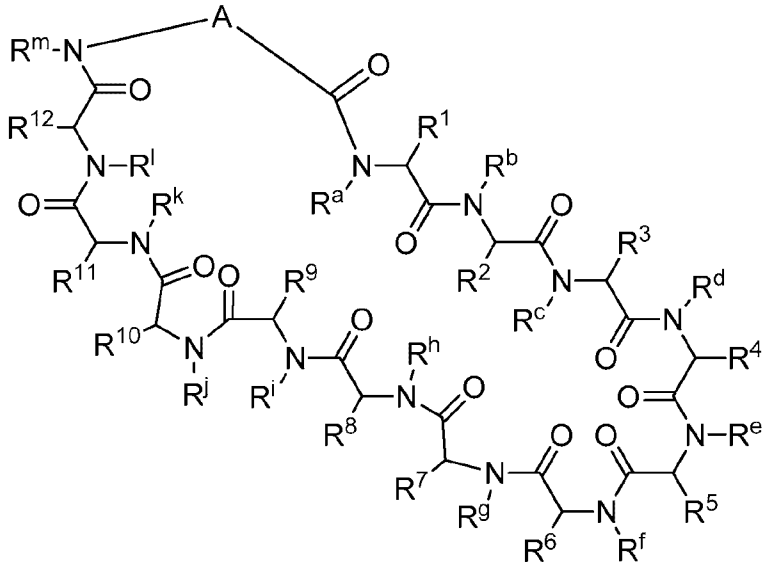
R^d and R^4, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, and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

R^g and R^7, together with the atoms to which they are attached, form a pyrollidine ring, wherein said ring is optionally substituted with one or two 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;

R^k is methyl; and

R^8 is 3-indolylC_1-C_3alkyl optionally substituted with one group selected from C_1-C_3alkyl, halo, hydroxy, or cyano.

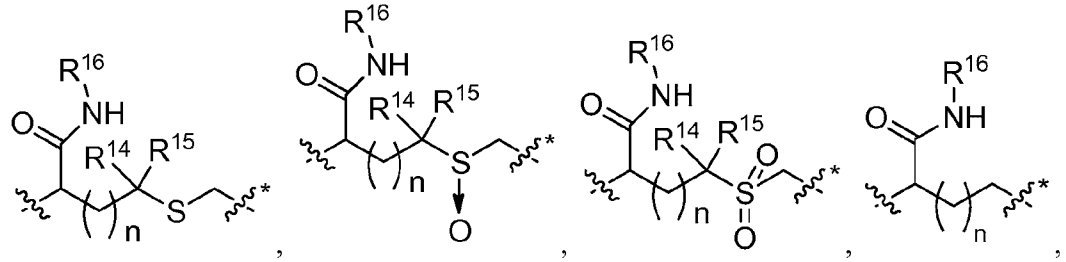
In another embodiment the present disclosure provides a compound of formula (II)



(II),

or a pharmaceutically acceptable salt thereof, wherein:

A is selected from a bond,



and ;

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;

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

R^{16} is selected from hydrogen, $-CHR^{17}C(O)NH_2$, $-CHR^{17}C(O)NHCHR^{18}C(O)NH_2$, and $-CHR^{17}C(O)NHCHR^{18}C(O)NHCH_2C(O)NH_2$;

wherein R^{17} is selected from hydrogen and $-CH_2OH$ and wherein R^{18} is selected from hydrogen and methyl;

R^a, R^f, R^j, R^k, R^l, and R^m are hydrogen;

R^b and R^c are methyl;

R^g is 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}, and R^{12} 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;

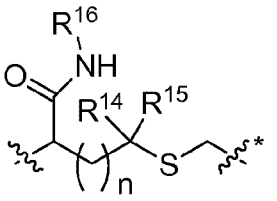
R^d is selected from hydrogen and methyl, or, R^d and R^4, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently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

R^e is selected from hydrogen and methyl, or, R^e and R^5, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy;

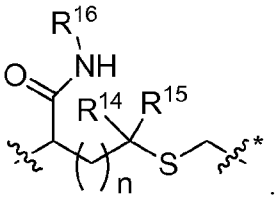
R^h is selected from hydrogen and methyl, or, R^h and R^8, together with the atoms to which they are attached, form a ring selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy; and

R^i is selected from hydrogen and methyl, or, R^i and R^9, together with the atoms to which they are attached selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one to four groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy.

In another embodiment the present disclosure provides a compound of formula (II), or a pharmaceutically acceptable salt thereof, wherein A is



In another embodiment the present disclosure provides a compound of formula (II), or a pharmaceutically acceptable salt thereof, wherein A is



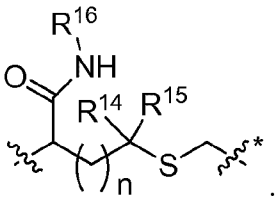
;

R^d is methyl, or, R^d and R^4, together with the atoms to which they are attached selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy;

R^g is methyl; and

R^i is methyl, or, R^i and R^9, together with the atoms to which they are attached selected from selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy.

In another embodiment the present disclosure provides a compound of formula (II), or a pharmaceutically acceptable salt thereof, wherein A is



;

R^d is methyl, or, R^d and R^4, together with the atoms to which they are attached selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy;

R^g is methyl;

R^i is methyl, or, R^i and R^9, together with the atoms to which they are attached selected from selected from azetidine, pyrollidine, morpholine, piperidine, piperazine, and tetrahydrothiazole; wherein each ring is optionally substituted with one or two groups independently selected from amino, cyano, methyl, halo, halomethyl, and hydroxy; and

R^7 is phenylC_1-C_3alkyl optionally substituted with a fluoro group.

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 at least one macrocyclic peptide described herein. In another embodiment the method further comprises administering an additional agent prior to, after, or simultaneously with the macrocyclic peptide or peptides described herein. 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 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 one or more macrocyclic peptides described herein. 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.

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 at least one macrocyclic peptide described herein. 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.

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.

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) and (II) where the R side chains are part of a ring that is 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 R^1 side chains are: phenylalanine, tyrosine, 3-thien-2-yl, 4-methylphenylalanine, 4-chlorophenylalanine, 3-methoxyphenylalananic, 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, biphenylalanine, and 3-chlorophenylalanine; and 2,4-diaminobutane.

In compounds of formula (I) where R^2 is not part of a ring, preferred R^2 side chains are: alanine, serine, and glycine.

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

In compounds of formula (I) where R^4 is not part of a ring, preferred R^4 side chains are: valine, alanine, isoleucine, and glycine.

In compounds of formula (I), preferred R^5 side chains are: histidine, asparagine, 2,3-diaminopropane, serine, glycine, 2,4-diaminobutane, threonine, alanine, lysine, aspartic acid, alanine, and 3-thiazolylalanine.

In compounds of formula (I), preferred R^6 side chains are: leucine, aspartic acid, asparagine, glutamic acid, glutamine, serine, lysine, 3-cyclohexane, threonine, ornithine, 2,4-diaminobutane, alanine, arginine, and ornithine ($COCH_3$).

In compounds of formula (I) where R^7 is not part of a ring, preferred R^7 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 R^8 side chains are tryptophan and 1,2-benzisothiazolinylalanine.

In compounds of formula (I) preferred R^9 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 R^{10} side chains are: tryptophan, benzisothiazolylalanine, 1-napththylalanine, 5-flurotryptophan, methionine, 7-methyltryptophan, 5-chlorotryptophan, and -methyltryptophan.

In compounds of formula (I) preferred R^{11} side chains are: norleucine, leucine, asparagine, phenylalanine, methionine, ethoxymethane, alanine, tryptophan, isoleucine, phenylpropane, glutamic acid, hexane, and heptane.

In compounds of formula (I) where R^{12} is not part of a ring, preferred R^{12} 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 R^{13} 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 compounds of formula (II) preferred R^1 side chains are: phenylalanine, 3-methoxyphenylalanine, 2-fluorophenylalanine, 3-fluorophenylalanine, 4-fluorophenylalanine, 3,4-difluorophenylalanine, 3,5-difluorophenylalanine, 3,4,5-trifluorophenylalanine, 3-fluro,4-chlorophenylalanine, 3-chloro,4-fluorophenylalanine, 3-chlorophenylalanine, 4-chlorophenylalanine, 3,4-dichlorophenylalanine, 3,5-dichlorophenylalanine, 3,5-dichloro,4-fluorophenylalanine, 3-chloro,4,5-difluorophenylalanine, 4-bromophenylalanine, 4-nitrophenylalanine, 3-trifluoromethylphenylalanine, 4-trifluoromethylphenylalanine, and 3-pyridylalanine.

In compounds of formula (II), preferred R^2 side chains are: phenylalanine, alanine, histidine, tyrosine, tryptophan, glutamic acid, 1-naphthylalanine, 2-naphthylalanine, 2-benzothiazolylalanine, 3-pyridinylalanine, and 4-pyridinylalanine.

In compounds of formula (II), preferred R^3 side chains are: norleucine, alanine, tyrosine, glutamic acid, leucine, and isoleucine.

In compounds of formula (II) where R^4 is not part of a ring, preferred R^4 side chains are: glycine, and alanine.

In compounds of formula (II) where R^5 is not part of a ring, preferred R^5 side chains are: aspartic acid, glutamic acid, arginine, lysine, asparagine, serine, 2,4-diaminobutane, 2,3-diaminopropane, and 2-aminobutane.

In compounds of formula (II) preferred R^6 side chains are: valine, leucine, isoleucine, N-methylthreonine, and cyclohexylmethane.

In compounds of formula (II) preferred R^7 side chains are: phenylalanine and 3-fluorophenylalanine.

In compounds of formula (II) where R^8 is not part of a ring, preferred R^8 side chains are: tyrosine, 3-iodotyrosine, leucine, arginine, glutamic acid, glutamine, pentafluorophenylalanine, 4-aminophenylalanine, 4-aminomethylphenylalanine, 3,4-dimethoxyphenylalanine, tryptophan, 5-chlorotryptophan, 5-hydroxytryptophan, isotryptophan, lysine, ornithine, and 2,3-diaminopropane.

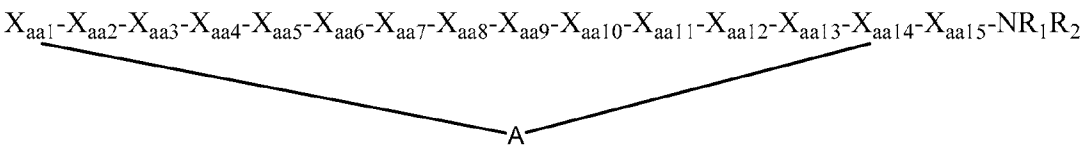
In compounds of formula (II) preferred R^{10} side chains are: tryptophan, 5-chlorotryptophan, 7-azatryptophan, isotryptophan, 3-benzothiazolylalanine, and 1-napththylalanine.

In compounds of formula (II) preferred R^{11} side chains are tyrosine, 4-fluorophenylalanine, 4-aminomethylphenylalanine, 4-aminophenylalanine, and 3,4-dihydroxyphenylalanine.

In compounds of formula (II) preferred R^{12} side chains are: leucine, tyrosine, arginine, lysine, ornithine, glutamic acid, phenylalanine, 4-methylphenylalanine, 4-chlorophenylalanine, 4-aminomethylphenyalanine, norleucine, cyclohexylalanine, 2,4-diaminobutane, and 2,3-diaminopropane.

In compounds of formula (II), when R^4 and R^9 are part of a ring the preferred stereochemistry is that of the D-isomer and when R^5 and R^8 are part of a ring the preferred stereochemistry is that of the L isomer.

One embodiment of the subject matter described herein is directed to a polypeptide comprising a sequence of Formula I(a):



wherein:

A is an organic or peptidic linker between X_{aa1} and X_{aa14}, thereby providing a macrocyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring aromatic or heteroaromatic or alkyl or heteroaryl alkyl amino acid;

X_{aa2} is a naturally or nonnaturally occurring alkyl or N-methylated alkyl amino acid;

X_{aa3} is a naturally or nonnaturally occurring hydrophilic or alkyl or polar amino acid;

X_{aa4} is a naturally or nonnaturally occurring amino acid, an alkyl amino acid or a N-methylated alkyl amino acid;

X_{aa5} is a naturally or nonnaturally occurring heteroaromatic amino acid or a positively charged amino acid or an alkyl amino acid;

X_{aa6} is a naturally or nonnaturally occurring hydrophilic or hydrophobic or positively or negatively charged amino acid;

X_{aa7} is a naturally or nonnaturally occurring N-methylated or non-N-methylated hydrophilic or hydrophobic or positively or negatively charged amino acid;

X_{aa8} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl amino acid;

X_{aa9} is a naturally or nonnaturally occurring hydrophilic or hydrophobic or positively or negatively charged amino acid;

X_{aa10} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or alkyl or heteroalkyl amino acid;

X_{aa11} is a naturally or nonnaturally N-methylated or non-N-methylated alkyl or heteroalkyl or aromatic or heteroaromatic occurring amino acid;

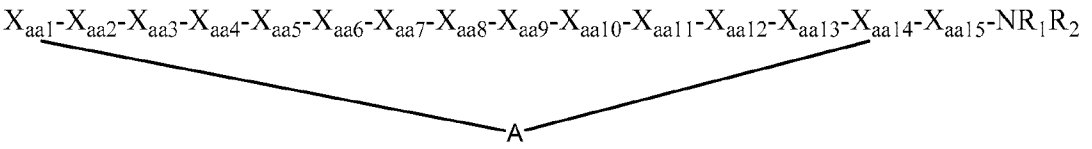
X_{aa12} is a naturally or nonnaturally N-methylated or non-N-methylated alkyl or heteroalkyl or aromatic or heteroaromatic occurring amino acid;

X_{aa13} is a naturally or nonnaturally occurring hydrophilic or hydrophobic or positively or negatively charged amino acid;

X_{aa14} is a naturally or nonnaturally occurring amino acid possessing a functional group that can be appropriately activated to react with one end of linker A to yield a cyclic peptide;

X_{aa15} is a naturally or nonnaturally occurring amino acid or a spacer followed by a tag or a spacer followed by a solubilizing or a PK-enhancing element.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula I(b):



wherein:

A is an electrophilic moiety such as a Michael acceptor or a chloro- or bromoacetyl group which is capable of reacting with a sulfhydryl group present on residue X_{aa14} to form a covalent thioether bond, thereby yielding a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

and wherein A can be optionally present; and wherein, if A is present, it can be a Gly or other spacer with a free amine, which can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa14}, thereby providing an N-terminus to side chain lactam cyclic peptide; and wherein, if A is not present, the N-terminal amino group of the X_{aa1} residue can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa14}, thereby providing an N-terminus to side chain lactam cyclic peptide;

and wherein, if A is present, it can be a Gly or other spacer with a free amine, which can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa15}, thereby providing a head-to-tail cyclic peptide; and wherein, if A is not present, the N-terminal amino group of the X_{aa1} amino acid can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa15}, thereby providing a head-to-tail cyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring amino acid comprising L-Phe, L-Ala, L-Trp, L-Tyr, L-Phe(4-OMe), L-Phe(4-F), L-Phe(4-Cl), L-Phe(4-Br), L-Phe(4-Me),

L-Phe(4-CF_3), L-Phe(4-*t*-Bu), L-Phe(penta-F), L-1-Nal, L-2-Nal, L-Bip, L-^{m}Phe, L-Tic, L-3-Pya, L-4-Pya, L-Tza, L-3-Tha;

X_{aa2} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-Ala, L-^{m}Ala, ^{m}Gly, L-^{m}Val;

X_{aa3} is selected from the group consisting of Gly, L-Asn and L-Ala;

X_{aa4} is a naturally or nonnaturally occurring amino acid comprising L-Pro, L-Ala, L-α-Me-Pro, L-Pro(4R-OH), L-Pro(4R-OBzl), L-Pro(4R-NH_2), L-Pro(3R-Ph), L-Pro(4S-Ph), L-Pro(5R-Ph), L-Azt, L-Pip, L-Oic, L-2,3-Methano-Pro, L-3,4-Methano-Pro, L-Val, L-Leu, L-Ile, L-^{m}Ala, L-^{m}Val, L-^{m}Leu, L-Tza;

X_{aa5} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-His, L-Ala, L-Tza, L-Arg, L-Lys, L-Orn, L-Dab and L-Dap;

X_{aa6} is a naturally or nonnaturally occurring amino acid comprising L-Leu, L-Ala, L-Arg, L-His, L-Glu and L-Asp;

X_{aa7} is a naturally or nonnaturally occurring amino acid comprising ^{m}Gly, Gly, L-^{m}Ala, D-^{m}Ala, L-Pro, L-Ser, L-^{m}Ser L-Dab, L-Arg and L-His;

X_{aa8} is L-Trp, L-Phe, L-Tyr, L-His, L-Phe(penta-F), L-Tza, L-Bzt, L-1-Nal, L-2-Nal, L-2-Pya, L-3-Pya, L-4-Pya;

X_{aa9} is a naturally or nonnaturally occurring amino acid comprising L-Ser, L-Ala, L-Arg and D-Asn;

X_{aa10} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-Trp, L-Ala, L-Met, L-Nle, L-Leu and L-Ile, L-Phe, L-Tyr, L-His, L-Phe(penta-F), L-Tza, L-Bzt, L-1-Nal, L-2-Nal, L-2-Pya, L-3-Pya, L-4-Pya;

X_{aa11} is a naturally or nonnaturally occurring amino acid comprising L-^{m}Nle, L-Nle, L-^{m}Ala, L-Ala, L-Phe, L-^{m}Phe and L-^{m}Leu, L-Ser, D-Nle and L-Pro;

X_{aa12} is a naturally or nonnaturally occurring amino acid comprising L-^{m}Nle, L-Nle, L-^{m}Ala, L-Ala, L-Phe, L-^{m}Phe, L-^{m}Leu and L-Pro;

X_{aa13} is a naturally or nonnaturally occurring amino acid comprising L-Arg, L-Ala, L-Leu, L-Lys, L-Asp, L-Glu, L-His;

X_{aa14} is selected from the group consisting of L-Cys, D-Cys, Asp, Glu, Gly, L-homo-Cys, D-homo-Cys, L-Pen, D-Pen, L-^{m}Cys and D-^{m}Cys;

X_{aa15} is Gly or Gly followed by a PEG spacer comprised of at least two ethylene glycol units, or Gly followed by a PEG spacer comprised of at least two ethylene glycol

units followed by a tag such as biotin, or Gly followed by a spacer followed by a PK-enhancing element;

wherein X_{aa15} is optionally present and wherein the C-terminal carbonyl carbon of said amino acid is attached to a hydroxyl group to form a carboxylic acid or to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group;

wherein, if X_{aa15} is not present, the C-terminal carbonyl carbon of X_{aa14} is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula I(c):

$$X_{aa1}\text{-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-}X_{aa6}\text{-}X_{aa7}\text{-Trp-}X_{aa9}\text{-}X_{aa10}\text{-}X_{aa11}\text{-}X_{aa12}\text{-}X_{aa13}\text{-}X_{aa14}\text{-}X_{aa15}\text{-}NR_1R_2$$

A

wherein:

A is an electrophilic moiety such as a Michael acceptor or a chloro- or bromoacetyl group which is capable of reacting with a sulfhydryl group present on residue X_{aa14} to form a covalent thioether bond, thereby yielding a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

and wherein A can be optionally present; and wherein, if A is present, it can be a Gly or other spacer with a free amine, which can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa14}, thereby providing an N-terminus to side chain lactam cyclic peptide; and wherein, if A is not present, the N-terminal amino group of the X_{aa1} residue can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa14}, thereby providing an N-terminus to side chain lactam cyclic peptide;

and wherein, if A is present, it can by a Gly or other spacer with a free amine, which can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa15}, thereby providing a head-to-tail cyclic peptide; and wherein, if

A is not present, the N-terminal amino group of the X_{aa1} amino acid can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa15}, thereby providing a head-to-tail cyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring amino acid comprising L-Phe, L-Ala, L-Trp, L-Tyr, L-Phe(4-OMe), L-Phe(4-F), L-Phe(4-Cl), L-Phe(4-Br), L-Phe(4-Me), L-Phe(4-CF_3), L-Phe(4-*t*-Bu), L-Phe(penta-F), L-1-Nal, L-2-Nal, L-Bip, L-^{m}Phe, L-Tic, L-3-Pya, L-4-Pya, L-Tza, L-3-Tha;

X_{aa2} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-Ala, L-^{m}Ala, ^{m}Gly, L-^{m}Val;

X_{aa3} is selected from the group consisting of Gly, L-Asn and L-Ala;

X_{aa4} is a naturally or nonnaturally occurring amino acid comprising L-Pro, L-Ala, L-α-Me-Pro, L-Pro(4R-OH), L-Pro(4R-NH_2), L-Pro(4S-Ph), L-Azt, L-Pip and L-Oic;

X_{aa5} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-His and L-Ala;

X_{aa6} is a naturally or nonnaturally occurring amino acid comprising L-Leu, L-Ala, L-Arg and L-Asp;

X_{aa7} is a naturally or nonnaturally occurring amino acid comprising ^{m}Gly, Gly, L-^{m}Ala, D-^{m}Ala, L-Pro, L-Ser, L-^{m}Ser L-Dab, L-Arg and L-His;

X_{aa9} is a naturally or nonnaturally occurring amino acid comprising L-Ser, L-Ala, L-Arg and D-Asn;

X_{aa10} is a naturally or nonnaturally occurring amino acid selected from the group consisting of L-Trp, L-Ala, L-Met, L-Leu and L-Ile;

X_{aa11} is a naturally or nonnaturally occurring amino acid comprising L-^{m}Nle, L-Nle, L-^{m}Ala, L-Ala, L-Phe, L-^{m}Phe and L-^{m}Leu, L-Ser and D-Nle;

X_{aa12} is a naturally or nonnaturally occurring amino acid comprising L-^{m}Nle, L-Nle, L-^{m}Ala and L-Ala;

X_{aa13} is a naturally or nonnaturally occurring amino acid comprising L-Arg, L-Ala and L-Leu;

X_{aa14} is selected from the group consisting of L-Cys, D-Cys, Asp, Glu and Gly;

X_{aa15} is Gly or Gly followed by a PEG spacer comprised of at least two ethylene glycol units, or Gly followed by a PEG spacer comprised of at least two ethylene glycol units followed by a tag such as biotin;

wherein X_{aa15} is optionally present and wherein the C-terminal carbonyl carbon of said amino acid is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group;

wherein, if X_{aa15} is not present, the C-terminal carbonyl carbon of X_{aa14} is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula II(a):

$$X_{aa1}\text{-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-}X_{aa6}\text{-}X_{aa7}\text{-}X_{aa8}\text{-}X_{aa9}\text{-}X_{aa10}\text{-}X_{aa11}\text{-}X_{aa12}\text{-}X_{aa13}\text{-}X_{aa14}\text{-}NR_1R_2$$

A

wherein:

A is an organic or peptidic linker between X_{aa1} and X_{aa13}, thereby providing a macrocyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring aromatic or heteroaromatic or alkyl or heteroaryl alkyl amino acid;

X_{aa2} is a naturally or nonnaturally occurring alkyl or aromatic N-methylated amino acid;

X_{aa3} is a naturally or nonnaturally occurring hydrophobic N-methylated amino acid;

X_{aa4} is a naturally or nonnaturally occurring hydrophobic N-methylated amino acid;

X_{aa5} is a naturally or nonnaturally occurring alkyl amino acid or a positively or negatively charged amino acid;

X_{aa6} is a naturally or nonnaturally occurring hydrophobic amino acid;

X_{aa7} is a naturally or nonnaturally occurring N-methylated or non-N-methylated aromatic or heteroaromatic or alkyl or heteroarylalkyl amino acid;

X_{aa8} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl amino acid or an alkyl amino acid;

X_{aa9} is a naturally or nonnaturally occurring N-methylated or non-N-methylated aromatic or heteroaromatic or alkyl or heteroarylalkyl amino acid;

X_{aa10} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or alkyl or heteroalkyl amino acid;

X_{aa11} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or alkyl or heteroalkyl amino acid;

X_{aa12} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or alkyl or heteroalkyl amino acid;

X_{aa13} is a naturally or nonnaturally occurring amino acid possessing a functional group that can be appropriately activated to react with one end of linker A to yield a cyclic peptide;

X_{aa14} is a naturally or nonnaturally occurring amino acid or a spacer or a spacer followed by a tag or a spacer followed by a solubilizing or a PK-enhancing element.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula II(b):

$$X_{aa1}\text{-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-}X_{aa6}\text{-}X_{aa7}\text{-Trp-}X_{aa9}\text{-}X_{aa10}\text{-}X_{aa11}\text{-}X_{aa12}\text{-}X_{aa13}\text{-}X_{aa14}\text{-}X_{aa15}\text{-}NR_1R_2$$
$$\text{A}$$

wherein:

A is an electrophilic moiety such as a Michael acceptor or a chloro- or bromoacetyl group which is capable of reacting with a sulfhydryl group present on residue X_{aa13} to form a covalent thioether bond, thereby yielding a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

and wherein A can be optionally present; and wherein, if A is present, it can be a Gly or other spacer with a free amino terminus, which can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa13}, thereby providing an N-terminus to side chain lactam cyclic peptide; and wherein, if A is not present, the N-terminal amino group of the X_{aa1} residue can be used to cyclize the peptide via amide bond formation with a carboxyl group on the side chain of X_{aa13}, thereby providing an N-terminus to side chain lactam cyclic peptide;

and wherein, if A is present, it can be a Gly or other spacer with a free amino terminus, which can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa14}, thereby providing a head-to-tail cyclic peptide; and

wherein, if A is not present, the N-terminal amino group of the X_{aa1} amino acid can be used to cyclize the peptide via amide bond formation with the C-terminal α-carboxyl group of X_{aa14}, thereby providing a head-to-tail cyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring amino acid comprising Phe and Ala;

X_{aa2} is a naturally or nonnaturally occurring amino acid comprising ^{m}Phe and ^{m}Ala;

X_{aa3} is a naturally or nonnaturally occurring amino acid comprising ^{m}Nle and ^{m}Ala;

X_{aa4} is a naturally or nonnaturally occurring amino acid comprising ^{m}Gly, Gly and ^{m}Ala;

X_{aa5} is a naturally or nonnaturally occurring amino acid comprising Asp and Ala;

X_{aa6} is a naturally or nonnaturally occurring amino acid comprising Val (preferred) and Ala;

X_{aa7} is a naturally or nonnaturally occurring amino acid comprising ^{m}Phe and Phe;

X_{aa8} is a naturally or nonnaturally occurring amino acid selected from the group consisting of Tyr and Ala;

X_{aa9} is a naturally or nonnaturally occurring amino acid comprising ^{m}Gly ^{m}Ala and Gly;

X_{aa11} is a naturally or nonnaturally occurring amino acid comprising Tyr and Ala;

X_{aa12} is a naturally or nonnaturally occurring amino acid comprising Leu and Ala;

X_{aa13} is a naturally or nonnaturally occurring amino acid comprising L-Cys, D-Cys, Asp, Glu and Gly;

X_{aa14} is Gly or Gly followed by a PEG spacer comprised of at least two ethylene glycol units, or Gly followed by a PEG spacer comprised of at least two ethylene glycol units followed by a tag such as biotin,

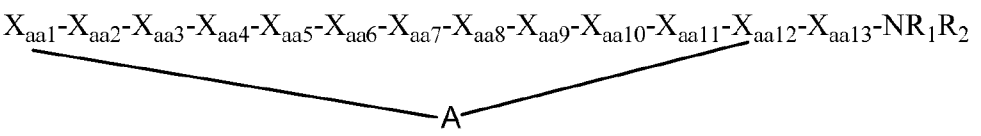
wherein X_{aa14} is optionally present and wherein the C-terminal carbonyl carbon of said amino acid is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group;

wherein, if X_{aa14} is not present, the C-terminal carbonyl carbon of X_{aa13} is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula III(a):



wherein:

A is an organic or peptidic linker between X_{aa1} and X_{aa12}, thereby providing a macrocyclic peptide;

X_{aa1} is a naturally or nonnaturally occurring aromatic or heteroaromatic or alkyl or heteroaryl alkyl amino acid;

X_{aa2} is a naturally or nonnaturally occurring alkyl or aromatic or charged amino acid;

X_{aa3} is a naturally or nonnaturally occurring aromatic or heteroaromatic or alkyl or heteroaryl alkyl amino acid;

X_{aa4} is a naturally or nonnaturally occurring aromatic or heteroaromatic or alkyl or heteroarylalkyl amino acid;

X_{aa5} is a naturally or nonnaturally occurring alkyl or heteroalkyl or aromatic or heteroaromatic or heteroarylalkyl amino acid;

X_{aa6} is a naturally or nonnaturally occurring heteroaromatic or positively charged amino acid;

X_{aa7} is a naturally or nonnaturally occurring polar or charged amino acid;

X_{aa8} is a naturally or nonnaturally occurring positively charged amino acid;

X_{aa9} is a naturally or nonnaturally occurring alkyl or heteroalkyl or aromatic or heteroaromatic or heteroarylalkyl amino acid;

X_{aa10} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or alkyl or heteroalkyl amino acid;

X_{aa11} is a naturally or nonnaturally occurring aromatic or heteroaromatic or arylalkyl or heteroarylalkyl or heteroalkyl or a positively charged amino acid;

X_{aa12} is a naturally or nonnaturally occurring amino acid possessing a functional group that can be appropriately activated to react with one end of linker A to yield a cyclic peptide;

X_{aa13} is a naturally or nonnaturally occurring amino acid or a spacer or a spacer followed by a tag or a spacer followed by a solubilizing or a PK-enhancing element.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula III(b):

$$X_{aa1}\text{-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-}X_{aa6}\text{-Asp-}X_{aa8}\text{-}X_{aa9}\text{-Phe-}X_{aa11}\text{-Cys-}X_{aa13}\text{-}NR_1R_2$$

A

wherein:

A is an electrophilic moiety such as a Michael acceptor or a chloro- or bromoacetyl group which is capable of reacting with the sulfhydryl group of Cys^{12} to form a covalent thioether bond, thereby yielding a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

X_{aa1} is selected from Phe and D-Phe;

X_{aa2} is selected from Leu, Arg and Phe;

X_{aa3} is selected from Ile, Leu and Phe;

X_{aa4} is selected from Val, Tyr and Phe;

X_{aa5} is selected from Ile and Val;

X_{aa6} is selected from Arg and His;

X_{aa8} is selected from Arg;

X_{aa9} is selected from Val, Leu, Tyr and Phe;

X_{aa11} is selected from Arg and Tyr;

X_{aa13} is Gly or Gly followed by a PEG spacer comprised of at least two ethylene glycol units,

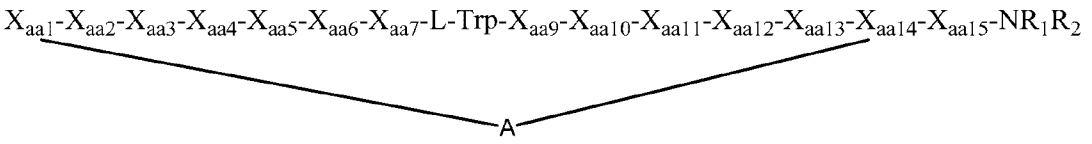
wherein X_{aa13} is optionally present and wherein the C-terminal carbonyl carbon of said amino acid is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group;

wherein, if X_{aa13} is not present, the C-terminal carbonyl carbon of Cys^{12} is attached to a nitrogen to form a carboxamide (NH_2), an alkyl carboxamide (NHR_1), or a dialkylcarboxamide (NR_1R_2);

wherein each of R_1 and R_2 is an alkyl or arylalkyl group.

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula I(d):



wherein:

A is a chloroacetyl group attached to the α-amine of the N-terminal X_{aa1} residue which is capable of reacting with a sulfhydryl group present on residue X_{aa14} to form a covalent thioether bond, thereby providing a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

and wherein,

X_{aa1} is selected from the group consisting of L-Phe, L-Trp, L-Tyr, L-Phe(4-OMe), L-Phe(4-F), L-Phe(4-Cl), L-Phe(4-Br), L-Phe(4-Me), L-Phe(4-CF_3), L-1-Nal, L-2-Nal, L-Bip, L-3-Pya, L-4-Pya, L-3-Tha;

X_{aa2} is selected from the group consisting of L-Ala, L-^{m}Ala, ^{m}Gly;

X_{aa3} is selected from the group consisting of L-Ala and L-Asn;

X_{aa4} is selected from the group consisting of L-Pro, L-Ala, L-α-Me-Pro, L-Pro(4-OH), L-Pro(4-NH_2), L-Pro(4S-Ph), L-Azt, L-Pip and L-Oic;

X_{aa5} is selected from the group consisting of L-Ala, L-His and L-Leu;

X_{aa6} is selected from the group consisting of L-Ala, L-Arg, L-Asp, L-His and L-Leu;

X_{aa7} is selected from the group consisting of ^{m}Gly, Gly, L-^{m}Ala, D-^{m}Ala, L-Pro, L-Ser, L-^{m}Ser, L-Dab, L-Arg and L-His;

X_{aa9} is selected from the group consisting of L-Ala, L-Arg and L-Ser;

X_{aa10} selected from the group consisting of L-Trp, L-Met and L-Bzt;

X_{aa11} is selected from the group consisting of L-Nle, L-^{m}Nle, L-^{m}Ala, L-Phe, L-^{m}Phe and L-^{m}Leu and L-^{m}Ser;

X_{aa12} is selected from the group consisting of L-^{m}Nle and L-^{m}Ala;

X_{aa13} is selected from the group consisting of L-Ala, L-Arg and L-Leu;

X_{aa14} is selected from the group consisting of L-Cys and D-Cys;

X_{aa15} is Gly or Gly followed by a PEG spacer comprised of twelve ethylene glycol units;

wherein X_{aa15} is optionally present and wherein the C-terminal carbonyl carbon of said amino acid is attached to a nitrogen to form a carboxamide ($CONH_2$);

wherein, if X_{aa15} is not present, the C-terminal carbonyl carbon of X_{aa14} is attached to a nitrogen to form a carboxamide ($CONH_2$).

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula II(c):

$$\underbrace{\text{L-Phe-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-}X_{aa6}\text{-}X_{aa7}\text{-}X_{aa8}\text{-}X_{aa9}\text{-L-Trp-L-Tyr-}X_{aa12}\text{-L-Cys}}_{A}\text{-}X_{aa14}\text{-}NR_1R_2$$

wherein:

A is a chloroacetyl group attached to the α-amine of the N-terminal X_{aa1} residue which is capable of reacting with a sulfhydryl group present on the Cys^{13} residue to form a covalent thioether bond, thereby yielding a macrocyclic peptide; wherein such thioether bond may or may not be oxidized to the corresponding diastereomeric sulfoxides;

and wherein,

X_{aa2} is selected from the group consisting of L-^{m}Ala and L-^{m}Phe;

X_{aa3} is selected from the group consisting of L-^{m}Ala and L-^{m}Nle;

X_{aa4} is selected from the group consisting of Gly, ^{m}Gly and L-^{m}Ala;

X_{aa5} is selected from the group consisting of L-Ala and L-Asp;

X_{aa6} is selected from the group consisting of L-Ala and L-Val;

X_{aa7} is selected from the group consisting of L-Phe and L-^{m}Phe;

X_{aa8} is selected from the group consisting of L-Ala and L-Tyr;

X_{aa9} is selected from the group consisting of Gly, ^{m}Gly and L-^{m}Ala;

X_{aa12} is selected from the group consisting of L-Leu and L-Ala;

X_{aa14} is Gly or Gly followed by a PEG spacer comprised of twelve ethylene glycol units,

wherein the C-terminal carbonyl carbon of X_{aa14} or of X_{aa14} followed by a PEG spacer is attached to a nitrogen to form a carboxamide ($CONH_2$).

In another embodiment, the subject matter described herein is directed to a polypeptide comprising a sequence of Formula III(c):

$$\underbrace{\text{L-Phe-}X_{aa2}\text{-}X_{aa3}\text{-}X_{aa4}\text{-}X_{aa5}\text{-L-Arg-L-Asp-L-Arg-}X_{aa9}\text{-Phe-L-Arg-L-Cys}}_{A}\text{-Gly-}NH_2$$

wherein:

A is a chloroacetyl group attached to the α-amine of the N-terminal L-Phe residue which is capable of reacting with a sulfhydryl group present on the L-Cys[12] residue to form a covalent thioether bond, thereby yielding a macrocyclic peptide;

and wherein,

X_{aa2} is selected from L-Leu, L-Arg and L-Phe;

X_{aa3} is selected from L-Ile and L-Phe;

X_{aa4} is selected from L-Phe, L-Tyr and L-Val;

X_{aa5} is selected from L-Ile and L-Val;

X_{aa9} is selected from L-Leu, L-Phe, L-Tyr and L-Val;

wherein the C-terminal carbonyl carbon of Gly[13] is attached to a nitrogen to form a carboxamide ($CONH_2$).

The present disclosure is also directed to a macrocyclic peptides comprising a sequence provided in Formula I.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula I(a).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula I(b).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula I(c).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula I(d).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula II.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula II(a).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula II(b).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula II(c).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula III.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula III(a).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula III(b).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula III(c).

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula IV.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula V.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula VI.

The present disclosure is also directed to macrocyclic peptides comprising a sequence provided in Formula VII.

The present disclosure is also directed to macrocyclic peptides comprising a sequence selected from the group consisting of: Compound Nos. 1, 2, 3, 4, 71, and 99.

The present disclosure is also directed to macrocyclic peptides comprising a sequence selected from those described herein.

The present disclosure is also directed to methods of using the macrocyclic peptides of the present disclosure to ameliorate and/or treat hyperproliferative disorders and/or viral disorders.

The present disclosure is also directed to a method of modulating an immune response in a subject comprising administering to the subject one or more macrocyclic peptides comprising the sequence selected from the peptides described herein.

The present disclosure is also directed to a method of enhancing, stimulating or increasing the immune response in the subject comprising administering to the subject one or more macrocyclic peptides comprising the sequence selected from those described herein.

The present disclosure is also directed to a method of promoting immune system inhibition of the growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of one or more macrocyclic peptides comprising the sequence selected from those peptides described herein.

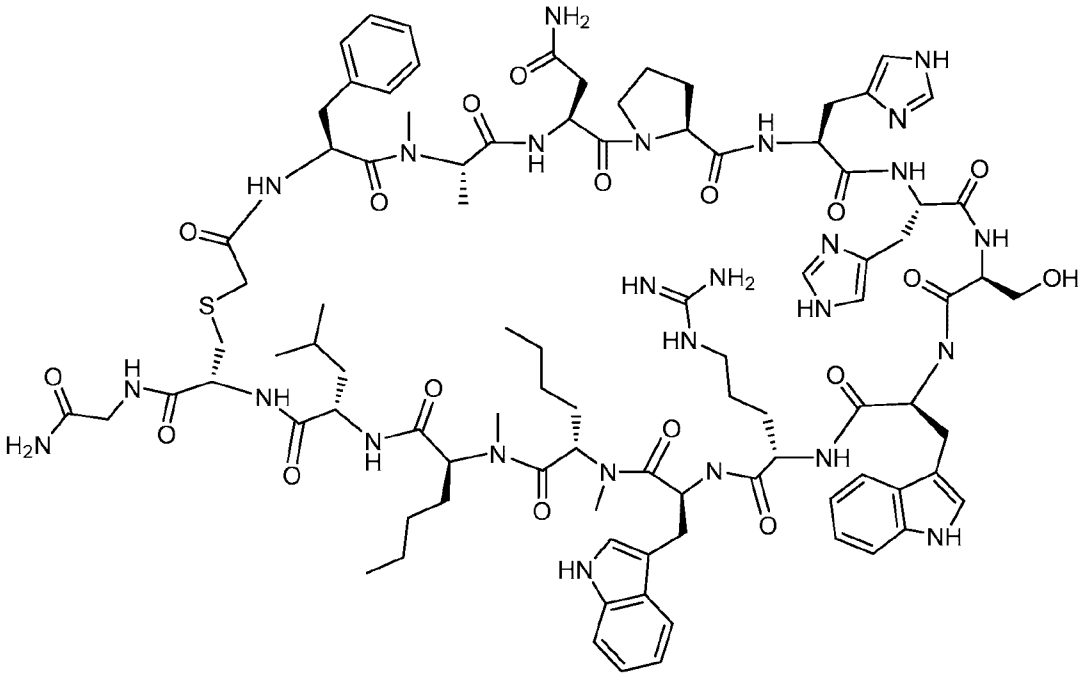
The present disclosure is also directed to a method of treating an infectious disease in a subject, comprising administering to a subject a therapeutically effective amount of one or more macrocyclic peptides comprising the sequence selected from those peptides described herein

The present disclosure is also directed to combinations comprising a sequence selected from the macrocyclic peptides described herein, with another agent, such an antimicrobial therapy, antiviral therapy, an additional immunomodulatory therapy, a vaccine, or a cancer chemotherapeutic agent.

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.

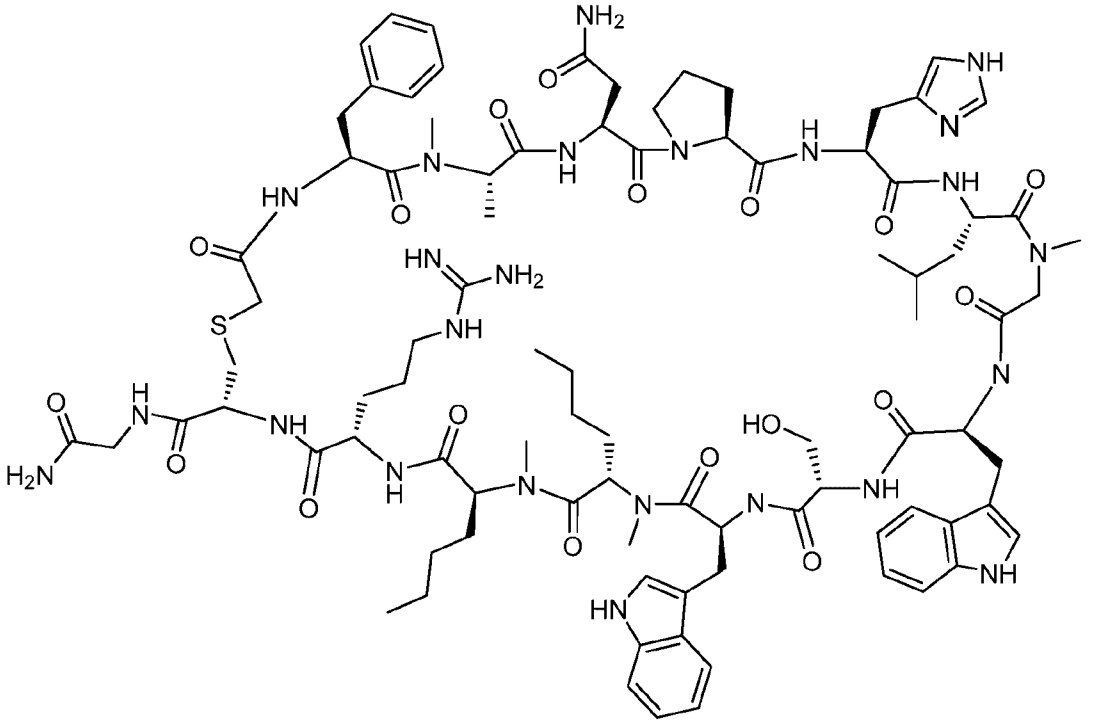
The terms "specific binding" or "specifically bind" refer to the interaction between a protein and a binding molecule, such as a compound or ligand. The interaction is dependent upon the presence of a particular structure (*i.e.*, an enzyme binding site, an antigenic determinant or epitope) of the protein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

Other embodiments include polypeptides comprising the following structures:



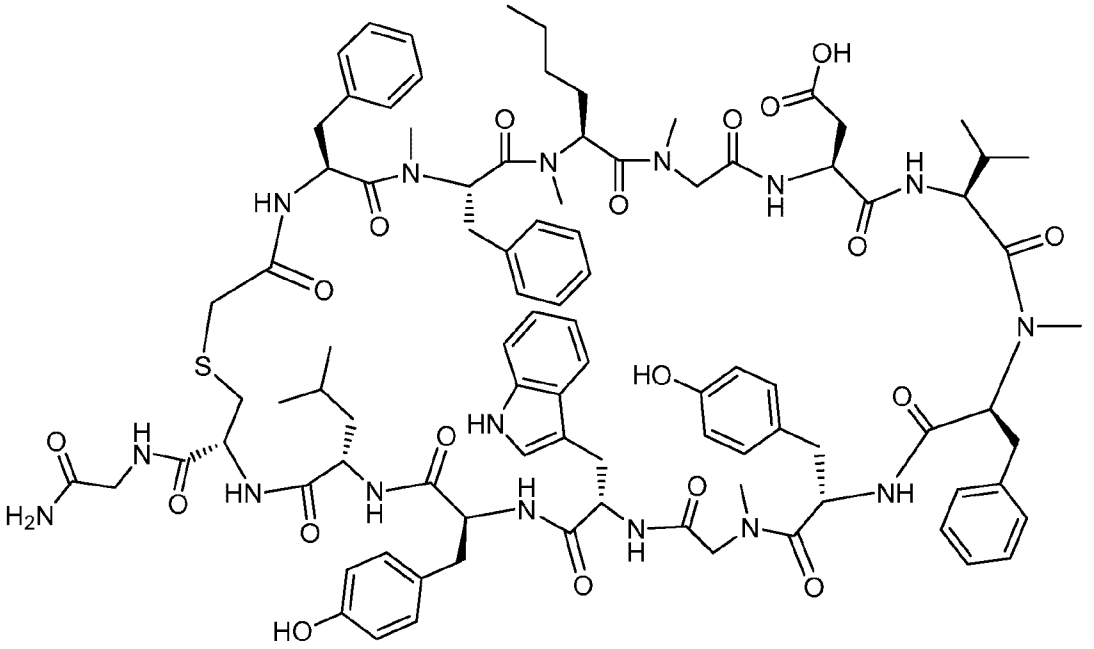
Formula IV

or



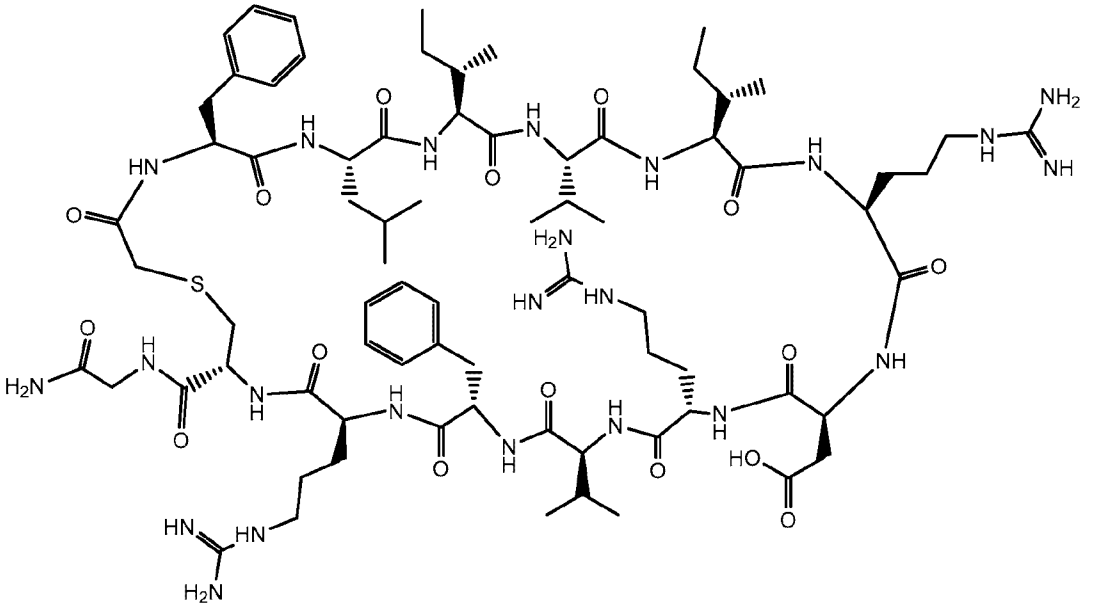
Formula V

or



Formula VI

or



Formula VII.

Another embodiment is a pharmaceutical composition comprising a polypeptide of Formula I(a), I(b), I(c), II(a), II(b), III(a), III(b), IV, V, VI, or VII, or a peptide comprising at least one of the macrocyclic peptides described herein.

Another embodiment is directed to a pharmaceutical combination comprising a polypeptide of Formula I(a), I(b), I(c), II(a), II(b), III(a), III(b), IV, V, VI, or VII or a macrocyclic peptide described herein, and at least one therapeutic agent selected from the group consisting of an antimicrobial, an antiviral, anti-cancer, anti-diabetic agent, an anti-obesity agent, an anti-hypertensive agent, an anti-atherosclerotic agent and a lipid-lowering agent.

Another embodiment is directed to a pharmaceutical combination of a polypeptide of Formula I(a), I(b), I(c), II(a), II(b), III(a), III(b), IV, V, VI, or VII, or a macrocyclic peptide described herein, with another agent disclosed herein.

Another embodiment is directed to a method for treating or delaying the progression or onset of cancer and/or virology disorder, which comprises administering to a mammalian species in need of treatment a therapeutically effective amount of a polypeptide of Formula I(a), I(b), I(c), II(a), II(b), III(a), III(b), IV, V, VI, or VII, or a macrocyclic peptide described herein.

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

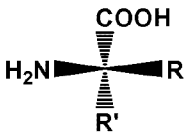
herein may be prepared using the radioactive isotope ^{125}I and the resulting radiolabeled peptide may be used to develop a binding assay or for metabolism studies. Alternatively, and for the same purpose, a macrocyclic peptide described herein may be converted to a radiolabeled form by catalytic tritiation 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.

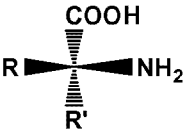
Preferred peptides include at least one of the macrocyclic peptides provided herein and these peptides may be included in pharmaceutical compositions and combinations.

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:



L- or S-α-amino acid
(if R=H)



D- or R-α-amino acid
(if R=H)

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 term "naturally occurring amino acid side chain", as used herein, refers 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 term "non-naturally occurring amino acid side chain", as used herein, refers 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:

C_2-C_7alkenyl, C_1-C_3alkoxyC_1-C_3alkyl, C_1-C_6alkoxycarbonylC_1-C_3alkyl, C_1-C_7alkyl, C_1-C_3alkylsulfanylC_1-C_3alkyl, amidoC_1-C_3alkyl, aminoC_1-C_3alkyl, azaindolylC_1-C_3alkyl, benzothiazolylC_1-C_3alkyl, benzothienylC_1-C_3alkyl, benzyloxyC_1-C_3alkyl, carboxyC_1-C_3alkyl, C_3-C_6cycloalkylC_1-C_3alkyl, diphenylmethyl, furanylC_1-C_3alkyl, imidazolylC_1-C_3alkyl, naphthylC_1-C_3alkyl, pyridinylC_1-C_3alkyl, thiazolylC_1-C_3alkyl, thienylC_1-C_3alkyl;

biphenylC_1-C_3alkyl wherein the biphenyl is optionally substituted with a methyl group;

indolylC_1-C_3alkyl, wherein the indolyl part is optionally substituted with one group selected from C_1-C_3alkyl, carboxyC_1-C_3alkyl, halo, hydroxy, and phenyl, wherein the phenyl is further optionally substituted by one, two, or three groups independently selected from C_1-C_3alkoxy, C_1-C_3alkyl, and halo;

NR^aR^b(C_1-C_7alkyl), wherein R^a and R^b are independently selected from hydrogen, C_2-C_4alkenyloxycarbonyl, C_1-C_3alkyl, C_1-C_3alkylcarbonyl, C_3-C_6cycloalkylcarbonyl, 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^dcarbonylC_1-C_3alkyl, wherein R^c and R^d are independently selected from hydrogen, C_1-C_3alkyl, and triphenylmethyl;

phenylC_1-C_3alkyl wherein the phenyl part is optionally substituted with one, two, three, four, or five groups independently selected from C_1-C_4alkoxy, C_1-C_4alkyl, C_1-C_3alkylsulfonylamino, amido, amino, aminoC_1-C_3alkyl, aminosulfonyl, carboxy, cyano, halo, haloC_1-C_3alkyl, hydroxy, -$NC(NH_2)_2$, nitro, and -$OP(O)(OH)_2$; and

phenoxyC_1-C_3alkyl wherein the phenyl is optionally substituted with a C_1-C_3alkyl group.

The term "C_2-C_4alkenyl", 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.

The term "C_2-C_7alkenyl", 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 "C_2-C_4alkenyloxy", as used herein, refers to a C_2-C_4alkenyl group attached to the parent molecular moiety through an oxygen atom.

The term "C_1-C_3alkoxy", as used herein, refers to aC_1-C_3alkyl group attached to the parent molecular moiety through an oxygen atom.

The term "C_1-C_4alkoxy", as used herein, refers to a C_1-C_4alkyl group attached to the parent molecular moiety through an oxygen atom.

The term "C_1-C_6alkoxy", as used herein, refers to a C_1-C_6alkyl group attached to the parent molecular moiety through an oxygen atom.

The term "C_1-C_3alkoxyC_1-C_3alkyl", as used herein, refers to a C_1-C_3alkoxy group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "C_1-C_6alkoxycarbonyl", as used herein, refers to a C_1-C_6alkoxy group attached to the parent molecular moiety through a carbonyl group.

The term "C_1-C_6alkoxycarbonylC_1-C_3alkyl", as used herein, refers to a C_1-C_6alkoxycarbonyl group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "C_1-C_3alkyl", as used herein, refers to a group derived from a straight or branched chain saturated hydrocarbon containing from one to three carbon atoms.

The term "C_1-C_4alkyl", as used herein, refers to a group derived from a straight or branched chain saturated hydrocarbon containing from one to four carbon atoms.

The term "C_1-C_6alkyl", 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 "C_1-C_3alkylcarbonyl", as used herein, refers to a C1-C3alkyl group attached to the parent molecular moiety through a carbonyl group.

The term "C_1-C_3alkylsulfanyl", as used herein, refers to a C_1-C_3alkyl group attached to the parent molecular moiety through a sulfur atom.

The term "C_1-C_3alkylsulfanylC_1-C_3alkyl", as used herein, refers to a C_1-C_3alkylsulfanyl group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "C_1-C_3alkylsulfonyl", as used herein, refers to a C_1-C_3alkyl group attached to the parent molecular moiety through a sulfonyl group.

The term "C_1-C_3alkylsulfonylamino", as used herein, refers to a C_1-C_3alkylsulfonyl group attached to the parent molecular moiety through an amino group.

The term "amido", as used herein, refers to -C(O)NH_2.

The term "amidoC_1-C_3alkyl", as used herein, refers to an amido group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "amino", as used herein, refers to -NH_2.

The term "aminoC_1-C_3alkyl", as used herein, refers to an amino group attached to the parent molecular moiety through a C_1-C_3alkyl group.

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

The term "azaindolylC_1-C_3alkyl", as used herein, refers to an azaindolyl group attached to the parent molecular through a C_1-C_3alkyl group. The azaindolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "benzothiazolylC_1-C_3alkyl", as used herein, refers to an benzothiazolyl group attached to the parent molecular through a C_1-C_3alkyl group. The benzothiazolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "benzothienylC_1-C_3alkyl", as used herein, refers to a benzothienyl group attached to the parent molecular through a C_1-C_3alkyl 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 "benzyloxyC_1-C_3alkyl", as used herein, refers to a benzyloxy group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "biphenylC_1-C_3alkyl", as used herein, refers to a biphenyl group attached to the parent molecular moiety through a C_1-C_3alkyl group. The biphenyl 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 -CO_2H.

The term "carboxyC_1-C_3alkyl", as used herein, refers to a carboxy group attached to the parent molecular moiety through a C_1-C_3alkyl group.

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

The term "C_3-C_6cycloalkyl", as used herein, refers to a saturated monocyclic, hydrocarbon ring system having three to six carbon atoms and zero heteroatoms.

The term "C_3-C_6cycloalkylC_1-C_3alkyl", as used herein, refers to a C_3-C_6cycloalkyl group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "C_3-C_6cycloalkylcarbonyl", as used herein, refers to a C_3-C_6 cycloalkyl group attached to the parent molecular moiety through a carbonyl group.

The term "furanylC_1-C_3alkyl", as used herein, refers to a furanyl group attached to the parent molecular moiety through a C_1-C_3alkyl 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 "haloC_1-C_3alkyl", as used herein, refers to a C_1-C_3alkyl group 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 "hydroxy", as used herein, refers to -OH.

The term "imidazolylC_1-C_3alkyl", as used herein, refers to an imidazolyl group attached to the parent molecular moiety through a C_1-C_3alkyl group. The imidazolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "indolylC_1-C_3alkyl", as used herein, refers to an indolyl group attached to the parent molecular moiety through a C_1-C_3alkyl group. The indolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

The term "naphthylC_1-C_3alkyl", as used herein, refers to a naphthyl group attached to the parent molecular moiety through a C_1-C_3alkyl 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 $-NO_2$.

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_4alkenyloxycarbonyl, C_1-C_3alkylcarbonyl, C_3-C_6cycloalkylcarbonyl, furanylcarbonyl, and phenylcarbonyl.

The term "$NR^aR^b(C_1\text{-}C_3)$alkyl", as used herein, refers to an NR^aR^b group attached to the parent molecular moiety through a C_1-C_3alkyl 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 independently selected from hydrogen, C_1-C_3alkyl, and triphenylmethyl.

The term "NR^cR^dcarbonyl", as used herein, refers to an NR^cR^d group attached to the parent molecular moiety through a carbonyl group.

The term "NR^cR^dcarbonylC_1-C_3alkyl", as used herein, refers to an NR^cR^dcarbonyl group attached to the parent molecular moiety through a C_1-C_3alkyl group.

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

The term "phenoxyC_1-C_3alkyl", as used herein, refers to a phenoxy group attached to the parent molecular moiety through a C_1-C_3alkyl group.

The term "phenylC_1-C_3alkyl", as used herein, refers to a phenyl group attached to the parent molecular moiety through a C_1-C_3alkyl group.

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

The term "pyridinylC_1-C_3alkyl", as used herein, refers to a pyridinyl group attached to the parent molecular moiety through a C_1-C_3alkyl 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 -S-.

The term "sulfonyl", as used herein, refers to $-SO_2-$.

The term "thiazolylC_1-C_3alkyl", as used herein, refers to a thiazolyl group attached to the parent molecular moiety through a C_1-C_3alkyl group. The thiazolyl group can be attached to the alkyl moiety through any substitutable atom in the group.

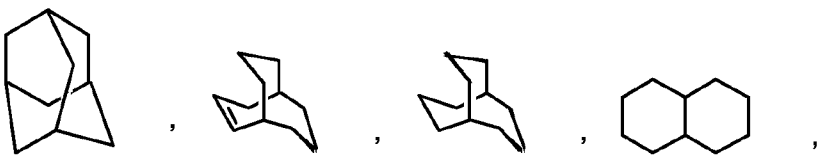
The term "thienylC_1-C_3alkyl", as used herein, refers to a thienyl group attached to the parent molecular moiety through a C_1-C_3alkyl group. The thienyl group can be attached to the alkyl moiety through any substitutable atom in the group.

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, 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.

Unless otherwise indicated, the term "alkyl" as employed herein alone or as part of another group includes, without limitation, both straight and branched chain hydrocarbons, containing 1 to 40 carbons, preferably 1 to 20 carbons, more preferably 1 to 8 carbons, in the normal chain, such as methyl, ethyl, propyl, isopropyl, butyl, *t*-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl, the various branched chain isomers thereof, and the like. Further, alkyl groups, as defined herein, may optionally be substituted on any available carbon atom with one or more functional groups commonly attached to such chains, such as, but not limited to alkyl, aryl, alkenyl, alkynyl, hydroxy, arylalkyl, cycloalkyl, cycloalkylalkyl, alkoxy, arylalkyloxy, heteroaryloxy, heteroarylalkyloxy, alkanoyl, halo, hydroxyl, thio, nitro, cyano, carboxyl, carbonyl (), carboxamido, amino, alkylamino, dialkylamino, amido, alkylamino, arylamido, heteroarylamido, azido, guanidino, amidino, phosphonic, phosphinic, sulfonic, sulfonamido, haloaryl, CF_3, OCF_2, OCF_3, aryloxy, heteroaryl, cycloalkylalkoxyalkyl, cycloheteroalkyl and the like to form alkyl groups such as trifluoro methyl, 3-hydroxyhexyl, 2-carboxypropyl, 2-fluoroethyl, carboxymethyl, cyanobutyl and the like.

Unless otherwise indicated, the term "cycloalkyl" as employed herein alone or as part of another group includes, without limitation, saturated or partially unsaturated (containing 1 or 2 double bonds) cyclic hydrocarbon groups containing 1 to 3 rings, appended or fused, including monocyclic alkyl, bicyclic alkyl and tricyclic alkyl, containing a total of 3 to 20 carbons forming the rings, preferably 4 to 7 carbons, forming each ring; which may be fused to 1 aromatic ring as described for aryl, which include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, cyclohexenyl,



any of which groups may be optionally substituted through any available carbon atoms with 1 or more groups selected from hydrogen, halo, haloalkyl, alkyl, haloalkyl, alkoxy, haloalkoxy, alkenyl, trifluoromethyl, trifluoromethoxy, alkynyl, cycloalkylalkyl,

fluorenyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylthio, arylazo, heteroarylalkyl, heteroarylalkenyl, heteroarylheteroaryl, heteroaryloxy, hydroxy, nitro, oxo, cyano, carboxyl, carbonyl (), carboxamido, amino, substituted amino wherein the amino includes 1 or 2 substituents (which are alkyl, aryl or any of the other aryl compounds mentioned in the definitions), amido, azido, guanidino, amidino, phosphonic, phosphinic, sulfonic, sulfonamido, thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkoxyarylthio, alkylcarbonyl, arylcarbonyl, alkylaminocarbonyl, arylaminocarbonyl, alkoxycarbonyl, aminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonylamino, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonylamino or arylsulfonaminocarbonyl, or any of alkyl substituents as set out above.

The term "aryl" as employed herein alone or as part of another group refers, without limitation, to monocyclic and bicyclic aromatic groups containing 6 to 10 carbons in the ring portion (such as phenyl or naphthyl) and may optionally include one to three additional rings fused to "aryl" (such as aryl, cycloalkyl, heteroaryl or heterocycloalkyl rings) and may be optionally substituted through any available carbon atoms with 1 or more groups selected from hydrogen, alkyl, halo, haloalkyl, alkoxy, haloalkoxy, alkenyl, trifluoromethyl, trifluoromethoxy, alkynyl, cycloalkylalkyl, fluorenyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylthio, arylazo, heteroarylalkyl, heteroarylalkenyl, heteroaryloxy, heteroarylalkyloxy, heteroarylalkyloxyalkyl, hydroxy, nitro, oxo, cyano, amino, substituted amino wherein the amino includes 1 or 2 substituents (which are alkyl, cycloalkyl, heterocycloalkyl, heteroaryl, or aryl or any of the other aryl compounds mentioned in the definitions), thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkoxyarylthio, alkylcarbonyl, arylcarbonyl, alkylaminocarbonyl, cycloalkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, heteroarylalkylaminocarbonyl alkoxycarbonyl, aminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonylamino, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonylamino or arylsulfonaminocarbonyl, or any of alkyl substituents as set out above.

The term "arylalkyl" as used herein alone or as part of another group refers, without limitation, to alkyl groups as defined above having an aryl substituent, such as

benzyl, phenethyl or naphthylpropyl, wherein said aryl and/or alkyl groups may optionally be substituted as defined above.

The term "alkoxy", "aryloxy", "heteroaryloxy", "arylalkyloxy", or "heteroarylalkyloxy" as employed herein alone or as part of another group includes, without limitation, an alkyl or aryl group as defined above linked through an oxygen atom.

The term "heterocyclo", "heterocycle", "heterocyclyl" or "heterocyclic", as used herein, represents, without limitation, an unsubstituted or substituted stable 4-, 5-, 6-, or 7-membered monocyclic ring system which may be saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from nitrogen, sulfur, oxygen and/or a SO or SO_2 group, wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic groups include, but are not limited to, tetrahydrofuranyl, tetrahydrothiophenyl pyrrolidinyl, piperidinyl, piperazinyl, oxopyrrolidinyl, oxopiperazinyl, oxopiperidinyl and oxadiazolyl. Optionally a heterocyclo group may be substituted with one or more functional groups, such as those described for "alkyl" or "aryl".

The term "heterocycloalkyl" as used herein alone or as part of another group refers, without limitation, to alkyl groups as defined above having a heterocycloalkyl substituent, wherein said "heterocyclo" and/or alkyl groups may optionally be substituted as defined above.

The term "heteroaryl" as used herein refers, without limitation, to a 5-, 6- or 7-membered aromatic heterocyclic ring which contains one or more heteroatoms selected from nitrogen, sulfur, oxygen and/or a SO or SO_2 group. Such rings may be fused to another aryl or heteroaryl ring and include possible N-oxides; examples of such heteroaryl groups include, but are not limited to, furan, pyrrole, thiophene, pyridine, pyrimidine, pyrazine, pyridazine, isoxazole, oxazole, imidazole and the like. Optionally a heteroaryl group may be substituted with one or more functional groups commonly attached to such chains, such as those described for "alkyl" or "aryl".

The term "heteroarylalkyl" as used herein alone or as part of another group refers, without limitation, to alkyl groups as defined above having a heteroaryl substituent,

wherein said heteroaryl and/or alkyl groups may optionally be substituted as defined above.

The "inhibitory concentration" of PD-1/PD-L1 inhibitor is intended to mean the concentration at which a compound screened in an assay of the disclosure inhibits a measurable percentage of the interaction of PD-1 with PD-L1. Examples of "inhibitory concentration" values range from IC_{50} to IC_{90}, and are preferably, IC_{50}, IC_{60}, IC_{70}, IC_{80}, or IC_{90}, which represent 50%, 60%, 70%, 80% or 90% reduction in PD-1/PD-L1 binding activity, respectively. More preferably, the "inhibitory concentration" is measured as the IC_{50} value. It is understood that another designation for IC_{50} is the half-maximal inhibitory concentration.

Binding of the macrocyclic peptides to PD-L1 can be measured, for example, by methods such as homogeneous time-resolved fluorescence (HTRF), Surface 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 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 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 administration. Thus, it is not useful to specify an exact effective amount in advance.

The macrocyclic peptides of the present disclosure show potent binding activity to PD-L1, both in HTRF assays, as well as cellular binding assays. In addition, the macrocyclic peptides also demonstrate biological activity in CMV recall and HIV Elispot

assays demonstrating their utility in ameliorating and/or treating hyperproliferative disorders, such as cancer, and virology indications, including HIV.

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 IFNγ secretion, and enhancement of HIV-specific T cell IFNg 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).

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" 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 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", "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® 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.

A "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present disclosure is the PD-1 receptor.

The term "macrocyclic peptide derivatives" refers to any modified form of the macrocyclic peptides disclosed herein, *e.g.*, mutations, isoforms, peptides with altered linker backbones, conjugates with an antibody and/or another agent, etc..

As used herein, a macrocyclic peptide of the present disclosure that "specifically binds to human PD-L1" is intended to refer to a macrocyclic peptide that binds to human PD-L1 with an IC_{50} of less than about 200 nM, less than about 150 nM, less than about 100 nM, less than about 80 nM, less than about 60 nM, less than about 40 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 5 nM, less than about 1 nM, or less. In this context, the term "about" shall be construed to mean anywhere between ± 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nM more or less than the cited amount.

The term "treatment" or "therapy" refers to administering an active agent with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a condition (*e.g.*, a disease), the symptoms of the condition, or to prevent or delay the onset of the symptoms, complications, biochemical indicia of a disease, or otherwise arrest or inhibit further development of the disease, condition, or disorder in a statistically significant manner.

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

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 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 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 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), 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 (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.

Variant Macrocyclic Peptides

In yet another embodiment, a macrocyclic peptide of the disclosure comprises amino acid sequences that are homologous to the amino acid sequences of the macrocyclic peptides described herein, and wherein the macrocyclic peptides retain the

desired functional and/or biological properties of the macrocyclic peptide of the disclosure.

For example, the disclosure provides a macrocyclic peptide, or antigen-binding portion thereof, comprising: an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the compounds described herein; and the macrocyclic peptide exhibits one or more of the following properties:

(a) the macrocyclic peptide binds to human PD-L1 with an IC_{50} of 200 nM or less;

(b) the macrocyclic peptide does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) the macrocyclic peptide increases CMV-specific T cell IFNγ secretion;

(d) the macrocyclic peptide increases HIV-specific T cell IFNγ secretion;

(e) the macrocyclic peptide binds to human PD-1 and one or more of the following: cynomolgus monkey PD-1; woodchuck PD-1, and/or mouse PD-1;

(f) the macrocyclic peptide inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(g) the macrocyclic peptide is capable of competing with binding of anti-PD-1 monoclonal antibodies, including nivolumab (BMS-936558, MDX-1106);

(h) the macrocyclic peptide inhibits tumor cell growth in a cellular assay and/or *in vivo* assay; and/or

(i) the macrocyclic peptide inhibits HIV in a cellular assay and/or *in vivo* assay.

In other embodiments, the macrocyclic peptide amino acid sequences may be about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% homologous to the sequences set forth above. In this context, the term "about" shall be construed to mean anywhere between 1, 2, 3, 4, or 5 percent more or less than the cited amount. A macrocyclic peptide of the present disclosure having sequences with high identity (*i.e.*, 80% or greater) to the sequences set forth above, can be obtained by mutating the sequences during chemical synthesis, for example, followed by testing of the altered macrocyclic peptide for retained function (*i.e.*, the functions set forth in (a)

through (i) above) using the functional assays described herein. The biological and/or functional activity of the variant macrocyclic peptide amino acid sequences may be at least about 1x, 2x, 3x, 4x, 5x, 6x,7x, 8x, 9x, or 10x more than the reference macrocyclic peptide on which the variant is based. In this context, the term "about" shall be construed to mean anywhere between 0.1x, 0.2x, 0.3x, 0.4x, 0.5x, 0.6x, 0.7x, 0.8x, or 0.9x more or less than the cited amount.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions / total # of positions.times.100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of Meyers E. et al., (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman et al. (*J. Mol. Biol.*, 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG® software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Macrocyclic Peptides with Conservative Modifications

In yet another embodiment, a macrocyclic peptide of the disclosure comprises amino acid sequences that are homologous to the amino acid sequences of the macrocyclic peptides described herein, and wherein the macrocyclic peptides retain the desired functional and/or biological properties of the macrocyclic peptide of the disclosure.

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For example, the disclosure provides a macrocyclic peptide, or antigen-binding portion thereof, comprising: an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the macrocyclic peptides described herein, wherein one or more amino acids have been substituted with a conservative amino acid; and the macrocyclic peptide exhibits one or more of the following properties:

(a) the macrocyclic peptide binds to human PD-L1 with an IC_{50} of 200 nM or less

(b) the macrocyclic peptide does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) the macrocyclic peptide increases CMV-specific T cell IFNγ secretion;

(d) the macrocyclic peptide increases HIV-specific T cell IFNγ secretion;

(e) the macrocyclic peptide binds to human PD-L1 and one or more of the following: cynomolgus monkey PD-L1; woodchuck PD-L1, and/or mouse PD-L1;

(f) the macrocyclic peptide inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(g) the macrocyclic peptide is capable of competing with binding of anti-PD-1 monoclonal antibodies, including nivolumab (BMS-936558, MDX-1106);

(h) the macrocyclic peptide inhibits tumor cell growth in a cellular assay and/or *in vivo* assay; and/or

(i) the macrocyclic peptide inhibits HIV in a cellular assay and/or *in vivo* assay.

As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the macrocyclic peptide containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the disclosure by standard techniques known in the art, such as substitution of peptide amidites during chemical synthesis, site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side

chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the antigen binding regions of macrocyclic peptides of the disclosure can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (a) thru (i) above) using the functional assays described herein. Conservative amino acid substitutions may also be selected from one or more non-naturally occurring amino acids disclosed herein.

Pharmaceutical Compositions

The disclosure further relates to the polypeptides described herein wherein the sequence comprises one or more amino acid deletions from either the C-terminus and/or the N-terminus.

In preferred embodiments, the following N-terminal Compound No. 99 deletion polypeptides are encompassed by the present disclosure: X1-X13, X2-X13, X3-X13, X4-X13, X5-X13, X6-X13, X7-X13, X8-X13, X9-X13, X10-X13, X11-X13, and/or X12-X13 of Compound No. 99, wherein each X is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In preferred embodiments, the following C-terminal Compound No. 99 deletion polypeptides are encompassed by the present disclosure: X1-X13, X1-X12, X1-X11, X1-X10, X1-X9, X1-X8, X1-X7, X1-X6, X1-X5, X1-X4 and/or X1-X3 of Compound No. 99, wherein each X is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In preferred embodiments, the following N-terminal Compound No. 1 deletion polypeptides are encompassed by the present disclosure: X1-X15, X2-X15, X3-X15, X4-X15, X5-X15, X6-X15, X7-X15, X8-X15, X9-X15, X10-X15, X11-X15, and/or X12-

X15 of Compound No. 1, wherein each 1 is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In preferred embodiments, the following C-terminal Compound No. 1 deletion polypeptides are encompassed by the present disclosure: X1-X15, X1-X14, X1-X13, X1-X12, X1-X11, X1-X10, X1-X9, X1-X8, X1-X7, X1-X6, X1-X5, X1-X4 and/or X1-X3 of Compound No. 1, wherein each X is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In preferred embodiments, the following N-terminal Compound No. 71 deletion polypeptides are encompassed by the present disclosure: X1-X14, X2-X14, X3-X14, X4-X14, X5-X14, X6-X14, X7-X14, X8-X14, X9-X14, X10-X14, X11-X14, and/or X12-X14 of Compound No. 71, wherein each X is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In preferred embodiments, the following C-terminal Compound No. 71 deletion polypeptides are encompassed by the present disclosure: X1-X14, X1-X13, X1-X12, X1-X11, X1-X10, X1-X9, X1-X8, X1-X7, X1-X6, X1-X5, X1-X4 and/or X1-X3 of Compound No. 71, wherein each X is representative of an amino acid at the indicated position for each peptide as outlined herein. The present disclosure also encompasses cyclic forms of these deletion mutants using the linking chemistries described elsewhere herein.

In another aspect, the present disclosure provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of macrocyclic peptides, or antigen-binding portion(s) thereof, 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" 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 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, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of the disclosure also may include a 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, 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 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 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. 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 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 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 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, 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 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 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 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 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 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 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 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, 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 disclosure include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody 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 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 antibody concentration of about 1-1000 .mu.g/ml and in some methods about 25-300 .mu.g/ml.

Alternatively, the macrocyclic peptide can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the macrocyclic peptide in the patient. 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

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. 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 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 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 anti-CTLA-4 antibody, 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 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 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 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, 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 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. 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

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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 antibody, or antigen-binding portion thereof, 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 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 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 monoclonal antibody, or an antigen-binding portion thereof, which specifically binds to human, woodchuck, cyno, and/or mouse PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof 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 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.

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 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, or antigen-binding portion thereof.

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 differentiation 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 a 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.

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 (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 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 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 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* 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.

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, or antigen-binding portion thereof, such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human PD-L1 macrocyclic peptide (such as any of the macrocyclic peptides described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Similar to its application to tumors as discussed above, antibody mediated 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 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. 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, 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, 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, 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 lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, 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 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

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 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 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 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 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 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 (*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, or antigen-binding portion thereof, 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. 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.*, 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 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 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 every 21 days. Co-administration of the macrocyclic peptides, or antigen binding fragments thereof, 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 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 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

The combination of the macrocyclic peptides of the present disclosure with another PD-L1 antagonist and/or CTLA-4 antagonist is useful for enhancement of an immune response against a hyperproliferative disease by blockade of PD-L1 and CTLA-4. 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 an antibody combination, or a combination of antigen-binding portions thereof, 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 hyperproliferative disease with an immunostimulatory therapeutic agent, comprising administering a macrocyclic peptide of the present disclosure and a subtherapeutic dose of anti-CTLA-4 antibody to a subject.

Blockade of PD-L1 and CTLA-4 by macrocyclic peptides can enhance the immune response to cancerous cells in the patient. Cancers whose growth may be inhibited 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 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 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.

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, an anti-CTLA-4 antibody and a macrocyclic peptide of the present disclosure can be administered sequentially, such as anti-CTLA-4 being administered first and the macrocyclic peptide second, or the macrocyclic peptide being administered first and anti-CTLA-4 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 combination anti-CTLA-4 antibody and the macrocyclic peptide may be concurrent, the second administration may be sequential with anti-CTLA-4 first and the macrocyclic peptide second, and the third administration may be sequential with the macrocyclic peptide first and anti-CTLA-4 second, etc. Another representative dosing scheme may involve a first administration that is sequential with the macrocyclic peptide first and anti-CTLA-4 second, and subsequent administrations may be concurrent.

Optionally, the combination of the macrocyclic peptide and anti-CTLA-4 agent 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 tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

A combined PD-L1 macrocyclic peptide and CTLA-4 blockade can be further 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 et al., Cancer Vaccines, Chapter 61, pp. 3023-3043 in DeVita 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, *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, 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 CTLA-4 blockade using the antibody compositions described herein 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 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 a 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 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 (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 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 CTLA-4 blockade to activate more potent anti-tumor responses.

A combined anti-PD-L1 macrocyclic peptide and CTLA-4 blockade may also be further combined with standard cancer treatments. For example, a combined macrocyclic peptide and CTLA-4 blockade may be effectively combined with chemotherapeutic regimes. In these instances, as is observed with the combination of a macrocyclic peptide and anti-CTLA-4 agent, 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 anti-CTLA-4 agent further in combination with decarbazine for the treatment of melanoma. Another example is a combination of a macrocyclic peptide and anti-CTLA-4 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 CTLA-4 blockade 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 CTLA-4 blockade 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 CTLA-4 blockade. 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 CTLA-4 blocking agents 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-1 and CTLA-4 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.

In another example, a combination of a macrocyclic peptide and anti-CTLA-4 agent can be used in conjunction with anti-neoplastic macrocyclic agents, such as RITUXAN® (rituximab), HERCEPTIN® (trastuzumab), BEXXAR® (tositumomab), ZEVALIN® (ibritumomab), CAMPATH® (alemtuzumab), Lymphocide (eprtuzumab), 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 CTLA-4 or PD-L1. In an exemplary embodiment, a treatment of a hyperproliferative disease (*e.g.*, a cancer tumor) may include an anti-cancer antibody in combination with a macrocyclic peptide and anti-CTLA-4 agents, 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. 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 (Hahne, M. et al.,

Science, 274:1363-1365 (1996)). In another example, antibodies to each of these entities may be further combined with a macrocyclic peptide and anti-CTLA-4 combination 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. 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. 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.

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 CTLA-4 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* 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 an anti-CTLA-4 antagonist, 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 anti-CTLA-4 antibody 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 CTLA-4 blockade, 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 CTLA-4 blockade (*i.e.*, immunostimulatory therapeutic macrocyclic peptides anti-PD-L1 and anti-CTLA-4) 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, intraoccular, intravenous, or intestinal administration. The compound is formulated 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 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 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.

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 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 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.

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.

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.

The compositions described herein may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropyl- methacrylamide-phenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compositions described herein may be 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.

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.

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.

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 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 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 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 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, 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 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 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 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 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 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

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.

Peptide Synthesis

The macrocyclic peptides of the present disclosure can be produced by methods known in the art, such as they can be synthesized chemically, recombinantly in a cell free system, recombinantly within a cell or can be isolated from a biological source. 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 methyl- oxycarbonyl) group for temporary protection of the α-amino

group, in combination 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, Undenfriend, S. et al., eds., Academic Press, San Diego (1987).

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.

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 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 purified by reverse phase HPLC.

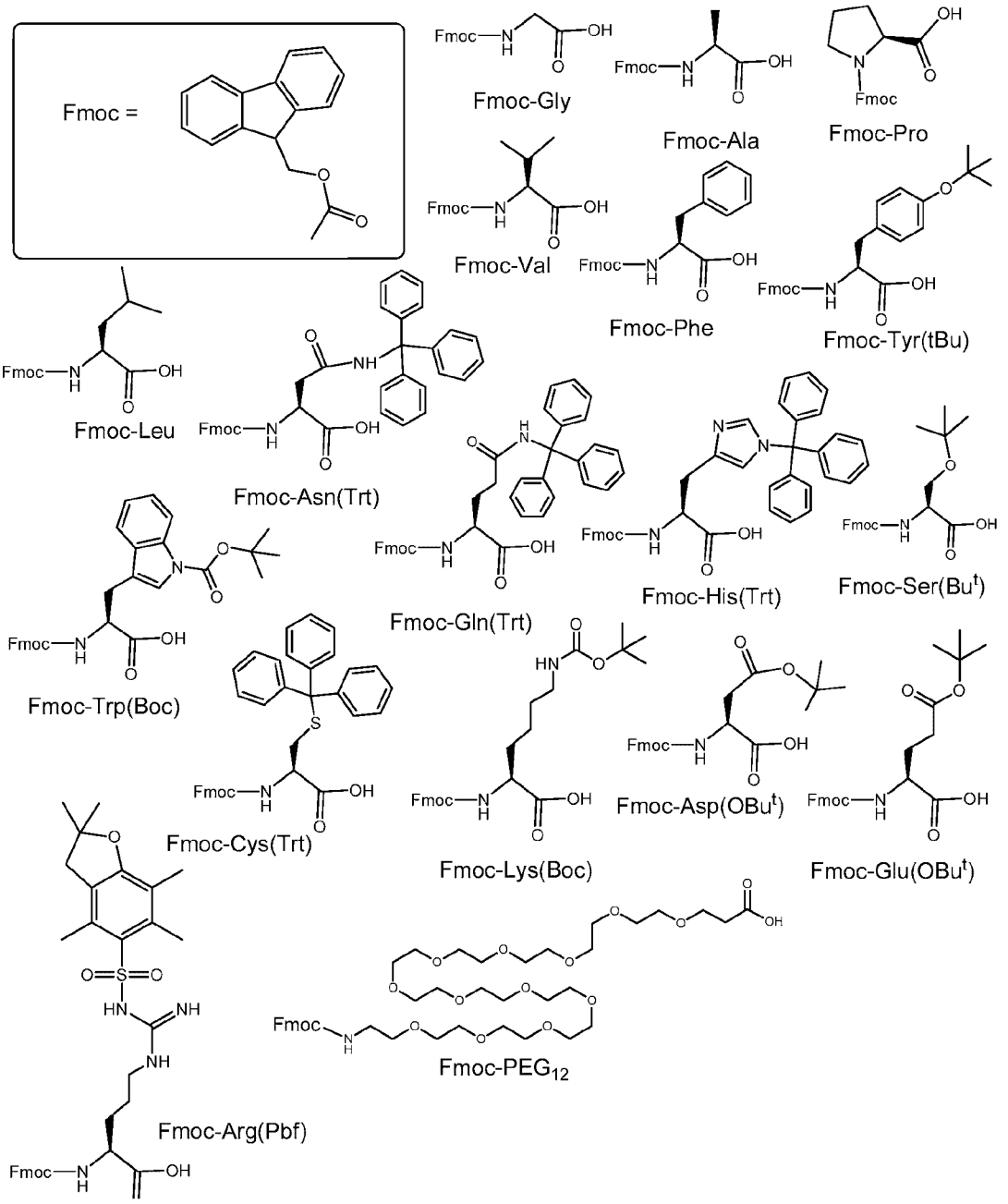
The synthesis of the peptidyl-resins required as precursors to the final peptides utilizes commercially available cross-linked polystyrene polymer resins (Novabiochem, San Diego, CA; Applied Biosystems, Foster City, CA). Preferred solid supports are: 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetyl-p-methyl 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 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.

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.

Useful Fmoc amino acids derivatives are shown below.

Examples of Orthogonally Protected Amino Acids used in Solid Phase Synthesis

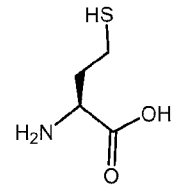


The peptidyl-resin precursors for their respective peptides may be cleaved and deprotected using any standard procedure (see, for example, King, D.S. et al., *Int. J. Peptide Protein Res.*, 36:255-266 (1990)). A desired method is the use of TFA in the presence of water and TIS as scavengers. Typically, 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 or

dried under reduced pressure. The resulting crude peptide is either precipitated and washed with Et_2O 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-8A liquid chromatograph. The solution of crude peptide is injected into a YMC S5 ODS (20 x 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.

List of non-naturally occurring amino acids referred to herein is provided below.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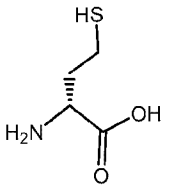
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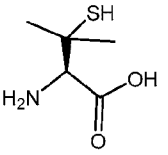
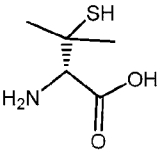

L-Pen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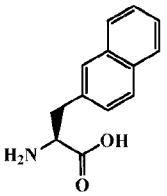
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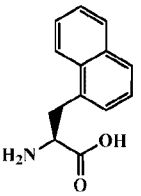
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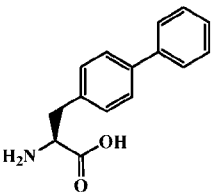
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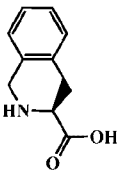
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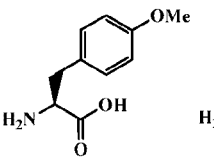
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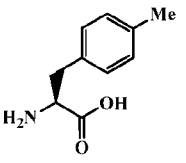
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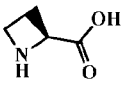
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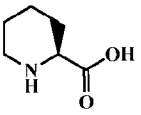
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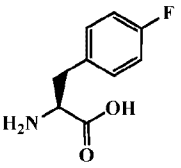
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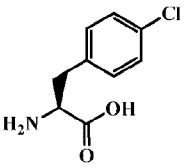
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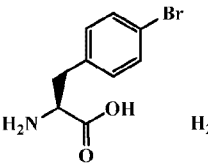
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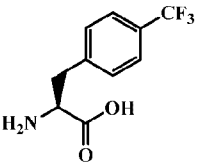
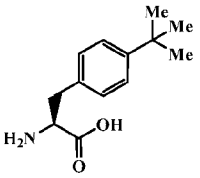
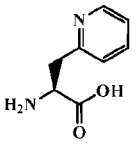
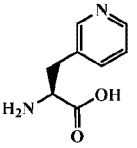
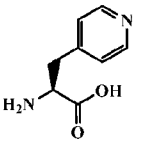
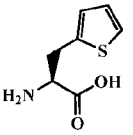

Phe(4-CF_3)

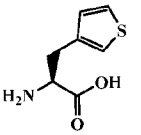

Phe(4-*t*Bu)

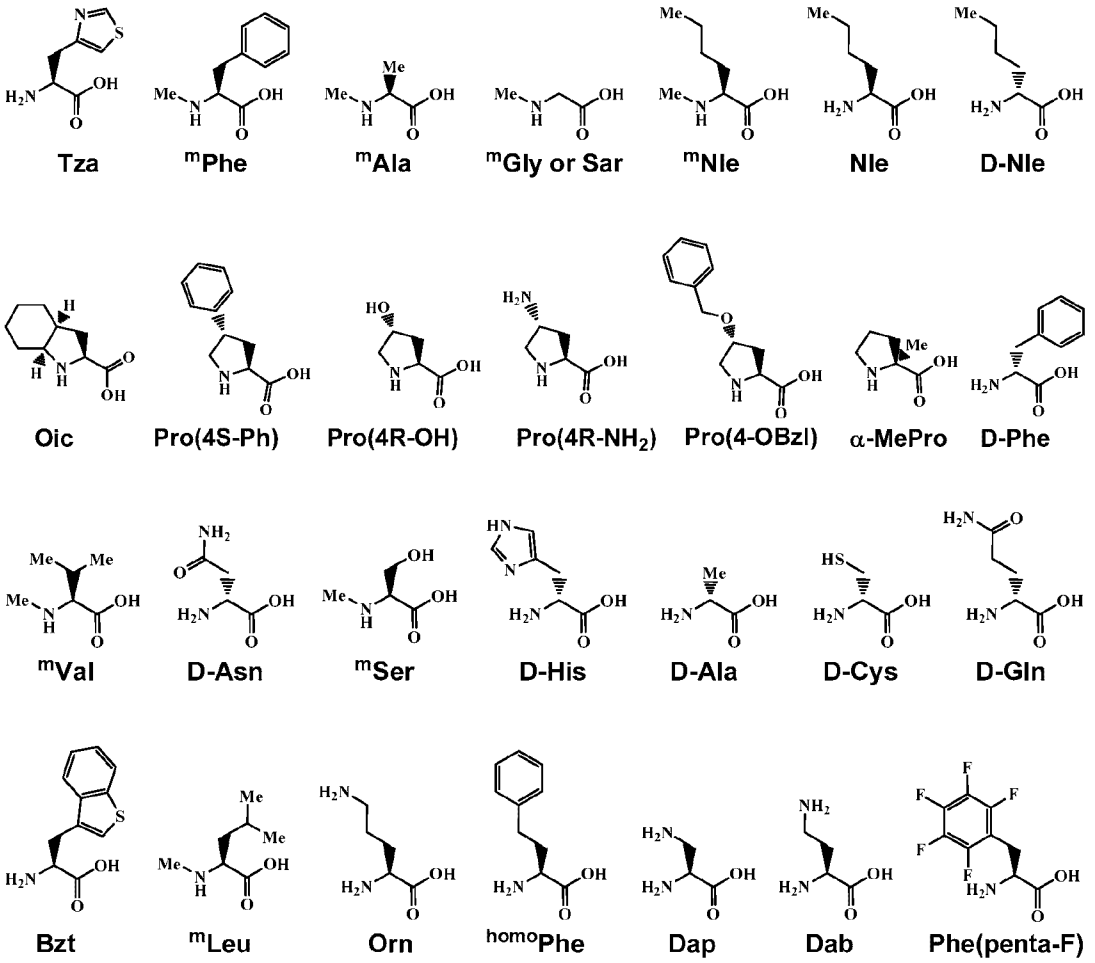

[2-PyrAla]


[3-PyrAla]


[4-PyrAla]


[2-ThienylAla]


[3-ThienylAla]



The following abbreviations are employed in the Examples and elsewhere herein:

Ph = phenyl

Bn = benzyl

i-Bu = iso-butyl

i-Pr = iso-propyl

Me = methyl

Et = ethyl

Pr = n-propyl

Bu = n-butyl

t-Bu = *tert*-butyl

Trt = trityl

TMS = trimethylsilyl

TIS =triisopropylsilane

Et_2O = diethyl ether

HOAc or AcOH = acetic acid

MeCN or AcCN = acetonitrile

DMF = N,N-dimethylformamide

EtOAc = ethyl acetate

THF = tetrahydrofuran

TFA = trifluoroacetic acid

TFE = α,α,α-trifluoroethanol

Et_2NH = diethylamine

NMM = N-methylmorpholine

NMP = N-methylpyrrolidone

DCM = dichloromethane

TEA = triethylamine

min. = minute(s)

h or hr = hour(s)

L = liter

mL or ml = milliliter

μL = microliter

g = gram(s)

mg = milligram(s)

mol = mole(s)

mmol = millimole(s)

meq = milliequivalent

rt or RT = room temperature

sat or sat'd = saturated

aq. = aqueous

mp = melting point

BOP reagent = benzotriazol-1-yloxy-tris-dimethylamino-phosphonium hexafluorophosphate (Castro's reagent)

PyBOP reagent = benzotriazol-1-yloxy-tripyrrolidino phosphonium hexafluorophosphate

HBTU = 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronim hexafluorophosphate

HATU = O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronim hexafluorophosphate

HCTU = 2-(6-Chloro-1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

T3P = 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide

DMAP = 4-(dimethylamino)pyridine

DIEA = diisopropylethylamine

Fmoc or FMOC = fluorenylmethyloxycarbonyl

Boc or BOC = *tert*-butyloxycarbonyl

HOBT or HOBT•H_2O = 1-hydroxybenzotriazole hydrate

Cl-HOBt = 6-Chloro-benzotriazole

HOAT = 1-hydroxy-7-azabenzotriazole

HPLC = high performance liquid chromatography

LC/MS = high performance liquid chromatography/mass spectrometry

MS or Mass Spec = mass spectrometry

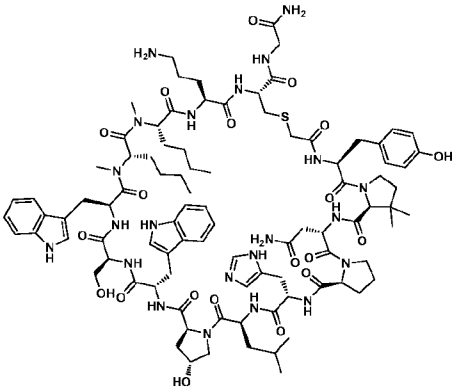
NMR = nuclear magnetic resonance

Sc or SC = sub-cutaneous

IP or ip = intra-peritoneal

EXAMPLES

Preparation of Example 1240



Example 1240 was prepared on Rink Resin following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Manual Coupling procedure A*",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

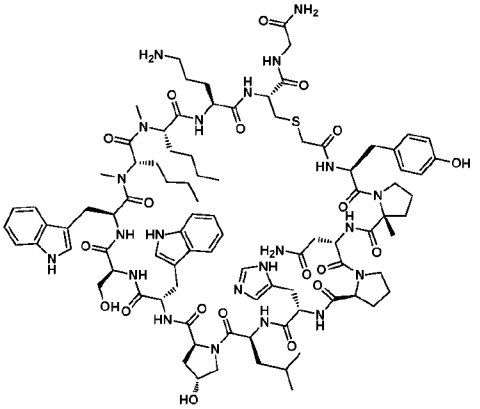
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-65% B over 25 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 11.5 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.45 min; ESI-MS(+) *m/z* 955.0 (M+2H).

Analysis LCMS Condition E: Retention time = 1.39 min; ESI-MS(+) *m/z* 954.7 (M+2H).

ESI-HRMS(+) *m/z:*Calculated: 954.4849(M+2H; Found: 954.4816 (M+2H)

Preparation of Example 1241



Example 1241 was prepared on Rink Resin following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Manual Coupling procedure A*",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

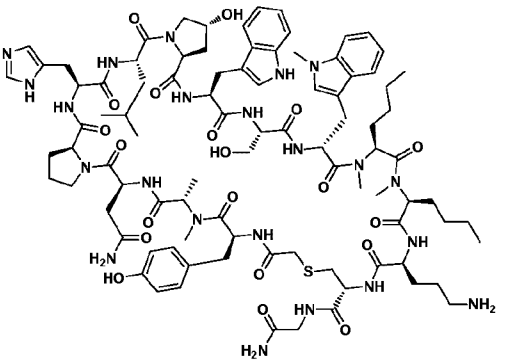
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-65% B over 25 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 11.3 mg, and its estimated purity by LCMS analysis was 92%.

Analysis LCMS Condition D: Retention time = 1.37 min; ESI-MS(+) *m/z* 947.8 (M+2H).

Analysis LCMS Condition E: Retention time = 1.30 min; ESI-MS(+) *m/z* 948.0 (M+2H).

ESI-HRMS(+) *m/z:*Calculated: 947.4771(M+2H); Found: 947.4755 (M+2H).

Preparation of Example 1244

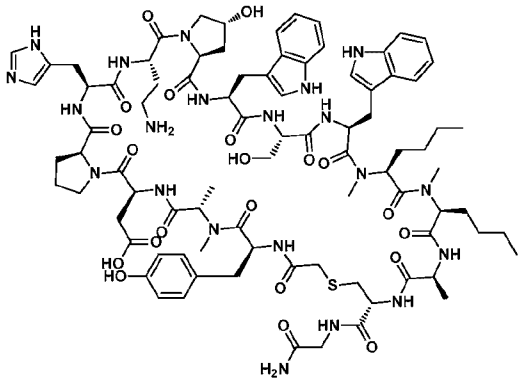


Example 1244 was prepared on Rink Resin following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Manual Coupling procedure A*",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-65% B over 25 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 21.9 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition D: Retention time = 1.49 min; ESI-MS(+) *m/z* 942.0 (M+2H).
Analysis LCMS Condition E: Retention time = 1.56 min; ESI-MS(+) *m/z* 942.0 (M+2H).
ESI-HRMS(+) *m/z:; Calculated*: 941.4771(M+2H)*; Found*: 941.4757 (M+2H)

Preparation of Example 1245

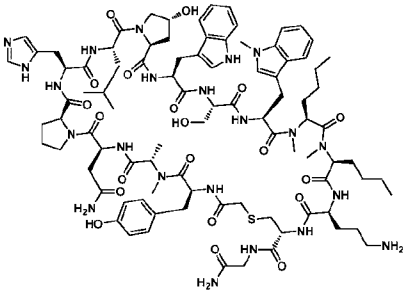


Example 1245 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method C*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-65% B over 25 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 90%.

Analysis LCMS Condition D: Retention time = 1.48 min; ESI-MS(+) *m/z* 907.3 (M+2H).
Analysis LCMS Condition E: Retention time = 1.42 min; ESI-MS(+) *m/z* 907.3 (M+2H).

Preparation of Example 1246



Example 1246 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method C*", and "*Cyclization Method D*".

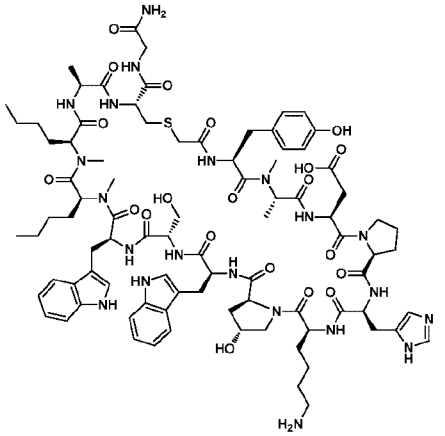
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-65% B over 25 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 15.5 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition D: Retention time = 1.57 min; ESI-MS(+) *m/z* 941.9 (M+2H).

Analysis LCMS Condition E: Retention time = 1.51 min; ESI-MS(+) *m/z* 942.2 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 941.4771(M+2H)*; Found*: 941.4755(M+2H)

Preparation of Example 1247



Example 1247 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method C*", and "*Cyclization Method D*".

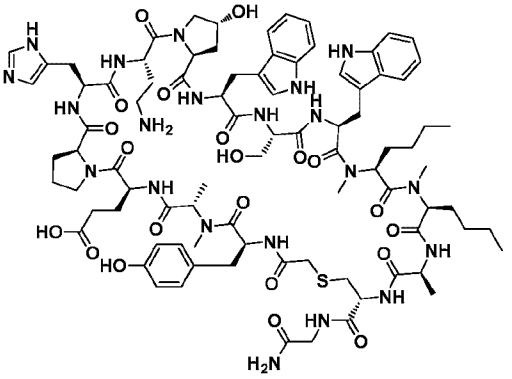
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-65% B over 25 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 94%.

Analysis LCMS Condition D: Retention time = 1.49 min; ESI-MS(+) *m/z* 921.1 (M+2H).

Analysis LCMS Condition E: Retention time = 1.42 min; ESI-MS(+) *m/z* 921.4 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 920.9456(M+2H)*; Found*: 920.9436(M+2H)

Preparation of Example 1248



Example 1248 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method C*", and "*Cyclization Method D*".

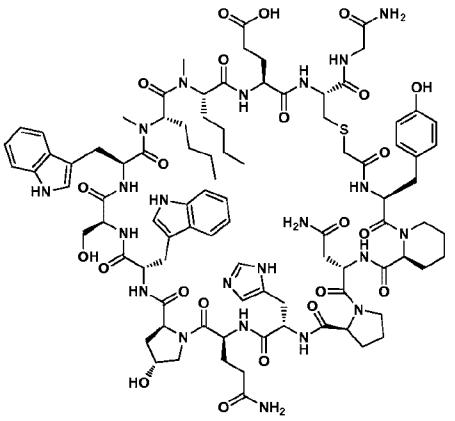
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-65% B over 25 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 4.7 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition D: Retention time = 1.45 min; ESI-MS(+) *m/z* 914.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.40 min; ESI-MS(+) m/z 914.1 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 913.9378(M+2H)*; Found*: 913.9372(M+2H)

Preparation of Example 1250



Example 1250 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the

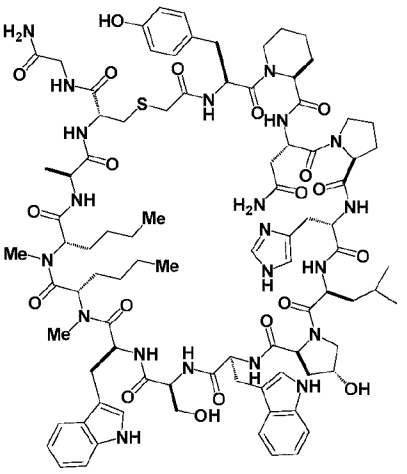
desired product were combined and dried via centrifugal evaporation. The yield of the product was 11.4 mg, and its estimated purity by LCMS analysis was 96%.

Analysis LCMS Condition H: retention time = 1.46 min.; ESI-MS(+) *m/z* 962.7 (M+2H).

Analysis LCMS Condition I: retention time = 2.10 min.; ESI-MS(+) *m/z* 962.5 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 962.4460(M+2H)*; Found*: 962.4459(M+2H)

Preparation of Example 1251



Example 1251 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

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

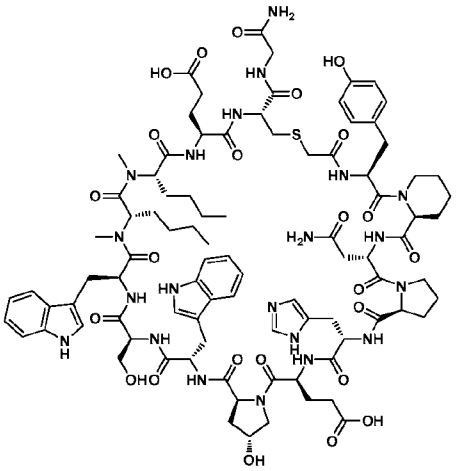
Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-85% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 7.0 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: retention time = 1.68 min.; ESI-MS(+) *m/z* 926.4 (M+2H).

Analysis LCMS Condition I: retention time = 3.10 min.; ESI-MS(+) *m/z* 926.4 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 925.9560(M+2H)*; Found*: 925.9548(M+2H).

Preparation of Example 1252

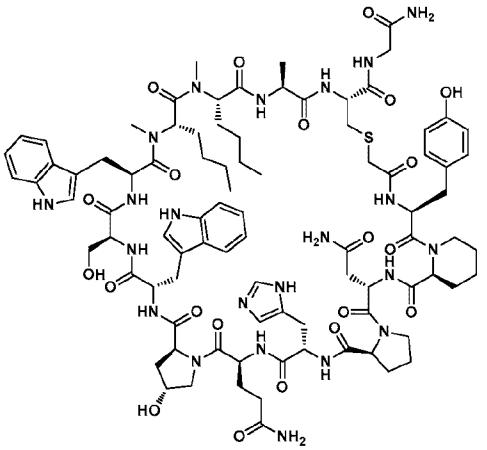


Example 1252 was prepared on Rink resin following the general synthetic sequence described for the preparation of Example 0002, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Single-coupling procedure", "CEM Method A: Double-coupling procedure", "Chloroacetyl chloride coupling procedure A", "Global Deprotection Method F", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 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: 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. The yield of the product was 16.6 mg, and its estimated purity by LCMS analysis was 100%.
Analysis LCMS Condition H: retention time = 1.48 min.; ESI-MS(+) *m/z* 963.1 (M+2H).
Analysis LCMS Condition I: retention time = 2.08 min.; ESI-MS(+) *m/z* 963.5 (M+2H).
ESI-HRMS(+) *m/z:; Calculated*: 962.9380(M+2H)*; Found*: 962.9370(M+2H).

Preparation of Example 1255



Example 1255 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

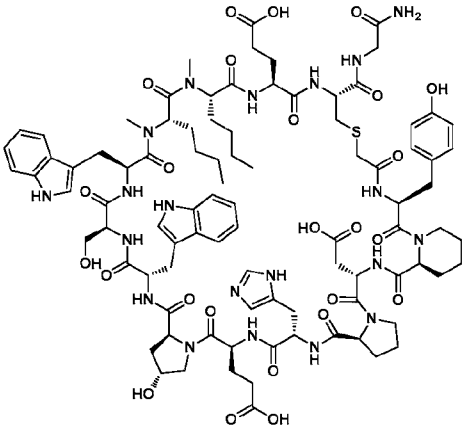
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 20-60% B over 30 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 8.6 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: retention time = 1.58 min.; ESI-MS(+) *m/z* 933.6 (M+2H).

Analysis LCMS Condition I: retention time = 2.25 min.; ESI-MS(+) *m/z* 933.5 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 933.4419(M+2H)*; Found*: 933.4432(M+2H).

Preparation of Example 1256



Example 1256 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

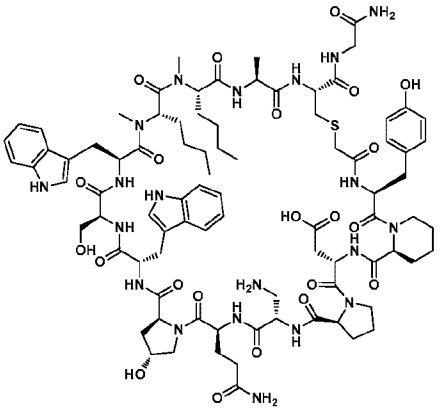
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 35-75% B over 30 minutes, 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 100%.

Analysis LCMS Condition H: retention time = 1.37 min.; ESI-MS(+) *m/z* 963.6 (M+2H).

Analysis LCMS Condition I: retention time = 1.98 min.; ESI-MS(+) *m/z* 963.6 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 963.4300(M+2H)*; Found*: 963.4295(M+2H).

Preparation of Example 1257

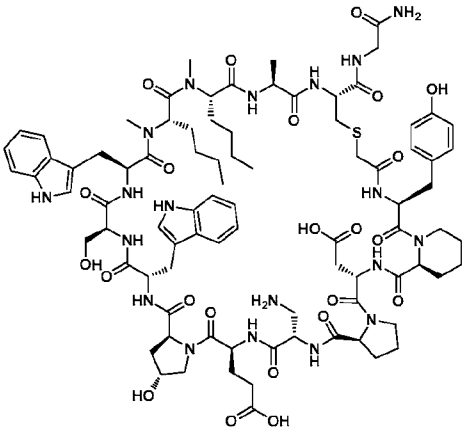


Example 1257 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

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 3.6 mg, and its estimated purity by LCMS analysis was 99%. Analysis LCMS Condition H: retention time = 1.60 min.; ESI-MS(+) *m/z* 908.6 (M+2H). Analysis LCMS Condition I: retention time = 3.12 min.; ESI-MS(+) *m/z* 908.6 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 908.4298(M+2H)*; Found*: 908.4283(M+2H).

Preparation of Example 1258



Example 1258 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

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

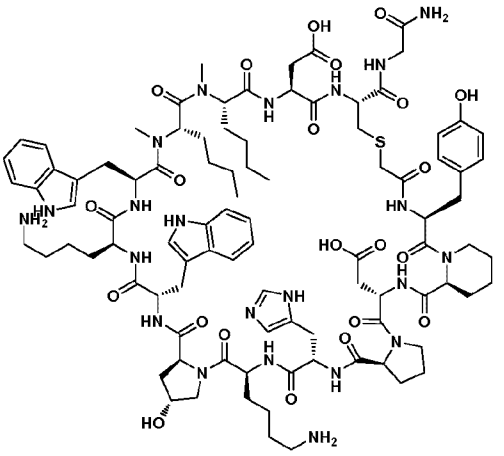
Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 15-55% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-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: 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. The yield of the product was 4.8 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: retention time = 1.61 min.; ESI-MS(+) *m/z* 909.0 (M+2H).

Analysis LCMS Condition I: retention time = 2.66 min.; ESI-MS(+) *m/z* 909.6 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 908.9218(M+2H)*; Found*: 908.9206(M+2H).

Preparation of Example 1259



Example 1259 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

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

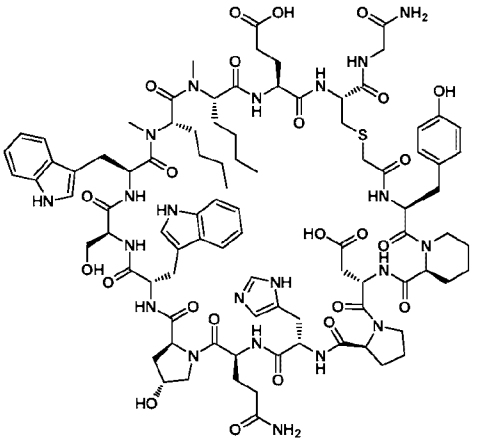
Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-80% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further 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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; 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 100%.

Analysis LCMS Condition H: retention time = 1.49 min.; ESI-MS(+) *m/z* 976.7 (M+2H).

Analysis LCMS Condition I: retention time = 2.15 min.; ESI-MS(+) *m/z* 976.7 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 976.4798(M+2H)*; Found*: 976.4781(M+2H).

Preparation of Example 1260



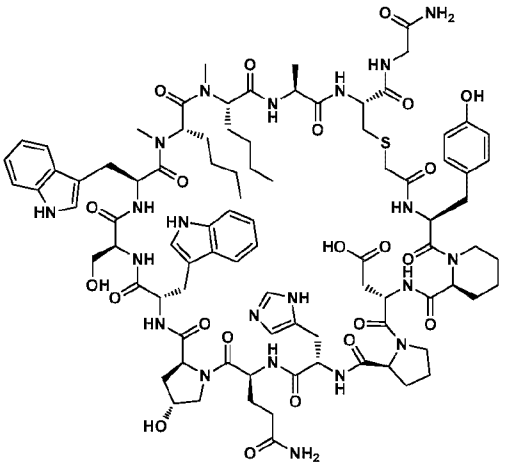
Example 1260 was prepared on Rink resin following the general synthetic sequence described for the preparation of Example 0002, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Single-coupling procedure", "CEM Method A: Double-coupling procedure", "Chloroacetyl chloride coupling procedure A", "Global Deprotection Method F", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 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: 35-75% 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 35.2 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: retention time = 1.51 min.; ESI-MS(+) *m/z* 963.2 (M+2H).

Analysis LCMS Condition I: retention time = 2.05 min.; ESI-MS(+) *m/z* 962.9 (M+2H).
ESI-HRMS(+) *m/z:; Calculated*: 962.9380(M+2H)*; Found*: 962.9367(M+2H).

Preparation of Example 1261



Example 1261 was prepared on Rink resin following the general synthetic sequence described for the preparation of Example 0002, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Single-coupling procedure", "CEM Method A: Double-coupling procedure", "Chloroacetyl chloride coupling procedure A", "Global Deprotection Method F", and "Cyclization Method C".

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

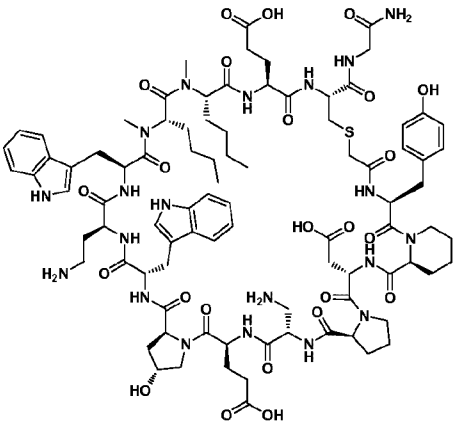
Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 35-75% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 20.2 mg, and its estimated purity by LCMS analysis was 96%.

Analysis LCMS Condition H: retention time = 1.55 min.; ESI-MS(+) *m/z* 934.1 (M+2H).

Analysis LCMS Condition I: retention time = 3.02 min.; ESI-MS(+) *m/z* 934.1 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 933.9352(M+2H)*; Found*: 933.9344(M+2H).

Preparation of Example 1262



Example 1262 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method D*", and "*Cyclization Method D*".

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

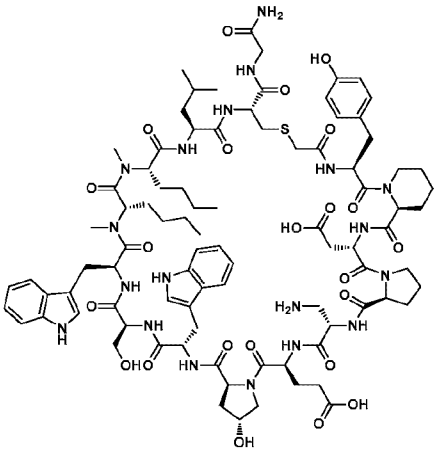
Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-80% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further 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: 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 3.8 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: retention time = 1.65 min.; ESI-MS(+) *m/z* 944.8 (M+2H).

Analysis LCMS Condition I: retention time = 2.59 min.; ESI-MS(+) *m/z* 945.0 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 944.4404(M+2H)*; Found*: 944.4388(M+2H).

Preparation of Example 1272



Example 1272 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method F*", and "*Cyclization Method D*"

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

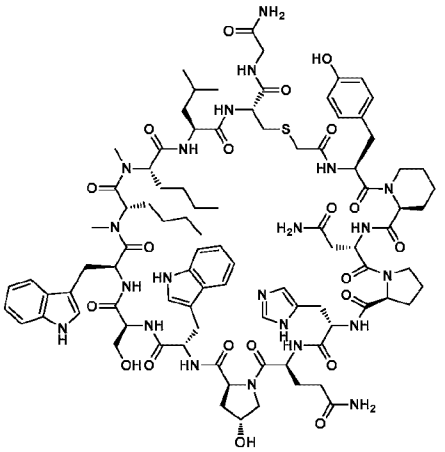
Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-80% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The material was further 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: 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 30.6 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: retention time = 1.65 min.; ESI-MS(+) *m/z* 930.4 (M+2H).

Analysis LCMS Condition I: retention time = 3.08 min.; ESI-MS(+) *m/z* 930.4 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 929.9453(M+2H)*; Found*: 929.9429(M+2H).

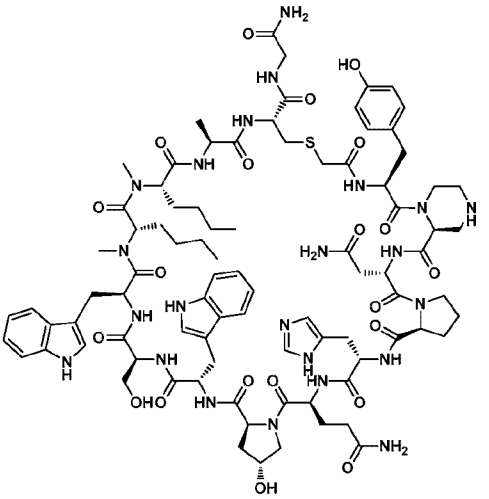
Preparation of Example 1273



Example 1273 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Prelude Method C: *Resin-swelling procedure*", "Prelude Method C: *Single-coupling procedure*", "Prelude Method C: *Secondary amine-coupling procedure*", "Prelude Method C: *Final Wash procedure*", *Chloroacetic acid coupling procedure B* "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 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 20.5 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.66 min.; ESI-MS(+) *m/z* 954.8 (M+2H). Analysis LCMS Condition I: retention time = 3.14 min.; ESI-MS(+) *m/z* 954.8 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 954.4667(M+2H)*; Found*: 954.4644(M+2H).

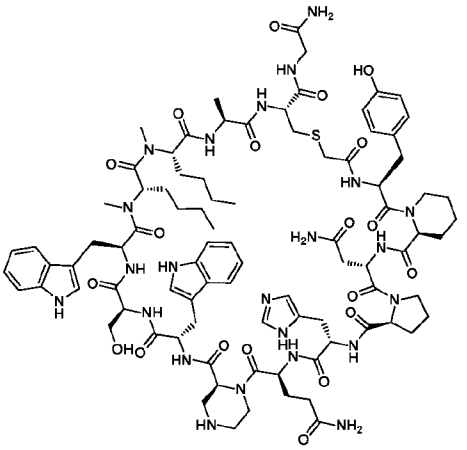
Preparation of Example 1275



Example 1275 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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 material was further 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: 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 0.4 mg, and its estimated purity by LCMS analysis was 97%. Analysis LCMS Condition H: retention time = 1.48 min.; ESI-MS(+) *m/z* 934.4 (M+2H). Analysis LCMS Condition I: retention time = 2.97 min.; ESI-MS(+) *m/z* 934.4 (M+2H).

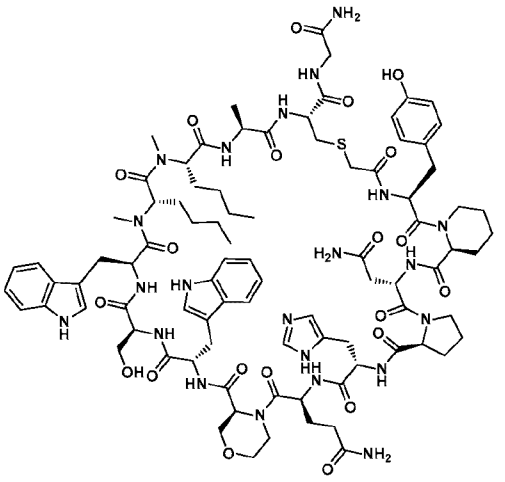
Preparation of Example 1276



Example 1276 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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: 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 10.6 mg, and its estimated purity by LCMS analysis was 97%. Analysis LCMS Condition H: retention time = 1.54 min.; ESI-MS(+) *m/z* 933.7 (M+2H). Analysis LCMS Condition I: retention time = 3.02 min.; ESI-MS(+) *m/z* 933.4 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 932.9512(M+2H)*; Found*: 932.9524(M+2H).

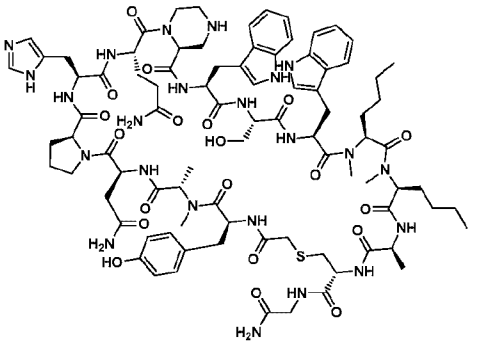
Preparation of Example 1277



Example 1277 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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: 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 7.0 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.61 min.; ESI-MS(+) *m/z* 933.9 (M+2H). Analysis LCMS Condition I: retention time = 3.07 min.; ESI-MS(+) *m/z* 933.9 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 933.4432(M+2H)*; Found*: 933.4416(M+2H).

Preparation of Example 1278



Example 1278 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

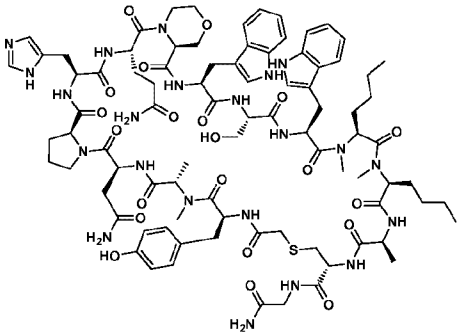
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 minutes, 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 3.3 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition H: retention time = 1.52 min.; ESI-MS(+) *m/z* 920.3 (M+2H).

Analysis LCMS Condition I: retention time = 2.97 min.; ESI-MS(+) *m/z* 920.4 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 919.9434(M+2H)*; Found*: 919.9422(M+2H).

Preparation of Example 1279



Example 1279 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

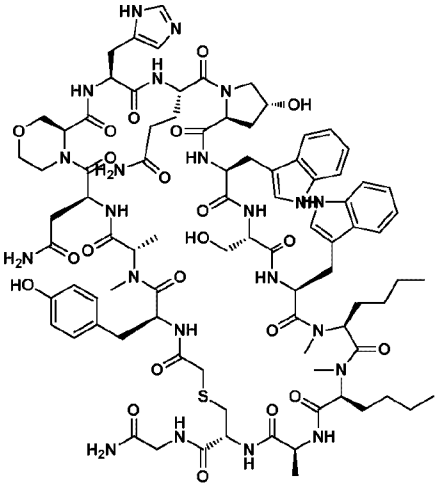
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 45-85% B over 30 minutes, 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 9.1 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition H: retention time = 1.55 min.; ESI-MS(+) *m/z* 921.0 (M+2H).

Analysis LCMS Condition I: retention time = 2.99 min.; ESI-MS(+) *m/z* 920.9 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 920.4354(M+2H)*; Found*: 920.4340(M+2H).

Preparation of Example 1280



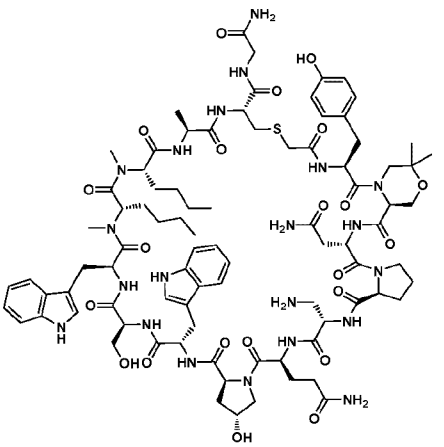
Example 1280 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*",

"Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 6.0 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition H: retention time = 1.48 min.; ESI-MS(+) *m/z* 929.0 (M+2H).
Analysis LCMS Condition I: retention time = 2.93 min.; ESI-MS(+) *m/z* 928.9 (M+2H).
ESI-HRMS(+) *m/z:; Calculated*: 928.4329(M+2H)*; Found*: 928.4324(M+2H).

Preparation of Example 1281



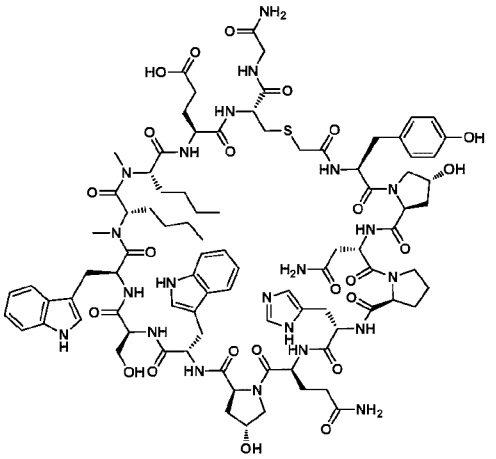
Example 1281 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 250 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 4.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: retention time = 1.81 min.; ESI-MS(+) *m/z* 923.7 (M+2H).
Analysis LCMS Condition I: retention time = 2.63 min.; ESI-MS(+) *m/z* 924.1 (M+2H).
ESI-HRMS(+) *m/z:; Calculated*: 922.9431 (M+2H)*; Found*: 922.9422 (M+2H).

Preparation of Example 1282



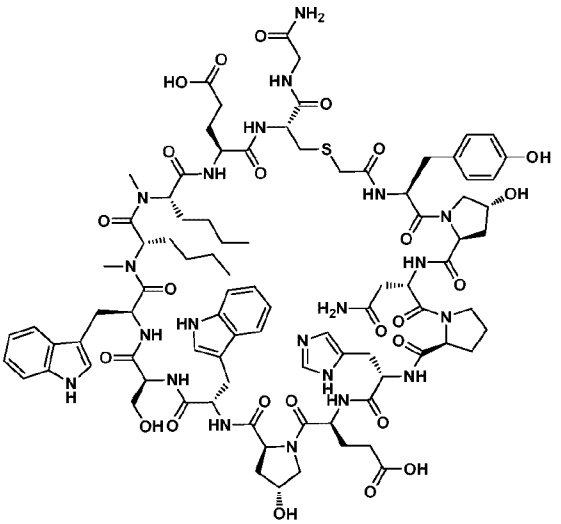
Example 1282 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desiredproduct were combined and dried via centrifugal evaporation. The yield of the product was 5.6 mg, and its estimated purity by LCMS analysis was 95%.

Analysis LCMS Condition H: retention time = 1.51 min.; ESI-MS(+) *m/z* 964.3 (M+2H).

Analysis LCMS Condition I: retention time = 2.27 min.; ESI-MS(+) *m/z* 964.7 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 963.4356 (M+2H)*; Found*: 963.4356 (M+2H).

Preparation of Example 1283



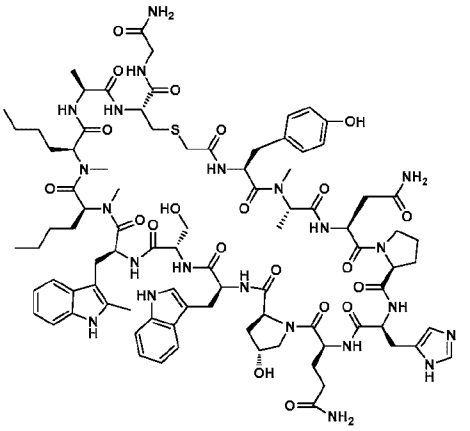
Example 1283 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% B over 30 minutes, then a 8-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 3.0 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: retention time = 1.32 min.; ESI-MS(+) *m/z* 963.7 (M+2H).

Analysis LCMS Condition I: retention time = 2.67 min.; ESI-MS(+) *m/z* 963.8 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 963.9276 (M+2H)*; Found*: 963.9270 (M+2H).

Preparation of Example 1285

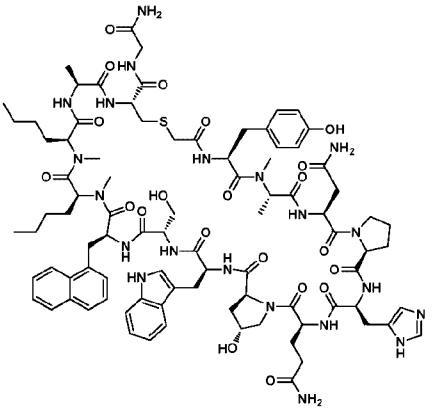


Example1285 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Manual Coupling procedure A"*,* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The yield of the product was 4.4 mg, and its estimated purity by LCMS analysis was 95%.

Analysis LCMS Condition H: retention time = 1.86 min.; ESI-MS(+) *m/z* 928.5 (M+2H). ESI-HRMS(+) *m/z:; Calculated*: 927.4432(M+2H)*; Found*: 927.4426 (M+2H).

Preparation of Example 1289



Example 1289 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

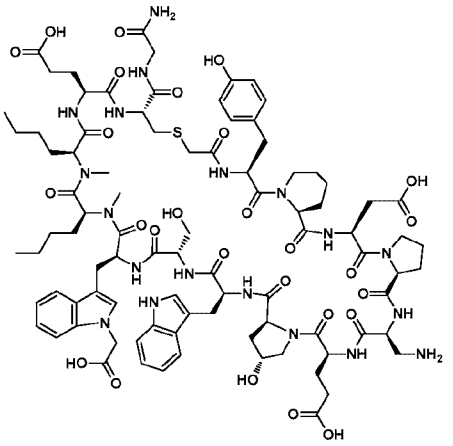
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 45-85% B over 30 minutes, 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 5.7 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition H: retention time = 1.74 min.; ESI-MS(+) *m/z* 926.4 (M+2H).

Analysis LCMS Condition I: retention time = 3.32 min.; ESI-MS(+) *m/z* 926.2 (M+2H).

ESI-HRMS(+) *m/z:; Calculated*: 925.9378(M+2H)*; Found*: 925.9350(M+2H).

Preparation of Example 1290



Example 1290 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

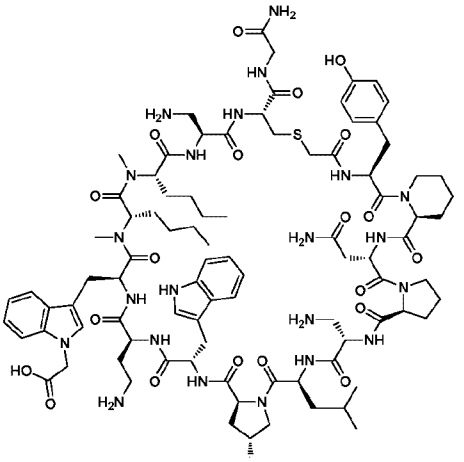
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile:water with 0.1% trifluoroacetic acid; Gradient: 5-45% B over 30 minutes, 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 7.7 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition H: retention time = 1.24 min.; ESI-MS(+) *m/z* 967.3 (M+2H).

Analysis LCMS Condition I: retention time = 2.66 min.; ESI-MS(+) *m/z* 967.4 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 966.9273(M+2H)*; Found*: 966.9273(M+2H)

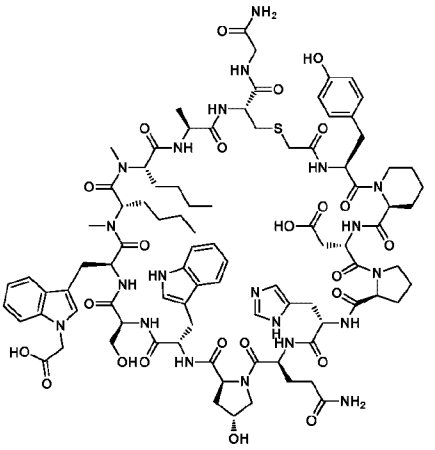
Preparation of Example 1291



Example 1291 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 50-100% 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 material was further 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: 5-45% 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 93%. Analysis LCMS Condition H: retention time = 1.39 min.; ESI-MS(+) *m/z* 944.1(M+2H). Analysis LCMS Condition I: retention time = 2.99 min.; ESI-MS(+) *m/z* 943.8 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 943.4745(M+2H)*; Found*: 943.4714(M+2H).

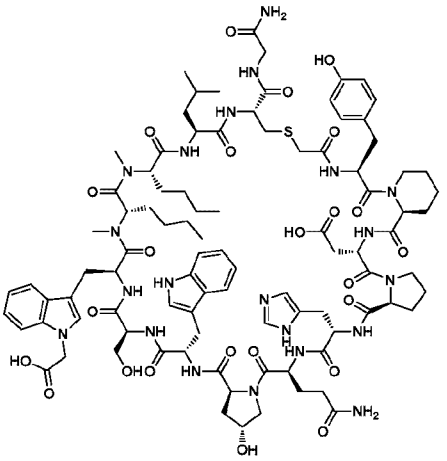
Preparation of Example 1292



Example 1292 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 5-45% 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 100%. Analysis LCMS Condition H: retention time = 1.20 min.; ESI-MS(+) *m/z* 963.3 (M+2H). Analysis LCMS Condition I: retention time = 2.52 min.; ESI-MS(+) *m/z* 963.5 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 962.9380(M+2H)*; Found*: 962.9357(M+2H).

Preparation of Example 1293



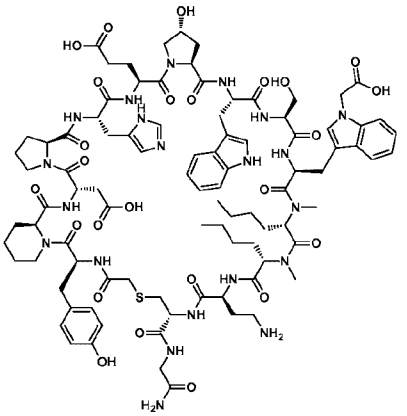
Example 1293 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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 2.1 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: retention time = 1.48 min.; ESI-MS(+) *m/z* 983.9 (M+2H).

Analysis LCMS Condition I: retention time = 2.12 min.; ESI-MS(+) *m/z* 984.1 (M+2H).

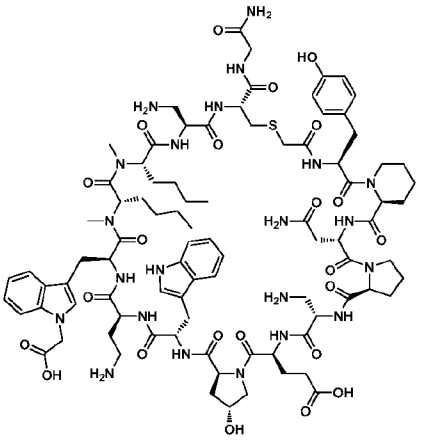
Preparation of Example 1294



Example 1294 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 0-40% 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 4.1 mg, and its estimated purity by LCMS analysis was 94%. Analysis LCMS Condition H: retention time = 1.22 min.; ESI-MS(+) *m/z* 978.8 (M+2H). Analysis LCMS Condition I: retention time = 2.56 min.; ESI-MS(+) *m/z* 979.0 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 977.9433(M+2H)*; Found*: 977.9417(M+2H).

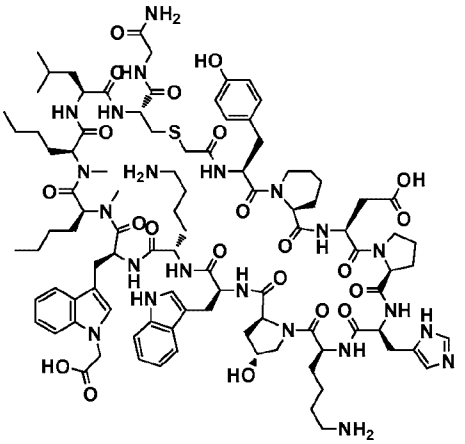
Preparation of Example 1295



Example 1295 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 5-45% B over 20 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 4.4 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.38 min.; ESI-MS(+) *m/z* 951.6 (M+2H). Analysis LCMS Condition I: retention time = 2.96 min.; ESI-MS(+) *m/z* 952.0 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 951.4538(M+2H)*; Found*: 951.4508(M+2H).

Preparation of Example 1296



Example 1296 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

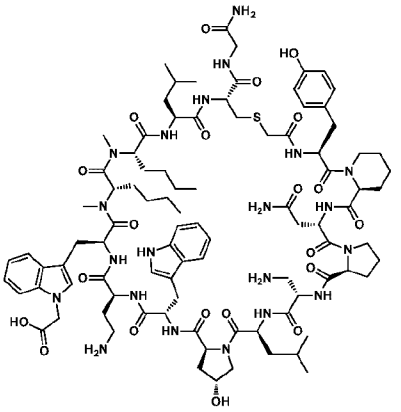
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 45-85% B over 30 minutes, 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-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: 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 1.4 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition H: retention time = 1.35 min.; ESI-MS(+) *m/z* 1005.2 (M+2H).

Analysis LCMS Condition I: retention time = 2.75 min.; ESI-MS(+) *m/z* 1005.3 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 1004.5111(M+2H)*; Found*: 1004.5078(M+2H).

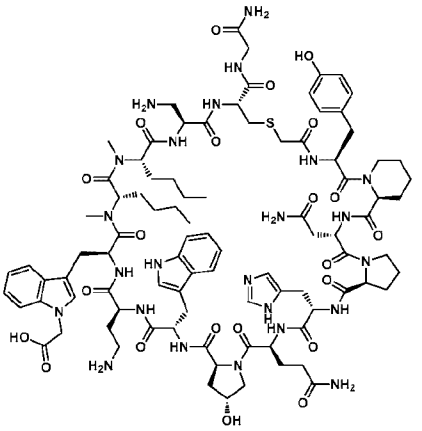
Preparation of Example 1297



Example 1297 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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: 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 4.9 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.58 min.; ESI-MS(+) *m/z* 957.5 (M+2H). Analysis LCMS Condition I: retention time = 3.18 min.; ESI-MS(+) *m/z* 957.5 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 956.9826(M+2H)*; Found*: 956.9896(M+2H).

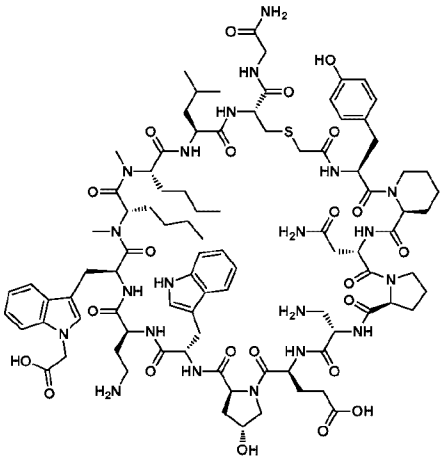
Preparation of Example 1298



Example 1298 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 0-40% B over 20 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 9.4 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.33 min.; ESI-MS(+) *m/z* 977.3 (M+2H). Analysis LCMS Condition I: retention time = 2.71 min.; ESI-MS(+) *m/z* 977.3 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 976.4672(M+2H)*; Found*: 976.4644(M+2H*).*

Preparation of Example 1299



Example 1299 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

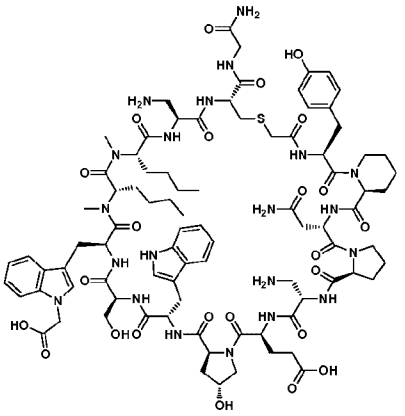
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 40-85% B over 30 minutes, 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 92%.

Analysis LCMS Condition H: retention time = 1.40 min.; ESI-MS(+) *m/z* 965.7 (M+2H).

Analysis LCMS Condition I: retention time = 2.79 min.; ESI-MS(+) *m/z* 965.3 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 964.9718(M+2H)*; Found*: 964.9680(M+2H*)*.

Preparation of Example 1300

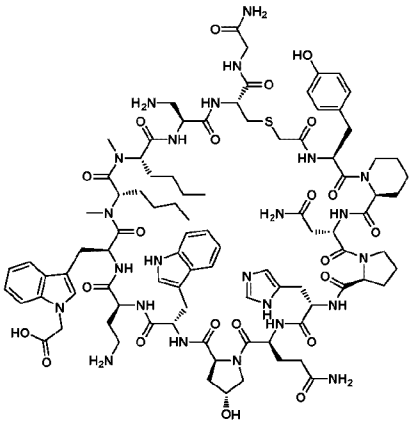


Example 1300 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% 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 97%.

Analysis LCMS Condition I: retention time = 2.65 min.; ESI-MS(+) *m/z* 944.8 (M+2H).

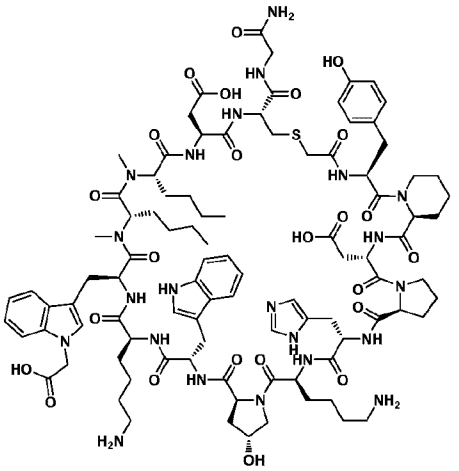
Preparation of Example 1301



Example 1301 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The material was further 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: 5-45% 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 2.1 mg, and its estimated purity by LCMS analysis was 97%. Analysis LCMS Condition H: retention time = 1.33 min.; ESI-MS(+) *m/z* 977.3 (M+2H). Analysis LCMS Condition I: retention time = 2.71 min.; ESI-MS(+) *m/z* 977.3 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 976.4672(M+2H)*; Found*: 976.4645(M+2H).

Preparation of Example 1302



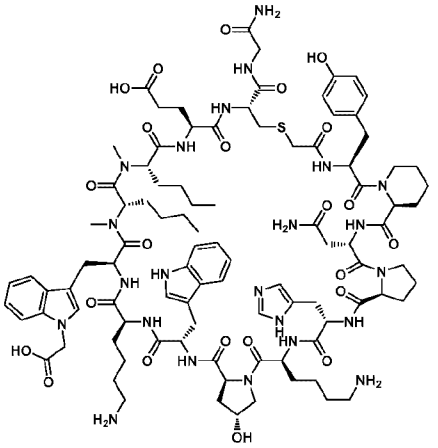
Example 1302 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% 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-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: 5-45% 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 1.0 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: retention time = 1.20 min.; ESI-MS(+) *m/z* 1006.3 (M+2H).

Analysis LCMS Condition I: retention time = 2.47 min.; ESI-MS(+) *m/z* 1006.3 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 1005.4826(M+2H)*; Found*: 1005.4786 (M+2H).

Preparation of Example 1303



Example 1303 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

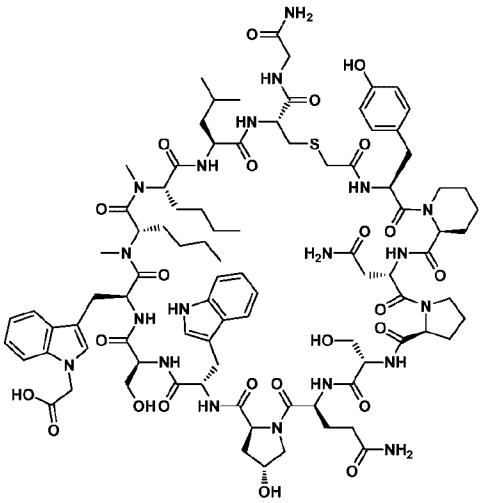
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 35-75% B over 30 minutes, 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 8.0 mg, and its estimated purity by LCMS analysis was 93%.

Analysis LCMS Condition H: retention time = 1.25 min.; ESI-MS(+) *m/z* 1012.9 (M+2H).

Analysis LCMS Condition I: retention time = 2.52 min.; ESI-MS(+) *m/z* 1013.3 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 1011.9984(M+2H)*; Found*: 1011.9941 (M+2H).

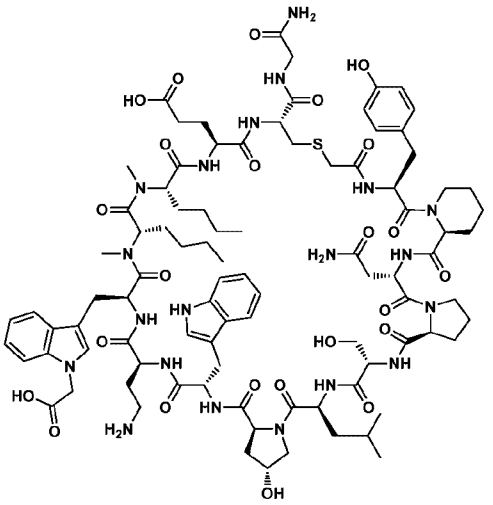
Preparation of Example 1304



Example 1304 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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: 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 1.1 mg, and its estimated purity by LCMS analysis was 95%. Analysis LCMS Condition H: retention time = 1.34 min.; ESI-MS(+) *m/z* 959.0 (M+2H). Analysis LCMS Condition I: retention time = 2.98 min.; ESI-MS(+) *m/z* 959.0 (M+2H). ESI-HRMS(+) *m/z:*; Calculated: 958.4560(M+2H)*; Found*: 958.4542 (M+2H).

Preparation of Example 1305

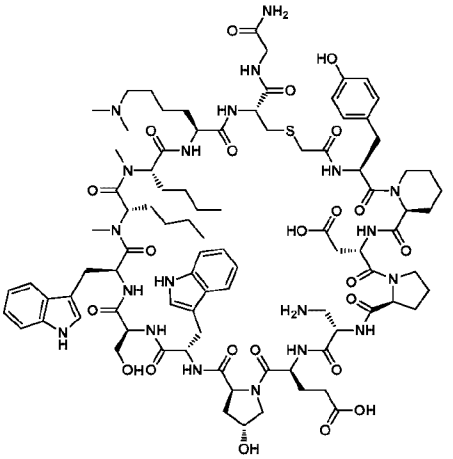


Example 1305 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 35-75% 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-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: 0-40% 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%. Analysis LCMS Condition H: retention time = 1.25 min.; ESI-MS(+) *m/z* 966.4 (M+2H). Analysis LCMS Condition I: retention time = 2.91 min.; ESI-MS(+) *m/z* 965.7 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 965.4638(M+2H)*; Found*: 965.4619 (M+2H).

Preparation of Example 1306



Example 1306 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

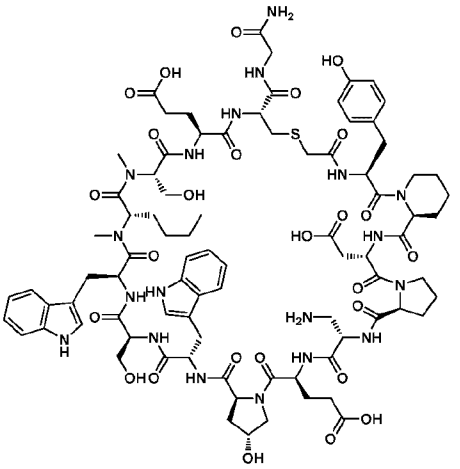
The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 45-85% B over 30 minutes, 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%.

Analysis LCMS Condition H: retention time = 1.57 min.; ESI-MS(+) *m/z* 951.9 (M+2H).

Analysis LCMS Condition I: retention time = 3.07 min.; ESI-MS(+) *m/z* 951.9 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 951.4664(M+2H)*; Found*: 951.4633 (M+2H).

Preparation of Example 1309



Example 1309 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure*", "Symphony Method B: *Custom amino acids-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 0-50% B over 25 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: waters xbridge c-18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 15-55% B over 25 minutes, then a 5-minute hold at 55% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation.

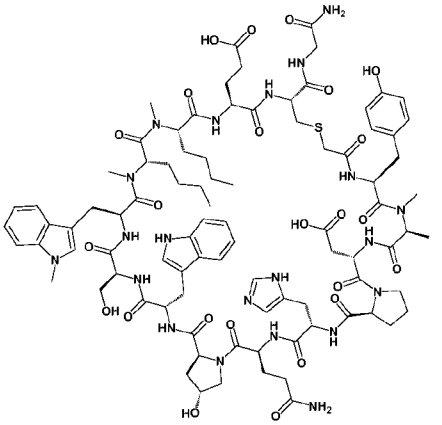
The yield of the product was 3.6 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition D: Retention time = 1.1 min; ESI-MS(+) *m/z* 925.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.25 min; ESI-MS(+) *m/z* 925.4 (M+2H).

ESI-HRMS(+) *m/z:*; Calculated: 924.8985(M+2H)*; Found*: 950.8961(M+2H).

Preparation of Example 1500



Molecular Weight: 1913.12

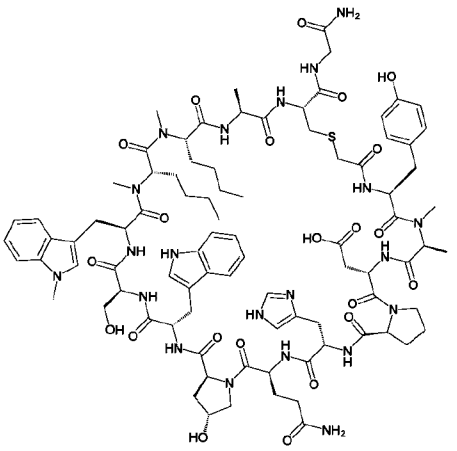
Example 1500 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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 0.6 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.60 min; ESI-MS(+) *m/z* 957.00 (M+2H).

Analysis LCMS Condition I: Retention time = 3.06 min; ESI-MS(+) *m/z* 957.00 (M+2H).

Preparation of Example 1501



Molecular Weight: 1855.08

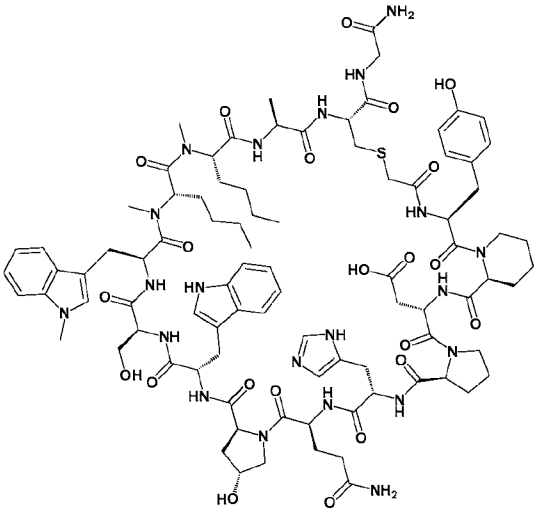
Example 1501 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 6.4 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition H: Retention time = 1.76 min; ESI-MS(+) *m/z* 928.15 (M+2H).

Analysis LCMS Condition I: Retention time = 3.20 min; ESI-MS(+) *m/z* 928.20 (M+2H).

Preparation of Example 1502



Molecular Weight: 1881.12

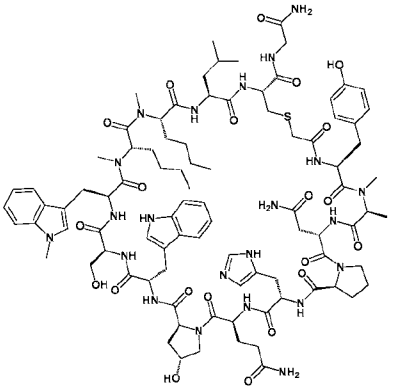
Example 1502 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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 5.4 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.796 min; ESI-MS(+) *m/z* 941.20 (M+2H).

Analysis LCMS Condition I: Retention time = 2.389 min; ESI-MS(+) *m/z* 940.95 (M+2H).

Preparation of Example 1503



Molecular Weight: 1896.17

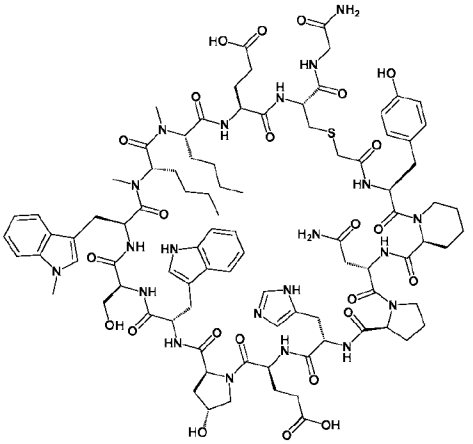
Example 1503 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 material was further 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 3.1 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.870 min; ESI-MS(+) *m/z* 948.75 (M+2H).

Analysis LCMS Condition I: Retention time = 3.358 min; ESI-MS(+) *m/z* 948.60 (M+2H).

Preparation of Example 1504



Molecular Weight: 1939.15

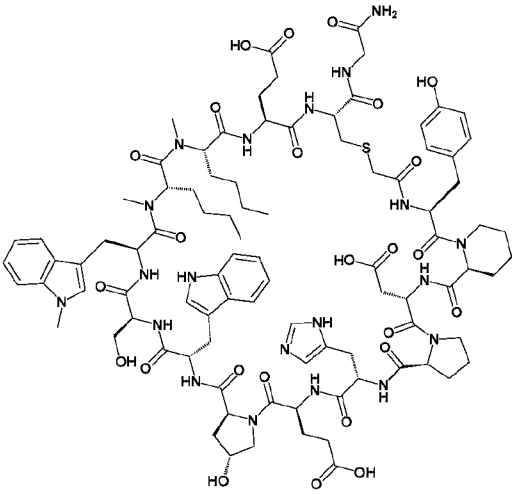
Example 1504 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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 8.4 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: Retention time = 1.447 min; ESI-MS(+) *m/z* 970.80 (M+2H).

Analysis LCMS Condition I: Retention time = 2.838 min; ESI-MS(+) *m/z* 970.20 (M+2H).

Preparation of Example 1505



Molecular Weight: 1940.14

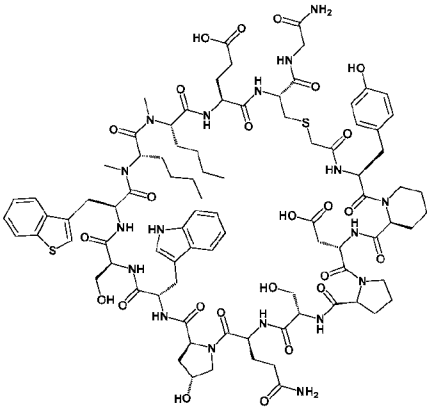
Example 1505 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 8.9 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: Retention time = 1.624 min; ESI-MS(+) *m/z* 970.95 (M+2H).

Analysis LCMS Condition I: Retention time = 3.075 min; ESI-MS(+) *m/z* 970.65 (M+2H).

Preparation of Example 1506



Molecular Weight: 1892.11

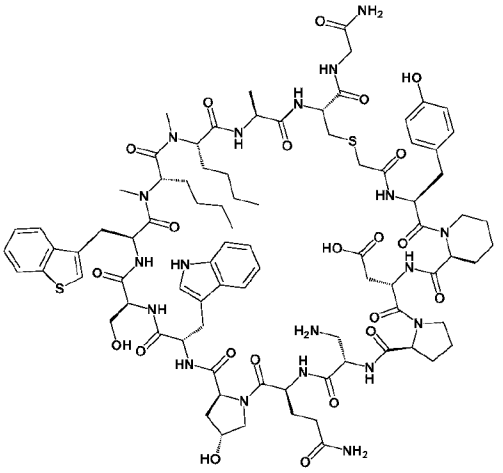
Example 1506 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 2.7 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: Retention time = 1.566 min; ESI-MS(+) *m/z* 947.20 (M+2H).

Analysis LCMS Condition I: Retention time = 3.069 min; ESI-MS(+) *m/z* 946.90 (M+2H).

Preparation of Example 1507



Molecular Weight: 1833.09

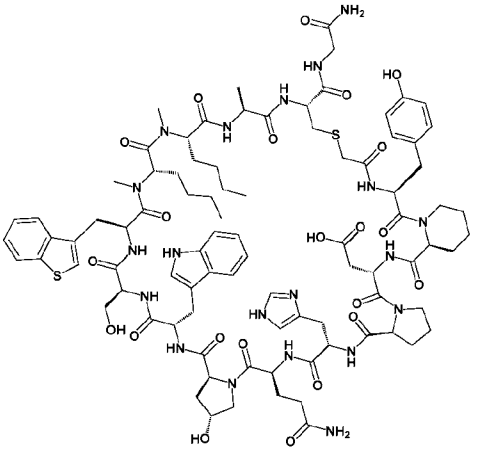
Example 1507 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 LCMS Condition H: Retention time = 1.761 min; ESI-MS(+) *m/z* 917.60 (M+2H).

Analysis LCMS Condition I: Retention time = 3.321 min; ESI-MS(+) *m/z* 917.40 (M+2H).

Preparation of Example 1508



Molecular Weight: 1884.14

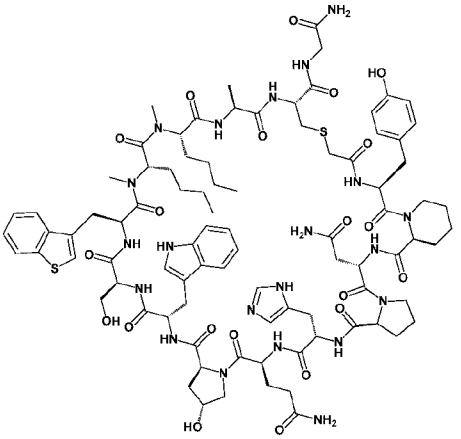
Example 1508 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The yield of the product was 2.2 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.702 min; ESI-MS(+) *m/z* 942.90 (M+2H).

Analysis LCMS Condition I: Retention time = 3.245 min; ESI-MS(+) *m/z* 942.95 (M+2H).

Preparation of Example 1509



Molecular Weight: 1883.16

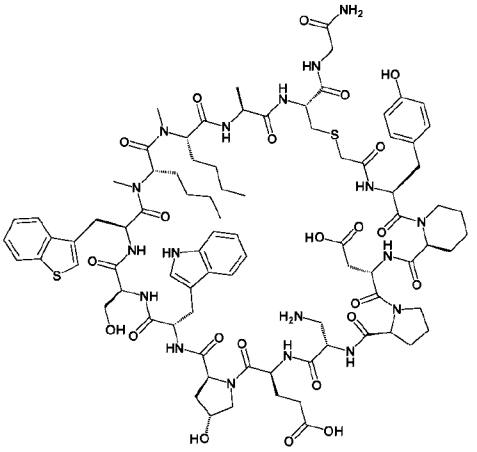
Example 1509 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 8.9 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition H: Retention time = 1.702 min; ESI-MS(+) *m/z* 942.90 (M+2H).

Analysis LCMS Condition I: Retention time = 3.245 min; ESI-MS(+) *m/z* 942.95 (M+2H).

Preparation of Example 1510



Molecular Weight: 1834.08

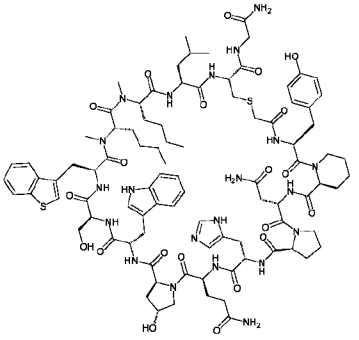
Example 1510 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 99%.

Analysis LCMS Condition H: Retention time = 1.706 min; ESI-MS(+) *m/z* 917.55 (M+2H).

Analysis LCMS Condition I: Retention time = 3.271 min; ESI-MS(+) *m/z* 917.90 (M+2H).

Preparation of Example 1511



Molecular Weight: 1926.24

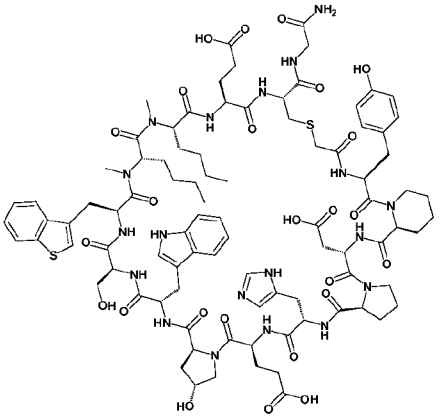
Example 1511 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 7.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.819 min; ESI-MS(+) *m/z* 963.45 (M+2H).

Analysis LCMS Condition I: Retention time = 3.377 min; ESI-MS(+) *m/z* 963.45 (M+2H).

Preparation of Example 1512



Molecular Weight: 1943.16

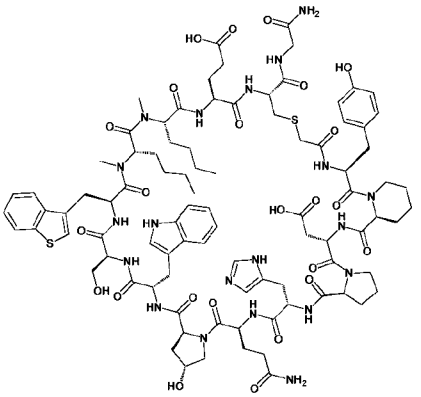
Example 1512 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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: 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 2.4 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.479 min; ESI-MS(+) *m/z* 972.25 (M+2H).

Analysis LCMS Condition I: Retention time = 3.000 min; ESI-MS(+) *m/z* 972.40 (M+2H).

Preparation of Example 1513



Molecular Weight: 1942.18

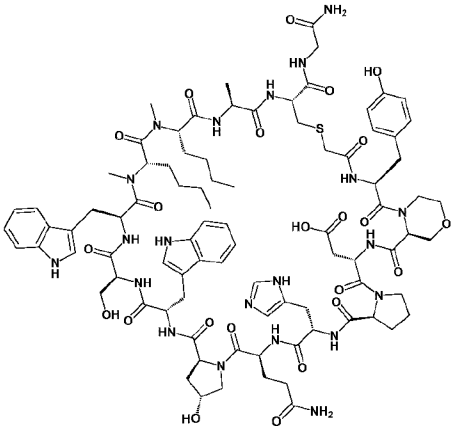
Example 1513 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 98%.

Analysis LCMS Condition H: Retention time = 1.539 min; ESI-MS(+) *m/z* 971.90 (M+2H).

Analysis LCMS Condition I: Retention time = 3.071 min; ESI-MS(+) *m/z* 971.85 (M+2H).

Preparation of Example 1514

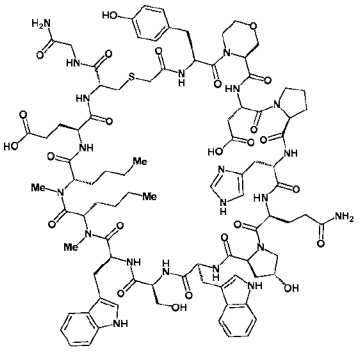


Molecular Weight: 1869.06

Example 1514 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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-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 24.3 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: Retention time = 1.66 min; ESI-MS(+) *m/z* 935.5 (M+2H). Analysis LCMS Condition I: Retention time = 2.53 min; ESI-MS(+) *m/z* 935.7 (M+2H).

Preparation of Example 1515



Molecular Weight: 1927.10

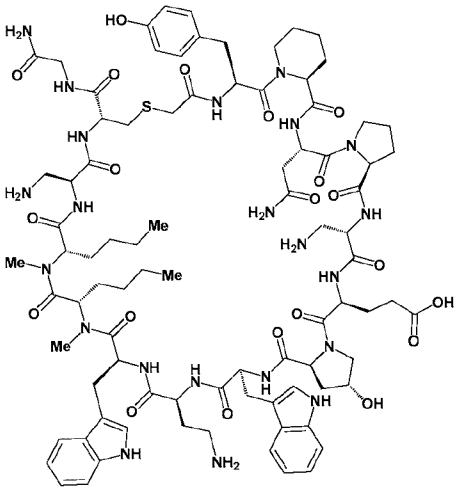
Example 1515 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The yield of the product was 17.0 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.455 min; ESI-MS(+) *m/z* 964.65 (M+2H).

Analysis LCMS Condition I: Retention time = 2.809 min; ESI-MS(+) *m/z* 964.75 (M+2H).

Preparation of Example 1519



Molecular Weight: 1844.10

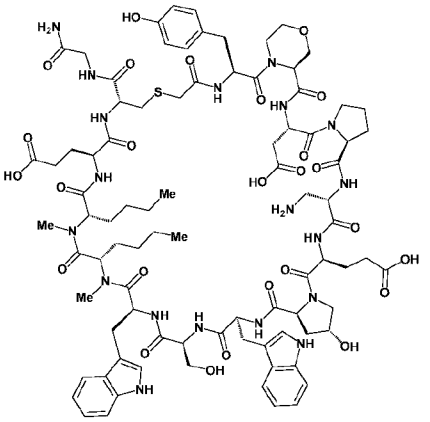
Example 1519 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 45-85% 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 28.3 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.551 min; ESI-MS(+) *m/z* 922.85 (M+2H).

Analysis LCMS Condition I: Retention time = 3.012 min; ESI-MS(+) *m/z* 922.90 (M+2H).

Preparation of Example 1520



Molecular Weight: 1877.04

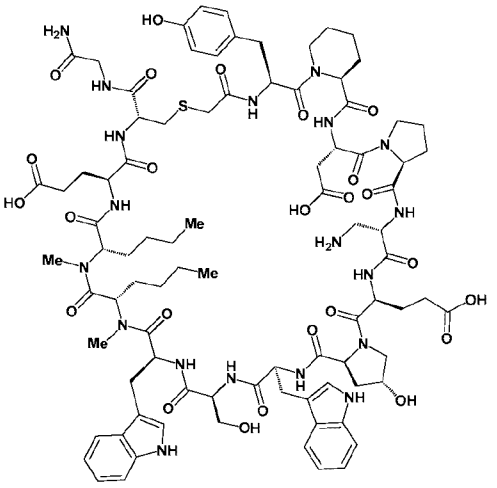
Example 1520 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-65% B over 25 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 8.4 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.30 min; ESI-MS(+) *m/z* 939.6 (M+2H).

Analysis LCMS Condition E: Retention time = 1.40 min; ESI-MS(+) *m/z* 939.4 (M+2H).

Preparation of Example 1521



Molecular Weight: 1875.06

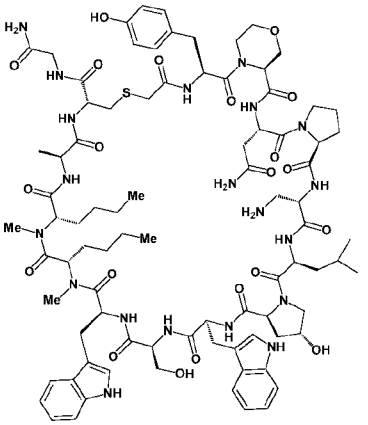
Example 1521 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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. The yield of the product was 8.9 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition H: Retention time = 1.525 min; ESI-MS(+) *m/z* 938.15 (M+2H).

Analysis LCMS Condition I: Retention time = 2.936 min; ESI-MS(+) *m/z* 938.05 (M+2H).

Preparation of Example 1522



Molecular Weight: 1802.06

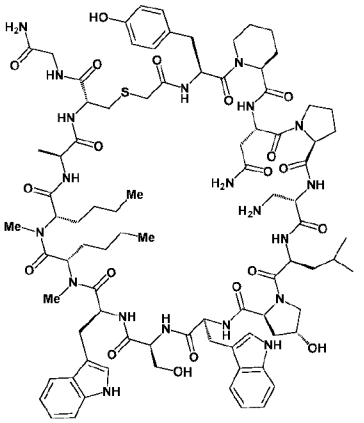
Example 1522 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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: 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-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: 45-85% 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 11.0 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.637 min; ESI-MS(+) *m/z* 901.95 (M+2H).

Analysis LCMS Condition I: Retention time = 3.038 min; ESI-MS(+) *m/z* 901.75 (M+2H).

Preparation of Example 1523



Molecular Weight: 1800.09

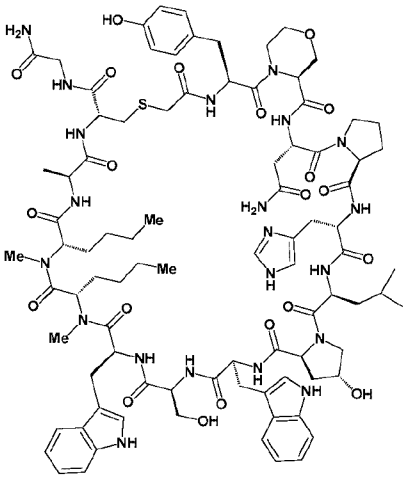
Example 1523 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 2.6 mg, and its estimated purity by LCMS analysis was 92%.

Analysis LCMS Condition H: Retention time = 1.683 min; ESI-MS(+) *m/z* 900.80 (M+2H).

Analysis LCMS Condition I: Retention time = 3.098 min; ESI-MS(+) *m/z* 900.60 (M+2H).

Preparation of Example 1525



Molecular Weight: 1853.11

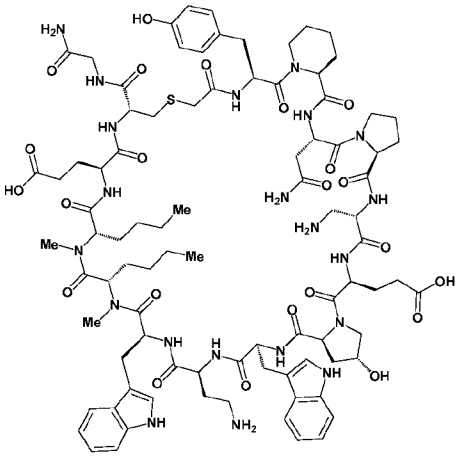
Example 1525 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 16.0 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.643 min; ESI-MS(+) *m/z* 927.20 (M+2H).

Analysis LCMS Condition I: Retention time = 2.250 min; ESI-MS(+) *m/z* 926.85 (M+2H).

Preparation of Example 1526



Molecular Weight: 1887.12

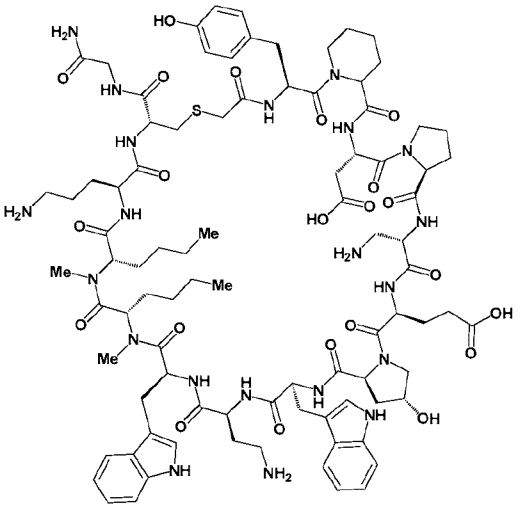
Example 1526 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-65% B over 25 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 3.0 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition D: Retention time = 1.39 min; ESI-MS(+) *m/z* 944.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.44 min; ESI-MS(+) *m/z* 944.2 (M+2H).

Preparation of Example 1528



Molecular Weight: 1873.14

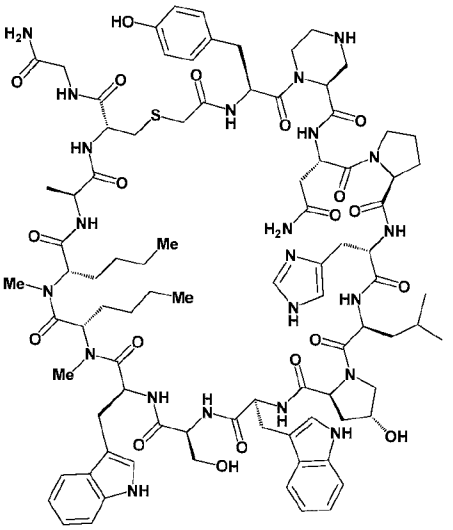
Example 1528 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-60% B over 25 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 15-60% B over 25 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 2.1 mg, and its estimated purity by LCMS analysis was 95%.

Analysis LCMS Condition D: Retention time = 1.43 min; ESI-MS(+) *m/z* 937.1 (M+2H).

Analysis LCMS Condition E: Retention time = 1.41 min; ESI-MS(+) *m/z* 937.5 (M+2H).

Preparation of Example 1529



Molecular Weight: 1852.12

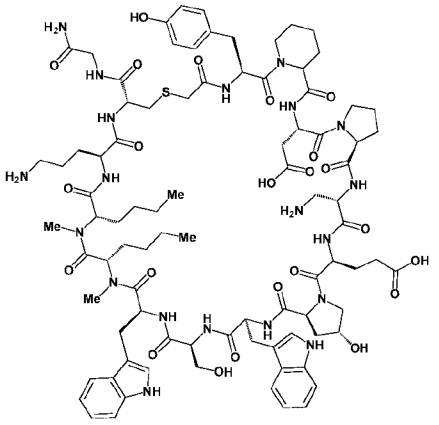
Example 1529 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 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 13.1 mg, and its estimated purity by LCMS analysis was 91%.

Analysis LCMS Condition D: Retention time = 1.44 min; ESI-MS(+) *m/z* 927.0 (M+2H).

Analysis LCMS Condition E: Retention time = 1.35 min; ESI-MS(+) *m/z* 926.8 (M+2H).

Preparation of Example 1530

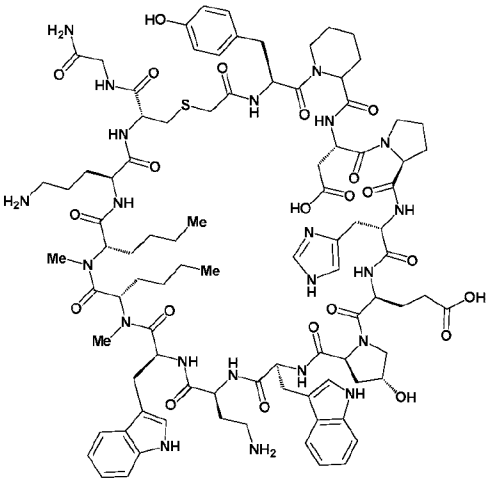


Molecular Weight: 1860.10

Example 1530 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: waters xbridge c-18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 methanol: water with 0.1% trifluoroacetic acid; 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. The material was further 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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; 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 11.9 mg, and its estimated purity by LCMS analysis was 100%. Analysis LCMS Condition H: Retention time = 1.57 min; ESI-MS(+) *m/z* 931.9 (M+2H). Analysis LCMS Condition I: Retention time = 2.70 min; ESI-MS(+) *m/z* 931.2 (M+2H).

Preparation of Example 1531



Molecular Weight: 1924.18

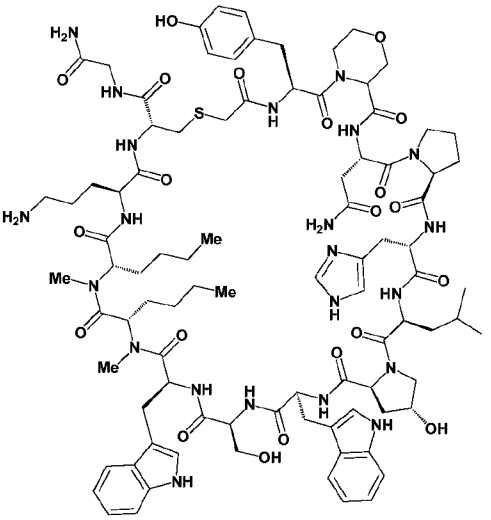
Example 1531 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-60% B over 25 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 1.2 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition D: Retention time = 1.34 min; ESI-MS(+) *m/z* 963.0 (M+2H).

Analysis LCMS Condition E: Retention time = 1.35 min; ESI-MS(+) *m/z* 962.6 (M+2H).

Preparation of Example 1532



Molecular Weight: 1896.17

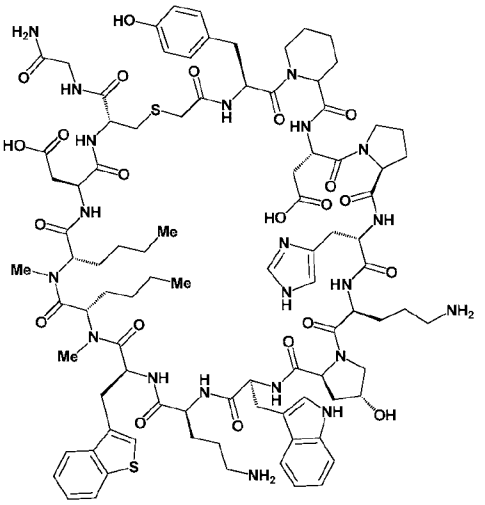
Example 1532 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: waters xbridge c-18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 methanol: water with 0.1% trifluoroacetic acid; 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. The yield of the product was 12.2 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition H: Retention time = 1.48 min; ESI-MS(+) *m/z* 949.4 (M+2H).

Analysis LCMS Condition I: Retention time = 2.57 min; ESI-MS(+) *m/z* 949.0 (M+2H).

Preparation of Example 1533



Molecular Weight: 1941.23

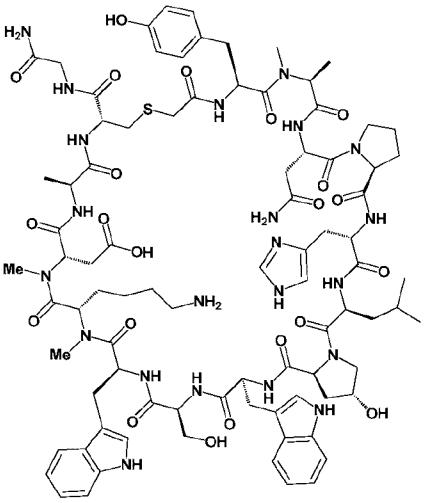
Example 1533 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: waters xbridge c-18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 methanol: water with 0.1% trifluoroacetic acid; Gradient: 45-85% 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 6.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition H: Retention time = 1.63 min; ESI-MS(+) *m/z* 971.6 (M+2H).

Analysis LCMS Condition I: Retention time = 2.87 min; ESI-MS(+) *m/z* 971.9 (M+2H).

Preparation of Example 1534



Molecular Weight: 1842.04

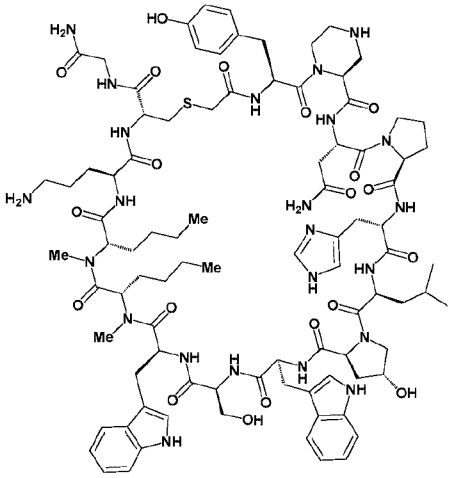
Example 1534 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 0-50% B over 25 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 0-45% B over 25 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 95%.

Analysis LCMS Condition D: Retention time = 0.94 min; ESI-MS(+) *m/z* 922.1 (M+2H).

Analysis LCMS Condition E: Retention time = 0.97 min; ESI-MS(+) *m/z* 921.5 (M+2H).

Preparation of Example 1535



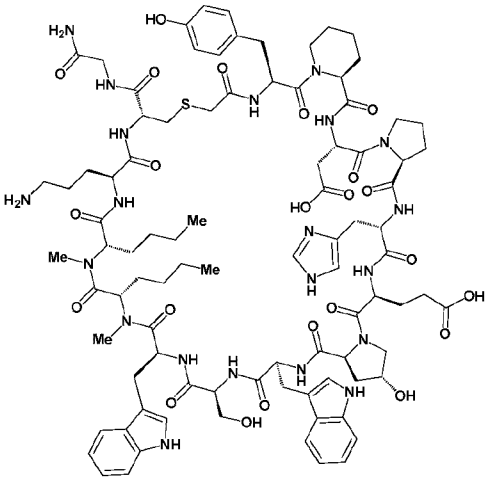
Molecular Weight: 1895.19

Example 1535 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-60% B over 25 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 5-50% B over 25 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 3.0 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.32 min; ESI-MS(+) *m/z* 948.5 (M+2H).
Analysis LCMS Condition E: Retention time = 1.21 min; ESI-MS(+) *m/z* 948.7 (M+2H).

Preparation of Example 1536



Molecular Weight: 1911.14

Example 1536 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

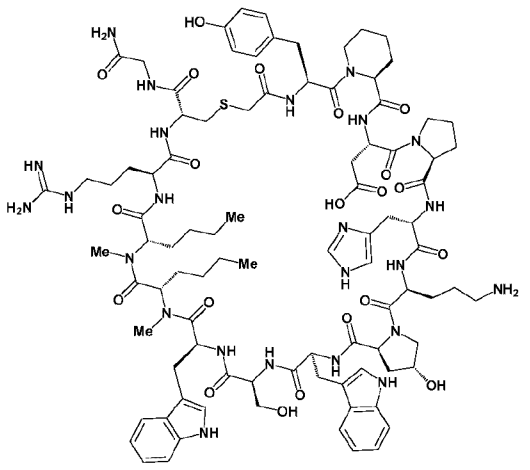
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-60% B over 25 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 15-60% B over 25 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 99%.

Analysis LCMS Condition D: Retention time = 1.32 min; ESI-MS(+) *m/z* 956.7 (M+2H).

Analysis LCMS Condition E: Retention time = 1.43 min; ESI-MS(+) *m/z* 956.7 (M+2H).

Preparation of Example 1537



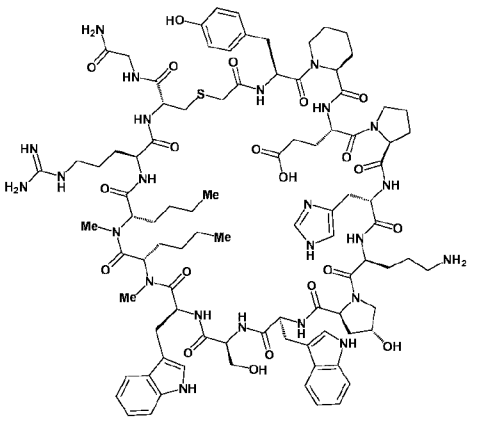
Molecular Weight: 1938.21

Example 1537 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-60% B over 25 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-55% B over 25 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 99%.
Analysis LCMS Condition D: Retention time = 1.42 min; ESI-MS(+) *m/z* 969.7 (M+2H).
Analysis LCMS Condition E: Retention time = 1.40 min; ESI-MS(+) *m/z* 969.7 (M+2H).

Preparation of Example 1538

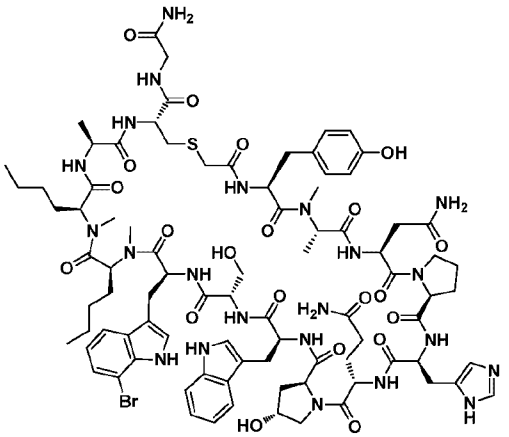


Molecular Weight: 1952.24

Example 1538 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 10-60% B over 25 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were comb ined and dried via centrifugal evaporation. The yield of the product was 5.5 mg, and its estimated purity by LCMS analysis was 100%.
Analysis LCMS Condition D: Retention time = 1.38 min; ESI-MS(+) *m/z* 977.6 (M+2H).
Analysis LCMS Condition E: Retention time = 1.31 min; ESI-MS(+) *m/z* 976.7 (M+2H).

Preparation of Example 1541



Molecular Weight: 1918.96

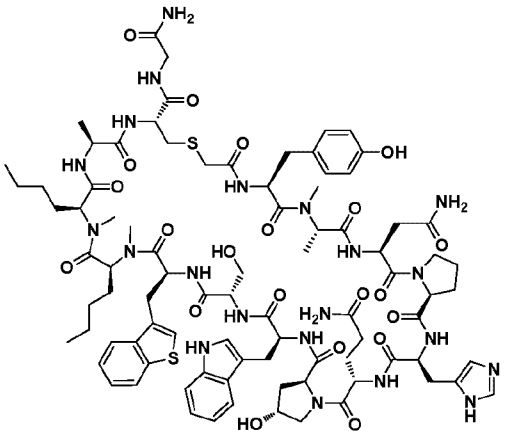
Example 1541 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic

acid; 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 2.4 mg, and its estimated purity by LCMS analysis was 96%.

Analysis LCMS Condition I: Retention time = 2.87 min; ESI-MS(+) *m/z* 960.7 (M+2H).

Preparation of Example 1542



Molecular Weight: 1857.12

Example 1542 was prepared following the general synthetic sequence described for the preparation of Example 0001, composed of the following general procedures: "Symphony Method B: *Resin-swelling procedure*", "Symphony Method B: *Standard-coupling procedure"*, "Symphony Method B*: Secondary amine-coupling procedure",* "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method F*", and "*Cyclization Method D*".

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 5.7 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition H: Retention time = 1.769 min; ESI-MS(+) *m/z* 929.30 (M+2H).

Analysis LCMS Condition I: Retention time = 3.313 min; ESI-MS(+) *m/z* 929.30 (M+2H).

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.

Analysis LCMS Condition A:

Column: Waters BEH C18, 2.1 x 50 mm, 1.7-μm particles; Mobile Phase A: water with 0.05% TFA; Mobile Phase B:Acetonitrile with 0.05% TFA; Temperature: 50 °C; Gradient: 2% B to 98% B over 2 min., then a 0.5 min. hold at 98% B; Flow: 0.8 mL/min; Detection: UV at 220 nm.

Analysis LCMS Condition C:

Column: Waters BEH C18, 2.1 x 50 mm, 1.7-μm particles; Mobile Phase A: water with 0.2% Formic Acid and 0.01% TFA; Mobile Phase B: Acetonitrile with 0.2% Formic acid an 0.01% TFA; Temperature: 50 °C; Gradient: 2% B to 80% B over 2 min., 80% B to 98% B over 0.1 minute then a 0.5 min. hold at 98% B; Flow: 0.8 mL/min; Detection: UV at 220 nm.

Analysis LCMS Condition D:

Column: Waters BEH C18, 2.1 x 50 mm, 1.7-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; Temperature: 50 °C; Gradient: 0-100% B over 3 min., then a 0.75-minute hold at 100% B; Flow: 1.0 mL/min; Detection: UV at 220 nm.

Analysis LCMS Condition E:

Column: Waters BEH C18, 2.1 x 50 mm, 1.7-μm particles; Mobile Phase A: 5:95 acetonitrile:water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile:water with 0.1% trifluoroacetic acid; temperature: 50 °C; Gradient: 0-100% B over 3 min., then a 0.75-minute hold at 100% B; Flow: 1.11 mL/min; Detection: UV at 220 nm.

Analysis LCMS Condition F:

Column: Waters XBridge C18, 2.1 x 50 mm; 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: 35 °C; Gradient: 0-100% B over 4 min., then a 1-minute hold at 100% B; Flow: 4 mL/min; Detection: UV at 220 nm.

Analysis LCMS Condition G:

Column: Waters BEH C18, 2.0 x 50 mm, 1.7-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; Temperature: 50 °C; Gradient: 0-100% B over 3 min., then a 0.5-minute hold at 100% B; Flow: 0.5 mL/min; Detection: UV at 220 nm.

Analysis HPLC Condition B:

Column: YMC Pack ODS-AQ 3um 150x4.6mm; Mobile Phase A: water with 0.1% TFA; Mobile Phase B: Acetonitrile with 0.1% TFA; Temperature: 40 °C; Gradient: from 10% B to 100% B over 10 to 40min.; Flow rate: 1 mL/min; Detection: UV at 220 nm.

General Procedures:

Prelude Method A:

All manipulations were performed under automation on a Prelude peptide synthesizer (Protein Technologies). All procedures unless noted were performed in a 10 or 45 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 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. Amino acid solutions were generally not used beyond three weeks from preparation. HATU solution was used within 5 days of preparation. DMF = dimethylformamide; HCTU = 2-(6-Chloro-1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; NMM = N-methylmorpholine; Sieber = Fmoc-amino-xanthen-3-yloxy, where "3-yloxy" describes the position and type of connectivity to the polystyrene resin. The resin used is Merrifield polymer (polystyrene) with a Sieber linker (Fmoc-protected at nitrogen); 100-200 mesh, 1% DVB, 0.71 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; 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-Ile-OH; Fmoc-Leu-OH; Fmoc-Lys(Boc)-OH; Fmoc-Nle-OH; Fmoc-[N-Me]Ala-OH; Fmoc-[N-Me]Nle-OH; Fmoc-Phe-OH; Fmoc-Pro-OH; Fmoc-(D)-cis-Pro(4-OtBu)-OH; Fmoc-(D)-trans-Pro(4-OtBu)-OH; Fmoc-Sar-OH; Fmoc-Ser(tBu)-OH; Fmoc-Thr(tBu)-OH; Fmoc-Trp(Boc)-OH; Fmoc-Tyr(tBu)-OH; Fmoc-Val-OH.

The procedures of "Prelude Method A" describe an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin. This scale corresponds to approximately 140 mg of the Sieber-Merrifield resin described above. All procedures can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale. 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 "Secondary amine-coupling procedure" described below. Coupling of chloroacetyl group to the N-terminus of the peptide is described by the "Chloroacetyl chloride coupling procedure" or "Chloroacetic acid coupling procedure" detailed below.

Resin-swelling procedure:

To a 40 mL polypropylene solid-phase reaction vessel was added Merrifield Sieber resin (140 mg, 0.100 mmol). The resin was washed (swelled) three times as follows: to the reaction vessel was added DMF (5.0 mL) and DCM (5.0 mL), upon which the mixture was periodically agitated with N2 bubbling from the bottom of the reaction vessel for 10 min. before the solvent was drained through the frit.

Single-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5 min. and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5 min. and then the solution was drained through the frit. The resin was washed successively five times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 60 seconds before the solution was drained through the frit. To the reaction vessel was added the amino acid (0.2M in DMF, 5.0 mL, 10 eq), then HATU or HCTU (0.2M in DMF, 5.0 mL, 10 eq), and finally NMM (0.8M in DMF, 2.5 mL, 20 eq). The mixture was periodically agitated for 60 min., then the reaction solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically agitated for 10 min., then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Secondary amine-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5

min. and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5 min. and then the solution was drained through the frit. The resin was washed successively five times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel 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, 2.5 mL, 5 eq), then HATU (0.2M in DMF, 2.5 mL, 5 eq), and finally NMM (0.8M in DMF, 1.5 mL, 12 eq). The mixture was periodically agitated for 300 min., then the reaction solution was drained through the frit. The resin was twice washed as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically agitated for 10 min., then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Custom amino acids-coupling procedure:

To the reaction vessel containing resin from the previous step was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5 min. and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 or 5 min. and then the solution was drained through the frit. The resin was washed successively five times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel 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, 0.5 to 2.5 mL, 1 to 5 eq), then HATU (0.2M in DMF, 0.5 to 2.5 mL, 1 to 5 eq), and finally DIPEA (0.8M in DMF, 0.5 to 1.5 mL, 4 to 12 eq). The mixture was periodically agitated for 60 min. to 600 min., 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 through the top of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically agitated for 10 min., then the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Chloroacetyl chloride coupling procedure A:

To the reaction vessel containing the resin from the previous step was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. The resin was washed successively five times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added 3.0 mL of a solution of DIPEA (4.0 mmol, 0.699 mL, 40 eq), and chloroacetyl chloride (2.0 mmol, 0.160 mL, 20 eq) in DMF. The mixture was periodically agitated for 12 to 18 hours, then the solution was drained through the frit. The resin was washed successively three times as follows: for each wash, DMF (4.0 mL) was added to top of the vessel 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, CH_2Cl_2 (2.0 mL) was added to top of the vessel and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit.

Chloroacetic acid coupling procedure A:

To the reaction vessel containing the resin from the previous step was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min.

and then the solution was drained through the frit. The resin was washed successively five times as follows: for each wash, DMF (4.0 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added DMF (2.0 mL), chloroacetic acid (1.2 mmol, 113 mg, 12 eq), and N,N'-Diisopropylcarbodiimide (1.2 mmol, 0.187 mL, 12 eq). The mixture was periodically agitated for 12 to 18 hours, then the solution was drained through the frit. The resin was washed successively three times as follows: for each wash, DMF (4.0 mL) was added to top of the vessel 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, CH_2Cl_2 (2.0 mL) was added to top of the vessel and the resulting mixture was periodically agitated for 90 seconds before the solution was drained through the frit.

CEM Method A:

All manipulations were performed under automation on a CEM Liberty microwave peptide synthesizer (CEM Corporation). All procedures unless noted were performed in a 30 or 125 mL polypropylene tube fitted with a bottom frit to a CEM Discovery microwave unit. The tube connects to the CEM Liberty synthesizer through both the bottom and the top of the tube. DMF and DCM can be added through the top and bottom of the tube, which washes down the sides of the tube equally. All solutions are removed through the bottom of the tube except while transferring resin from the top. "Periodic bubbling" describes a brief bubbling of N2 gas through the bottom frit. Amino acid solutions were generally not used beyond three weeks from preparation. HATU solution was used within 5 days of preparation. DMF = dimethylformamide; HCTU = 2-(6-Chloro-1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; DIPEA = diisopropylethylamine; Sieber = Fmoc-amino-xanthen-3-yloxy, where "3-yloxy" describes the position and type of connectivity to the polystyrene resin. The resin used is Merrifield polymer (polystyrene) with a Sieber linker (Fmoc-protected at nitrogen); 100-200 mesh, 1% DVB, 0.71 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; 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

The procedures of "CEM Method A" describe an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin. This scale corresponds to approximately 140 mg of the Sieber-Merrifield resin described above. All procedures can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale. 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 "Secondary amine-coupling procedure" described below. Coupling of chloroacetyl group to the N-terminus of the peptide is described by the "Chloroacetyl chloride coupling procedure" or "Chloroacetic acid coupling procedure" detailed above.

Resin-swelling procedure:

To 50 mL polypropylene conical tube was added Merrifield Sieber resin (140 mg, 0.100 mmol). Then DMF (7 mL) was added to the tube followed by DCM (7 mL). The resin was then transferred to the reaction vessel from top of the vessel. The procedure is repeated additionally two times. DMF (7 mL) was added followed by DCM (7 mL). The resin was allowed to swell with N2 bubbling from the bottom of the reaction vessel for 15 min. before the solvent was drained through the frit.

Standard Coupling procedure:

To the reaction vessel containing resin from the previous step was added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. To the reaction vessel was

added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added the amino acid (0.2M in DMF,2.5 mL, 5 eq), HATU (0.5M in DMF, 1.0 mL, 5 eq), and DIPEA (2M in NMP, 0.5 mL, 10 eq). The mixture was mixed by N2 bubbling for 5 min. at 75 °C for all amino acids, except Fmoc-Cys(Trt)-OH and Fmoc-His(Trt)-OH which are coupled at 50 °C, the reaction solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically bubbled for 2 min. at 65 °C, then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. The resulting resin was used directly in the next step.

Double-couple Coupling procedure:

To the reaction vessel containing resin from the previous step was added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. To the reaction vessel was added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added the amino acid (0.2M in DMF,2.5 mL, 5 eq), HATU (0.5M in DMF, 1.0 mL, 5 eq), and DIPEA (2M in NMP, 0.5 mL, 10 eq). The mixture was mixed by N2 bubbling for 5 min. at 75 °C for all amino acids, except Fmoc-Cys(Trt)-OH and Fmoc-His(Trt)-OH which are coupled at 50 °C, the reaction solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added the amino acid (0.2M in DMF,2.5 mL,

5 eq), HATU (0.5M in DMF, 1.0 mL, 5 eq), and DIPEA (2M in NMP, 0.5 mL, 10 eq). The mixture was mixed by N2 bubbling for 5 min. at 75 °C for all amino acids, except Fmoc-Cys(Trt)-OH and Fmoc-His(Trt)-OH which are coupled at 50 °C, the reaction solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically bubbled for 2 min. at 65 °C, then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. The resulting resin was used directly in the next step.

Custom amino acids-coupling procedure:

To the reaction vessel containing resin from the previous step was added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. To the reaction vessel was added a solution of piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was periodically agitated for 3 min. and then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added the amino acid solution (1.25 mL to 5 mL, 2.5 eq to 10 eq) containing HATU (2.5 eq to 10 eq), and finally DIPEA (2M in NMP, 0.5 mL to 1 mL, 20 eq). The mixture was mixed by N2 bubbling for 5 min. to 2 hours at 25 °C to 75 °C, then the reaction solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. To the reaction vessel was added a solution of acetic anhydride:DIEA:DMF (10:1:89 v/v/v, 5.0 mL). The mixture was periodically bubbled for 2 min. at 65 °C, then the solution was drained through the frit. The resin was washed successively three times as follows: DMF (7 mL) wash from top, followed by DMF (7 mL) wash from bottom and finally with DMF (7 mL) wash from top. The resulting resin was used directly in the next step.

Symphony Method A:

All manipulations were performed under automation on a Symphony peptide synthesizer (Protein Technologies). All procedures unless noted were performed in a Symphony polypropylene tube fitted with a bottom frit. The tube connects to the Symphony peptide synthesizer through both the bottom and the top of the tube. All Solvents, DMF, DCM, amino acids and reagents are added through the bottom of the 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 15 seconds. Amino acid solutions were generally not used beyond three weeks from preparation. HATU solution was used within 5 days of preparation. DMF = dimethylformamide; HCTU = 2-(6-Chloro-1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; NMM= n-Methyl morpholine; DIPEA = diisopropylethylamine; Sieber = Fmoc-amino-xanthen-3-yloxy, where "3-yloxy" describes the position and type of connectivity to the polystyrene resin. The resin used is Merrifield polymer (polystyrene) with a Sieber linker (Fmoc-protected at nitrogen); 100-200 mesh, 1% DVB, 0.71 mmol/g loading. Other common Acid sensitive resins can also be used in the synthesis such as Rink or functionalized Chloro trityl Resin. 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; 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

The procedures of "Symphony Method A" describes an experiment performed on a 0.050-0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin. This scale corresponds to approximately 70-140 mg of the Sieber-Merrifield resin described above. All procedures can be scaled beyond the 0.050-0.100 mmol scale by adjusting the described volumes by the multiple of the scale. Prior to amino acid coupling, all peptide synthesis sequences began with a resin-swelling

procedure, described below as "Swelling procedure". Coupling of amino acids to a primary amine N-terminus used the "Standard-coupling procedure" described below. Coupling of amino acids to a secondary amine N-terminus used the "Double-coupling ", custom amino acids are coupled via a manual Blank addition of the amino acid "Blank coupling" described below.

Swelling procedure:

To a Symphony polypropylene solid-phase reaction vessel was added Merrifield Sieber resin (70 mg, 0.050 mmol or 140 mg, 0.100 mmol). The resin was washed (swelled) three times as follows: to the reaction vessel was added DMF (2.5 mL) upon which the mixture was periodically agitated with N2 bubbling from the bottom of the reaction vessel for 10 minutes before the solvent was drained through the frit.

Standard-coupling procedure:

The resin was washed three times as follows: to the reaction vessel was added DMF (2.5 mL) upon which the mixture was periodically agitated with N_2 bubbling from the bottom of the reaction vessel for 30 seconds before the solvent was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.5 mL). The mixture was periodically agitated for 5 minutes and then the solution was drained through the frit. The procedure was repeated one more time. The resin was washed 6 times as follows: for each wash, DMF (2.5 mL) was added through the bottom of the vessel 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, 1.25 mL, 5 eq), then HATU (0.2M in DMF, 1.25 mL, 5 eq), and finally NMM (0.8M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 10 minutes, then the reaction solution was drained through the frit. The resin was washed with DMF (6.25 mL) was added through the bottom of the vessel 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, 1.25 mL, 5 eq), then HATU (0.2M in DMF, 1.25 mL, 5 eq), and finally NMM (0.8M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 10 minutes, then the reaction solution was drained through the frit. The resin was washed successively three times as follows: for each

wash, DMF (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Secondary amine-coupling procedure:

The resin was washed three times as follows: to the reaction vessel was added DMF (2.5 mL) upon which the mixture was periodically agitated with N_2 bubbling from the bottom of the reaction vessel for 30 seconds before the solvent was drained through the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.5 mL). The mixture was periodically agitated for 5 minutes and then the solution was drained through the frit. The procedure was repeated one more time. The resin was washed 6 times as follows: for each wash, DMF (2.5 mL) was added through the bottom of the vessel 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, 1.25 mL, 5 eq), then HATU (0.2M in DMF, 1.25 mL, 5 eq), and finally NMM (0.8M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 300 minutes, then the reaction solution was drained through the frit. The resin was washed with DMF (6.25 mL) was added through the bottom of the vessel 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, 1.25 mL, 5 eq), then HATU (0.2M in DMF, 1.25 mL, 5 eq), and finally NMM (0.8M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 300 minutes, then the reaction solution was drained through the frit. The resin was washed successively three times as follows: for each wash, DMF (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. The resulting resin was used directly in the next step.

Symphony Method B

Final capping procedure:

The resin was washed three times as follows: to the reaction vessel was added DMF (2.5 mL) upon which the mixture was periodically agitated with N_2 bubbling from the bottom of the reaction vessel for 30 seconds before the solvent was drained through

the frit. To the reaction vessel was added piperidine:DMF (20:80 v/v, 2.5 mL). The mixture was periodically agitated for 2.5 minutes and then the solution was drained through the frit. The resin was washed 6 times as follows: for each wash, DMF (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added NMM (0.8M in DMF, 1.25 mL, 10 eq) followed by the addition of the Chloroacetic anhydride (0.4M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was washed with DMF (6.25 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added NMM (0.8M in DMF, 1.25 mL, 10 eq) followed by the addition of the Chloroacetic anhydride (0.4M in DMF, 1.25 mL, 10 eq). The mixture was periodically agitated for 15 minutes, then the reaction solution was drained through the frit. The resin was washed 6 times as follows: DMF (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. To the reaction vessel was added Ac_2O/DIPEA/DMF (v/v/v 1:1:3 2.5 mL) the mixture was periodically agitated for 10 minutes, then the reaction solution was drained through the frit. The resin was washed successively six times as follows: for each wash, DMF (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. The resin was washed successively four times as follows: for each wash, DCM (2.5 mL) was added through the bottom of the vessel and the resulting mixture was periodically agitated for 30 seconds before the solution was drained through the frit. The resulting resin was then dried with a stream of Nitrogen for 10 mins.

N-methylation on-resin (Turner, R. A.; Hauksson, N. E.; Gipe, J. H.; Lokey, R. S. *Org. Lett.* 2013, *15*(19), 5012-5015):

All manipulations were performed manually unless noted. The procedure of "N-methylation on-resin" describes an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin that was used to generate the peptide. This scale is not based on a direct determination of the quantity of

peptide used in the procedure. The procedure can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale.

The resin was transferred into a 25 mL syringe equipped with a frit. To the resin was added piperidine:DMF (20:80 v/v, 5.0 mL). The mixture was shaken for 3 min. and then the solution was drained through the frit. The resin was washed 3 times with DMF (4.0 mL). To the reaction vessel was added piperidine:DMF (20:80 v/v, 4.0 mL). The mixture was shaken for 3 min. and then the solution was drained through the frit. The resin was washed successively six times as follows: 3 times DMF (4.0 mL) was added and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit followed by 3 addition of DCM (4.0 mL) and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit.

The resin was suspended in DMF (2.0 mL) and ETHYL TRIFLUOROACETATE (0.119 ml, 1.00 mmol), 1,8-DIAZABICYCLO[5.4.0]UNDEC-7-ENE (0.181 ml, 1.20 mmol). The mixture was put on a shaker for 60 min.. The solution was drained through the frit. The resin was washed successively six times as follows: 3 times DMF (4.0 mL) was added and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit followed by 3 addition of DCM (4.0 mL) and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit. The resin was washed 3 times with dry THF (2.0 mL) to remove any residual water. In an oven dried 4.0 mL vial is added THF (1.0 mL), TRIPHENYLPHOSPHINE (131 mg, 0.500 mmol) on dry 4 Å molecular sieves (20 mg). The turbid solution is transferred on the resin and isopropyl azodicarboxylate (0.097 mL, 0.5 mmol) is added slowly. The resin is shaken for 15 min.. The solution was drained through the frit and the resin was washed with 3 times with dry THF (2.0 mL) to remove any residual water. In an oven dried 4.0 mL vial is added THF (1.0 mL), TRIPHENYLPHOSPHINE (131 mg, 0.500 mmol) on dry 4 Å molecular sieves (20 mg). The turbid solution is transferred on the resin and diisopropyl azodicarboxylate (0.097 mL, 0.5 mmol) is added slowly. The resin is shaken for 15 min.. The solution was drained through the frit. The resin was washed successively six times as follows: 3 times DMF (4.0 mL) was added and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit followed by 3 addition of DCM (4.0 mL) and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit.

The resin was suspended in Ethanol (1.0 mL) and THF (1.0 mL) and SODIUM BOROHYDRIDE (37.8 mg, 1.000 mmol) was added. The mixture was mixed on a shaker for 30 min.. Solution was drained through the frit and the resin was washed successively six times as follows: 3 times DMF (4.0 mL) was added and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit followed by 3 addition of DCM (4.0 mL) and the resulting mixture was shaken for 3 seconds before the solution was drained through the frit.

Global Deprotection Method B:

All manipulations were performed manually unless noted. The procedure of "Global Deprotection Method B" describes an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin. The procedure can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale. A "deprotection solution" was prepared using trifluoroacetic acid:triisopropylsilane:dithiothreitol (94:3:3 v:v:w). The resin was removed from the reaction vessel and transferred to a 25 mL syringe equipped with a frit. To the syringe was added the "deprotection solution" (5.0 mL). The mixture was mixed in a shaker for 5 min.. The solution was filtered through and diluted in diethyl ether (30 mL). The precipitated solid was centrifuged for 3 min.. The supernatant solution was decanted and the solid was re-suspended in diethyl ether (25 mL). The suspension was centrifuged for 3 min.. The supernatant was decanted and the remaining solid was suspended diethyl ether (25 mL). The suspension was centrifuged for 3 min.. The supernatant was decanted and the remaining solid was dried under high vacuum. The crude peptide was obtained as a white to off-white solid.

Global Deprotection Method G

All manipulations were performed manually unless noted. The procedure of "Global Deprotection Method G" describes an experiment performed on a 0.50 or 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin. The procedure can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale. A "deprotection solution" was prepared using trifluoroacetic acid:triisopropylsilane:water (95:2.5:2.5 v:v:v). To the resin was added the

"deprotection solution" (2.5 mL). The mixture was stirred for 5 min.. The solution was filtered and the filtrate was added to cold diethyl ether (40 mL). The resin was treated with an additional 2.5 mL of "deprotection solution" for 2 min and the filtrate was added to cold ether from the previous treatment. The precipitated solid was collected by centrifugation, washed twice with ether (40 mL) and dried under high vacuum to yield a white to off-white solid.

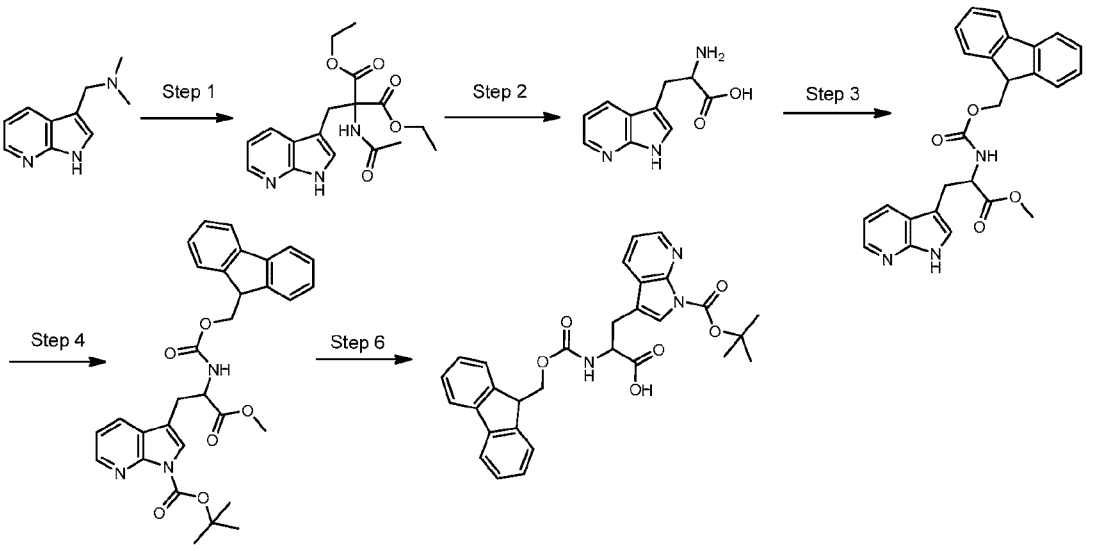
Cyclization Method C:

All manipulations were performed manually unless noted. The procedure of "Cyclization Method C" describes an experiment performed on a 0.100 mmol scale, where the scale is determined by the amount of Sieber linker bound to the resin that was used to generate the peptide. This scale is not based on a direct determination of the quantity of peptide used in the procedure. The procedure can be scaled beyond 0.100 mmol scale by adjusting the described volumes by the multiple of the scale. The crude peptide solids were dissolved in a solution of acetonitrile:aqueous 0.1M ammonium bicarbonate buffer (11 mL:24 mL), and the solution was then carefully adjusted to pH = 8.5-9.0 using aqueous NaOH (1.0 M). The solution was then mixed using a shaker for 12 to 18 hours. The reaction solution was concentrated and the residue was then dissolved in acetonitrile:water. This solution was subjected to reverse-phase HPLC purification to afford the desired cyclic peptide.

Preparation of racemic 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(tert-butoxycarbonyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid

(Robison, M. M and Robison, B. L . *J. Am.. Chem. Soc.*, 1955, *77*, 457-459).

Scheme:



Step 1:

A mixture of 7-azagramine (3.5 g, 19.97 mmol), diethyl acetamidomalonate (4.34 g, 19.97 mmol) and xylenes (35 ml) was treated with powder sodium hydroxide (0.080 g, 1.997 mmol) and stirred at reflux for 15 h under nitrogen. The hot solution was filtered to give a yellow filtrate. A yellow solid precipitated from the filtrate when cooled to room temperature. The solid mass was suspended in benzene (40 mL) and filtered. The collected solid was washed with cyclohexane (2 x 100 mL) to give diethyl 2-((1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)-2-acetamidomalonate (3.5 g, 50.4 %) as a white solid. Analysis LCMS Condition A: Retention time = 0.79 min; ESI-MS(+) *m/z* 348.3 (M+H).

Step 2:

A mixture of diethyl 2-((1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)-2-acetamidomalonate (3.5 g, 10.08 mmol) and hydrochloric acid, 37% (30 mL) was refluxed for 15 h an the concentrated to 10 mL. The product was treated with acetonitrile (5 mL) and lyophilized to give an off-white solid. This was re-dissolved in 15% NH4OH to pH 7 and the solution was then diluted with water (20 mL). The precipitated white solid was collected by filtration, washed with water and EtOH and dried to yield 2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid (1.5 g, 72.5 %) as a white solid. Analysis LCMS Condition A: Retention time = 0.29 min; ESI-MS(+) *m/z* 206.0 (M+H).

Step 3:

A solution of 2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid (1.3 g, 6.33 mmol) and triethylamine (1.766 ml, 12.67 mmol) in acetonitrile (20 ml) and water (15 ml) was treated with FMOC-OSu (2.137 g, 6.33 mmol) and the resulting solution was stirred at rt for 30 min. The mixture was concentrated to dryness to give a white foamy solid which was triturated with ether (50 mL). The solid was treated 1 M HCl (100 mL) and the gummy solid that formed was triturated with water, MeOH and ether and dried under vacuum. The resulting product was suspendend in anhydrous MeOH (50 mL) and 4 M HCl/dioxane (10 mL) and the solution was refluxed for 1 h. The mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc and saturated $NaHCO_3$ and filtered. The EtOAc phase was washed twice with brine, dried over sodium sulfate and concentrated under reduced pressure to give methyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoate (1.0 g, 28 % yield) as a foamy yellow solid.

Analysis LCMS Condition A: Retention time = 0.81 min; ESI-MS(+) *m/z* 442.5 (M+H).

Step 4:

A solution of methyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoate (1.0 g, 2.265 mmol) in anhydrous THF (10 mL) was treated with DMAP (0.028 g, 0.227 mmol), placed under nitrogen and cooled in an ice bath. A solution of Boc_2O (0.789 mL, 3.40 mmol) in THF (5 mL) was added to the mixture over 3 min and the mixture was stirred and allowed to warm up over 16 h. The reaction mixture was diluted with EtOAc (150 mL), washed with saturated NH4Cl (3 x 50 mL) and brine (50 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography using a 40 g ISCO silica gel cartridge eluted with 0-60% EtOAc/hexanes to give tert-butyl 3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)-1H-pyrrolo[2,3-b]pyridine-1-carboxylate (0.85 g, 69 % yield) as a white solid.

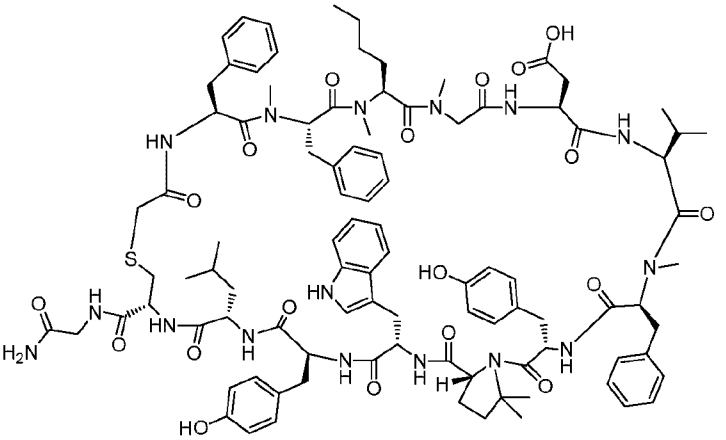
Analysis LCMS Condition A: Retention time = 1.01 min; ESI-MS(+) *m/z* 542.5 (M+H).

Step 5:

A solution of tert-butyl 3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)-1H-pyrrolo[2,3-b]pyridine-1-carboxylate (200 mg, 0.369 mmol) in anhydrous 1,2-Dichloroethane (5 mL) was treated with trimethyltin hydroxide (200 mg, 1.108 mmol) and the mixture was stirred at 65 °C for 1 h. The mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with 1 M HCl and brine, dried over sodium sulfate and concentrated under reduce pressure to yield 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(tert-butoxycarbonyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid as a white foamy solid. This was used without further purification. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.40 – 8.37 (m, 1H), 8.12 – 8.05 (m, 1H), 7.90-7.75 (m, 2H), 7.70 - 7.55 (m, 2H), 7.43 - 7.35 (m, 2H), 7.30-7.18 (m, 4H), 4.37-4.29 (m, 1H), 4.16 - 4.12 (m, 3H), 3.23-3.15 (m, 1H), 3.07-2.98 (m, 1H), 1.60 (s, 9H).

Analysis LCMS Condition A: Retention time = 0.93 min; ESI-MS(+) *m/z* 528.4 (M+H).

Preparation of Example 3210



Example 3210

To a 50 mL polypropylene tube was added Sieber resin (140 mg, 0.100 mmol), and the tube was placed on the CEM Liberty microwave peptide synthesizer. The following procedures were then performed sequentially:

"CEM Method A: Resin-swelling procedure" was followed;

"CEM Method A: Standard coupling procedure" was followed with Fmoc-Gly-OH;

"CEM Method A: Standard coupling procedure" was followed with Fmoc-Cys(Trt)-OH;

"CEM Method A: Standard coupling procedure" was followed with Fmoc-Leu-OH;

"CEM Method A: Standard coupling procedure" was followed with Fmoc-Tyr(tBu)-OH;
"CEM Method A: Standard coupling procedure" was followed with Fmoc-Trp(Boc)-OH;
Fmoc-D-Pro(5,5-di-Me) was coupled manually as follows: to the peptidyl-resin was added a 5 mL solution of Fmoc-D-Pro(5,5-di-Me)-OH (1.2 eq), HATU (1.2 eq) and DIEA (2.5 eq). The resin suspension was stirred for 16 hrs. The resin was washed with DMF (3 x 5 mL), DCM (3 x 5 mL) and once again with DMF (5 mL). The synthesis was then continued on the CEM synthesizer.
"CEM Method A: Standard coupling procedure" was followed with Fmoc-Tyr(tBu)-OH;
"CEM Method A: Standard coupling procedure" was followed with Fmoc-[N-Me]Phe-OH;
"CEM Method A: *Custom amino acids-coupling procedure*" was followed with Fmoc-Val-OH using 10 eq for 10 min at 75 °C, followed by 2 hours at room temperature;
"CEM Method A: Standard coupling procedure" was followed with Fmoc-Asp(OtBu)-OH;
"CEM Method A: Standard coupling procedure" was followed with Fmoc-Sar-OH;
"CEM Method A: *Custom amino acids-coupling procedure*" was followed with Fmoc-[N-Me]Nle-OH using 5 eq for 10 min;
"CEM Method A: *Custom amino acids-coupling procedure*" was followed with Fmoc-[N-Me]Phe-OH using 5 eq for 10 min;
"CEM Method A: *Custom amino acids-coupling procedure*" was followed with Fmoc-Phe-OH using 5 eq for 10 min;
"Prelude Method A: Chloroacetyl chloride coupling procedure A" was followed;
"Global Deprotection Method B" was followed;
"Cyclization Method C" was followed.

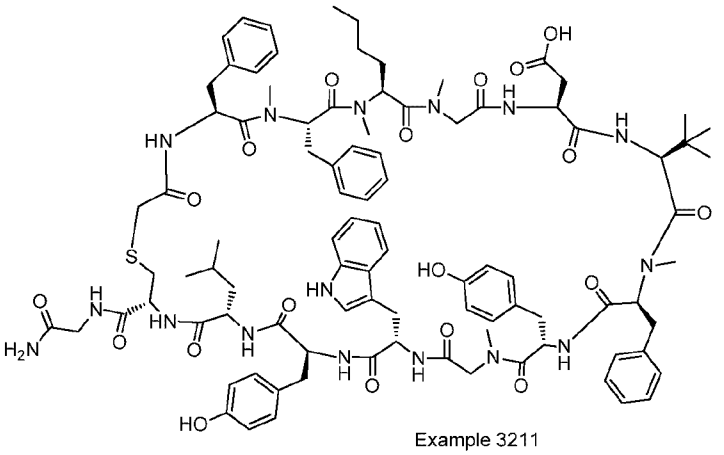
The crude material was purified via preparative LC/MS with the following conditions: Column: Phenomenex Luna 20x250 5µ particles; Mobile Phase A: water with 0.1% TFA; Mobile Phase B: Acetonitrile with 0.1% TFA; Gradient: 30-80% B over 50 min., then a 5-minute hold at 80% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 1.1 mg, and its estimated purity was 99% by "Analysis HPLC Condition B" using a gradient of 35% to 85% buffer B over 30 min.
Analysis LCMS Condition A: Retention time = 1.55 min; ESI-MS(+) *m/z* 925.8 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 925.4547 (M+2H)

Found: 925.4551 (M+2H)

Preparation of Example 3211



Example 3211

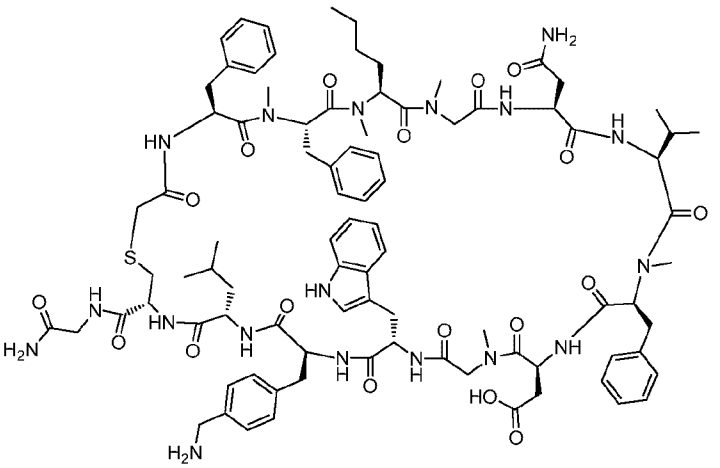
Example 3211 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard-coupling procedure", "*Custom amino acids-coupling procedure*", "CEM Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 15-65% B over 25 min., 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 3.4 mg, and its estimated purity by LCMS analysis was 97% using "Analysis conditions D and E".

Analysis LCMS Condition D: Retention time = 1.60 min; ESI-MS(+) *m/z* 905.9 (M+2H).

Analysis LCMS Condition E: Retention time = 1.82 min; ESI-MS(+) *m/z* 906.0 (M+2H).

Preparation of Example 3212



Example 3212

To a 40 mL polypropylene solid-phase reaction vessel was added Sieber resin (140 mg, 0.100 mmol), and the reaction vessel was placed on the Prelude peptide synthesizer. The following procedures were then performed sequentially:

"Prelude Method A: Resin-swelling procedure" was followed;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Gly-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Cys(Trt)-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Leu-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Phe(CH_2NH_2)-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Trp(Boc)-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Sar-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Asp(OtBu)-OH;;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-[N-Me]Phe-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Val-OH;

"Prelude Method A: Single-coupling procedure" was followed with Fmoc-Asn(Trt)-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Sar-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-[N-Me]Nle-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-[N-Me]Phe-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Phe-OH;

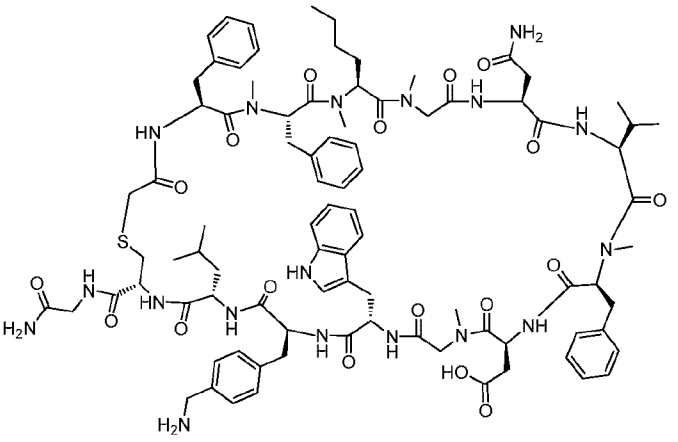
"Prelude Method A: Chloroacetyl chloride coupling procedure A" was followed;

"Global Deprotection Method B" was followed;

"Cyclization Method C" was followed.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., 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 6.8 mg, and its estimated purity by LCMS analysis was 97% by "Analysis conditions D and E". Analysis LCMS condition D: Retention time = 1.62 min; ESI-MS(+) *m/z* 880.7 (M+2H). Analysis LCMS condition E: Retention time = 1.61 min; ESI-MS(+) *m/z* 880.7 (M+2H).

Preparation of Example 3213



Example 3213

Example 3213 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling

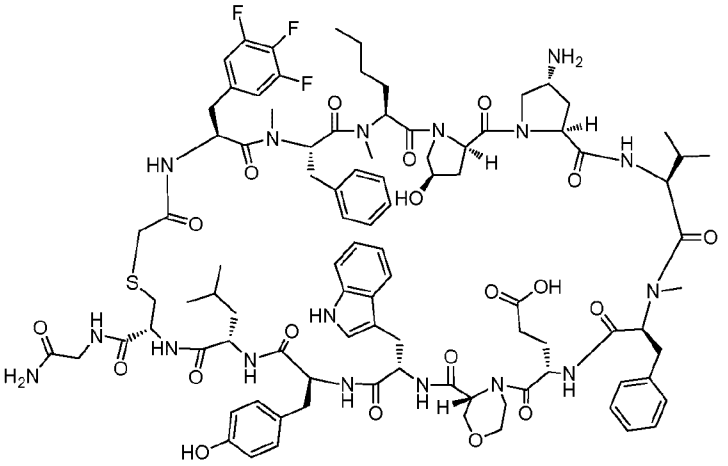
procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 15-65% B over 25 min., 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 9.5 mg, and its estimated purity by LCMS analysis was 99% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 1.60 min; ESI-MS(+) *m/z* 887.9 (M+2H).

Analysis LCMS Condition E: Retention time = 1.60 min; ESI-MS(+) *m/z* 887.7 (M+2H).

Preparation of Example 3216



Example 3216

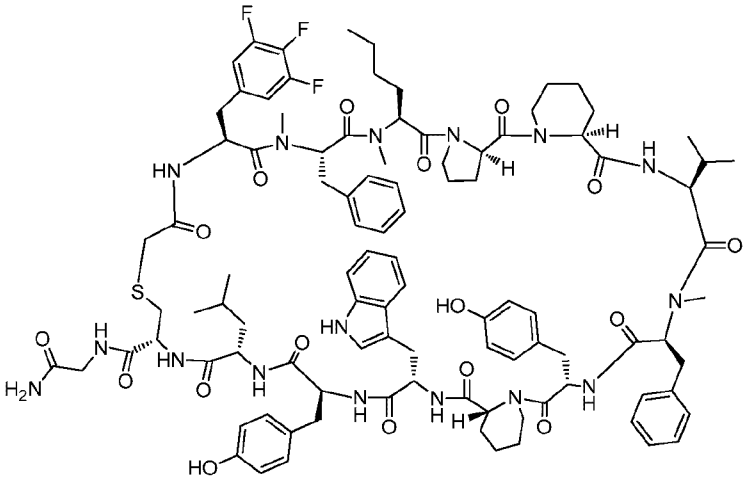
Example 3216 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 15-65% B over 25 min., 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 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 25-70% B over 25 min., 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 1.3 mg, and its estimated purity by LCMS analysis was 96% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 1.69 min; ESI-MS(+) *m/z* 949.2 (M+2H).

Analysis LCMS Condition E: Retention time = 1.70 min; ESI-MS(+) *m/z* 949.2 (M+2H).

Preparation of Example 3217



Example 3217

Example 3217 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude

Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 35-85% B over 25 min., 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 5.2 mg, and its estimated purity by LCMS analysis was 98% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 2.43 min; ESI-MS(+) *m/z* 956.8 (M+2H).

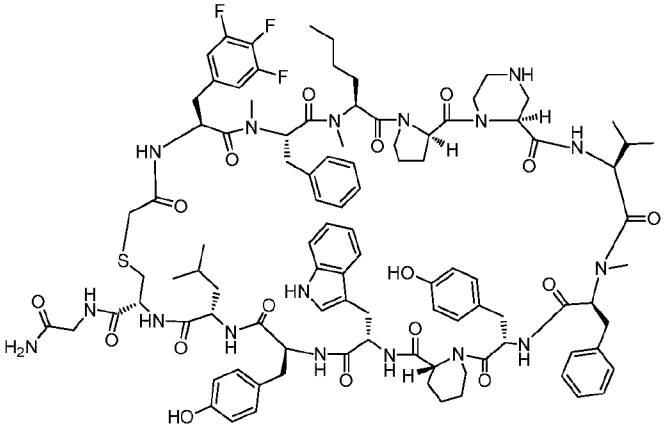
Analysis LCMS Condition E: Retention time = 2.43 min; ESI-MS(+) *m/z* 957.0 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 956.4613 (M+2H).

Found: 956.4604 (M+2H).

Preparation of Example 3218



Example 3218

Example 3218 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 20-70% B over 25 min., 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 7.6 mg, and its estimated purity by LCMS analysis was 94% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 2.12 min; ESI-MS(+) *m/z* 957.2 (M+2H).

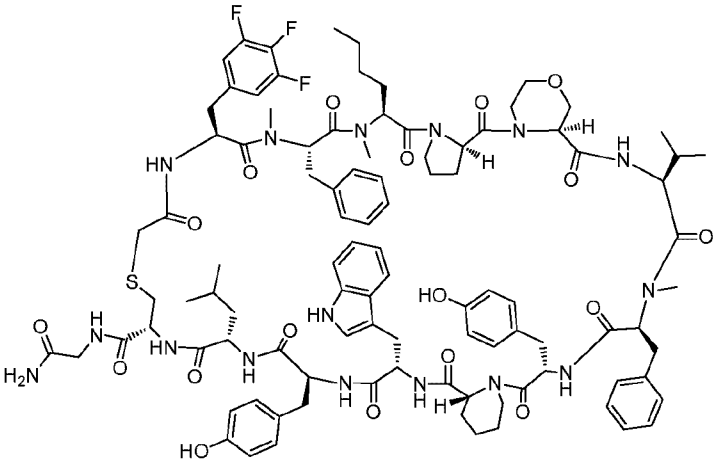
Analysis LCMS Condition E: Retention time = 1.83 min; ESI-MS(+) *m/z* 957.4 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 956.9590 (M+2H)

Found: 956.9582 (M+2H)

Preparation of Example 3219



Example 3219

Example 3219 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 30-80% B over 25 min., 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 7.9 mg, and its estimated purity by LCMS analysis was 96% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 2.26 min; ESI-MS(+) *m/z* 957.9 (M+2H).

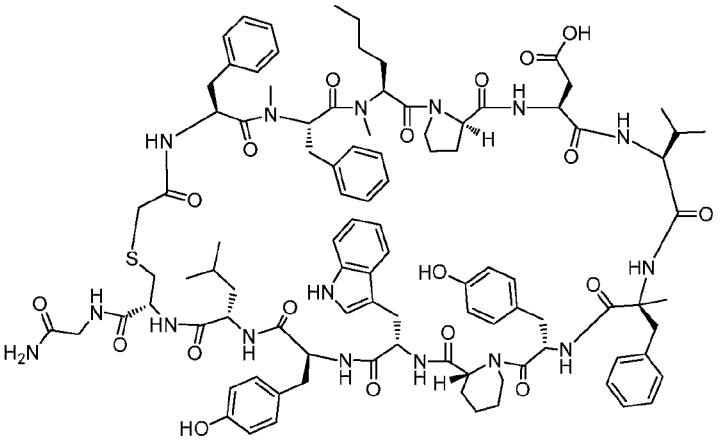
Analysis LCMS Condition E: Retention time = 2.26 min; ESI-MS(+) *m/z* 958.0 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 956.4510 (M+2H)

Found: 956.4493 (M+2H)

Preparation of Example 3220



Example 3220

Example 3220 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 25-75% B over 25 min., 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 8.4 mg, and its estimated purity by LCMS analysis was 92% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 1.71 min; ESI-MS(+) *m/z* 932.0 (M+2H).

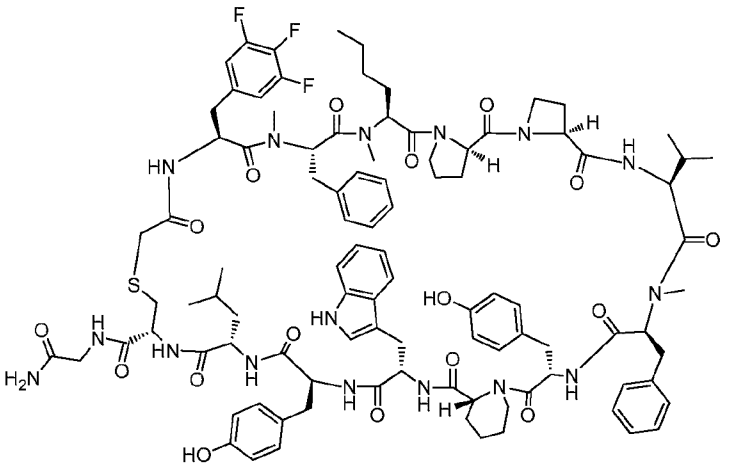
Analysis LCMS Condition E: Retention time = 1.94 min; ESI-MS(+) *m/z* 932.0 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 931.4547 (M+2H)

Found: 931.4536 (M+2H)

Preparation of Example 3221



Example 3221

Example 3221 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 30-80% B over 25 min., 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 7.2 mg, and its estimated purity by LCMS analysis was 94% by "Analysis Conditions D and E".

Analysis LCMS Condition D: Retention time = 2.22 min; ESI-MS(+) *m/z* 942.1 (M+2H).

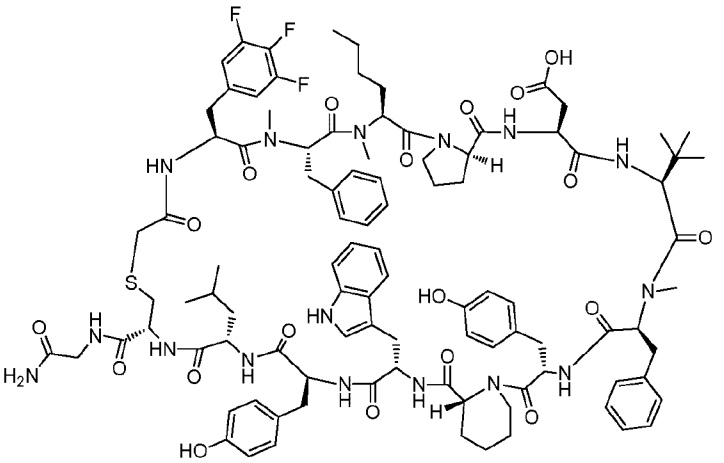
Analysis LCMS Condition E: Retention time = 2.20 min; ESI-MS(+) *m/z* 943.2 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 942.4457 (M+2H)

Found: 942.4445 (M+2H)

Preparation of Example 3222



Example 3222

Example 3222 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Phenomenex Luna 20x250 5u particles; Mobile Phase A: water with 0.1% TFA; Mobile Phase B: Acetonitrile with 0.1% TFA; Gradient: .35-95% B over 50 min., then a 5-minute hold at 95% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 8.9 mg, and its estimated purity was 99% by "Analysis HPLC Condition B" using a gradient of 35% to 85% buffer B over 30 min.

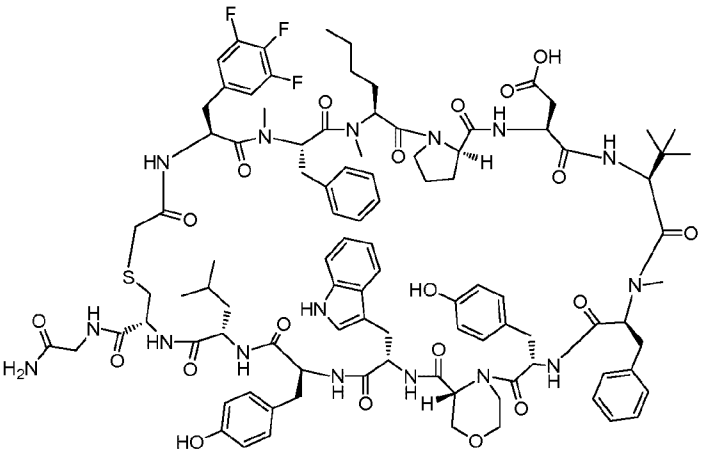
Analysis LCMS Condition A: Retention time = 1.65 min; ESI-MS(+) *m/z* 966.1 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 965.4484 (M+2H)

Found: 965.4473 (M+2H)

Preparation of Example 3223



Example 3223

Example 3223 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Phenomenex Luna 20x250 5u particles; Mobile Phase A: water with

0.1% TFA; Mobile Phase B: Acetonitrile with 0.1% TFA; Gradient: 35-95% B over 50 min., then a 5-minute hold at 95% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The yield of the product was 9.1 mg, and its estimated purity was 98% by "Analysis HPLC Condition B" using a gradient of 35% to 85% buffer B over 30 min.

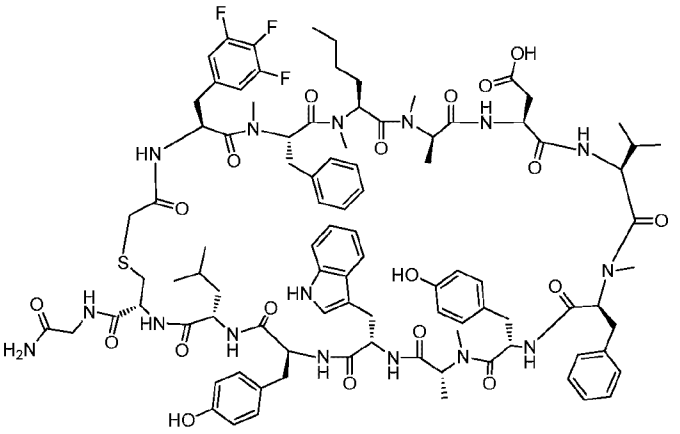
Analysis LCMS Condition A: Retention time = 1.65 min; ESI-MS(+) *m/z* 966.8 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 966.4381 (M+2H)

Found: 966.4375 (M+2H)

Preparation of Example 3224



Example 3224

Example 3224 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 min., 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-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: 25-65% B over 30 min., 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 9.7 mg, and its estimated purity by LCMS analysis was 97% by "Analysis LCMS Condition E".

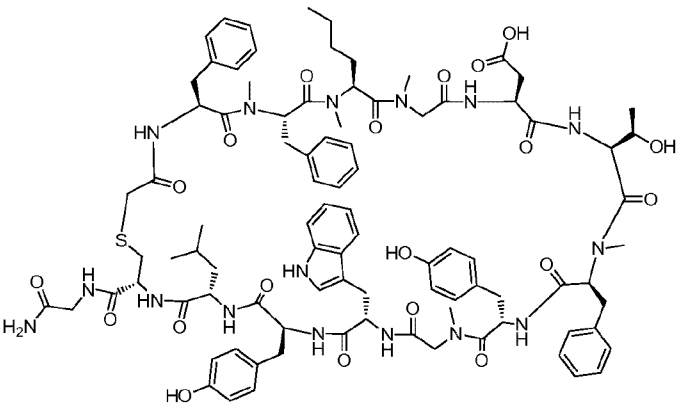
Analysis LCMS Condition E: Retention time = 1.93 min; ESI-MS(+) *m/z* 939.31 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 939.4328 (M+2H)

Found: 939.4322 (M+2H)

Preparation of Example 3225



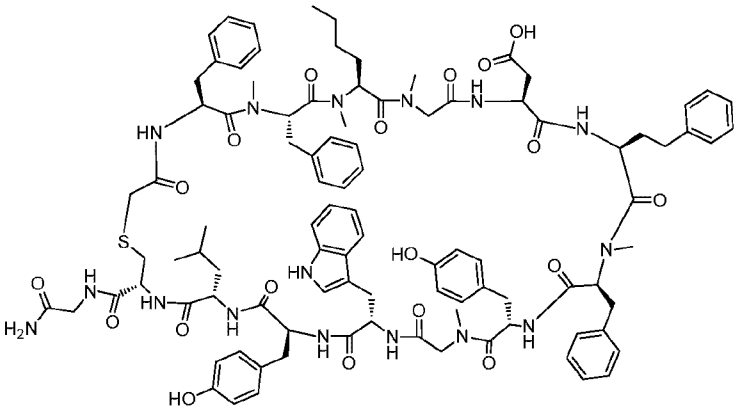
Example 3225

Example 3225 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 20-60% B over 30 min., 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 10.2 mg, and its estimated purity by LCMS analysis was 96% by "Analysis Conditions E and G". Analysis LCMS Condition E: Retention time = 1.59 min; ESI-MS(-) *m/z* 898.5 (M-2H). Analysis LCMS Condition G: Retention time = 3.18 min; ESI-MS(+) *m/z* 899.5 (M+2H).

Preparation of Example 3226

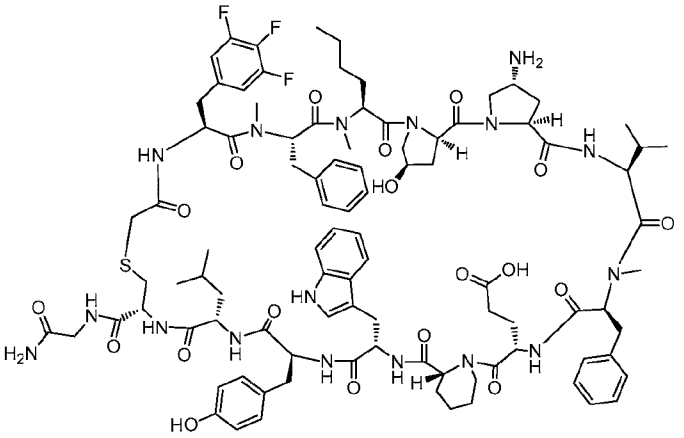


Example 3226

Example 3226 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 25-65% B over 30 min., 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 16.2 mg, and its estimated purity by LCMS analysis was 95% by "Analysis LCMS Condition E". Analysis LCMS Condition E: Retention time = 1.84 min; ESI-MS(-) *m/z* 928.9 (M-2H).

Preparation of Example 3227



Example 3227

Example 3227 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 35-75% B over 30 min., 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 97% by "Analysis LCMS Condition E and G".

Analysis LCMS Condition E: Retention time = 1.74 min; ESI-MS(+) *m/z* 947.7 (M+2H).

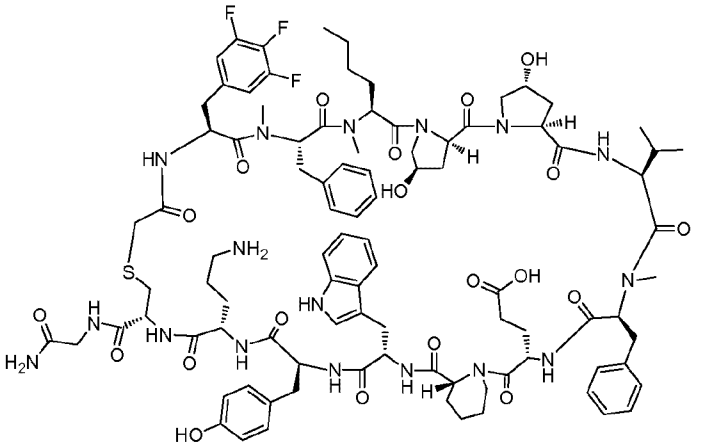
Analysis LCMS Condition G: Retention time = 3.37 min; ESI-MS(+) *m/z* 948.00 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 947.9461 (M+2H)

Found: 949.9449 (M+2H)

Preparation of Example 3228



Example 3228

Example 3228 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 35-75% B over 30 min., 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 5.2 mg, and its estimated purity by LCMS analysis was 98% by "Analysis LCMS Conditions E and G".

Analysis LCMS Condition E: Retention time = 1.80 min; ESI-MS(-) *m/z* 947.7 (M-2H).

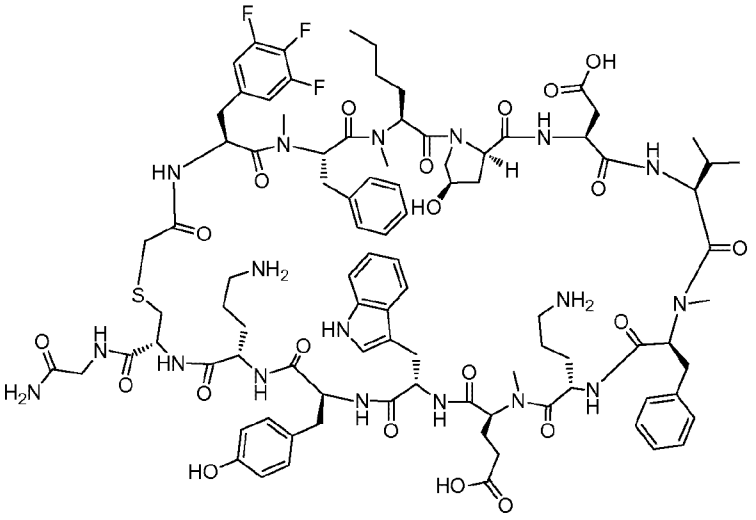
Analysis LCMS Condition G: Retention time = 3.41 min; ESI-MS(+) *m/z* 949.10 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 948.9357 (M+2H)

Found: 948.9354 (M+2H)

Preparation of Example 3229



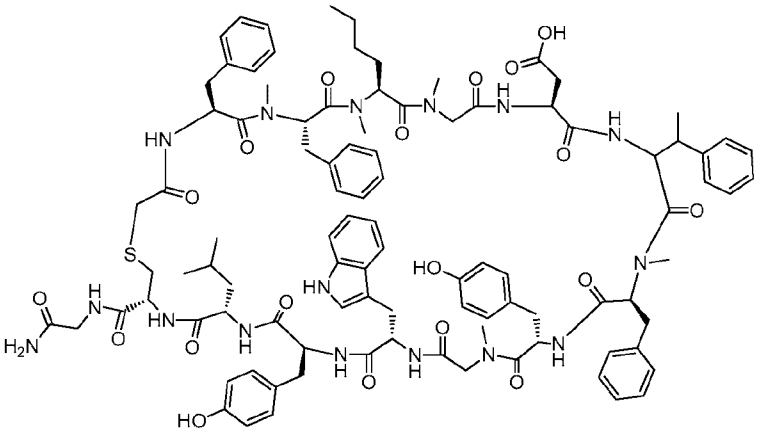
Example 3229

Example 3229 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

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 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 30-70% B over 30 min., 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 8.6 mg, and its estimated purity by LCMS analysis was 95% by "Analysis LCMS Conditions E and G".

Analysis LCMS Condition G: Retention time = 3.443 min; ESI-MS(+) *m/z* 965.55 (M+2H).

Preparation of Example 3230



Example 3230

Example 3230 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative HPLC with the following conditions: Column: Phenomenex Luna 20x250 5u particles; Mobile Phase A: water with 0.1% TFA; Mobile Phase B: Acetonitrile with 0.1% TFA; Gradient: 45-95% B over 55 min., then a 5-minute hold at 95% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. Four isomers (Isomers 3230-A, 3230-B, 3230-C and 3230-D) were obtained. The yields of the products isomer 3230-A, 3230-B, 3230-C and 3060-D were 7.5 mg, 9.3 mg, 0.66 mg and 0.72 mg, respectively, and their estimated purities were 97%, 97.5%, 99% and 84 % , respectively, by "Analysis HPLC Condition B" using a gradient of 35% to 90% buffer B over 30 min. at 60° C.

Analysis LCMS Condition A: isomer 3230-A: Retention time = 1.52 min; ESI-MS(+) *m/z* 930.1 (M+2H).

Analysis LCMS Condition A: isomer 3230-B: Retention time = 1.55 min; ESI-MS(+) *m/z* 930.0 (M+2H).

Analysis LCMS Condition A: isomer 3230-C: Retention time = 1.55 min; ESI-MS(+) *m/z* 929.8 (M+2H).

Analysis LCMS Condition A: isomer 3230-D: Retention time = 1.67 min; ESI-MS(+) *m/z* 930.2 (M+2H).

3230-A:

ESI-HRMS(+) *m/z:*

Calculated: 929.4391 (M+2H)

Found: 929.4371 (M+2H)

3230-B:

ESI-HRMS(+) *m/z:*

Calculated: 929.4391 (M+2H)

Found: 929.4372 (M+2H)

3230-C:

ESI-HRMS(+) *m/z:*

Calculated: 929.4391 (M+2H)

Found: 929.4380 (M+2H)

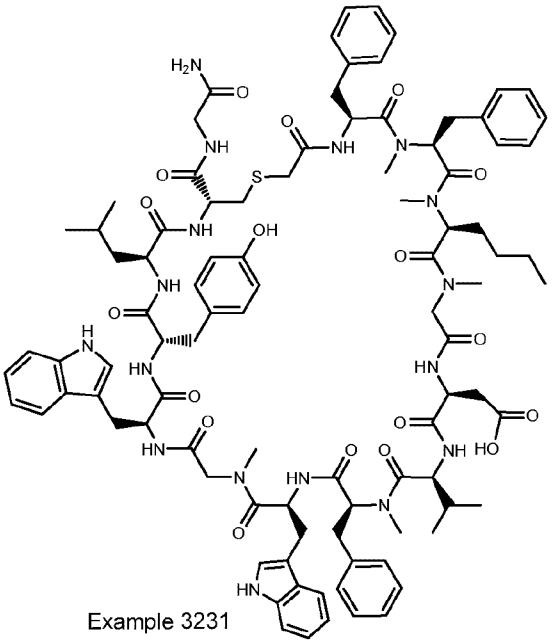
3230-D:

ESI-HRMS(+) *m/z:*

Calculated: 929.4391 (M+2H)

Found: 929.4379 (M+2H)

Preparation of Example 3231



Example 3231

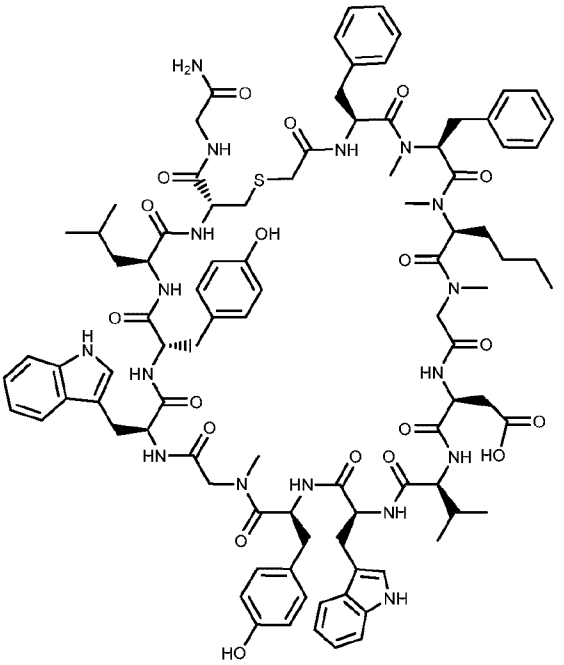
Example 3231 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 25-70% B over 25 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 5.2 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition D: Retention time = 1.652 min; ESI-MS(+) *m/z* 910.40 (M+2H).

Analysis LCMS Condition E: Retention time = 1.801 min; ESI-MS(+) *m/z* 910.75 (M+2H).

Preparation of Example 3232



Example 3232

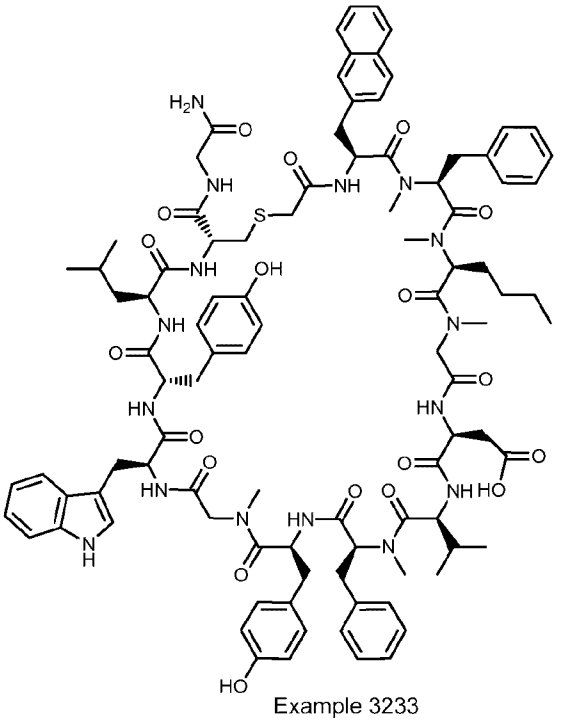
Example 3232 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 20-65% B over 25 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 11.1 mg, and its estimated purity by LCMS analysis was 95%.

Analysis LCMS Condition D: Retention time = 1.499 min; ESI-MS(+) *m/z* 911.70 (M+2H).

Analysis LCMS Condition E: Retention time = 1.633 min; ESI-MS(+) *m/z* 911.40 (M+2H).

Preparation of Example 3233



Example 3233

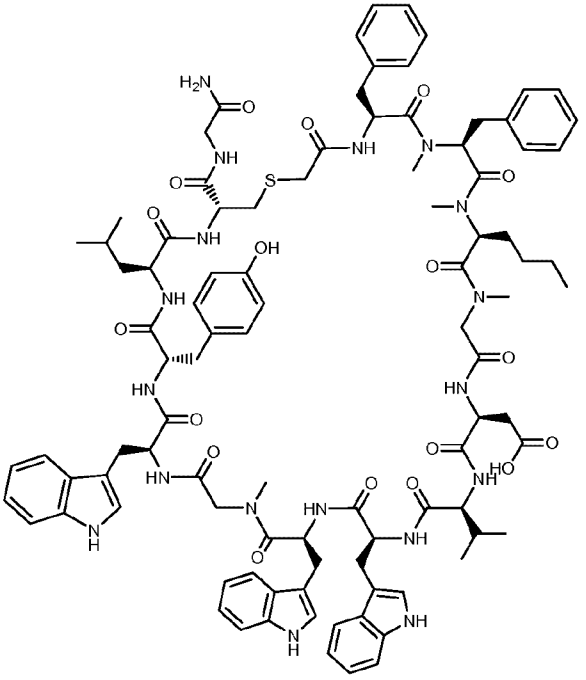
Example 3233 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 25-70% B over 25 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 94%.

Analysis LCMS Condition D: Retention time = 1.662 min; ESI-MS(+) *m/z* 923.70 (M+2H).

Analysis LCMS Condition E: Retention time = 1.823 min; ESI-MS(+) *m/z* 923.75 (M+2H).

Preparation of Example 3234



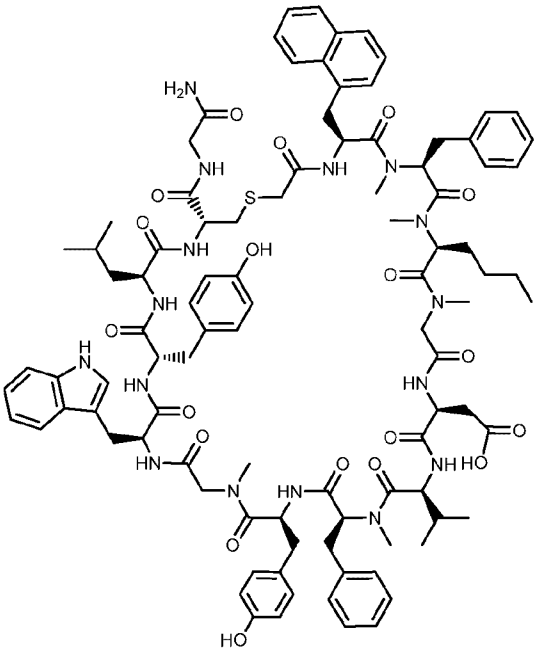
Example 3234

Example 3234 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 25-65% B over 25 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 9.0 mg, and its estimated purity by LCMS analysis was 99%.
Analysis LCMS Condition D: Retention time = 1.585 min; ESI-MS(+) *m/z* 922.60 (M+2H).

Analysis LCMS Condition E: Retention time = 1.724 min; ESI-MS(+) *m/z* 924.45 (M+2H).

Preparation of Example 3235



Example 3235

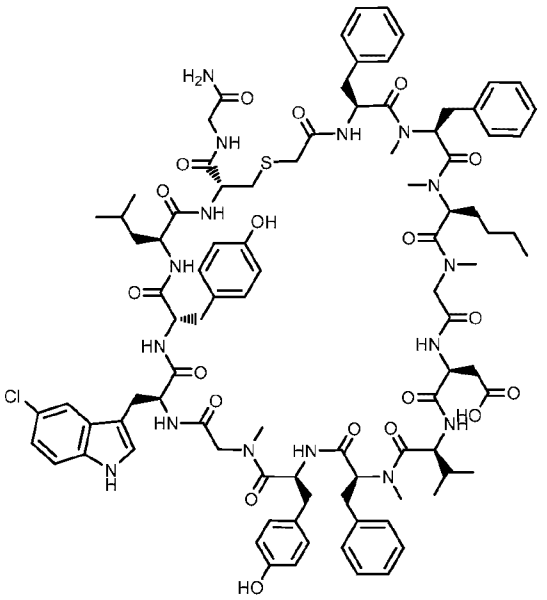
Example 3235 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 25-70% B over 25 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 4.0 mg, and its estimated purity by LCMS analysis was 98%.

Analysis LCMS Condition D: Retention time = 1.77 min; ESI-MS(+) *m/z* 923.8 (M+2H).

Analysis LCMS Condition E: Retention time = 1.99 min; ESI-MS(+) *m/z* 924.0 (M+2H).

Preparation of Example 3236



Example 3236

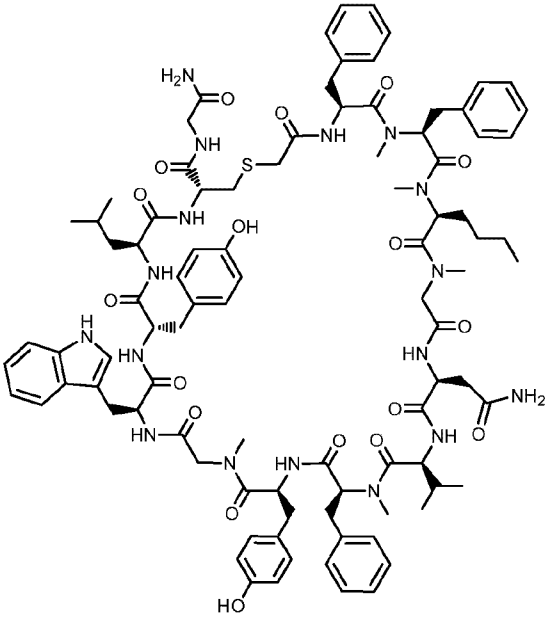
Example 3236 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-55% B over 25 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 3.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.69 min; ESI-MS(+) *m/z* 916.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.89 min; ESI-MS(+) *m/z* 916.3 (M+2H).

Preparation of Example 3237

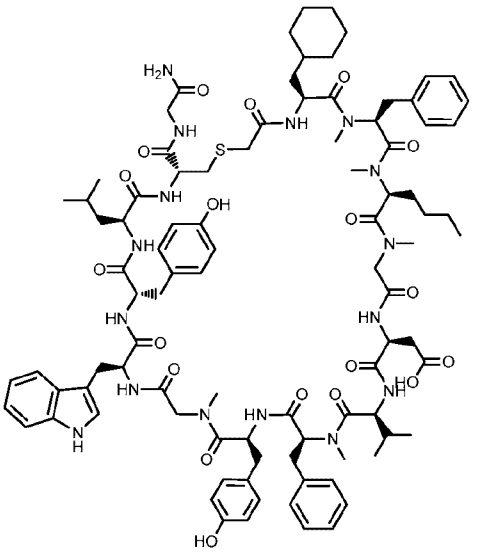


Example 3237

Example 3237 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 20-60% B over 25 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 9.9 mg, and its estimated purity by LCMS analysis was 100%.
Analysis LCMS Condition D: Retention time = 1.78 min; ESI-MS(+) *m/z* 898.4 (M+2H).
Analysis LCMS Condition E: Retention time = 1.78 min; ESI-MS(+) *m/z* 898.5 (M+2H).

Preparation of Example 3238



Example 3238

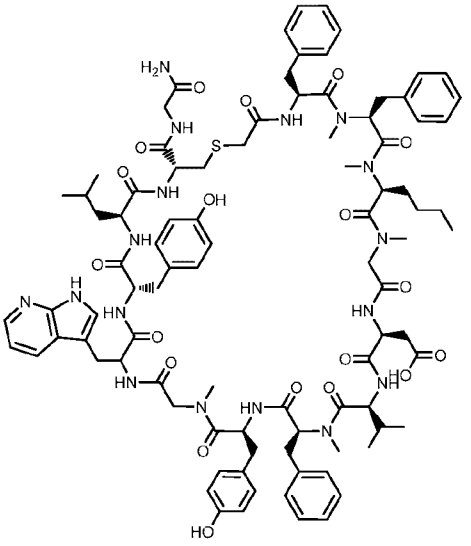
Example 3238 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 20-60% B over 25 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 10.3 mg, and its estimated purity by LCMS analysis was 91%.

Analysis LCMS Condition D: Retention time = 1.78 min; ESI-MS(+) *m/z* 902.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.98 min; ESI-MS(+) *m/z* 902.1 (M+2H).

Preparation of Example 3239

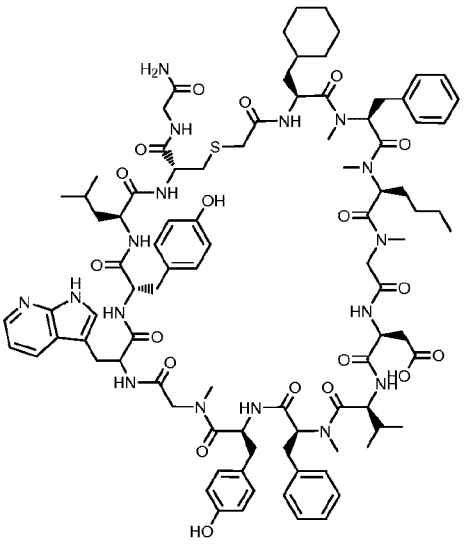


Example 3239

Example 3239 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C". The Fmoc-protected 7-aza-Trp residue was coupled as the racemate.

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-65% B over 25 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 product was 9.4 mg, and its estimated purity by LCMS analysis was 98% as a diastereomeric mixture. Analysis LCMS Condition D: Retention time = 1.51 min; ESI-MS(+) *m/z* 899.8 (M+2H). Analysis LCMS Condition E: Retention time = 1.61 min; ESI-MS(+) *m/z* 899.3 (M+2H).

Preparation of Example 3240

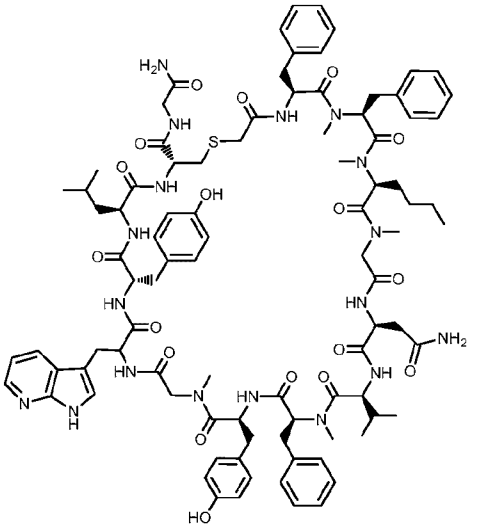


Example 3240

Example 3240 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C". The Fmoc-protected 7-aza-Trp residue was coupled as the racemate.

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-65% B over 25 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 product was 3.5 mg, and its estimated purity by LCMS analysis was 97% as a diastereomeric mixture. Analysis LCMS Condition D: Retention time = 1.67 min; ESI-MS(+) *m/z* 902.5 (M+2H). Analysis LCMS Condition E: Retention time = 1.77 min; ESI-MS(+) *m/z* 902.5 (M+2H).

Preparation of Example 3241

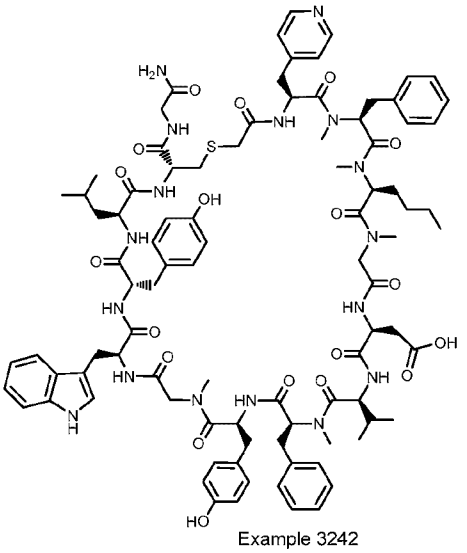


Example 3241

Example 3241 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C". The Fmoc-protected 7-aza-Trp residue was coupled as the racemate.

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-55% B over 25 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 product was 7.0 mg, and its estimated purity by LCMS analysis was 98% as a distereomeric mixture. Analysis LCMS Condition D: Retention time = 1.66 min; ESI-MS(+) *m/z* 898.6 (M+2H). Analysis LCMS Condition E: Retention time = 1.57 min; ESI-MS(+) *m/z* 899.2 (M+2H).

Preparation of Example 3242



Example 3242

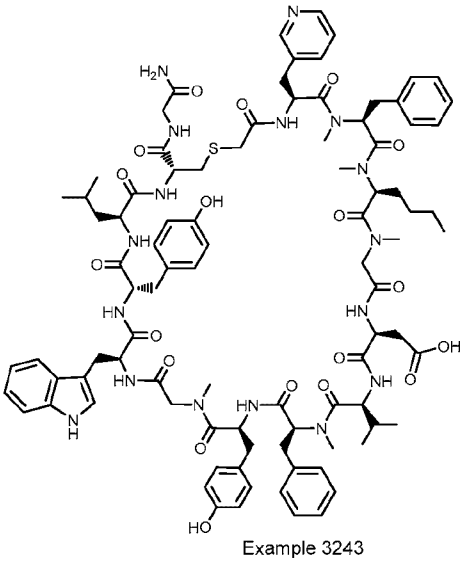
Example 3242 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-65% B over 25 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 2.3 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition D: Retention time = 1.43 min; ESI-MS(+) *m/z* 899.5 (M+2H).

Analysis LCMS Condition E: Retention time = 1.54 min; ESI-MS(+) *m/z* 899.6 (M+2H).

Preparation of Example 3243



Example 3243

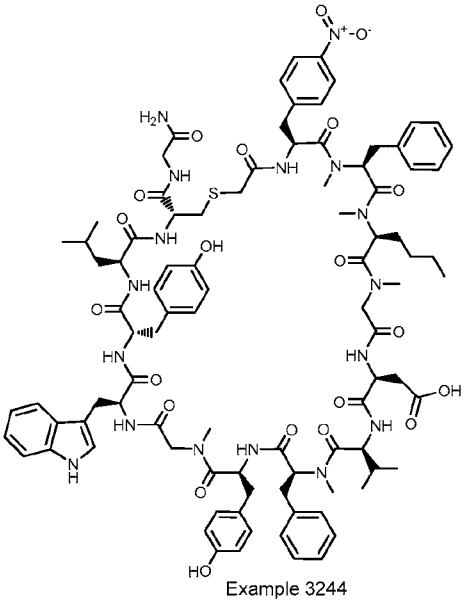
Example 3243 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-65% B over 25 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 4.8 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition D: Retention time = 1.44 min; ESI-MS(+) *m/z* 899.5 (M+2H).

Analysis LCMS Condition E: Retention time = 1.54 min; ESI-MS(+) *m/z* 899.9 (M+2H).

Preparation of Example 3244



Example 3244

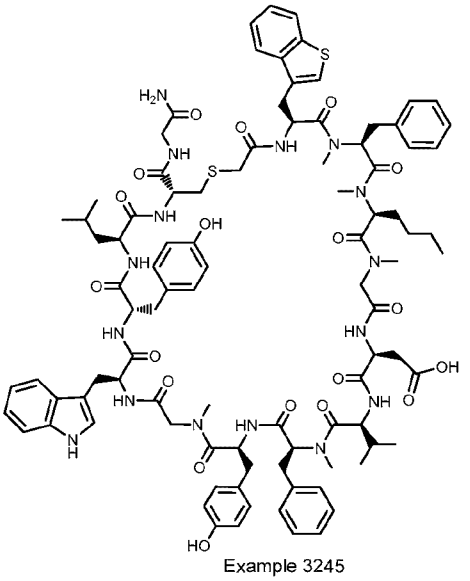
Example 3244 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 20-70% B over 25 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 4.6 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition D: Retention time = 1.60 min; ESI-MS(+) *m/z* 921.3 (M+2H).

Analysis LCMS Condition E: Retention time = 1.81 min; ESI-MS(+) *m/z* 922.0 (M+2H).

Preparation of Example 3245



Example 3245

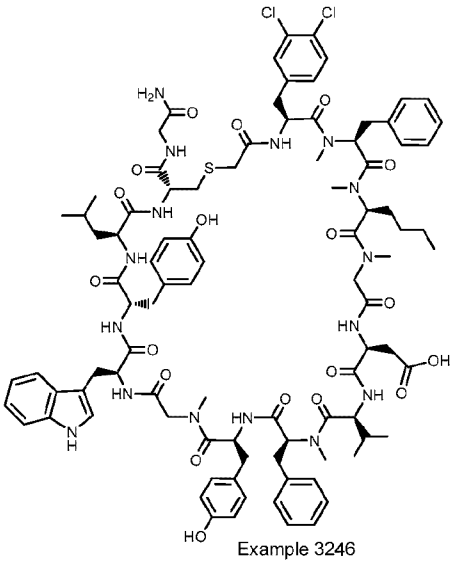
Example 3245 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 25-75% B over 25 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 2.3 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition D: Retention time = 1.73 min; ESI-MS(+) *m/z* 927.1 (M+2H).

Analysis LCMS Condition E: Retention time = 1.93 min; ESI-MS(+) *m/z* 927.4 (M+2H).

Preparation of Example 3246



Example 3246

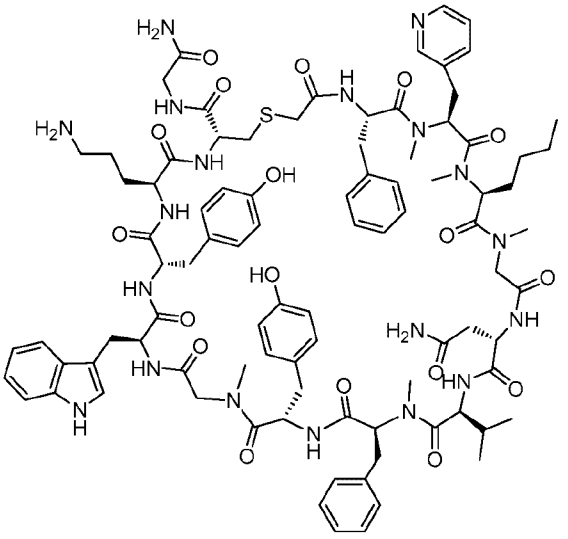
Example 3246 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method G", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: XBridge C18 300, 19 x 250 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-mMammonium acetate; Gradient: 15-65% B over 25 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 4.4 mg, and its estimated purity by LCMS analysis was 94%.

Analysis LCMS Condition D: Retention time = 1.78 min; ESI-MS(+) *m/z* 932.9 (M+2H).

Analysis LCMS Condition E: Retention time = 1.97 min; ESI-MS(+) *m/z* 932.9 (M+2H).

Preparation of Example 3614



Example 3614

Example 3614 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*", "N-Methylation on-resin procedure" for the N-methylation of the Fmoc-3-PyAla-OH, "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C". Fmoc-3-PyAla-OH (2.5 eq.) was coupled manually using HATU (2.5 eq.) and NMM (2.5 eq.) as the coupling method, followed by a second manual coupling step of Fmoc-Phe-OH (5 eq.) using HATU (5 eq.) and NMM (5 eq.) as the coupling method.

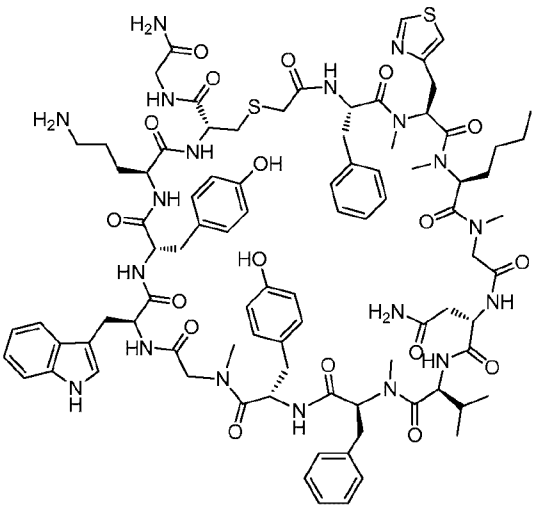
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 30-70% B over 25 min., 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 94%.

Analysis LCMS Condition D: Retention time = 1.53 min; ESI-MS(+) *m/z* 899.6 (M+2H).
Analysis LCMS Condition E: Retention time = 1.45 min; ESI-MS(+) *m/z* 899.4 (M+2H).
ESI-HRMS(+) *m/z:*

Calculated: 898.9345 (M+2H)

Found: 898.9345 (M+2H)

Preparation of Example 3616



Example 3616

Example 3616 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*", "N-Methylation on-resin procedure" for the N-methylation of the Fmoc-4-thiazole-ala-OH, "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C". Fmoc-3-(Thiazol-4yl)-Ala-OH (2.5 eq.) was coupled manually using HATU (2.5 eq.) and NMM (2.5 eq.) as the coupling method, followed by a second manual coupling step of Fmoc-Phe-OH (5 eq.) using HATU (5 eq.) and NMM (5 eq.) as the coupling method. The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., 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 11.8 mg, and its estimated purity by LCMS analysis was 97%.

Analysis LCMS Condition D: Retention time = 1.53 min; ESI-MS(+) *m/z* 902.1 (M+2H).

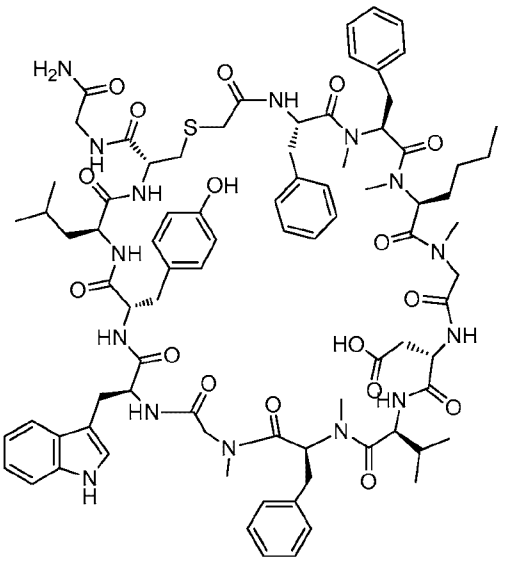
Analysis LCMS Condition E: Retention time = 1.52 min; ESI-MS(+) *m/z* 902.1 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 901.9127 (M+2H)

Found: 901.9130 (M+2H)

Preparation of Example 3617



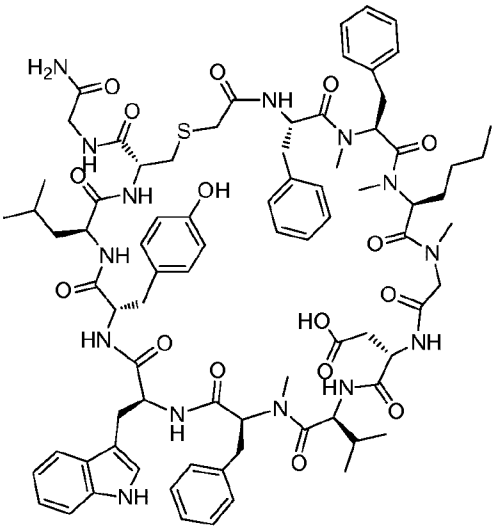
Example 3617

Example 3617 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative HPLC with the following conditions: Column: Phenomenex Luna 5u C18(2) 250 x 21.2 AXIA, 100A Ser.#520221-1; Mobile Phase A: 0.1% TFA in water; Mobile Phase B: 0.1% TFA in acetonitrile; Gradient: 35-75% B over 40 min., then a 5-minute gradient up to 85% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The product was dissolved in a minimum if acetonitrile and water, frozen and lyophilized to give a white amorphous solid. The yield of the product was 1.9 mg, and its estimated purity by LCMS analysis was 90%.

Analysis LCMS Condition A: Retention time = 1.42 min; ESI-MS(+) *m/z* 817.2 (M+2H).
Analysis LCMS Condition C: Retention time = 1.65 min; ESI-MS(+) *m/z* 1632.8 (M+H).
ESI-HRMS(+) *m/z:*
Calculated: 816.8996 (M+2H)
Found: 816.8968 (M+2H)

Preparation of Example 3618



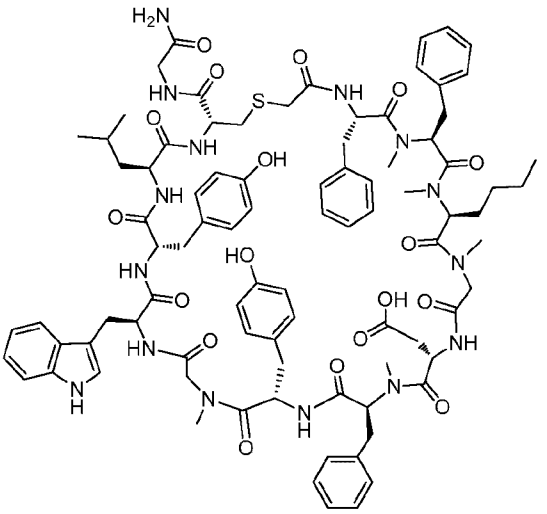
Example 3618

Example 3618 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative HPLC with the following conditions: Column: Phenom Luna 5u C18(2) 250 x 21.2 AXIA, 100A Ser.#520221-1; Mobile Phase A: 0.1% TFA in water; Mobile Phase B: 0.1% TFA in acetonitrile; Gradient: 35-75% B over 40 min., then a 5-minute gradient up to 85% B; Flow: 15 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. The product was dissolved in a minimum if acetonitrile and water, frozen and lyophilized to give a white amorphous solid. The yield of the product was 3.5 mg, and its estimated purity by LCMS analysis was 99%.

Analysis LCMS Condition A: Retention time = 1.49 min; ESI-MS(+) *m/z* 781.7 (M+2H).
Analysis LCMS Condition C: Retention time = 1.74 min; ESI-MS(+) *m/z* 1561.8 (M+H).
ESI-HRMS(+) *m/z:*
Calculated: 781.3810 (M+2H)
Found: 781.3778 (M+2H)

Preparation of Example 3628



Example 3628

Example 3628 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C".

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., 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 2.97 mg, and its estimated purity by LCMS analysis was 100%.
Analysis LCMS Condition D: Retention time = 1.45 min; ESI-MS(+) *m/z* 849.2 (M+2H).

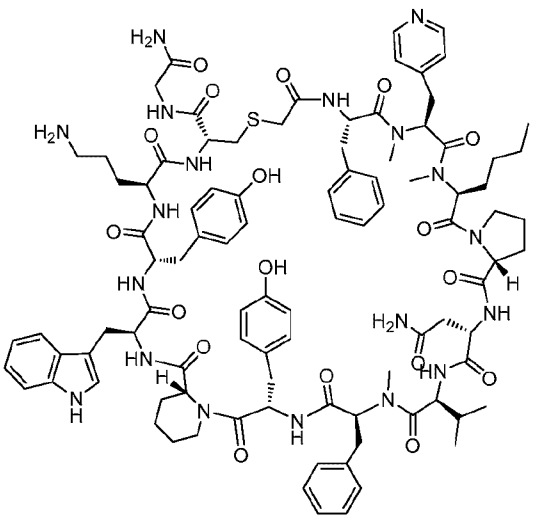
Analysis LCMS Condition E: Retention time = 1.66 min; ESI-MS(+) *m/z* 849.3 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 848.8971 (M+2H)

Found: 848.8962 (M+2H)

Preparation of Example 3637



Example 3637

Example 3637 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "N-Methylation on-resin procedure" for the N-methylation of the Fmoc-4-Py-ala-OH, "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C". Fmoc-4-PyAla-OH (10 eq.) was coupled manually using HATU (10 eq.) and NMM (20 eq.) as the coupling method, followed by a second manual coupling step of Fmoc-Phe-OH (5 eq.) using HATU (5 eq.) and NMM (10 eq.) as the coupling method.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., 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 2.78 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.74 min; ESI-MS(+) *m/z* 932.5 (M+2H).

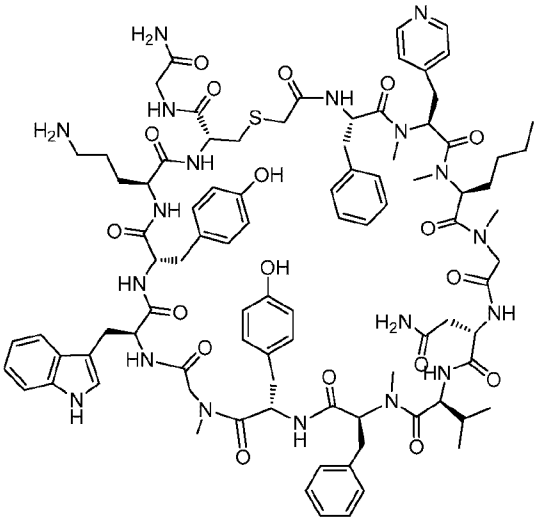
Analysis LCMS Condition E: Retention time = 1.61 min; ESI-MS(+) *m/z* 932.5 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 931.9580 (M+2H)

Found: 931.9557 (M+2H)

Preparation of Example 3638



Example 3638

Example 3638 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "N-Methylation on-resin procedure" for the N-methylation of the Fmoc-4-Py-ala-OH, "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C". Fmoc-4-PyAla-OH (10 eq.) was coupled manually using HATU (10 eq.) and NMM (20 eq.) as the coupling method, followed by a second manual coupling step of Fmoc-Phe-OH (5 eq.) using HATU (5 eq.) and NMM (10 eq.) as the coupling method.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase

A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., 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 separation led to two isomers. The yield of the first isomer, 3638-A, was 1.66 mg, and its estimated purity by LCMS analysis was 91%.

Analysis LCMS Condition D: Retention time = 1.07 min; ESI-MS(+) *m/z* 899.5 (M+2H).

Analysis LCMS Condition E: Retention time = 1.08 min; ESI-MS(+) *m/z* 899.4 (M+2H).

The yield of the second isomer, 3638-B, was 7.56 mg, and its estimated purity by LCMS analysis was 92%.

Analysis LCMS Condition D: Retention time = 1.52 min; ESI-MS(+) *m/z* 899.2 (M+2H).

Analysis LCMS Condition E: Retention time = 1.45 min; ESI-MS(+) *m/z* 899.5 (M+2H).

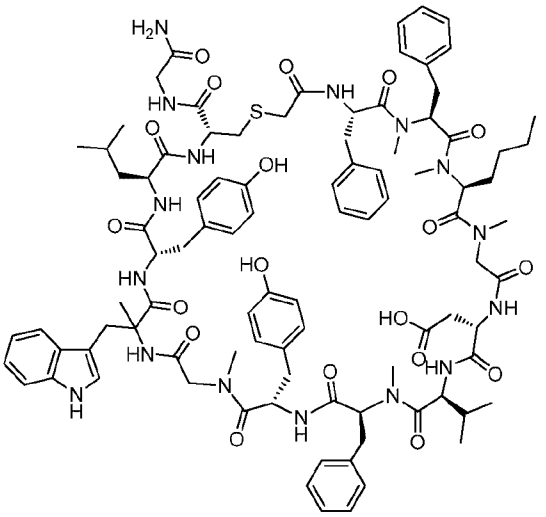
3638-B:

ESI-HRMS(+) *m/z:*

Calculated: 898.9345 (M+2H)

Found: 898.9331 (M+2H)

Preparation of Example 3639



Example 3639

Example 3639 was prepared following the general synthetic sequence described for the preparation of Example 3210, composed of the following general procedures: "CEM Method A: Resin-swelling procedure", "CEM Method A: Standard coupling

procedure", "CEM Method A: *Custom amino acids-coupling procedure*" , "*Chloroacetic acid coupling procedure A*", "Global Deprotection Method B", and "Cyclization Method C".

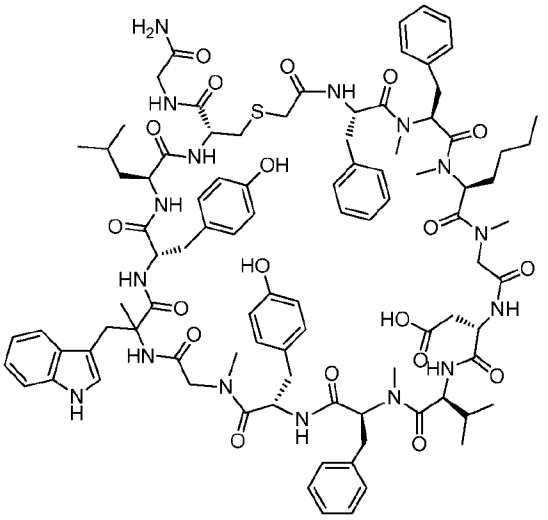
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 10-60% B over 25 min., then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product as a diastereomeric mixture were combined and dried via centrifugal evaporation. The yield of the product was 6.7 mg, and its estimated purity by LCMS analysis was 96%. Analysis LCMS Condition D: Retention time = 1.65 min; ESI-MS(+) *m/z* 903.45 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: 905.4391 (M+2H)

Found: 905.4376 (M+2H)

Preparation of Example 3640



Example 3640

The two diastereoisomers from Example 3639 were separated by Supercritical Fluid Chromatography (SFC) on a Berger SFC MGII system using the following conditions: Column: ES DEAP 25 X 2.1 cm 5-μm particles; Mobile Phase 44/55 of CO_2 / 95:5 MeOH: H_2O with 10 mM NH_4OAc.

The yield of the first isomer, 3640-A, was 2.39 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition A: Retention time = 1.43 min; ESI-MS(+) *m/z* 1812.1 (M+H).

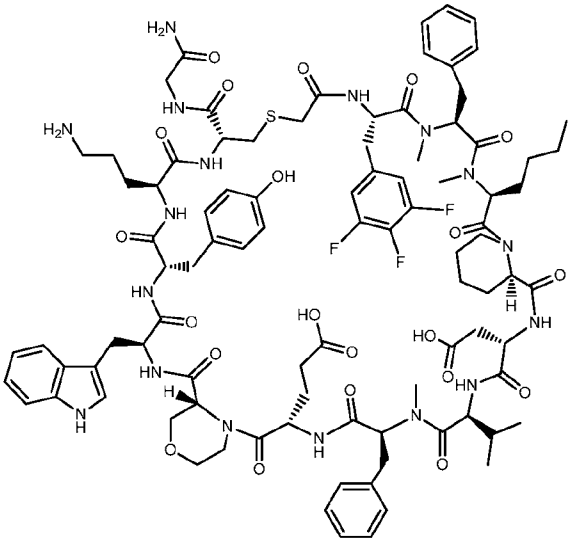
Analysis LCMS Condition C: Retention time = 1.67 min; ESI-MS(+) *m/z* 1810.2 (M+H).

The yield of the second isomer, 3640-B, was 2.15 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition A: Retention time = 1.42 min; ESI-MS(+) *m/z* 1812.1 (M+H).

Analysis LCMS Condition C: Retention time = 1.66 min; ESI-MS(+) *m/z* 1811.1 (M+H).

Preparation of Example 3641



Example 3641

Example 3641 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 40-90% 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% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.61 min; ESI-MS(-) *m/z* 948.7 (M-2H).

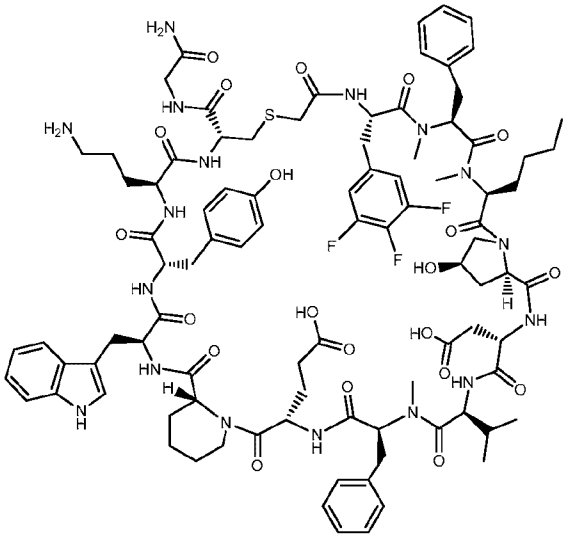
Analysis LCMS Condition G: Retention time = 2.98 min; ESI-MS(+) *m/z* 950.7 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 949.9253

Found: (M+2H) 949.9230

Preparation of Example 3642



Example 3642

Example 3642 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin

was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 40-90% 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 14.8 mg, and its estimated purity by LCMS analysis was 100% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.63 min; ESI-MS(+) *m/z* 950.8 (M-2H).

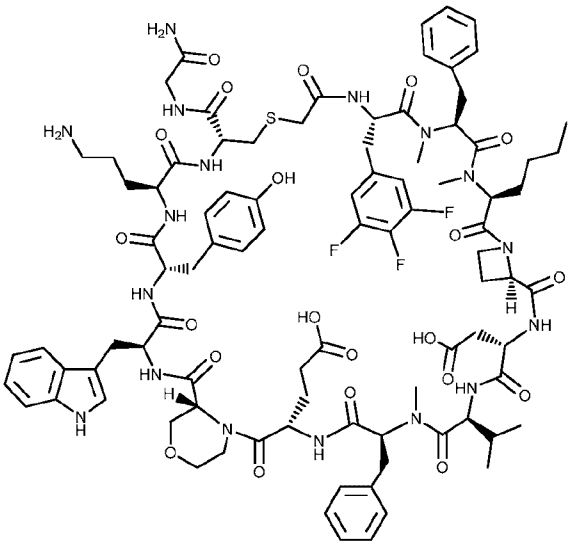
Analysis LCMS Condition G: Retention time = 2.98 min; ESI-MS(+) *m/z* 950.8 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 949.9253

Found: (M+2H) 949.9229

Preparation of Example 3643



Example 3643

Example 3643 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

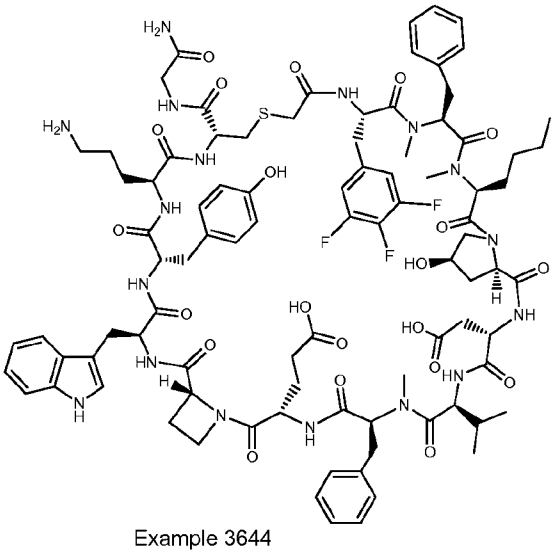
The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Mobile Phase A: 5:95 methanol: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 methanol: water with 10-mM ammonium acetate; Gradient: 50-90% B over 30 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 31.8 mg, and its estimated purity by LCMS analysis was 98% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.52 min; ESI-MS(+) *m/z* 936.9 (M+2H).
Analysis LCMS Condition G: Retention time = 2.90 min; ESI-MS(+) *m/z* 936.8 (M+2H).
ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 935.9097

Found: (M+2H) 935.9073

Preparation of Example 3644



Example 3644

Example 3644 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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 35.9 mg, and its estimated purity by LCMS analysis was 100% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.57 min; ESI-MS(+) *m/z* 936.5 (M+2H).

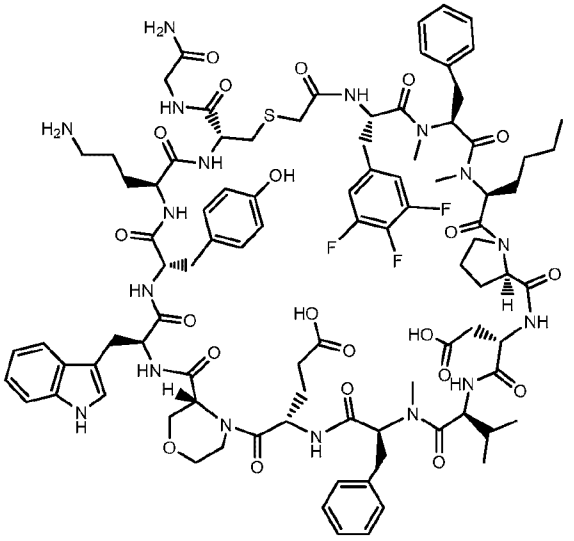
Analysis LCMS Condition G: Retention time = 2.93 min; ESI-MS(+) *m/z* 936.5 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 935.9097

Found: (M+2H) 935.9069

Preparation of Example 3645



Example 3645

Example 3645 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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: 50-100% 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 46.3 mg, and its estimated purity by LCMS analysis was 95% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.82 min; ESI-MS(-) *m/z* 941.05 (M-2H).

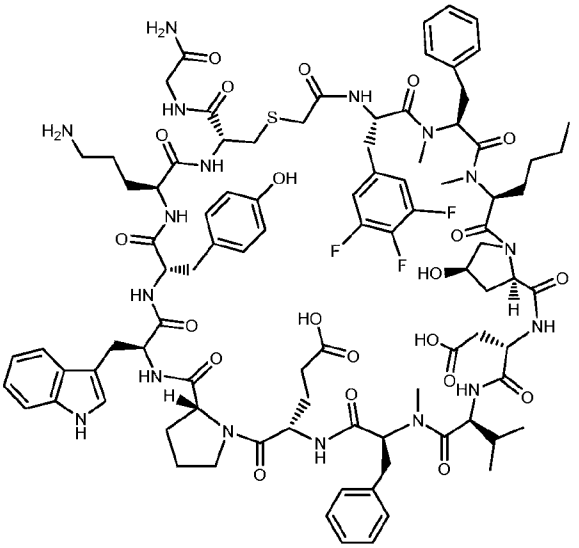
Analysis LCMS Condition G: Retention time = 3.06 min; ESI-MS(+) *m/z* 943.8 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 942.9175

Found: (M+2H) 942.9159

Preparation of Example 3646



Example 3646

Example 3646 was prepared following the general synthetic sequence described for the preparation of Example 3212, composed of the following general procedures: "Prelude Method A: Resin-swelling procedure", "Prelude Method A: Single-coupling procedure", "Prelude Method A: *Secondary amine-coupling procedure*", "Prelude Method A: Chloroacetyl chloride coupling procedure A", "Global Deprotection Method B", and "Cyclization Method C". The coupling of Fmoc-Val-OH to the peptidyl-resin was performed using 10 eq. of amino acid, 10 eq. of HATU and 20 eq. of NMM, and was extended to 10 hours. The final coupling of Fmoc-Phe(3,4,5-tri-F)-OH was preformed manually using 1.5 eq. of amino acid, 1.65 eq. of 7-aza-benzotriazole (HOAt) and 1.58 eq. of N,N-diisopropylcarbodiimide (DIC) in DMF, and was allowed to proceed for 60 hours.

The crude material was purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 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 28.3 mg, and its estimated purity by LCMS analysis was 98% by "Analysis LCMS Conditions D and G".

Analysis LCMS Condition D: Retention time = 1.57 min; ESI-MS(+) *m/z* 943.4 (M+2H).

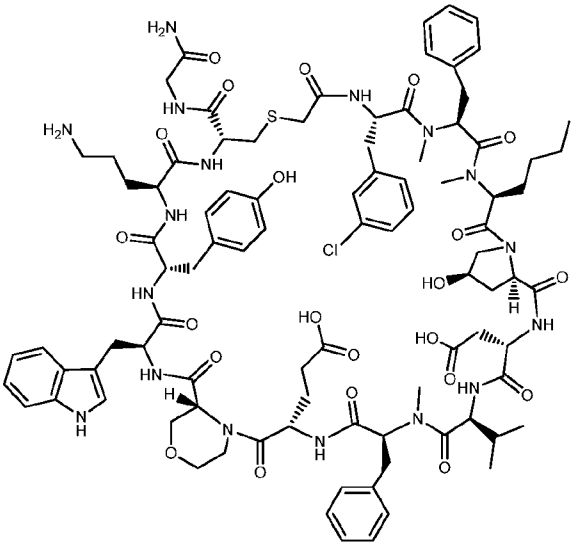
Analysis LCMS Condition G: Retention time = 2.93 min; ESI-MS(+) *m/z* 943.6 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 942.9175

Found: (M+2H) 942.9152

Preparation of Example 3647



Example 3647

To a Symphony polypropylene solid-phase reaction vessel was added Sieber resin (140 mg, 0.100 mmol), and the reaction vessel was placed on the Symphony peptide synthesizer. The following procedures were then performed sequentially:

"Symphony Method A: Resin-swelling procedure" was followed;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-Gly-OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-Cys(Trt)-

OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-Leu-OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-Tyr(tBu)-OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-Trp(Boc)-OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-(D)-Morpholino-3-carboxylic acid;

"Symphony Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Glu(OtBu)-OH;

"Symphony Method A: Standard-coupling procedure" was followed with Fmoc-[N-Me]Phe-OH;

"Symphony Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Val-OH;

"Symphony Method A: Single Standard-coupling procedure" was followed with Fmoc-Asp(OtBu)-OH;

"Symphony Method A: Single Standard-coupling procedure" was followed with Fmoc-(D)-cis-Pro(4-OH)-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-[N-Me]Nle-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-[N-Me]Phe-OH;

"Prelude Method A: *Secondary amine-coupling procedure*" was followed with Fmoc-Phe(3-Cl)-OH;

"Symphony Method B: *Final capping procedure*" was followed;

"Global Deprotection Method B" was followed;

"Cyclization Method C" was followed.

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 5.8 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.73 min; ESI-MS(-) *m/z* 939.8 (M-2H).

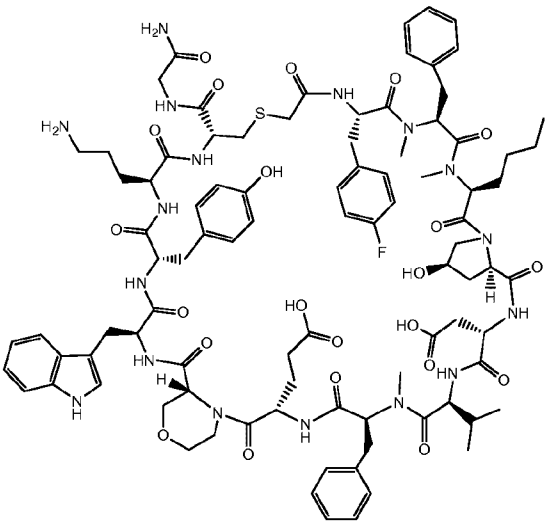
Analysis LCMS Condition G: Retention time = 2.9 min; ESI-MS(+) *m/z* 941.8 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 940.9096

Found: (M+2H) 940.9063

Preparation of Example 3648



Example 3648

Example 3648 was prepared on Rink Resin following the general synthetic sequence described for the preparation of Example 3647, composed of the following general procedures: "Symphony Method A: *Resin-swelling procedure*", "Symphony Method A: *Standard-coupling procedure"*, "Symphony Method A*: Secondary amine-coupling procedure*", "Symphony Method B: *Final capping procedure*", "*Global Deprotection Method B*", and "*Cyclization Method C*".

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 4.9 mg, and its estimated purity by LCMS analysis was 100%.

Analysis LCMS Condition D: Retention time = 1.67 min; ESI-MS(-) *m/z* 931.1 (M-2H).

Analysis LCMS Condition G: Retention time = 2.84 min; ESI-MS(+) *m/z* 933.3 (M+2H).

ESI-HRMS(+) *m/z:*

Calculated: (M+2H) 932.9244

Found: (M+2H) 932.9200

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.

Analysis Condition A:

Column: Waters BEH C18, 2.0 x 50 mm, 1.7-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; 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-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; 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.

DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS COMPREND PLUS D'UN TOME.

CECI EST LE TOME __1___ DE __4___

NOTE: Pour les tomes additionels, veillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

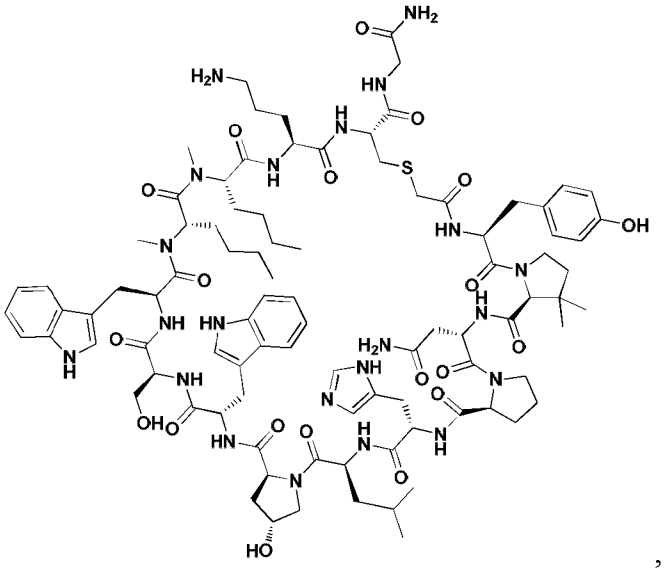
THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __4___

NOTE: For additional volumes please contact the Canadian Patent Office.

Claims:

1. A compound which is



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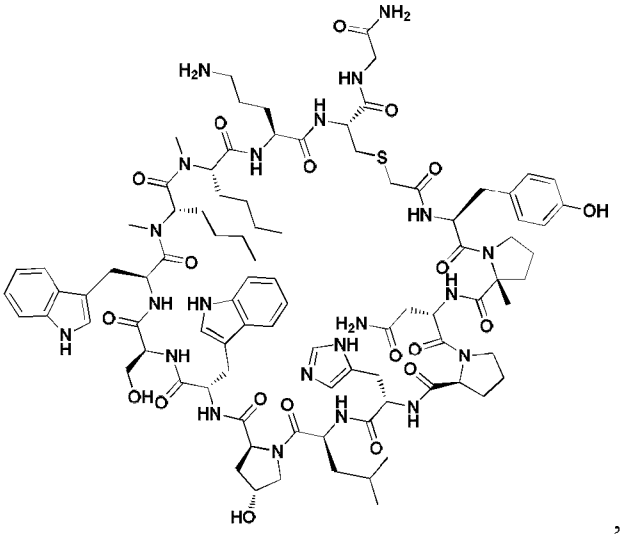

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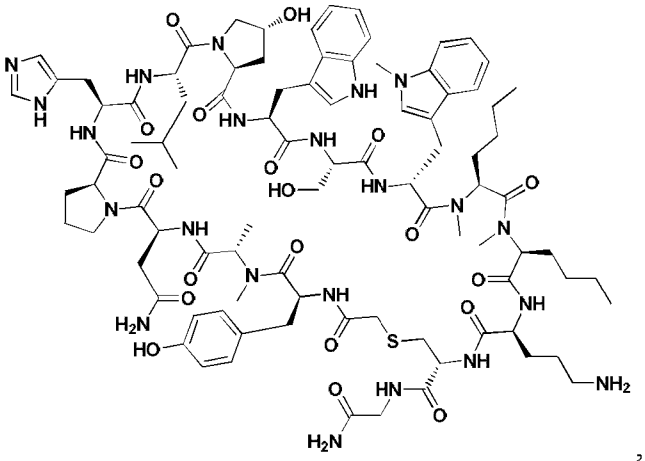

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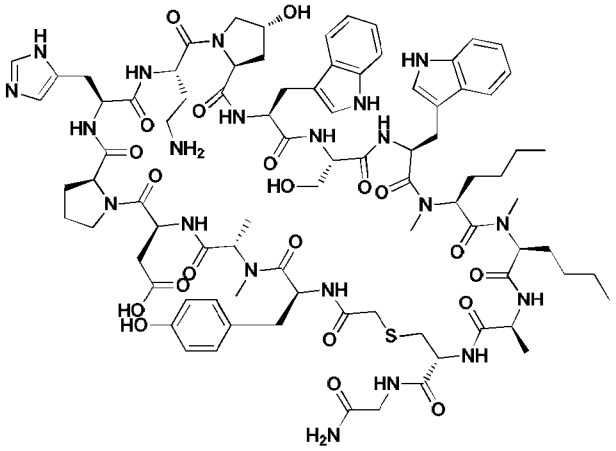

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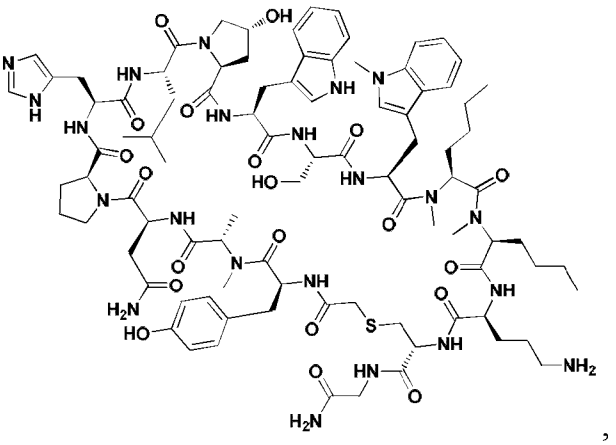

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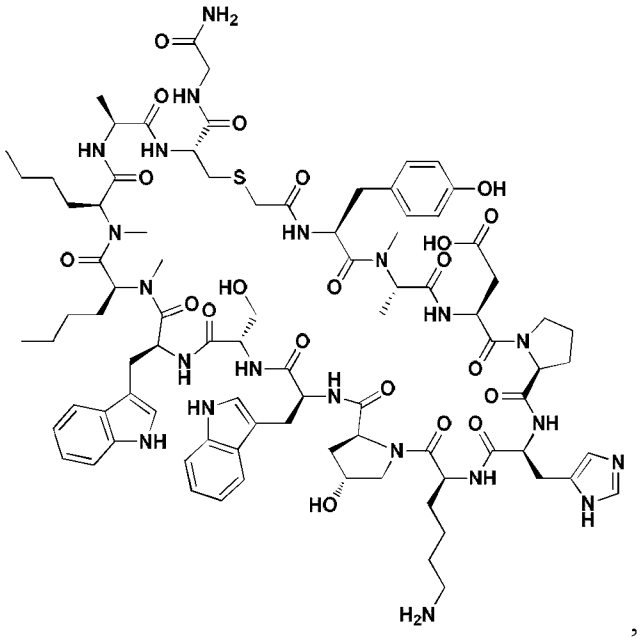

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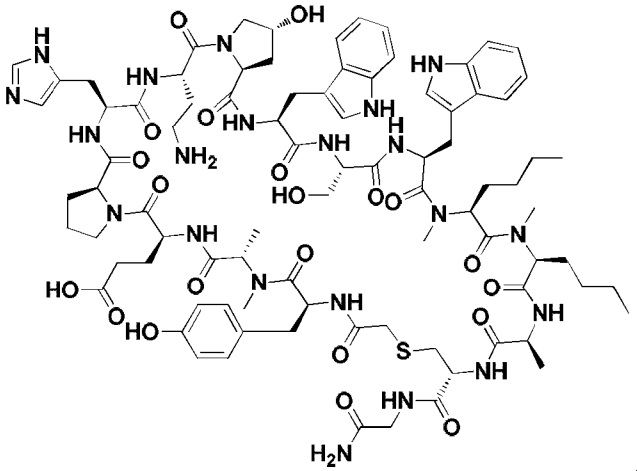

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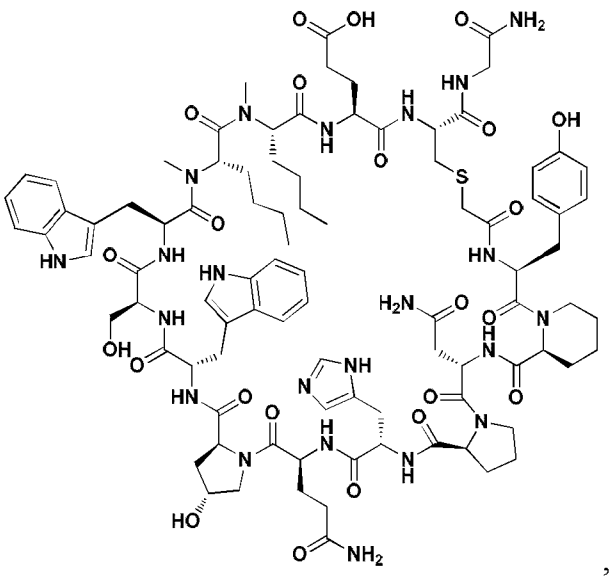

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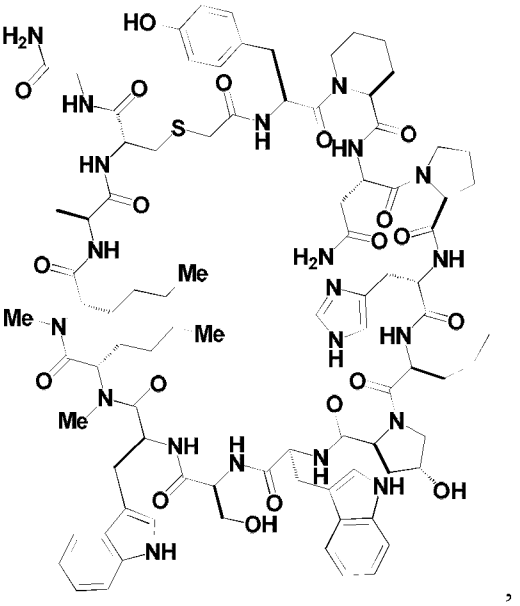

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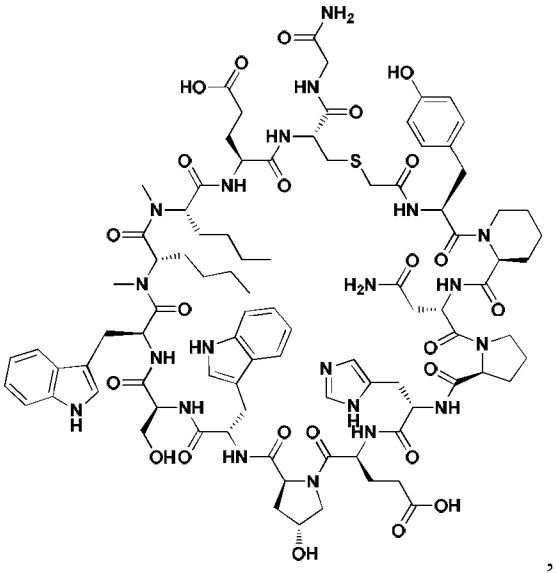

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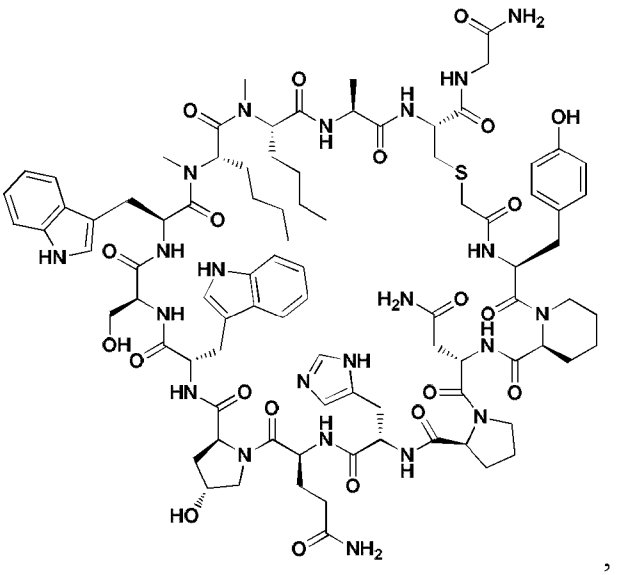

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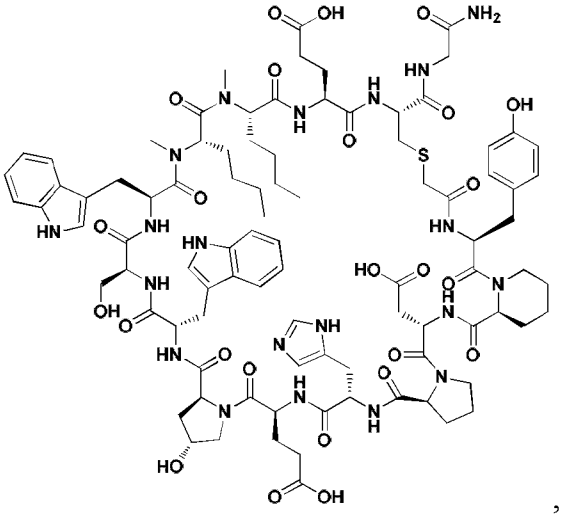
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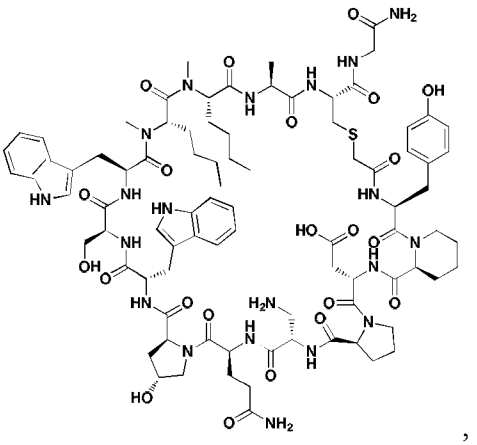
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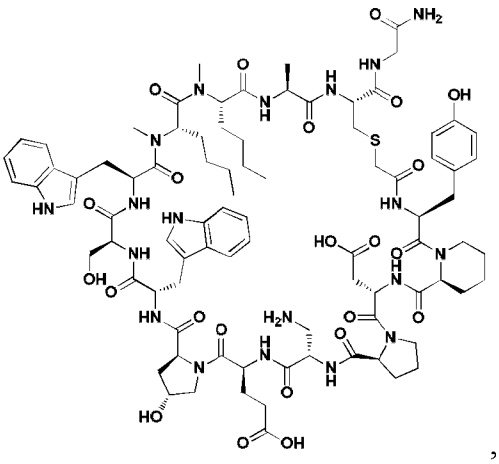
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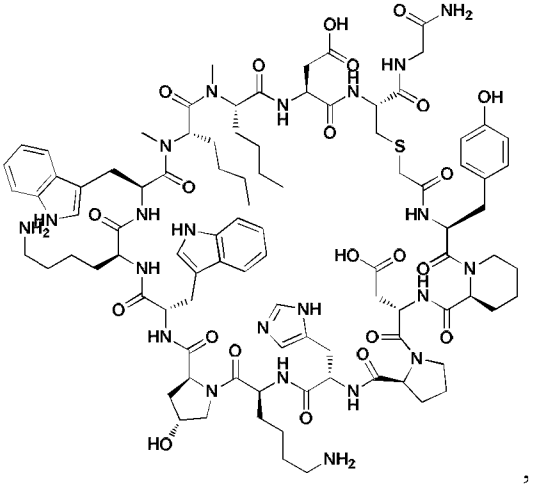
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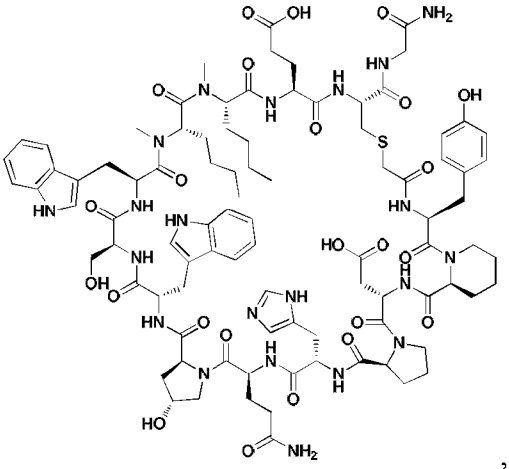
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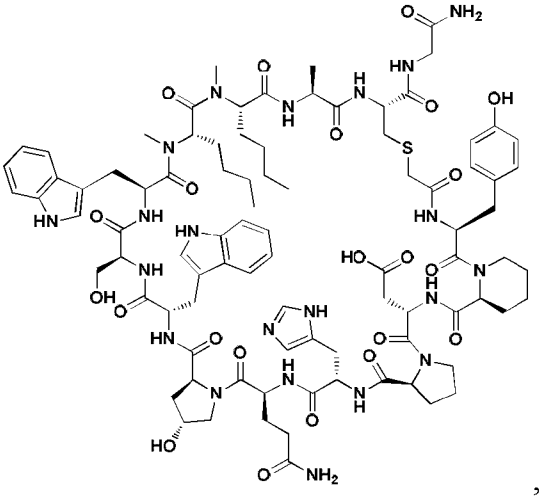
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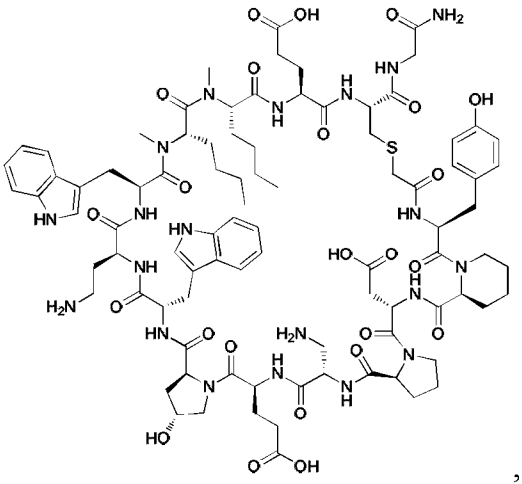
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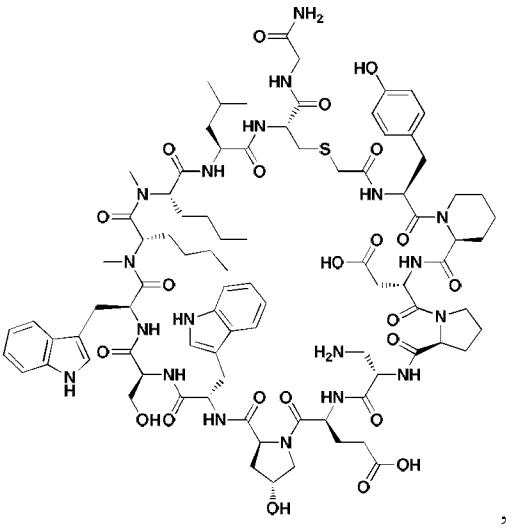
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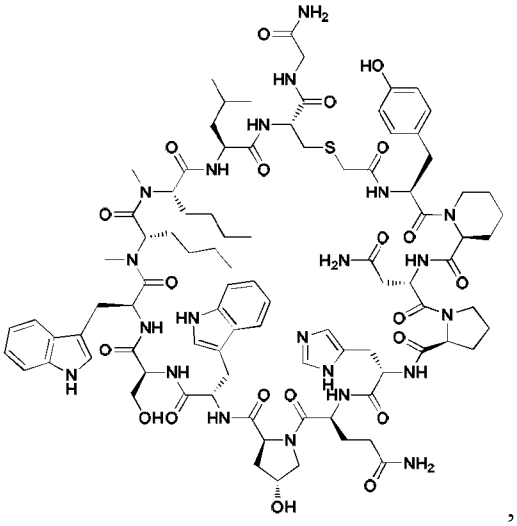
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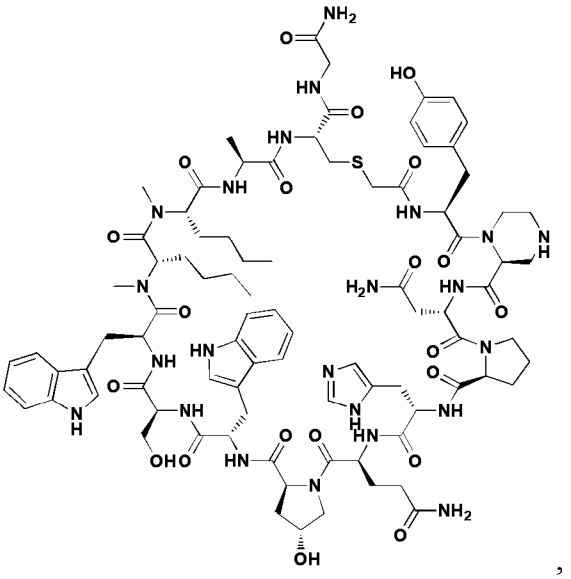
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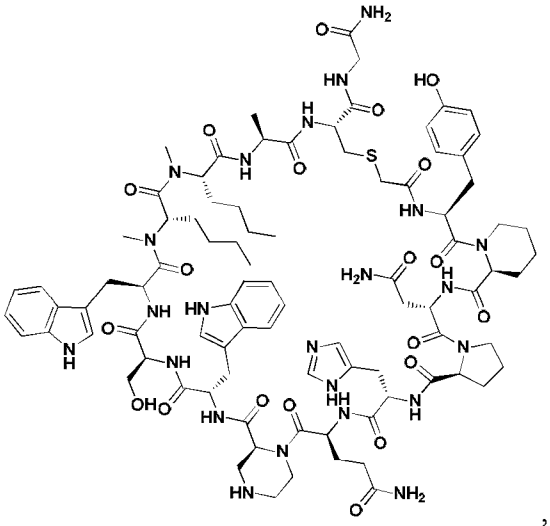
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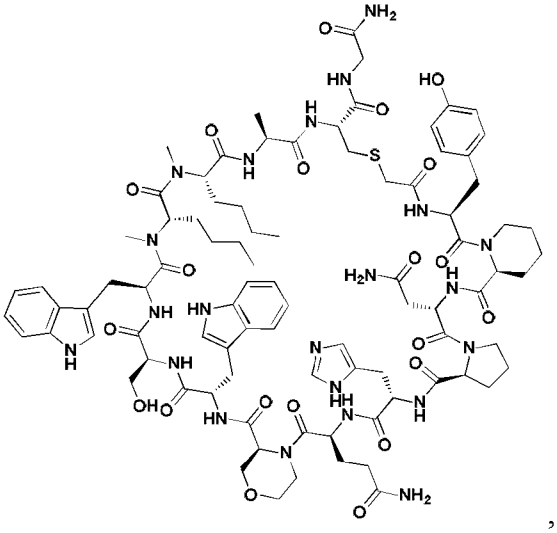
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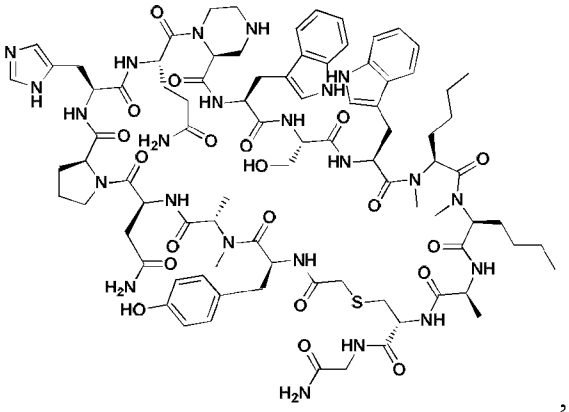
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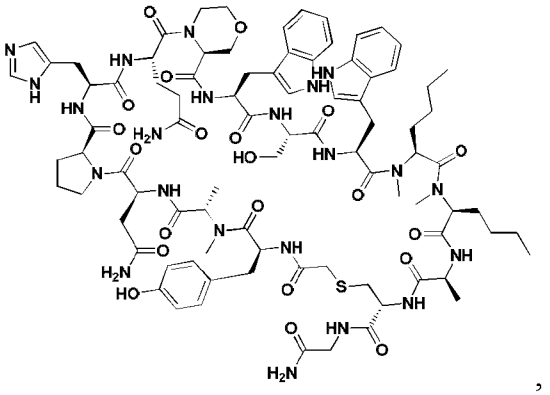
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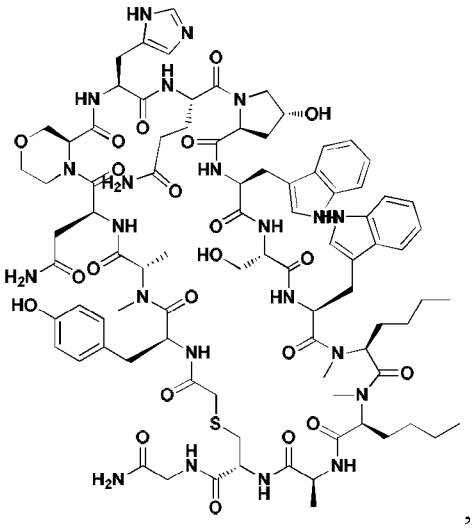
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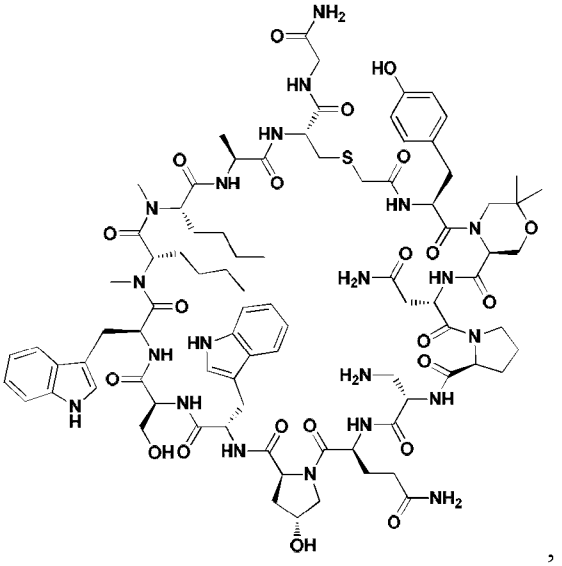
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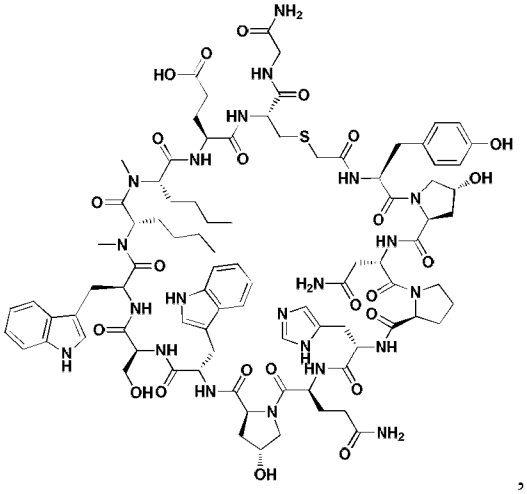
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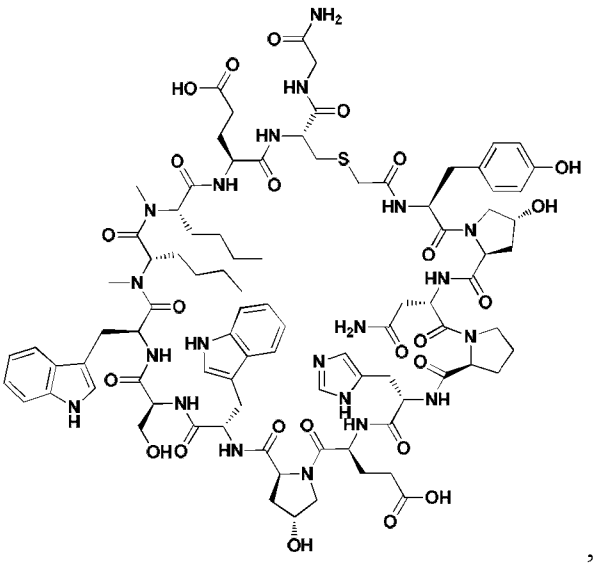
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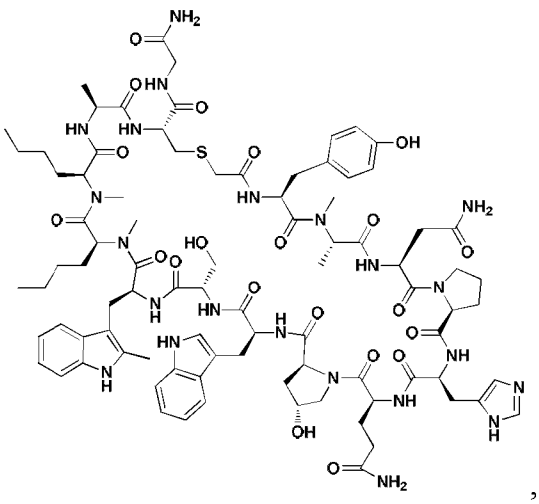
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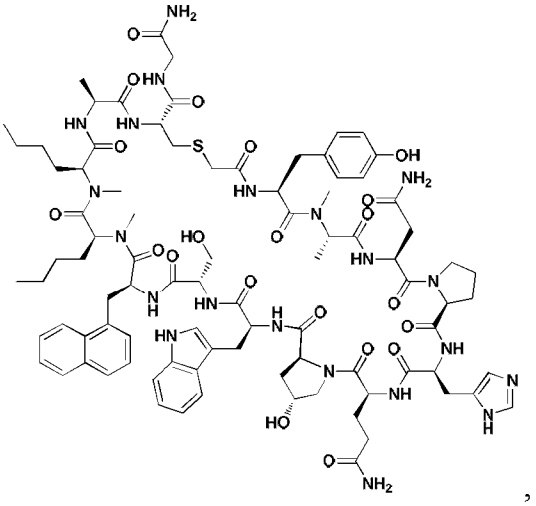
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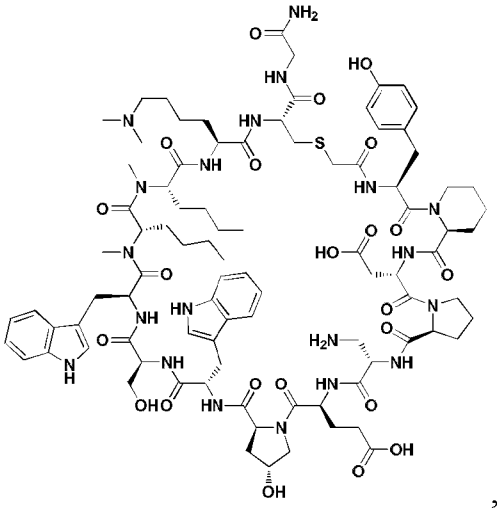
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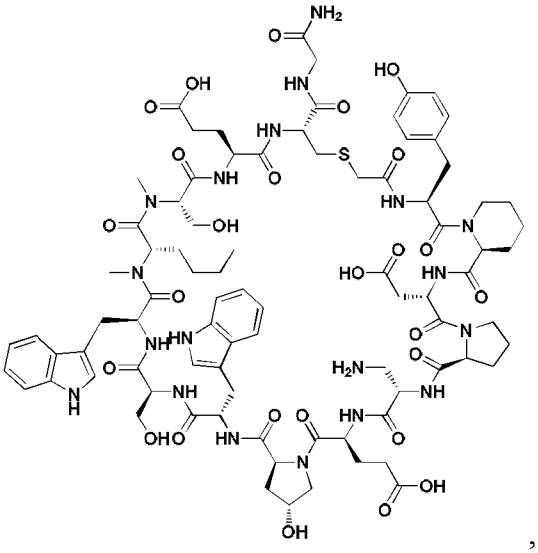
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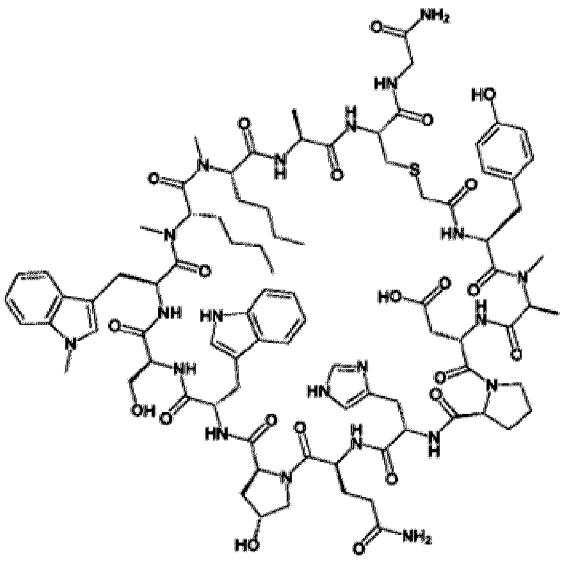
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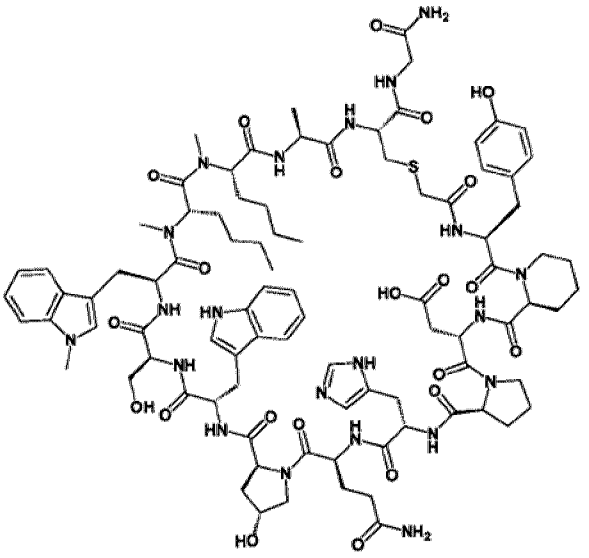
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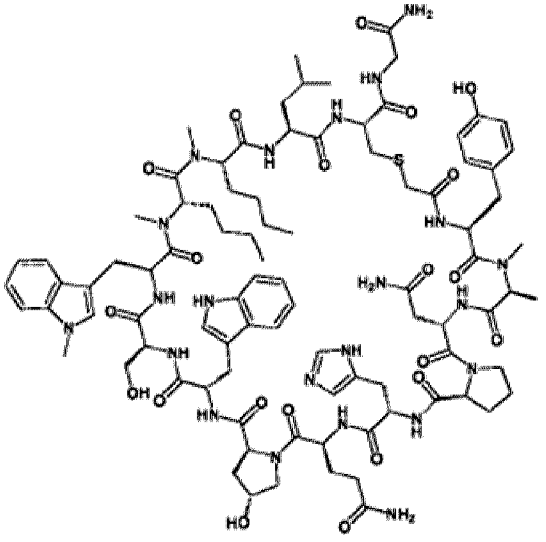
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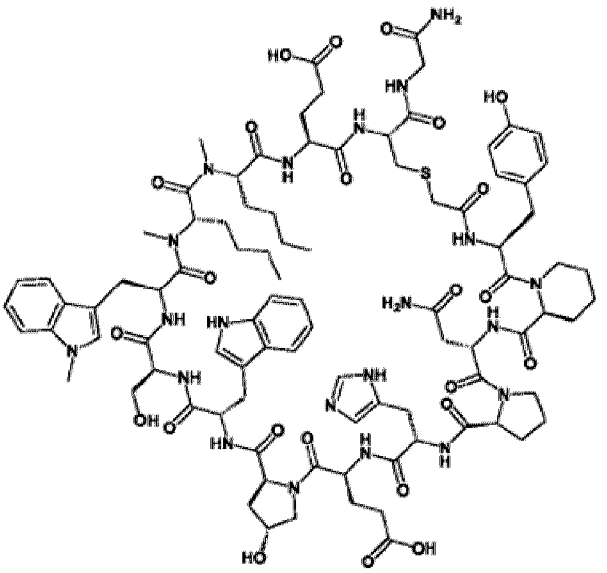
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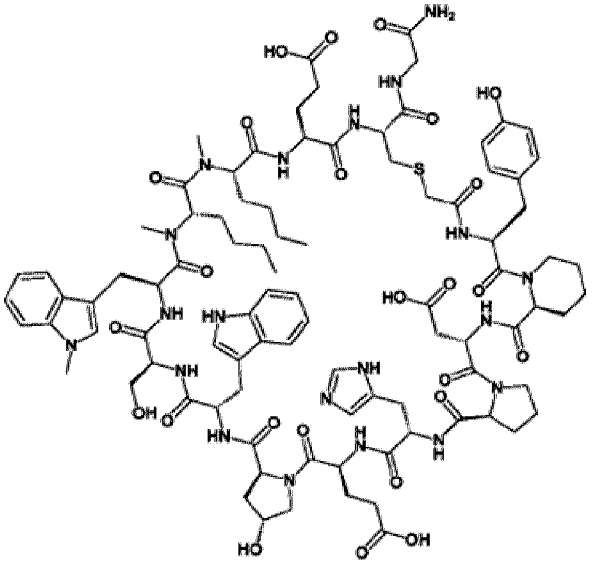
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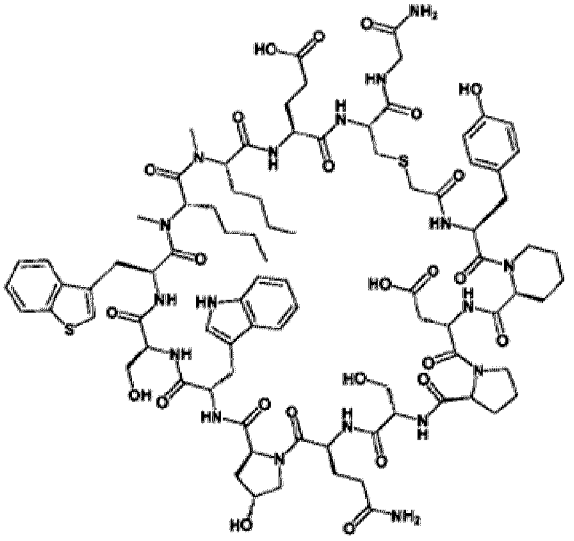
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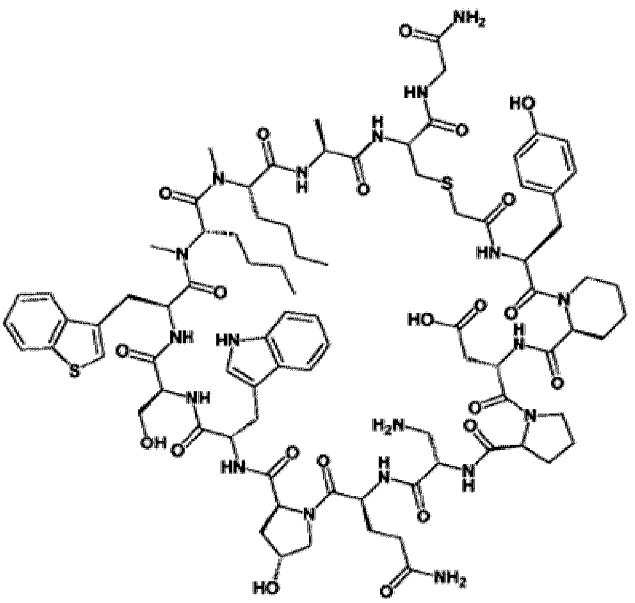

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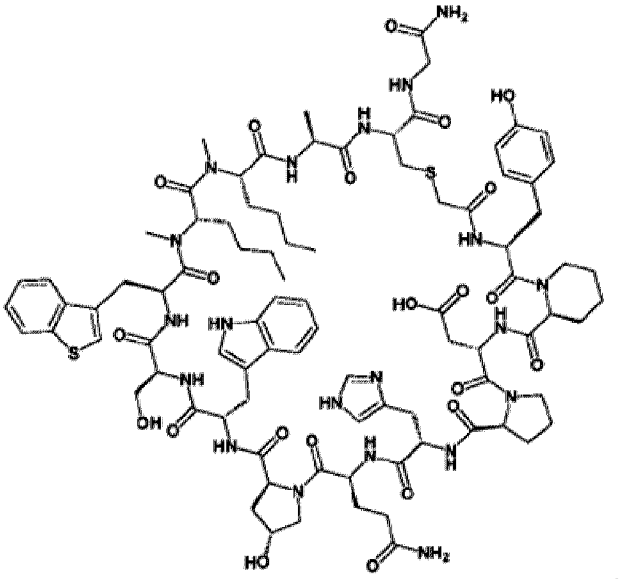

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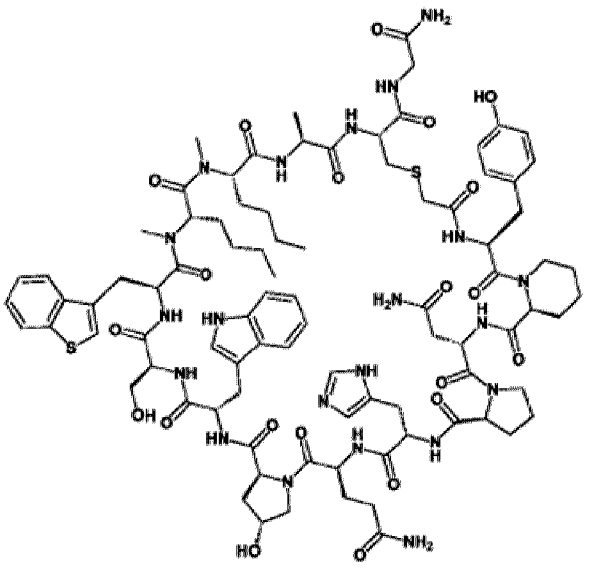

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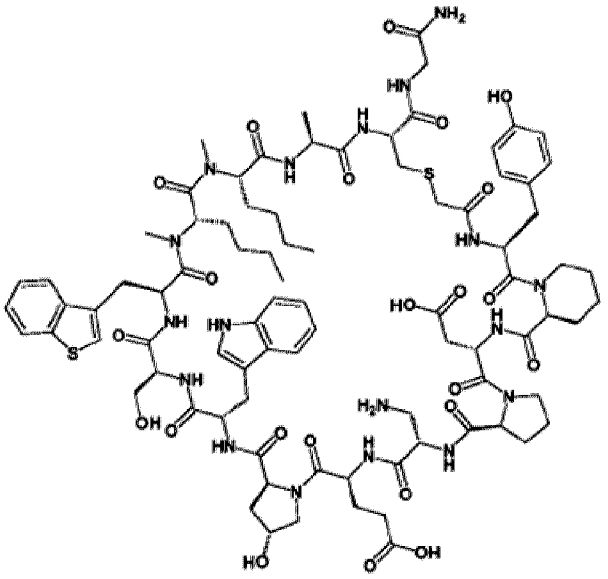

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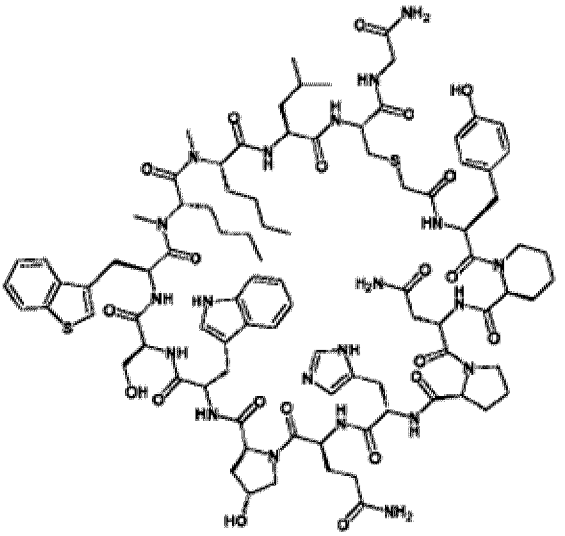

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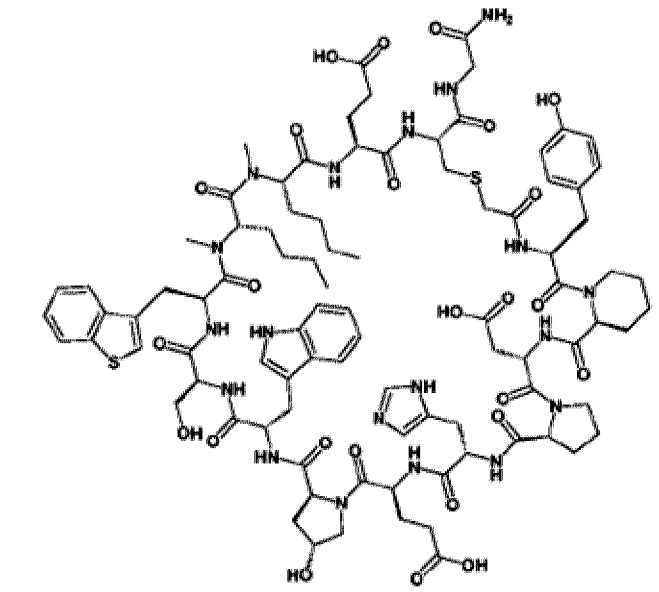
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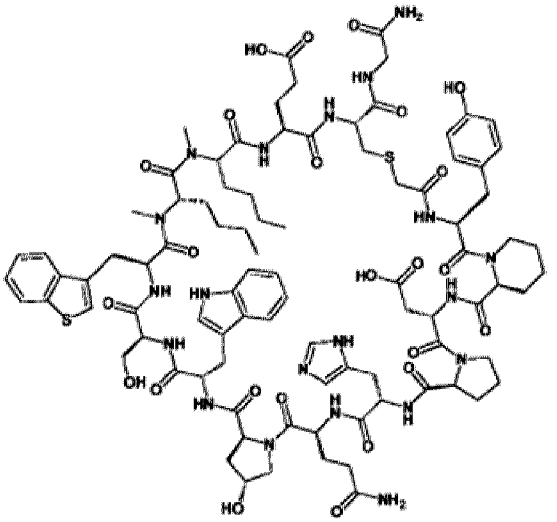
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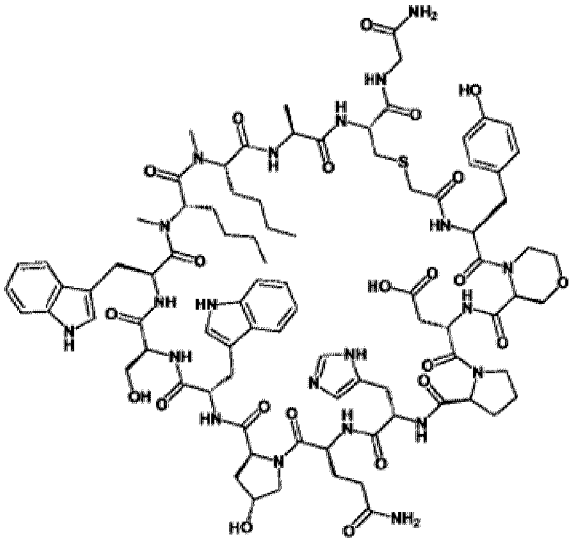
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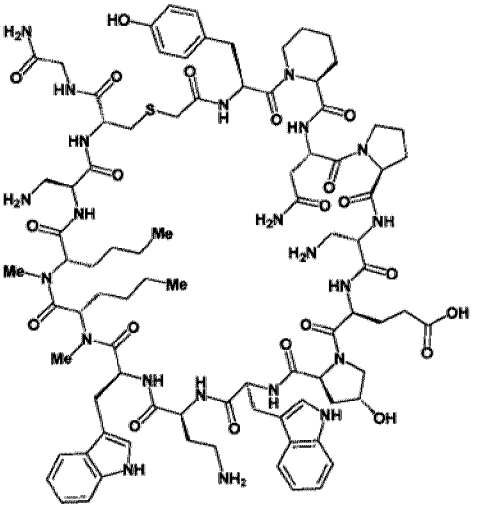
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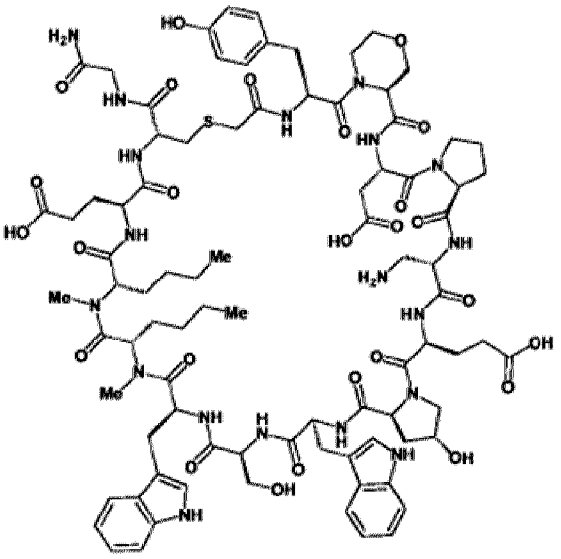
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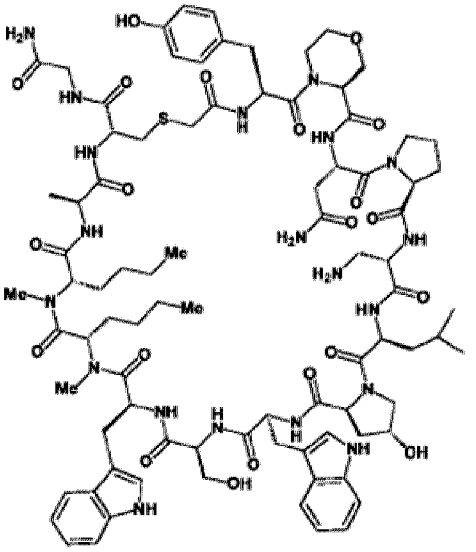
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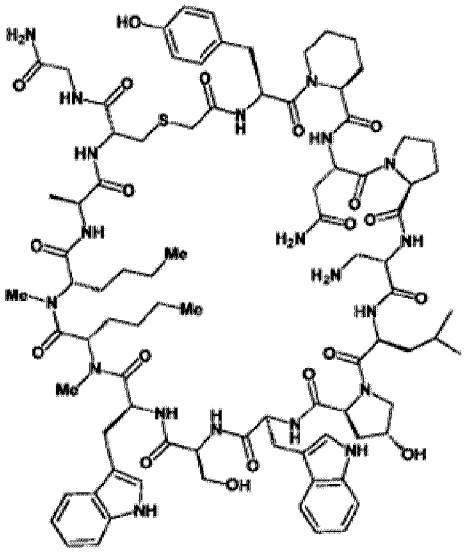
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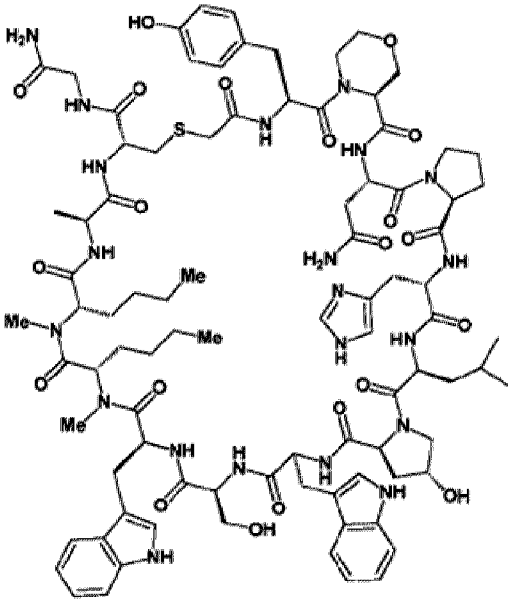
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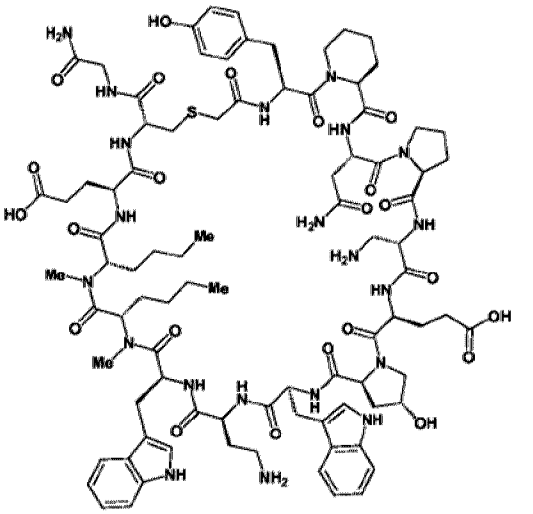

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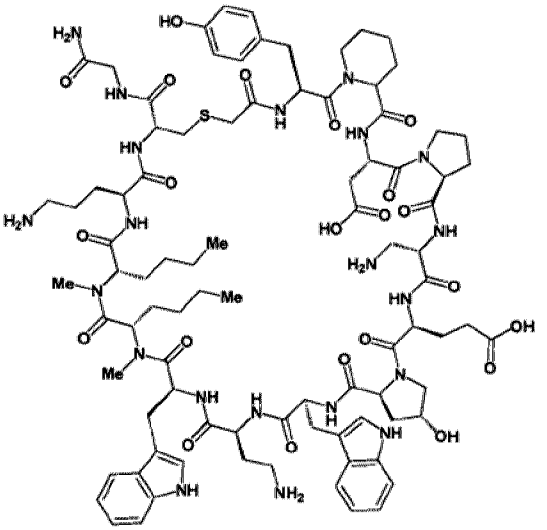

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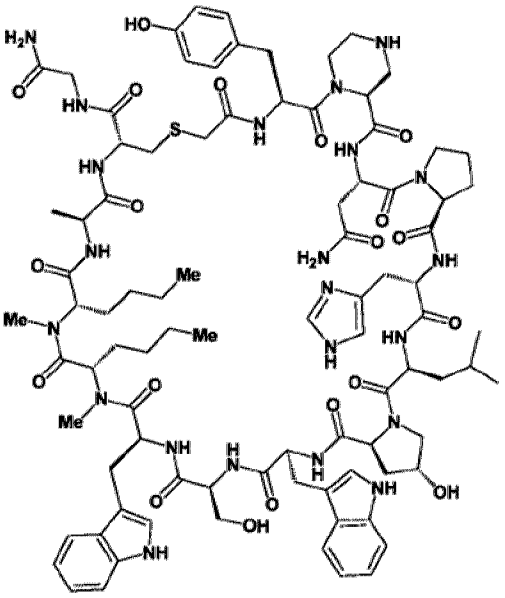

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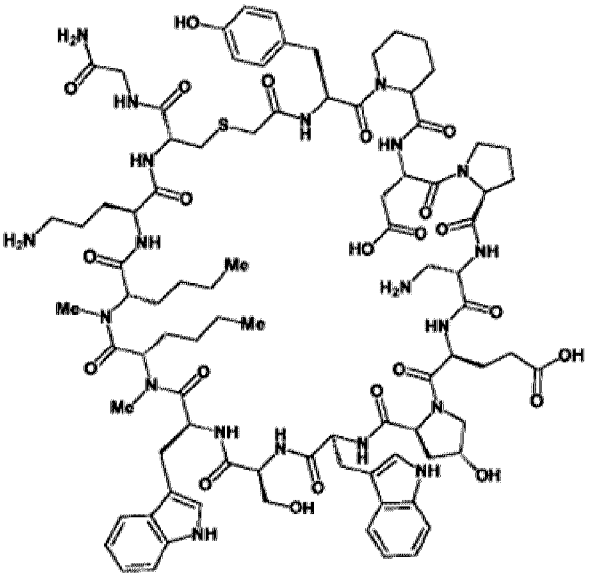

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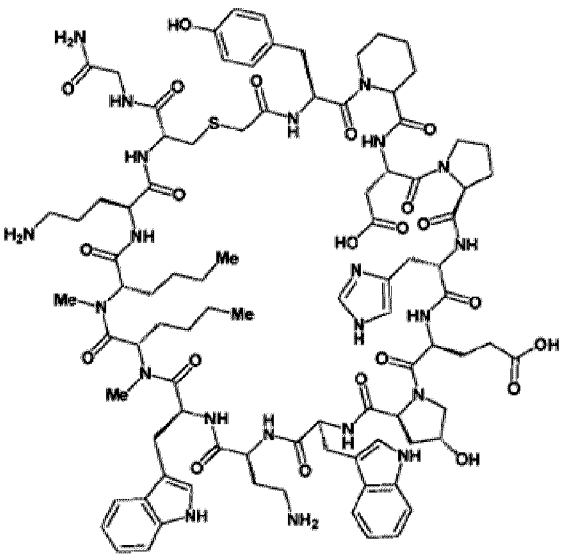

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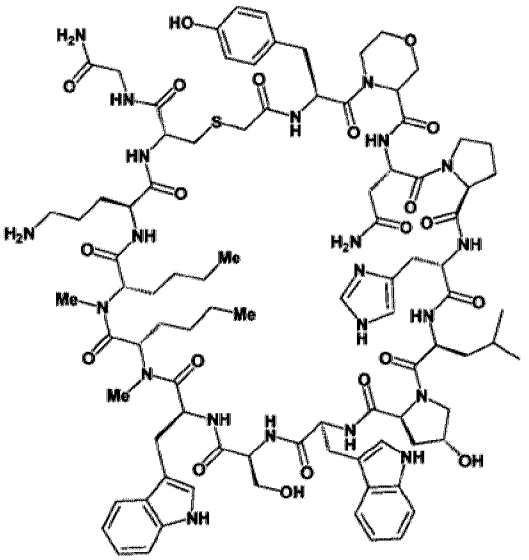

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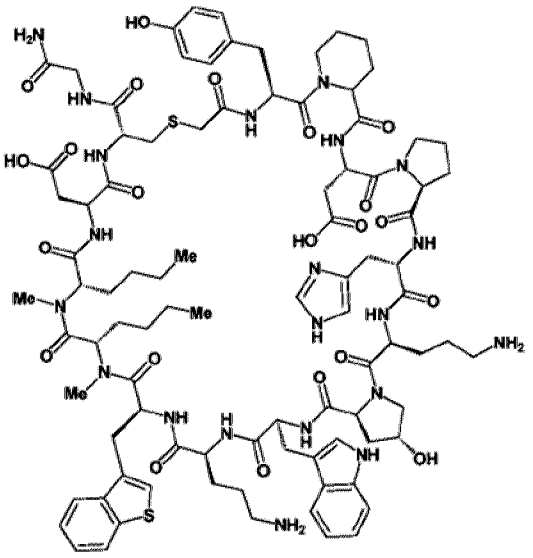

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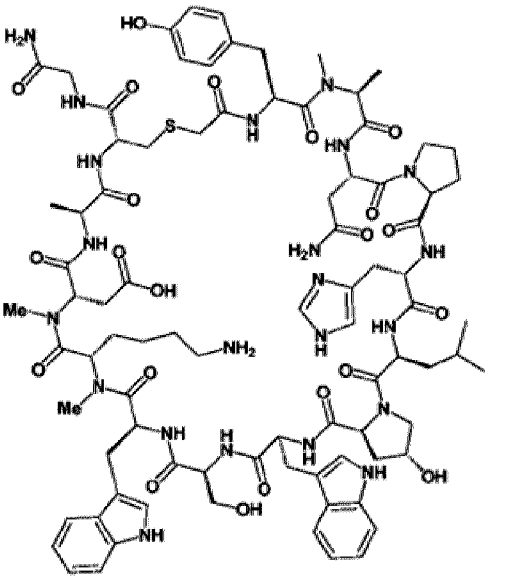

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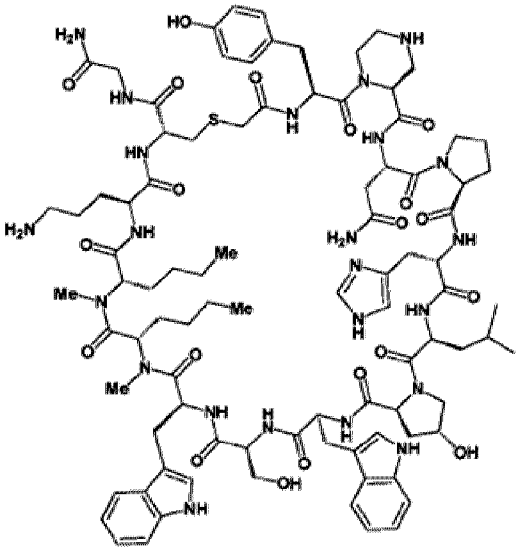
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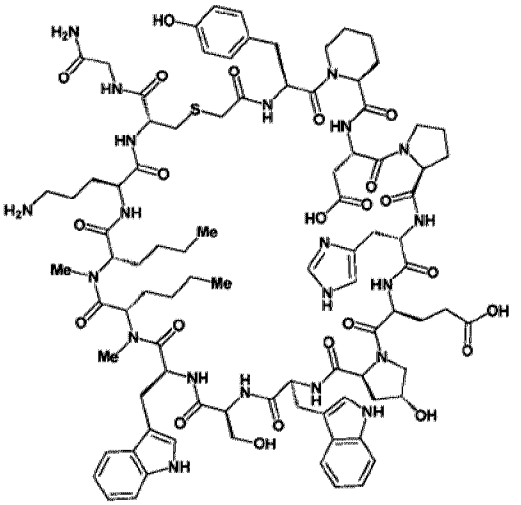
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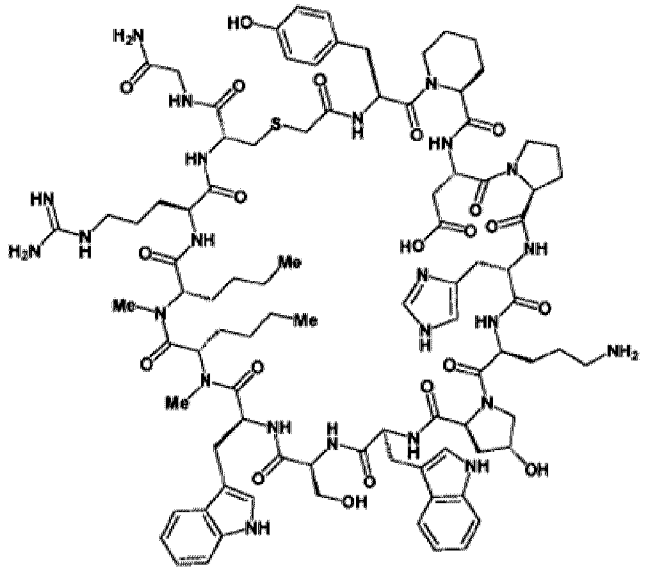
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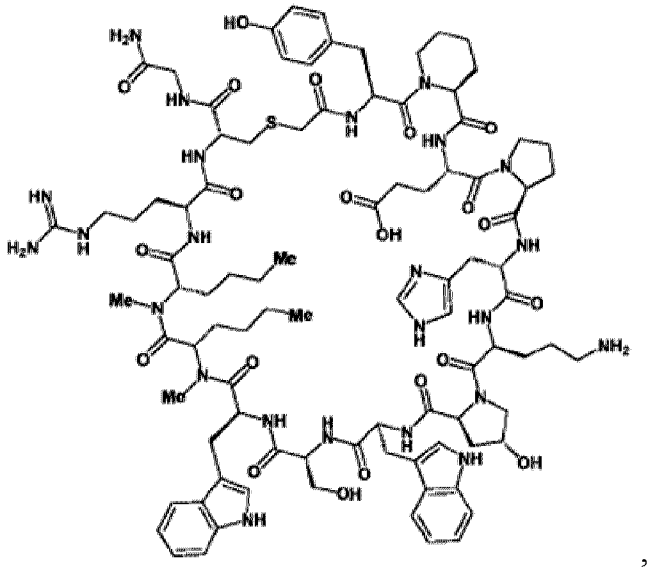

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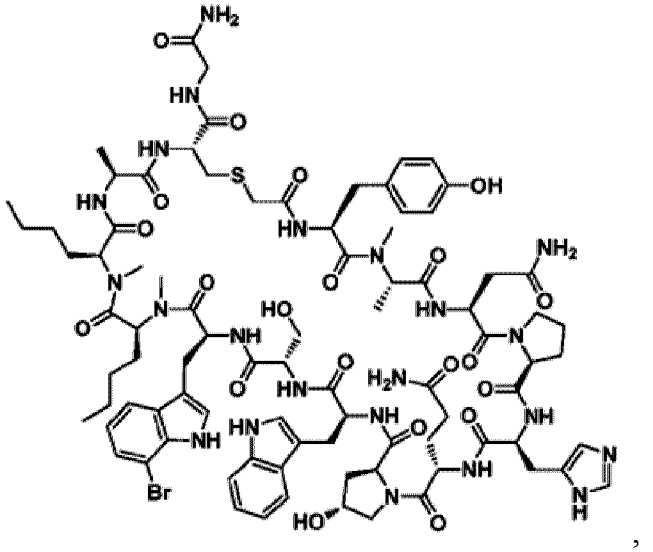

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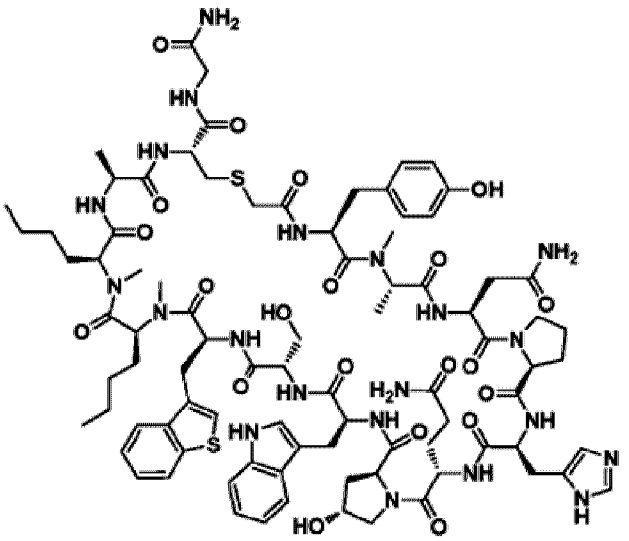

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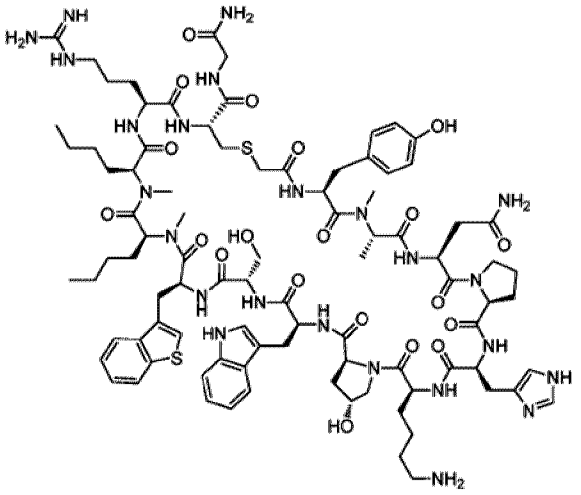

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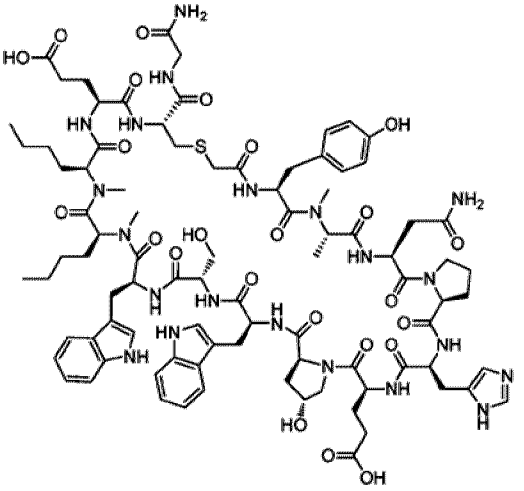

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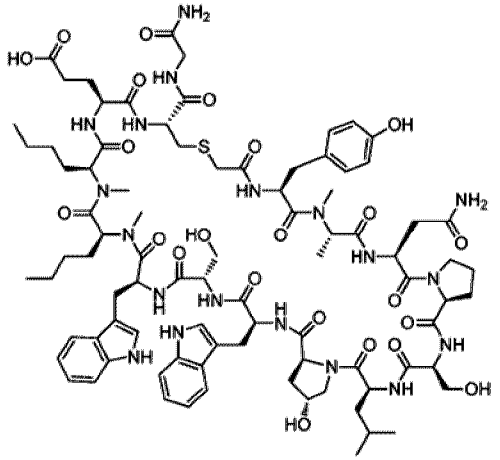

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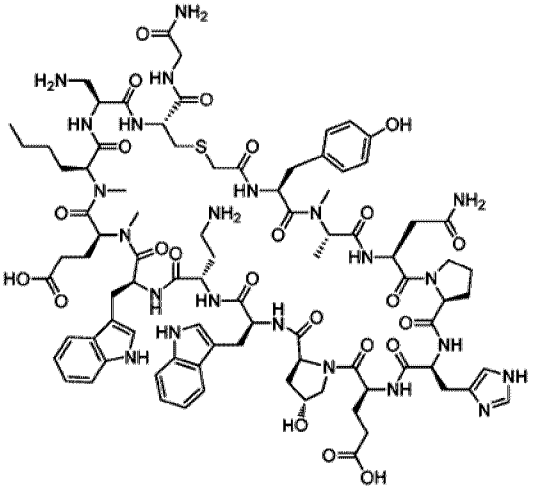

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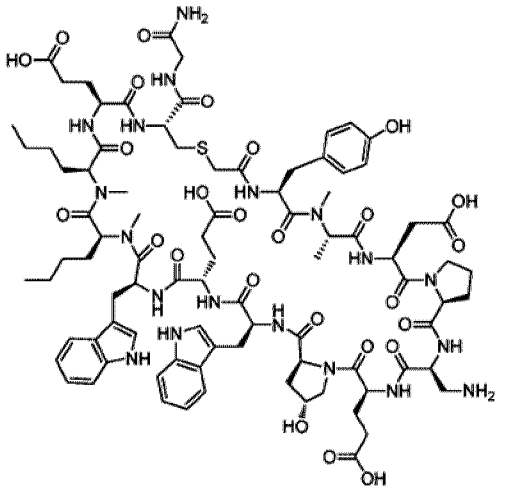

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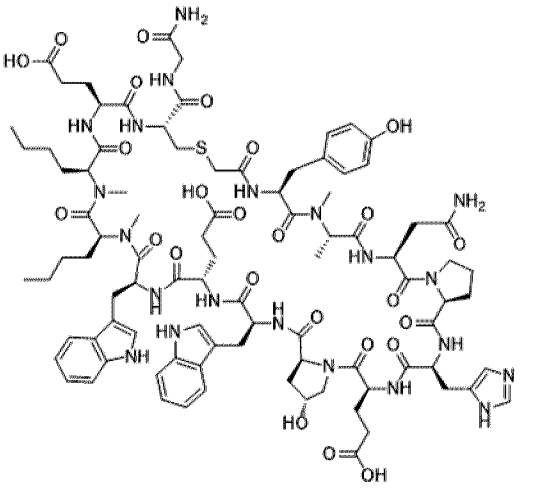

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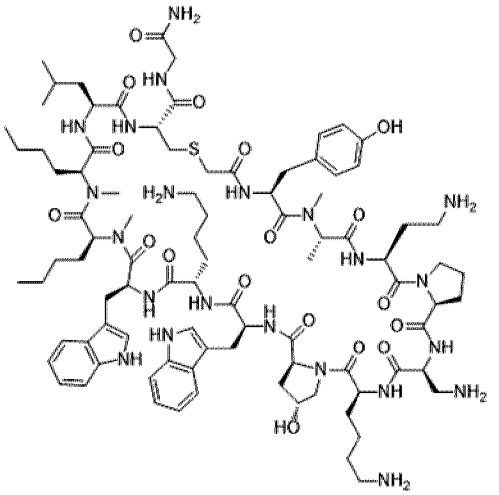

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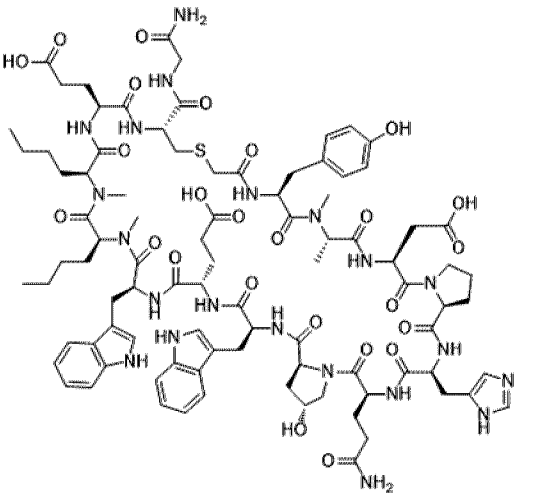

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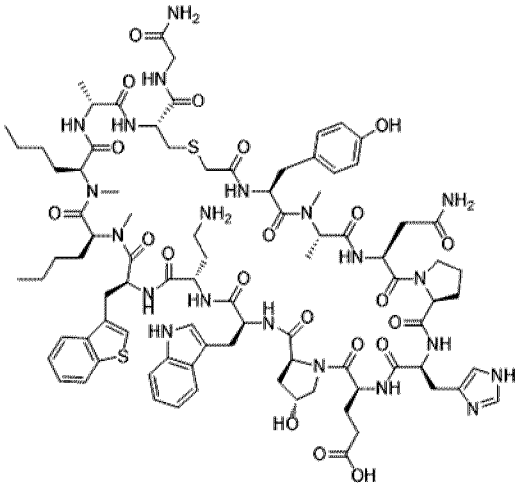

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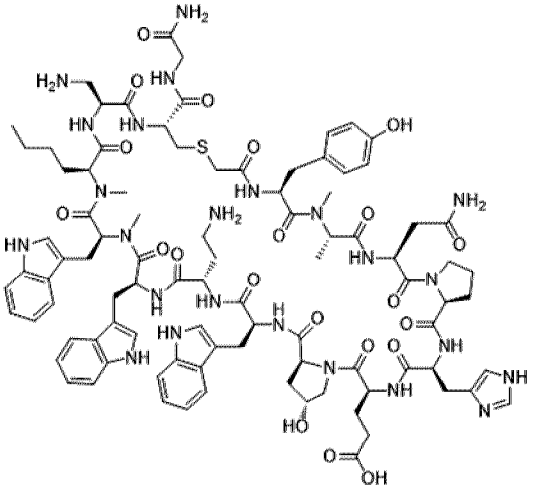

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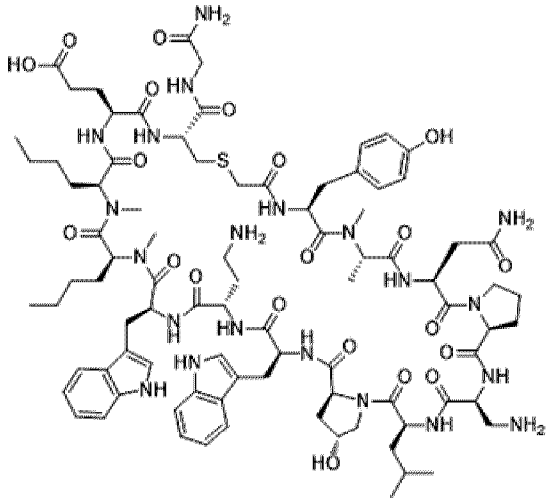
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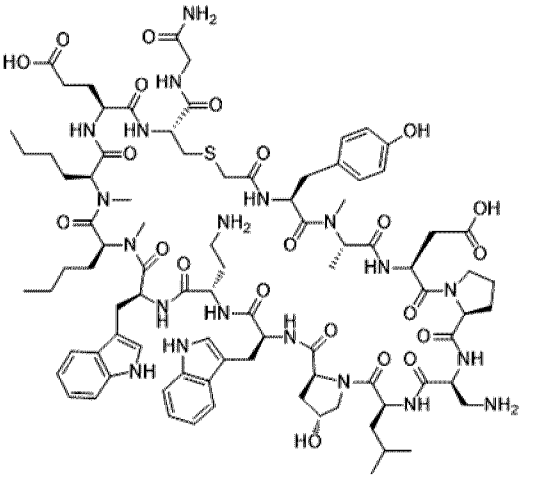
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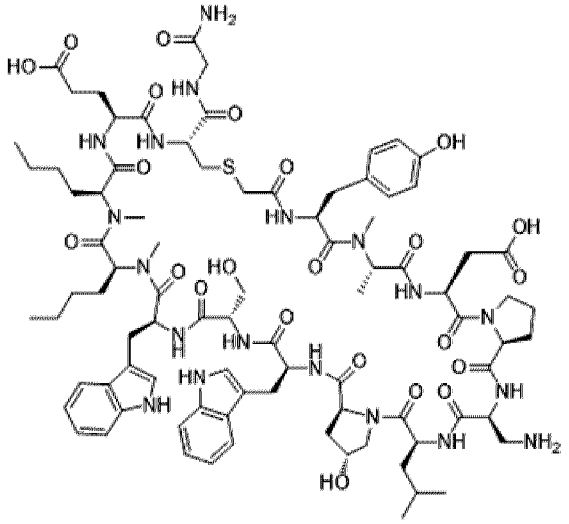
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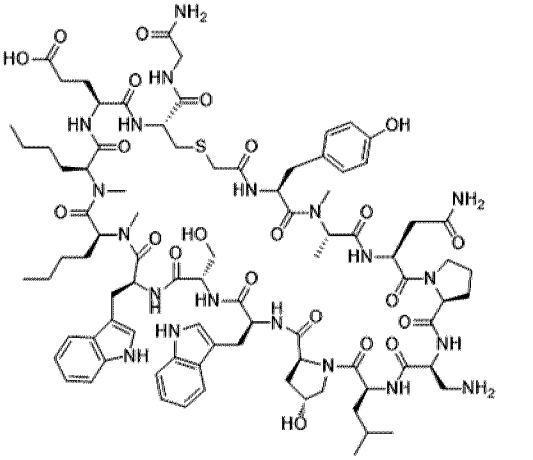
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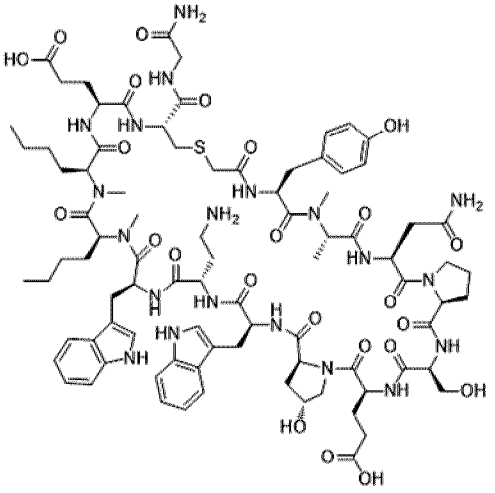
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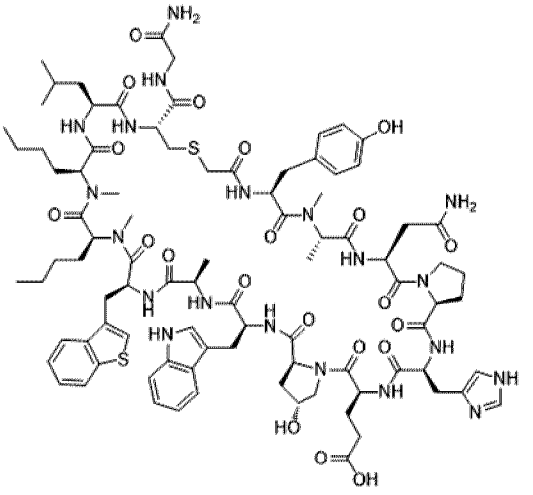
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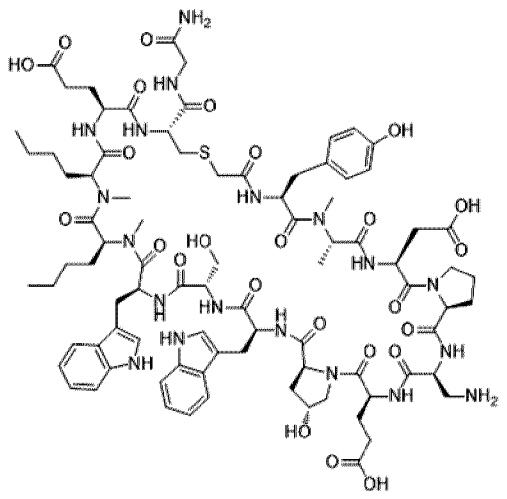
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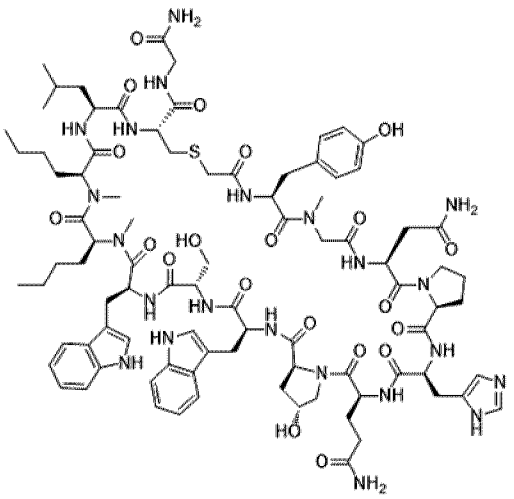
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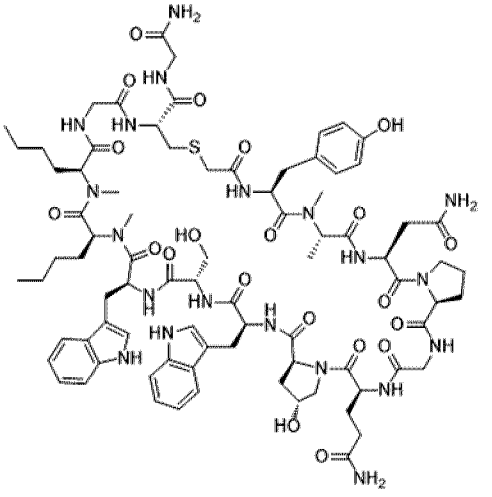
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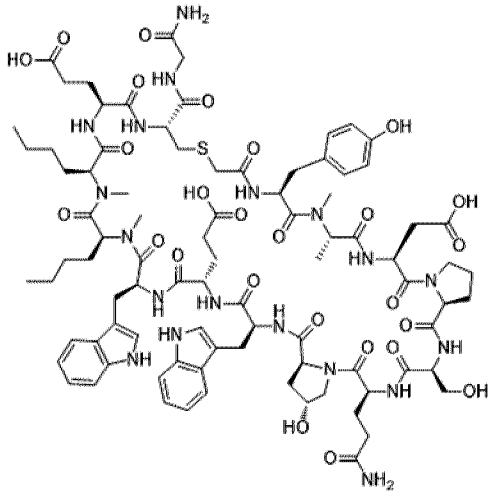
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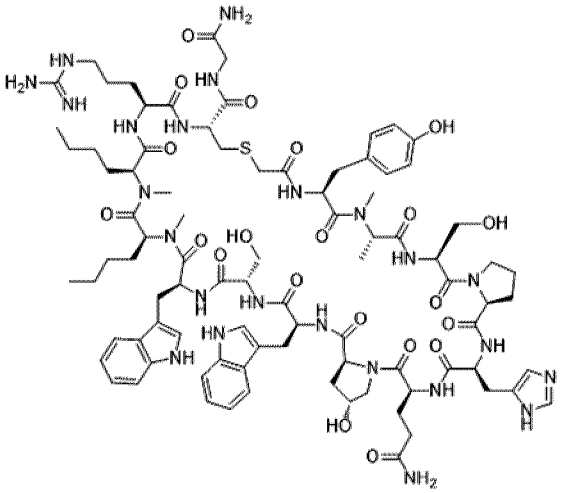
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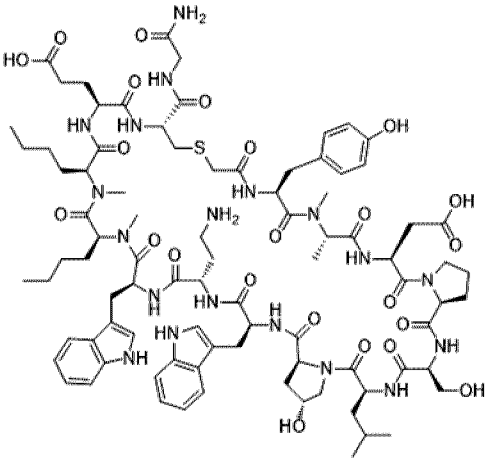
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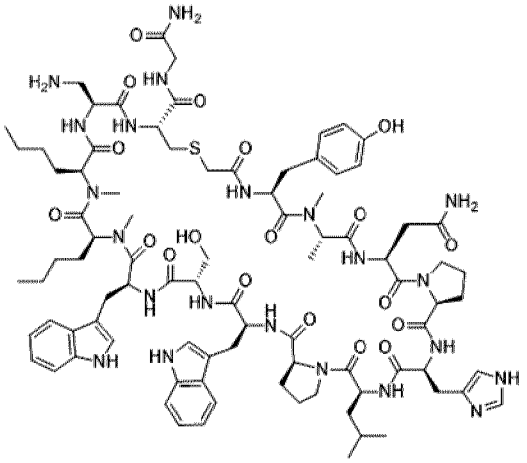

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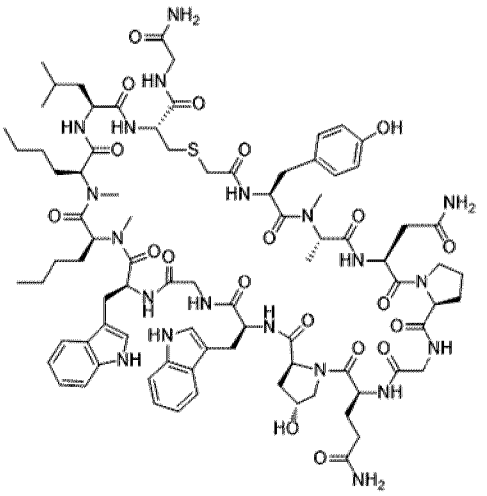

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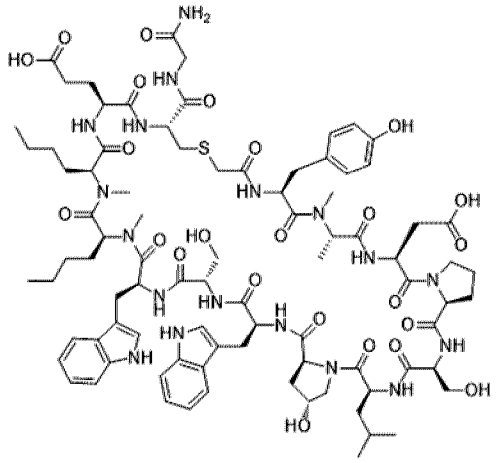

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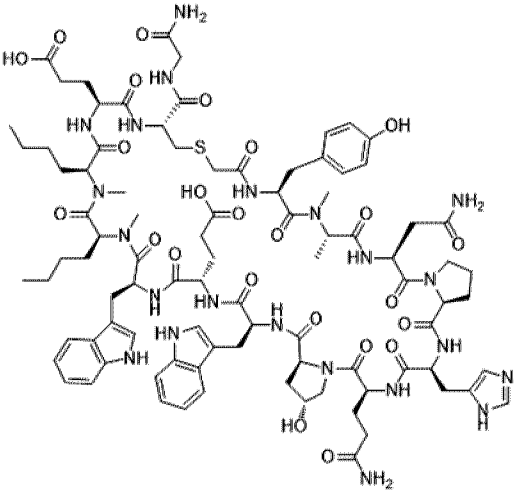

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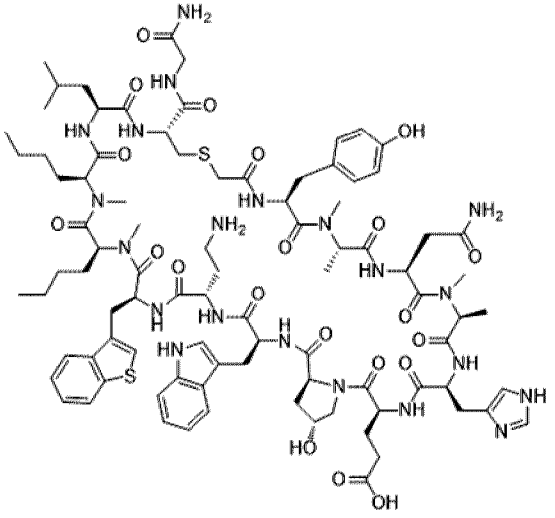

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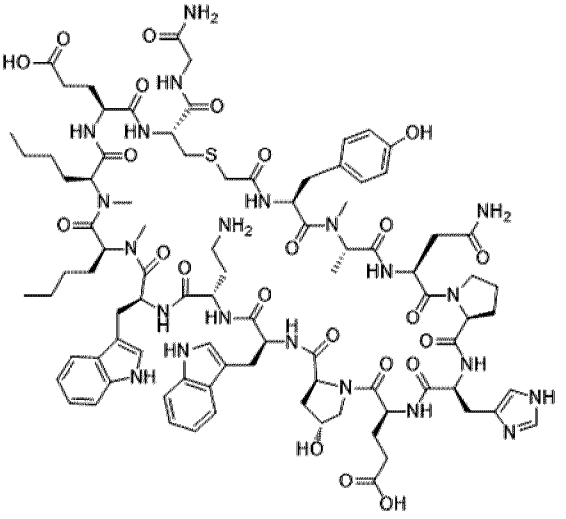

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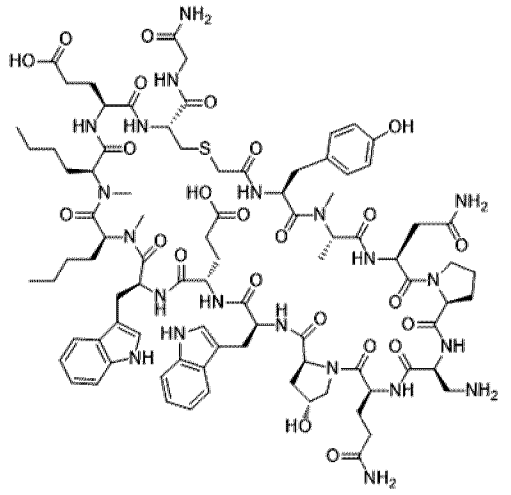

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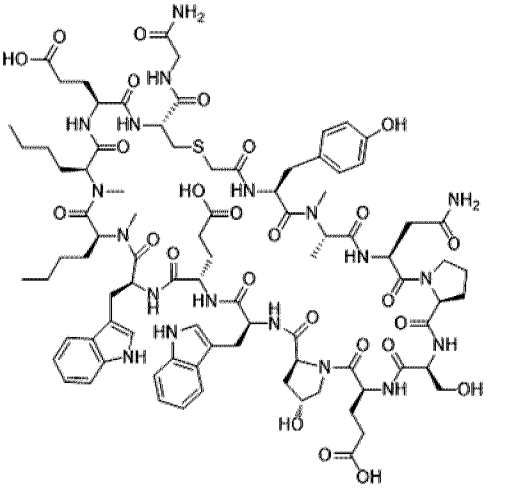

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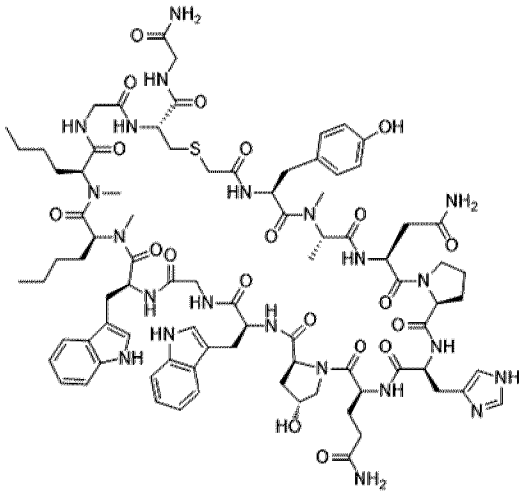

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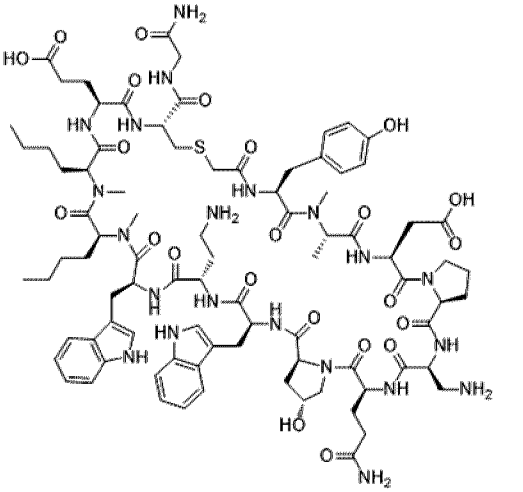

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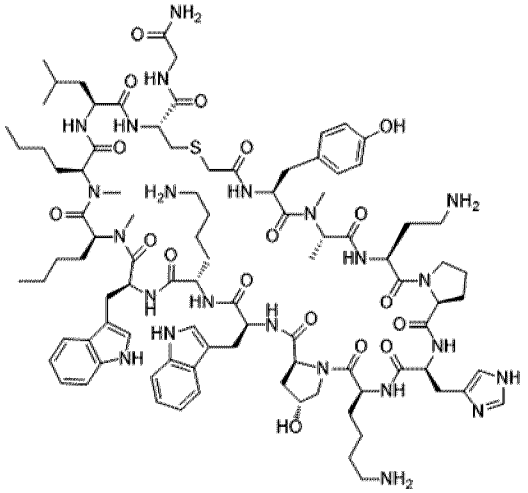

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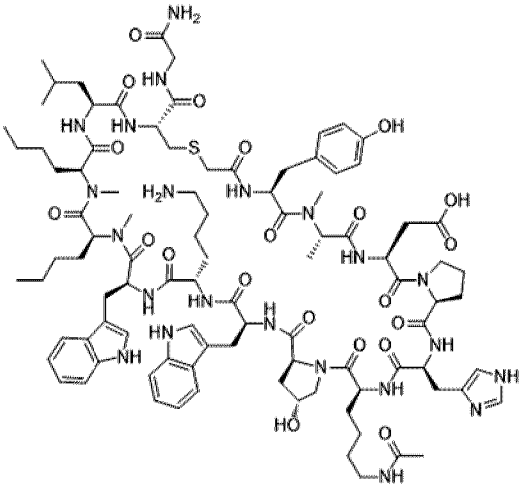

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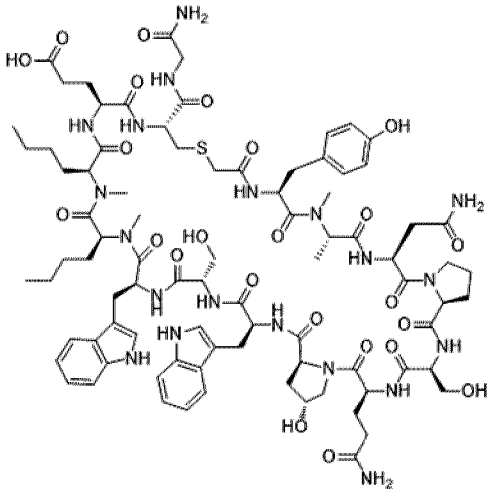
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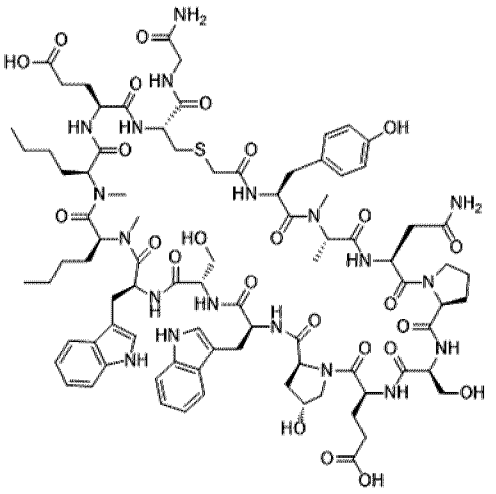
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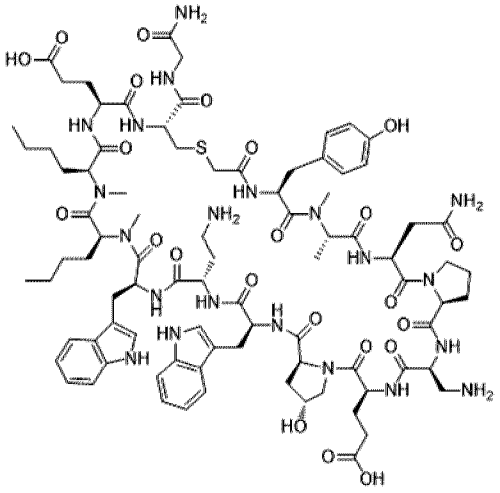
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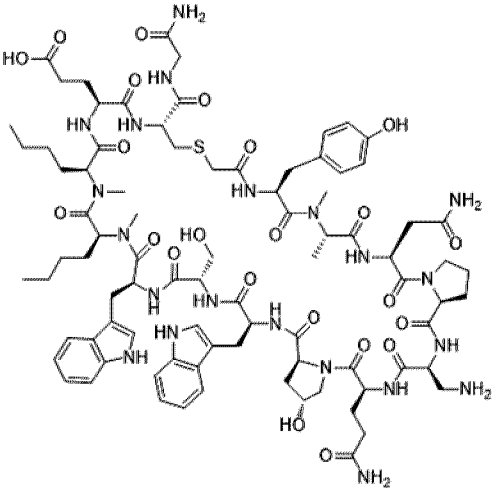
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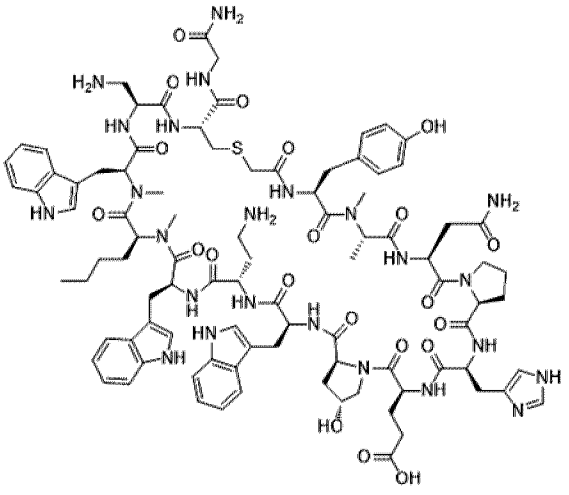
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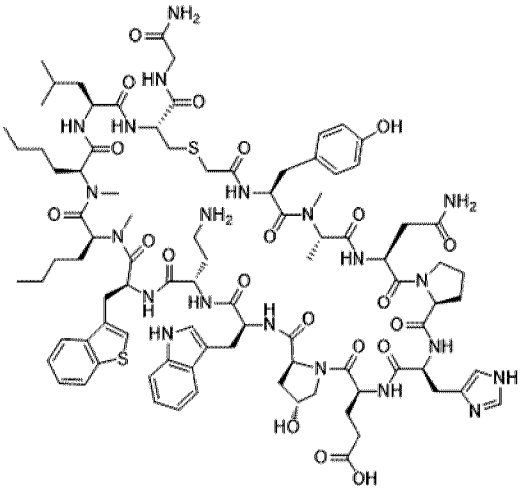
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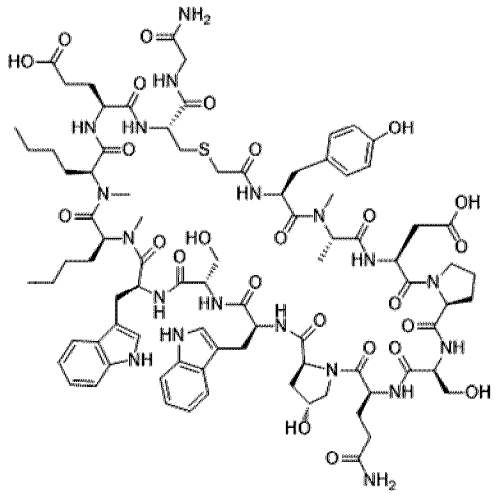
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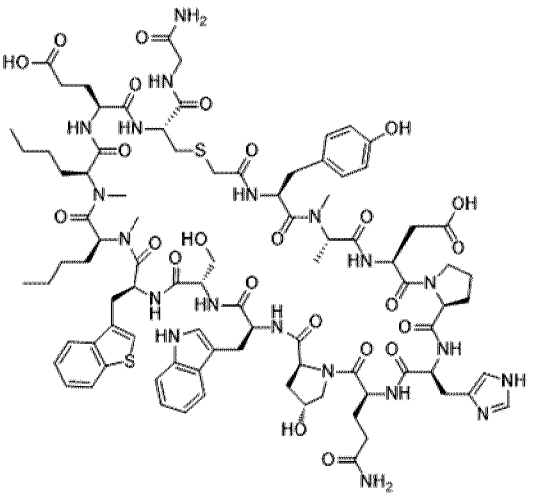
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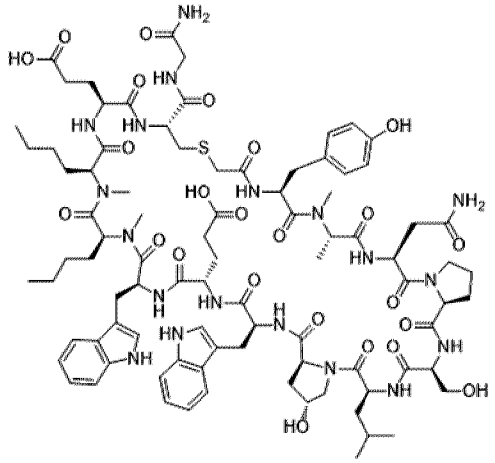
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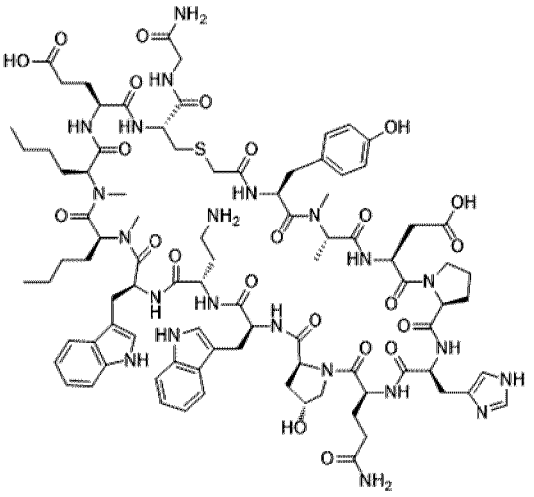
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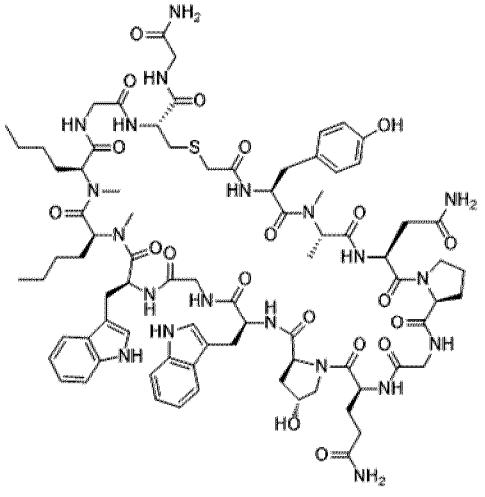
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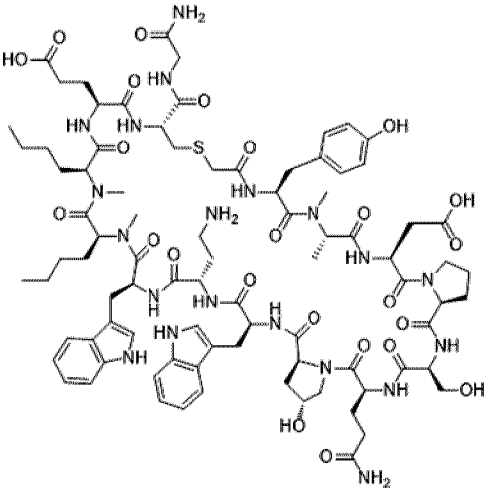
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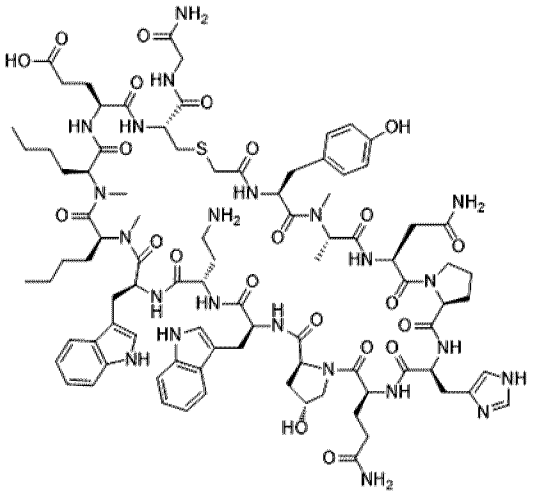
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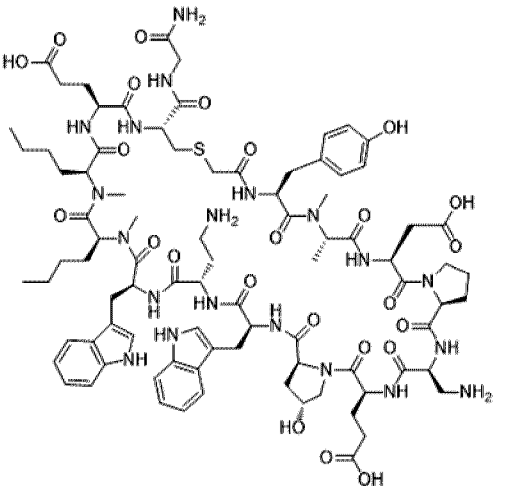
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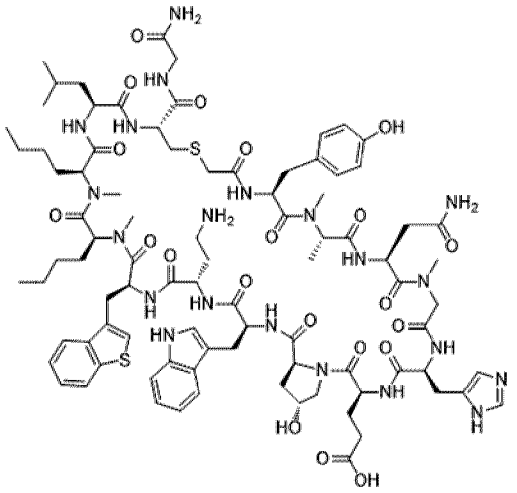
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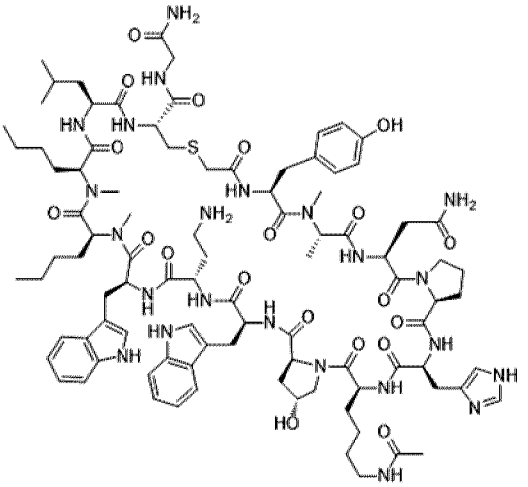
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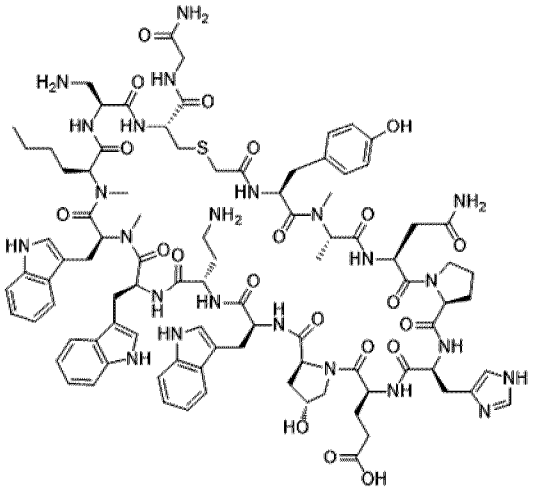
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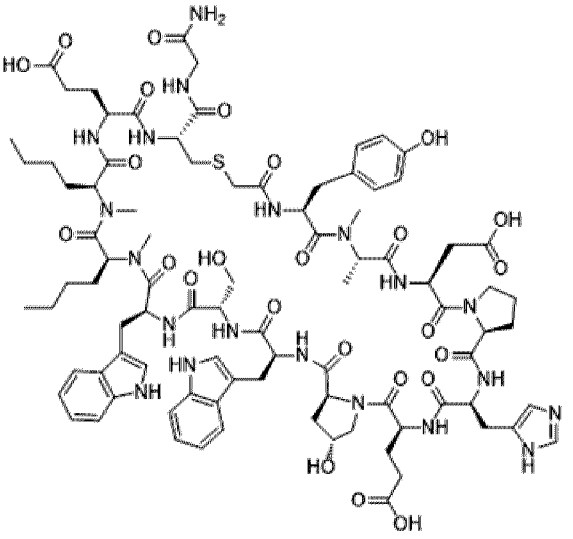
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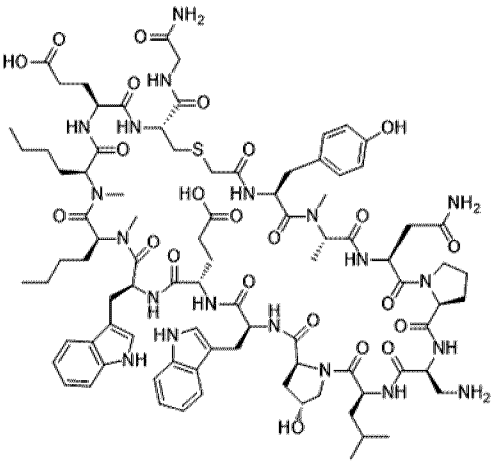
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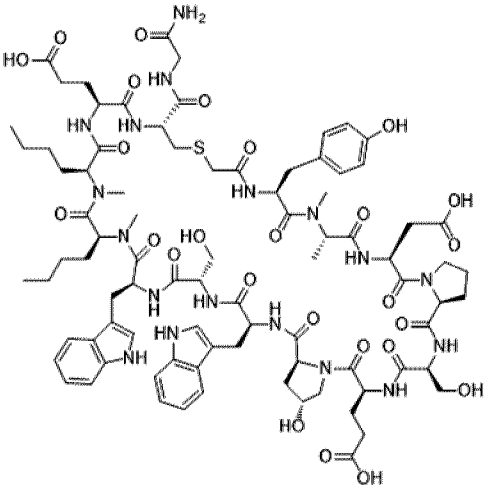
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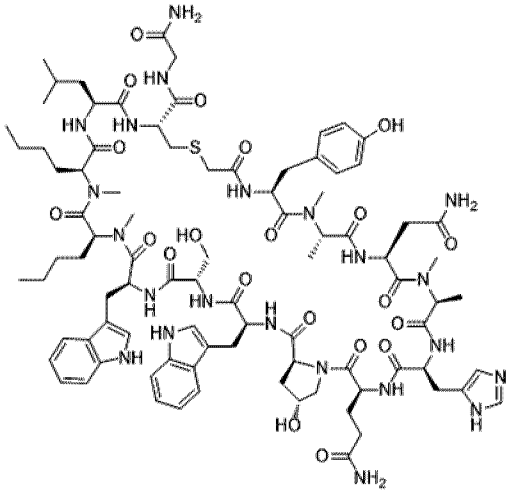
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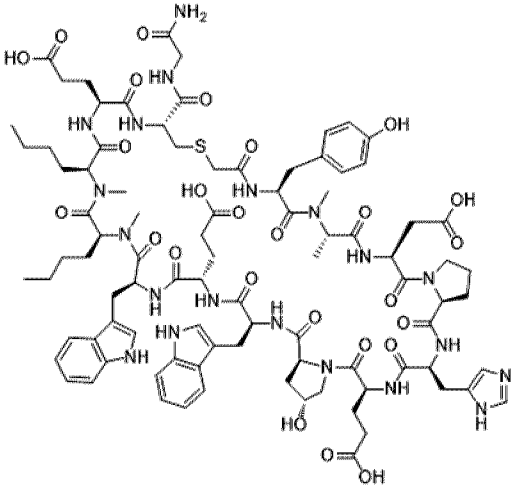
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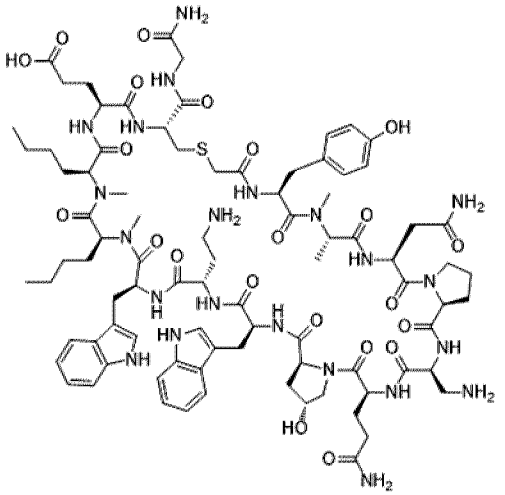
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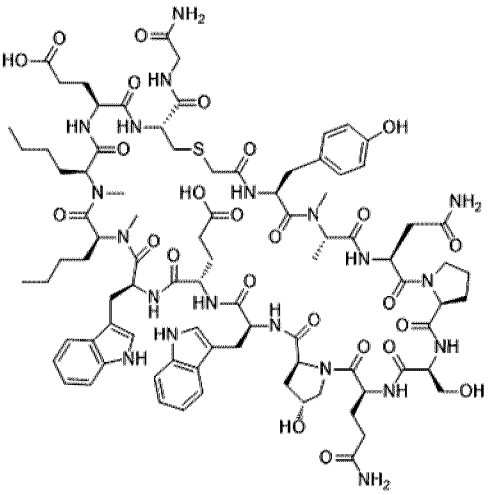
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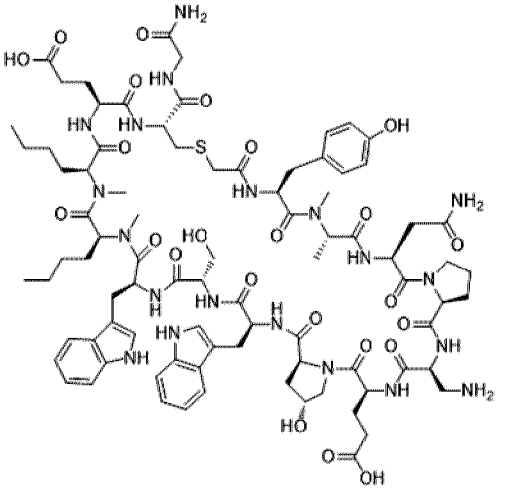
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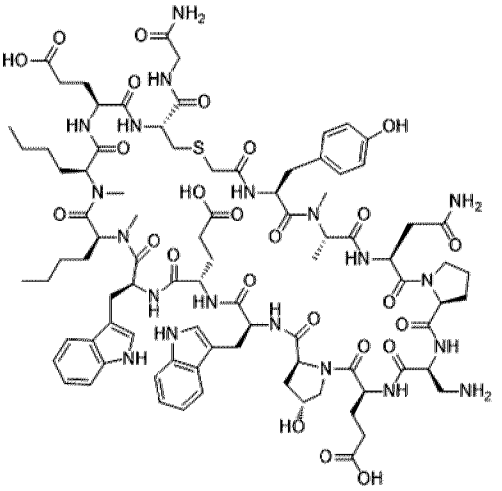
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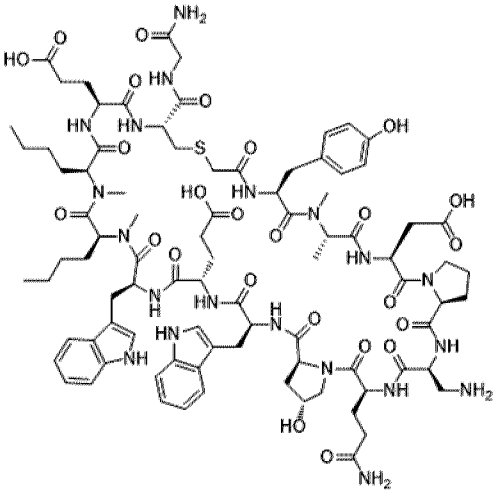
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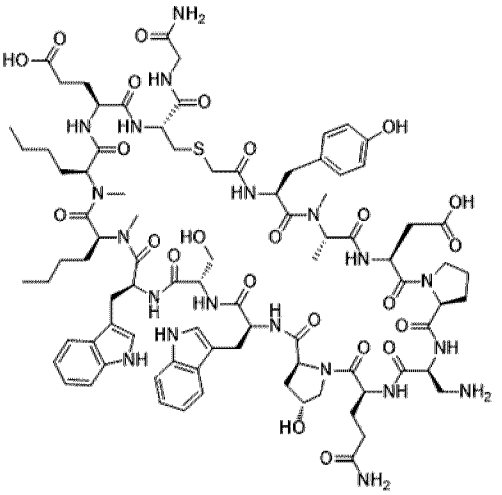
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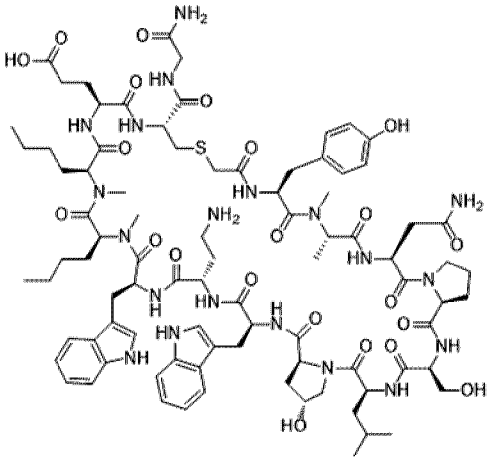
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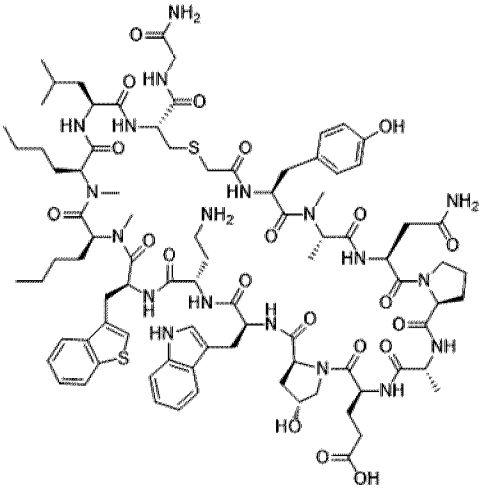
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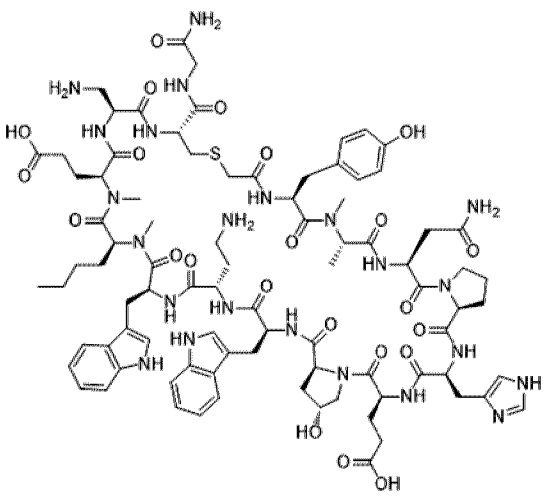

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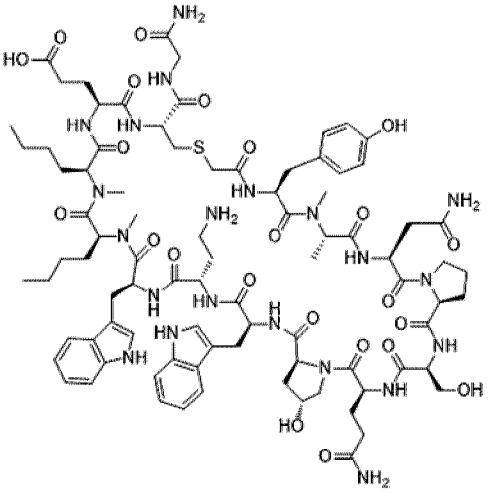

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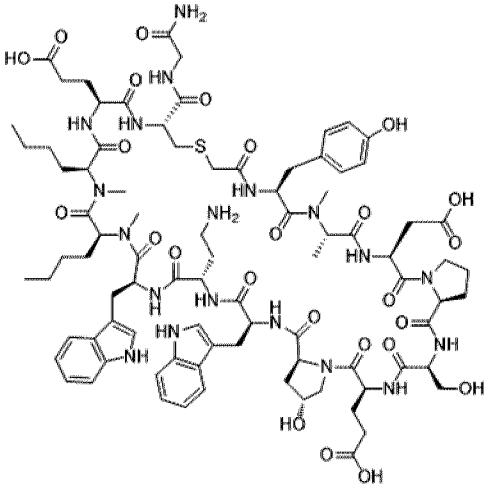

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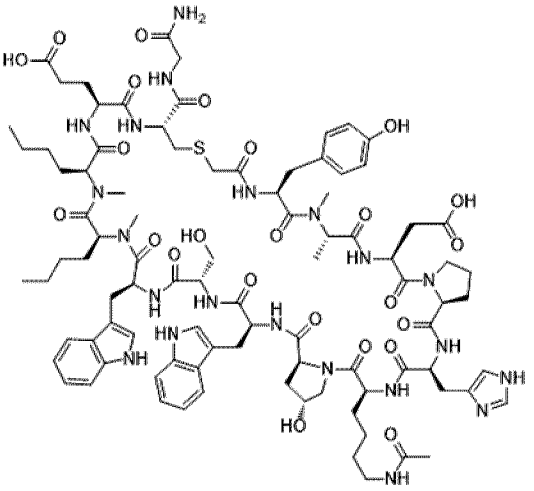

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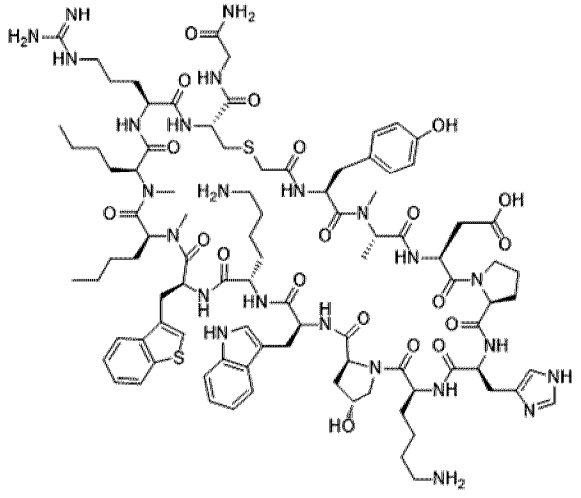

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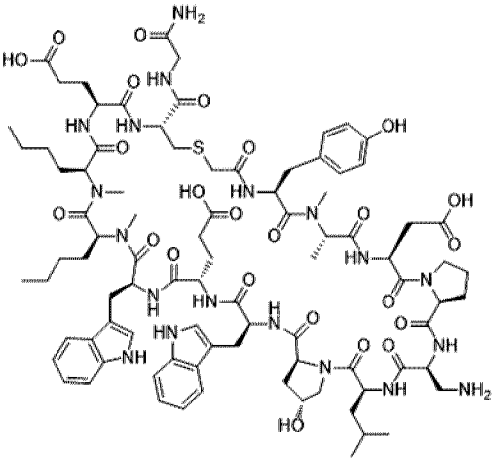

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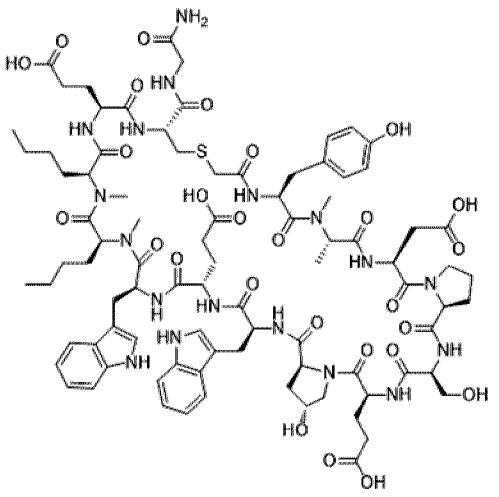

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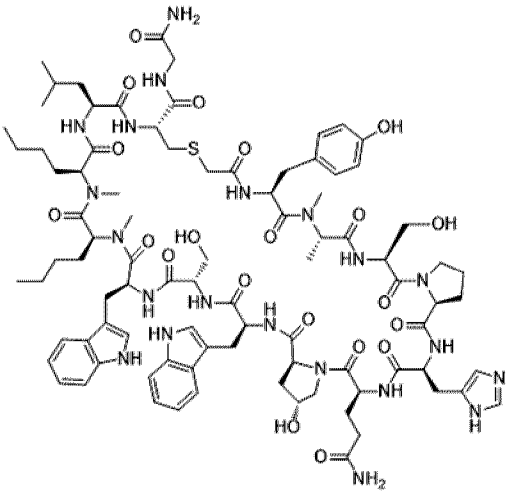

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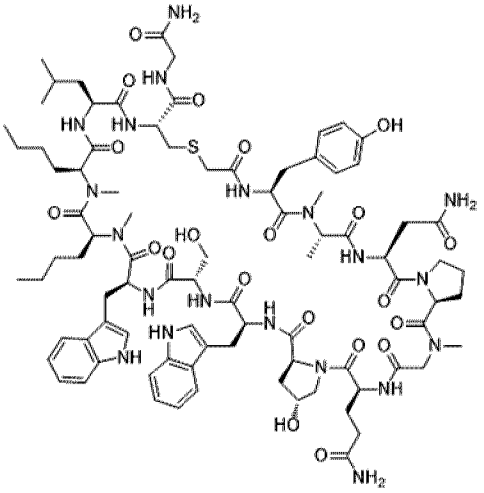

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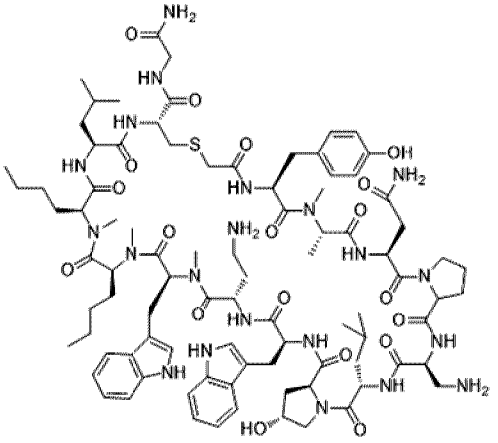

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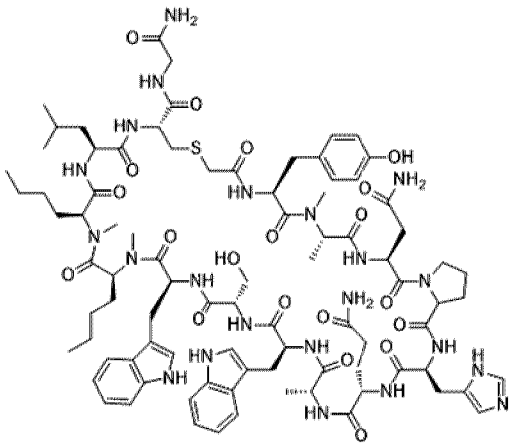

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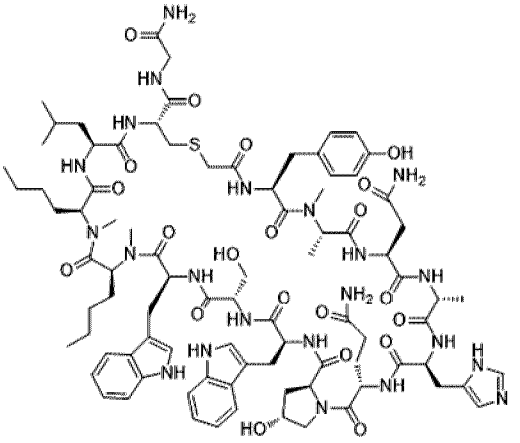

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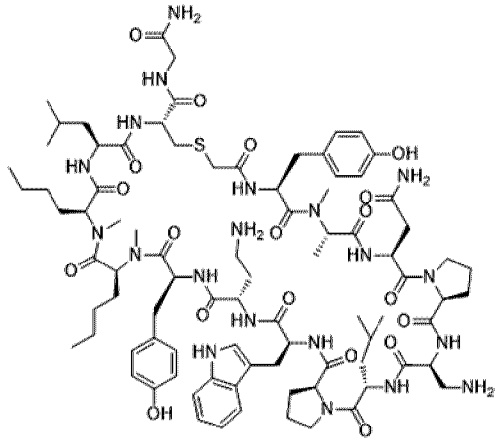

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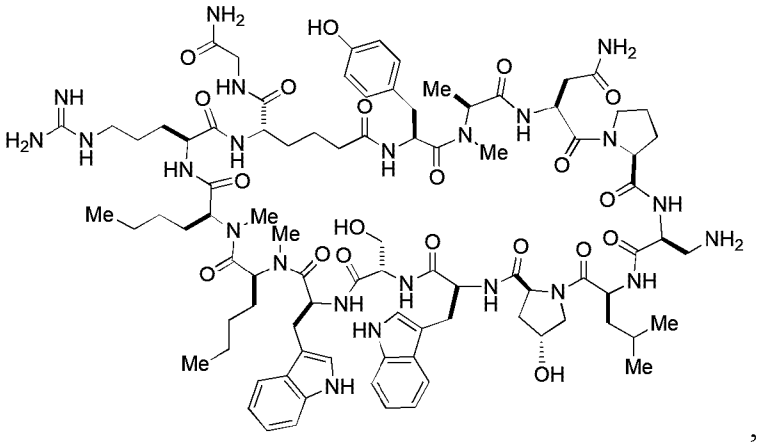

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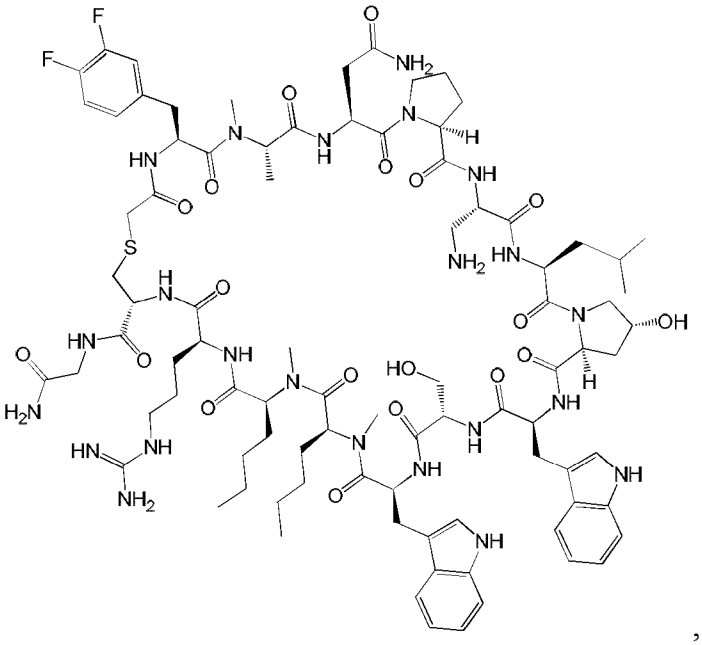

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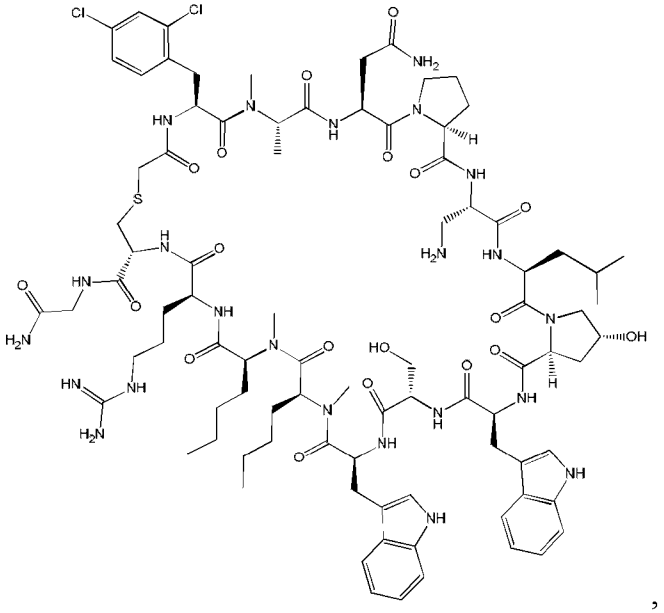

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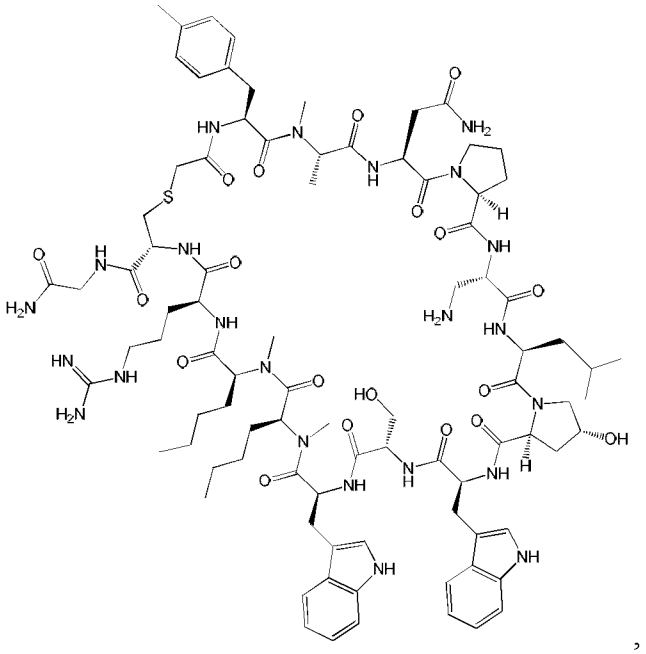

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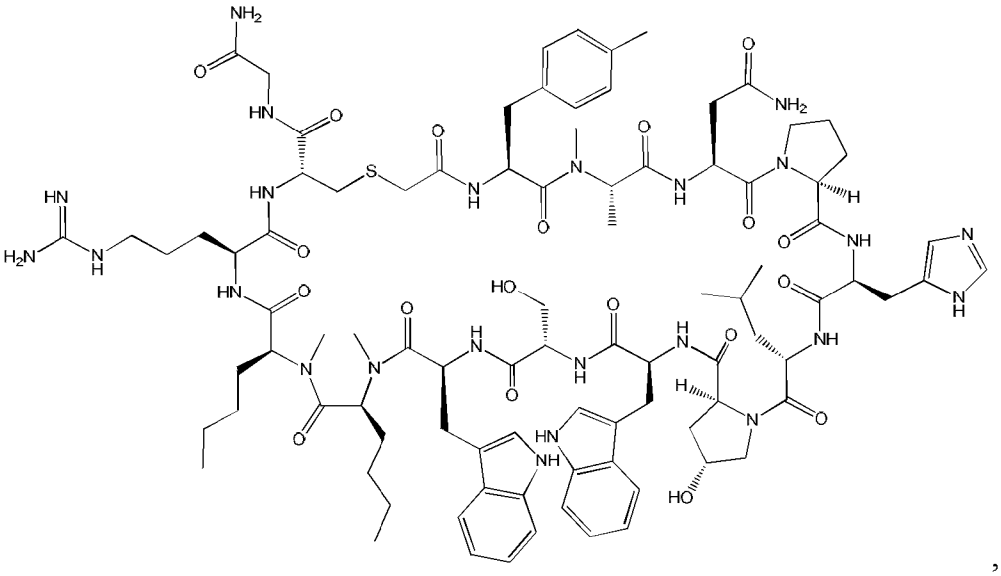

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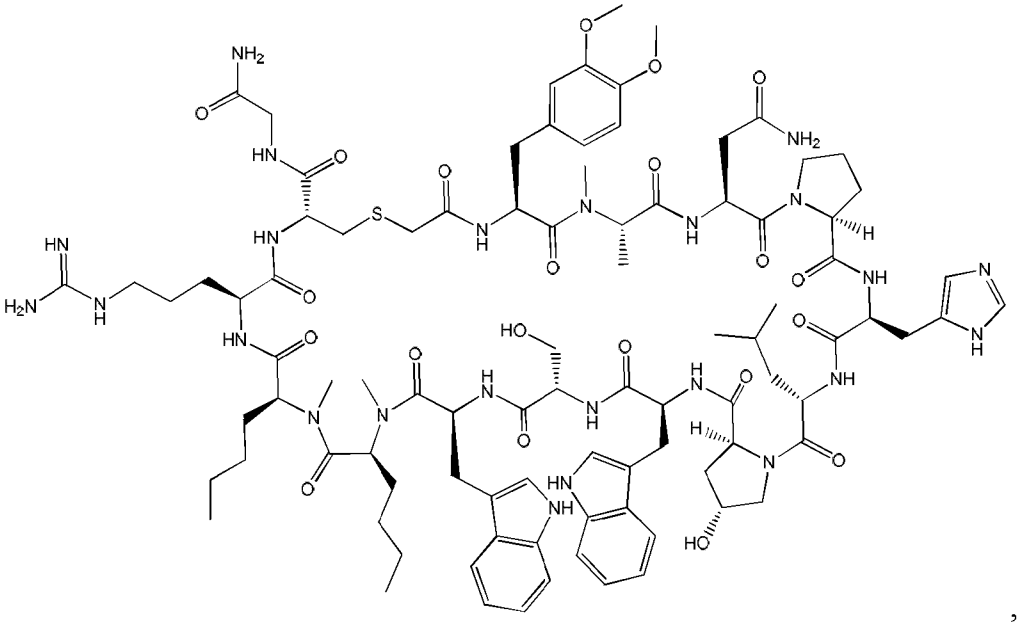

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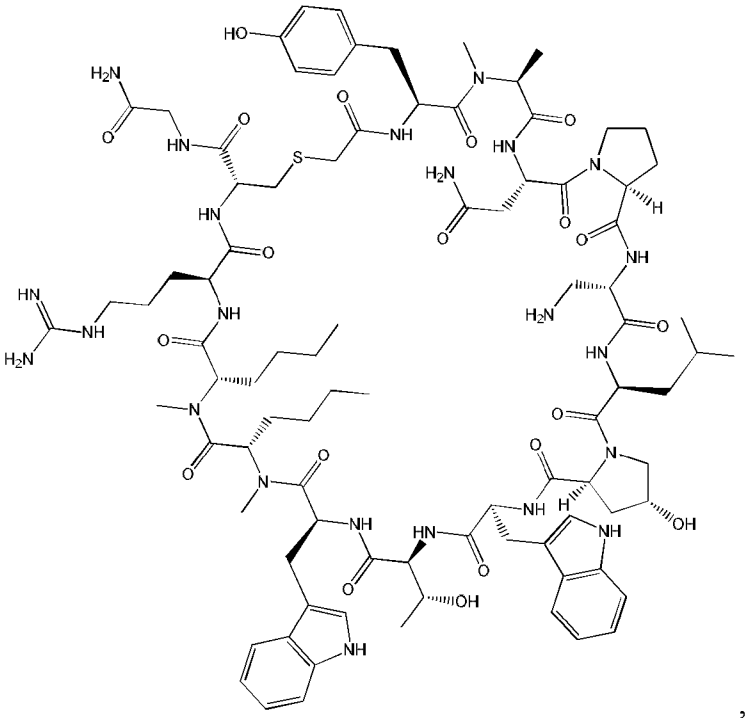

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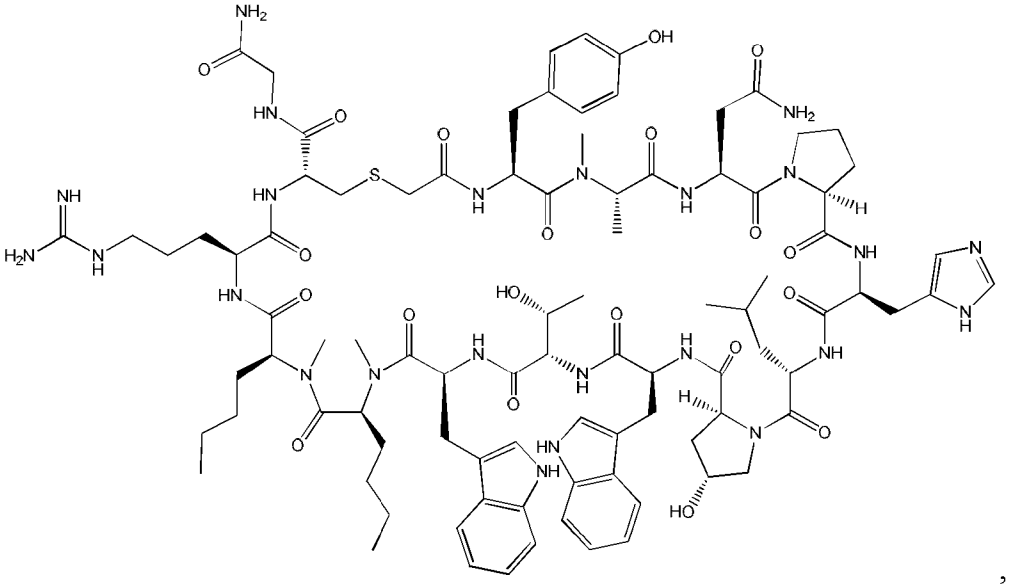

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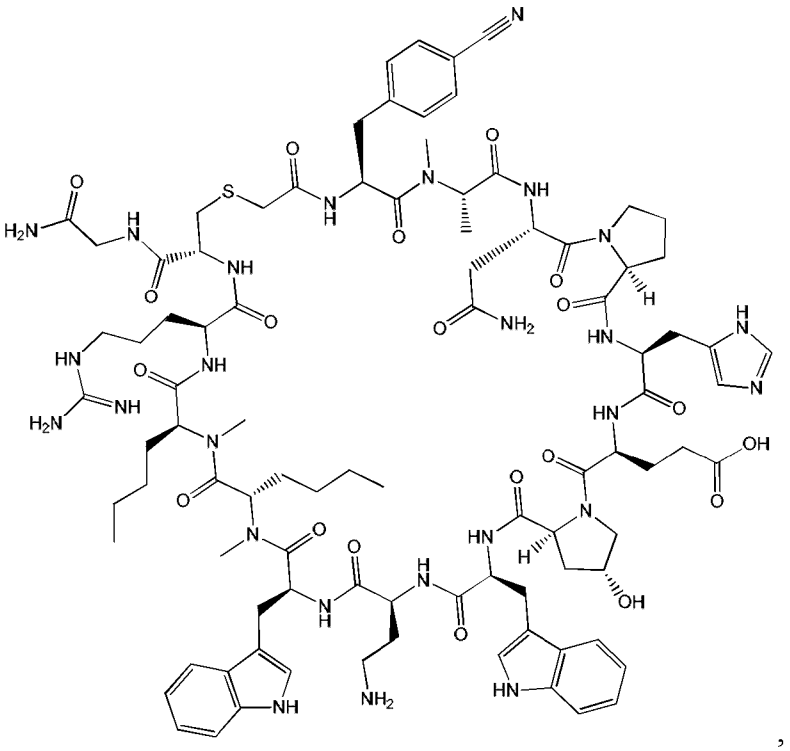

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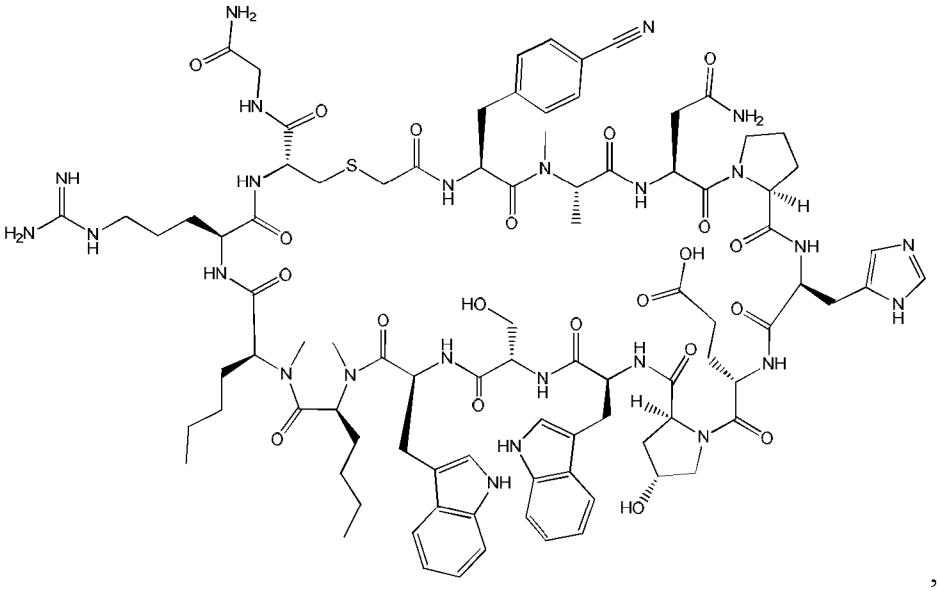

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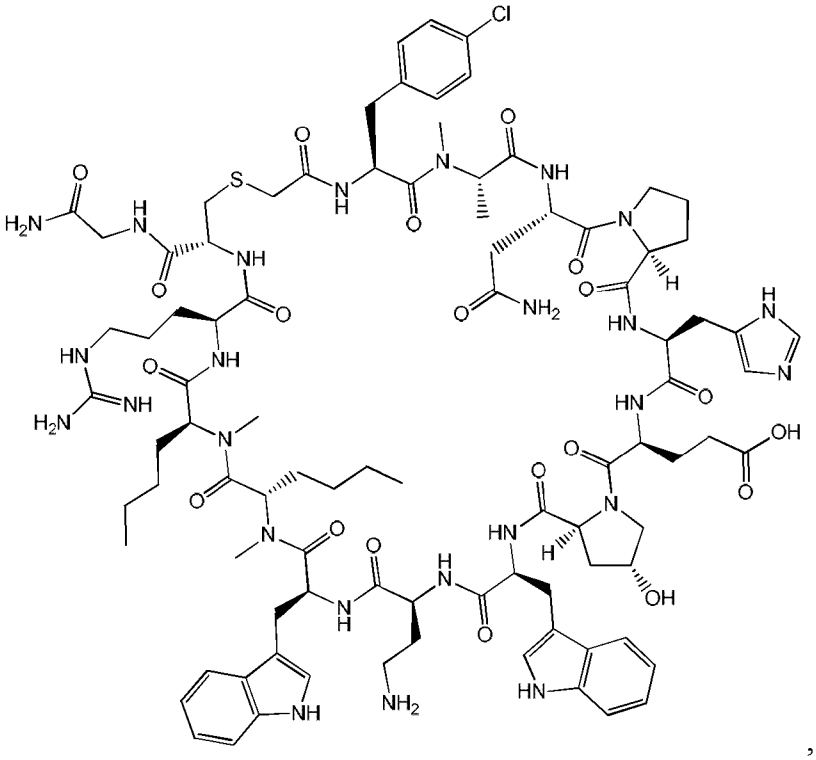

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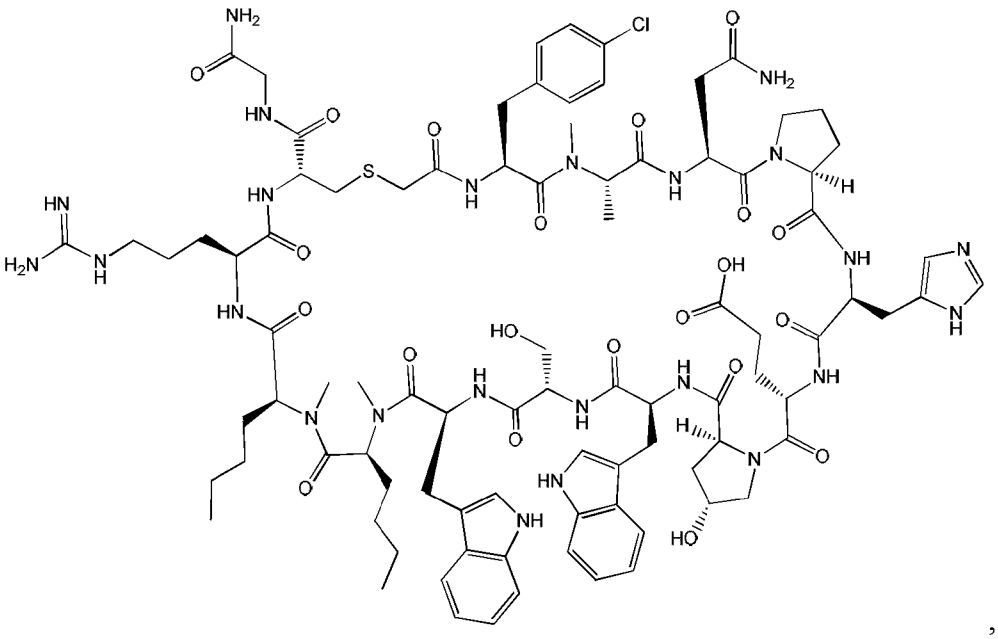

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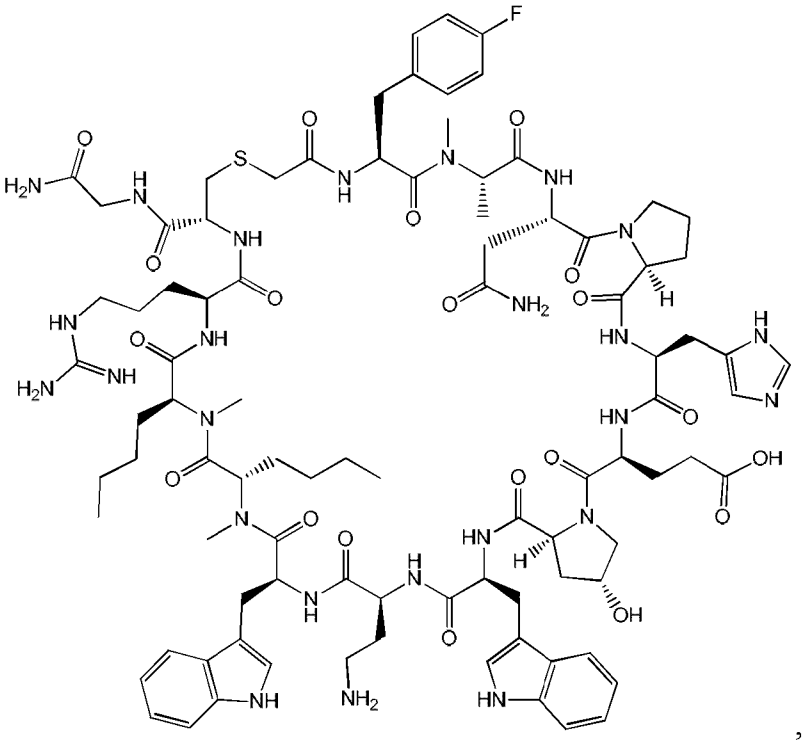

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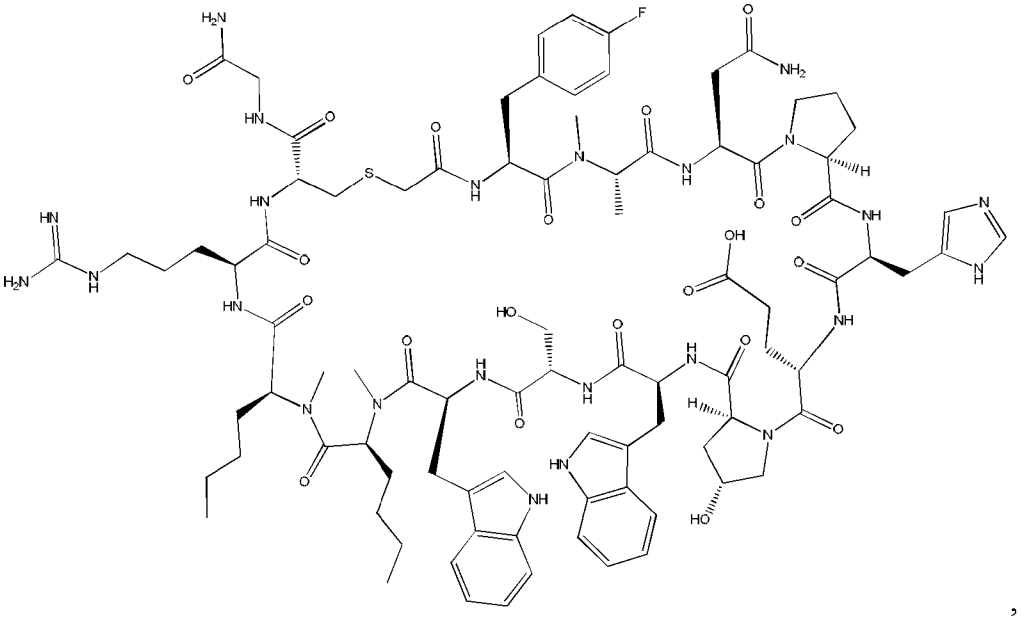

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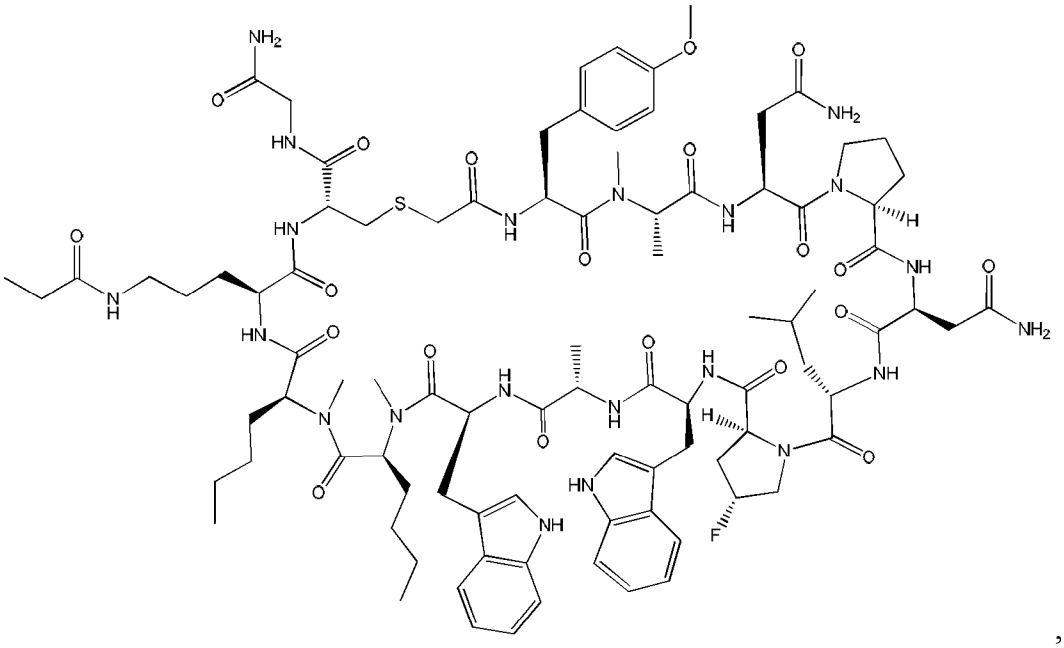

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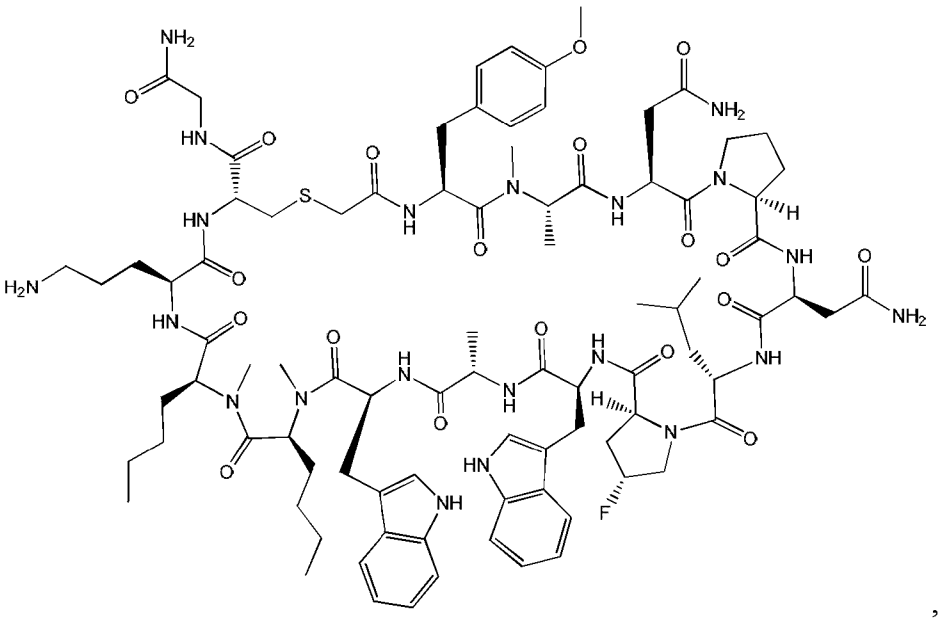

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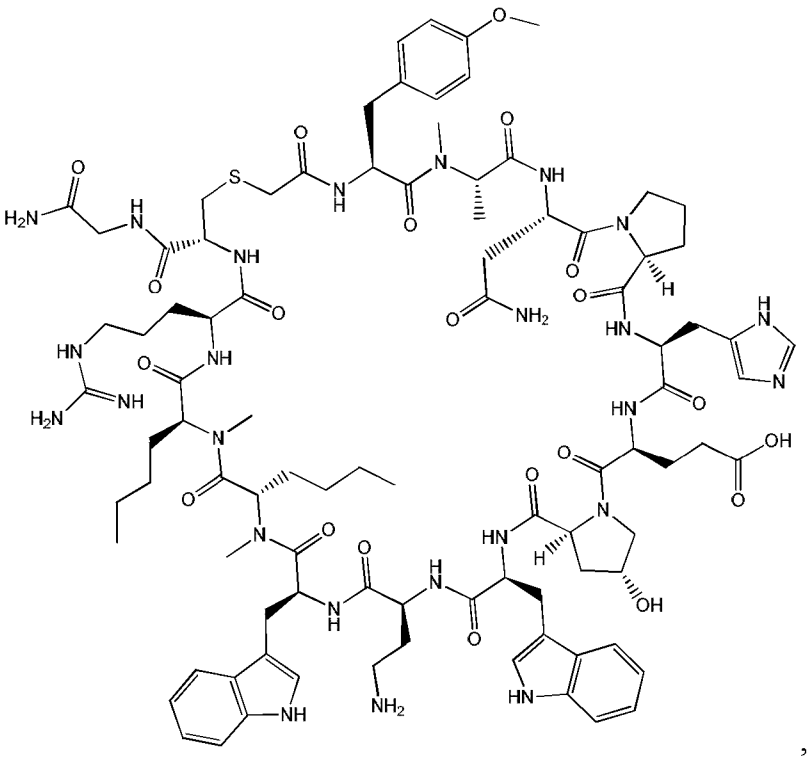

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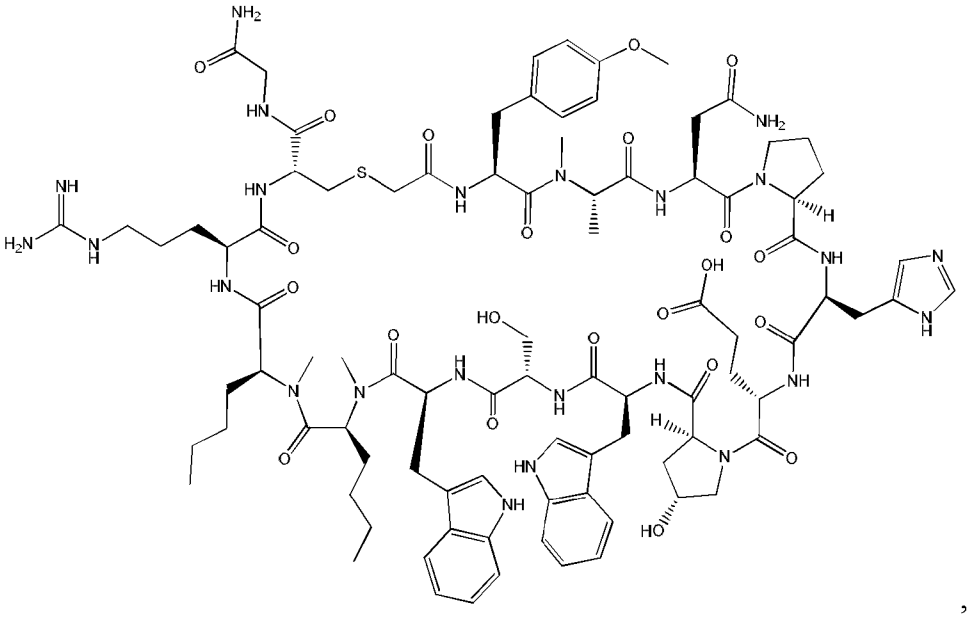

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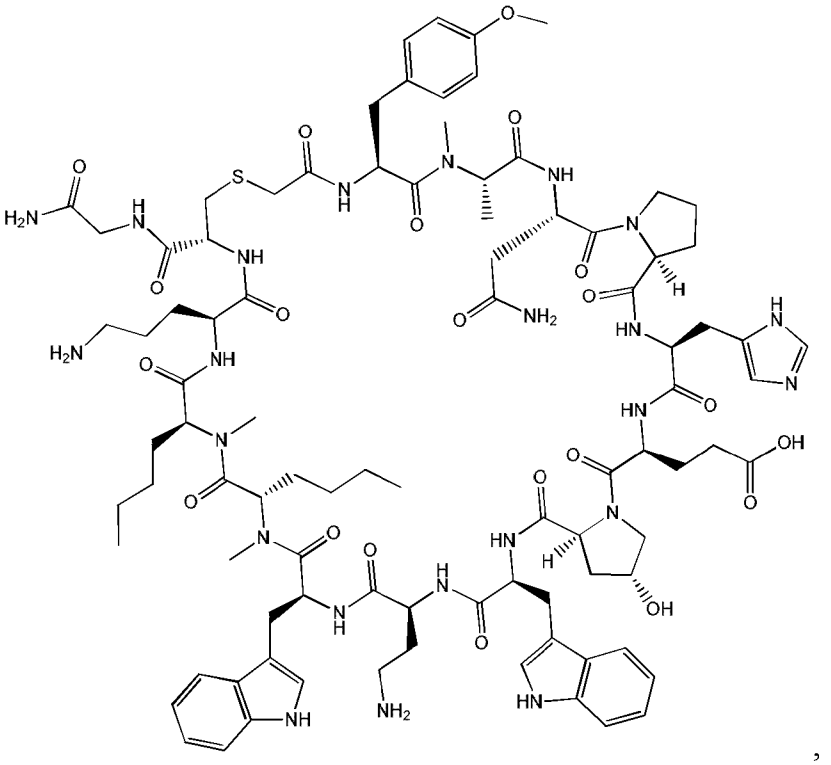

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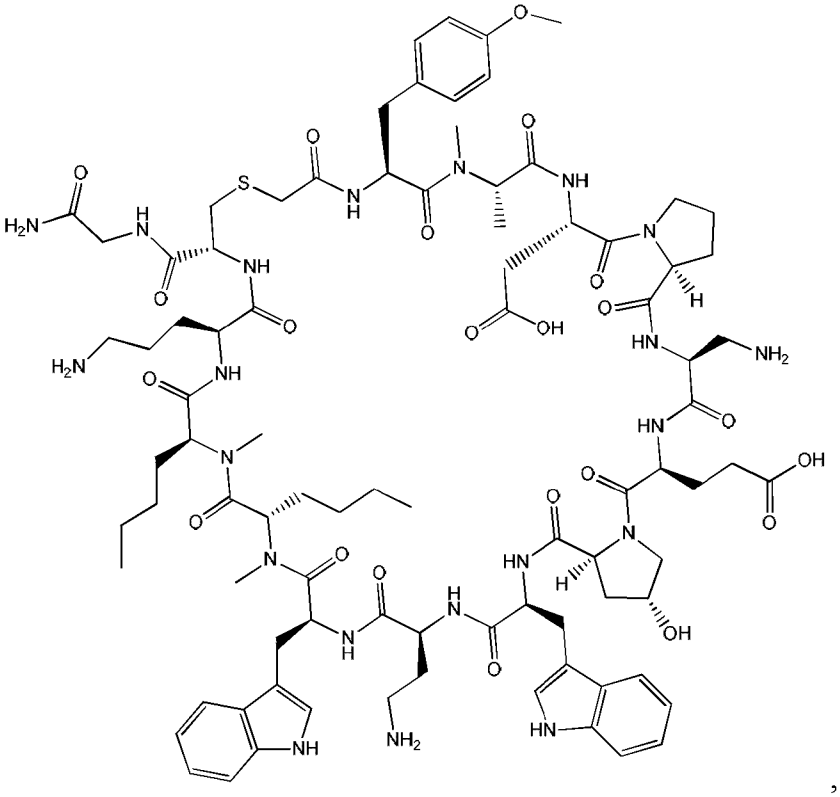

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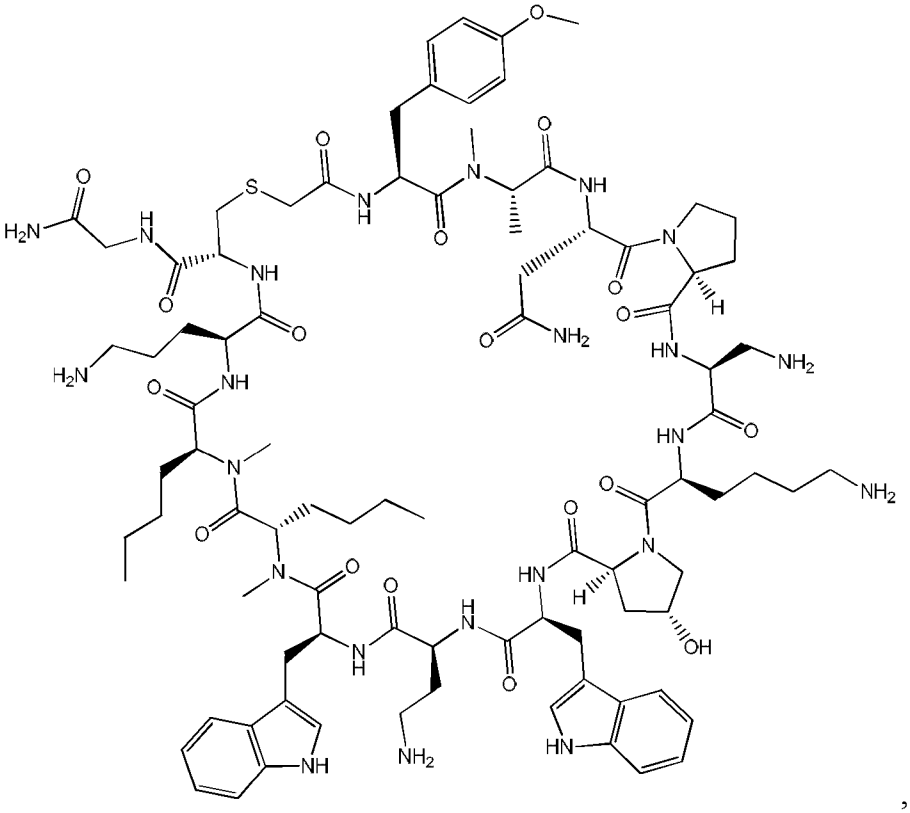

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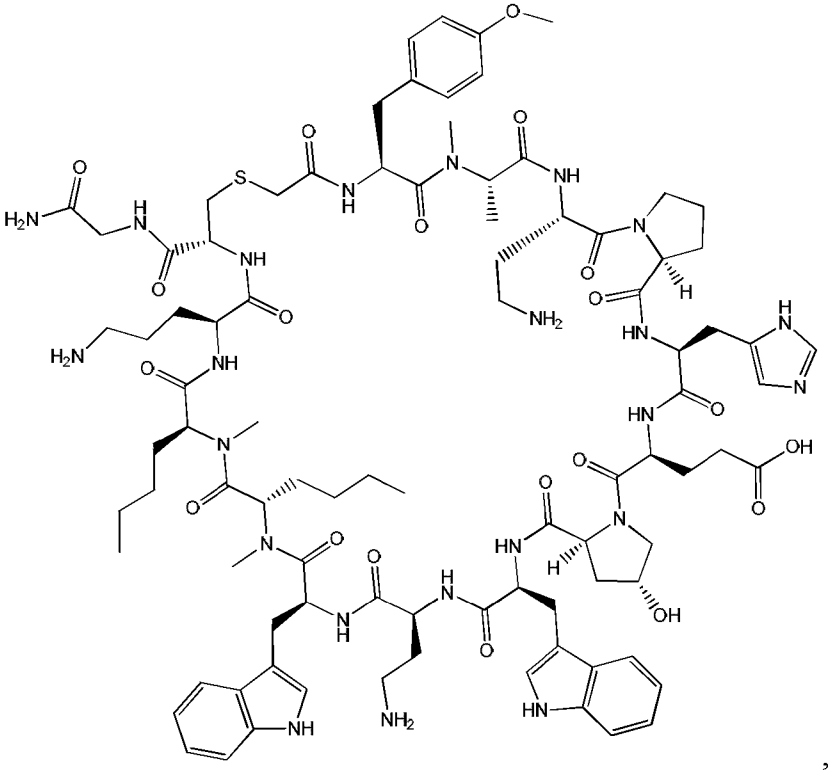

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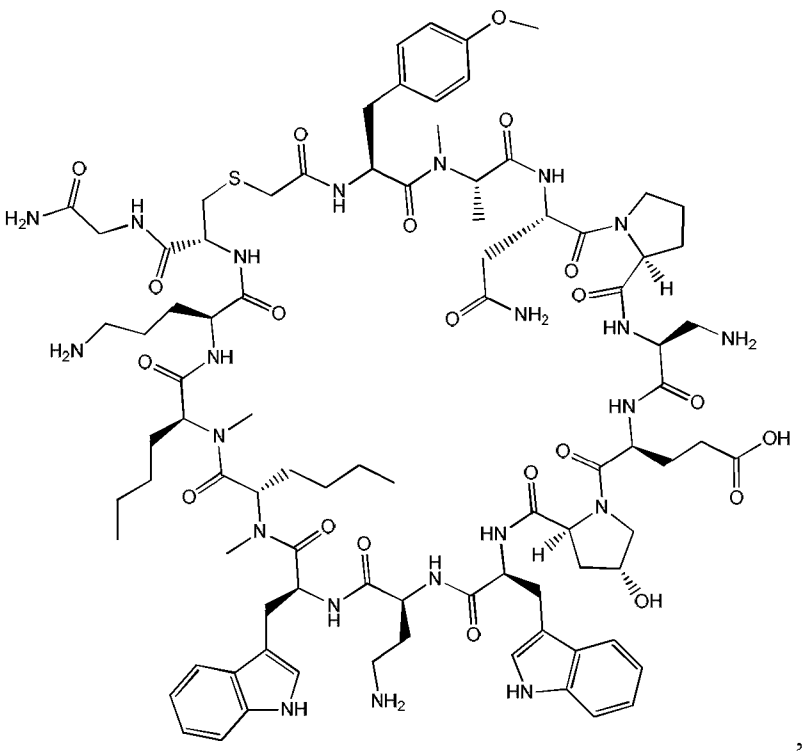

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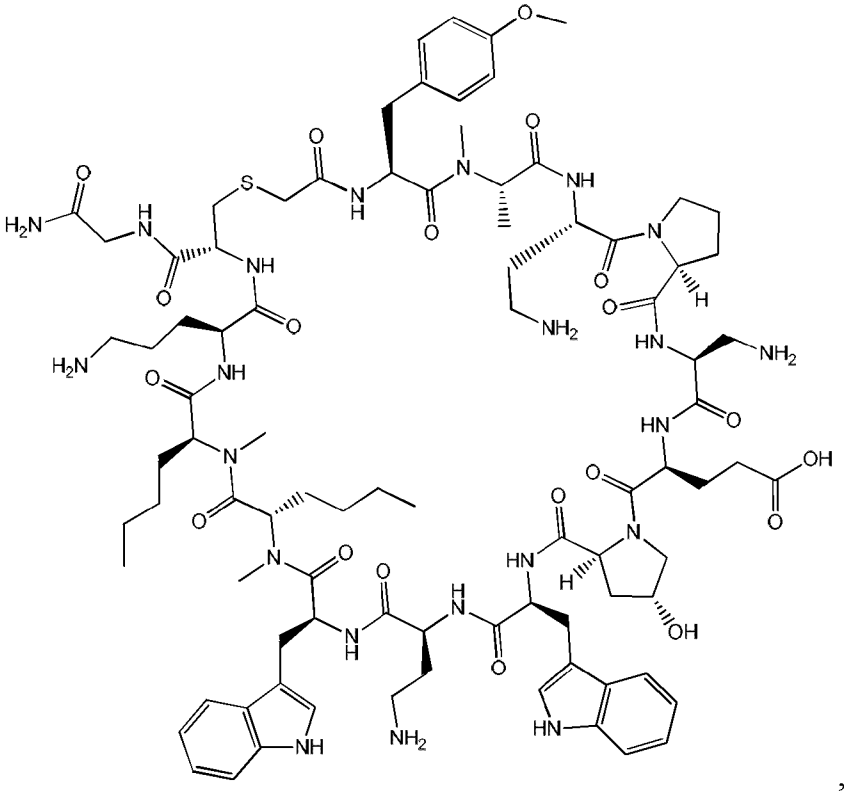

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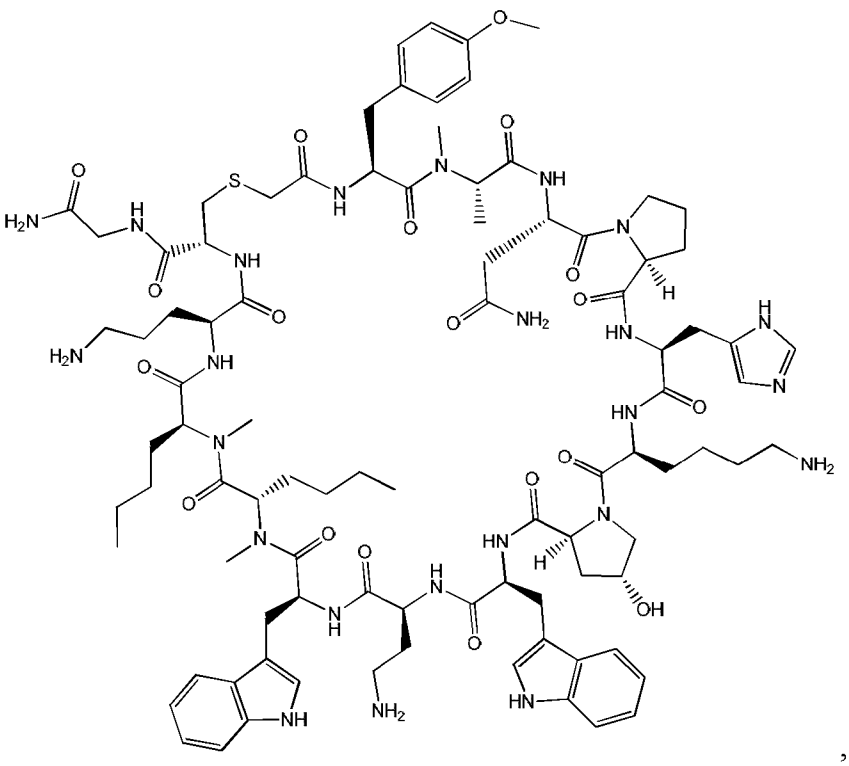

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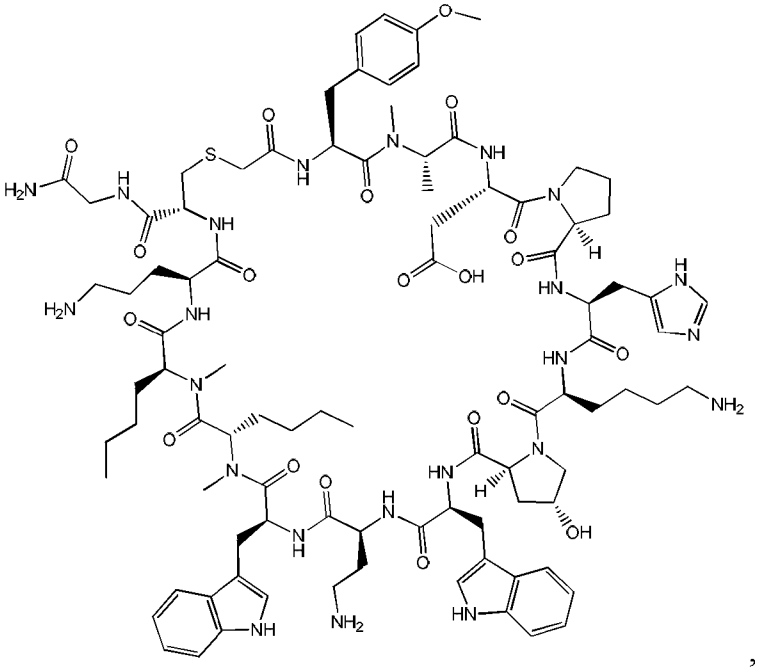

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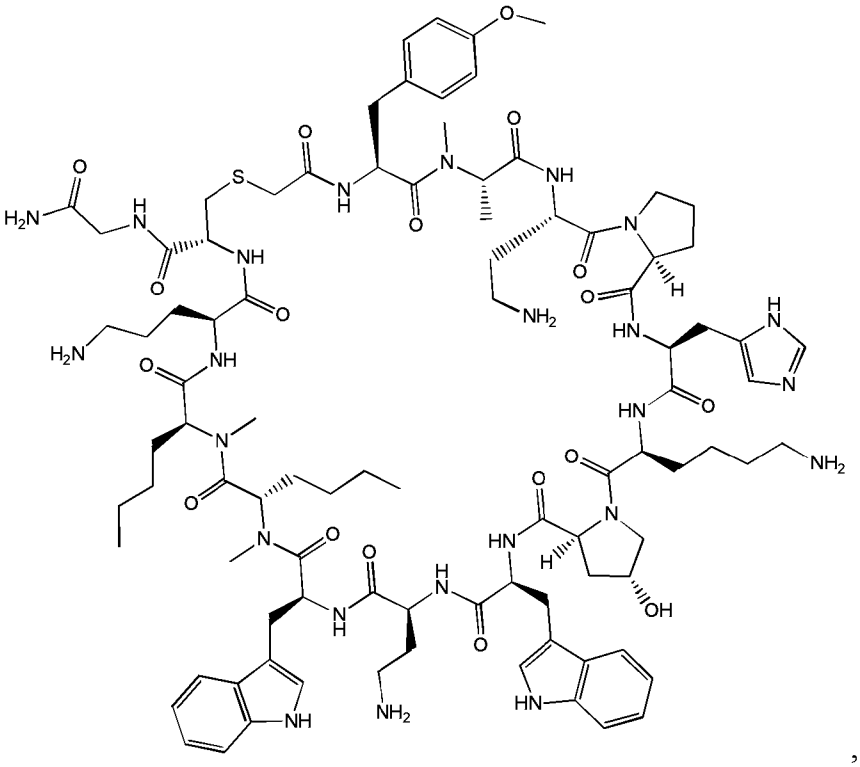

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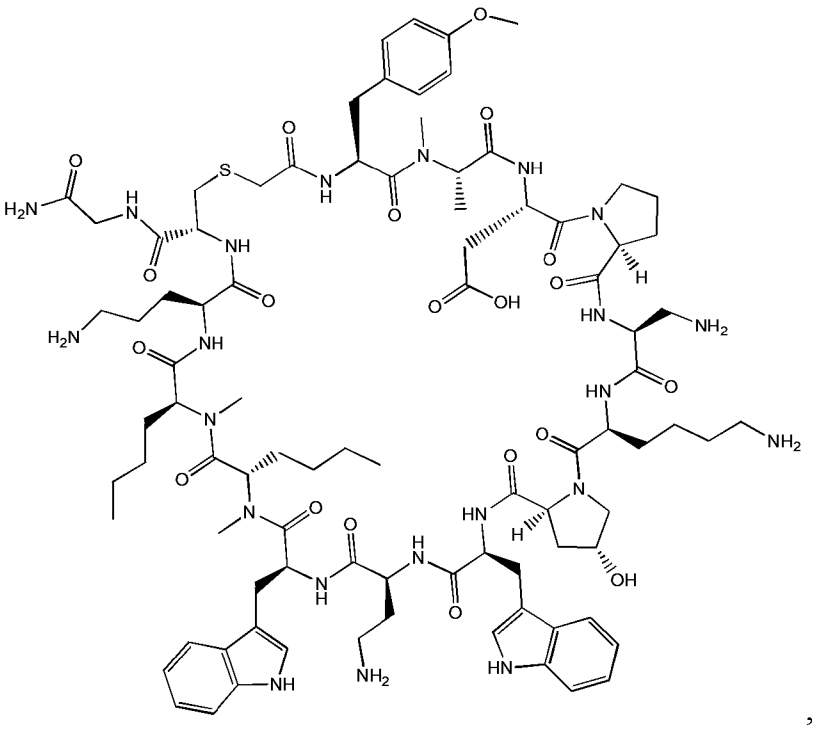

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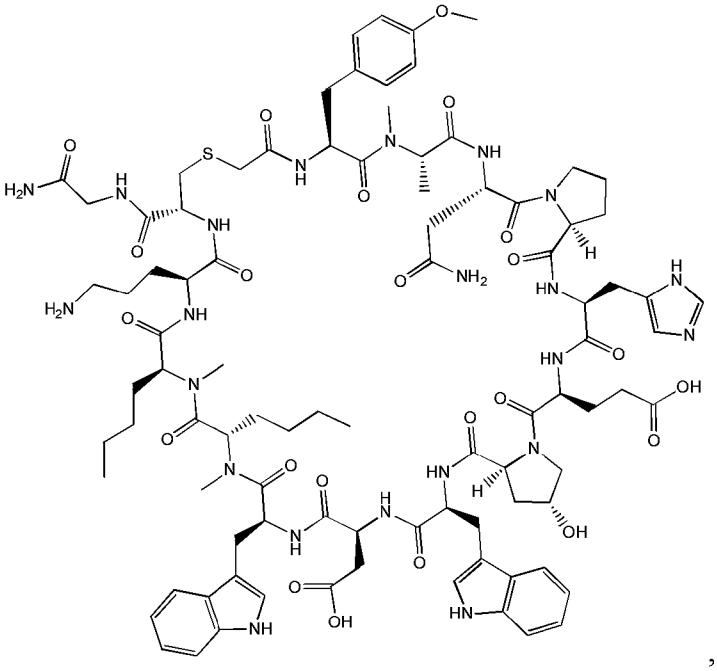

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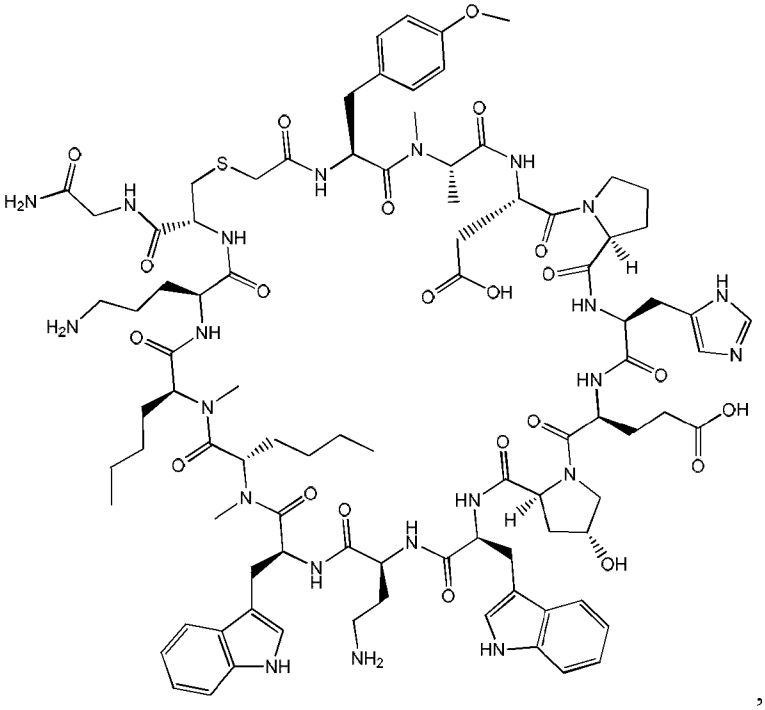

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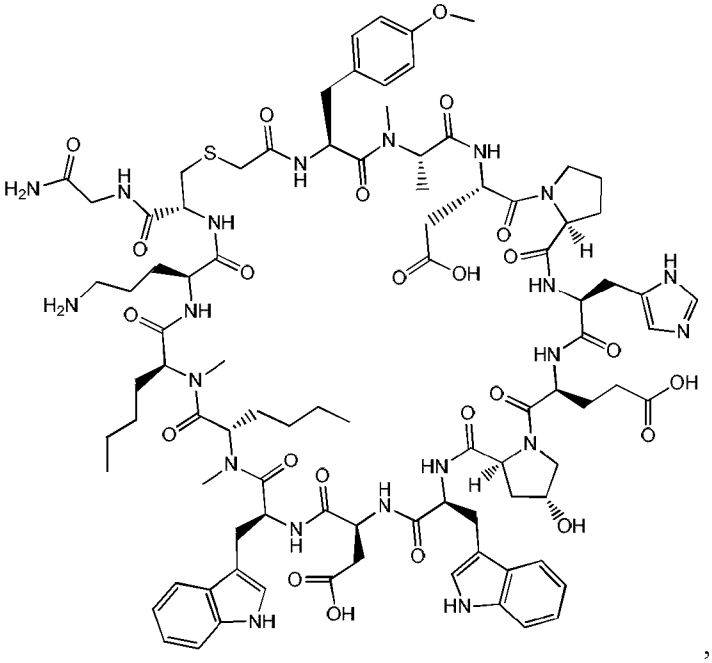

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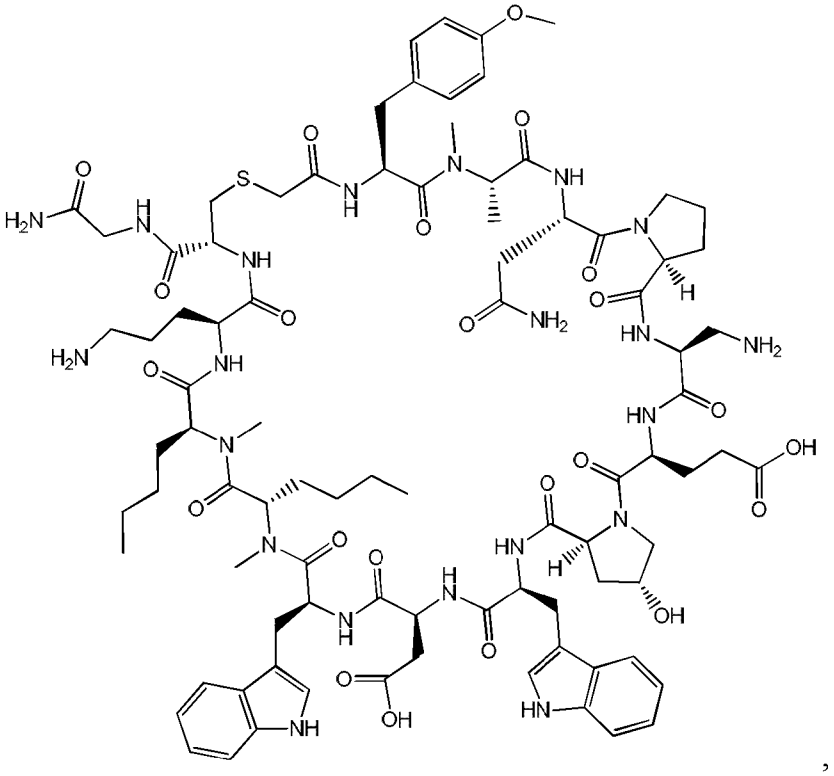

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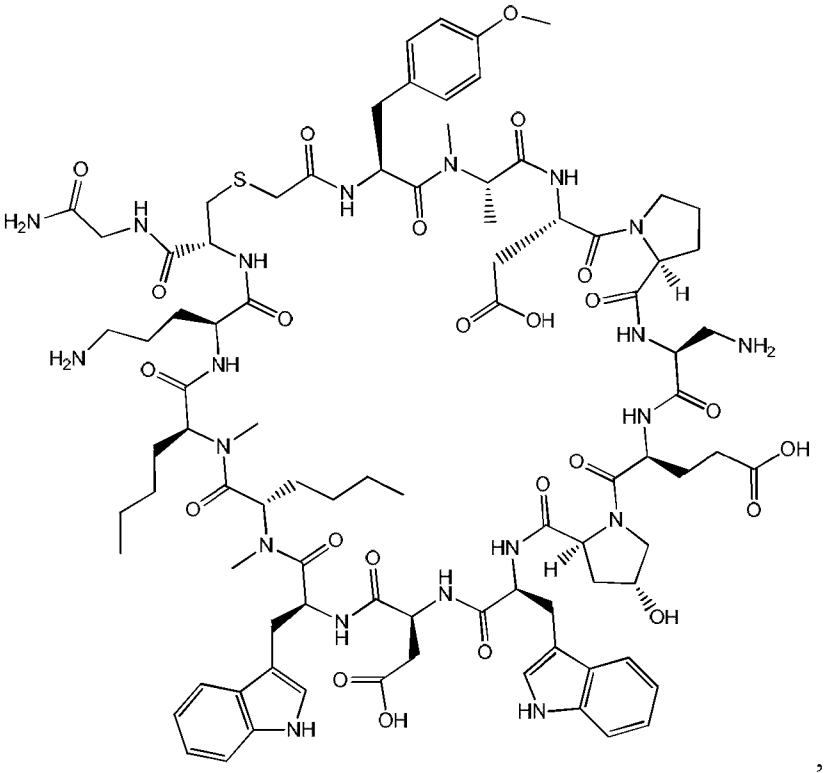

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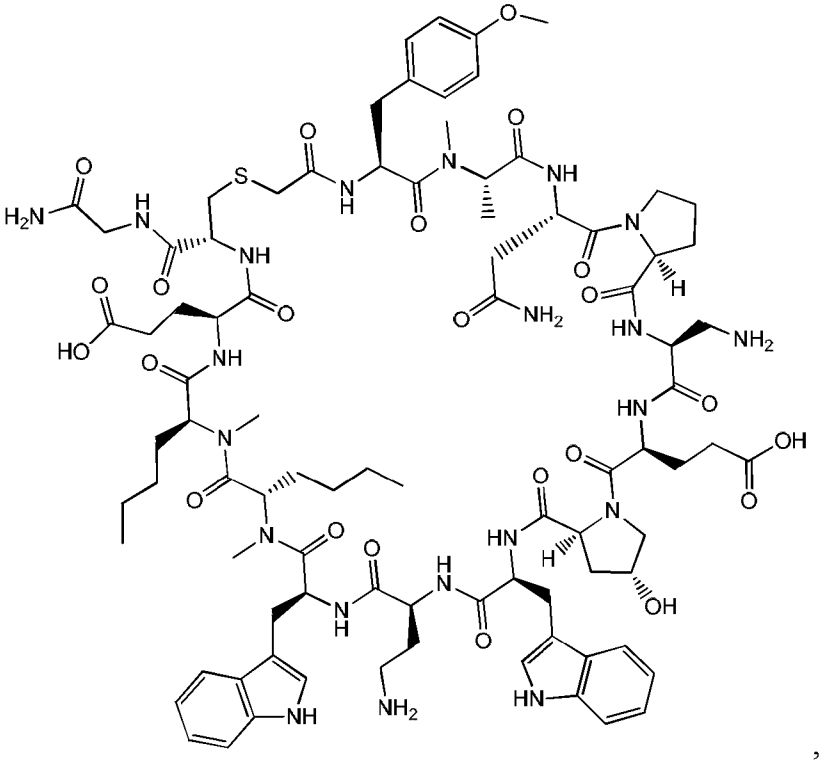

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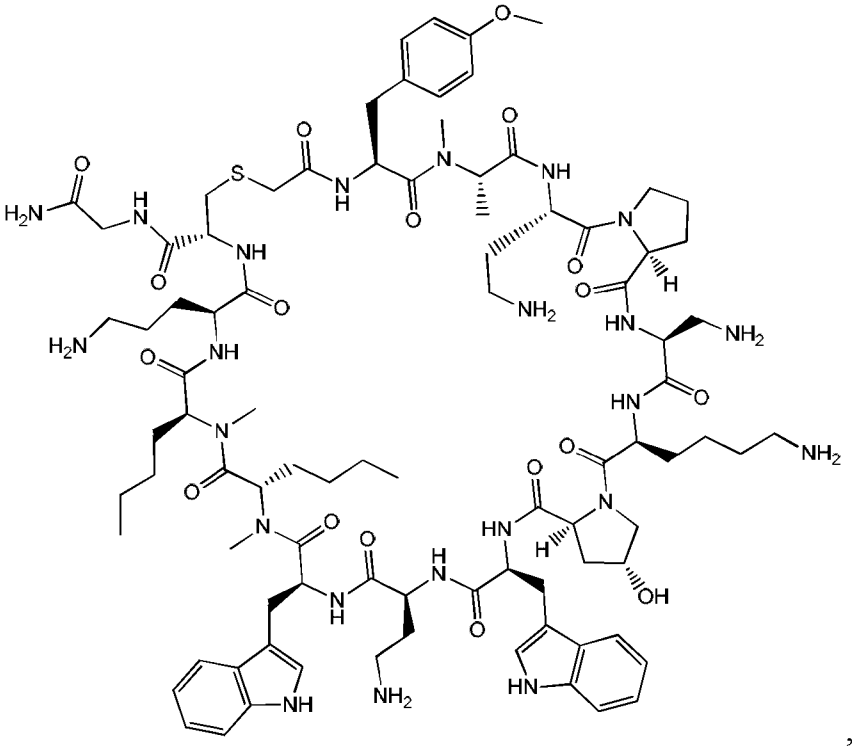

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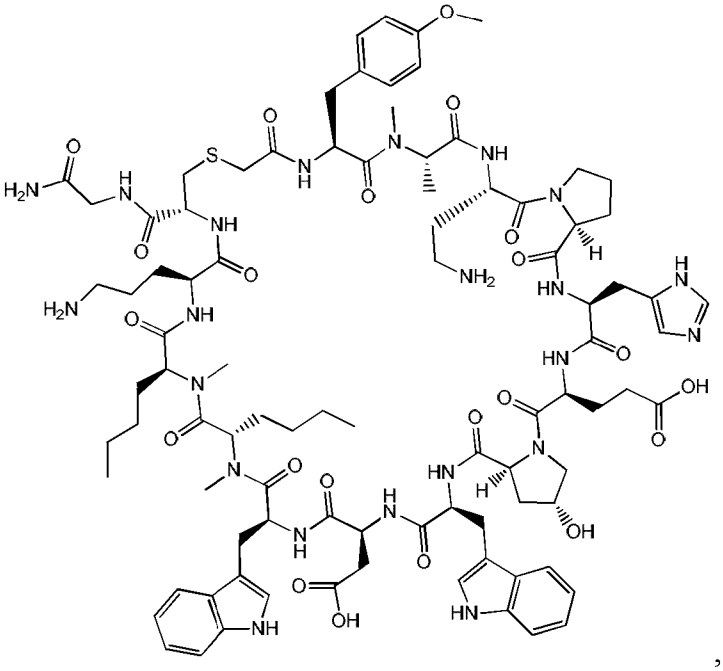

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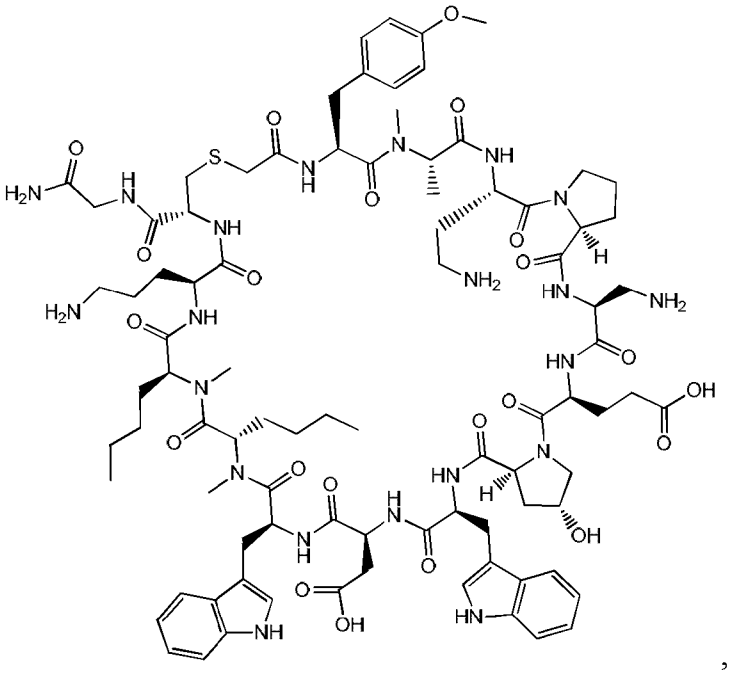

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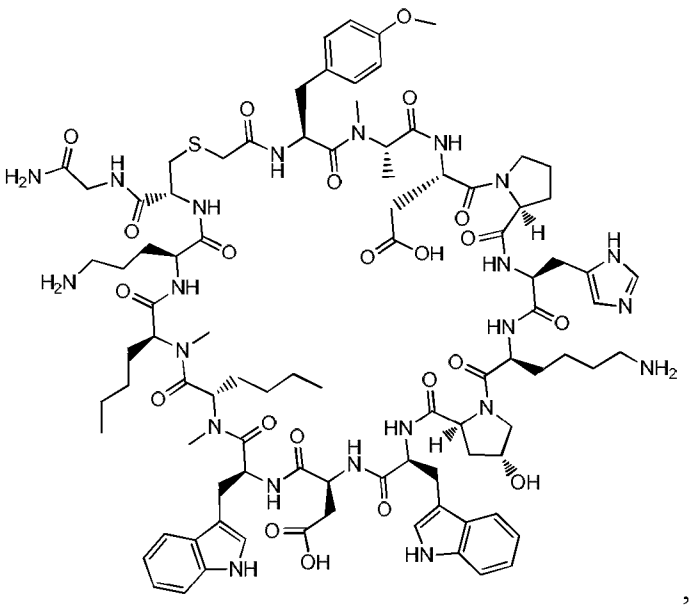

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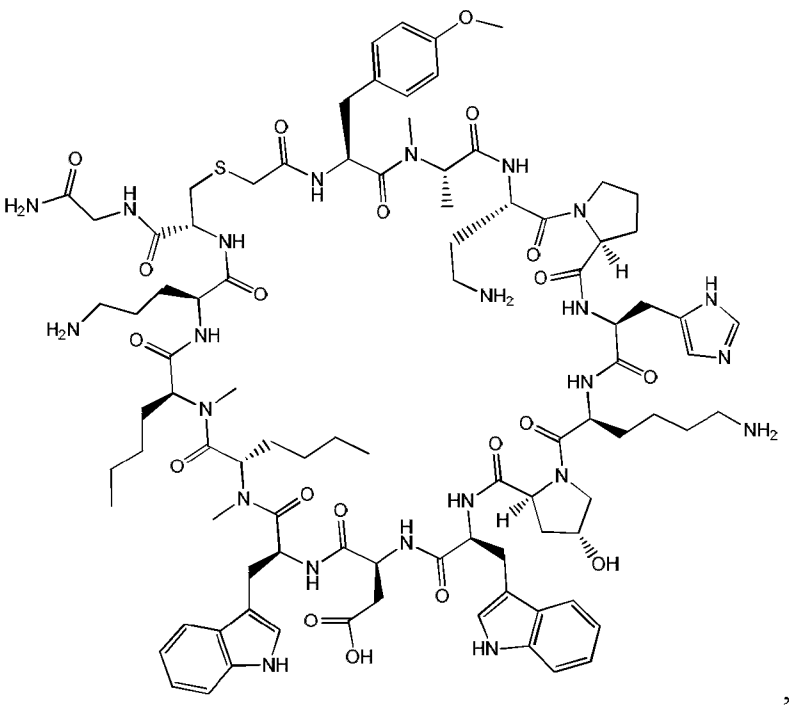

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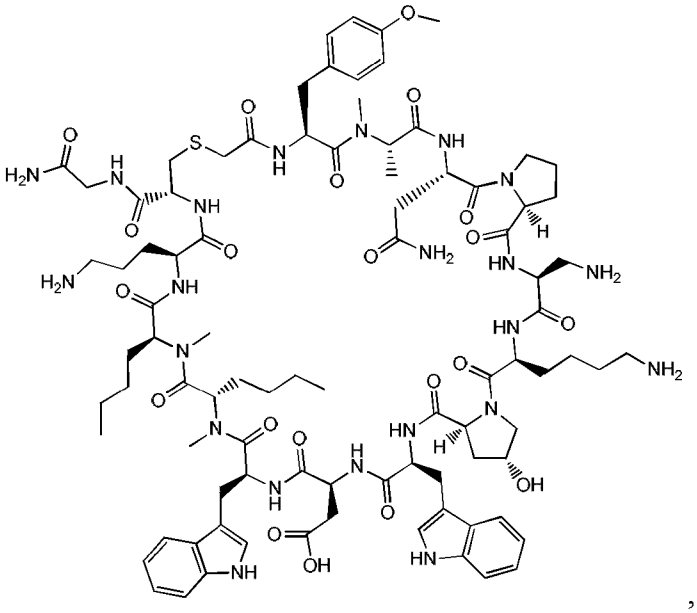

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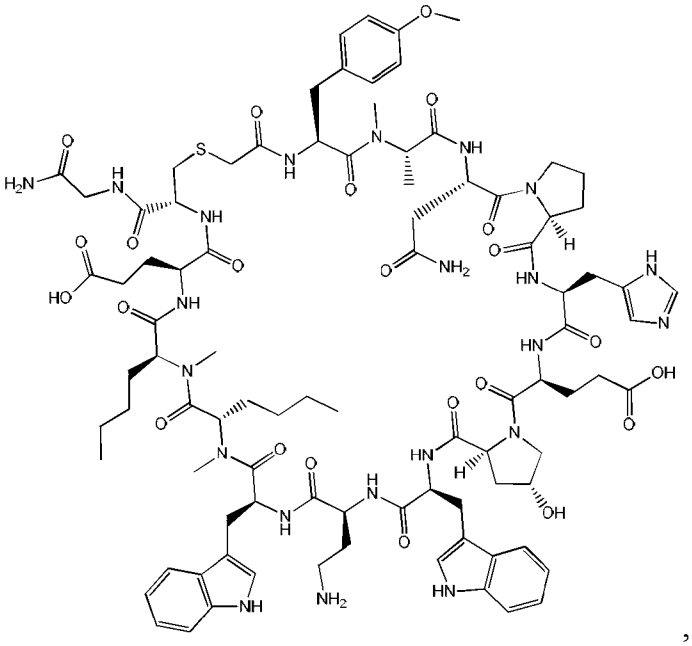

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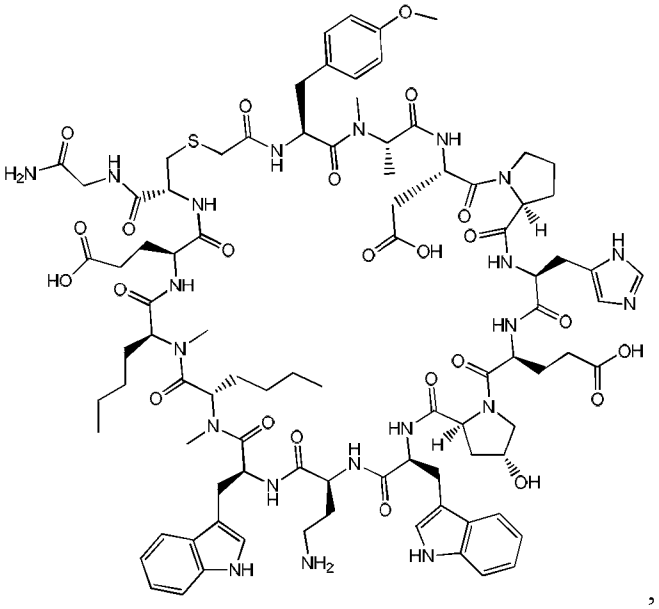

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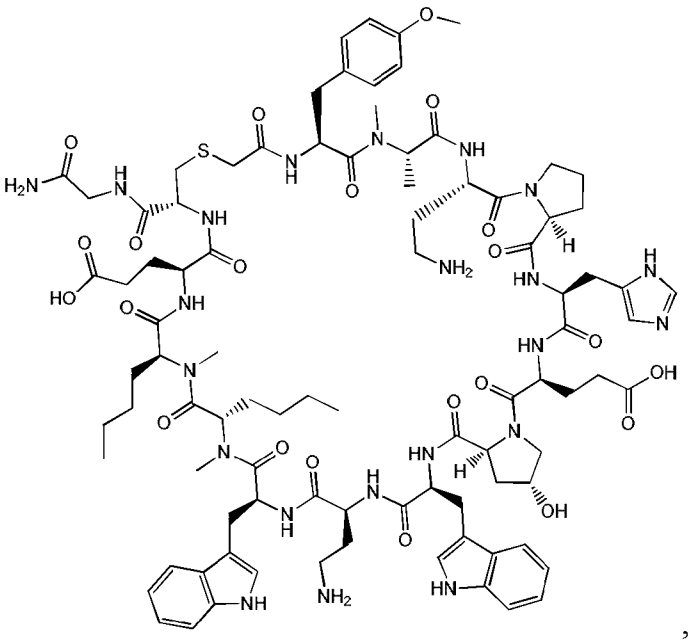

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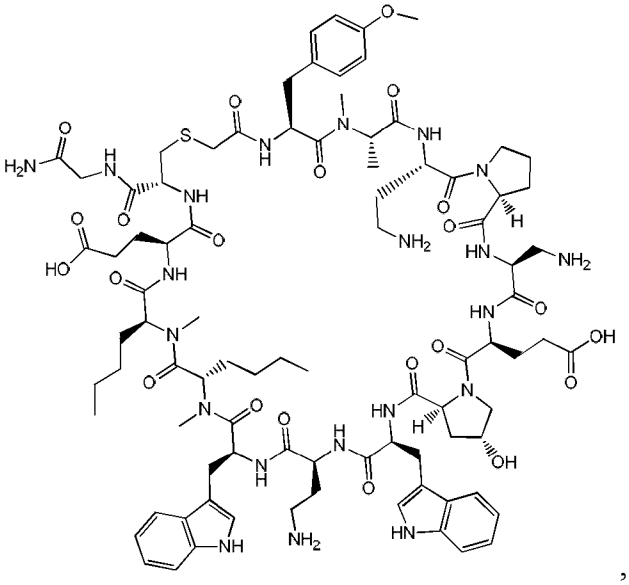

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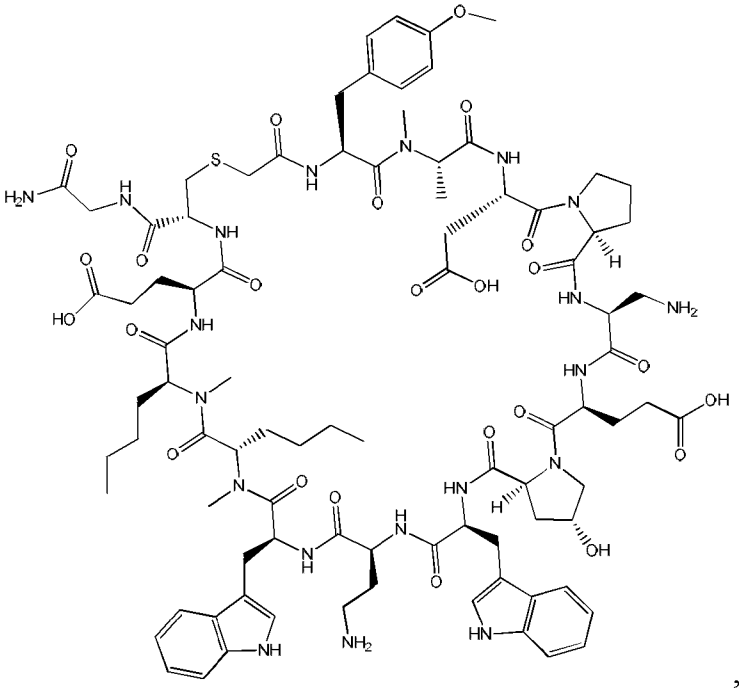

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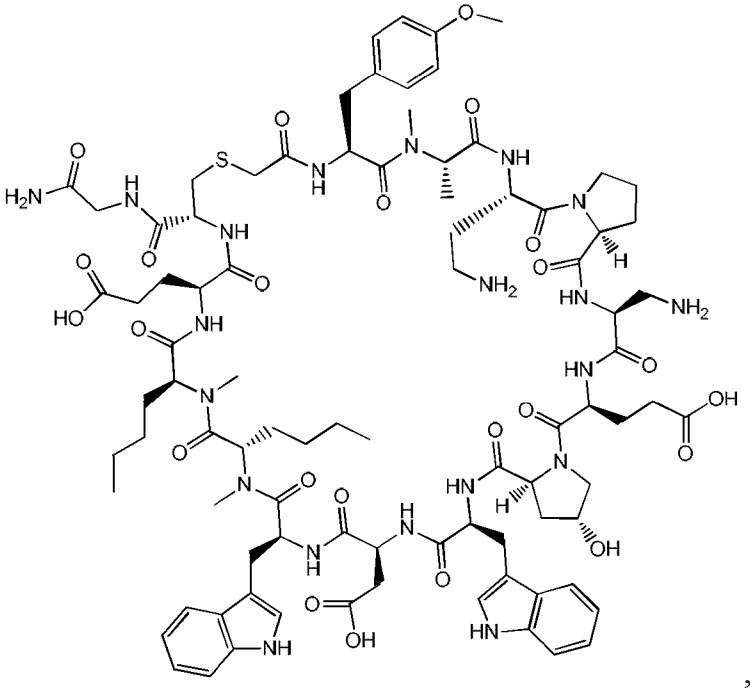

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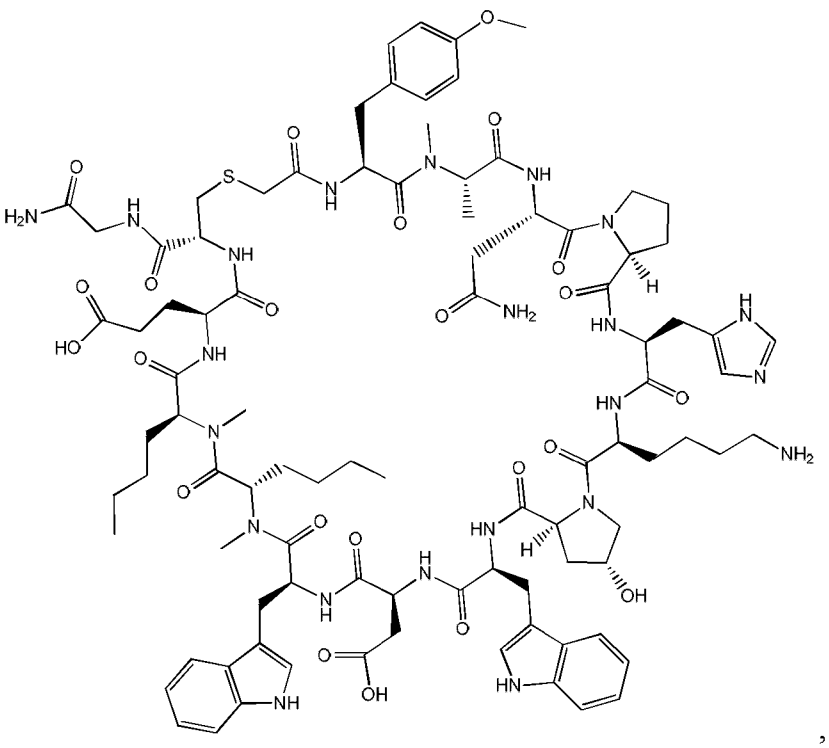

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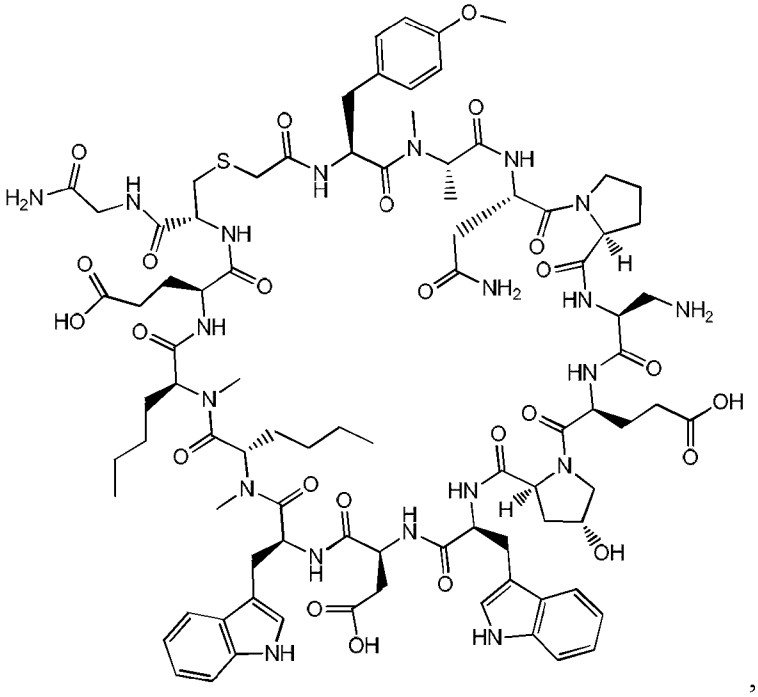

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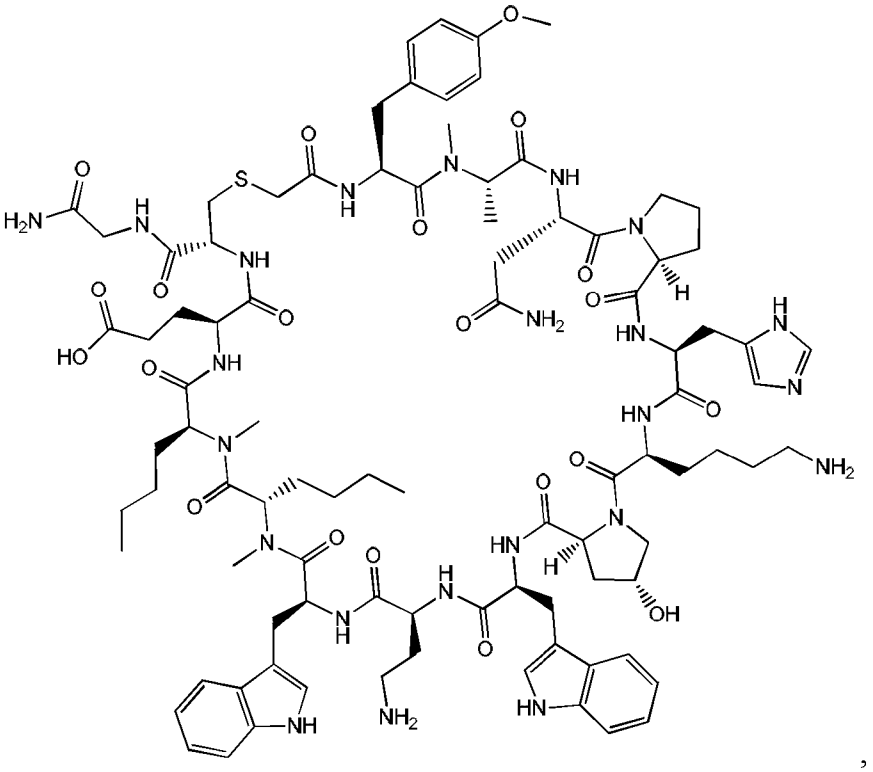

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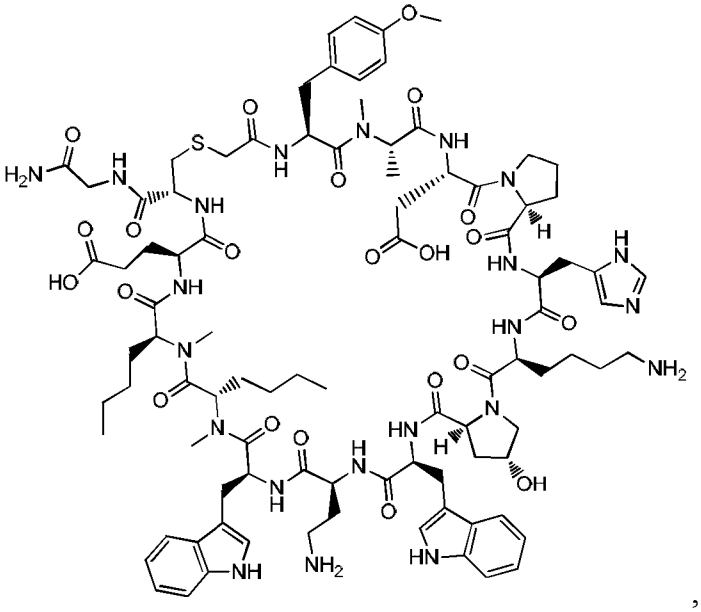

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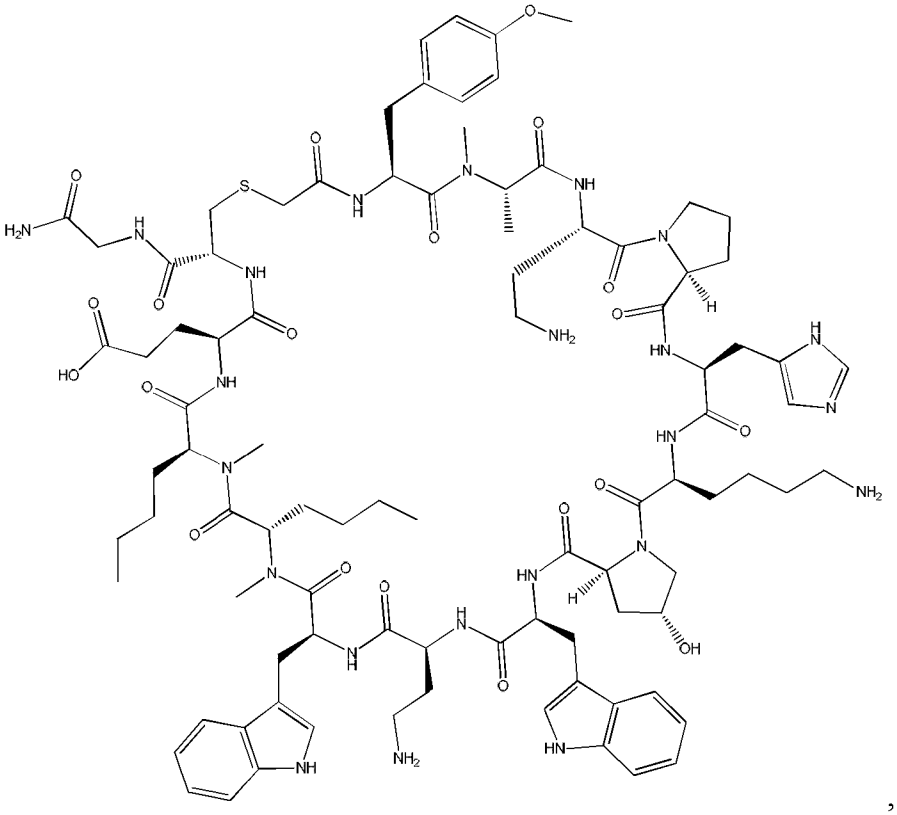

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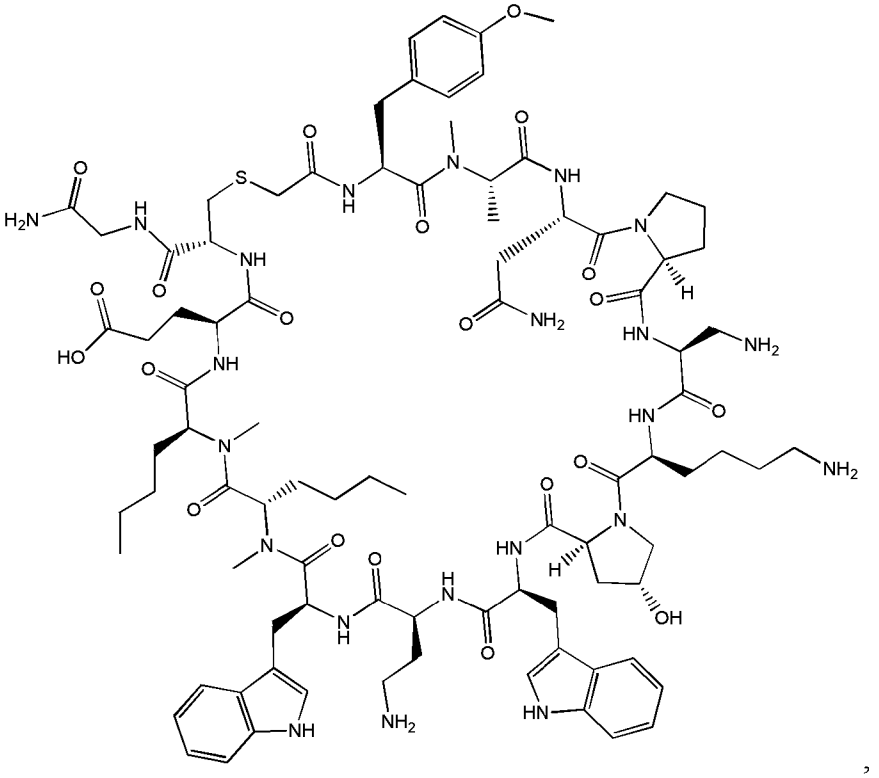

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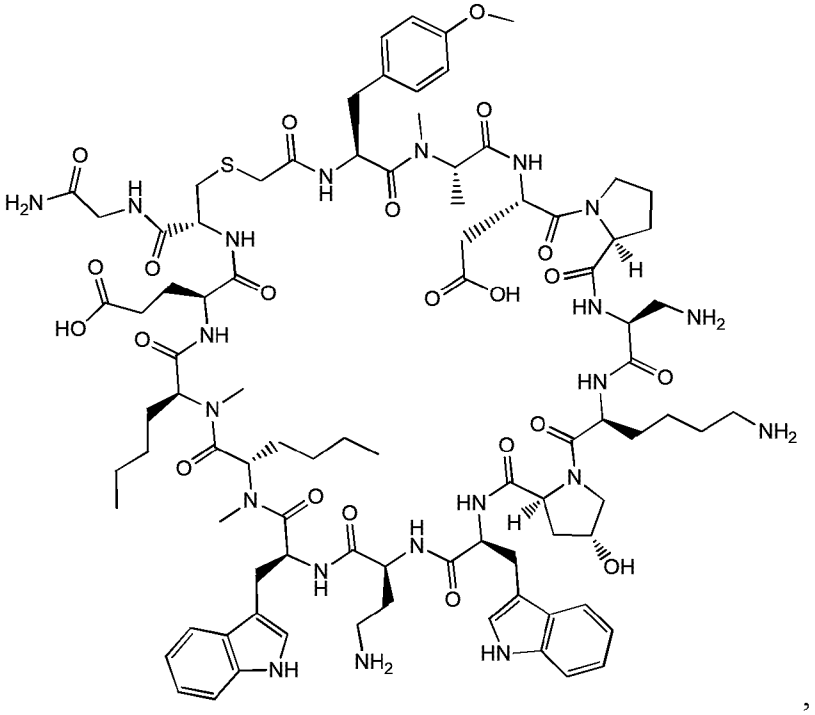

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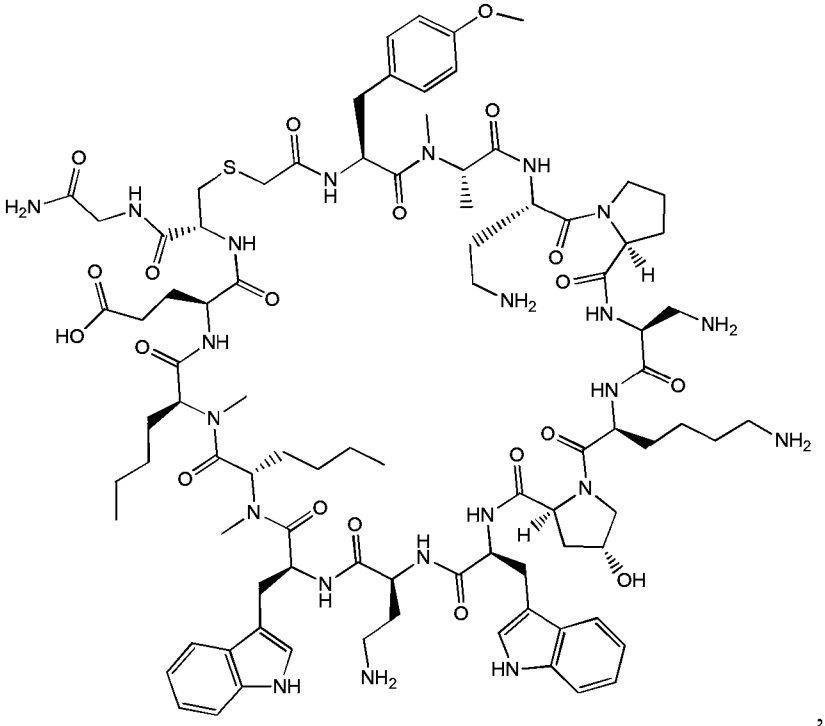

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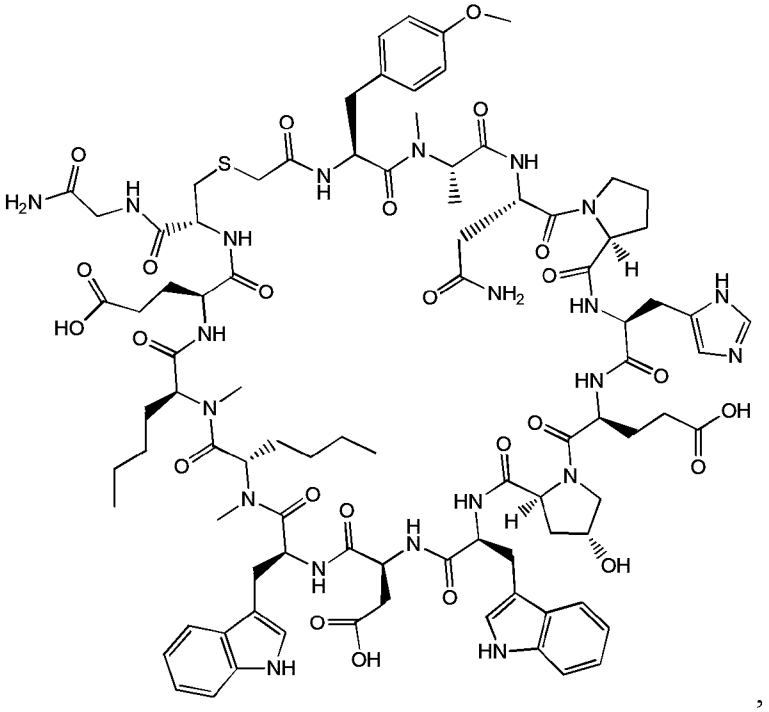

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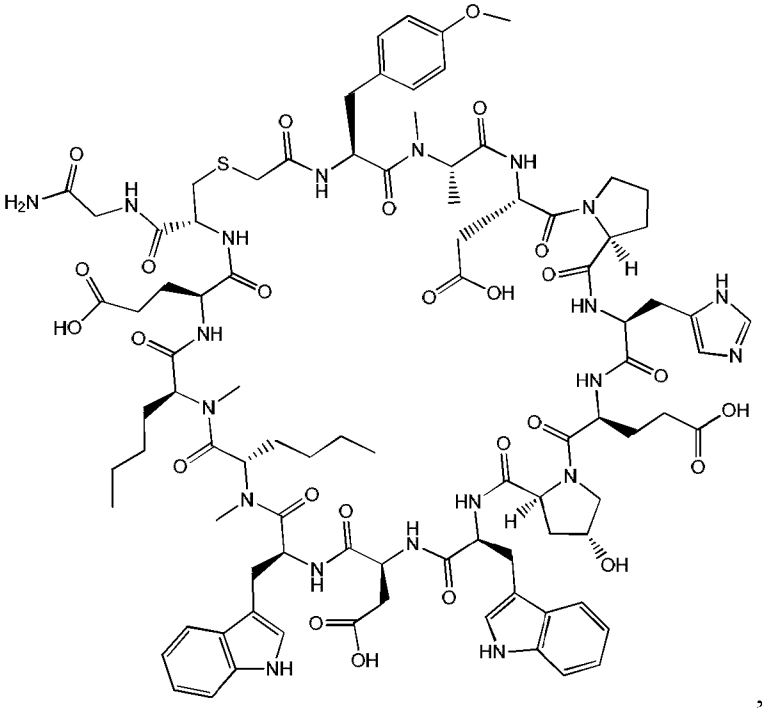

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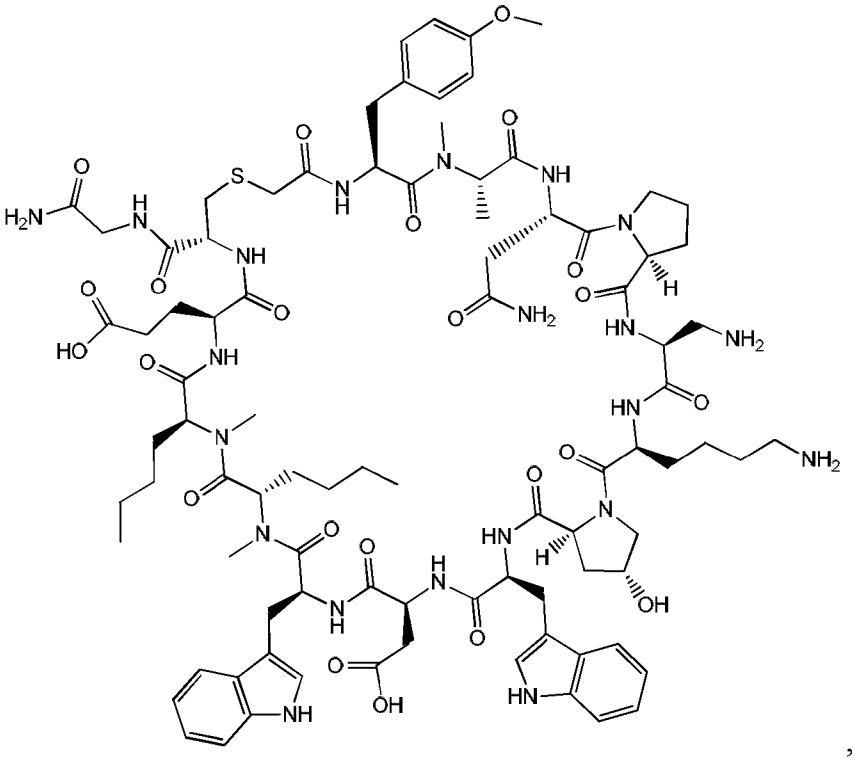

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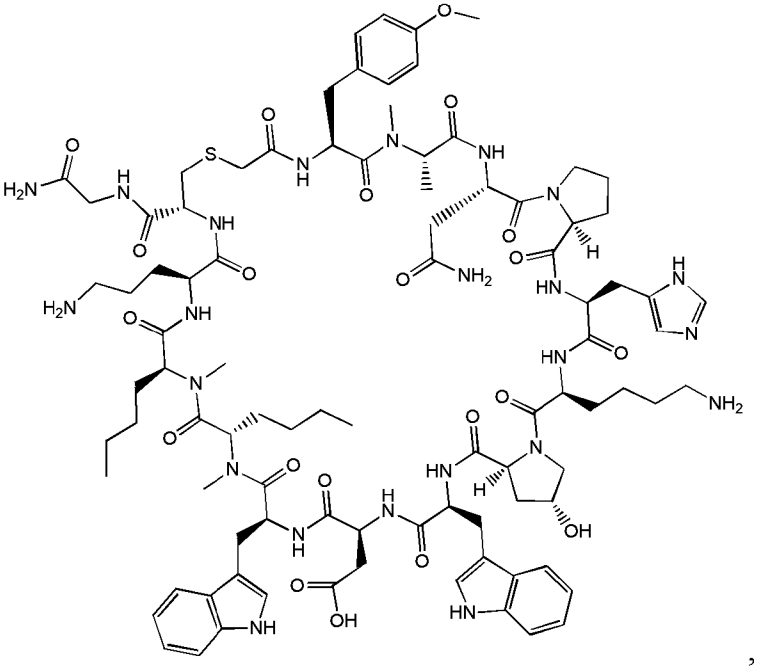

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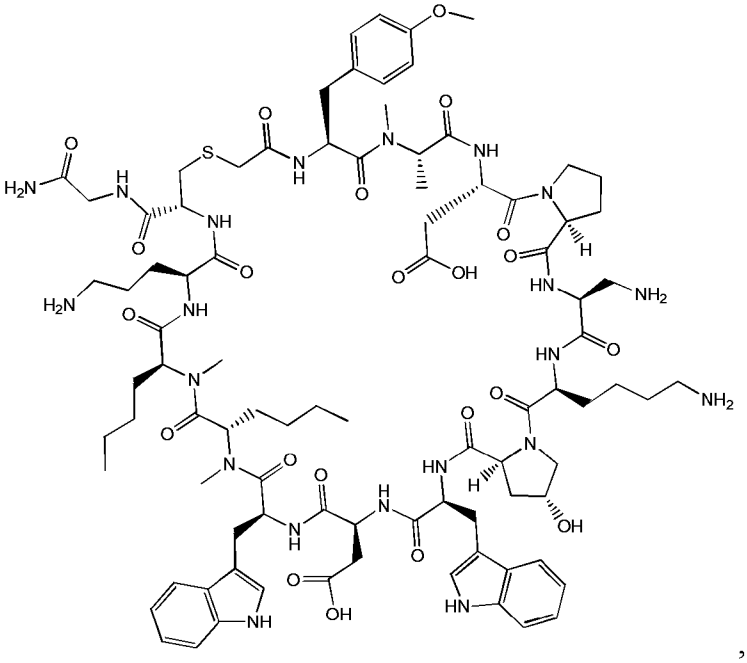

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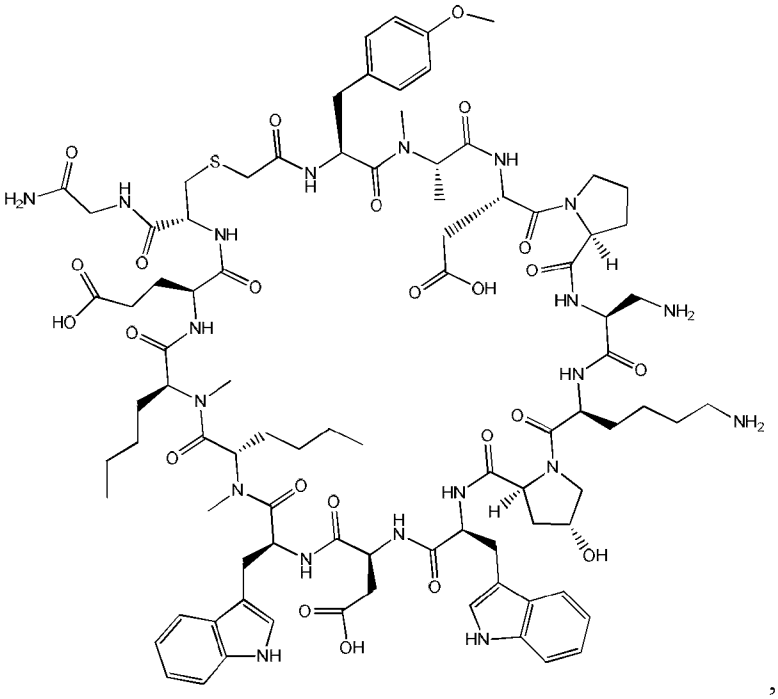

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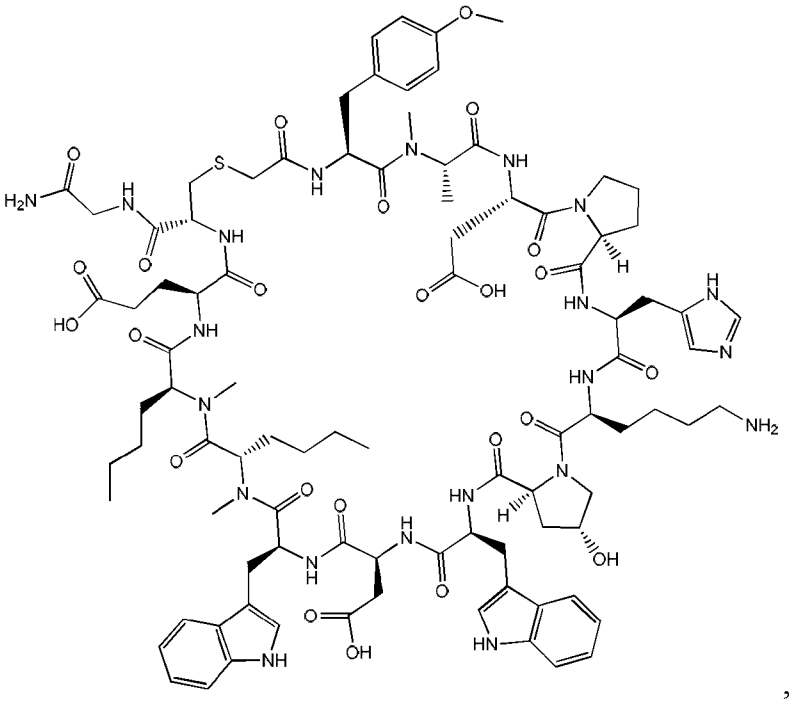

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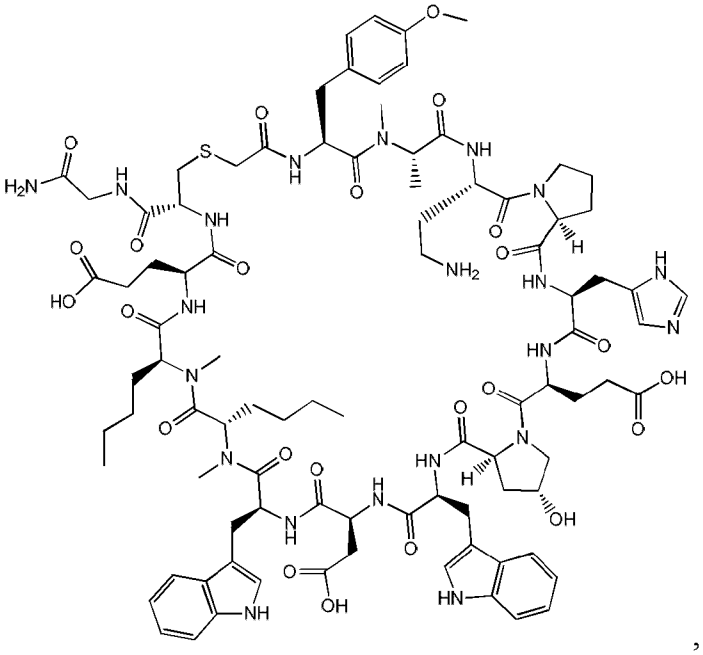

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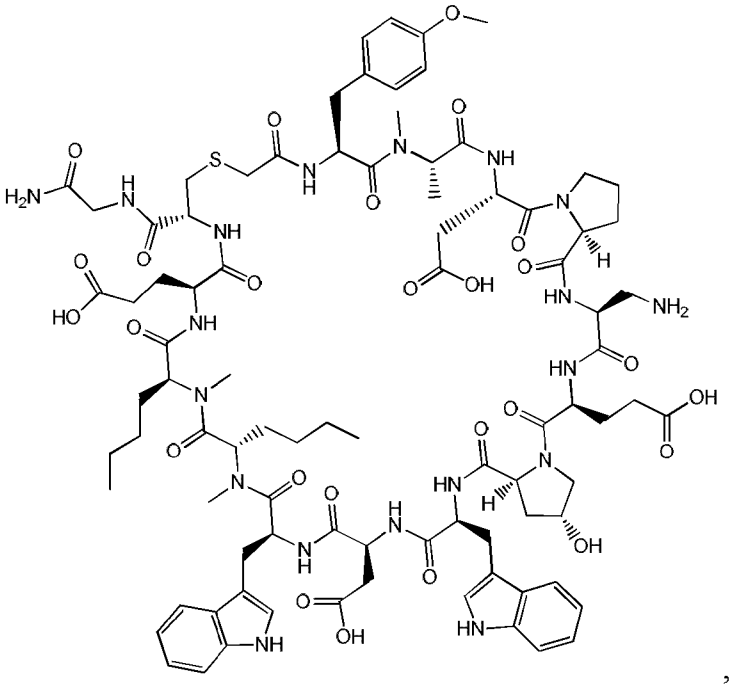

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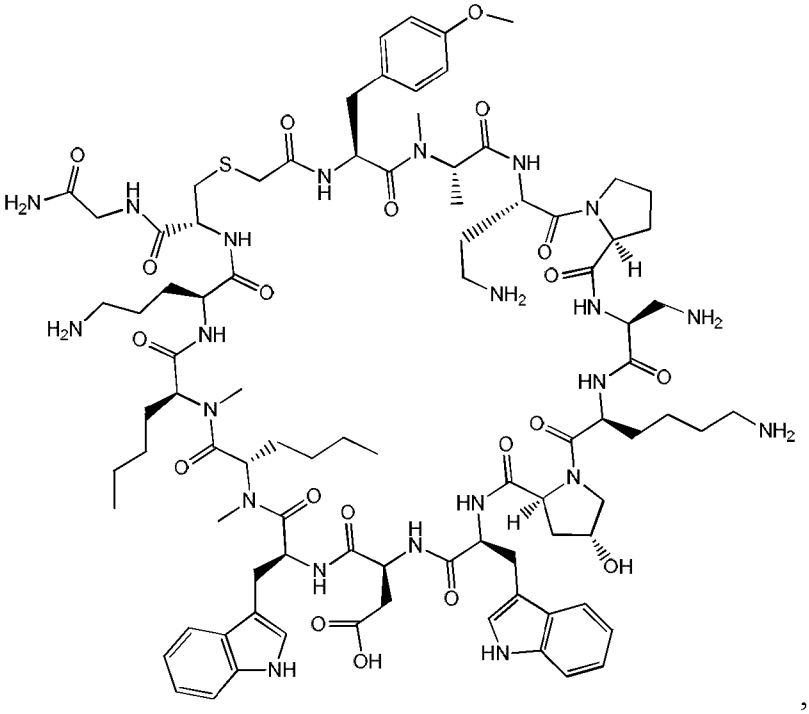

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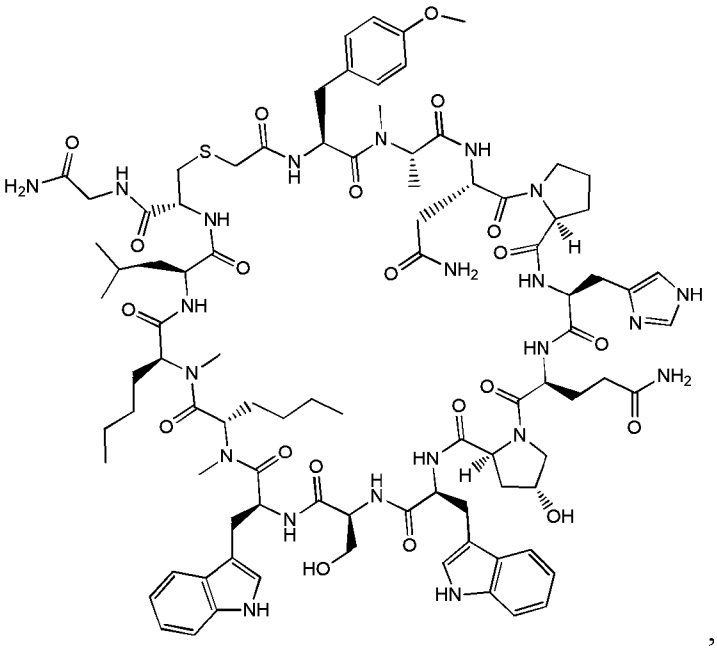

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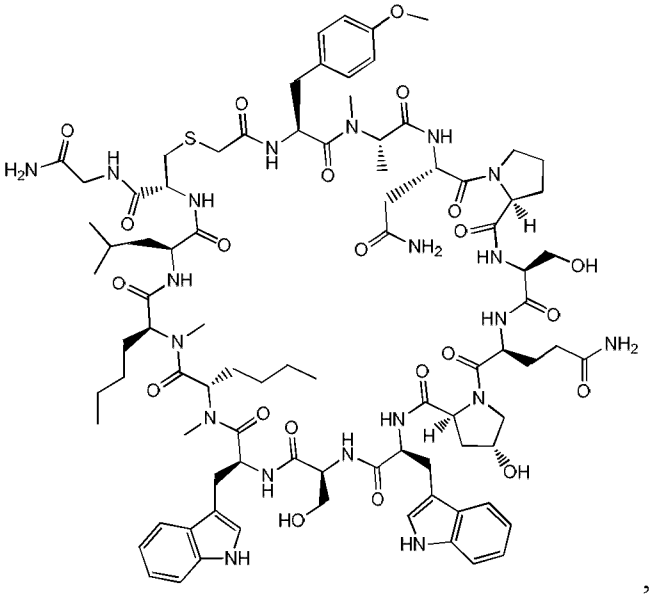

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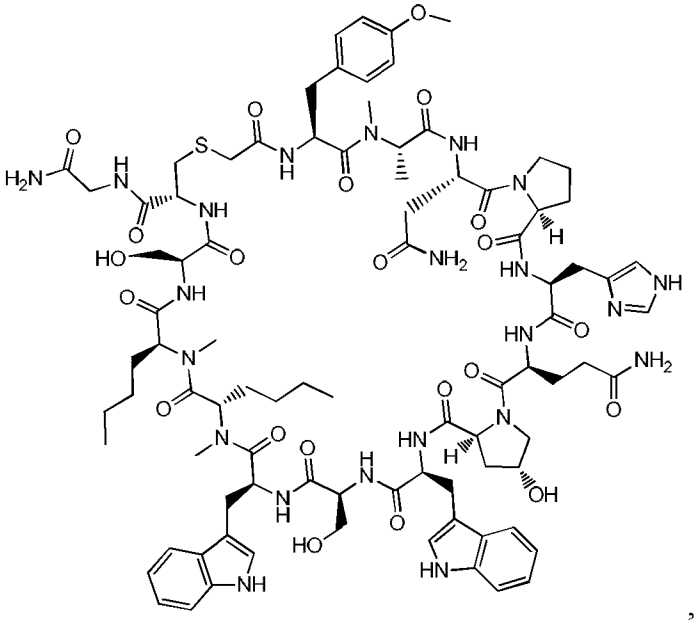

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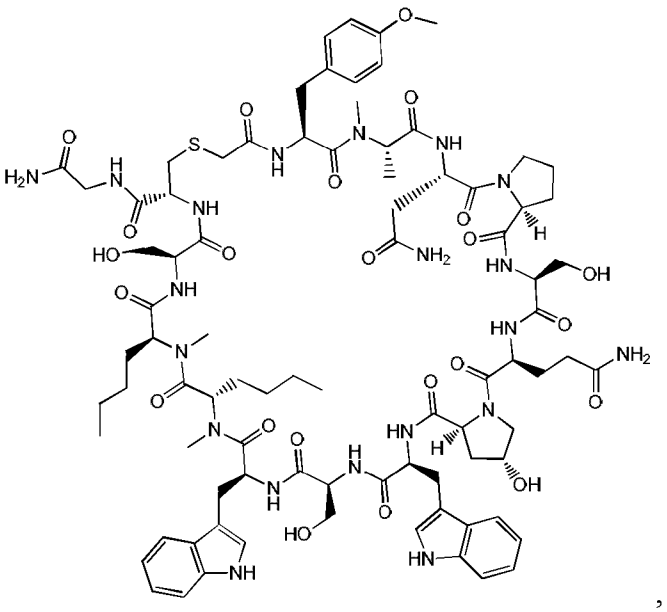

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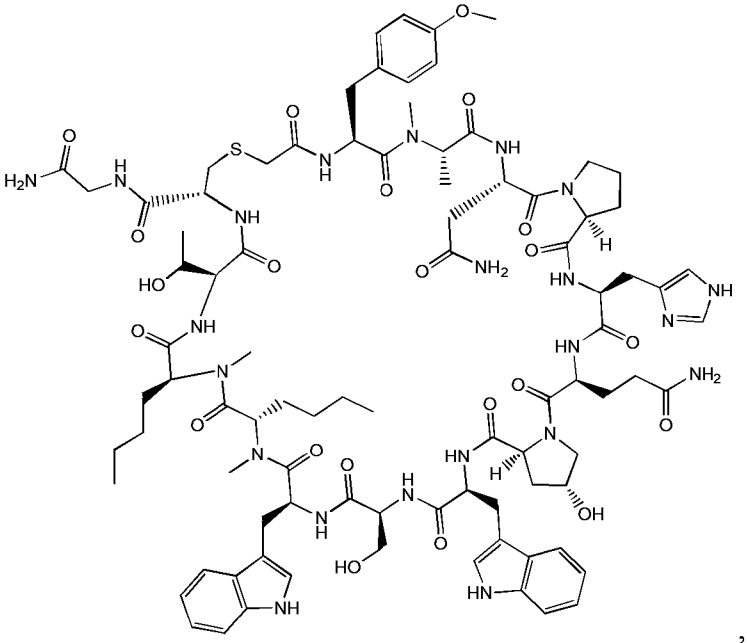

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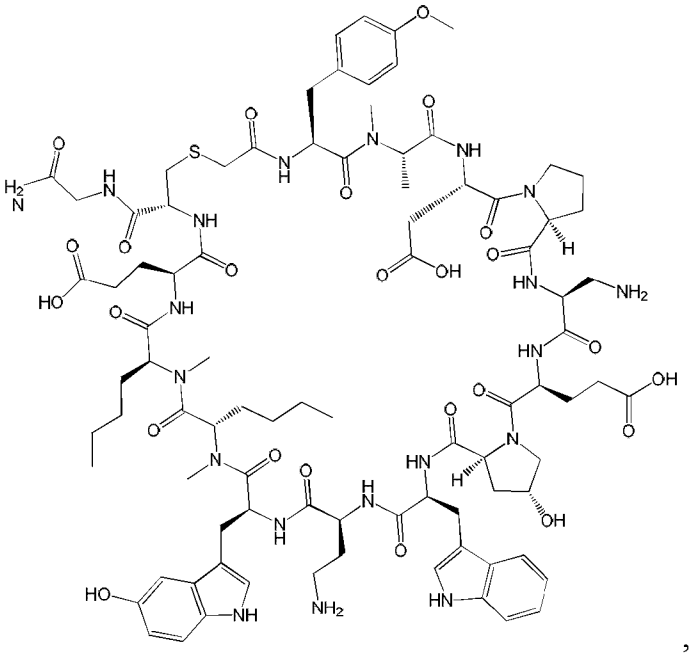

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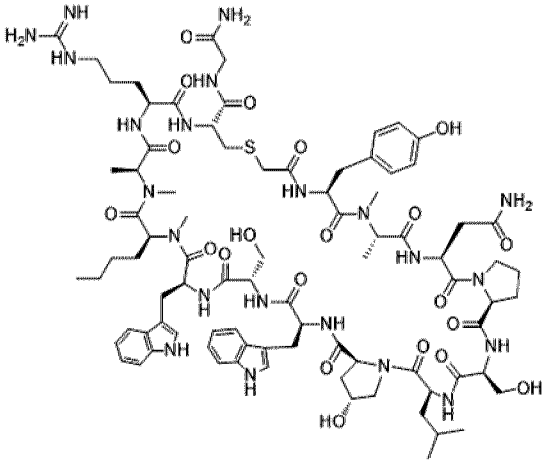

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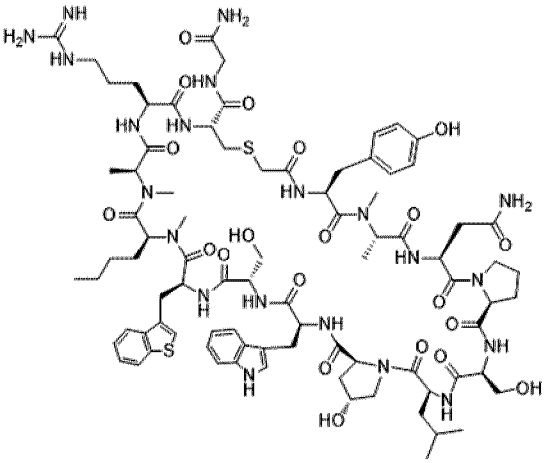

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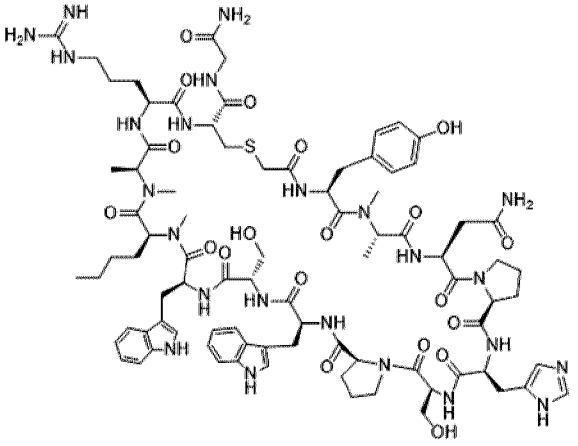

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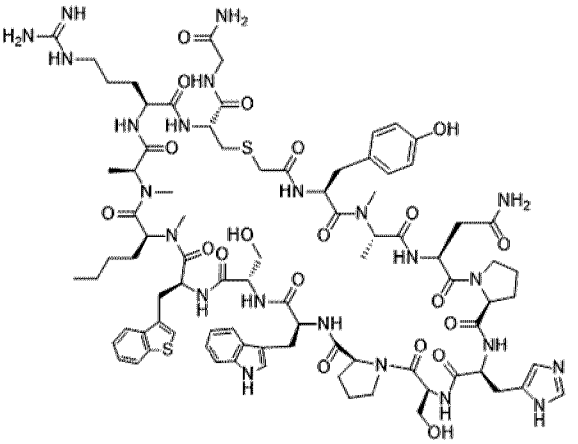

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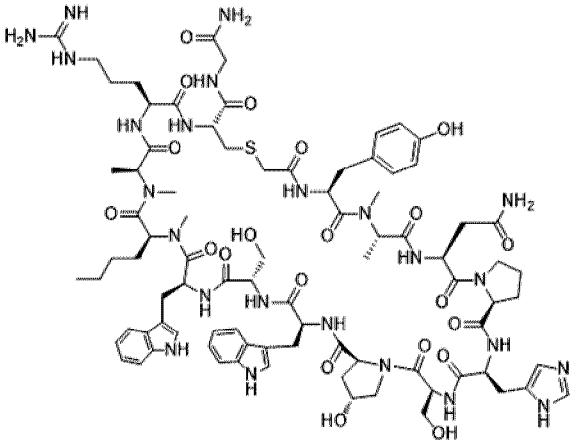

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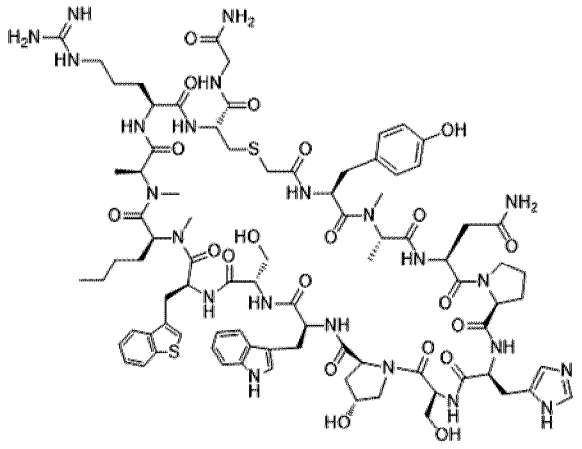

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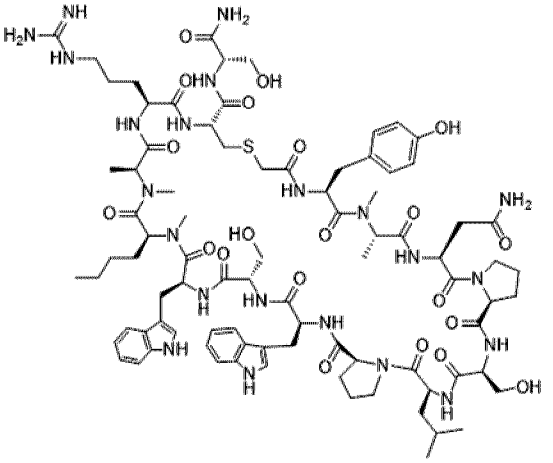

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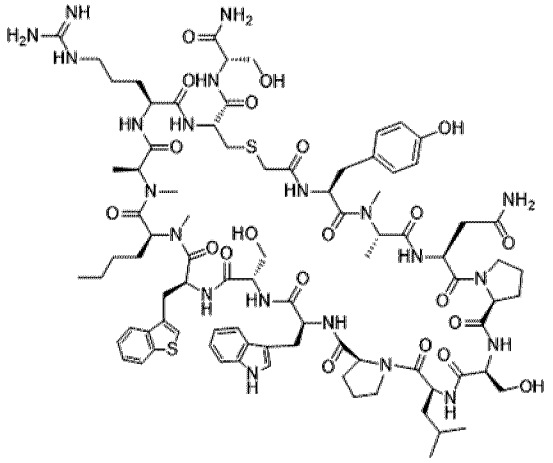

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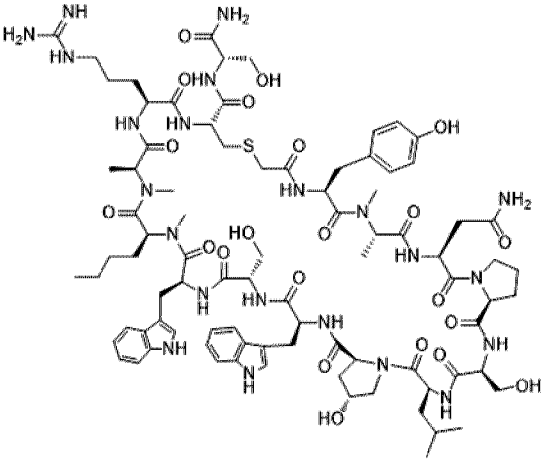

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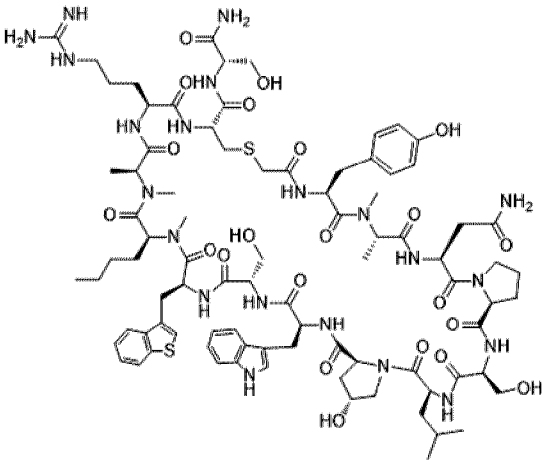

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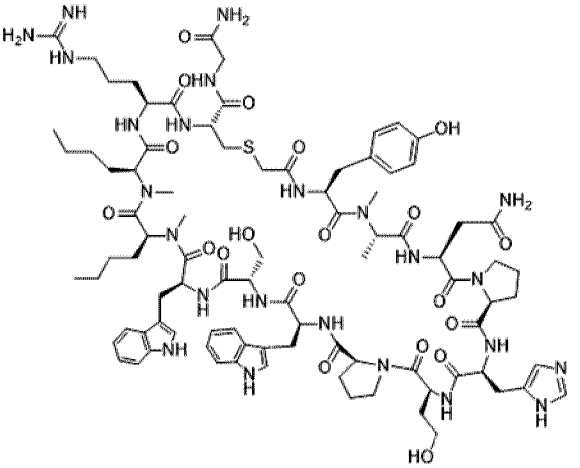

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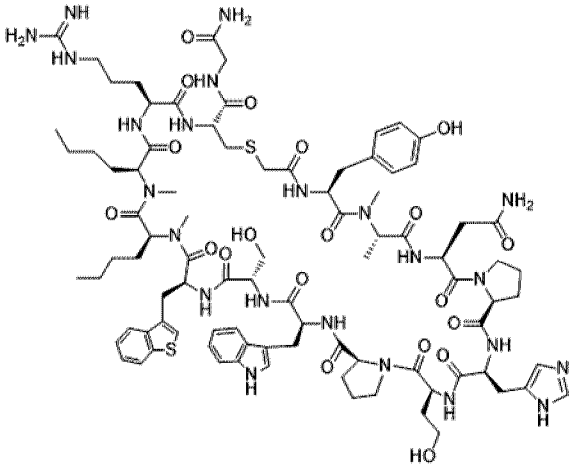

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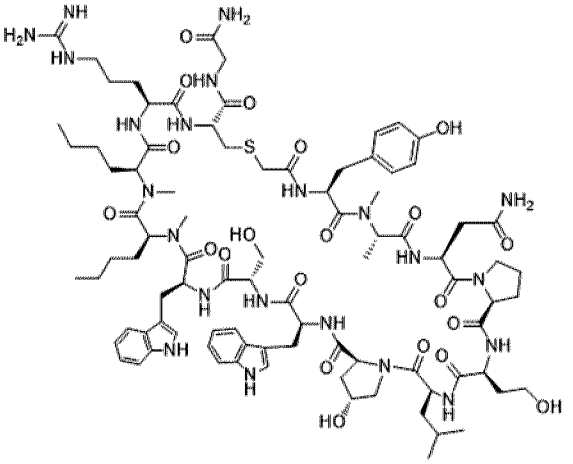

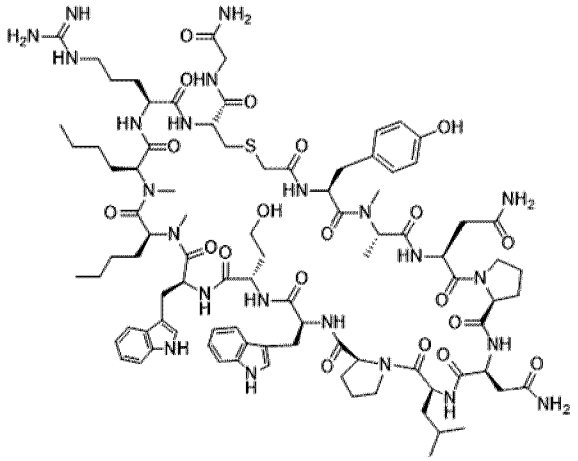

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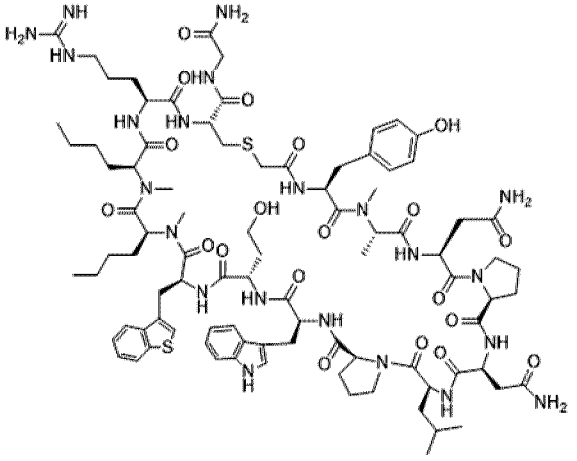

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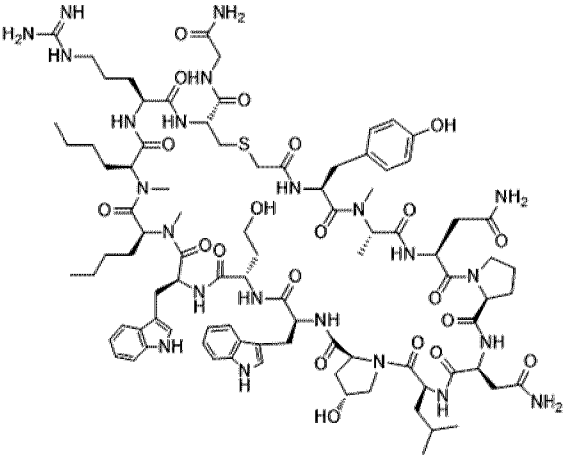

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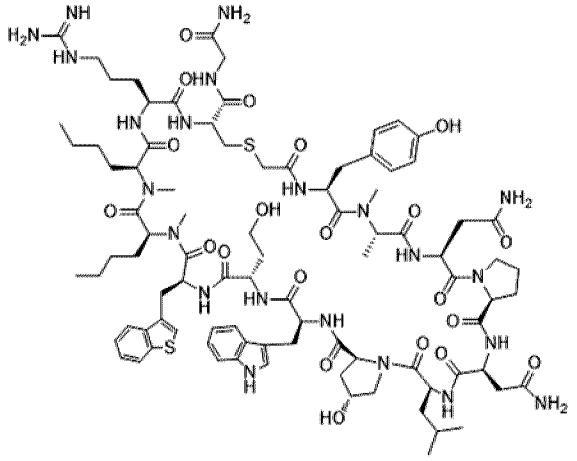

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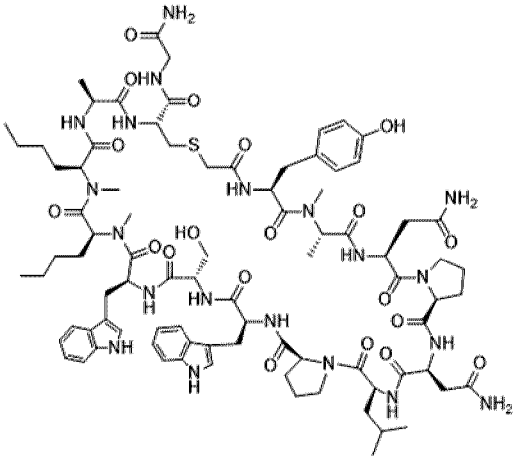

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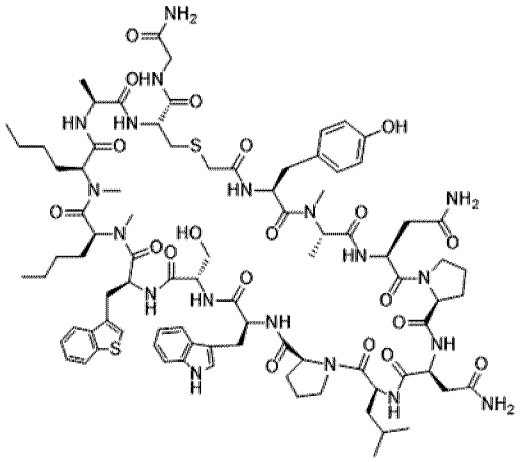

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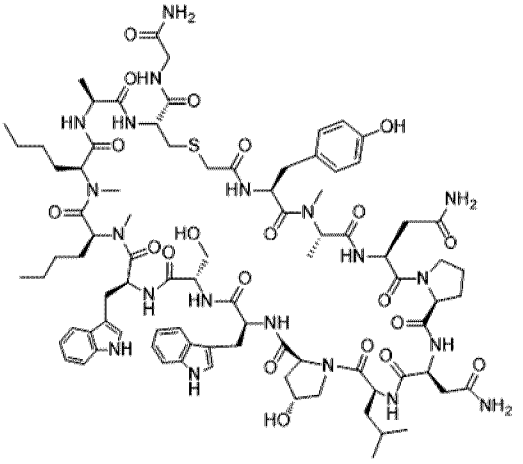

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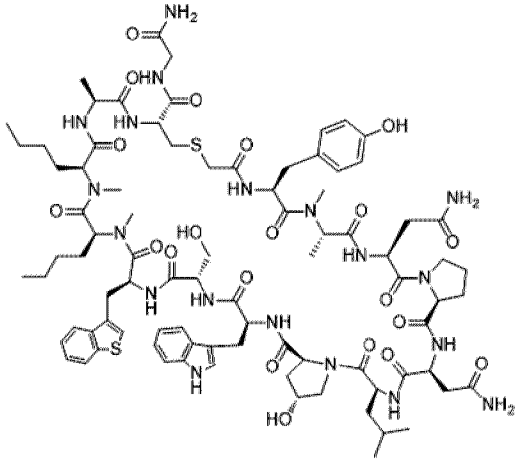

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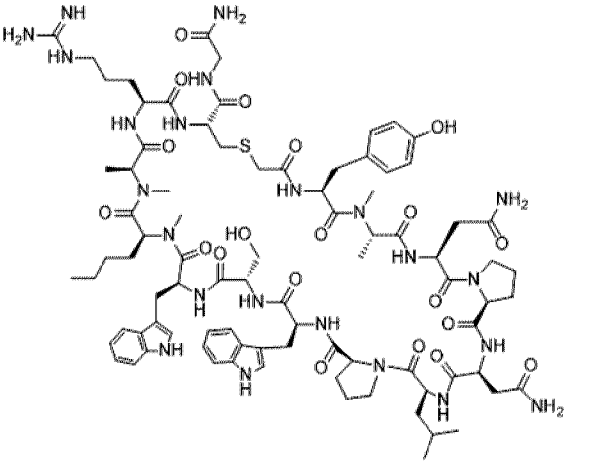

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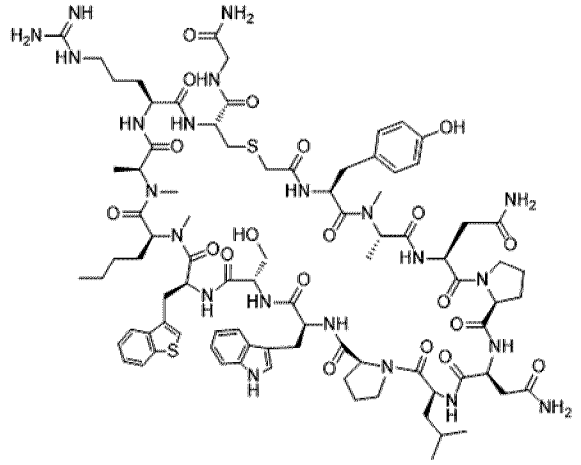
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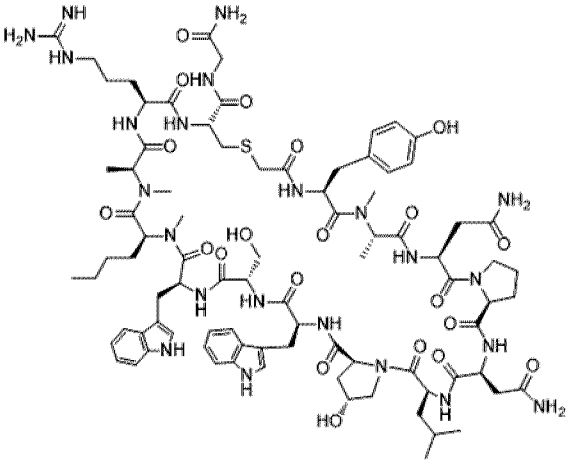
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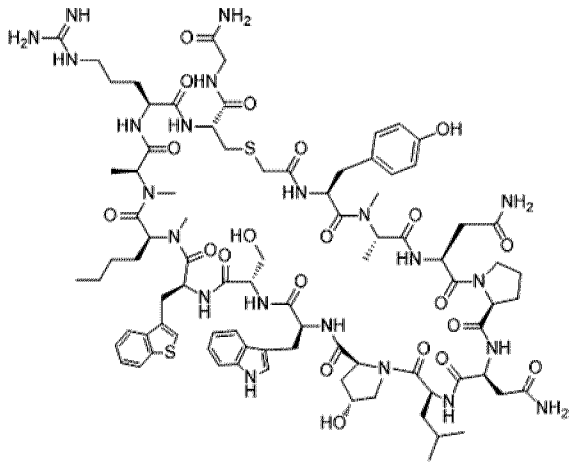
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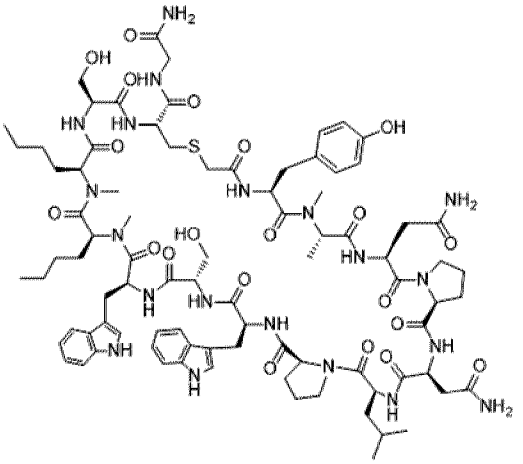
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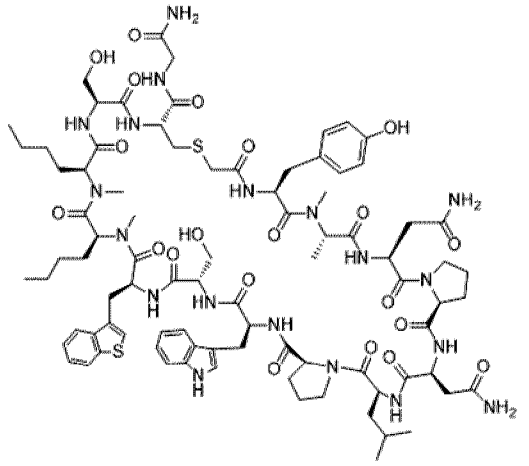
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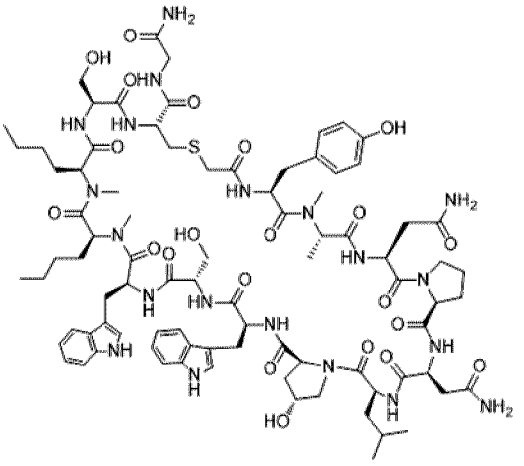
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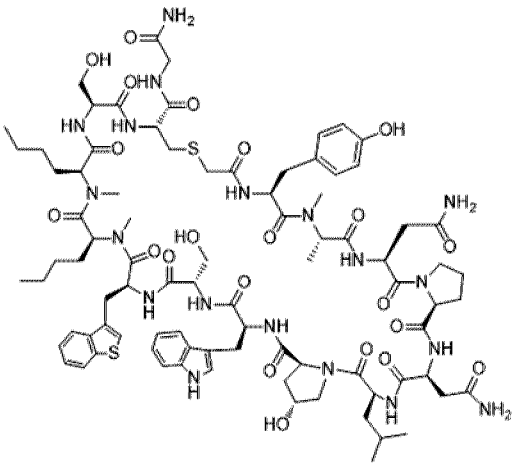
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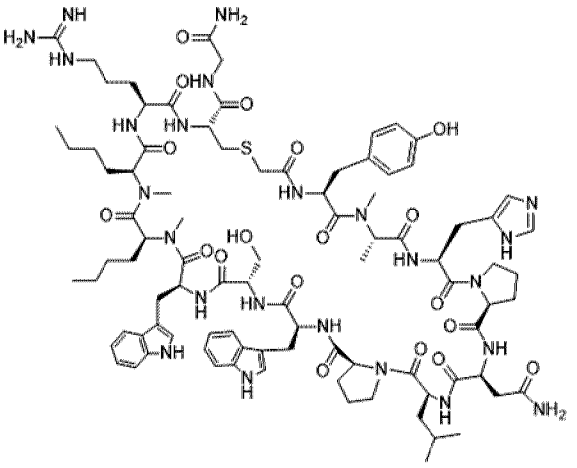
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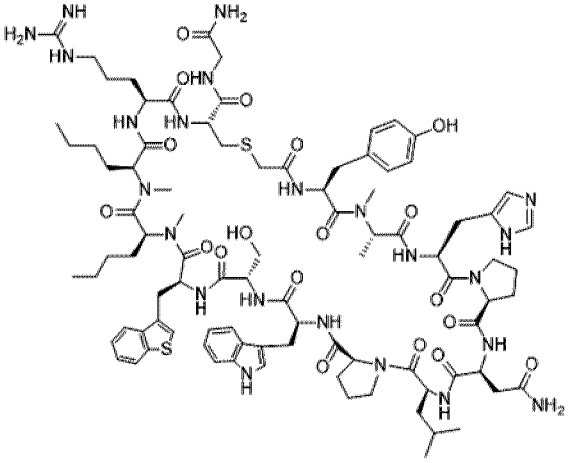
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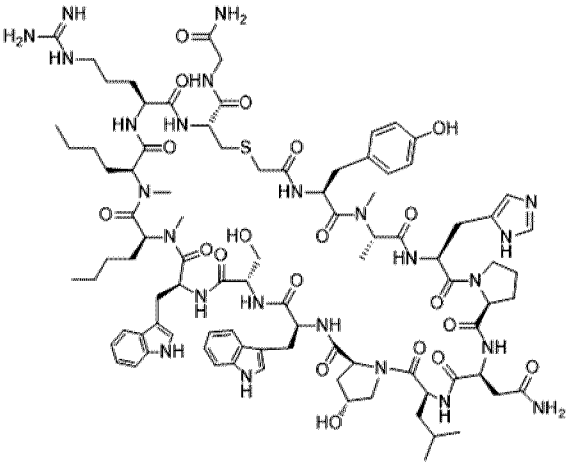
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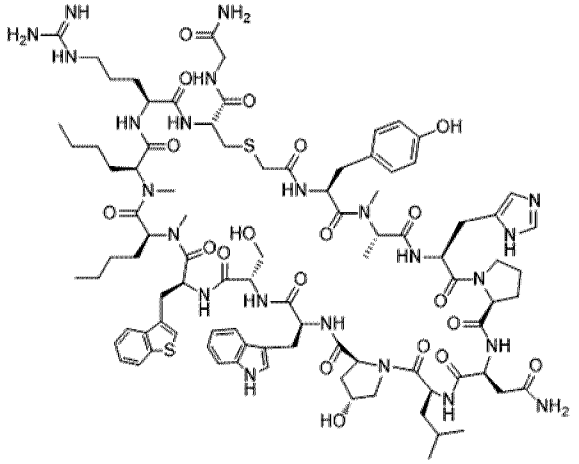
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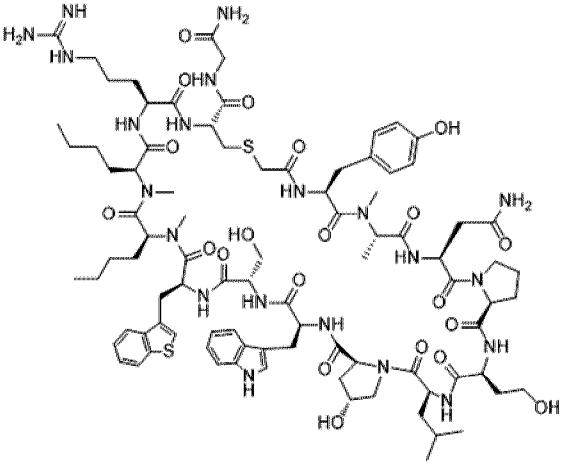
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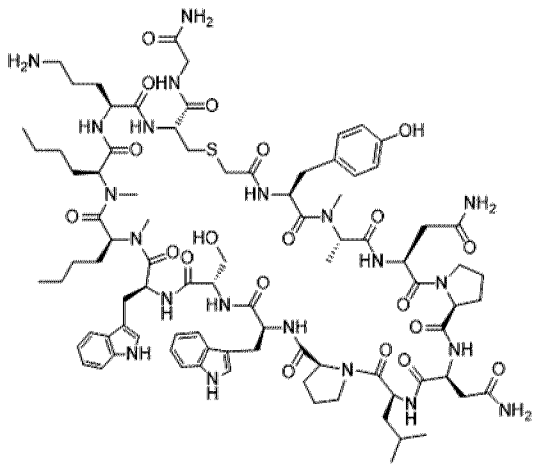
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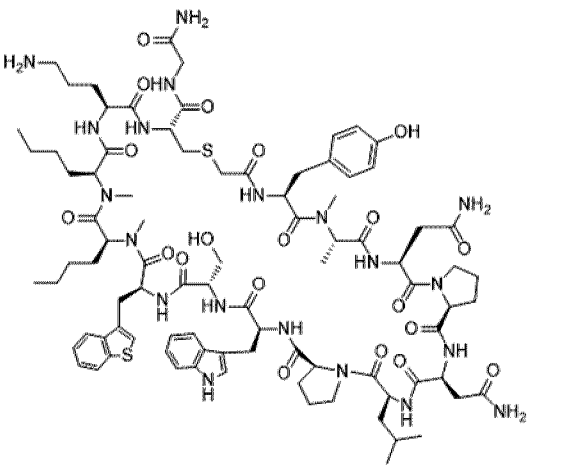
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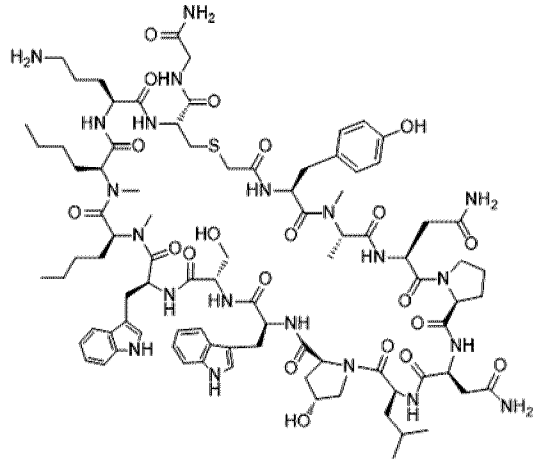
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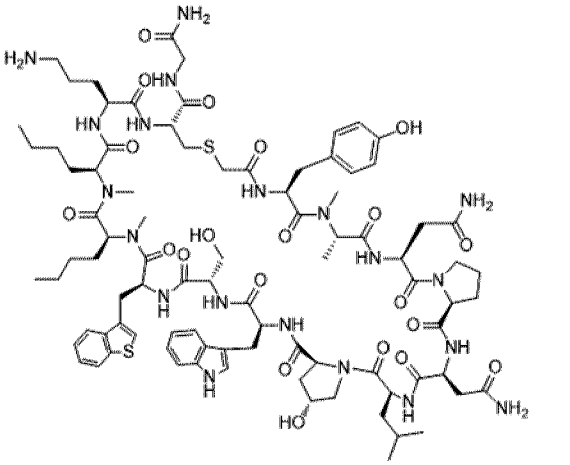
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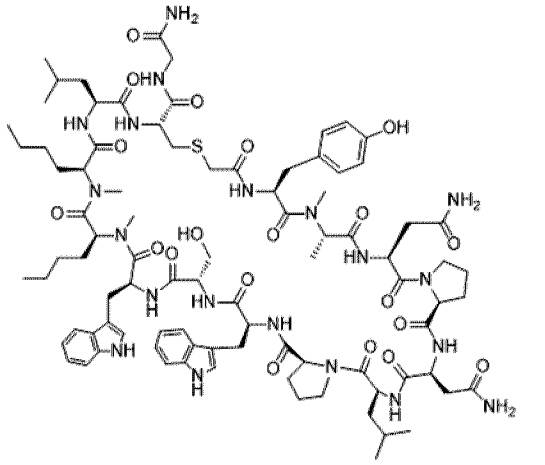
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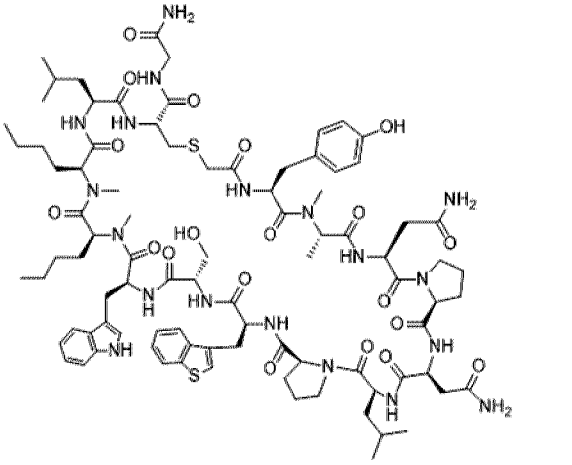
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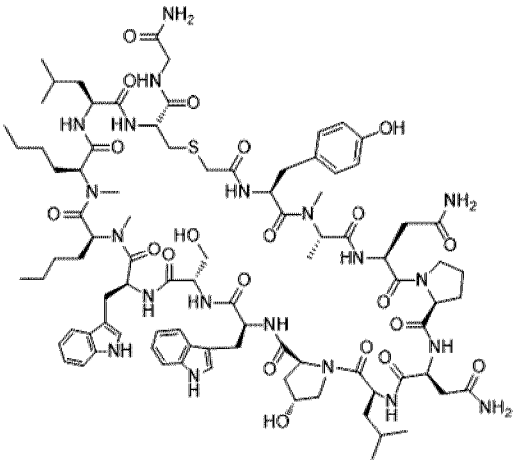
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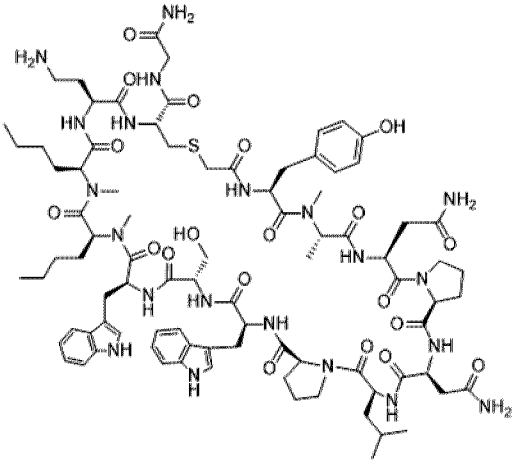
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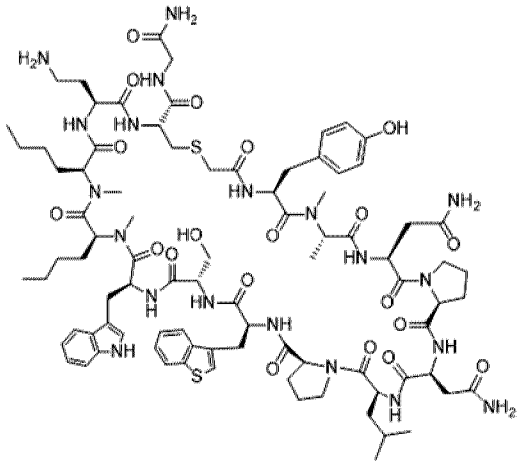
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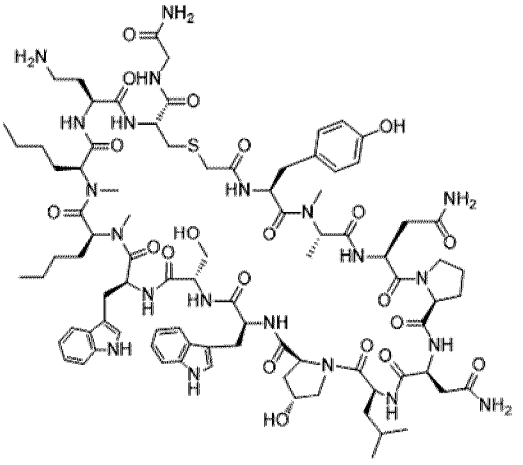
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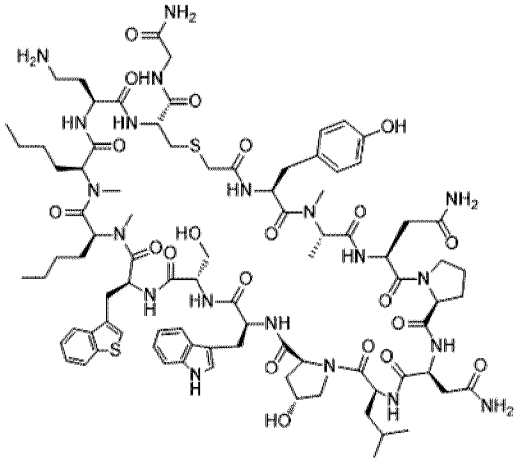
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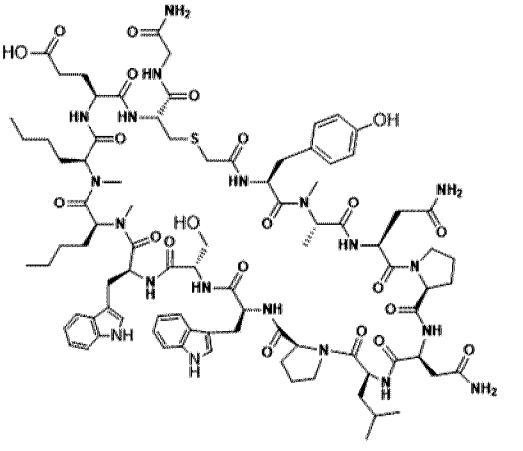
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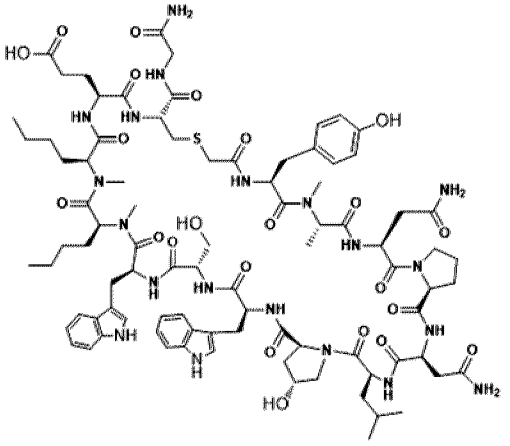
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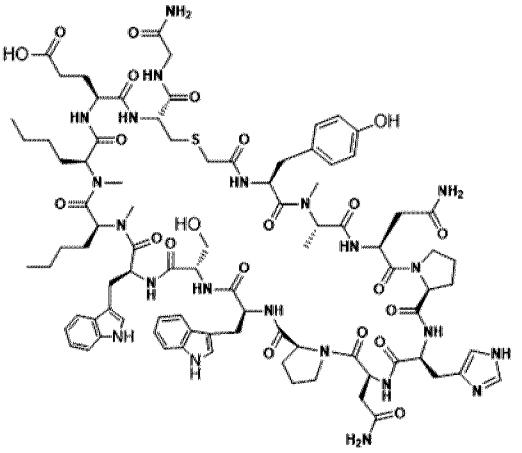
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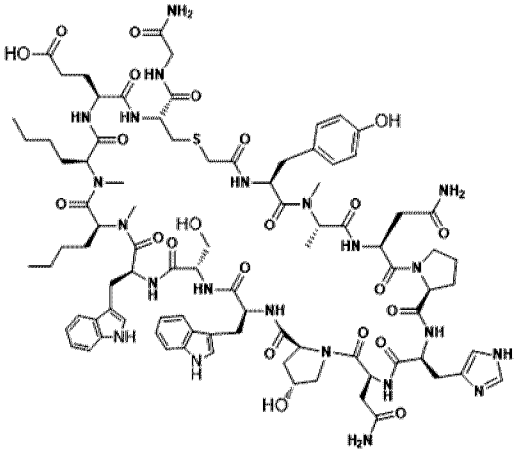
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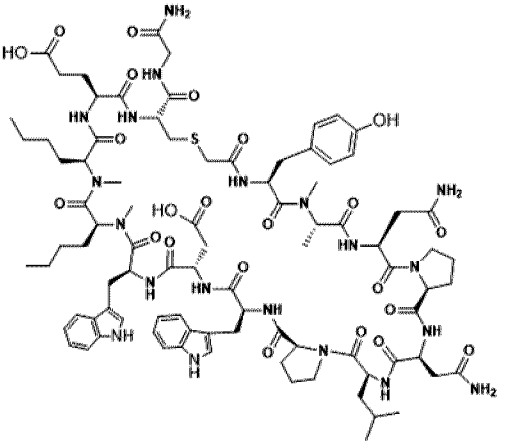
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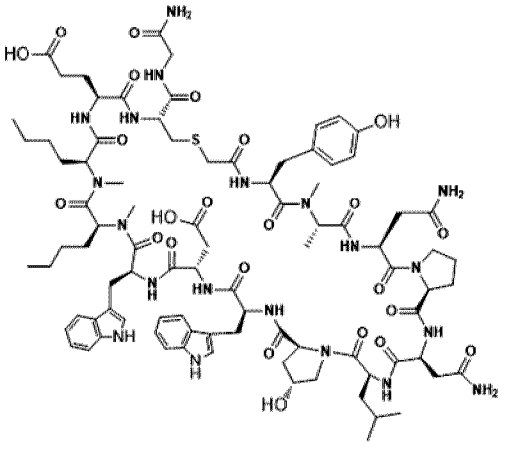
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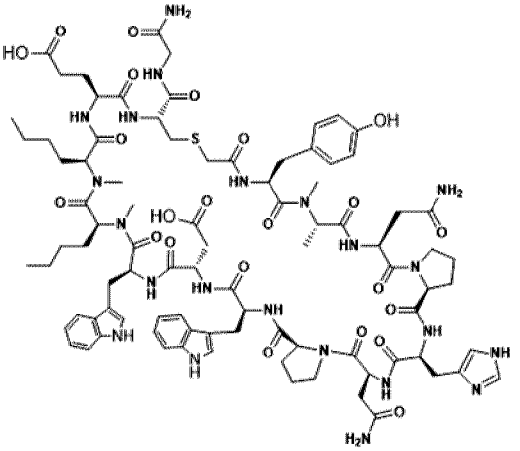
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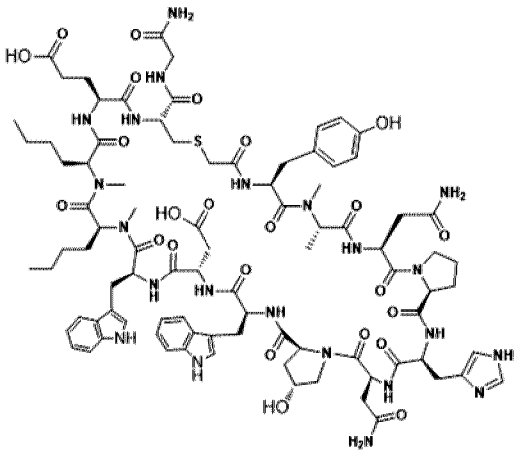

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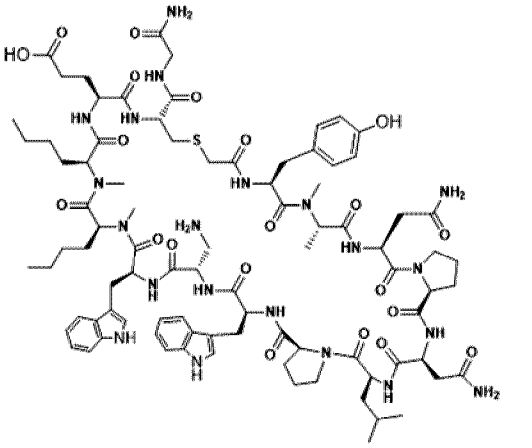

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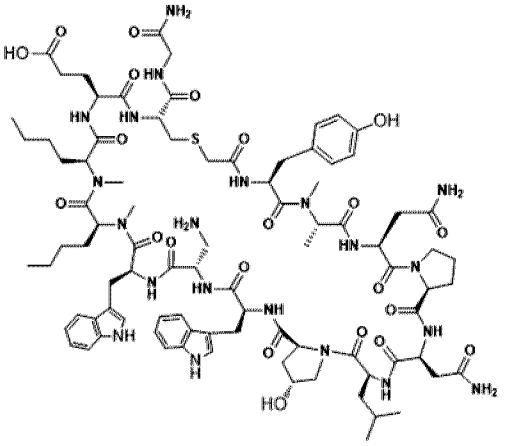

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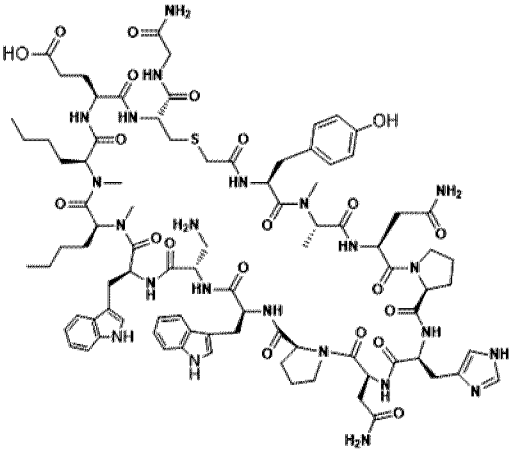

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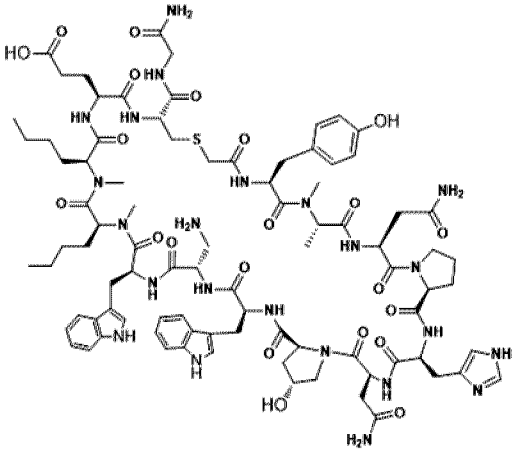

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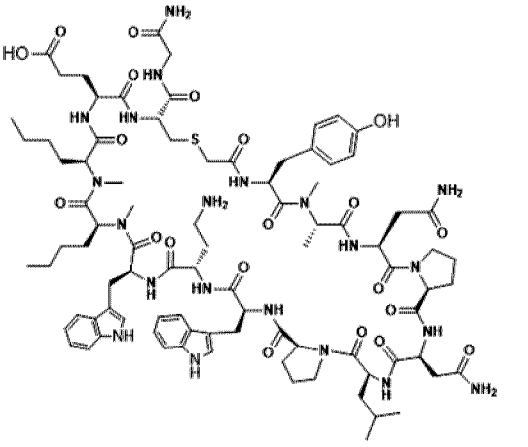

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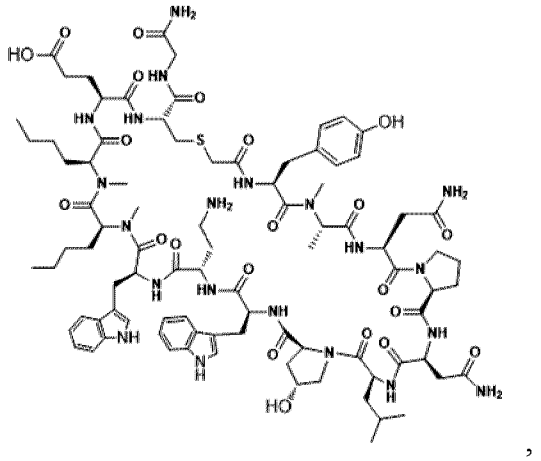
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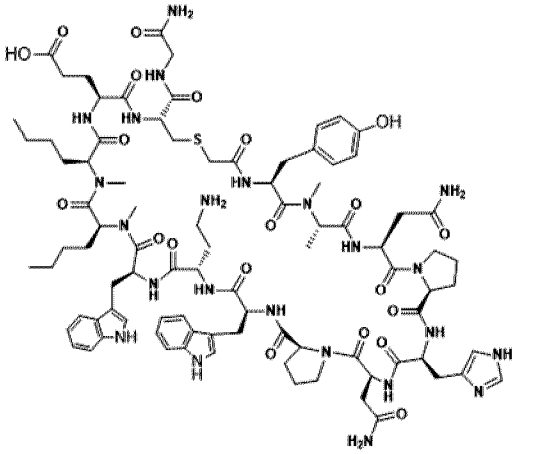
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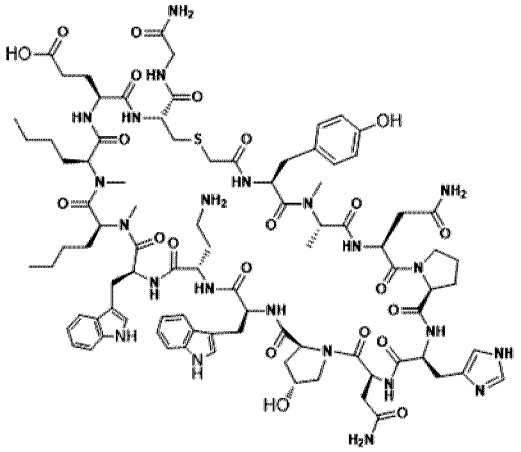
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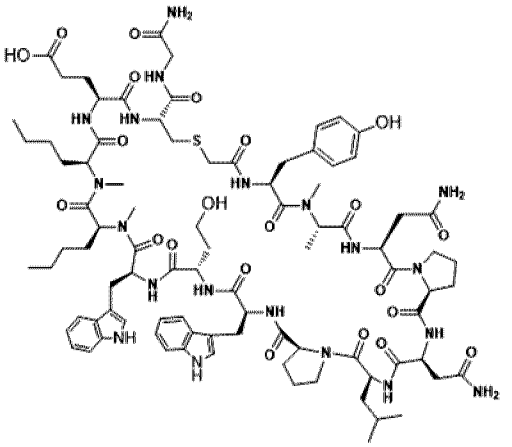
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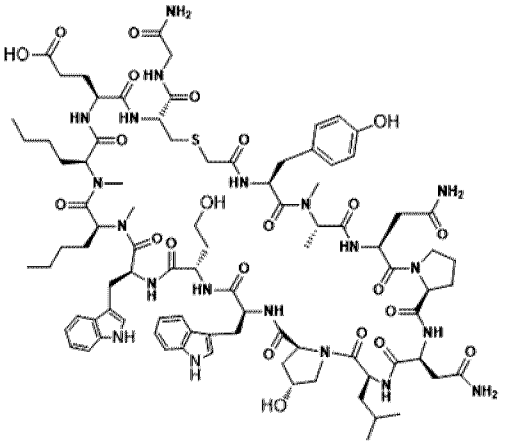
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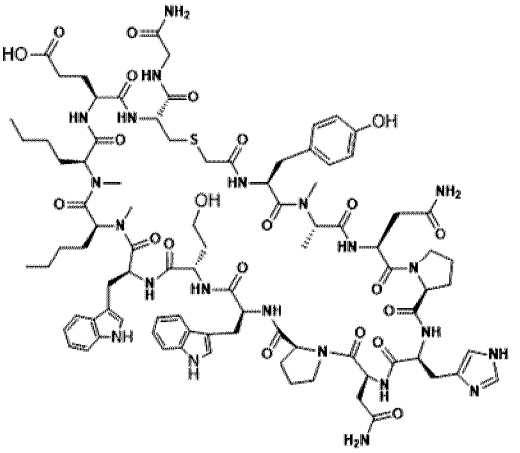
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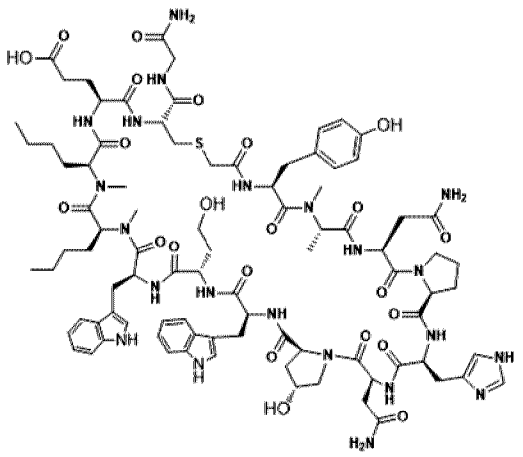

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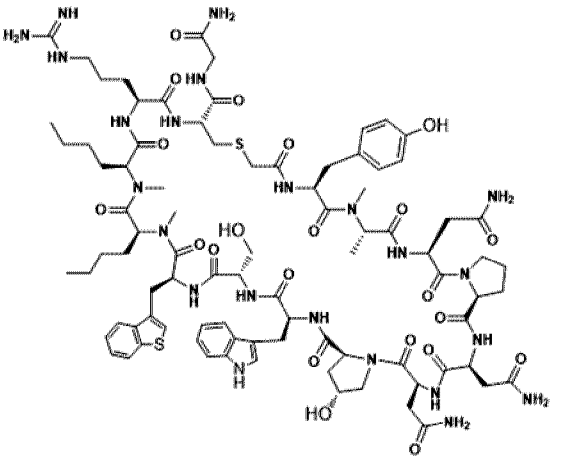

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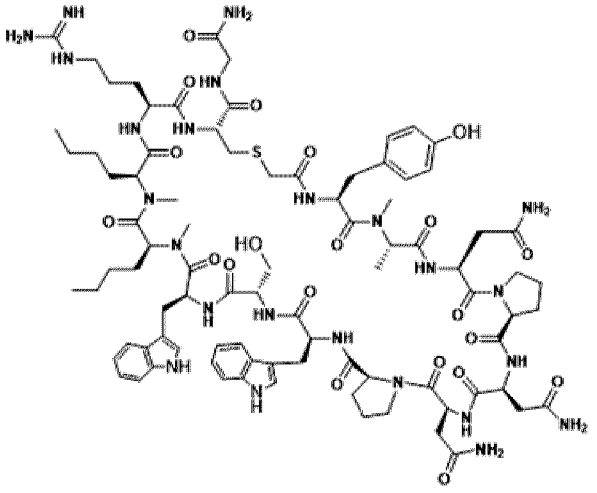

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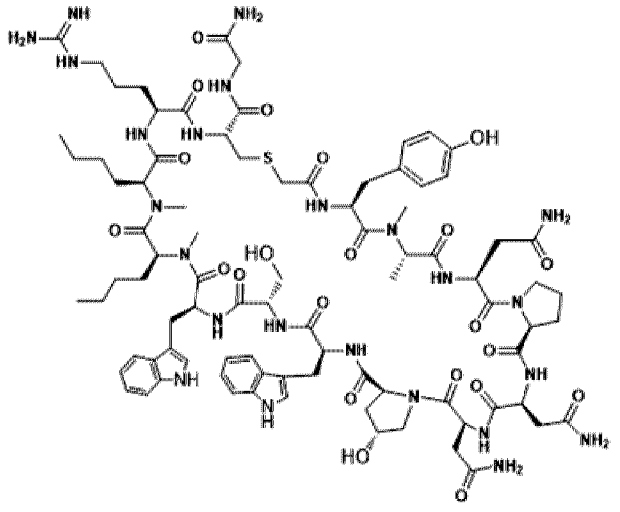

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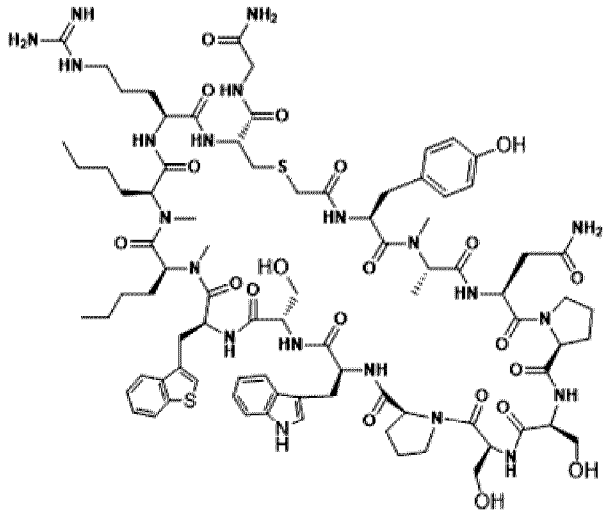

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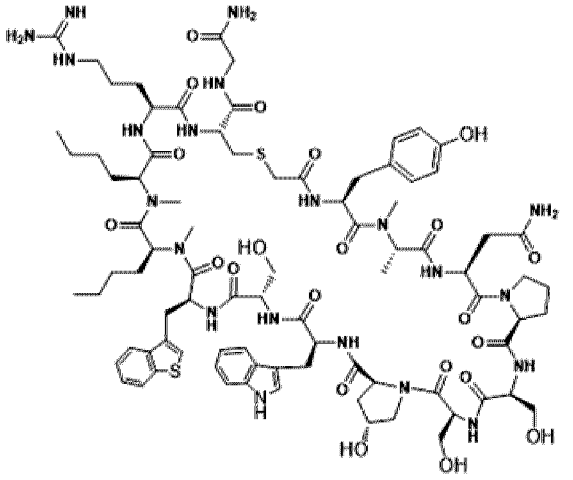

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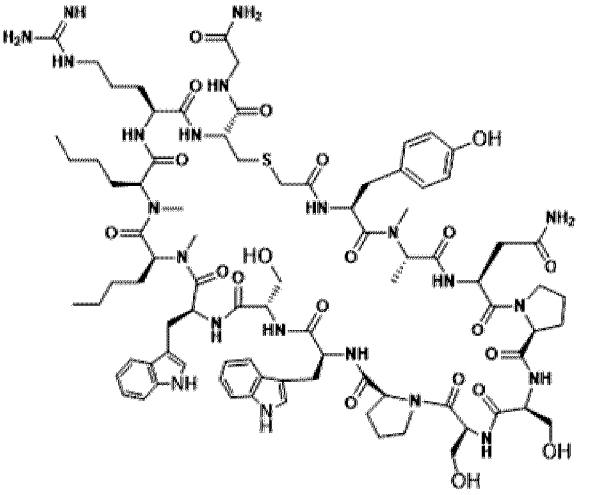

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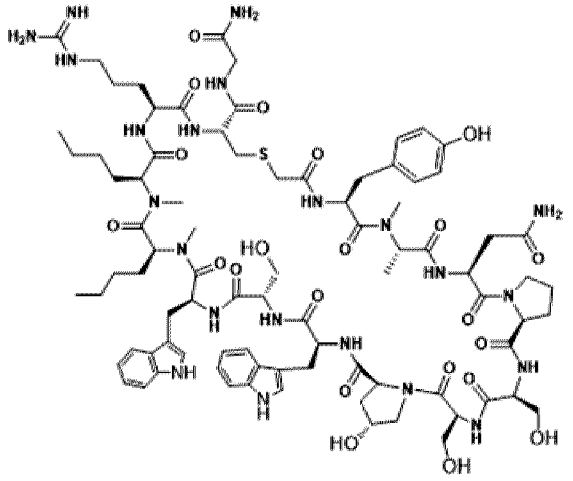

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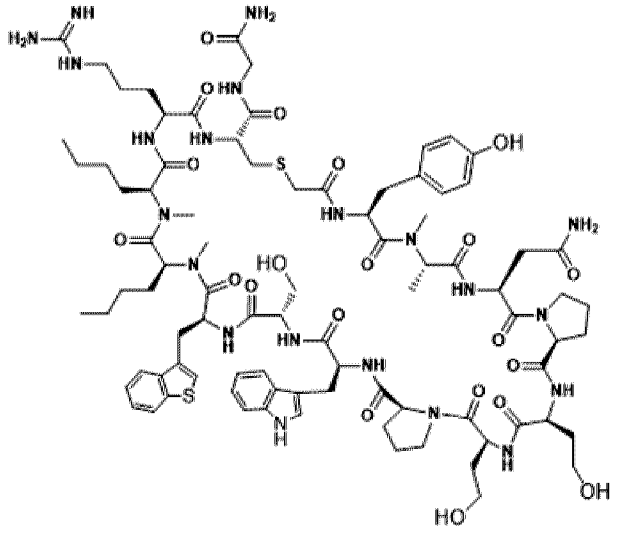

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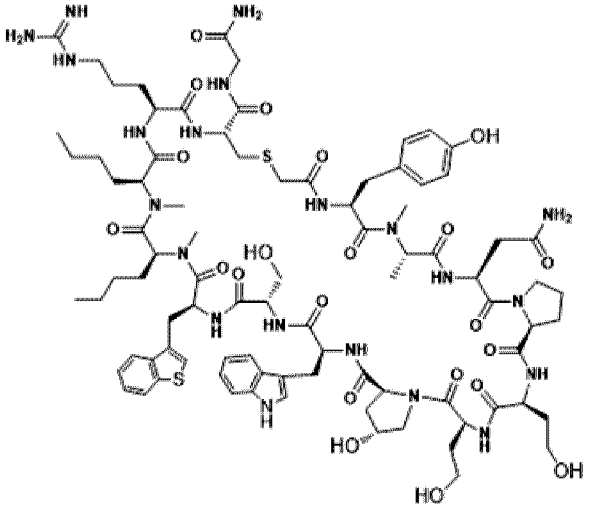

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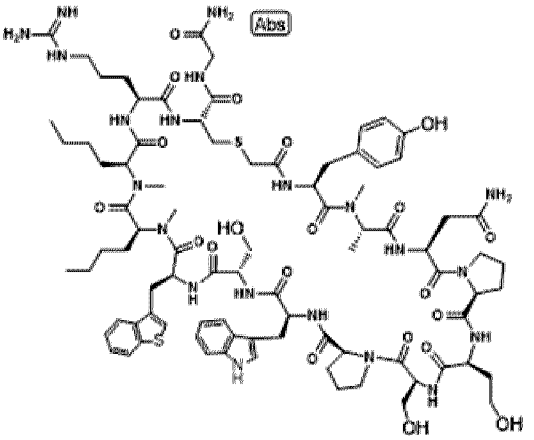

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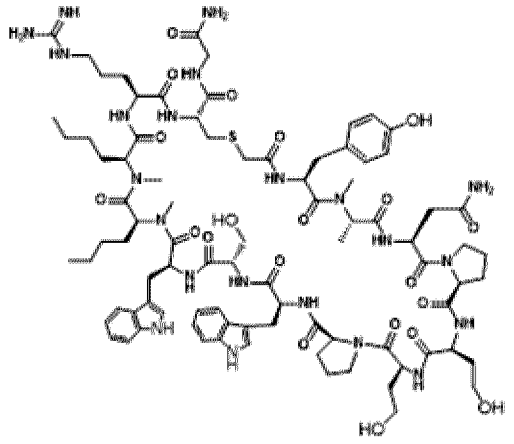

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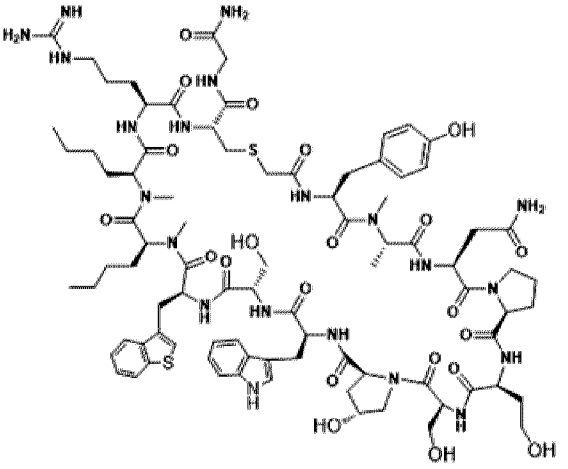

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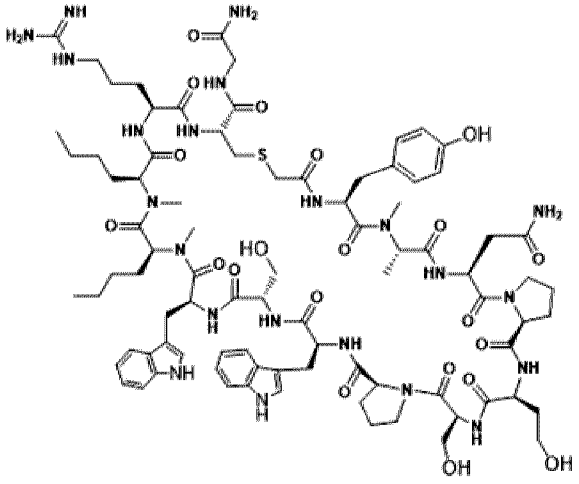

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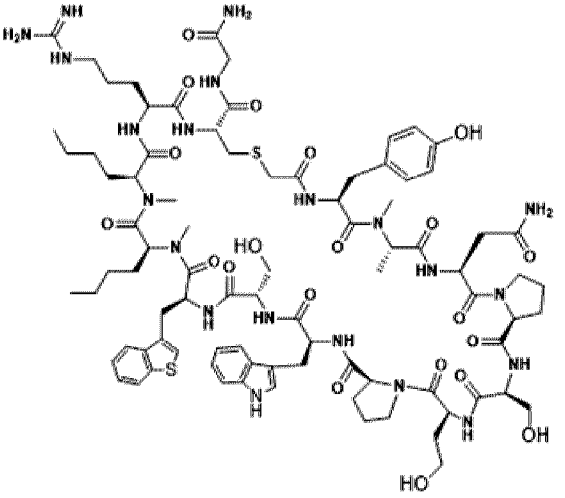

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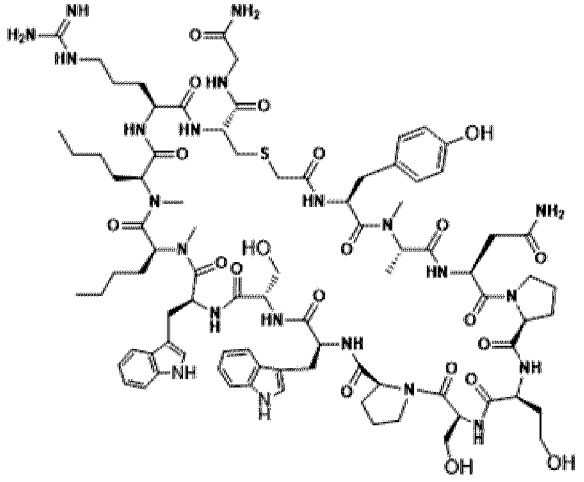

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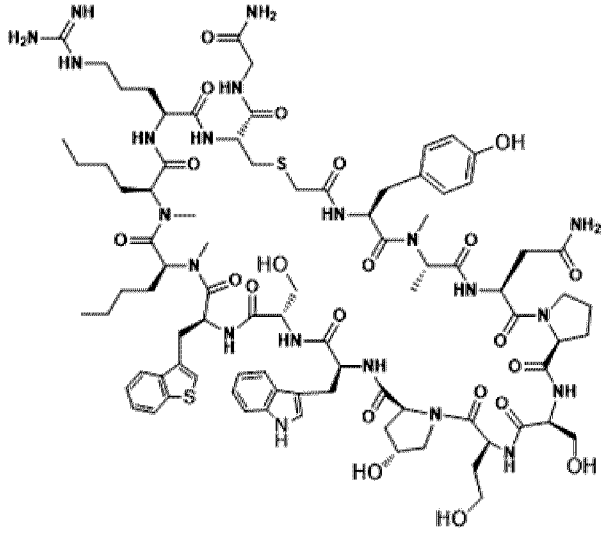

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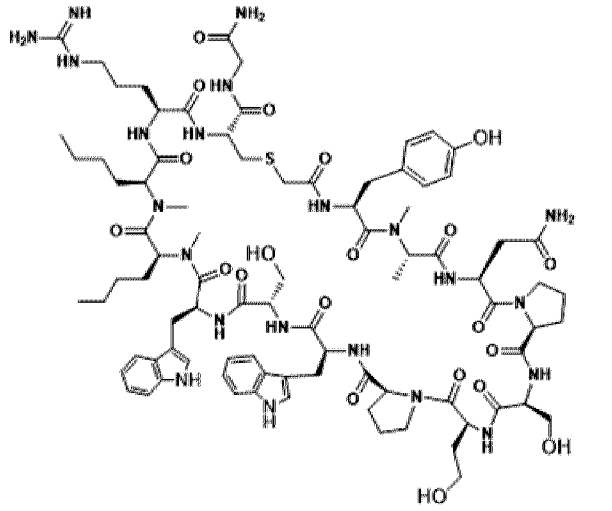

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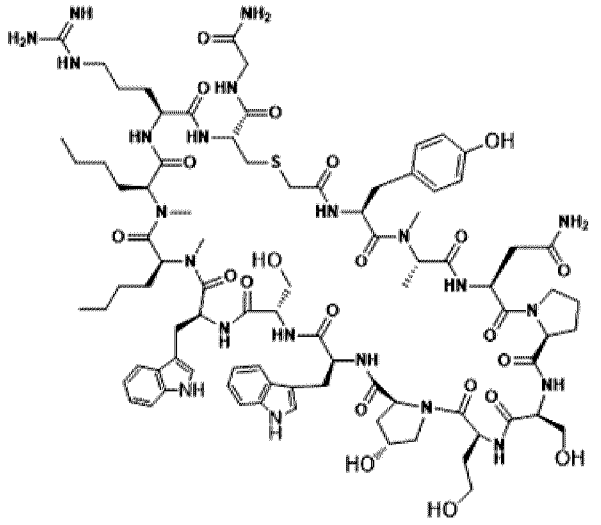

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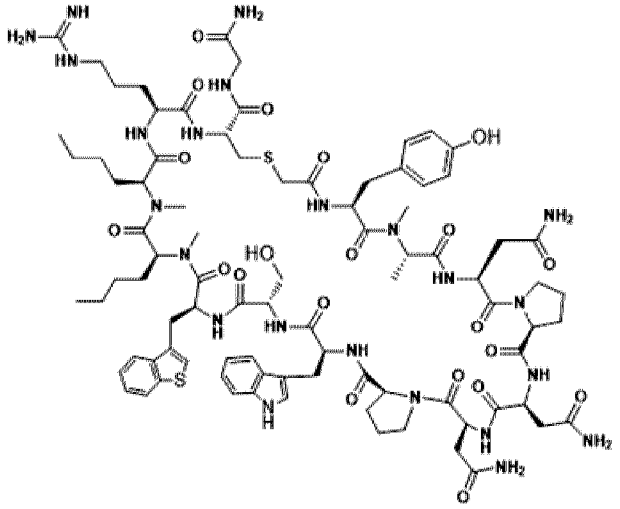

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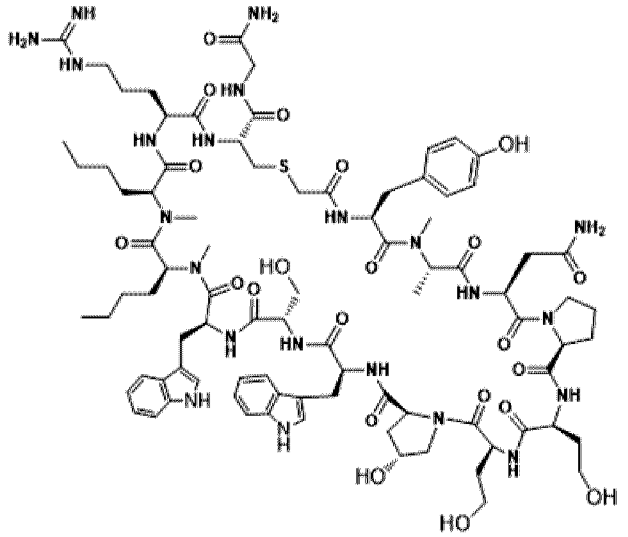

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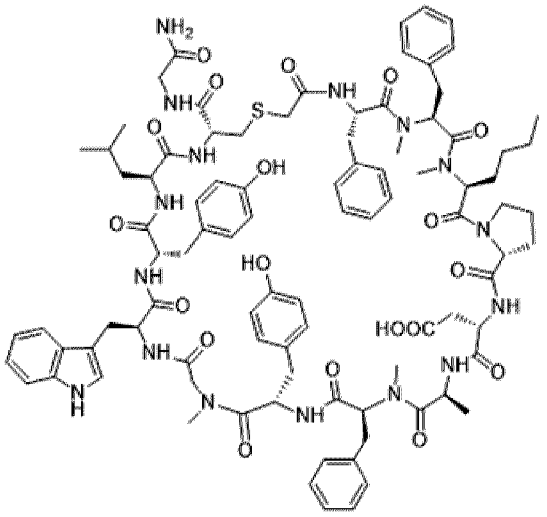

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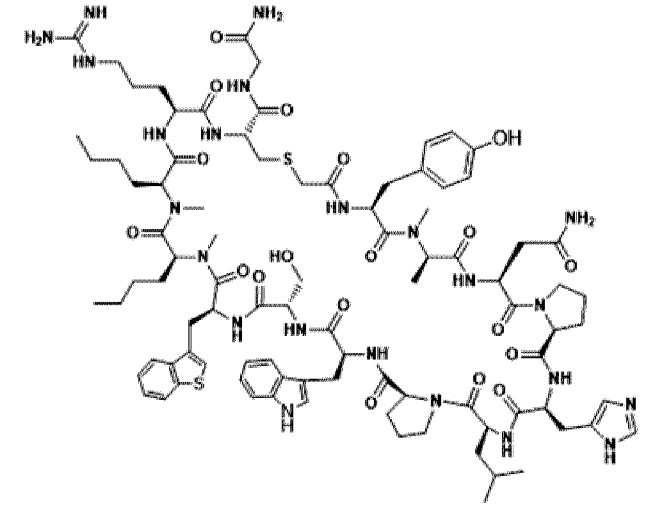

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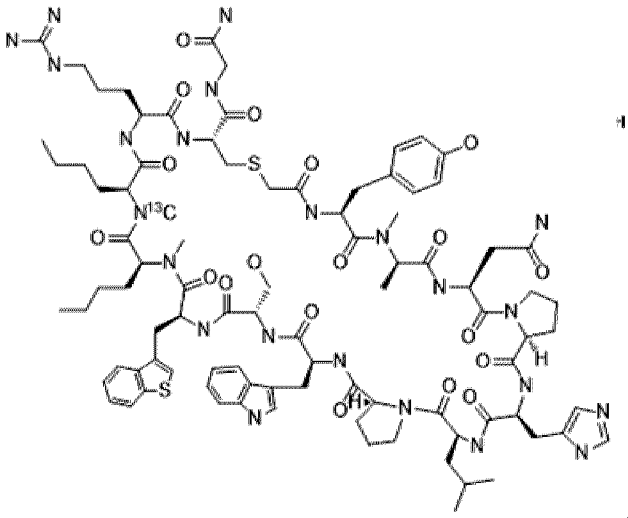

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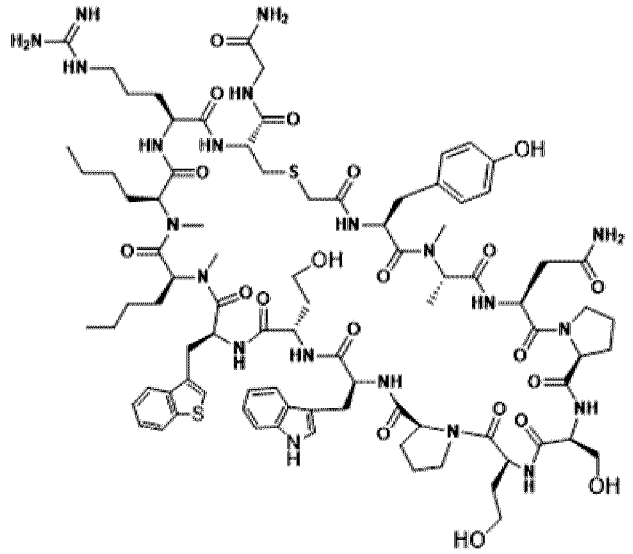

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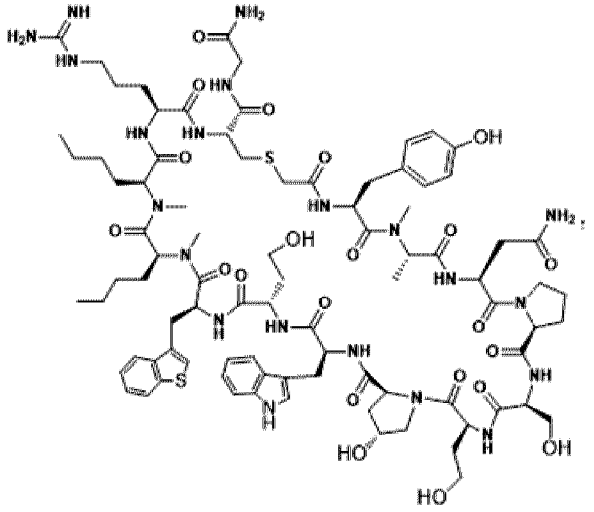

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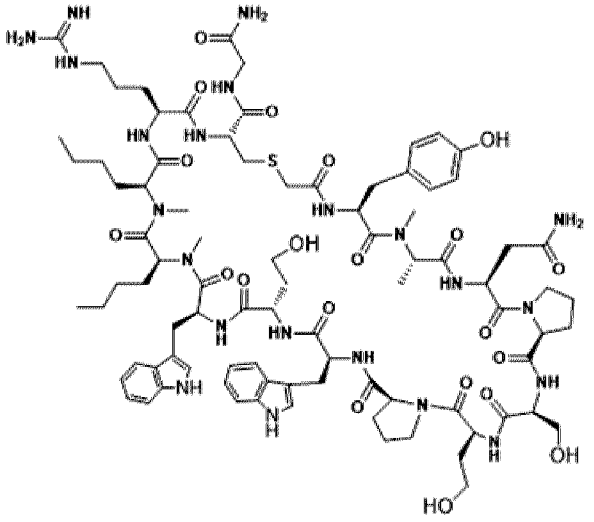

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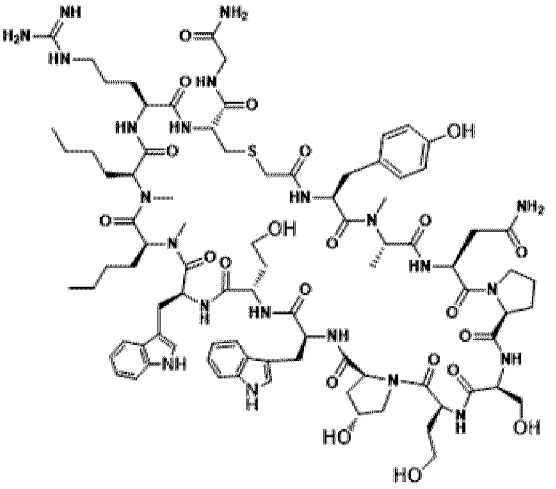

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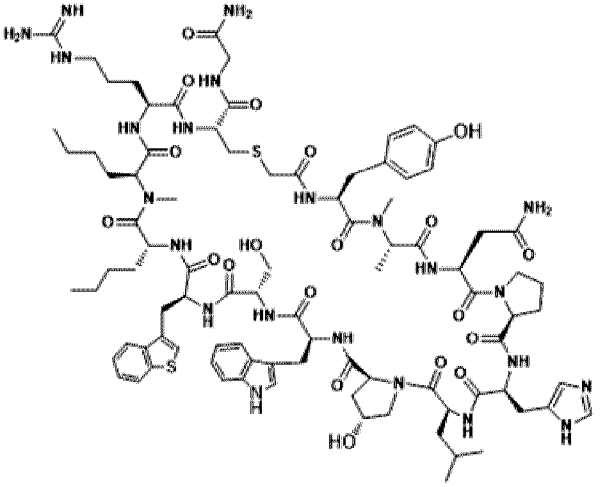

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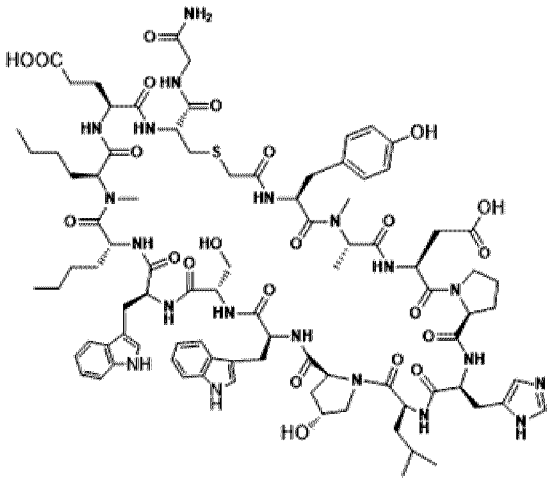

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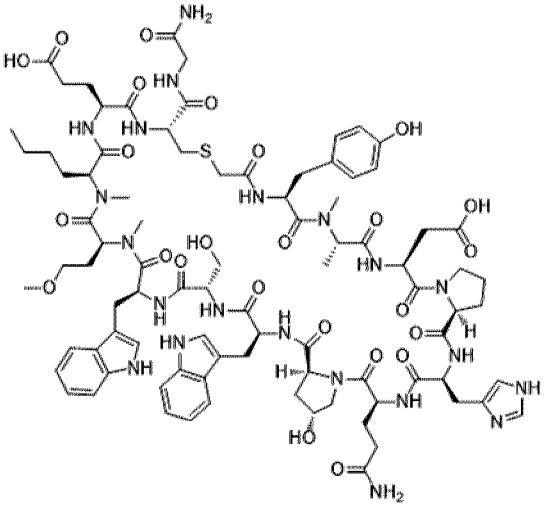

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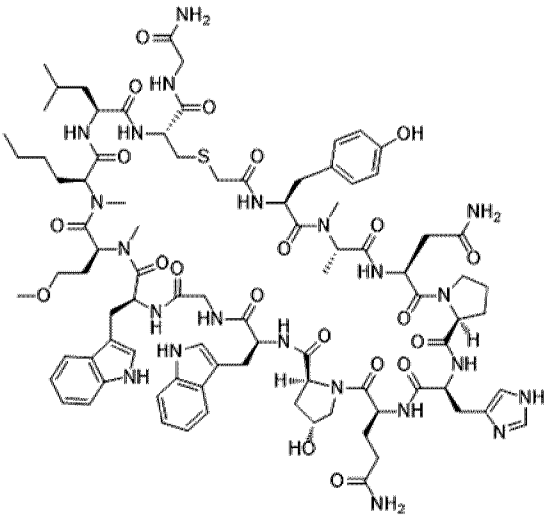

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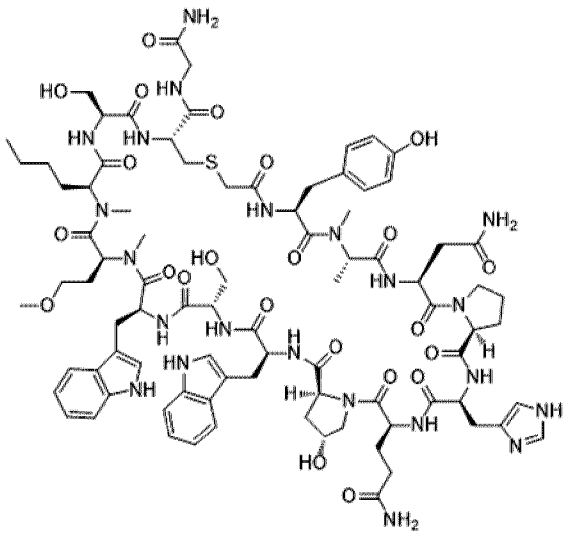

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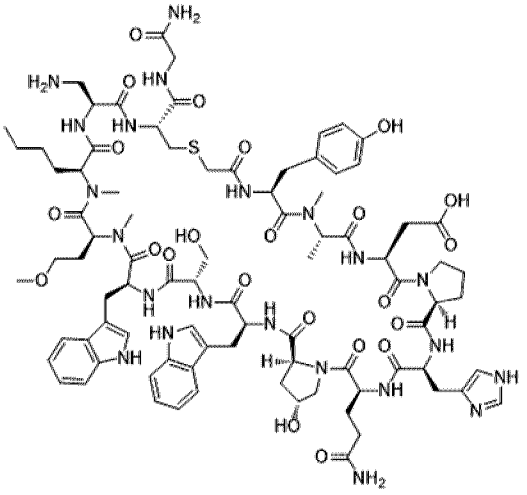

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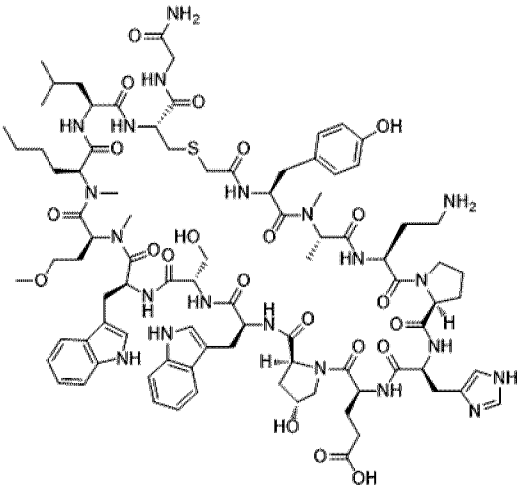

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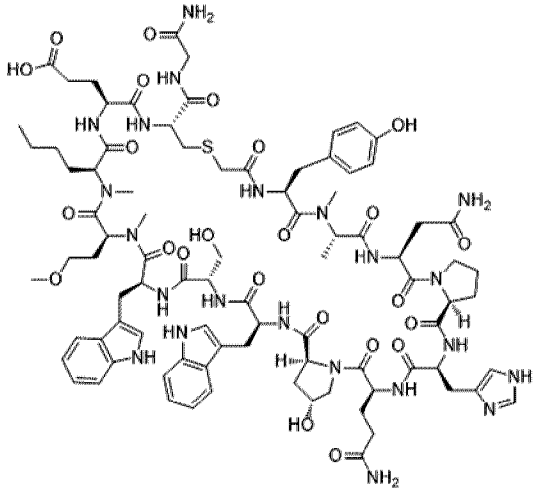

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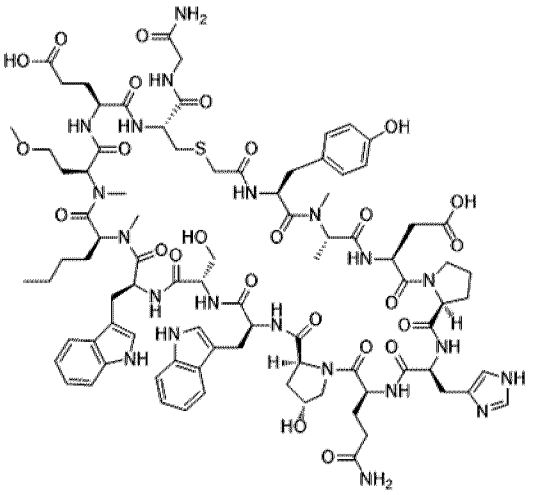

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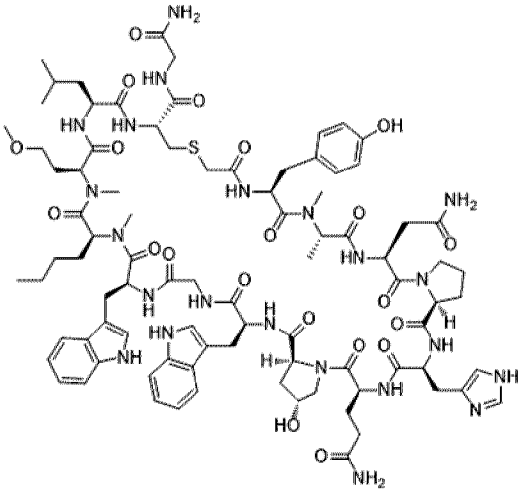

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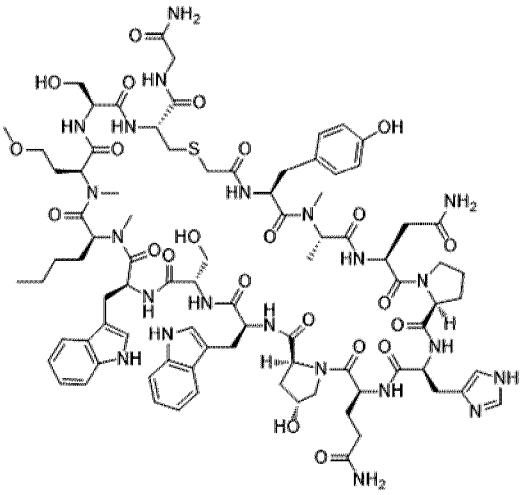

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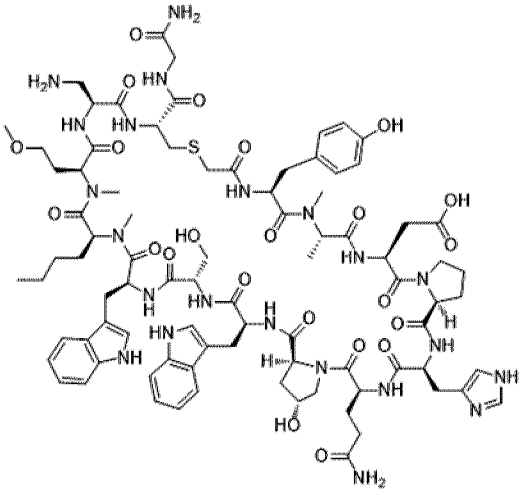

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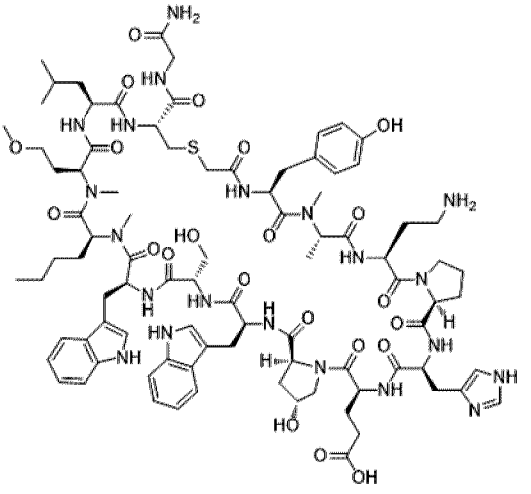

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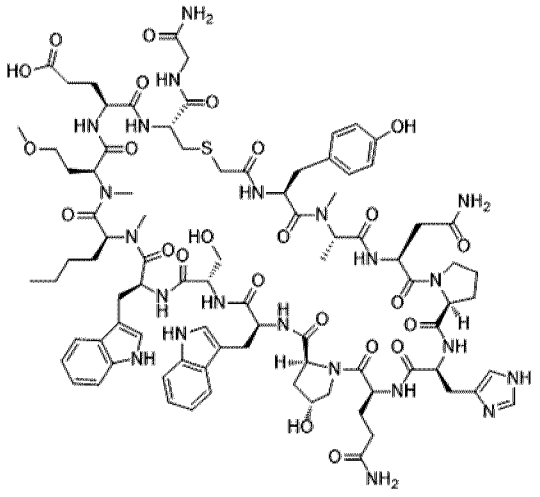

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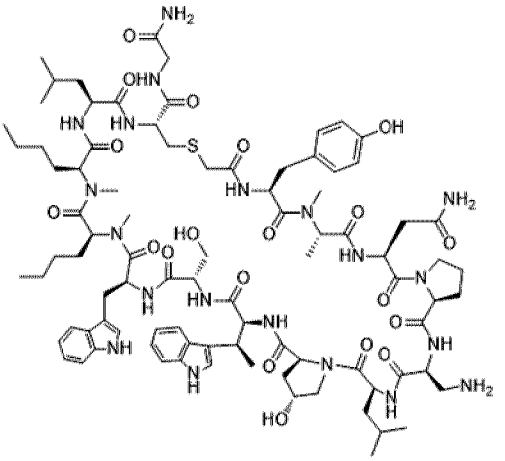

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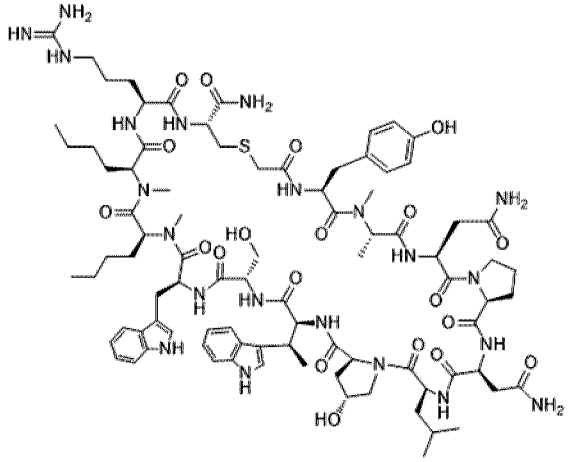

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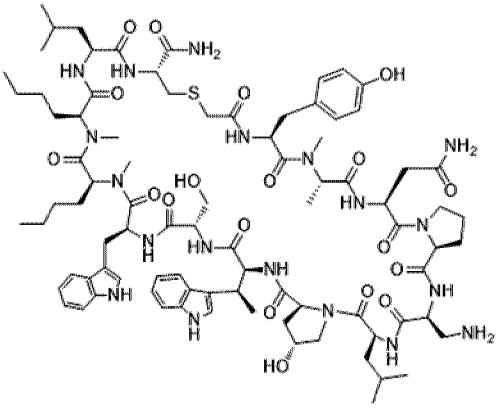

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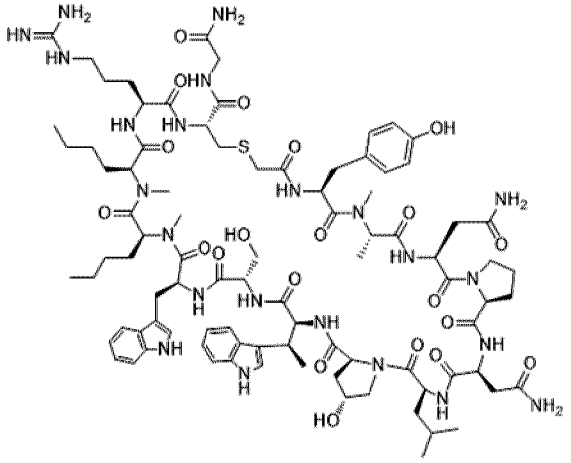

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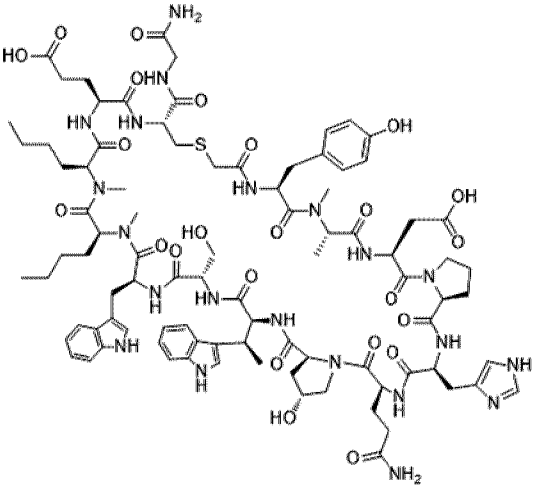

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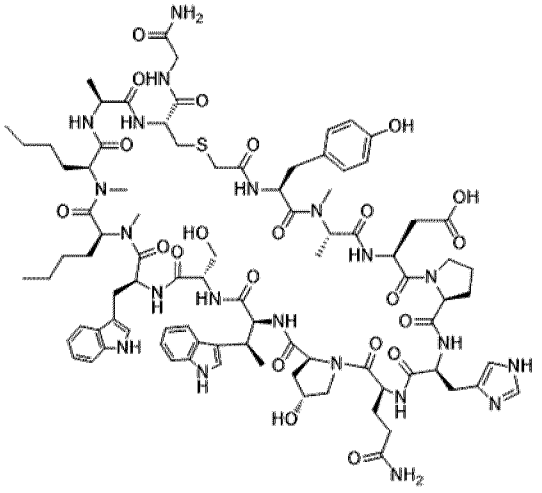

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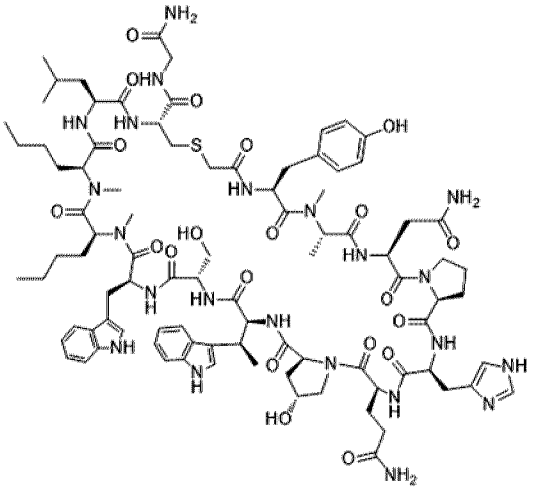

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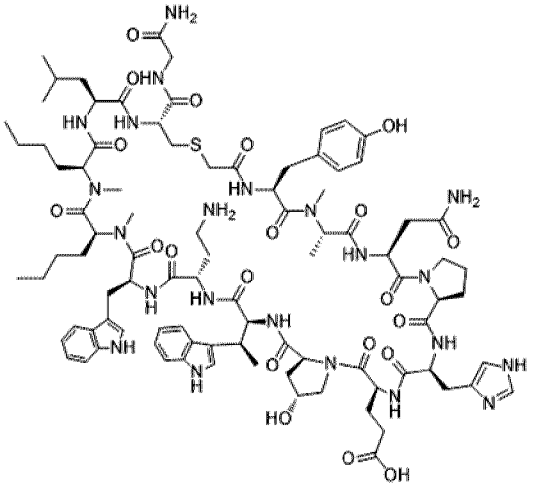

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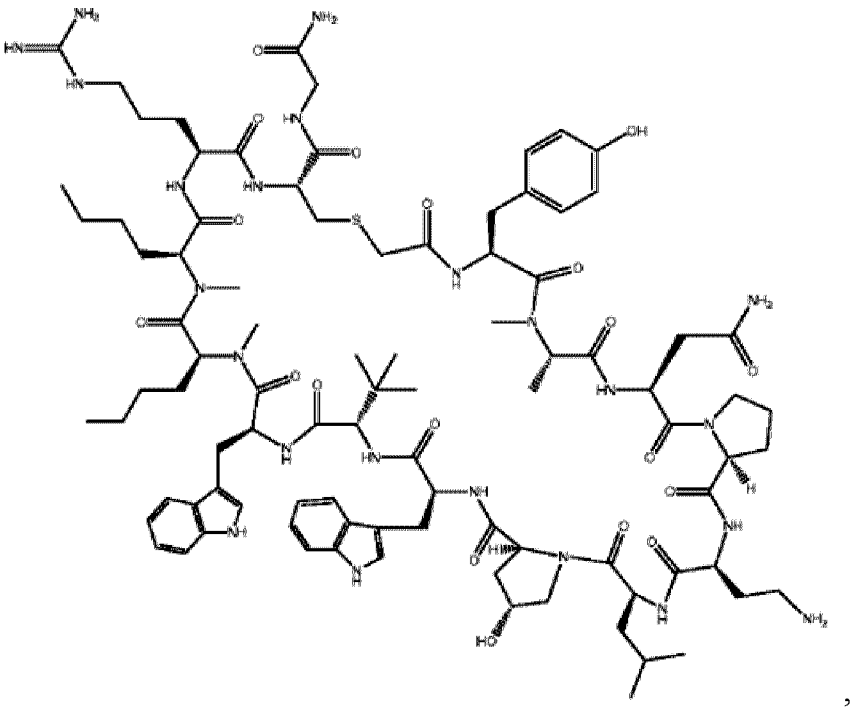

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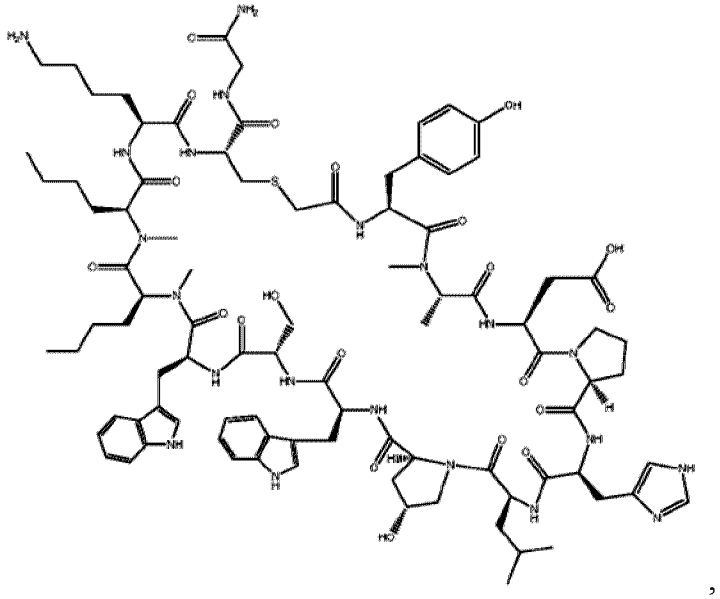

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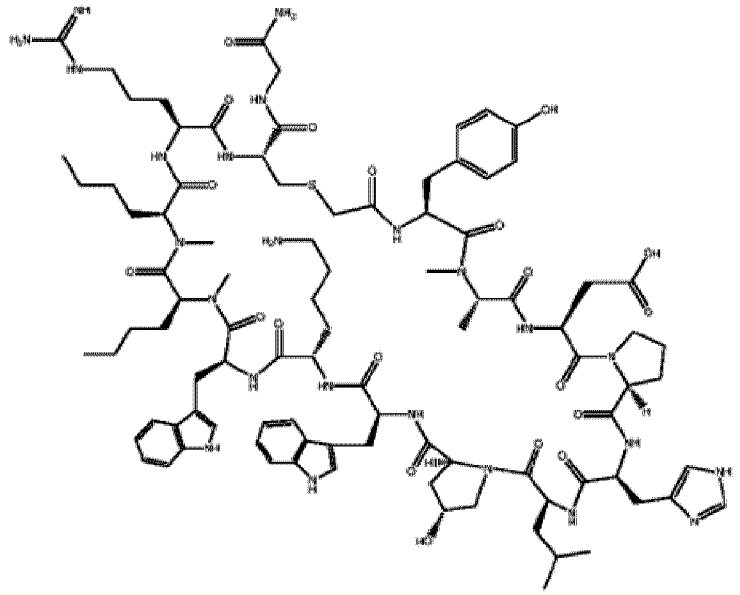

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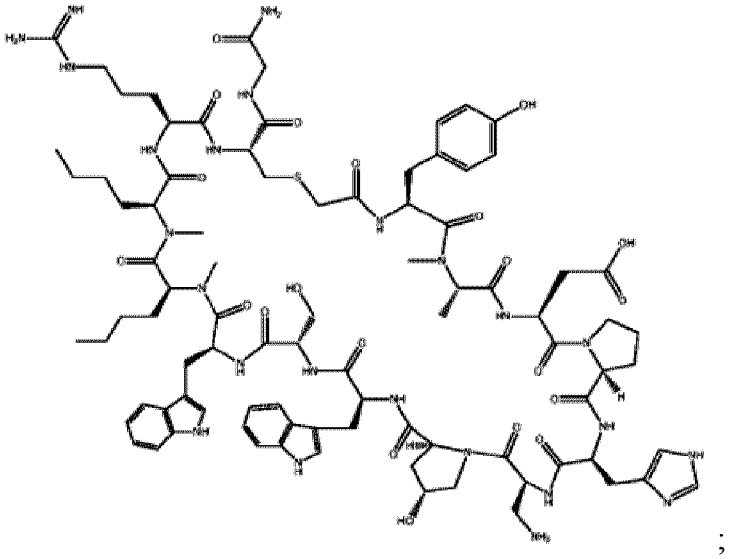

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or a pharmaceutically acceptable salt thereof.

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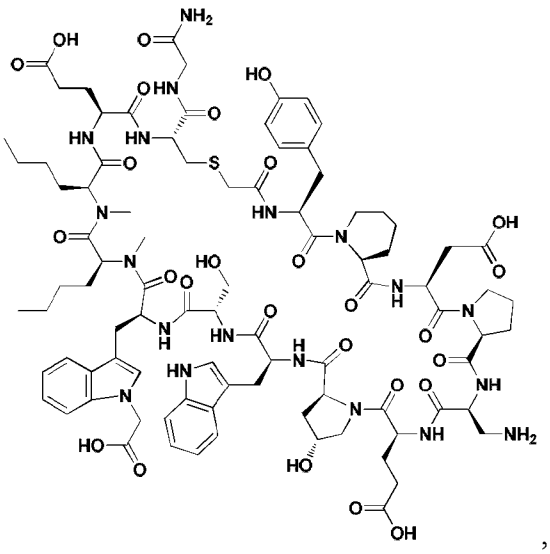
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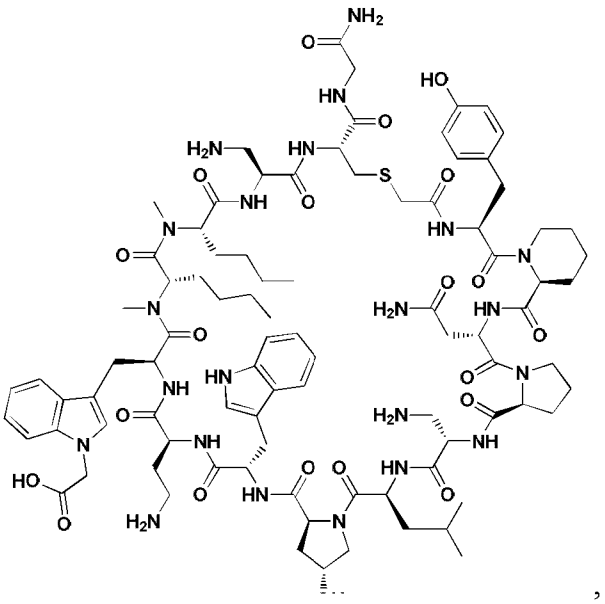
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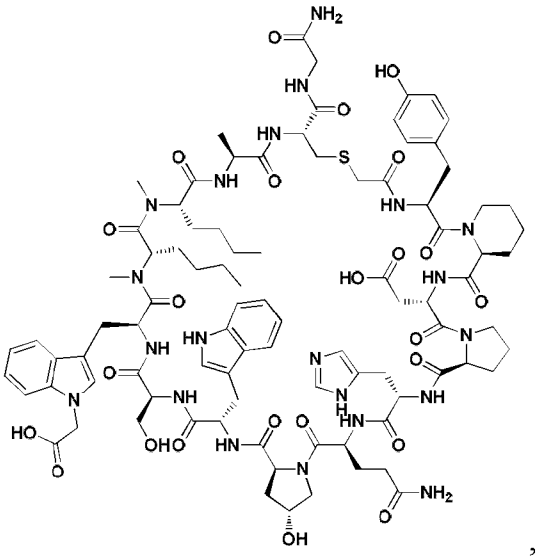
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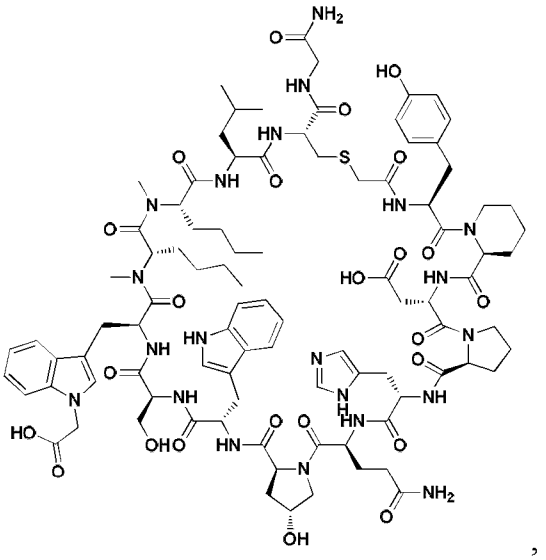
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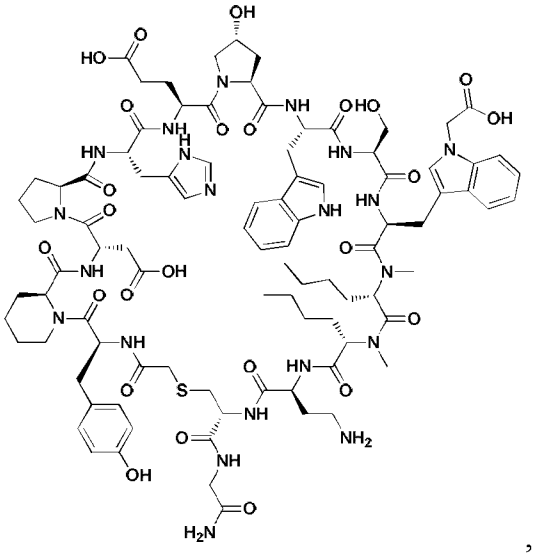
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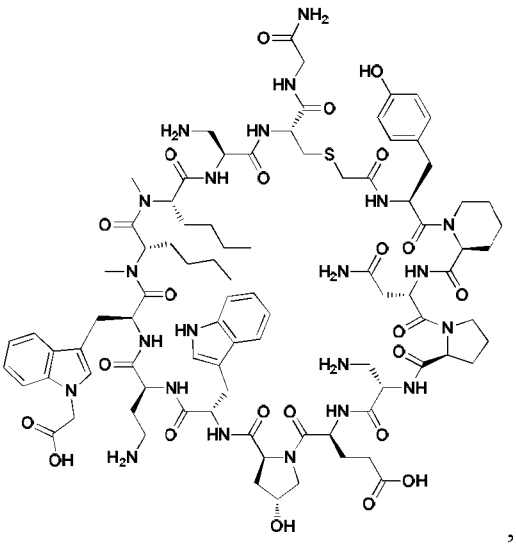
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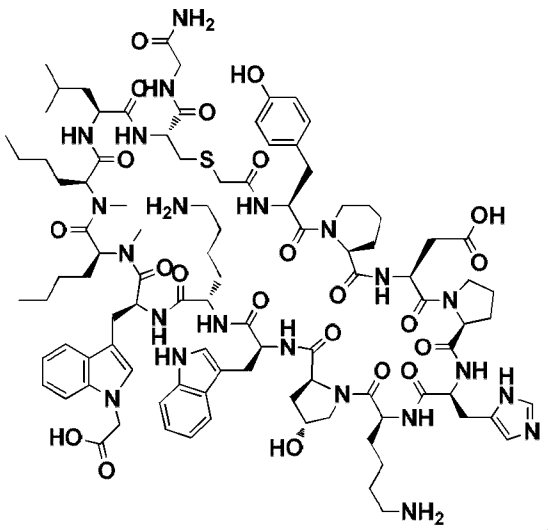
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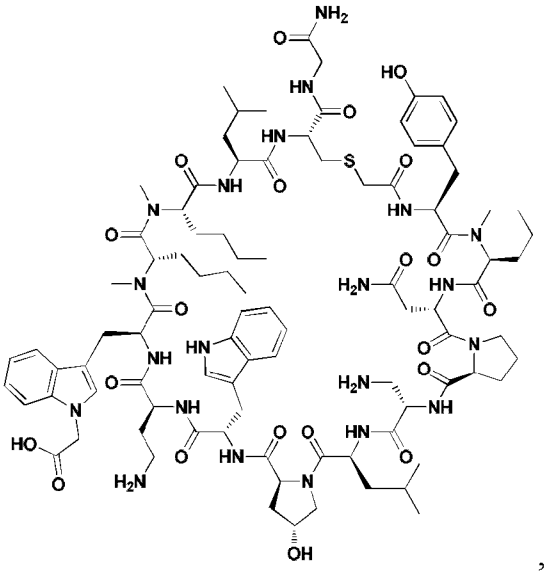
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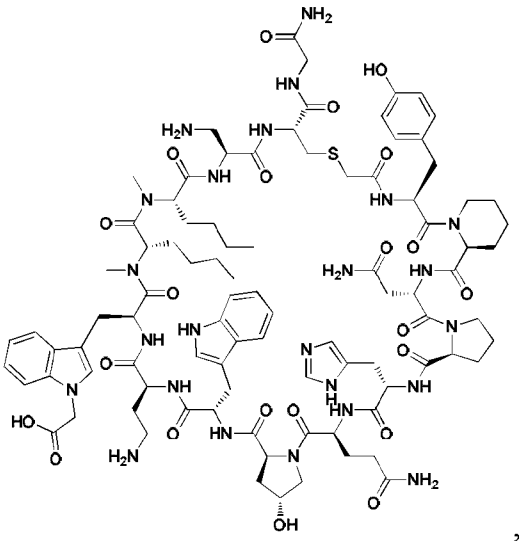
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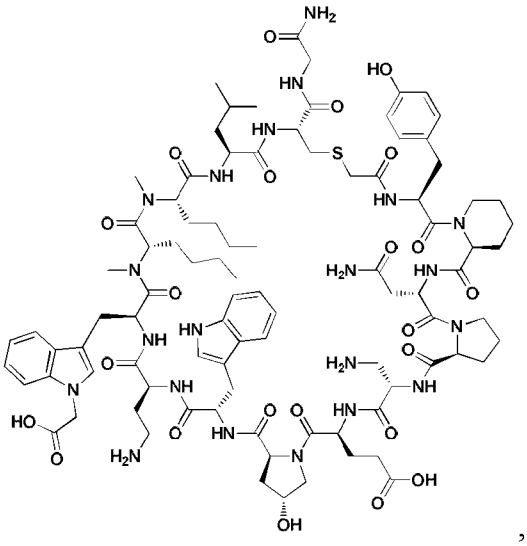
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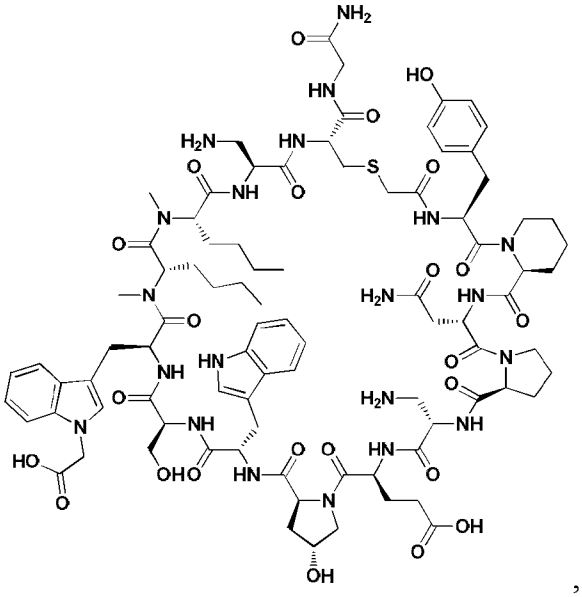
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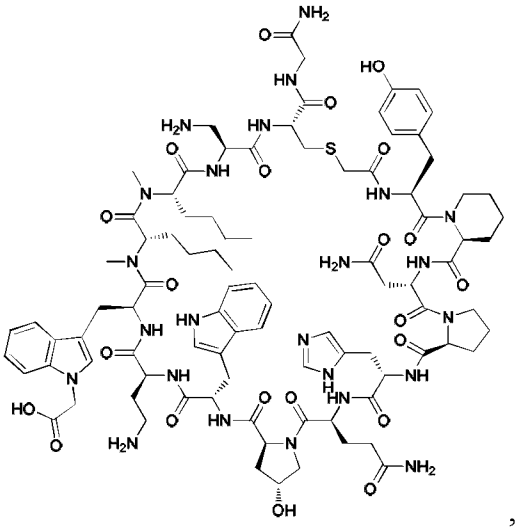
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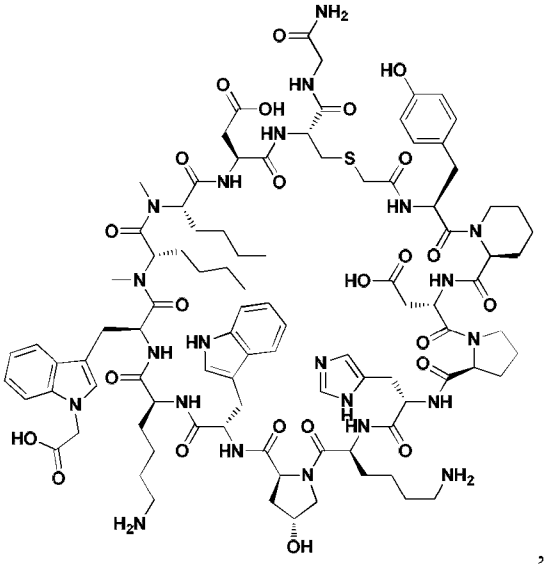
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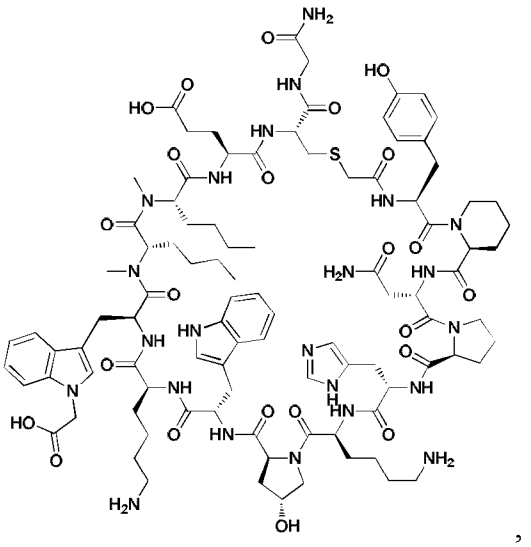
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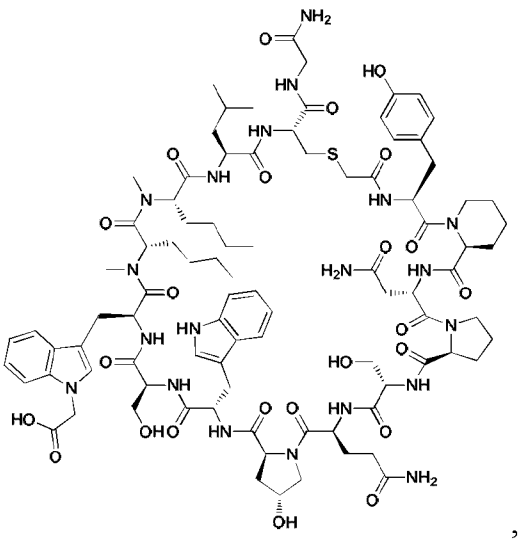
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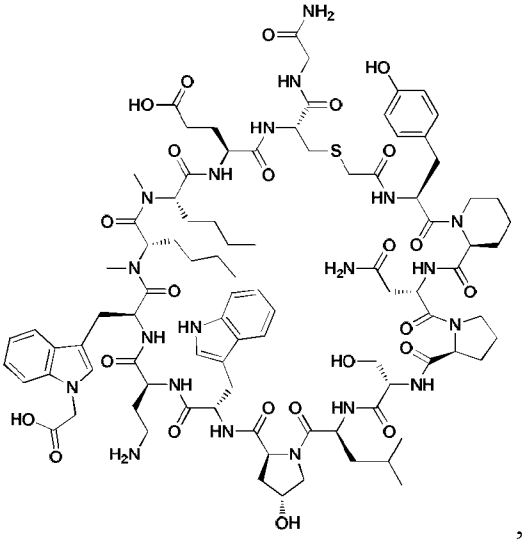
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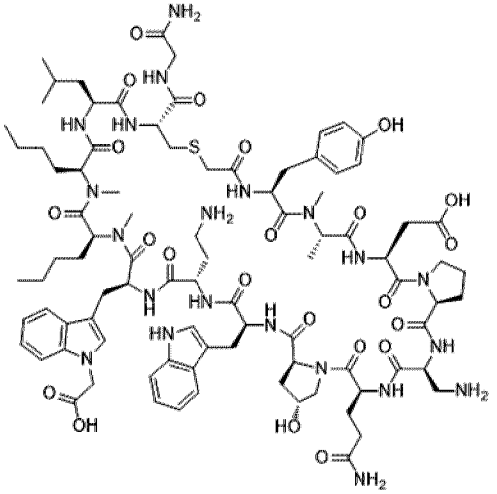
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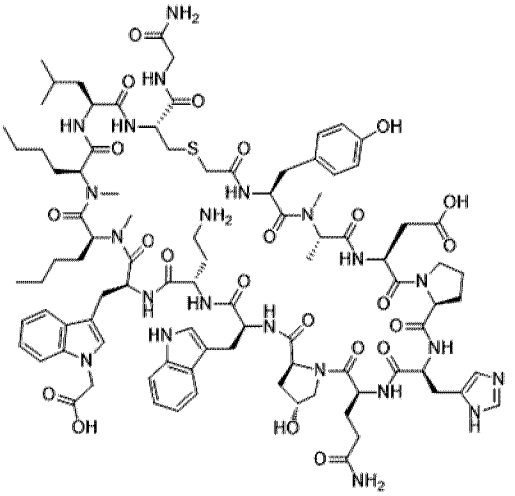
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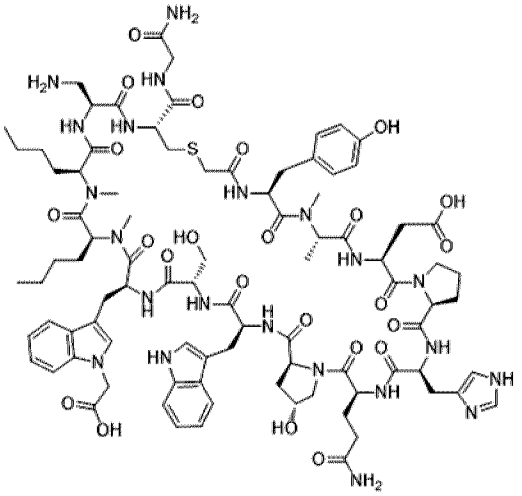
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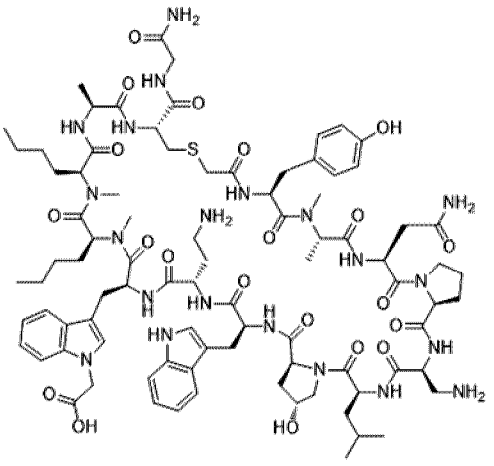
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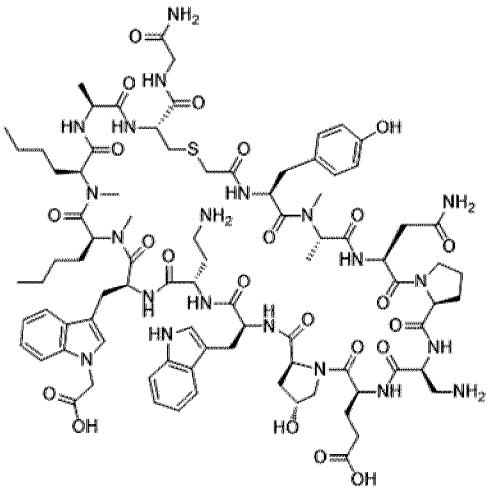
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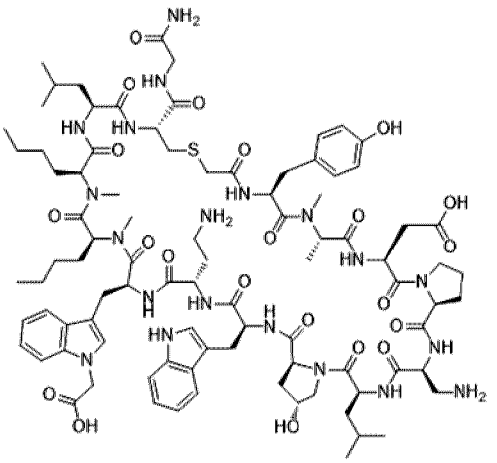
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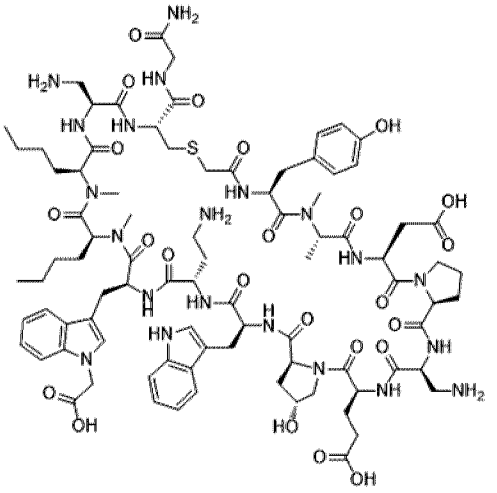
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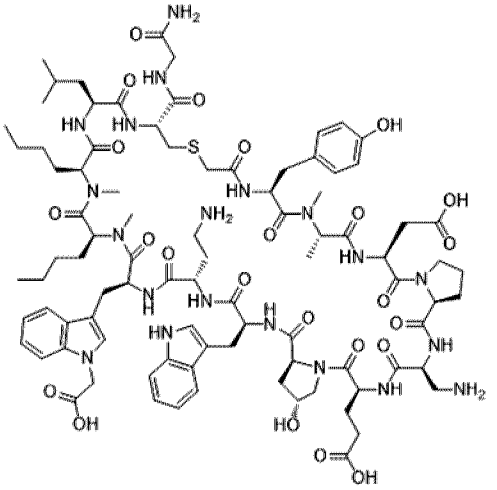
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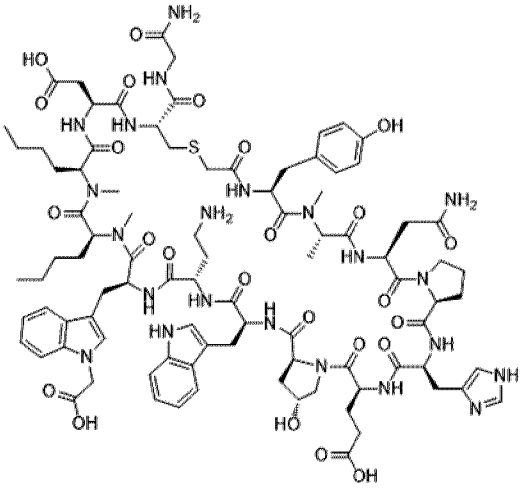
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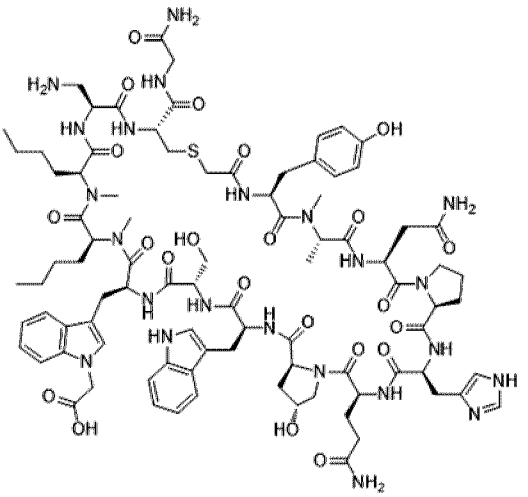
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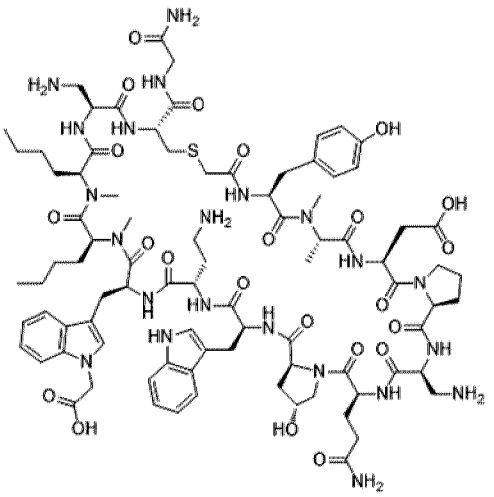
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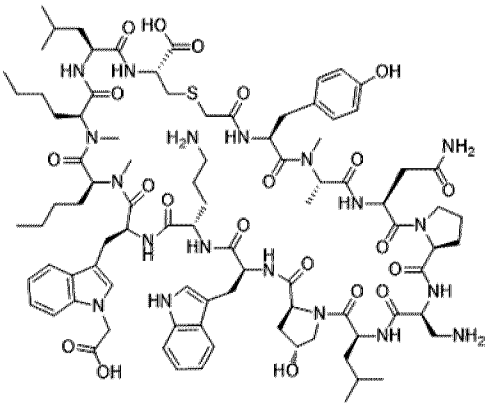
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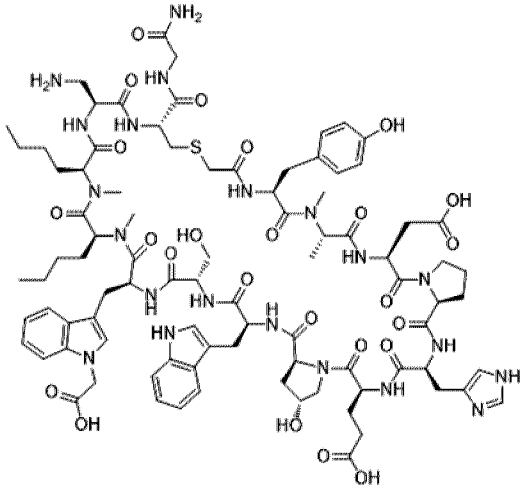
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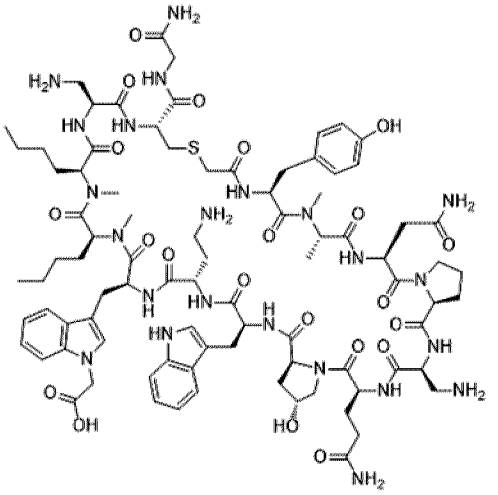
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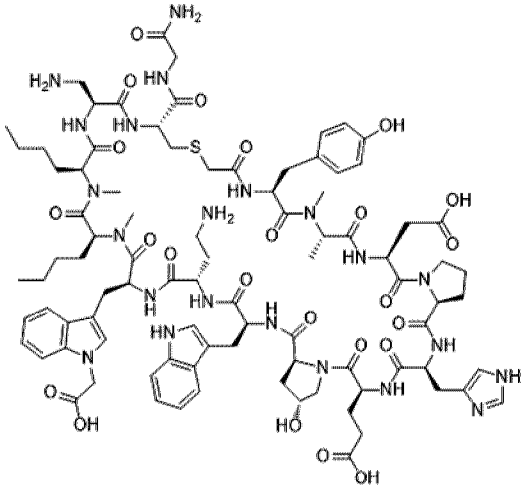
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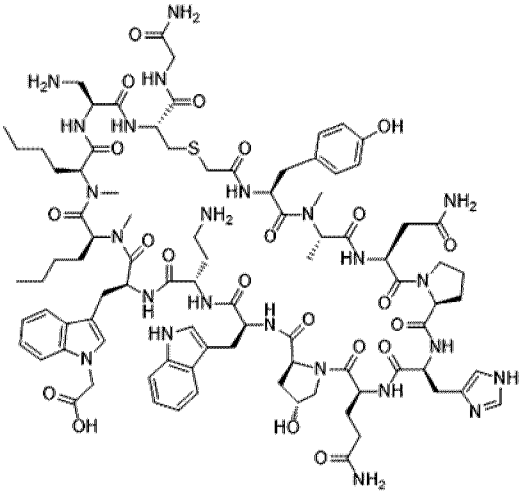
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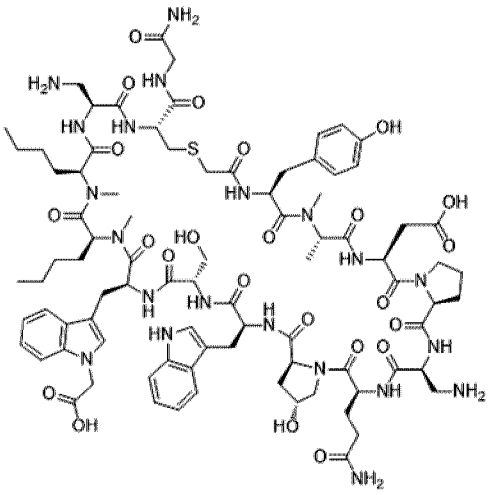

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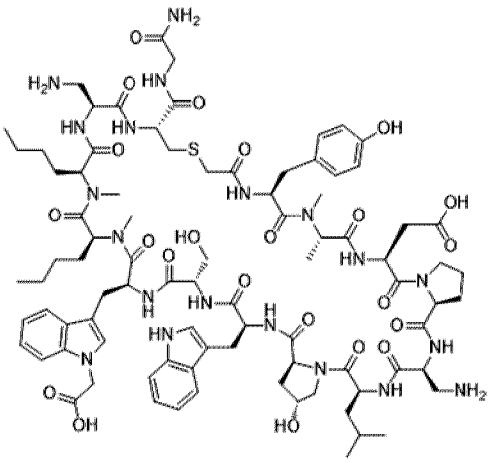

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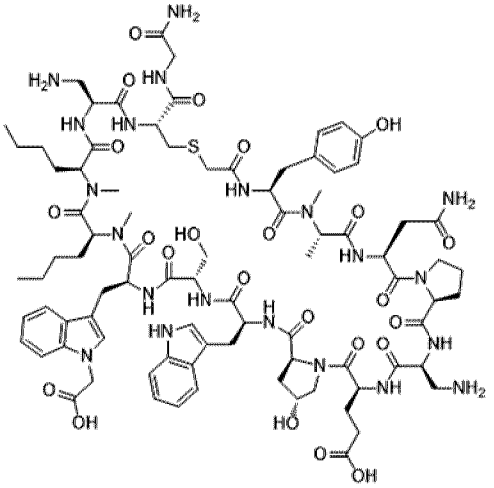

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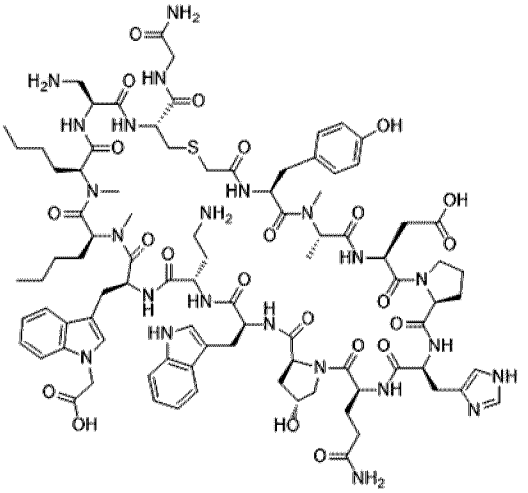

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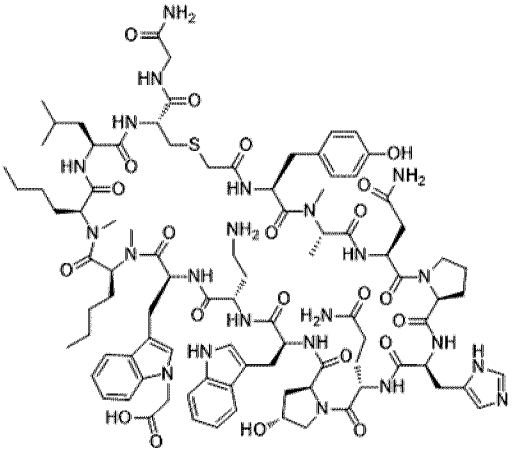

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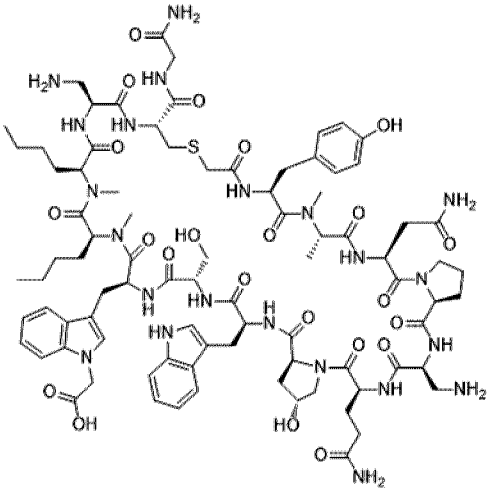

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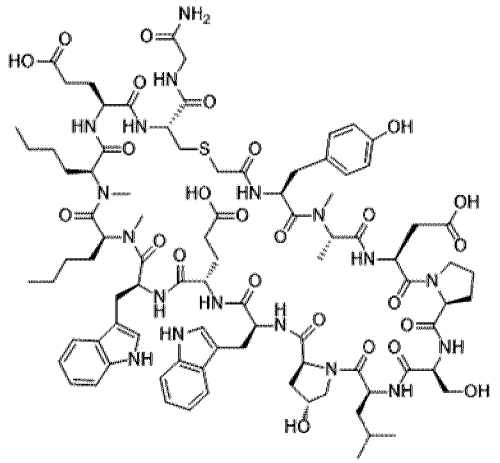

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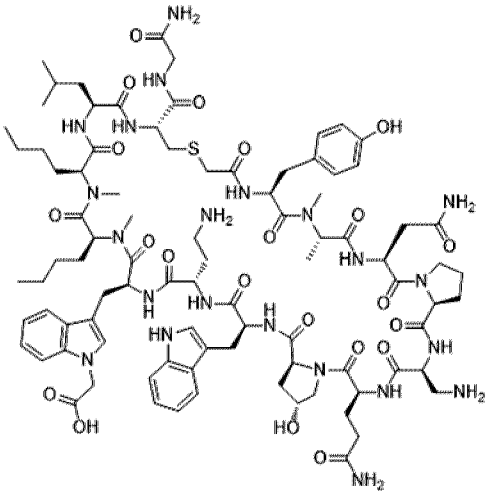

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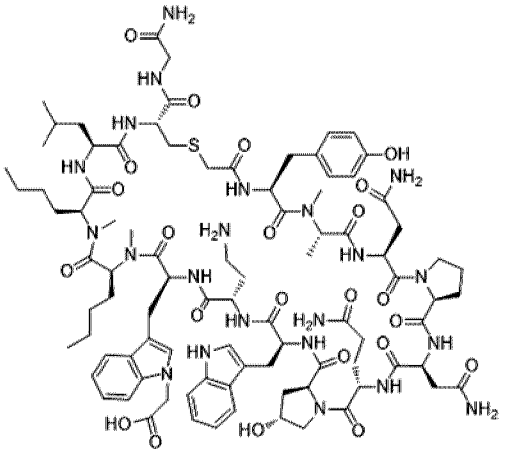

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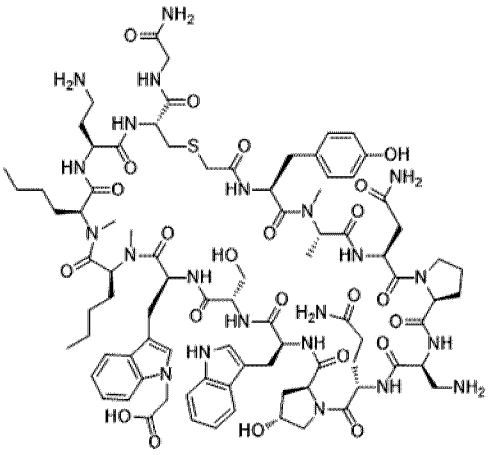

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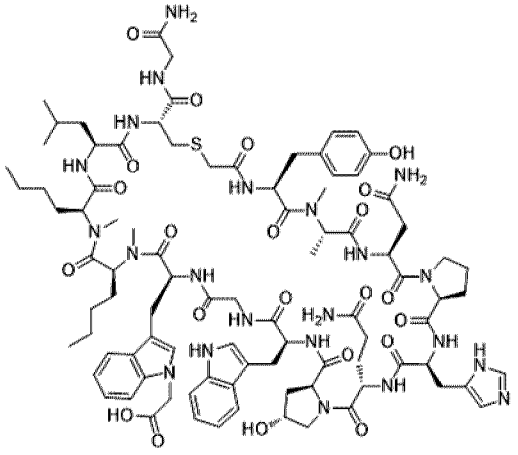

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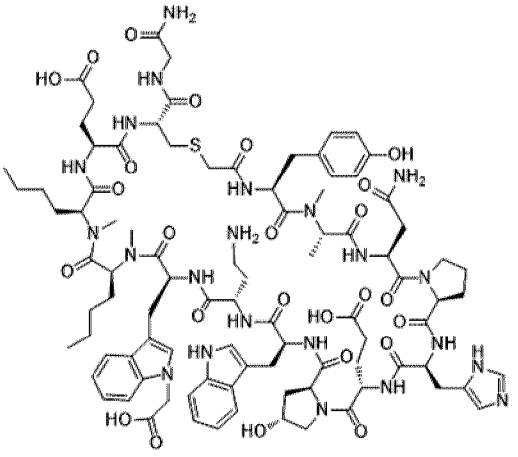

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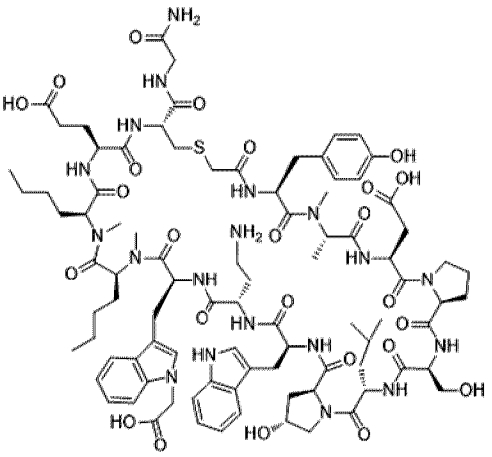

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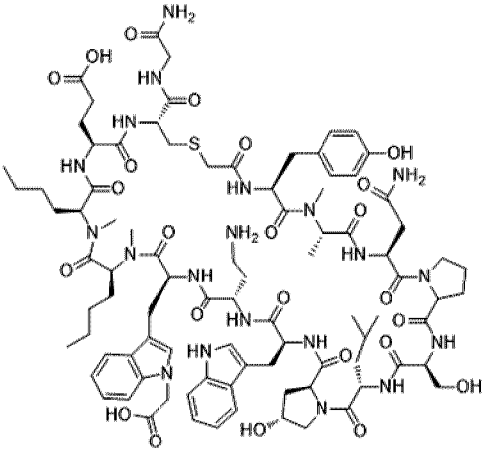

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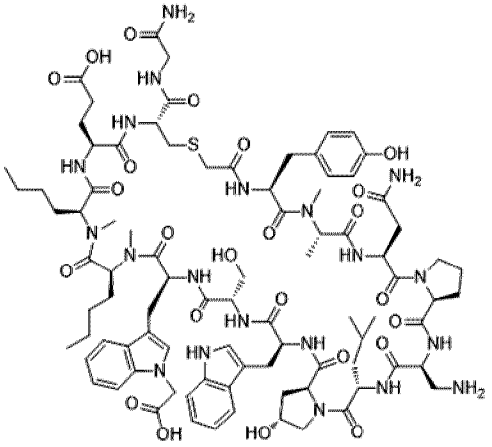

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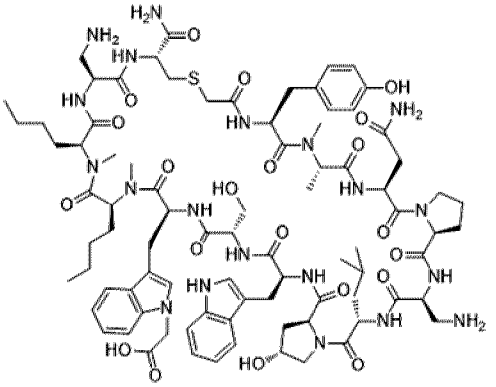

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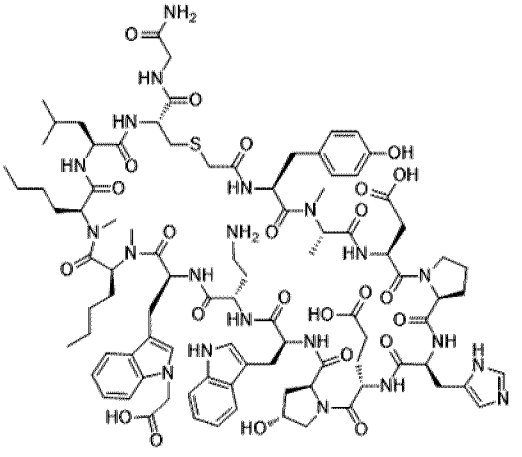

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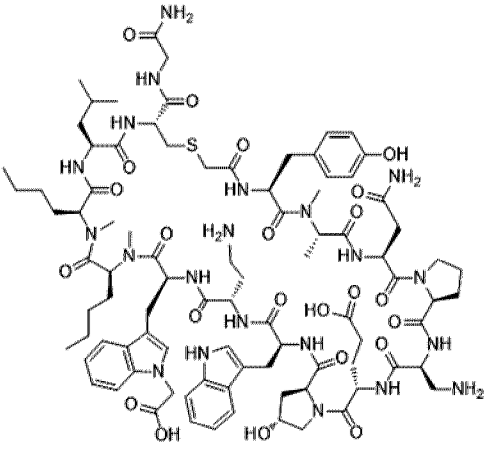

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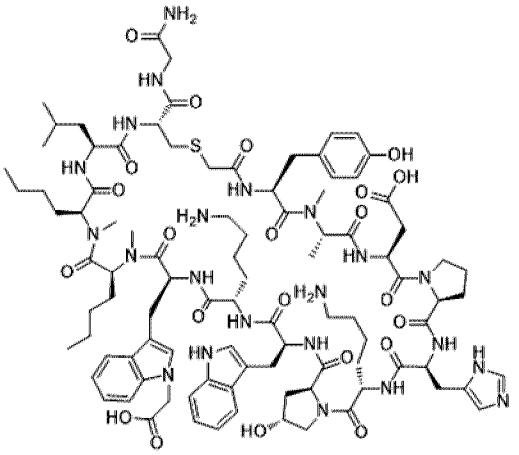
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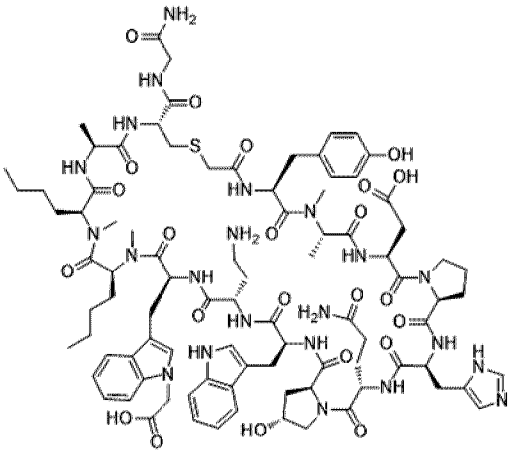
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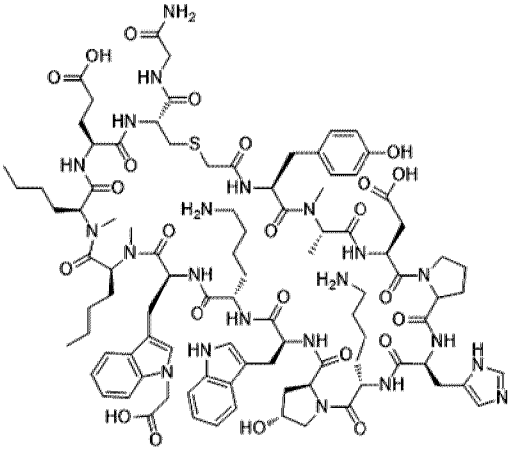
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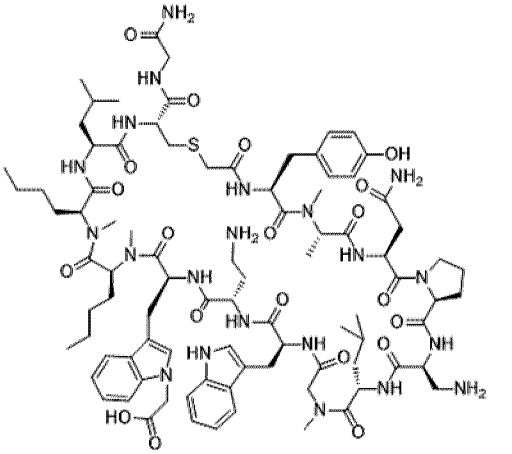
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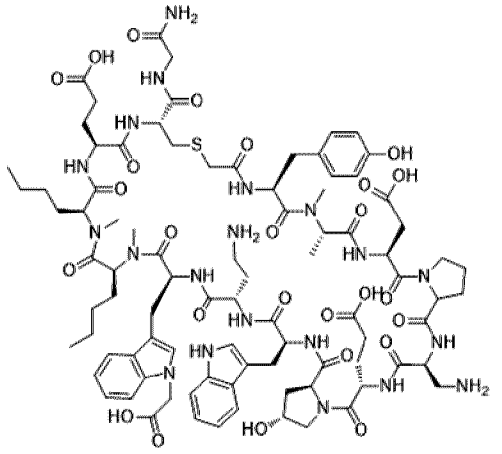
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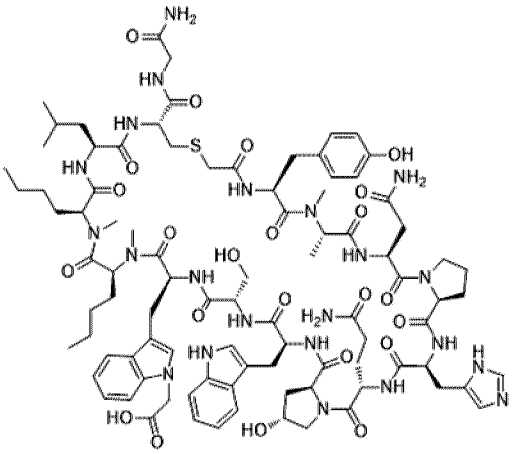
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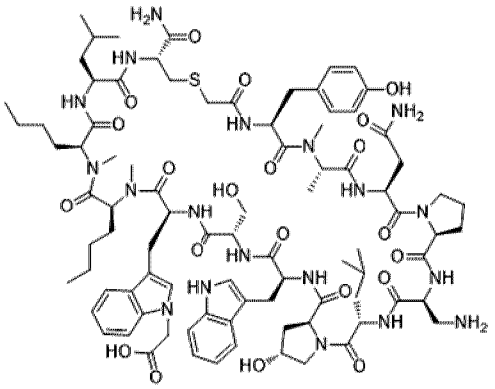

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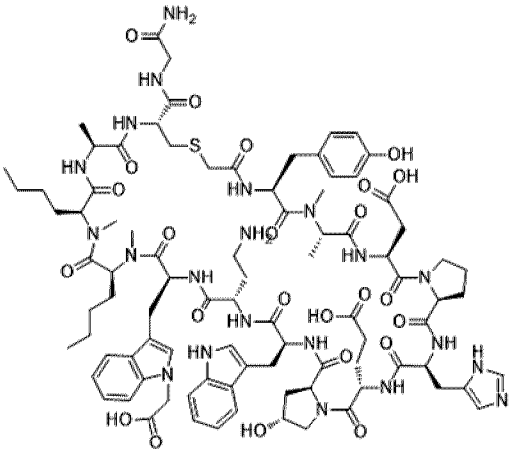

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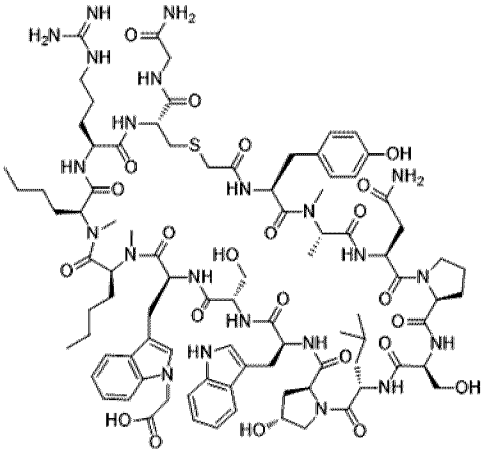

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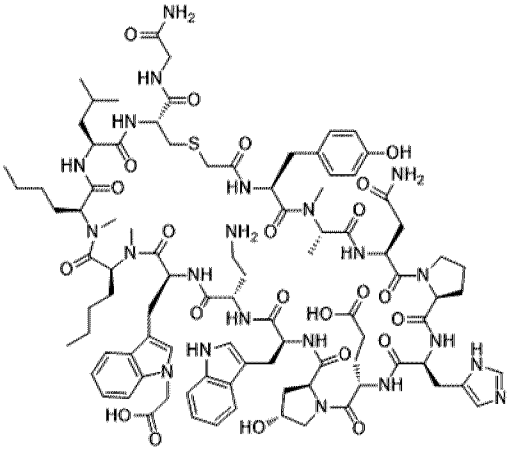

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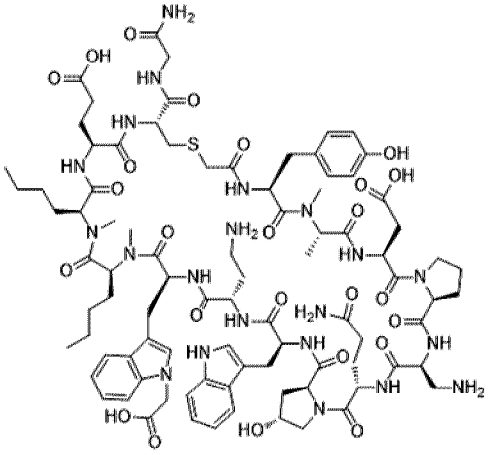

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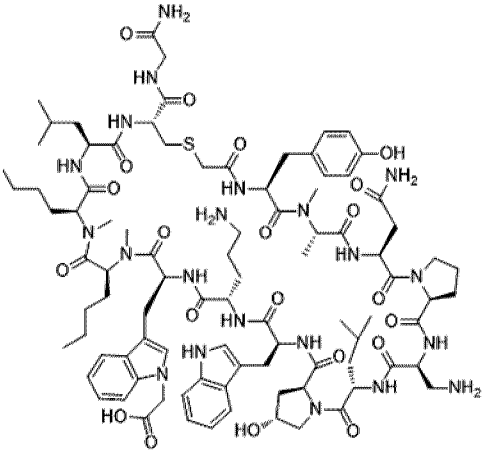

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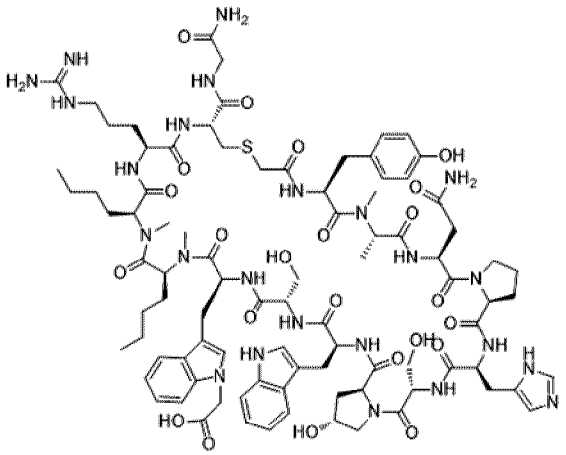

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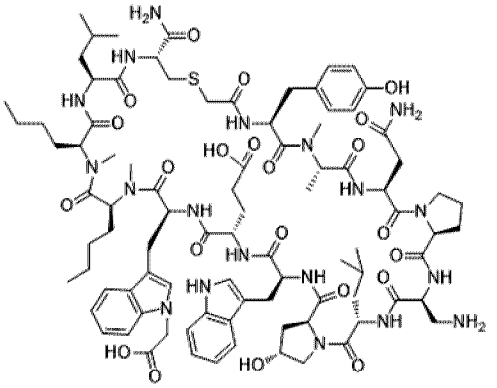

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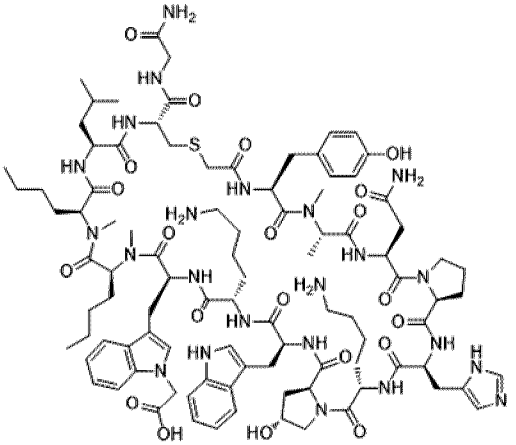

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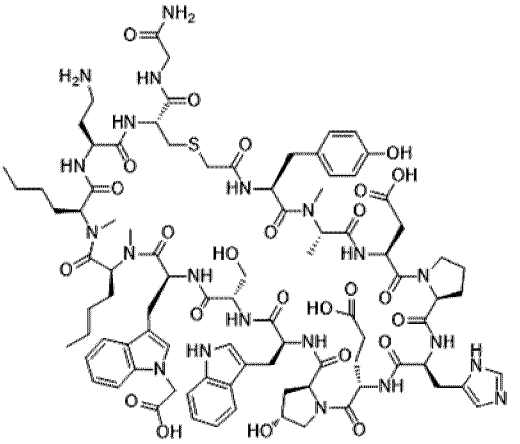

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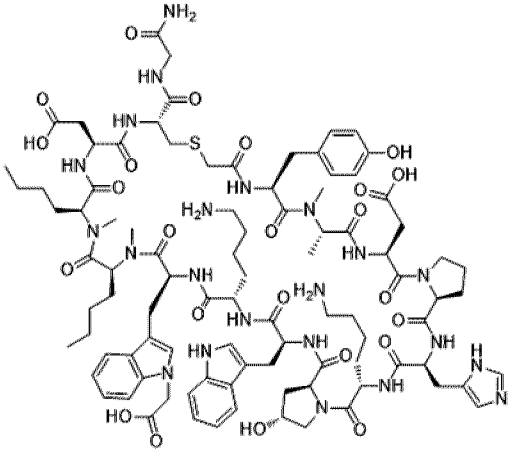

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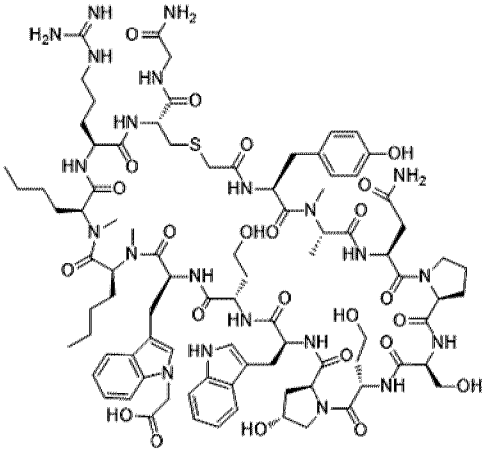

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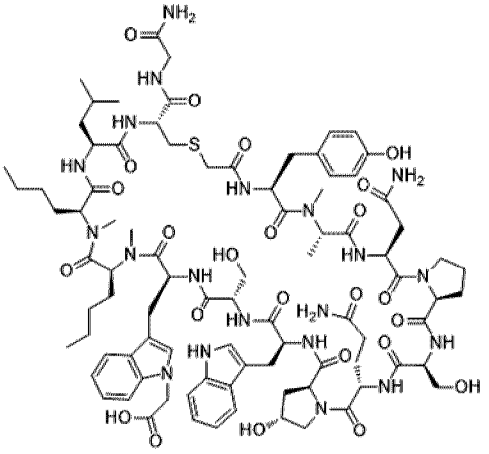

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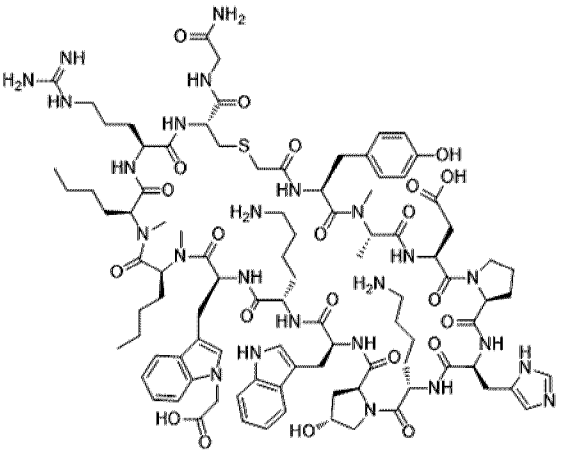

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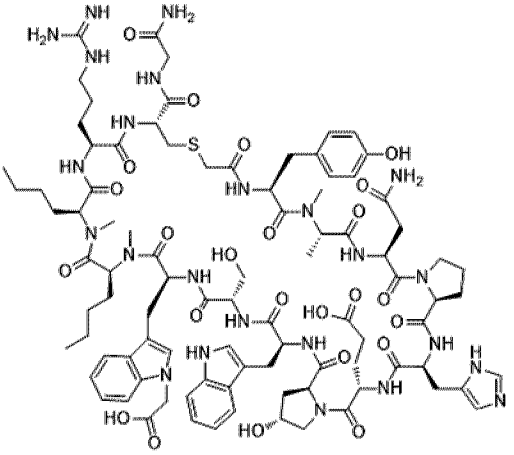

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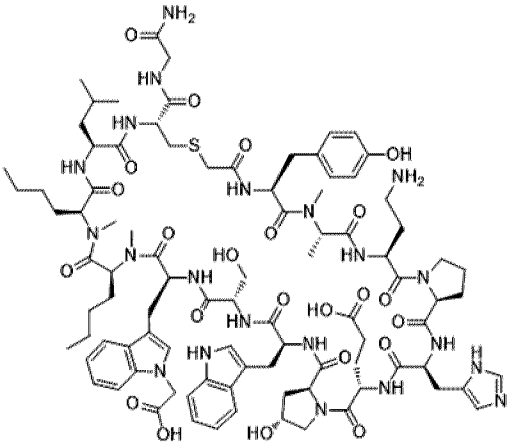

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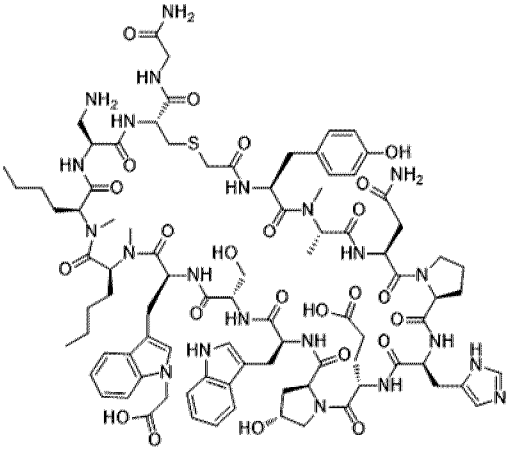

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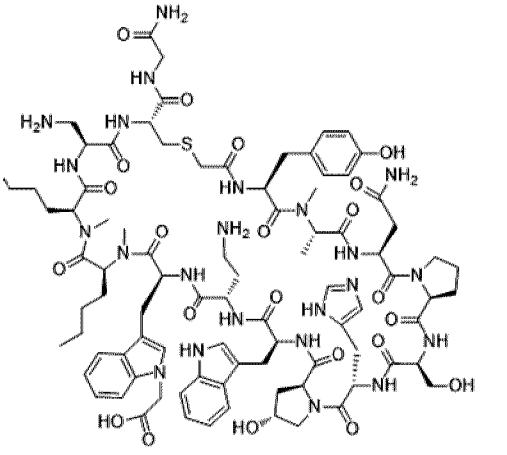

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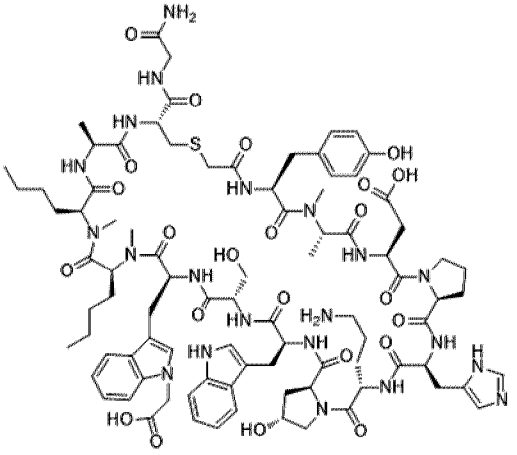

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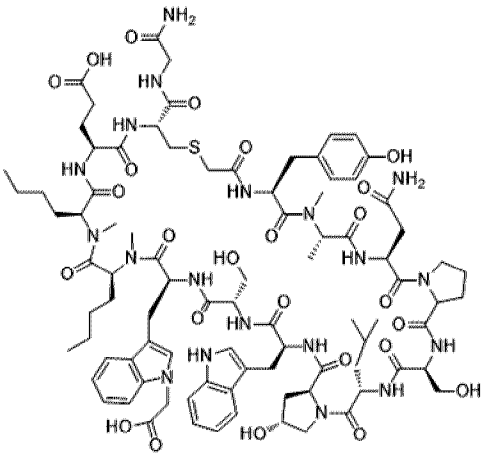

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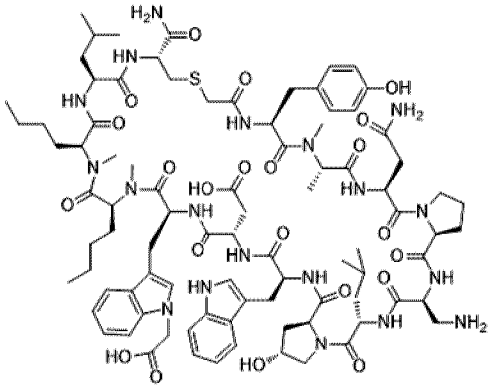

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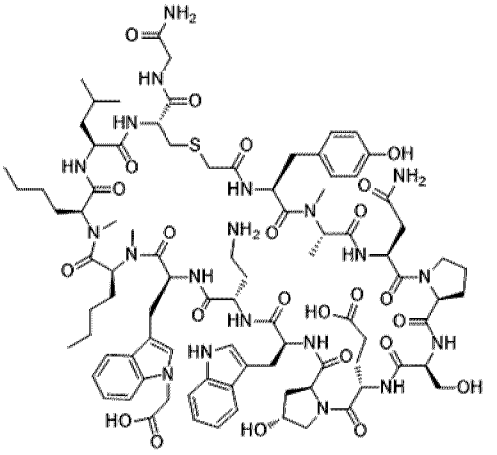

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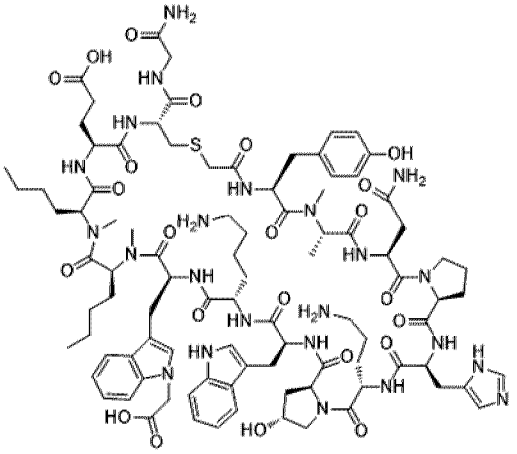

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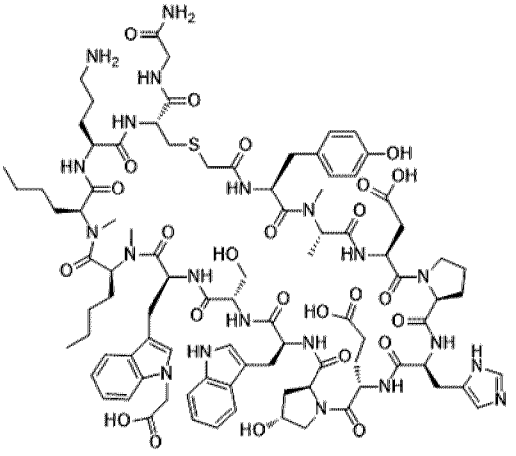

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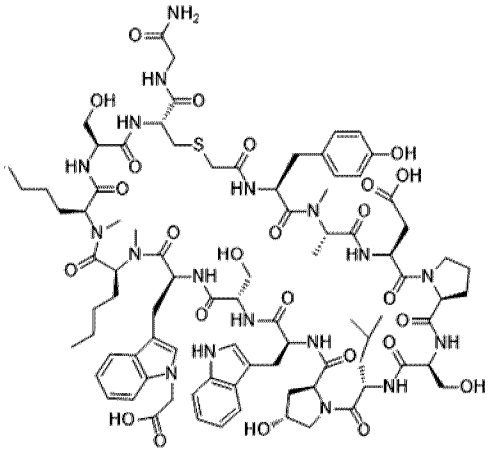
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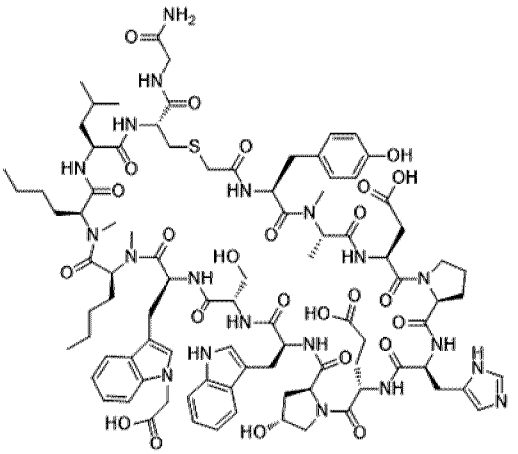
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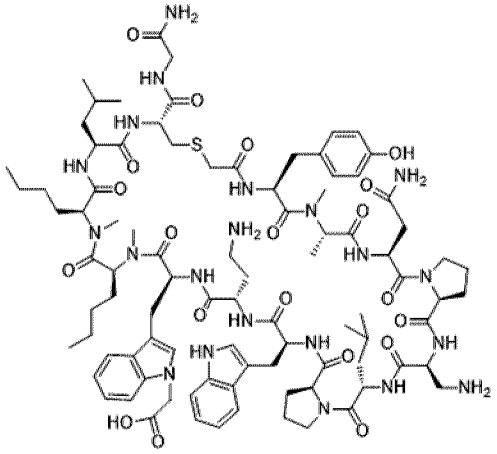
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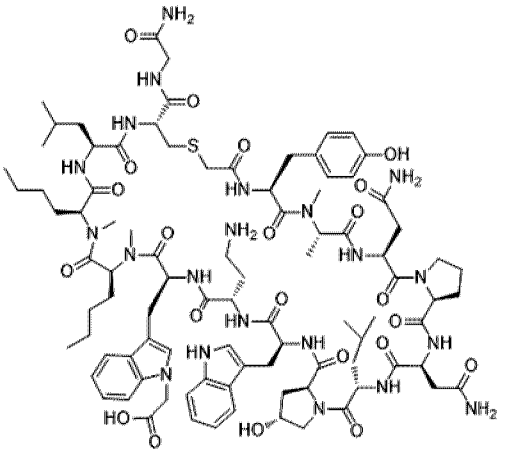
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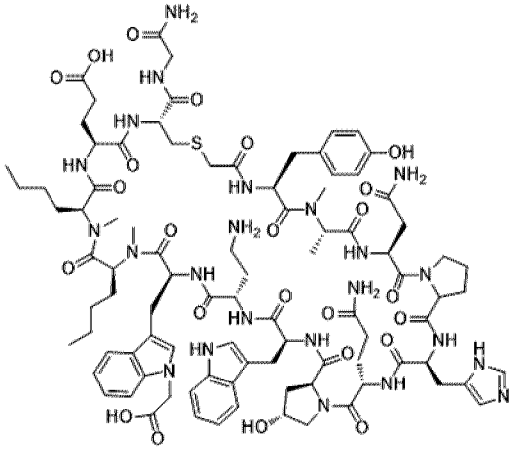
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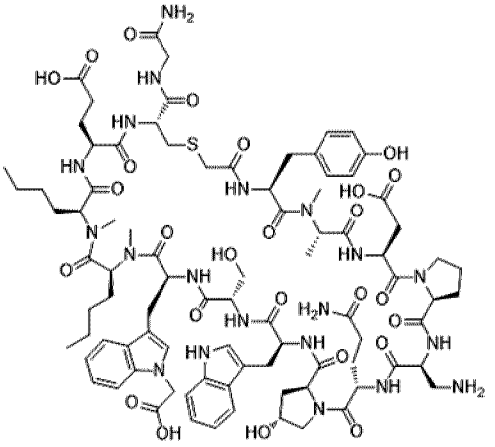
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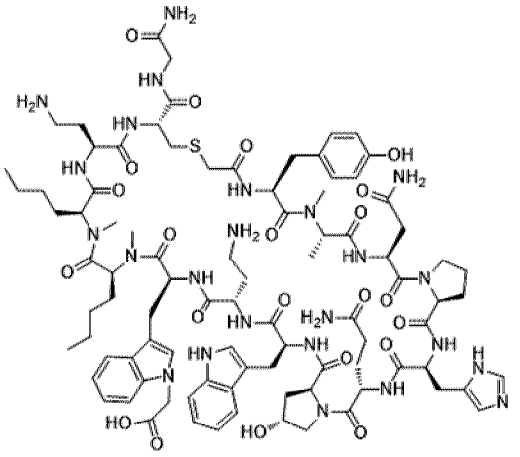
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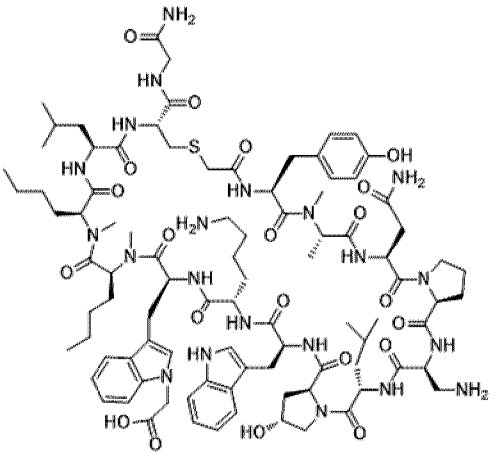
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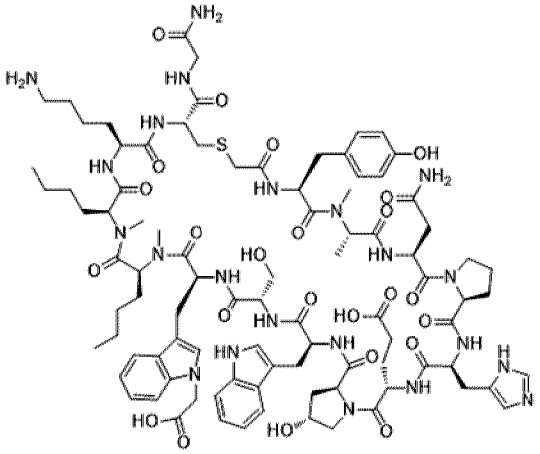
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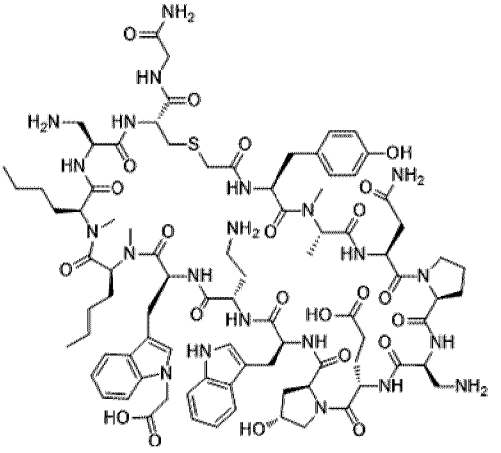
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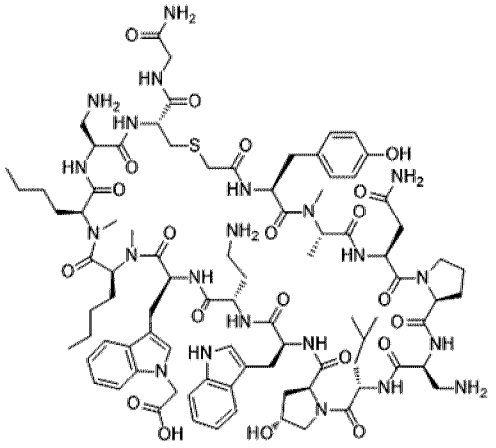
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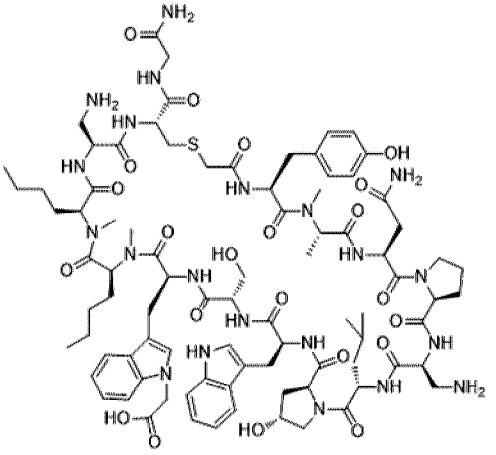
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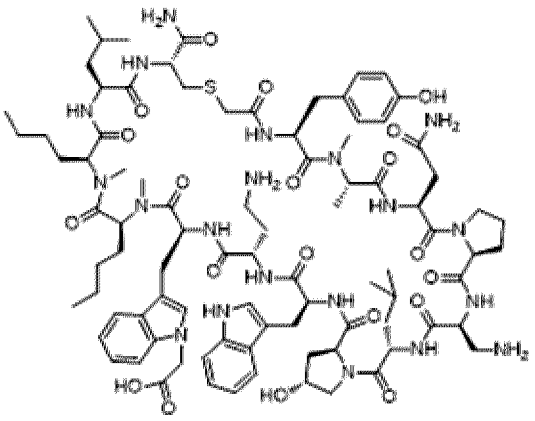

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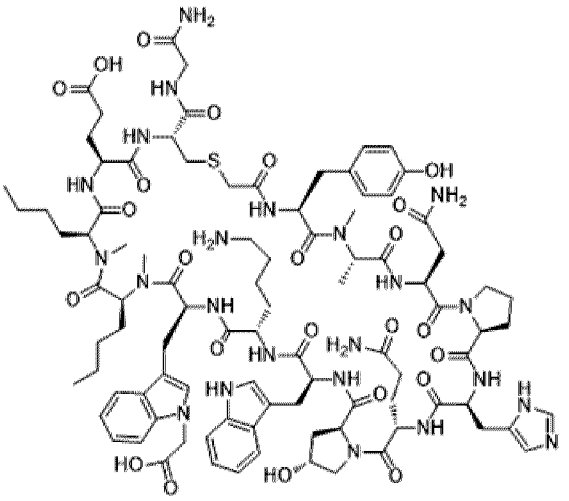

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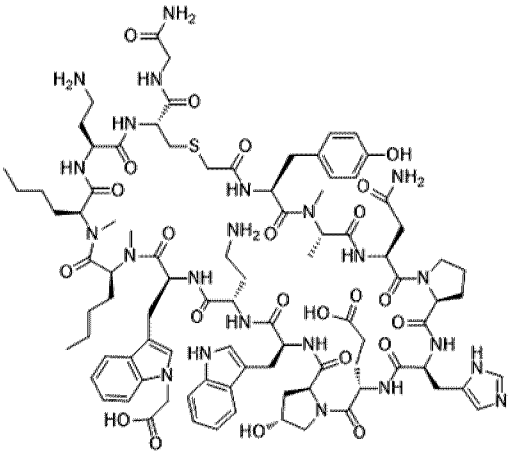

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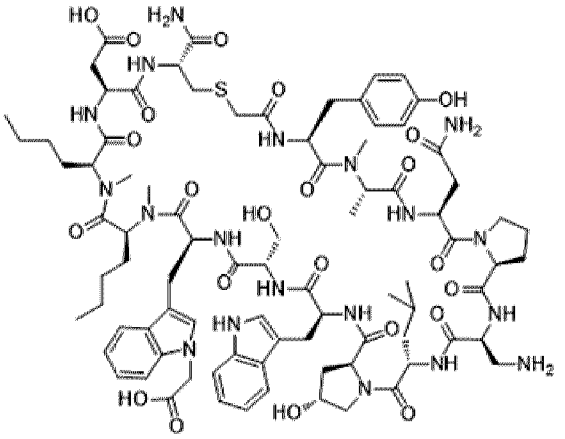

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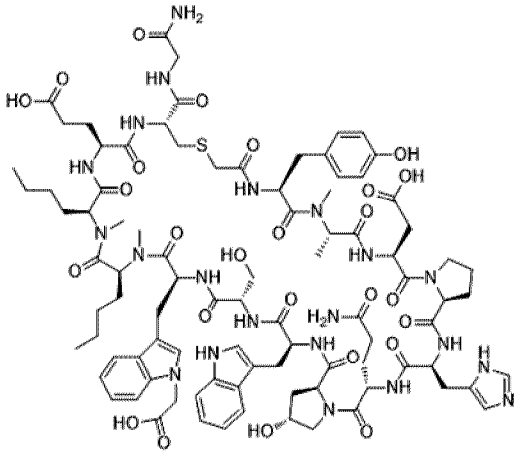

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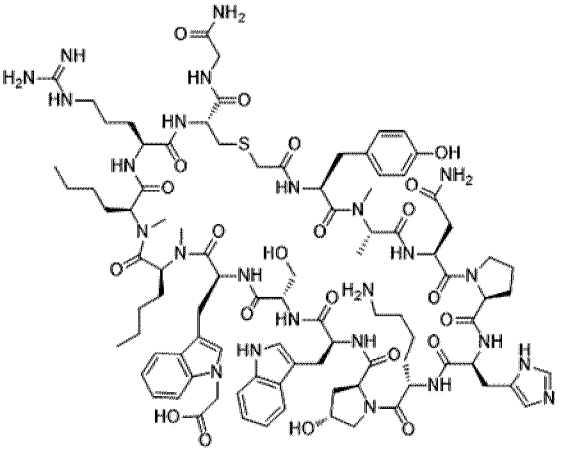

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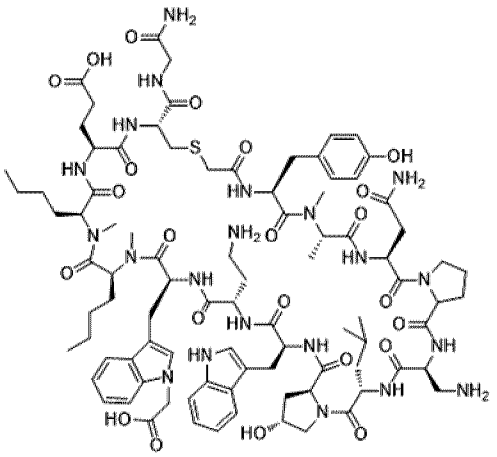

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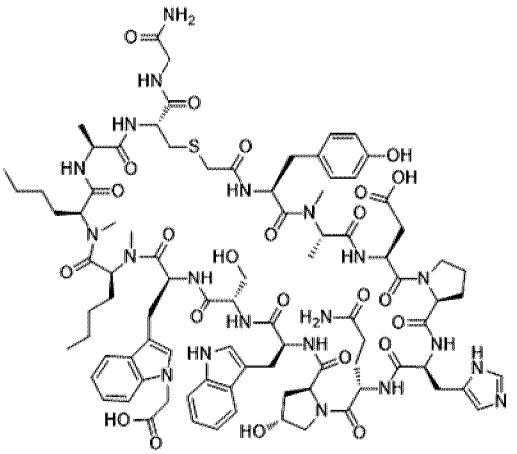

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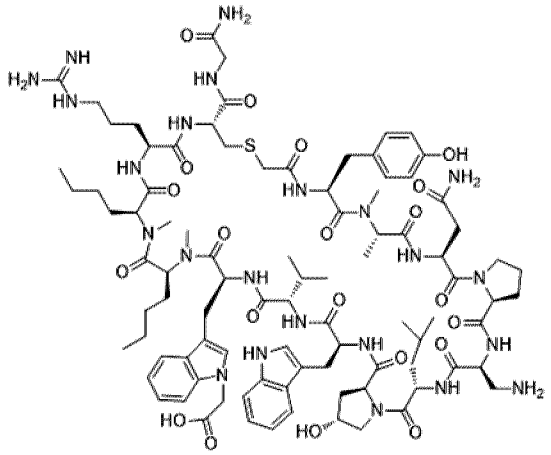

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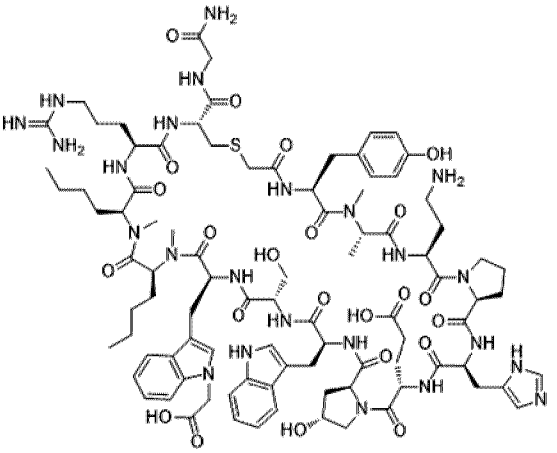

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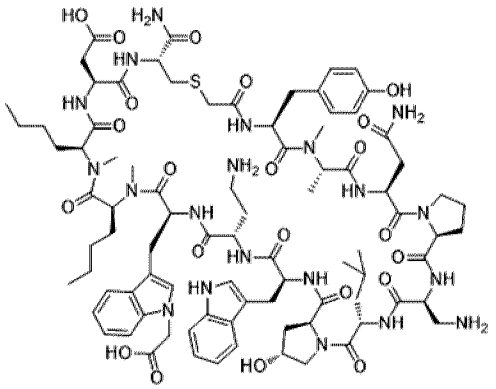

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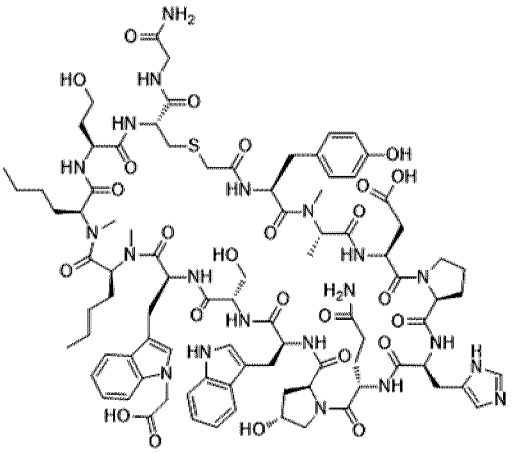

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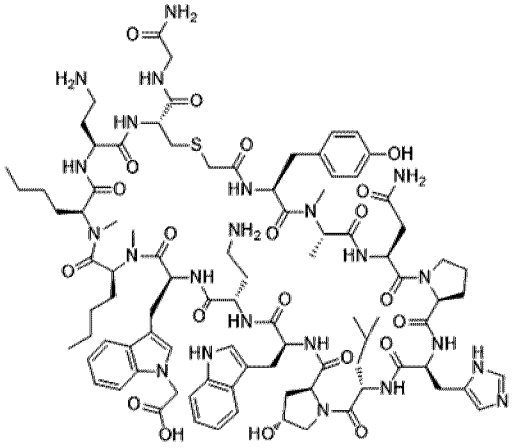

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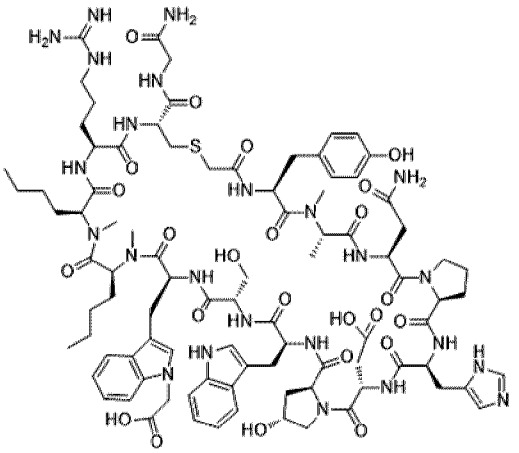

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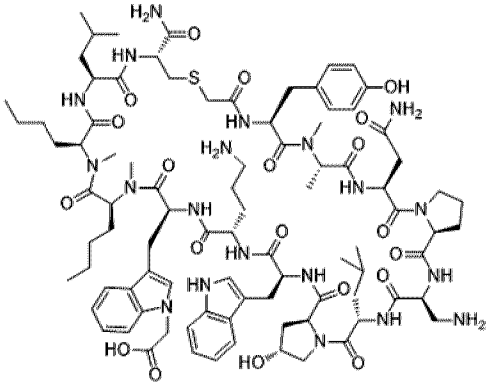

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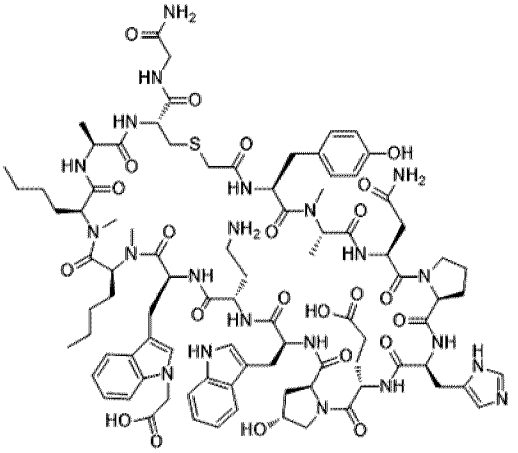

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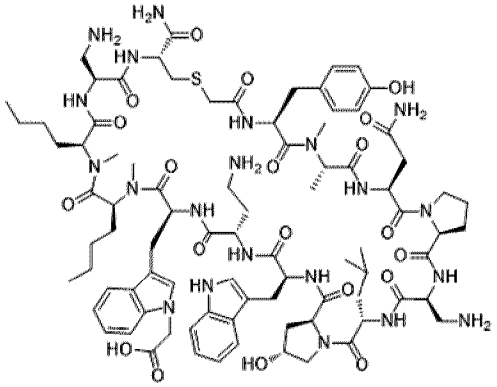

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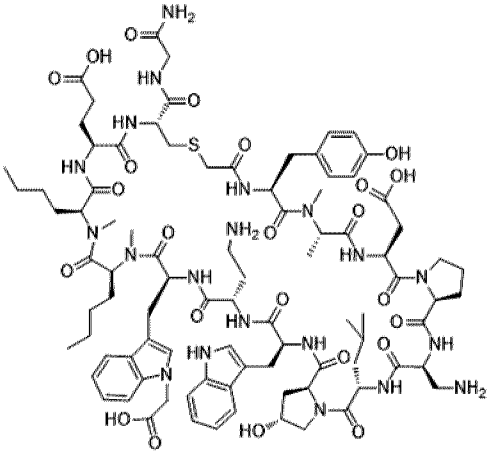

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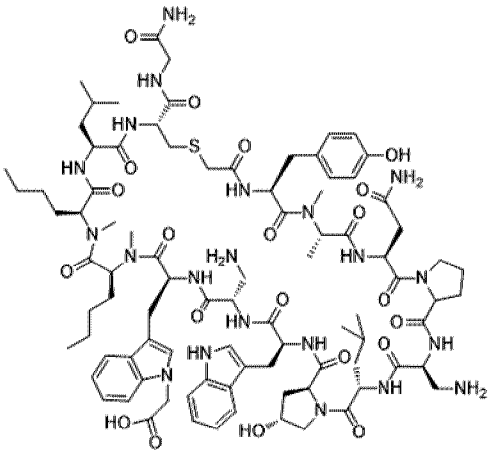

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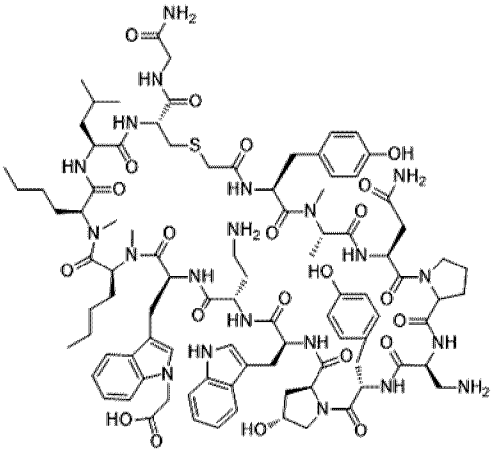

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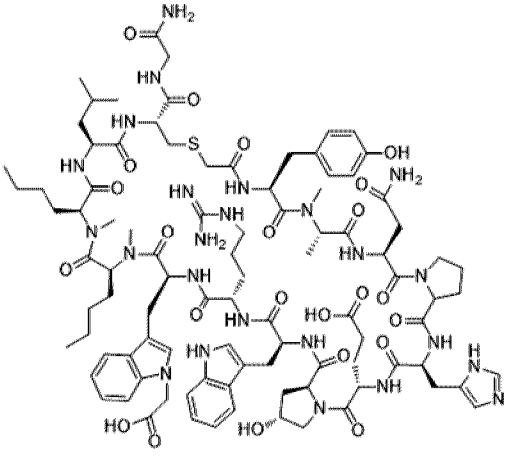

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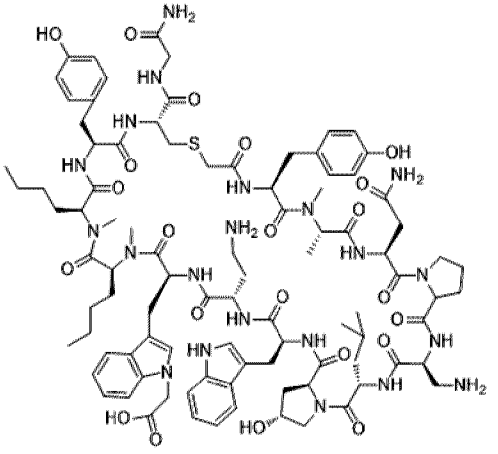

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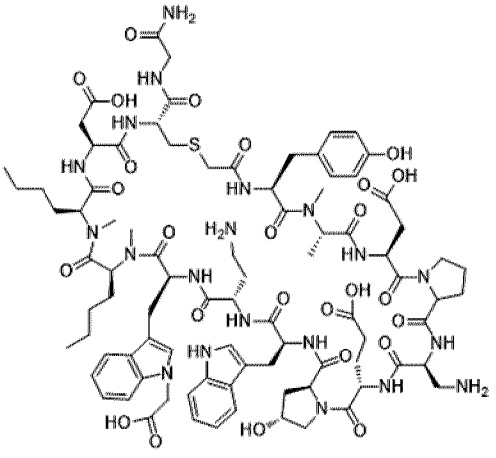

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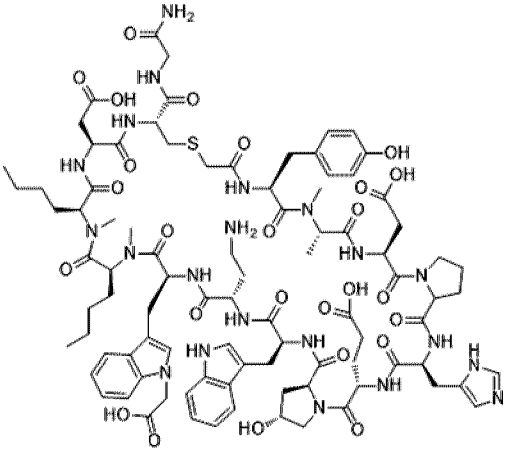

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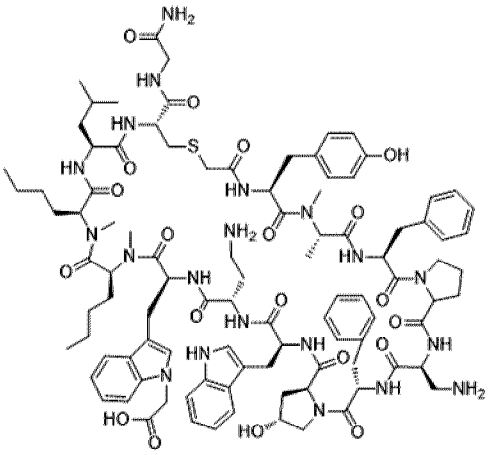

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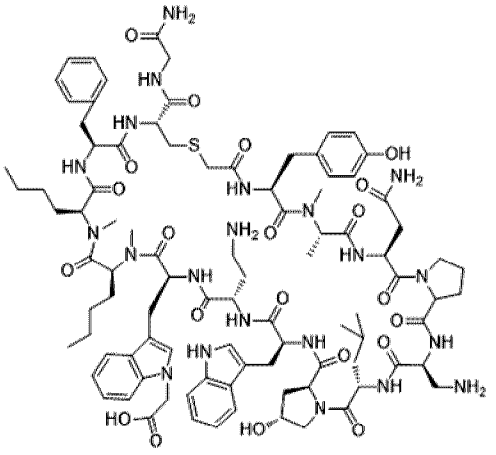

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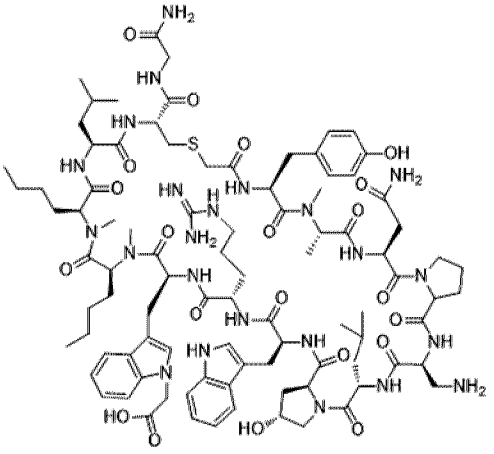

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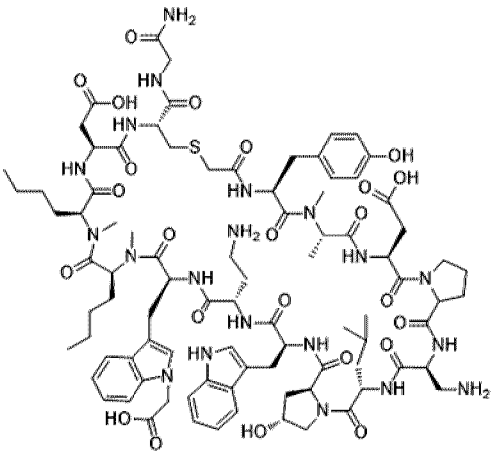

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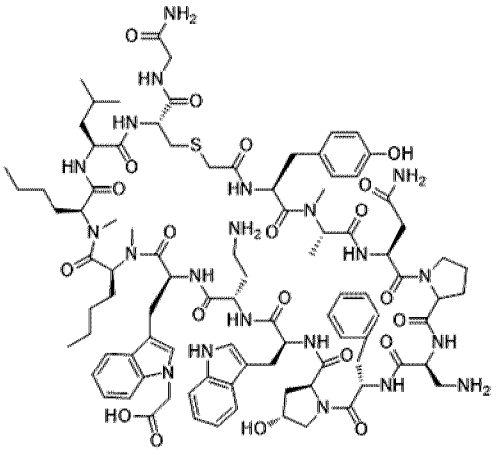

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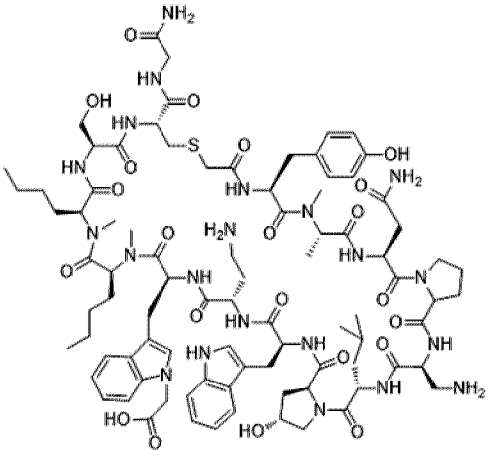

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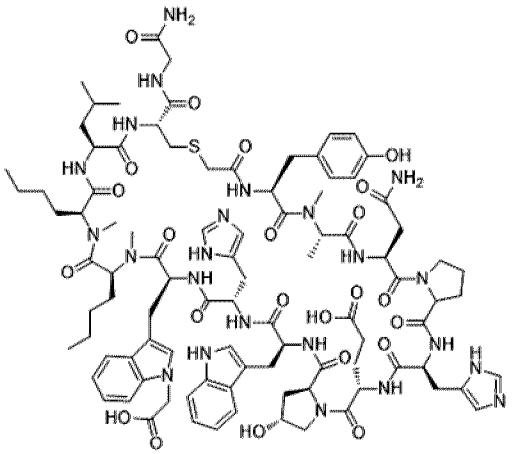

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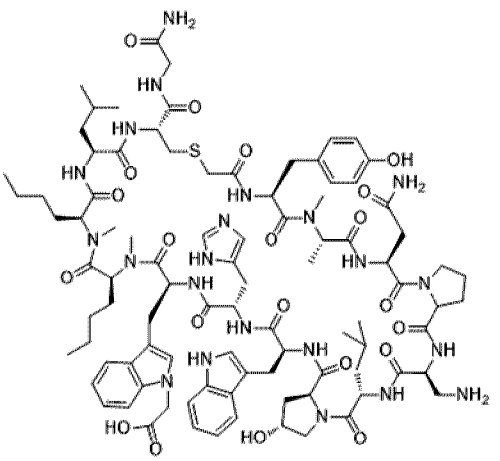

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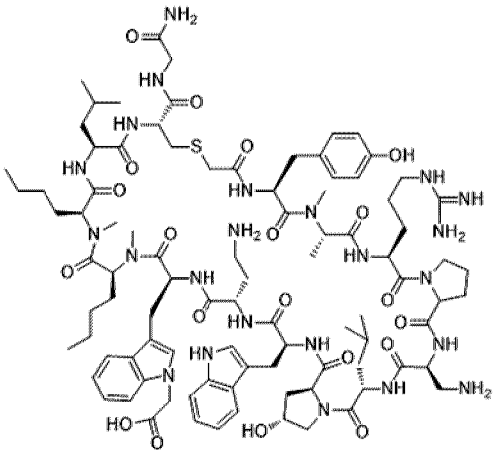

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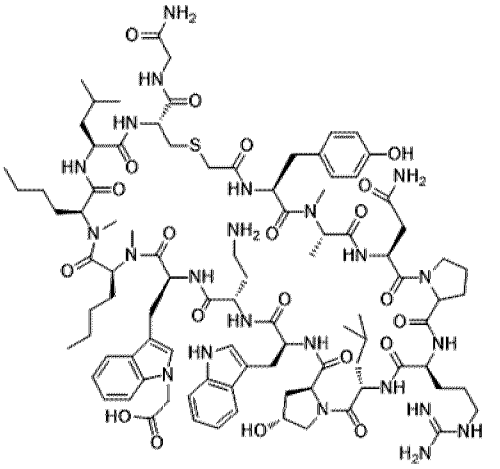

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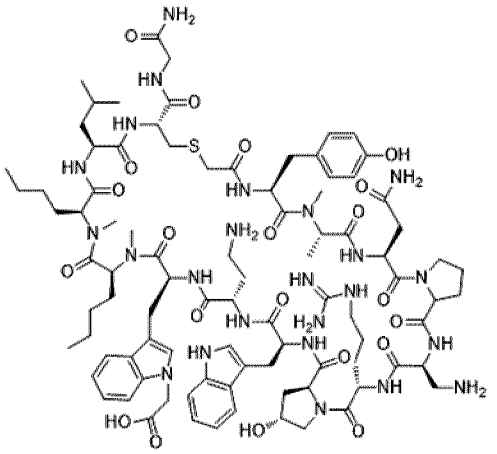

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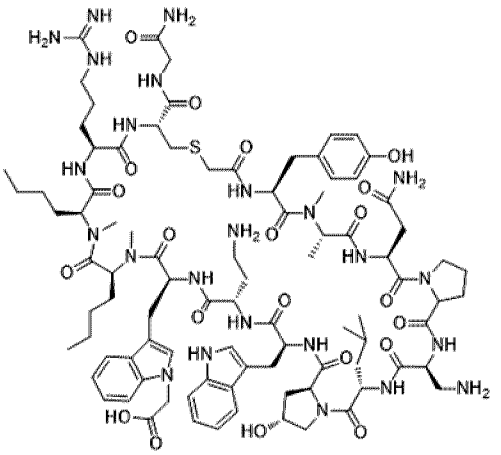

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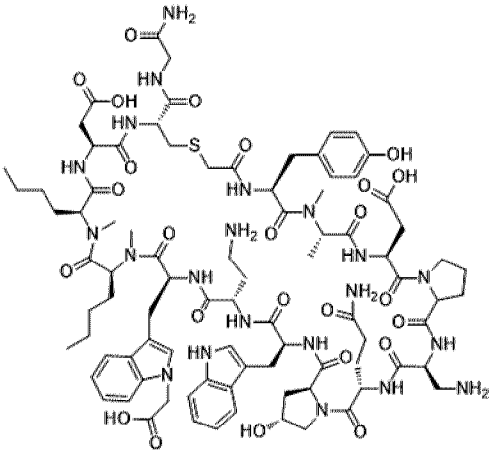

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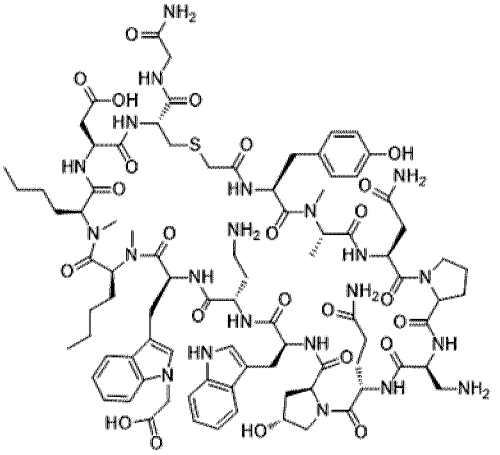

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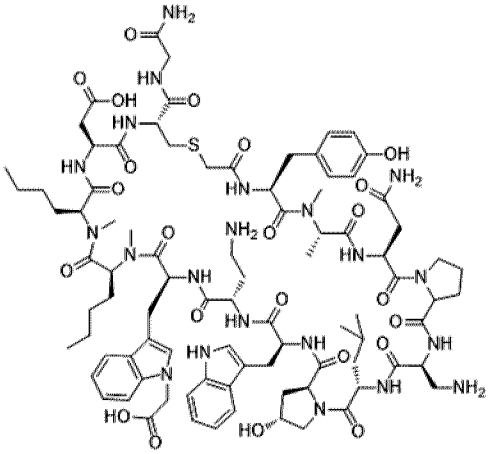

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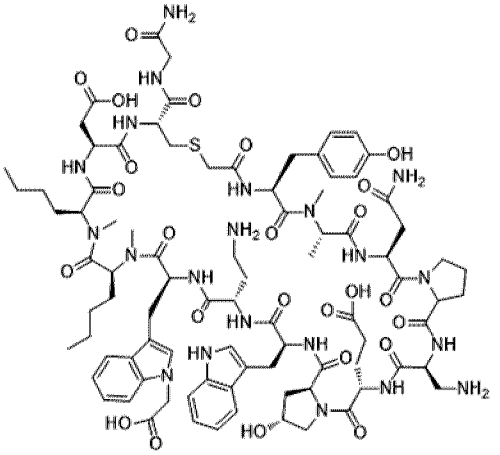

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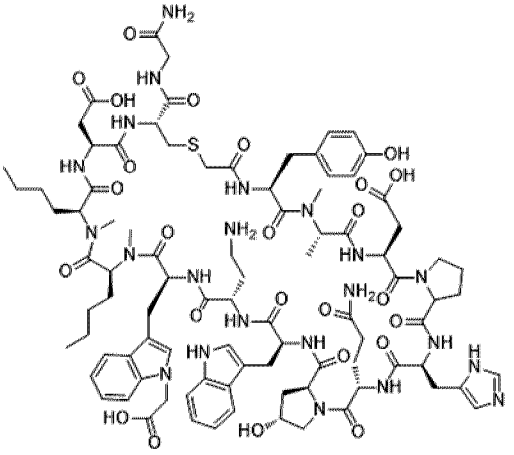

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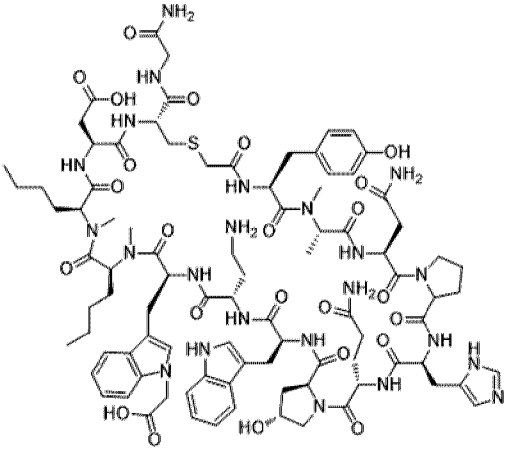

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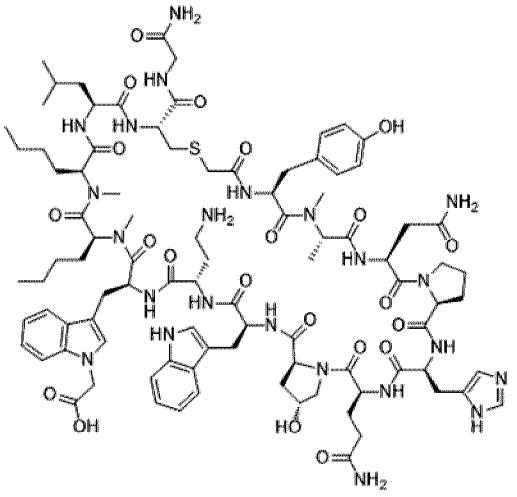

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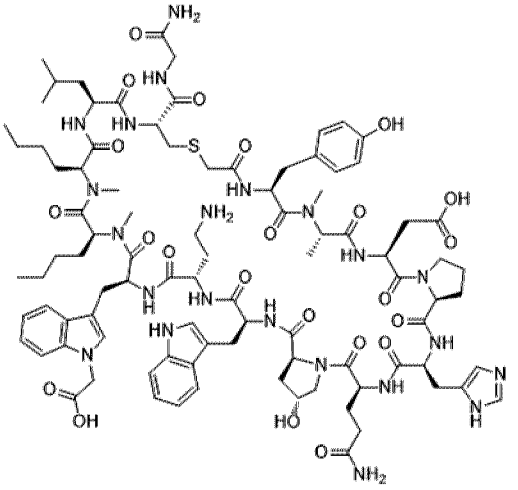

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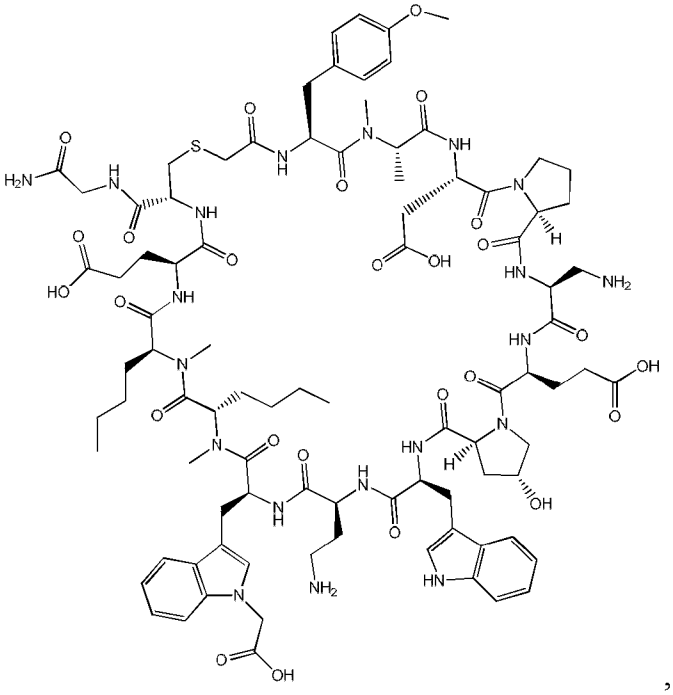

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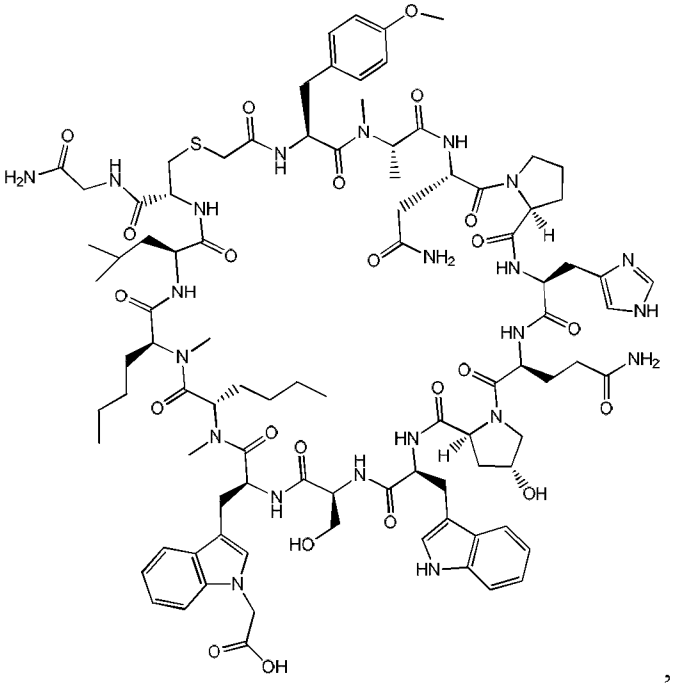

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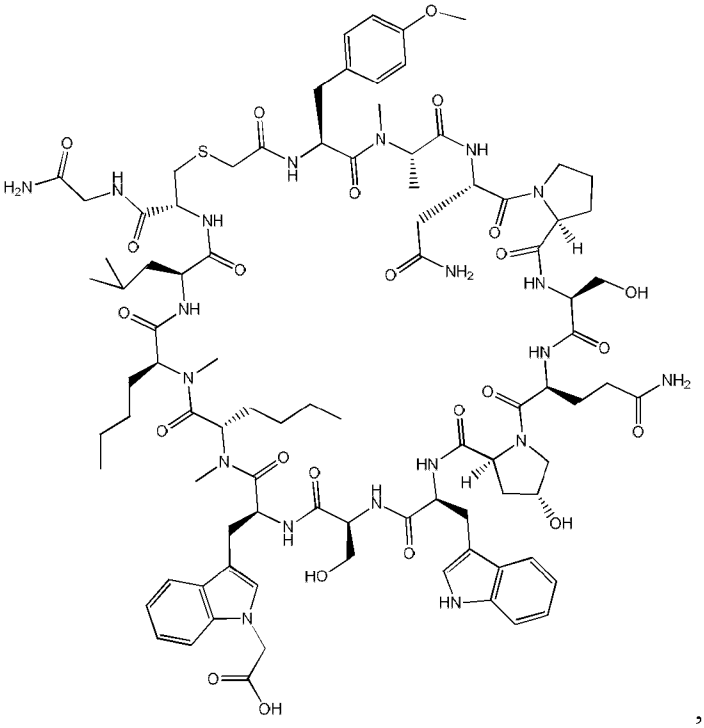

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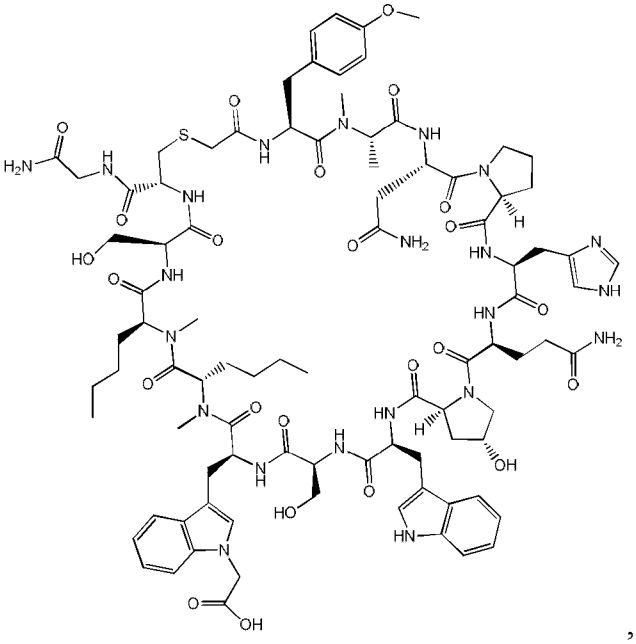

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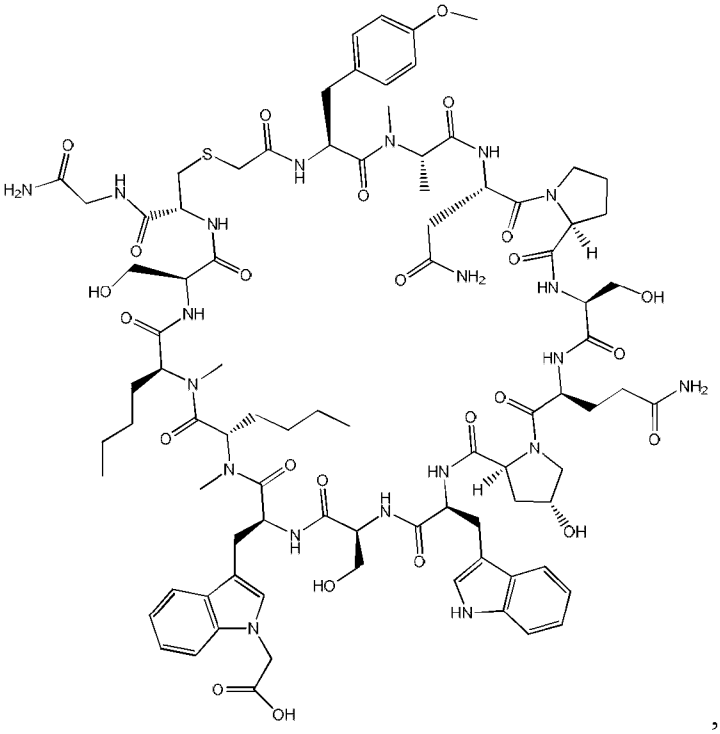

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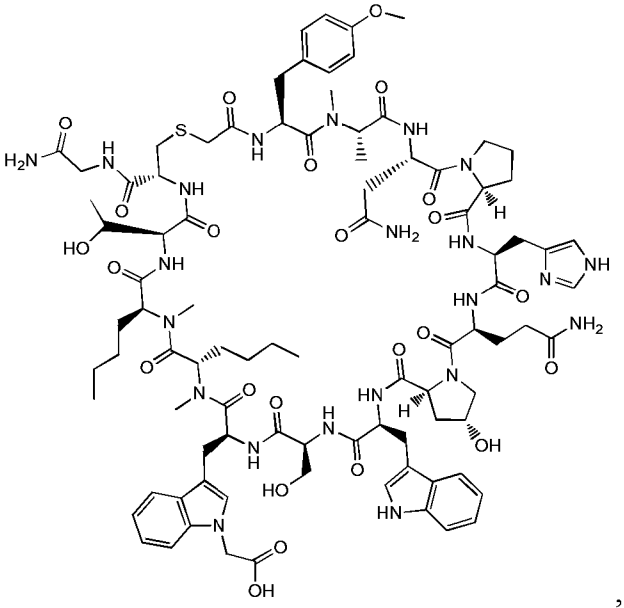

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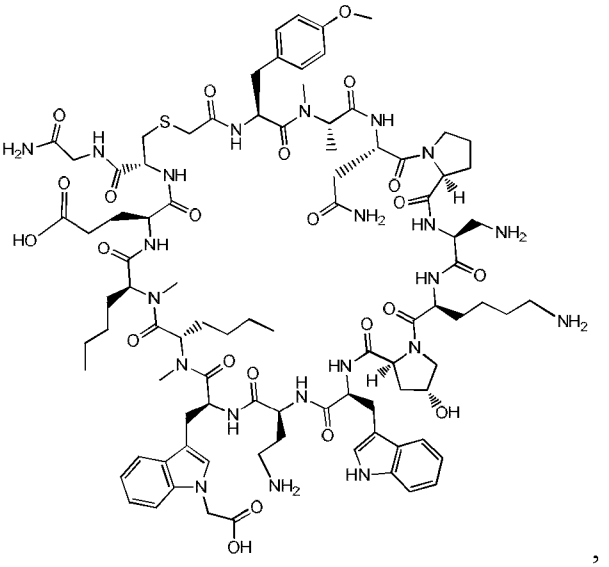

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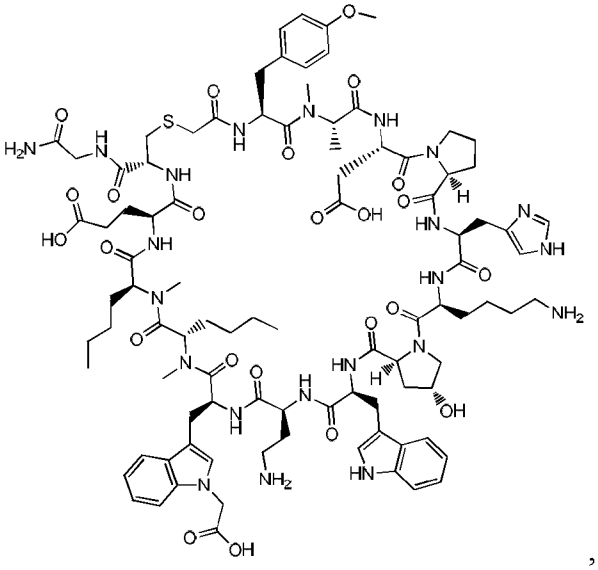

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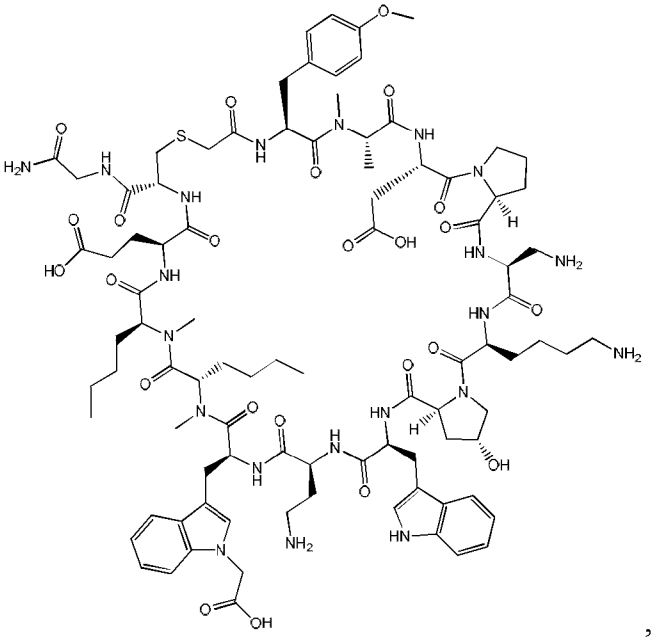

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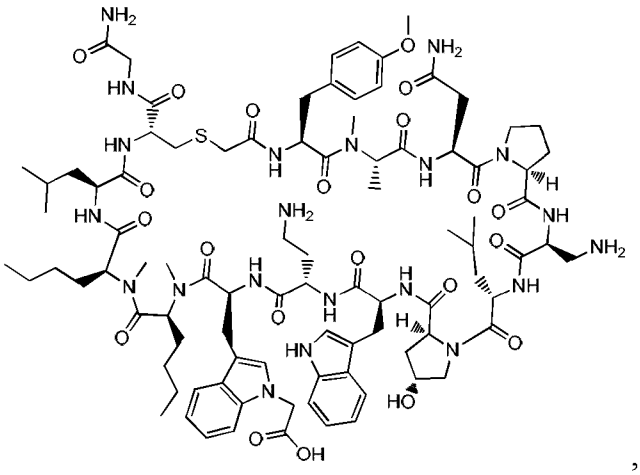

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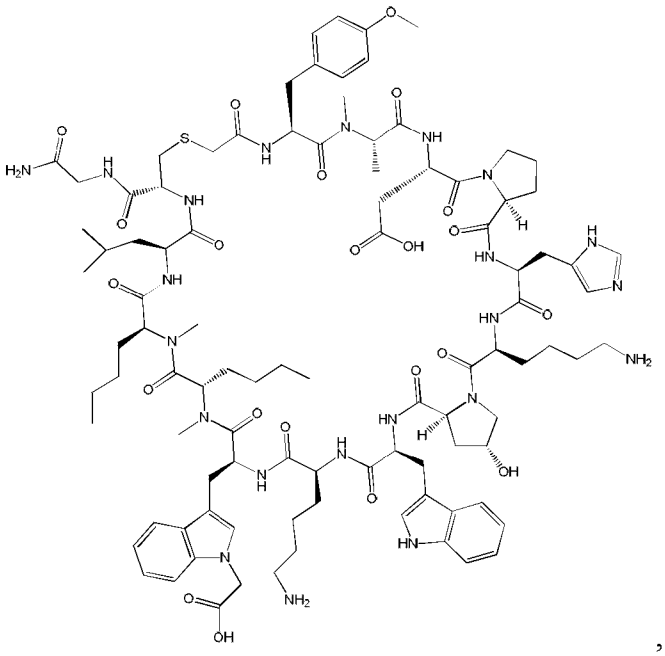

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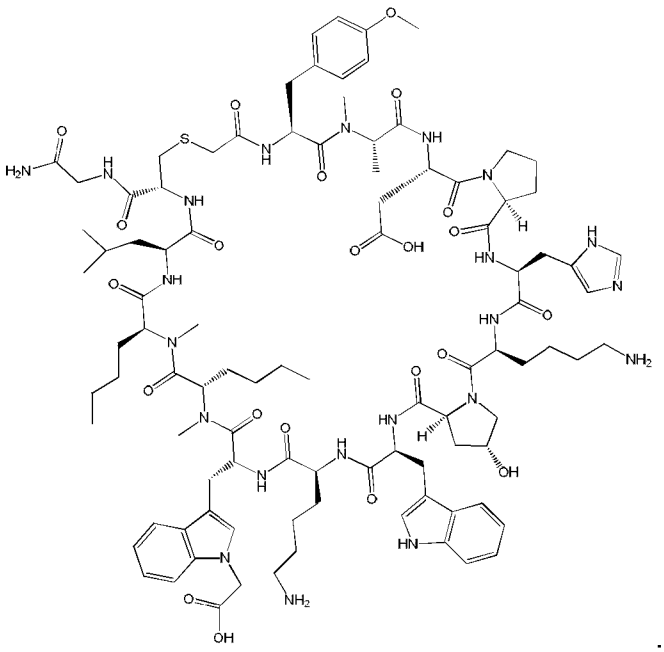

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3. Use of at least one compound of claim 1 or 2 for enhancing, stimulating, and/or increasing the immune response in a subject.

4. Use of at least one compound of claim 1 or 2 in the manufacture of a medicament for enhancing, stimulating, and/or increasing the immune response in a subject.

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5. The use of claim 3 or 4 in combination with an additional agent prior to, after, or simultaneously with the compound or compounds of claim 1 or 2.

6. The use of claim 5 wherein the additional agent is an antimicrobial agent, an antiviral agent, a cytotoxic agent, and/or an immune response modifier.

7. Use of one or more compounds of claim 1 or 2 for inhibiting growth, proliferation, or metastasis of cancer cells in a subject.

8. Use of one or more compounds of claim 1 or 2 in the manufacture of a medicament for inhibiting growth, proliferation, or metastasis of cancer cells in a subject.

9. The use of claim 7 or 8 wherein the cancer cell is 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, or a hematological malignancy.

10. Use of one or more compounds of claim 1 or 2 for treating septic shock in a subject.

11. Use of one or more compounds of claim 1 or 2 in the manufacture of a medicament for treating septic shock in a subject.

12. Use of one or more compounds of claim 1 or 2 for blocking the interaction of Programmed Death-Ligand 1 with Programmed Death-1 and/or CD80 in a subject.

13. Use of one or more compounds of claims 1 or 2 in the manufacture of a medicament for blocking the interaction of Programmed Death-Ligand 1 with Programmed Death-1 and/or CD80 in a subject.

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