OXYTOCIN PEPTIDE ANALOGS

CROSS REFERENCE TO RELATED APPLICATIONS

[0000] This application claims priority to and the benefit of U.S. Provisional Application No. 63/566,711, filed March 18, 2024, the disclosures of which, is incorporated by reference herein in its entirety.

BACKGROUND

[0001] Oxytocin (OT) is a nine-residue, cyclized peptide which, through the oxytocin receptor (OTR), acts as both a hormone, regulating diverse processes such as glucose metabolism, social bonding, lactation, and uterine contraction, as well as a neurotransmitter, modulating neural processes, including pain. Inadequate OTR activity is linked with postpartum hemorrhage, inadequate milk production, autism spectrum disorder, Prader–Willi syndrome, and migraine headaches (*see* Kabasakalian, A., et al., "Oxytocin and Prader-Willi Syndrome. Curr. Top. Behav." *Neurosci*. 2018, 35, 529–557; Klimek, R. et al., "Further studies on the oxytocin-oxytocinase system." *Am. J. Obs. Gynecol.* 1969, 105, 427–430; Aita, C., et al., "Oxytocin levels and sex differences in autism spectrum disorder with severe intellectual disabilities." *Psychiatry Res.* 2019, 273, 67–74; Bharadwaj, V.N.et al., "A new hypothesis linking oxytocin to menstrual migraine." *Headache* 2021, 61, 1051–1059). Studies in both animal models and patients have demonstrated that intranasal administration of OT can relieve core symptoms in ASD, schizophrenia, migraine, and anxiety disorders *(see* Yamasue, H., et al. "Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: A randomized clinical trial." *Mol. Psychiatry* 2020, 25, 1849–1858; Tzabazis, A.; et al., "Oxytocin and Migraine Headache." Headache 2017, 57 (Suppl. 2), 64–75; Gottschalk, M.G.; Domschke, K., Oxytocin and Anxiety Disorders." *Curr. Top. Behav. Neurosci*. 2018, 35, 467–498).

[0002] In particular use of oxytocin peptides in treatment of pain, such as headache pain, has been demonstrated. See WO 2007/025249 and WO 2007/025286, the disclosures of which are incorporated herein by reference. For example, OT has been shown to reduce pain, in particular chronic pain, associated with the trigeminal nerve, such as trigeminal neuralgia and migraine headache. And, human clinical trials have demonstrated efficacy of intranasal oxytocin in treating migraine headache. Similarly, OT has been shown to improve the core

symptoms of autism, in particular, social and communication deficits and associated anxiety symptoms. And, human clinical trials, have demonstrated efficacy of intranasal oxytocin in treating autism spectrum disorder, related disorders and symptoms of such disorders (*see*, e.g., Yatawara et al., *Mol. Psychiatry* 2015, 1-9; Gorka et al., *Neuropsychopharmacology* 2015, 40(2):278-286; Anagnostou et al., Mol. Autism 2012, 3(1):16; Guastella et al., *Psychoneuroendocrinology* 2009, 34(6):917-923. However, these trials have shown wide variability in the response that subjects with autism spectrum disorder and related disorders have to treatment with OT.

[0003] Several factors have been found to alter OTR activity including OT levels, OTR expression, and OTR affinity for its ligand. OTR affinity has also been shown to be closely modulated by the local concentration of the magnesium cation (Mg^{2+}), which acts as an essential cofactor, affecting the affinity of OT for OTR. For OT, Mg^{2+} dependency and craniofacial anti-nociception (pain) Mg^{2+} dependency increases with increasing OT concentration. In craniofacial anti-nociception, this increase in Mg^{2+} dependency with ligand (OT) dose results in an upside-down U-shaped dose response curve at low Mg^{2+} concentrations. *See* Bharadwaj, Yeomans et al., "Impact of Magnesium on Oxytocin Receptor Function," *Pharmaceutics* 2022, 14, 1105. This upside-down U-shaped dose response curve has also been observed in rat studies for reversal of opiate-induced respiratory depression and in clinical studies on autism (see Yamasue, et al., "Effect of a novel nasal oxytocin spray with enhanced bioavailability on autism-- a randomized trial," Brain 2022, 145(2), pp. 1-10). Magnesium cation's effect on OT activity has also been demonstrated in *in vivo* rat studies, specifically when measuring the head withdrawal response latencies for A-delta and C-fiber radiant heat stimulation of the cheek. (*See* Bharadwaj, et al.,).

[0004] This disclosure addresses the modulation of OT activity by local concentrations of Mg^{2+}. In that context, this disclosure provides oxytocin analogs capable of providing a pronounced OT effect independent of the concentration of Mg^{2+} and avoiding the complexities of dose determinations for two components (ligand (OT) and Mg^{2+}) that have distinct pharmacokinetic and biodistribution behavior. These analogs are useful themselves and in pharmaceutical compositions or medicaments for the treatment of various medical conditions associated with inadequate OTR activity.

SUMMARY OF THE DISCLOSURE

[0005] This disclosure provides oxytocin peptide analogs capable of binding to the oxytocin receptor with an affinity similar to or better than OT itself but with that affinity

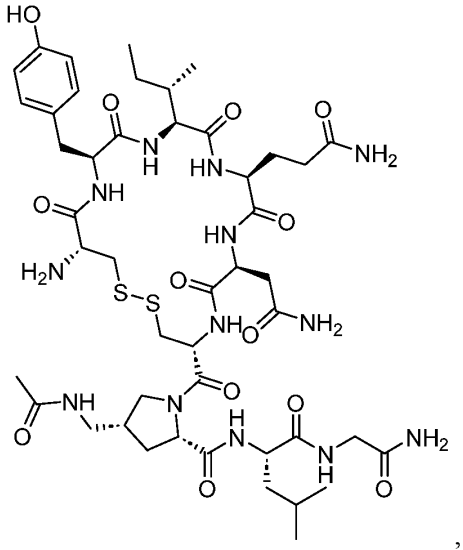
being independent of the local concentrations of Mg^{2+}. In certain embodiments, such analogs and compositions containing them are useful as therapeutic agents for the treatment of various medical conditions associated with inadequate OTR activity, such as headache pain, autism disorder related symptoms, insulin resistance disorders and feeding disorders, in patients in need thereof. In particular, in certain embodiments, such analogs and compositions containing them, are useful in those therapeutic methods in that they reduce the Mg^{+2} dependence on the binding affinity of naturally-occurring OT and other analogs thereof for the OTR, e.g., the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy.

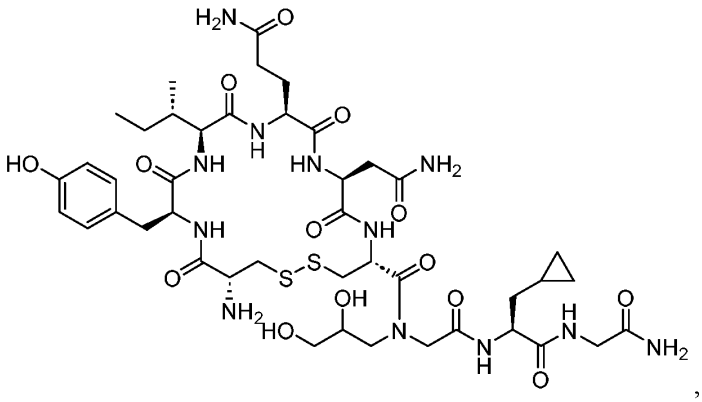
[0006] The methods of treatment and uses (*e.g.*, uses to prepare a medicament for treating) of this disclosure comprise the use of an oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, and pharmaceutically acceptable compositions of the oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, for the treatment of a subject suffering from various medical conditions associated with inadequate OTR activity. In various aspects, these conditions include, but are not limited to, headache disorders, insulin resistant disorders and feeding disorders. In some embodiments, the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache. In some embodiments, the insulin disorder is type II diabetes or obesity. In some embodiments, the feeding disorder is Prader-Willi Syndrome. In some embodiments, the feeding disorder is NOFITT. In some embodiments, the subject suffering from NOFITT is an adult. In some embodiments, the subject suffering from NOFITT is an infant. In some embodiments, the subject suffering from NOFITT is an infant in the first three months of life.

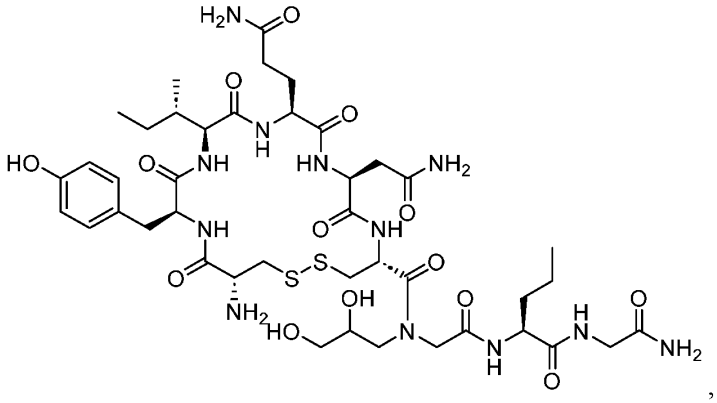
[0007] The methods of this disclosure, in some embodiments, comprise administration of an oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, and pharmaceutically acceptable salts of any of them, or a pharmaceutically acceptable composition comprising any of them via parenteral or enteral administration to a subject in need of treatment. In some embodiments, the administration is via craniofacial mucosal administration. In some embodiments, the craniofacial mucosal administration is intranasal administration.

[0008] Exemplary embodiments of the disclosure include:

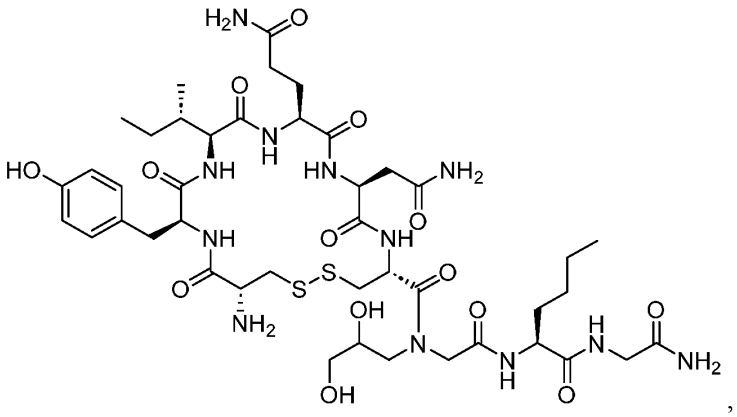
1. A compound selected from the group consisting of:

Compound 1 ,

Compound 2 ,

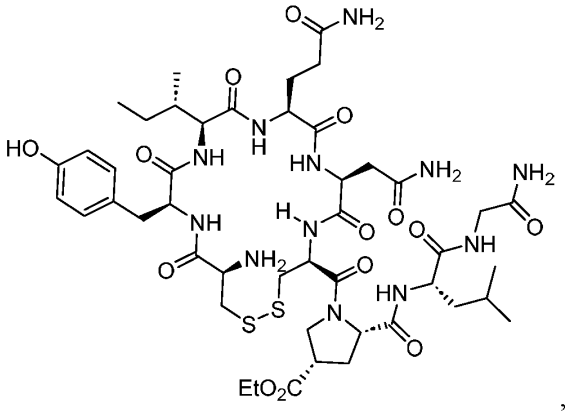
Compound 3 ,

Compound 4



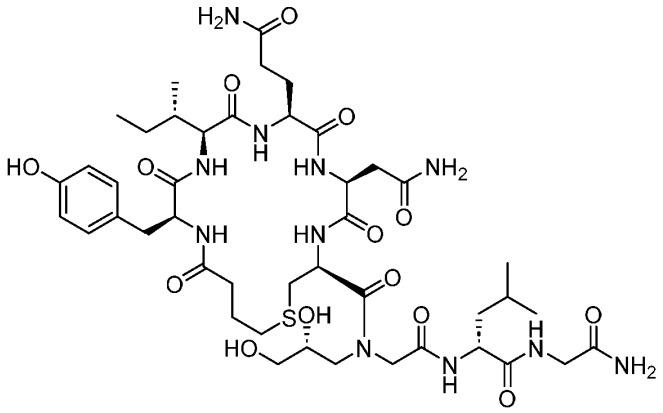
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Compound 5



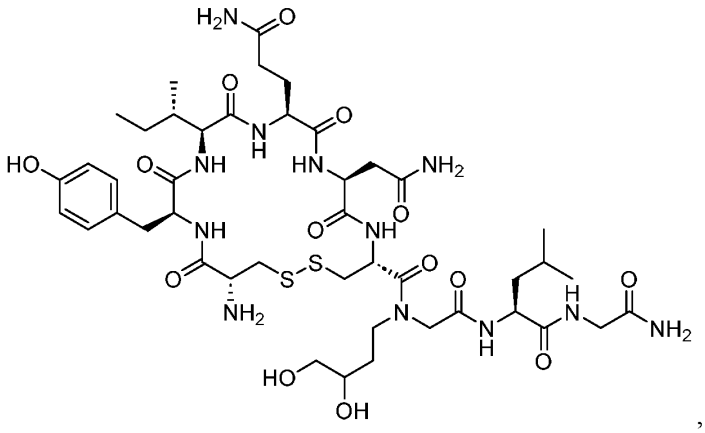
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Compound 6



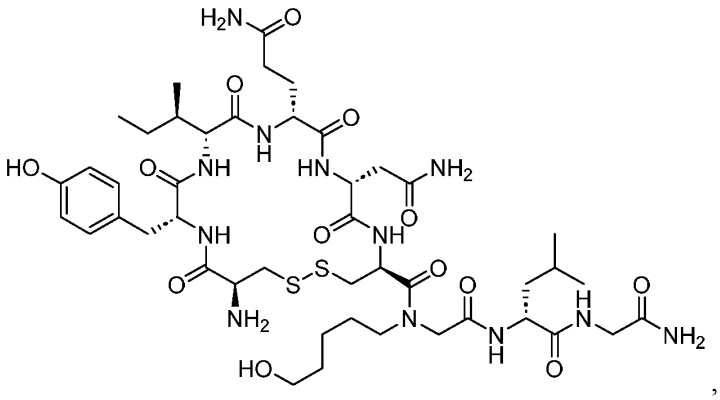
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Compound 7



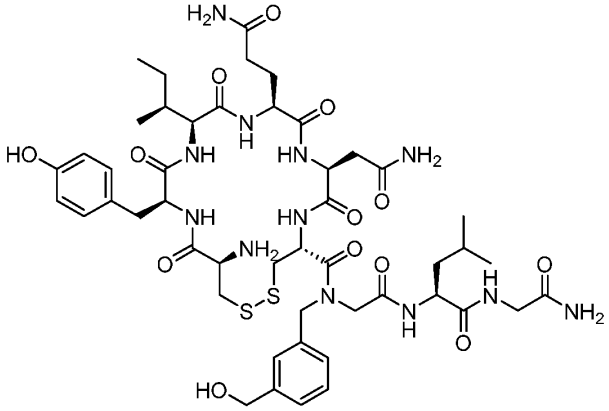
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Compound 8

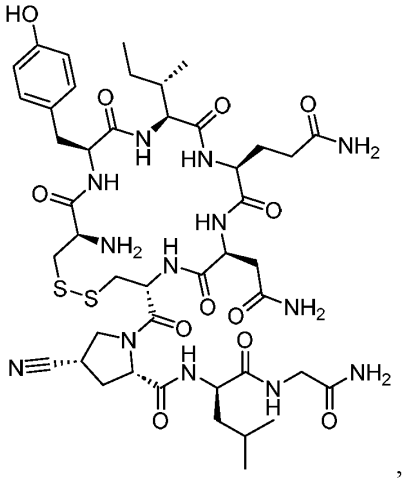


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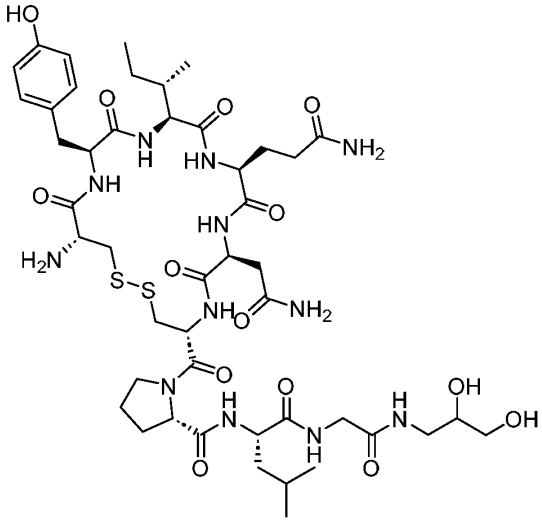
Compound 9



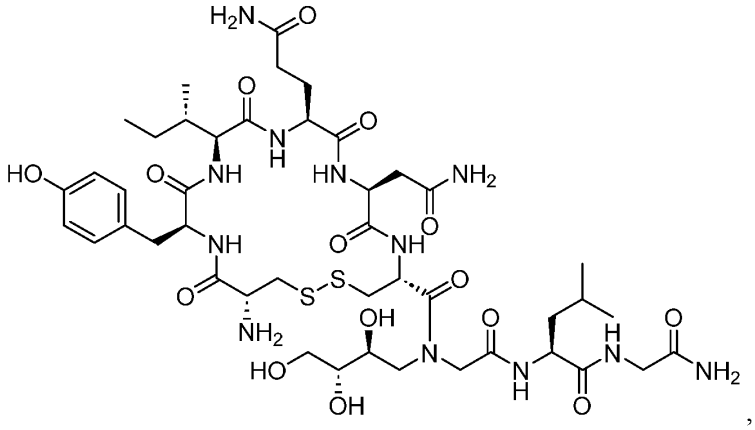
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Compound 10 

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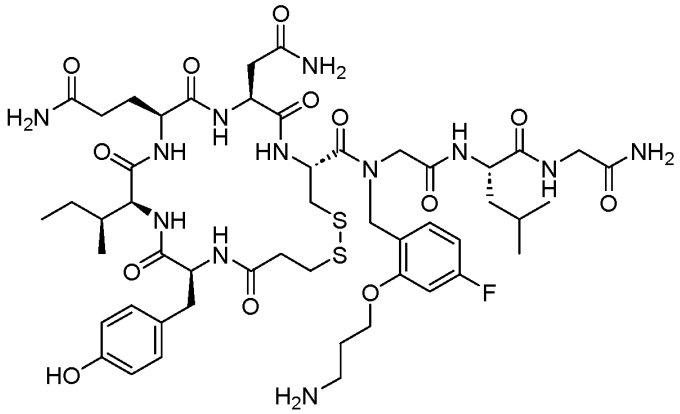
Compound 11 

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Compound 12 

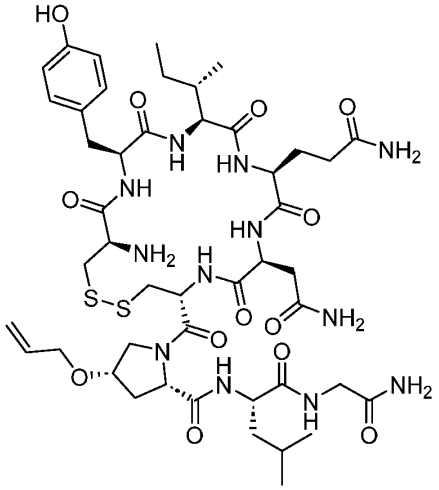
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Compound 13



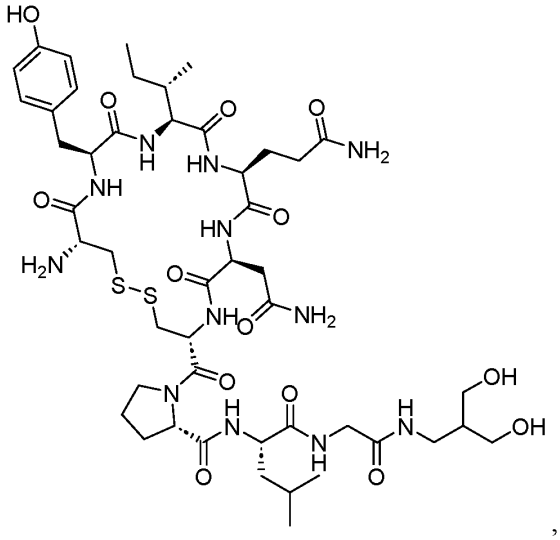
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Compound 14



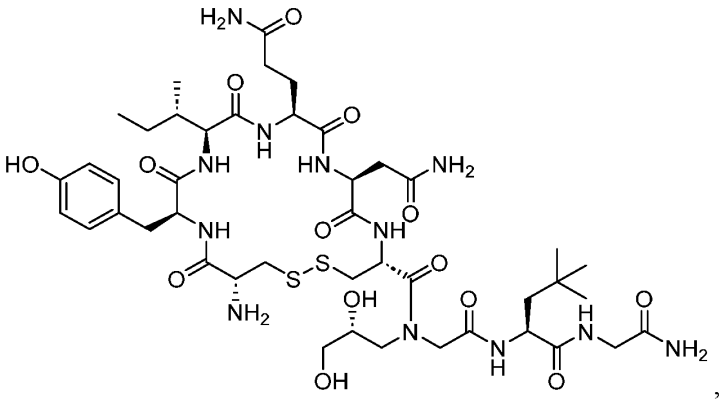
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Compound 15



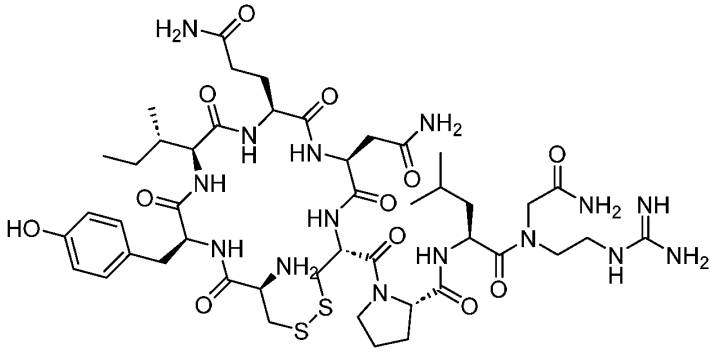
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Compound 16



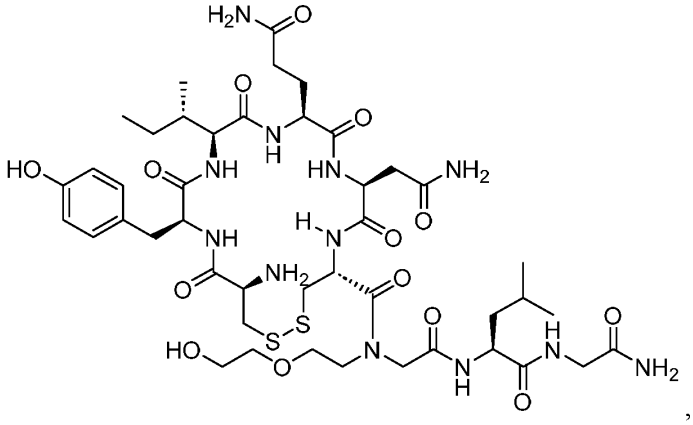
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Compound 17



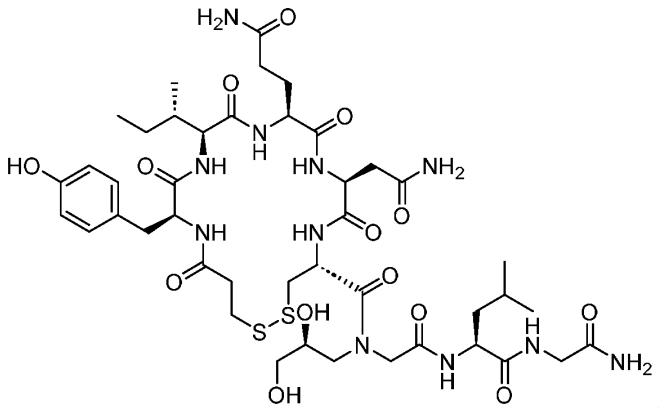
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Compound 18



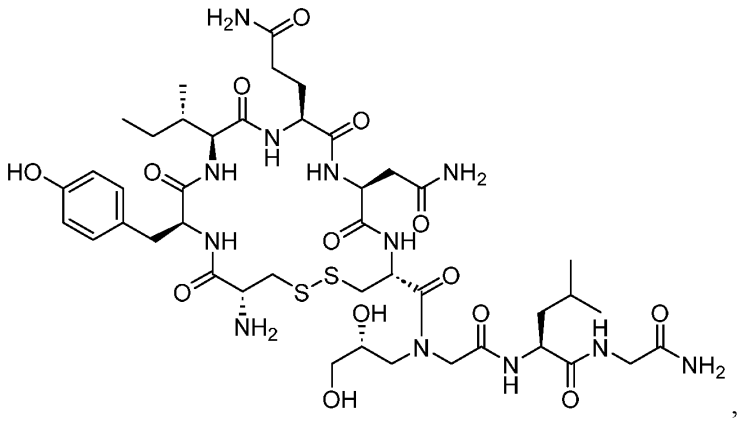
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Compound 19



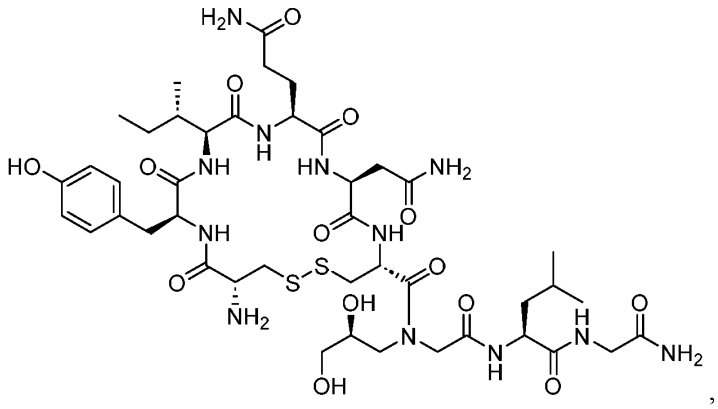
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Compound 20



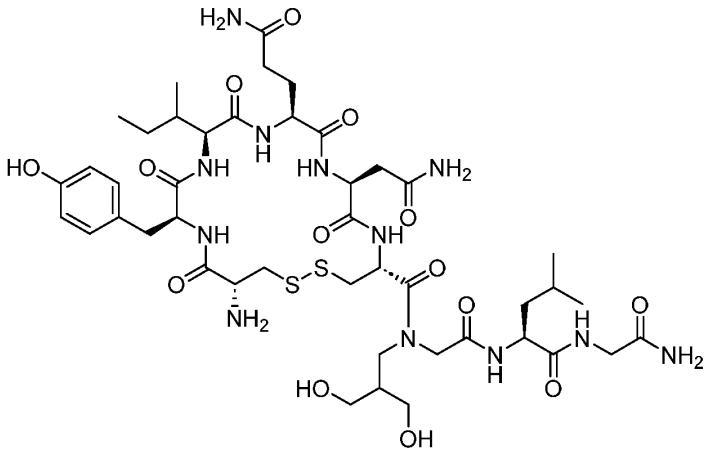
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Compound 21



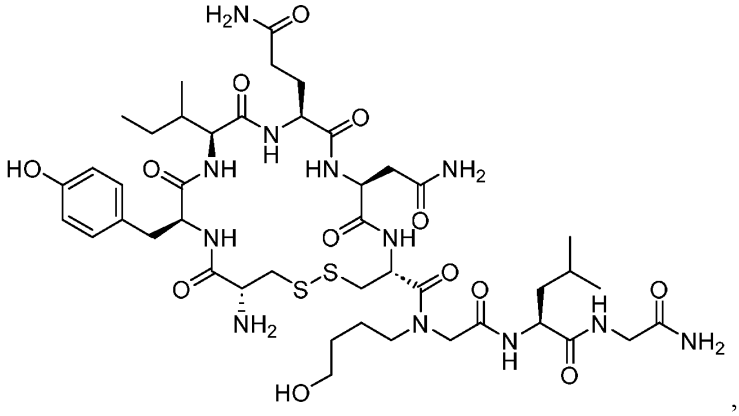
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Compound 22



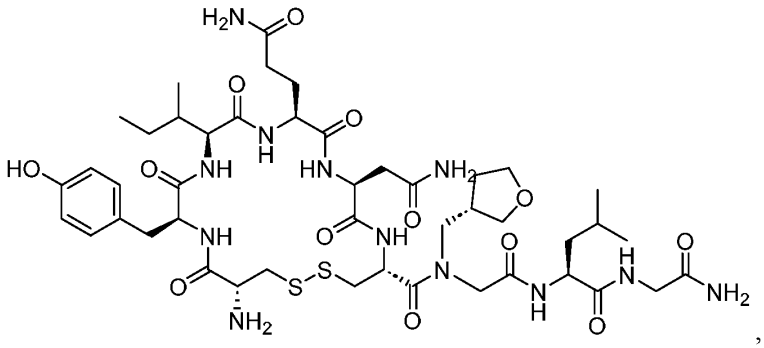
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Compound 23



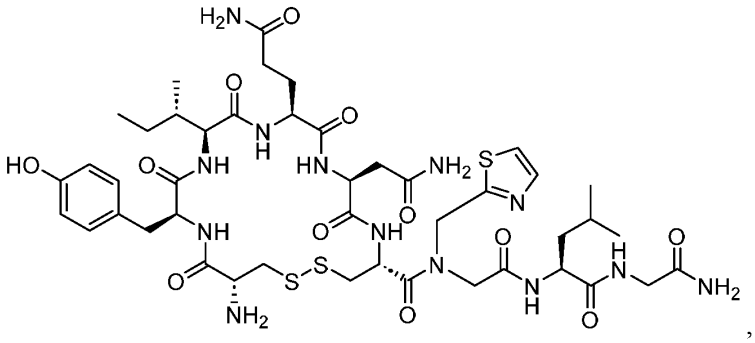
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Compound 24



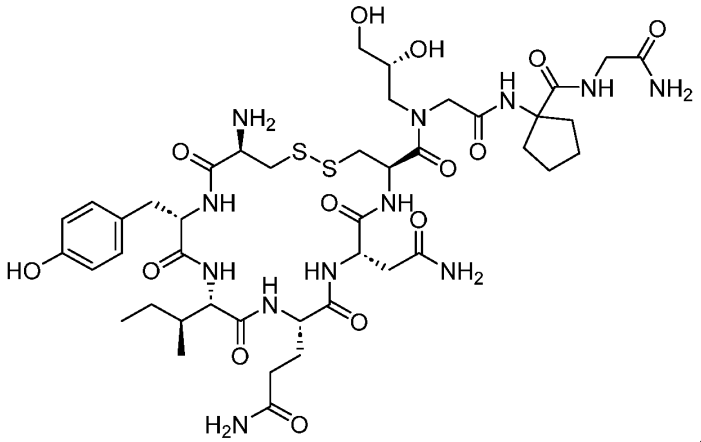
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Compound 25



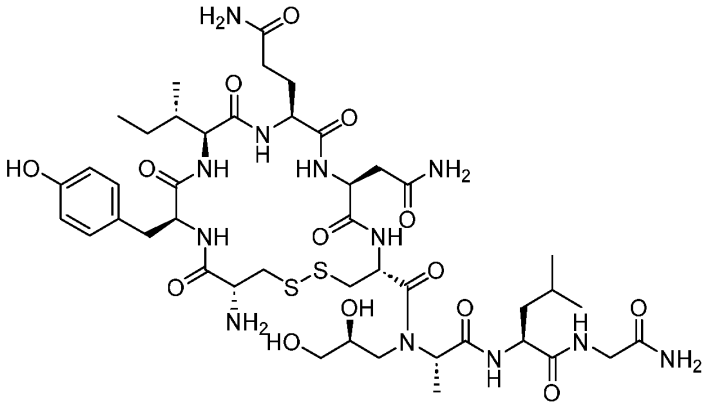
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Compound 26



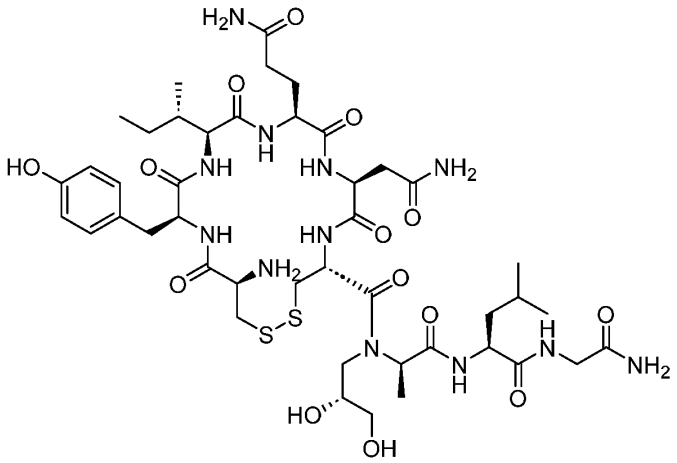
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Compound 27



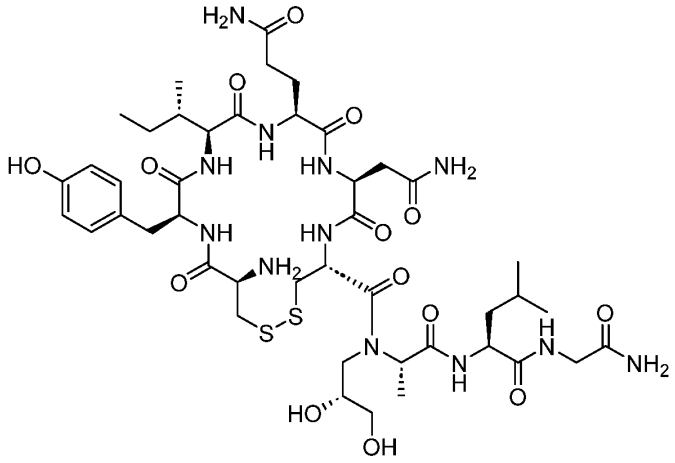
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Compound 28



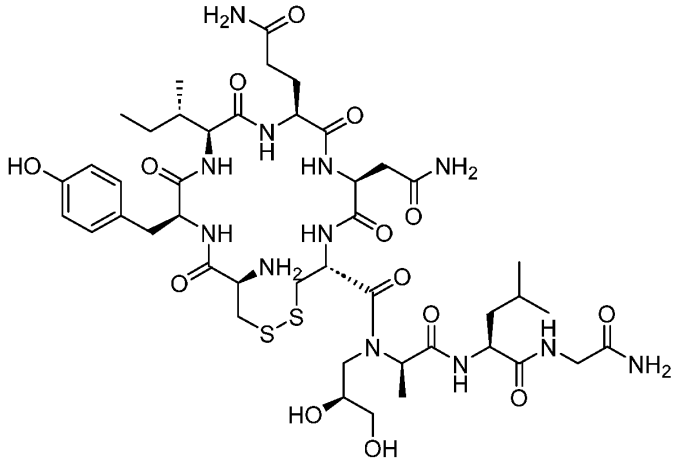
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Compound 29



, and

Compound 30

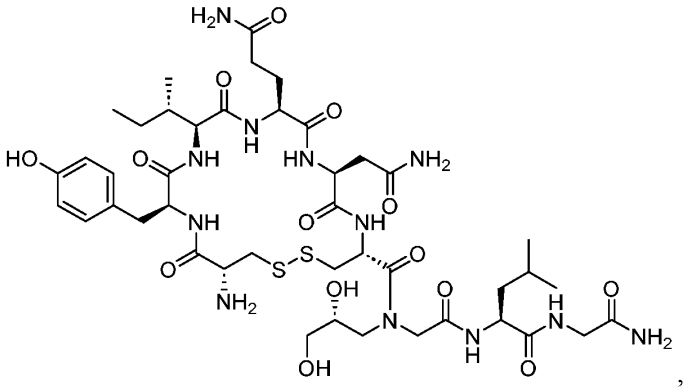


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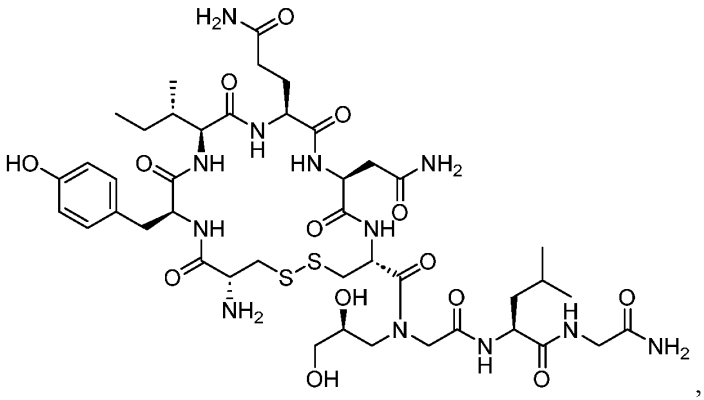
or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

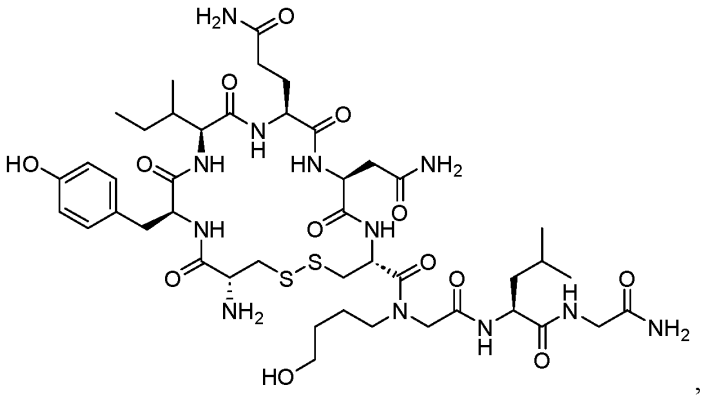
2. The compound of Embodiment 1, selected from the group consisting of:

Compound 20



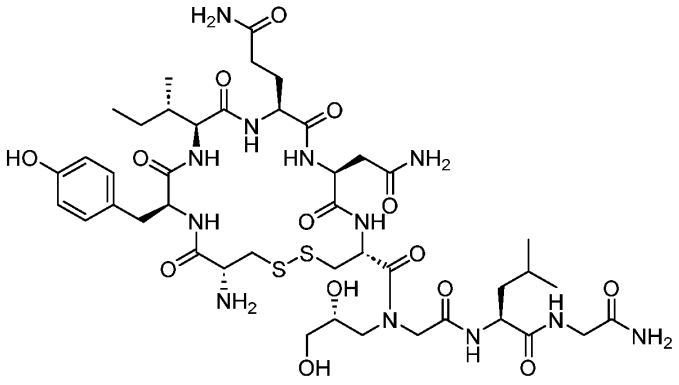
,

Compound 21 , and

Compound 23 ,

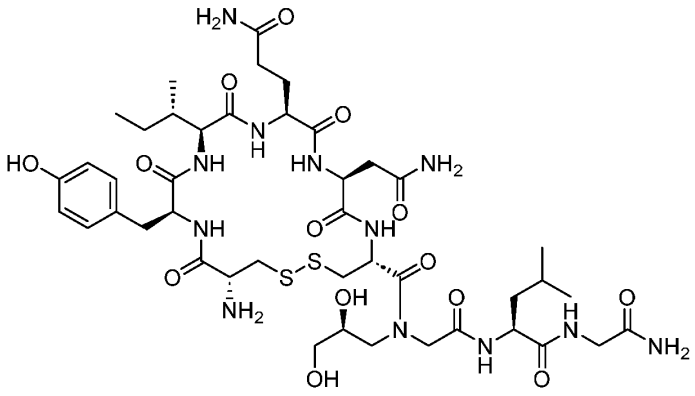
or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

3. The compound of Embodiment 2, wherein the compound is Compound 20:



Compound 20, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

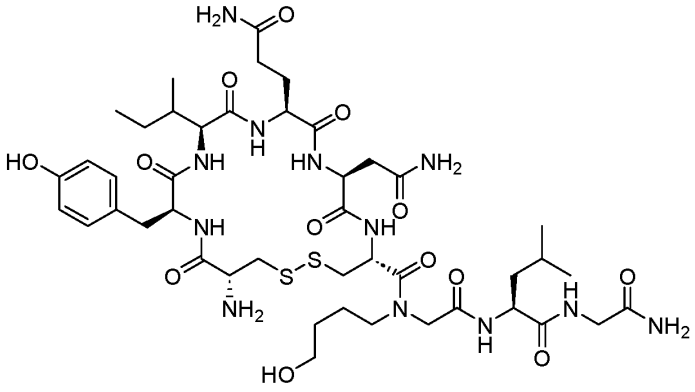
4. The compound of Embodiment 2, wherein the compound is Compound 21:



Compound 21,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

5. The compound of Embodiment 2, wherein the compound is Compound 23:



Compound 23,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

6. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to any one of the Embodiment 1-5 and a pharmaceutically acceptable carrier.

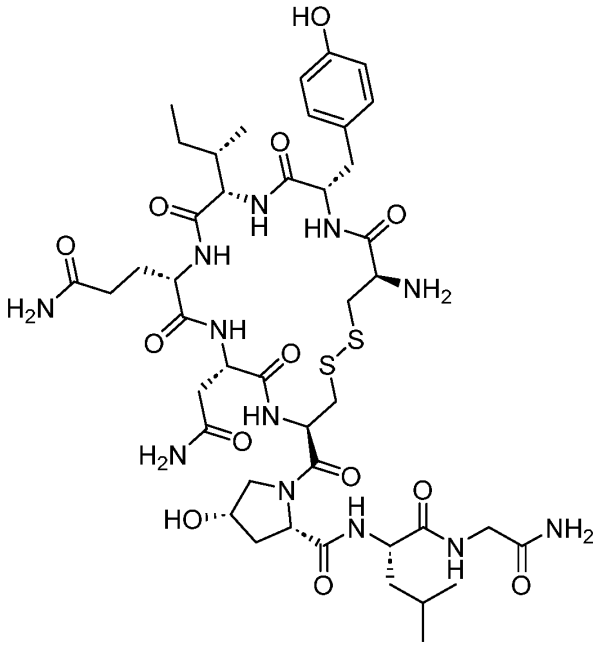
7. The pharmaceutical composition of Embodiment 7, wherein the pharmaceutical composition is a liquid formulation.

8. The pharmaceutical composition of Embodiment 8, wherein the liquid formulation is an aqueous solution.

9. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt

of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.

10. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B:



Compound B,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

11. The method of Embodiment 9 or 10, wherein the administration is via craniofacial mucosal administration.

12. The method of Embodiment 11, wherein the craniofacial mucosal administration is intranasal administration.

13. The method of Embodiment 9 or 10, wherein administration is via parenteral administration.

14. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 1-5, or a pharmaceutical composition of any one of Embodiments 6-8, wherein the

volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

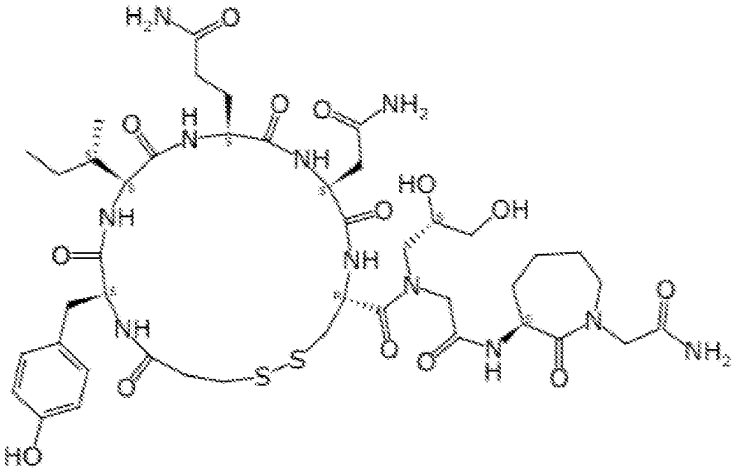
15. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
16. The method of either Embodiment 9, 10, 14, or 15, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.
17. The method of Embodiment 16, wherein the headache disorder is a migraine.
18. The method of Embodiment 16, wherein the headache disorder is a tension headache.
19. The method of Embodiment 16, wherein the headache disorder is a cluster headache.
20. The method of Embodiment 16, wherein the headache disorder is trigeminal neuralgia.
21. The method of Embodiment 16, wherein the headache disorder is a secondary headache.
22. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.
23. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them.
24. The method of Embodiment 22 or 23, wherein the administration is via craniofacial mucosal administration.
25. The method of Embodiment 24, wherein the craniofacial mucosal administration is intranasal administration.

26. The method of Embodiment 22 or 23, wherein the administration is via parenteral administration.
27. The method of Embodiment 22 or 23, wherein the administration is via enteral administration.
28. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
29. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
30. The method of either Embodiment 22, 23, 28 or 29, wherein the insulin resistant disorder is type II diabetes.
31. The method of either Embodiment 22, 23, 28 or 29, wherein the insulin resistant disorder is obesity.
32. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.
33. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them.

34. The method of Embodiment 32 or 33, wherein the administration is via craniofacial mucosal administration.
35. The method of Embodiment 34, wherein the craniofacial mucosal administration is intranasal administration.
36. The method of Embodiment 32 or 33, wherein the administration is via parenteral administration.
37. The method of Embodiment 29, wherein the administration is via enteral administration.
38. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any of Embodiments 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
39. A method for treating a feeding disorder with onset during neonate development comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
40. The method of either Embodiment 32, 33, 38, or 39, wherein the feeding disorder is Prader-Willi syndrome.
41. The method of either Embodiment 32, 33, 38, or 39, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).
42. The method of any of the Embodiments 9-41, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.
43. The method of any of the Embodiments 14, 15, 28, 29, 38 or 39, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.
44. The method of any of the Embodiments 14, 15, 28, 29, 38 or 34, wherein the liquid formulation is contained in a device for intranasal administration.

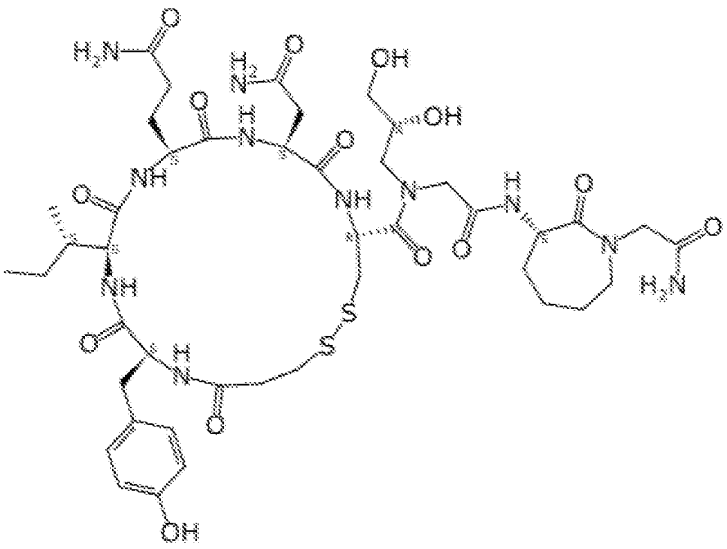
45. The method of Embodiment 44, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.
46. The method of Embodiment 44, wherein the device for intranasal administration is a nasal pump apparatus.
47. The method of Embodiment 46, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.
48. The method of Embodiment 47, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.
49. The method of Embodiment 46, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.
50. The method of any one of Embodiments 46-49, wherein the nasal pump apparatus comprises one of more of the following:
    i) a filter for preventing back flow,
    ii) a metal-tree fluid path, and
    iii) a plastic material stable to gamma-radiation.
51. A compound selected from the group consisting of:

Compound 31



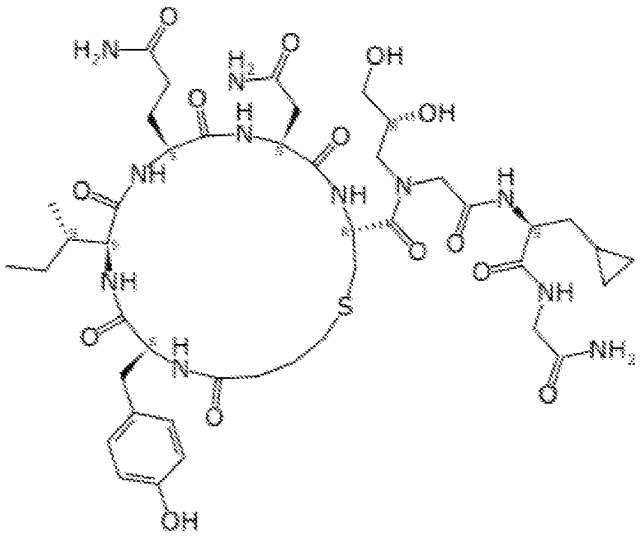
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Compound 32



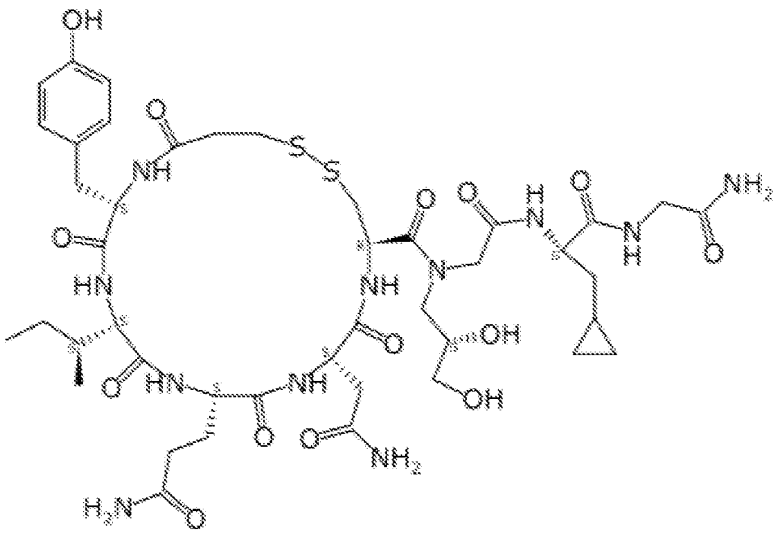
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Compound 33



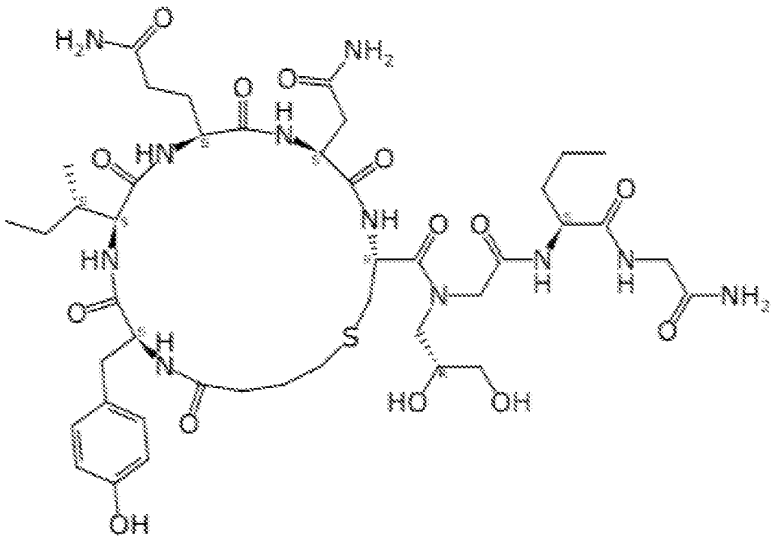
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Compound 34



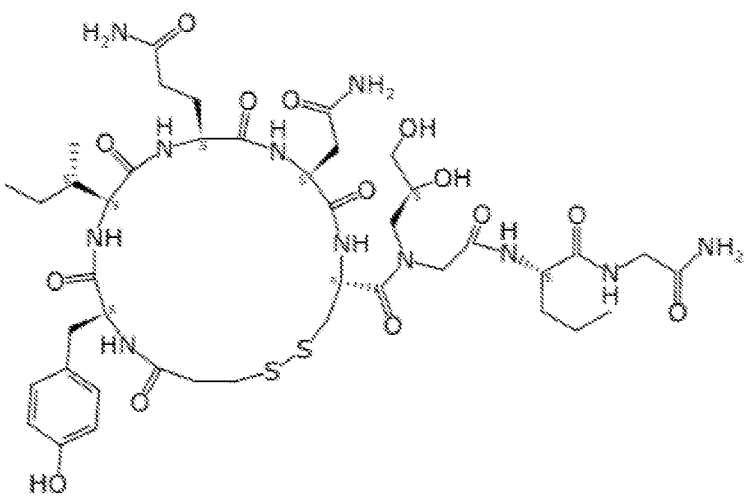
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Compound 35



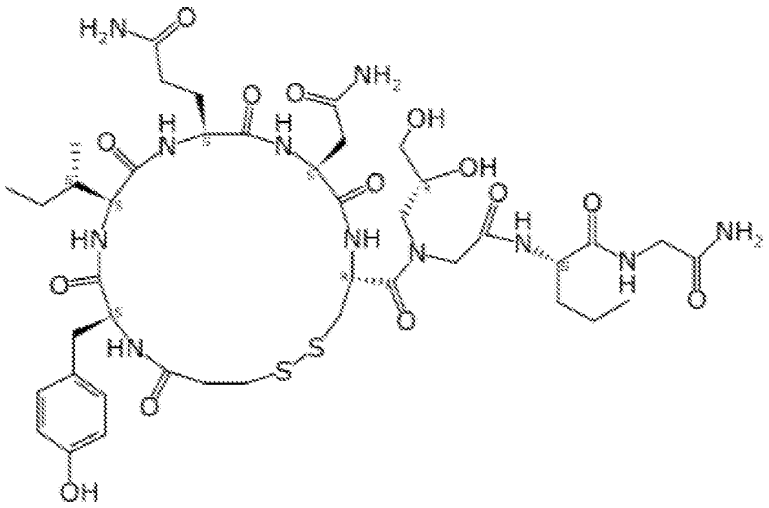
,

Compound 36



, and

Compound 37



,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

52. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to Embodiment 51 and a pharmaceutically acceptable carrier.
53. The pharmaceutical composition of Embodiment 52, wherein the pharmaceutical composition is a liquid formulation.
54. The pharmaceutical composition of Embodiment 53, wherein the liquid formulation is an aqueous solution.

55. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54.
56. The method of Embodiment 55, wherein the administration is via craniofacial mucosal administration.
57. The method of Embodiment 56, wherein the craniofacial mucosal administration is intranasal administration.
58. The method of Embodiment 57, wherein the administration is via parenteral administration.
59. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
60. The method of either Embodiment 55 or 59, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.
61. The method of Embodiment 60, wherein the headache disorder is a migraine.
62. The method of Embodiment 60, wherein the headache disorder is a tension headache.
63. The method of Embodiment 60, wherein the headache disorder is a cluster headache.
64. The method of Embodiment 50, wherein the headache disorder is trigeminal neuralgia.
65. The method of Embodiment 60, wherein the headache disorder is a secondary headache.
66. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiments 51, or a pharmaceutical composition of any one of Embodiments 52-54.

67. The method of Embodiment 66, wherein the administration is via craniofacial mucosal administration.
68. The method of Embodiment 67, wherein the craniofacial mucosal administration is intranasal administration.
69. The method of Embodiment 66, wherein administration is via parenteral administration.
70. The method of Embodiment 61, wherein the administration is via enteral administration.
71. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
72. The method of either Embodiment 66 or 71, wherein the insulin resistant disorder is type II diabetes.
73. The method of either Embodiment 66 or 71, wherein the insulin resistant disorder is obesity.
74. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54.
75. The method of Embodiment 74, wherein the administration is via craniofacial mucosal administration.
76. The method of Embodiment 75, wherein the craniofacial mucosal administration is intranasal administration.
77. The method of Embodiment 74, wherein the administration is via parenteral administration.
78. The method of Embodiment 74, wherein the administration is via enteral administration.

79. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiments 51, or a pharmaceutical composition of any of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
80. The method of either Embodiment 74 or 79, wherein the feeding disorder is Prader-Willi syndrome.
81. The method of either Embodiment 74 or 79, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).
82. The method of any of the Embodiments 55-81, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.
83. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of the compound of any one of Embodiments 1-5, or the pharmaceutical composition of Embodiments 6-8.
84. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a Compound B, an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.
85. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a compound of Embodiment 51, or a pharmaceutical composition of Embodiments 52-54.

BRIEF DESCRIPTION OF THE FIGURES

[0009] **Figure 1** depicts the efficacy of oxytocin in the presence of 0.5 mM and 1.75 mM Mg^{2+} using a rat TG electrophysiology assay.

[0010] **Figure 2** depicts the efficacy of Compound 20 in the presence of 0.5 mM and 1.75 mM Mg^{2+} using a rat TG electrophysiology assay.

[0011] **Figure 3** depicts the efficacy of Compound 21 in the presence of 0.5 mM and 1.75 mM Mg^{2+} using a rat TG electrophysiology assay.

[0012] **Figure 4** depicts the efficacy of Compound 23 in the presence of 0.5 mM and 1.75 mM Mg^{2+} using a rat TG electrophysiology assay.

[0013] **Figure 5** depicts the efficacy of Compound 20 in a delta A-fiber Heat Tolerance Test in the presence of 0.5 mM and 1.75 mM Mg^{2+}.

[0014] **Figure 6** depicts the efficacy of Compound 20 in the presence of 0.5 mM (low) and 1.75 mM (high) Mg^{2+} concentrations and the efficacy of oxytocin in the presence of 0.5 mM Mg^{2+} (low) concentration in a delta A-fiber test.

[0015] **Figure 7** depicts the efficacy of Compound 20 in the presence of 0.5 mM (low) and 1.75 mM Mg^{2+} (high)concentrations and the efficacy of oxytocin in the presence of 0.5 mM Mg^{2+} (low) concentration in a delta C-fiber Heat Tolerance Test.

[0016] **Figure 8** depicts the Mg^{2+} dependence of oxytocin and Compound 20 in Hyperpolarization electrophysiology test.

[0017] **Figure 9** depicts the antinociceptive Mg^{2+} dependence for Compound 20 (1.75 mM vs 0.5 mM Mg^{2+}) and OT (300 mM vs 0 mM Mg^{2+}).

DETAILED DESCRIPTION OF THE INVENTION

[0018] This disclosure provides, inter alia, oxytocin peptide analogs, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, pharmaceutical compositions comprising such oxytocin peptide analogs, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, and methods for treatment of various medical conditions associated with inadequate OTR activity in patients in need thereof, using those oxytocin peptide analogs or pharmaceutically acceptable compositions thereof.

Definitions

[0019] Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, molecular biology, cell and cancer biology, immunology, microbiology, pharmacology, and protein and nucleic acid chemistry, described herein, are those well known and commonly used in the art.

[0020] Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer (or

components) or group of integers (or components), but not the exclusion of any other integer (or components) or group of integers (or components).

[0021] The articles "a" and "an" may be used herein to refer to one or to more than one (*i.e.*, at least one) of the grammatical objects of the article. By way of example "an analog" means one analog or more than one analog.

[0022] The term "including" is used to mean "including but not limited to," "including," and "including but not limited to" are used interchangeably.

[0023] The term "or" as used herein should be understood to mean "and/or", unless the context clearly indicates otherwise.

[0024] Notwithstanding that the disclosed numerical ranges and parameters are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Accordingly, as used herein, the term "about" permits a variation of ±10%. As used herein, the term "about" refers to a value or parameter that includes (and describes) embodiments that are directed to that value or parameter per se.

[0025] Definitions of specific functional groups and chemical terms are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75thEd., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties, reactivity and synthetic methods, are described, for example, in Thomas Sorrell, Organic Chemistry, University Science Books, Sausalito, 1999; Smith and March, March's Advanced Organic Chemistry, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, Comprehensive Organic Transformations, VCH Publishers, Inc., New York, 1989; and Carruthers, Some Modern Methods of Organic Synthesis, 3rd Edition, Cambridge University Press, Cambridge, 1987.

[0026] The terms "magnesium" and "magnesium ions" refer to the Mg^{2+} cation and are used interchangeably. Any water-soluble magnesium salt may be used to provide the "magnesium," "magnesium ions" or Mg^{2+}. Examples of magnesium salts are $MgCl_2$, magnesium citrate, magnesium sulfate, etc. A magnesium salt in the magnesium-containing liquid may be a magnesium salt used initially or formed in situ during preparation of the magnesium containing liquid. For example, magnesium chloride may be used initially to

prepare the magnesium containing liquid, which upon addition of citric acid, may form magnesium citrate in situ. In such instance, the magnesium ions in the magnesium-containing liquid are provided by both the $MgCl_2$ and magnesium citrate.

[0027] "Oxytocin Peptide Analog" refers to the oxytocin peptide analogs of this disclosure, wherein one or more amino acids within the naturally-occurring OT peptide have been substituted, deleted, inserted, or derivatized, as disclosed herein. The term also refers to the oxytocin peptide analogs of this disclosure wherein one or more amino acids (for example one, two or three amino acids) of the naturally-occurring OT peptide have been modified, for example, by chemical modification. In general, the term covers all peptides analogs of this disclosure which exhibit oxytocin activity, but which may, if desired, have a different potency or pharmacological profile. The amino acid sequence for human oxytocin is Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ ID NO: 1). Human oxytocin can be synthesized according to known processes, see, for example, U.S. Pat. No. 2,938,891 and U.S. Pat. No. 3,076,797.

[0028] Oxytocin peptide analogs of this disclosure, or pharmaceutically acceptable salts thereof, can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, e.g., enantiomers and/or diastereomers, or tautomers. For example, in some embodiments, the oxytocin peptide analogs of this disclosure can be in the form of an individual enantiomer, racemate, tautomer, diastereomer or geometric isomer, *e.g.*, E (entgegen) and Z (zusammen) isomers of a double bond or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or a desired isomer can be prepared by asymmetric syntheses. *See*, for example, Jacques et al., Enantiomers, Racemates and Resolutions (Wiley Interscience, New York, 1981); Wilen et al., *Tetrahedron* 33:2725 (1977); Eliel, Stereochemistry of Carbon Compounds (McGraw-Hill, NY, 1962); and Wilen, Tables of Resolving Agents and Optical Resolutions p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind. 1972). In some embodiments, the disclosure additionally encompasses the analogs, forms and salts, described herein as individual isomers substantially free of other isomers, i.e., containing less that about 10%, less than about 5%, less than about 1%, less than about 0.5%, less than about 0.1 %, of the other enantiomer, and alternatively, as mixtures of various isomers.

[0029] The oxytocin peptide analogs, or pharmaceutically acceptable salts thereof, of this disclosure in various of its embodiments may also comprise one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D or deuterium), and ^{3}H (T or tritium); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

[0030] An "international unit" (IU, UI or IE) is an internationally accepted unit of activity used to quantify vitamins, hormones and vaccines. It defines the amount of a substance that gives a unit of activity as determined using a defined biological assay in order to standardize preparations from multiple source materials. Similarly, a USP unit is a defined dosage unit established by the United States Pharmacopeia in cooperation with the Food and Drug Administration in order to ensure the identity, strength, quality, purity and consistency of a drug product. In general, USP units are equal to International Units, due to harmonization efforts. By convention, for oxytocin, 1 unit of activity is generally defined as equal to approximately 2 micrograms of a synthetic naturally-occurring oxytocin peptide; or 1 mg is equal to 500 units (Stedman's Medical Dictionary). Therefore, as used herein, one "IU" or "International Unit" of an oxytocin peptide or analog thereof is the amount of the oxytocin peptide or analog that has the same biological activity or produces the same level of a biological effect (e.g. contractile response of rat uterine strips) as approximately 2 micrograms of the synthetic oxytocin peptide. An analogue with weaker activity would require more material to achieve the same level of biological effect. Determinations of drug potency are well known to those skilled in the art and may include either *in vitro* or *in vivo* assays using human oxytocin as a reference. Atke and Vilhardt *Acta Endocrinol.* 1987: 115(1):155-60; Engstrom et al. *Eur. J. Pharmacol.* 1998: 355(2-3):203-10.

[0031] When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example, if a range of 1 µg to 8 µg is stated, it is intended that 1 µg, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, and 8 µg are also explicitly disclosed, as well as the range of values in between (e.g., 1.1 µg, 1.2 µg, 1.3 µg, 1.4 µg, 1.5 µg, 1.6 µg, 1.7 µg, etc.). If a range of 10-14% is stated, it is intended that 10%, 11%, 12%, 13%, and 14% are also explicitly disclosed.

[0032] "Geometric isomer" refers to isomers that have the same molecular formula and sequence of bonded atoms (constitution), but differ in the relative orientation of functional groups within the molecule, for example, wherein one geometric isomer could theoretically be converted to another geometric isomer by rotation of one or more bonds which do not

freely rotate in practice (*e.g.*, double bonds). The IUPAC-endorsed convention of designating E (entgegen) and Z (zusammen) geometric isomers with respect to individual double bonds is well known in the art.

[0033] "Tautomers" refers to compounds whose structures differ markedly in the arrangement of atoms, but which exist in easy and rapid equilibrium. It is to be understood that compounds of present disclosure may be depicted as different tautomers. It should also be understood that when compounds have tautomeric forms, all tautomeric forms are intended to be within the scope of the disclosure, and the naming of the compounds does not exclude any tautomer form. Some compounds of the present disclosure can exist in a tautomeric form which is also intended to be encompassed within the scope of the present disclosure.

[0034] "Solvate" refers to an oxytocin peptide analog bound to a molecule of solvent.

[0035] "Hydrate" refers to the pharmaceutically acceptable salt of an oxytocin peptide analog when dissolved in water.

[0036] The term "pharmaceutically acceptable carrier" refers to a non-toxic carrier that may be administered to a patient, together with a therapeutically effective substance of this disclosure, and which does not destroy the pharmacological activity of the agent. The term "excipient" refers to an additive in a formulation or composition that is not a pharmaceutically active ingredient. In certain embodiments, a "pharmaceutically acceptable" substance is suitable for use in contact with cells, tissues or organs of animals or humans without excessive toxicity, irritation, allergic response, immunogenicity or other adverse reactions, in the amount used in the dosage form according to the dosing schedule, and commensurate with a reasonable benefit/risk ratio. In certain embodiments, a "pharmaceutically acceptable" substance that is a component of a pharmaceutical composition is, in addition, compatible with the other ingredient(s) of the composition. In certain embodiments, the terms "pharmaceutically acceptable excipient", "pharmaceutically acceptable carrier" and "pharmaceutically acceptable diluent" encompass, without limitation, pharmaceutically acceptable inactive ingredients, materials, compositions and vehicles, such as liquid fillers, solid fillers, diluents, excipients, carriers, solvents and encapsulating materials. Carriers, diluents and excipients also include all pharmaceutically acceptable dispersion media, coatings, buffers, isotonic agents, stabilizers, absorption delaying agents, antimicrobial agents, antibacterial agents, antifungal agents, adjuvants, and so on. Except

insofar as any conventional excipient, carrier or diluent is incompatible with the active ingredient, the present disclosure encompasses the use of conventional excipients, carriers and diluents in pharmaceutical compositions. See, e.g., Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins (Philadelphia, Pennsylvania, 2005); Handbook of Pharmaceutical Excipients, 5th Ed., Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association (2005); Handbook of Pharmaceutical Additives, 3rd Ed., Ash and Ash, Eds., Gower Publishing Co. (2007); and Pharmaceutical Preformulation and Formulation, Gibson, Ed., CRC Press LLC (Boca Raton, Florida, 2004).

[0037] "Pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds, wherein the parent compound is modified by making acid or base salts thereof.

[0038] The term "free base" refers to a compound having an amine (or other basic nitrogen) group, i.e., a primary, secondary, or tertiary amine, possesses a free electron pair. The amine is neutral, i.e., is uncharged, it is not protonated, it is not in the form of a salt.

[0039] A "subject" or "patient" as used herein refers to a mammal, including but not limited to a human. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as guinea pigs, cats, dogs, rabbits and horses), primates, mice and rats. In one embodiment, a subject is a human.

[0040] "Therapeutically effective amount" or "therapeutically effective dose" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment of a disease, disorder or condition, or to delay, modulate or reduce one or more symptoms associated with the disease, disorder or condition. A therapeutically effective dose of a peptide analog of this disclosure in any form or formulation means an amount of the analog, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the disorder or condition. The term "therapeutically effective dose" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disorder or condition, or enhances the therapeutic efficacy of another therapeutic agent. As will be appreciated by those of ordinary skill in the art, the effective amount of a compound of this disclosure may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound or composition containing it, the disorder being treated, the mode of administration, and the age, weight, health, and condition of the subject.

[0041] As used herein, the term "treating" or "treatment" includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to

improve or stabilize a subject's condition in the context of a condition related to inadequate OTR activity. As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation, amelioration, or slowing the progression, of one or more symptoms or conditions associated with a condition.

[0042] "Analgesia agent," "analgesic agent," or "analgesic" refers to any biomolecule, drug or active agent that alleviates or prevents pain.

[0043] "Nociception" refers to a neuronal signal or perception that denotes the induced pain or injury caused by noxious stimuli, such as pin-pricks or inflammation, exceeding a certain threshold stimulus level in nociceptive nerve fibers (nociceptors), which innervate the skin or other peripheral tissue. Pain is also experienced when peripheral or central neuronal structures involved in the processing of pain become hyperactive, e.g. as a result of trauma, ischemia or inflammation. Oxytocin has been shown to reduce pain (antinociceptive), in particular chronic pain, associated with the trigeminal nerve, such as trigeminal neuralgia and migraine headache.

[0044] "Headache disorder" includes a migraine, tension headache, cluster headache, trigeminal neuralgia, secondary headache, and miscellaneous-type headaches.

[0045] "Migraine" includes migraine headache, migraine without aura, migraine with aura, and migraine with aura but without headache.

[0046] "Secondary headache" include headaches attributed to head and/or neck traumas; headaches attributed to cranial and/or cervical vascular disorders; headaches attributed to non-vascular intracranial disorders; headaches attributed to drugs; headaches attributed to withdrawal from drugs; headaches attributed to infections, headaches attributed to disturbances of homeostasis, headaches or facial pain attributed to disorders of facial structures and/or cranial structures; or headaches attributed to a psychiatric disorders.

[0047] "Insulin resistance disorder" refers to a phenomenon wherein, even though insulin is normally secreted in the body, "supply of glucose into cells" performed by insulin does not work properly. Therefore, glucose in the blood cannot enter cells, thus causing hyperglycemia, and further, cells themselves cannot perform normal functions thereof due to a shortage of glucose, leading to the manifestation of metabolic syndrome.

[0048] "Administering" or "administration" of a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially,

intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods. In some aspects, the administration includes both direct administration, including self-administration, and indirect administration, including the act of prescribing a drug. For example, as used herein, a physician who instructs a patient to self-administer a drug, or to have the drug administered by another and/or who provides a patient with a prescription for a drug is administering the drug to the patient. When a method is part of a therapeutic regimen involving more than one agent or treatment modality, the disclosure contemplates that the agents may be administered at the same or differing times and via the same or differing routes of administration. Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age of the subject, whether the subject is active or inactive at the time of administering, whether the subject is cognitively impaired at the time of administering, the extent of the impairment, and the chemical and biological properties of the compound or agent (e.g. solubility, digestibility, bioavailability, stability and toxicity).

[0049] "Craniofacial mucosal administration" refers to delivery to the mucosal surfaces of the nose, nasal passageways, nasal cavity; the mucosal surfaces of the oral cavity including the gingiva (gums), the floor of the oral cavity, the lips, the tongue; and the mucosal surfaces of or around the eye including the conjunctiva, the lacrimal gland, the nasolacrimal ducts, and the mucosa of the upper or lower eyelid and the eye.

[0050] "Intranasal administration" or "administered intranasally" refers to delivery to the nose, nasal passageways or nasal cavity by spray, drops, powder, gel, film, inhalant or other means known in the art.

[0051] The "inferior region of the nasal cavity" refers generally to the portion of the nasal cavity where the middle and inferior turbinate bones protrude and is a region of the nasal cavity that is significantly innervated by the trigeminal nerve. The "superior region of the nasal cavity" is referred generally to the upper third and cribriform plate region of the nasal cavity wherein olfactory innervation is located.

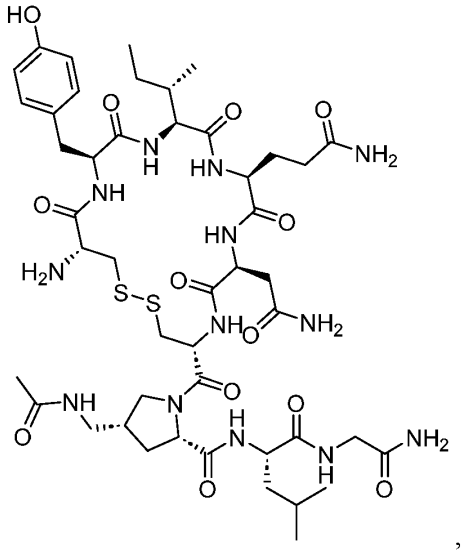
[0052] "Parenteral administration" refers to delivery not through the digestive tract, for example, via intravenous, intramuscular, subcutaneous, or intraperitoneal routes in the form of injectables, including liquid solutions or suspensions.

[0053] "Enteral administration" refers to delivery to the subject through the digestive tract, for example, orally, via the mouth in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

Compounds

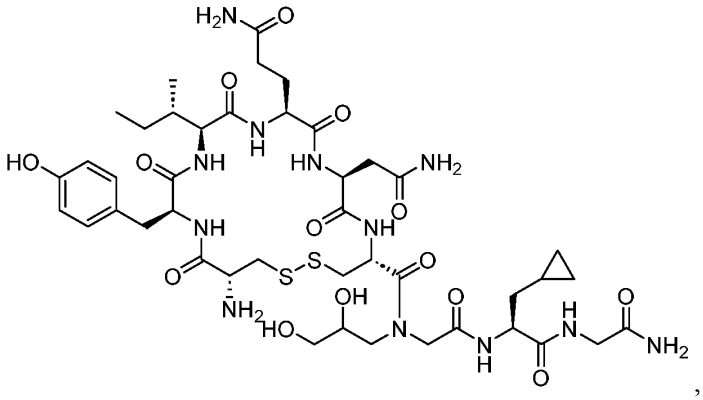
[0054] In one aspect, provided herein are compounds selected from the group consisting of:

Compound 1



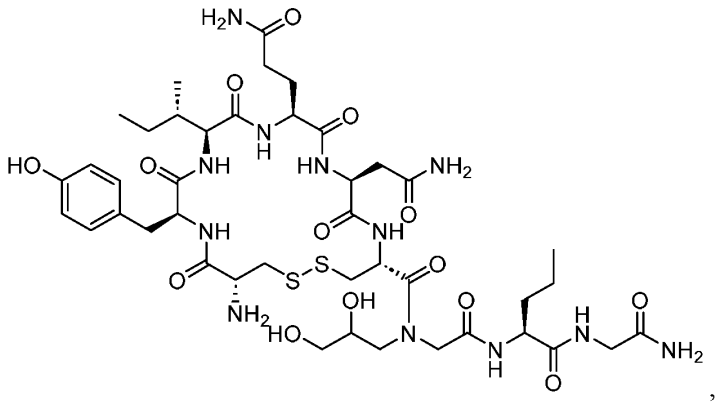
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Compound 2



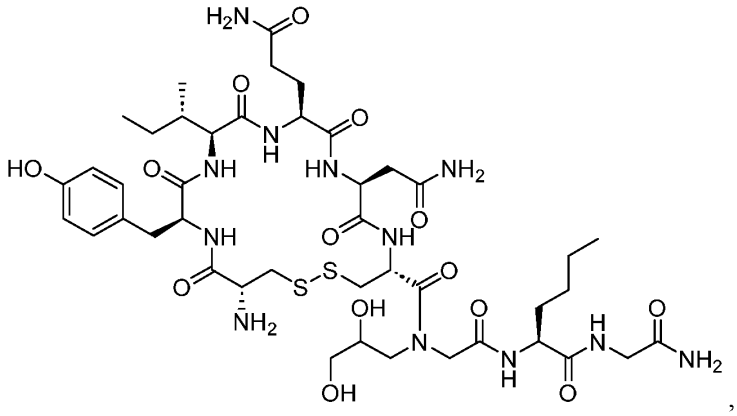
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Compound 3



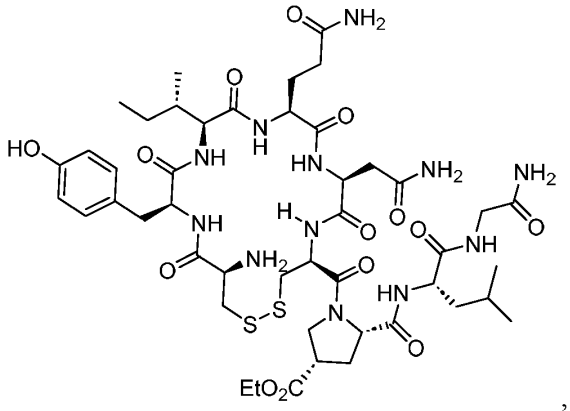
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Compound 4



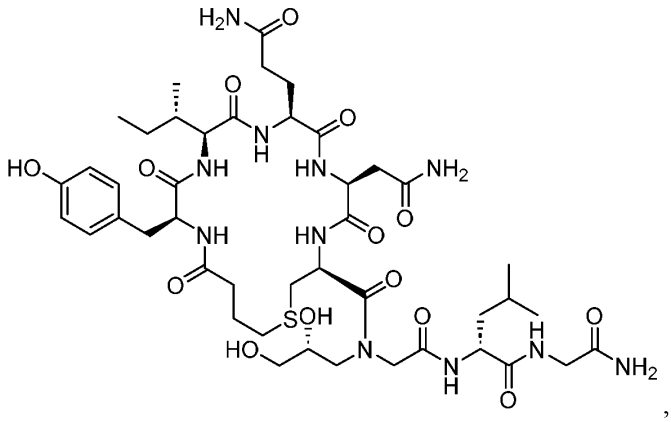
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Compound 5



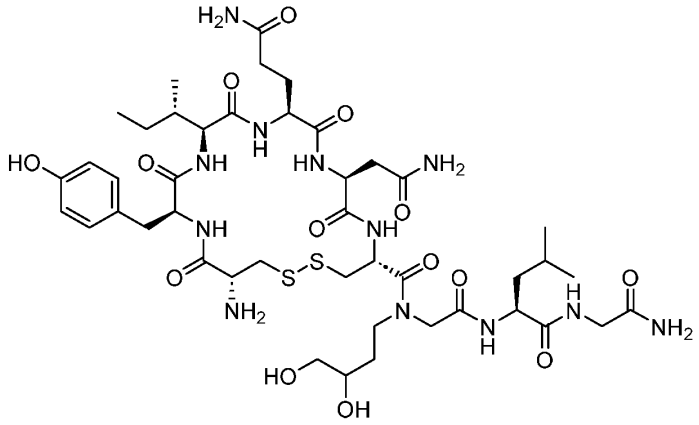
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Compound 6



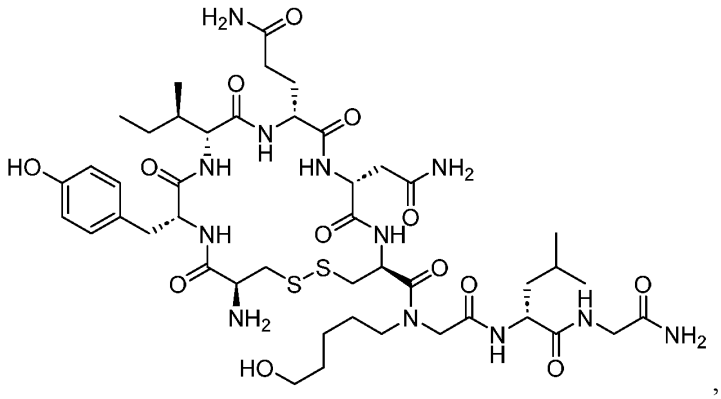
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Compound 7



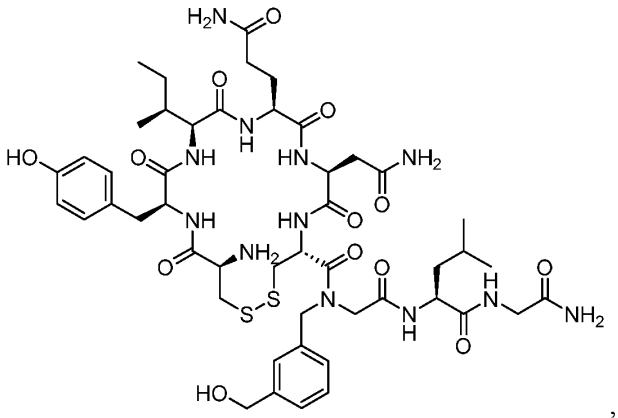
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Compound 8



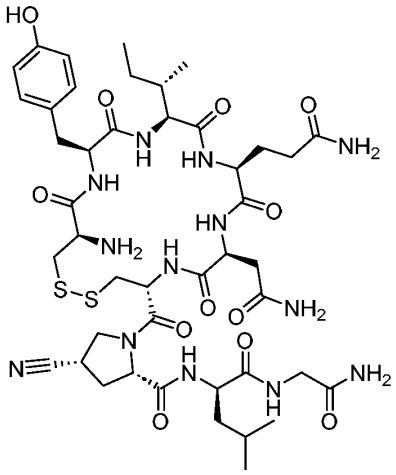
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Compound 9



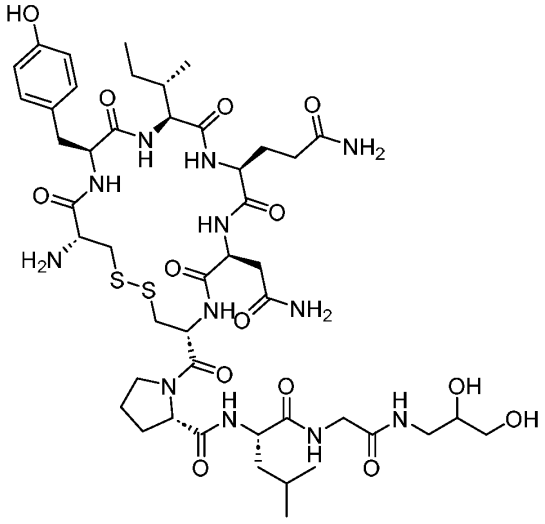
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Compound 10



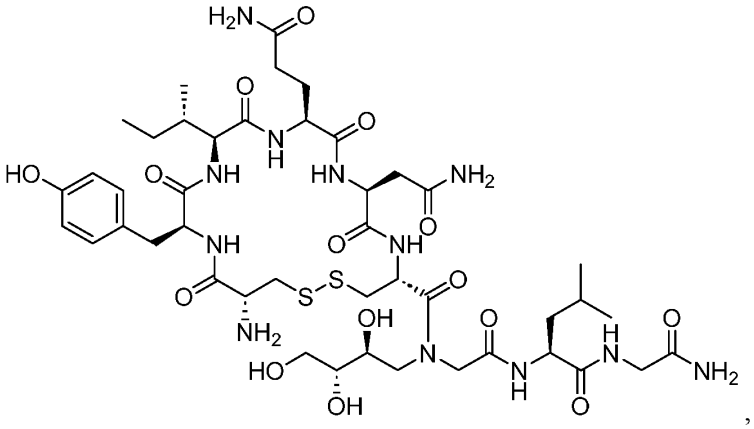
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Compound 11



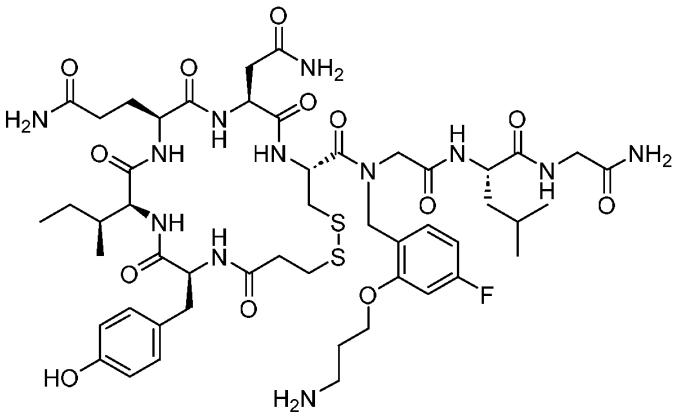
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Compound 12



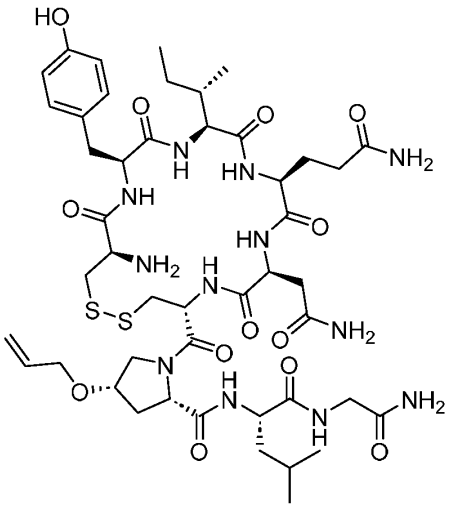
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Compound 13



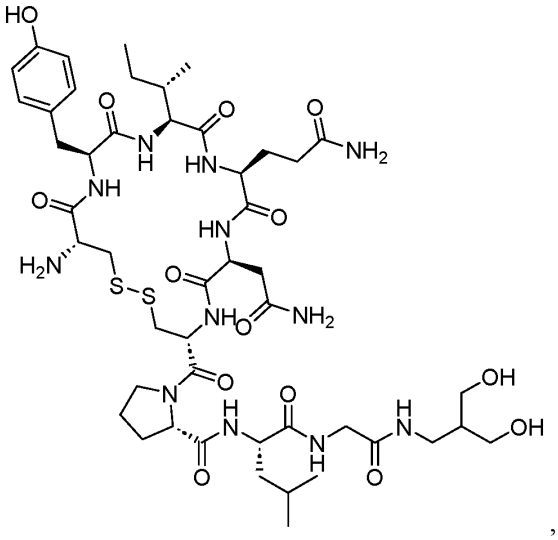
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Compound 14



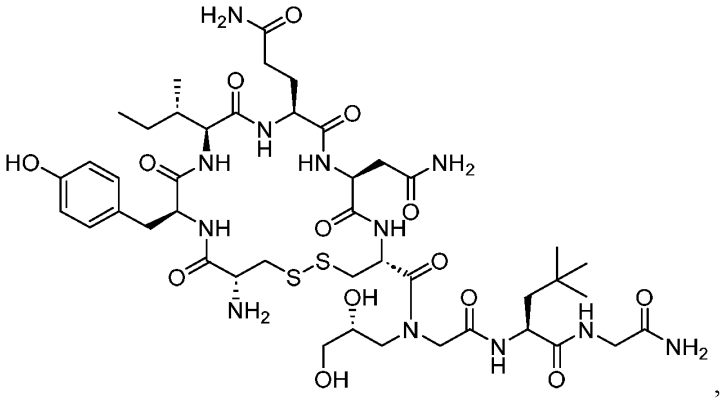
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Compound 15



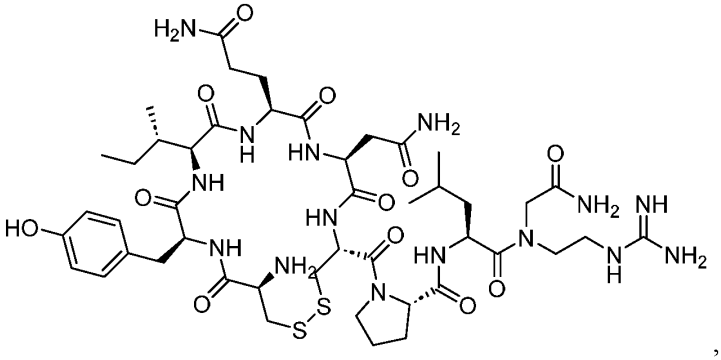
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Compound 16



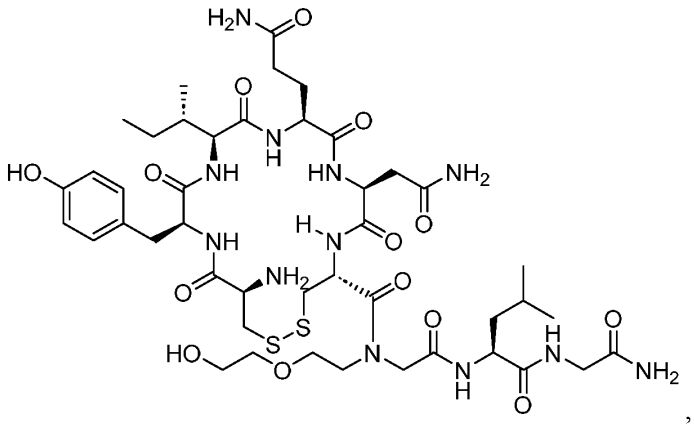
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Compound 17



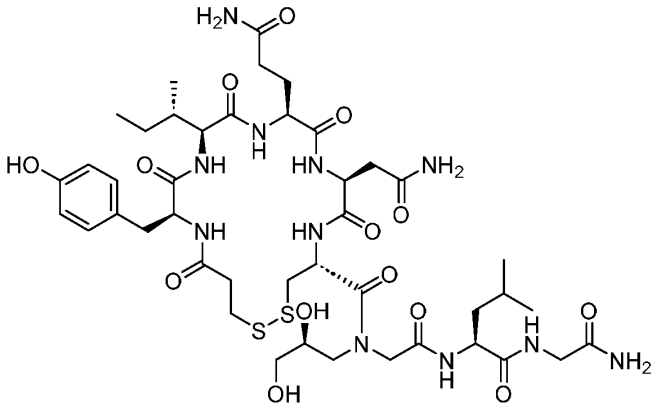
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Compound 18



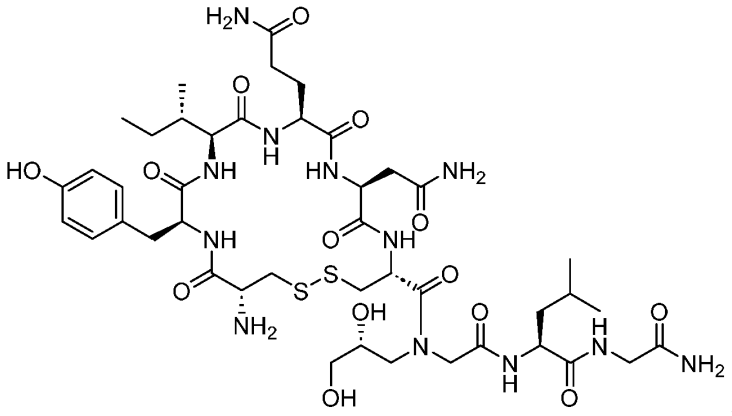
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Compound 19



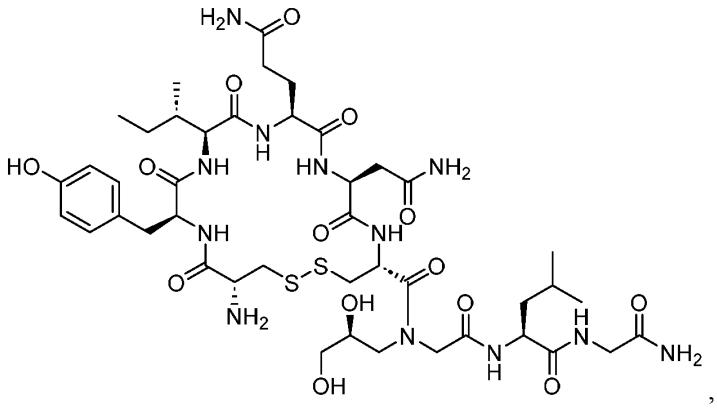
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Compound 20



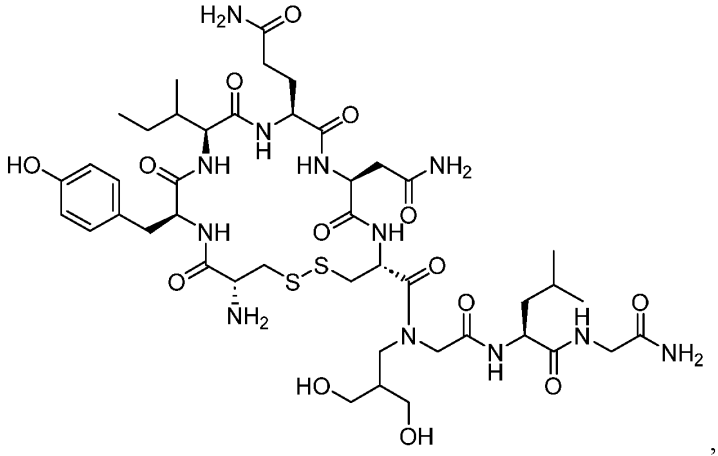
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Compound 21



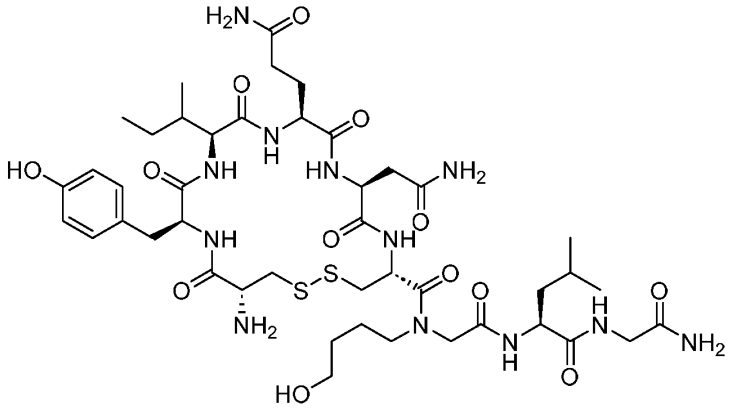
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Compound 22



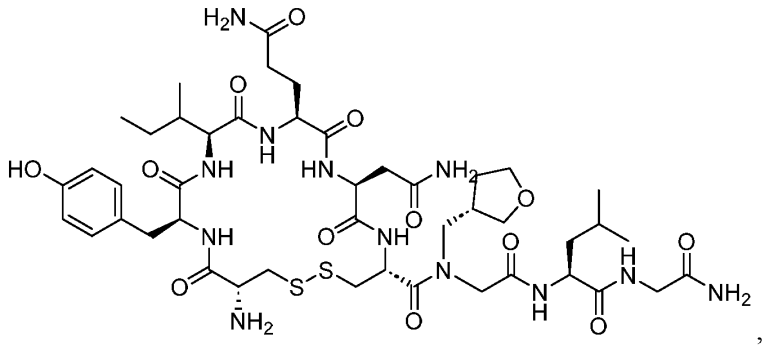
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Compound 23



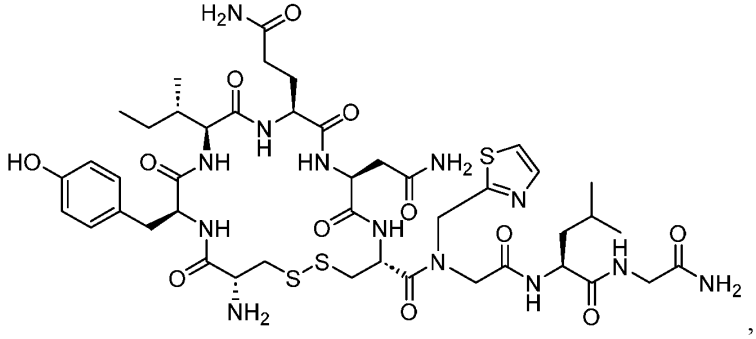
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Compound 24



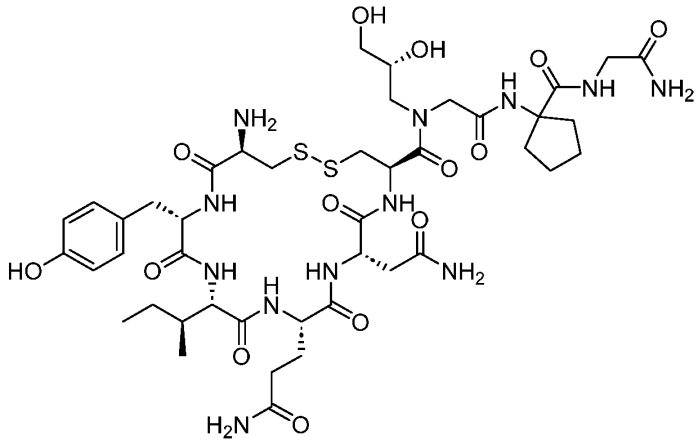
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Compound 25



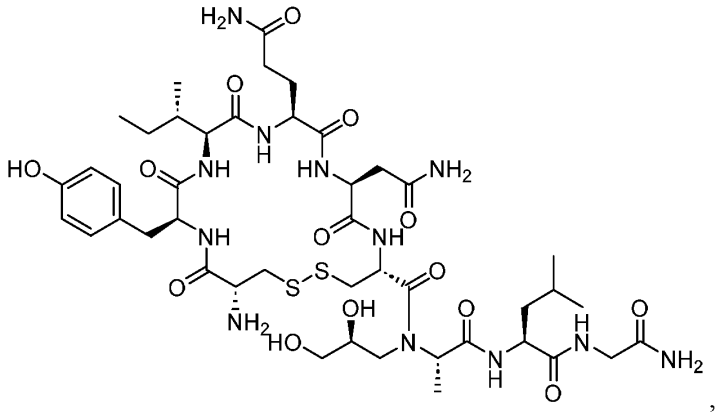
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Compound 26



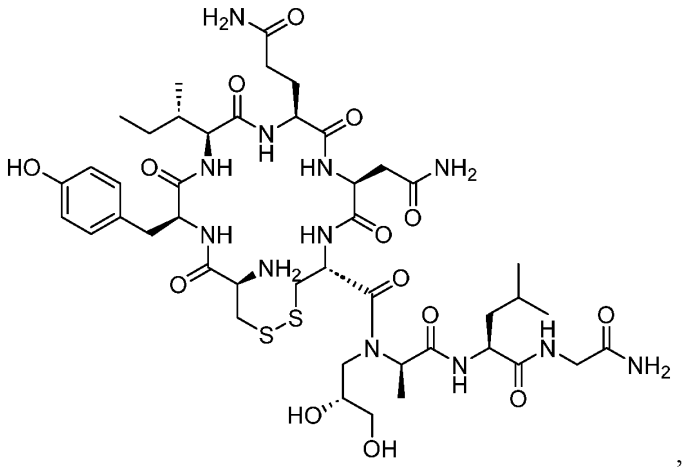
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Compound 27



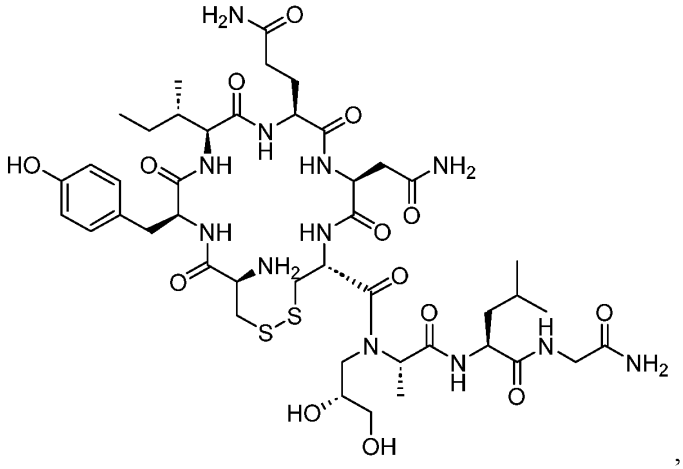
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Compound 28



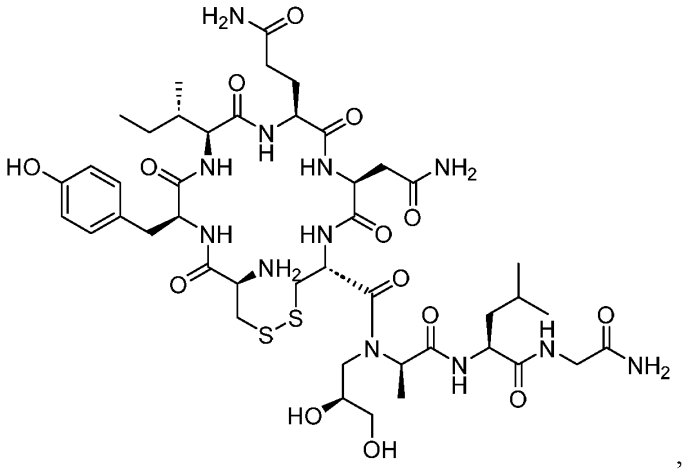
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Compound 29



, and

Compound 30

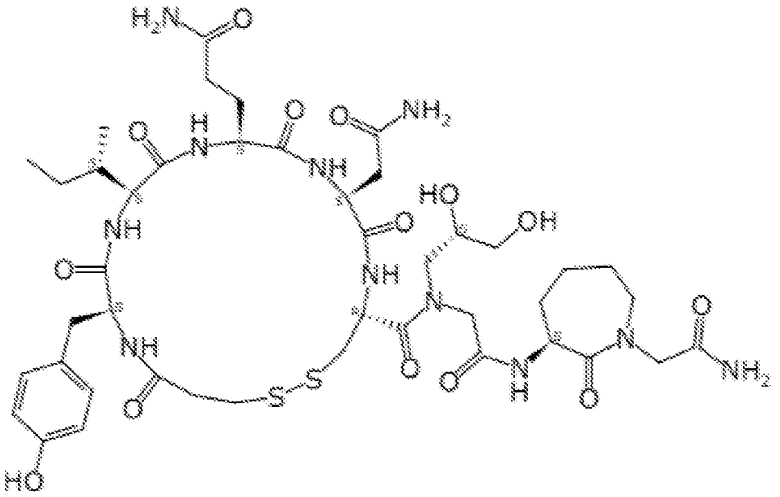


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or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

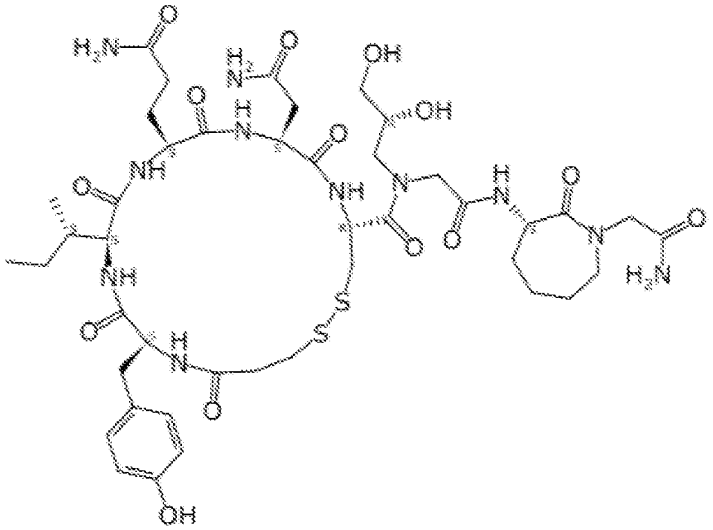
[0055] In one aspect, provided herein are compounds, selected from the group consisting of:

Compound 31



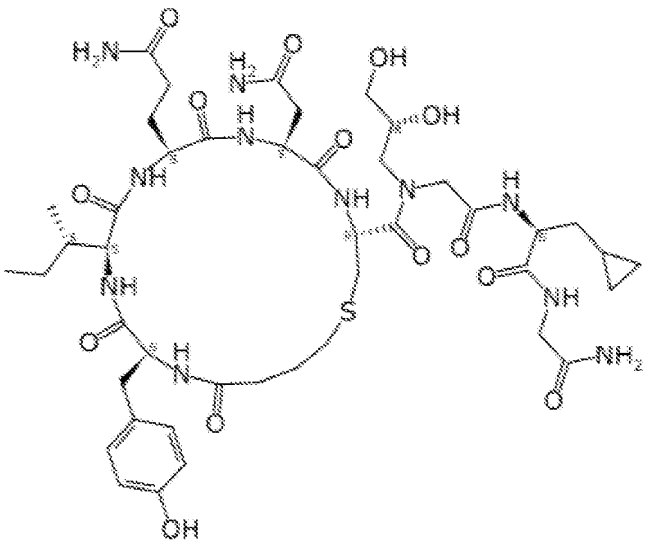
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Compound 32



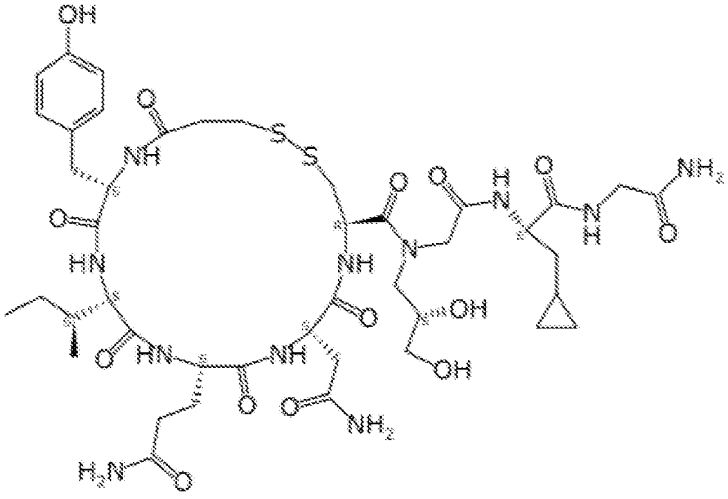
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Compound 33



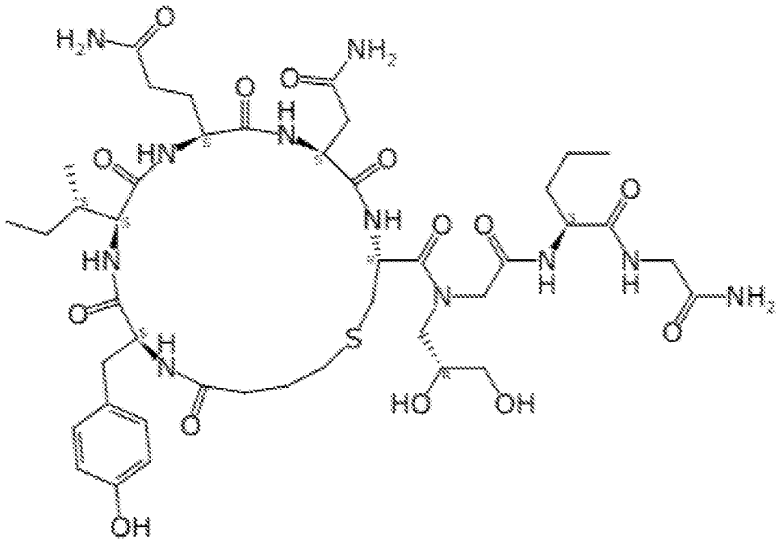
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Compound 34



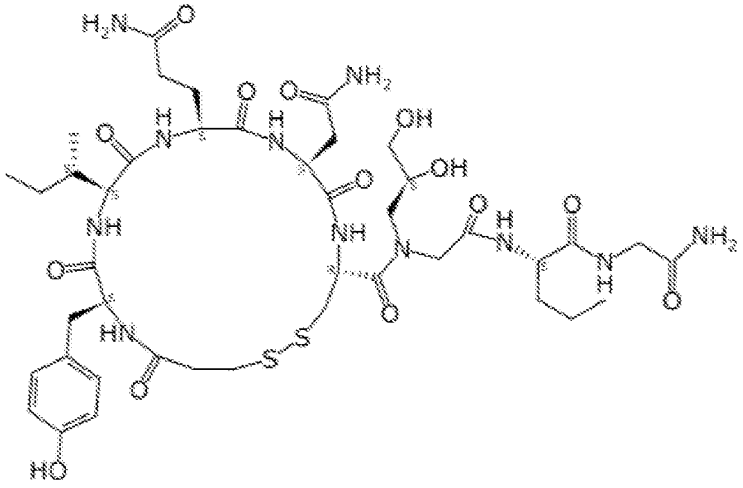
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Compound 35



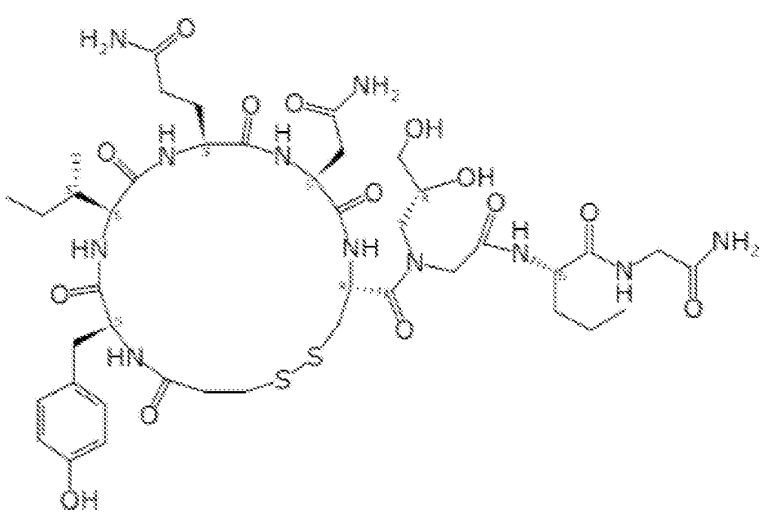
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Compound 36



, and

Compound 37

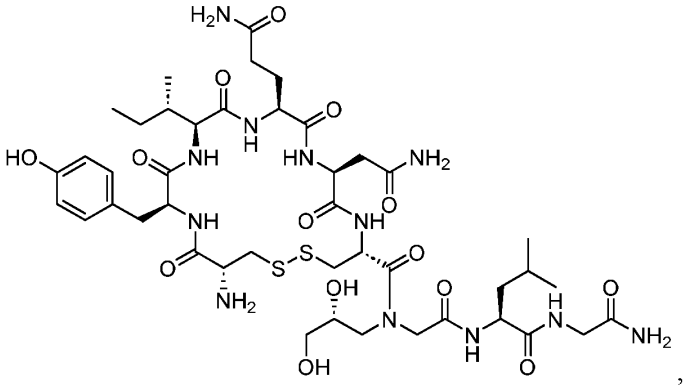


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or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

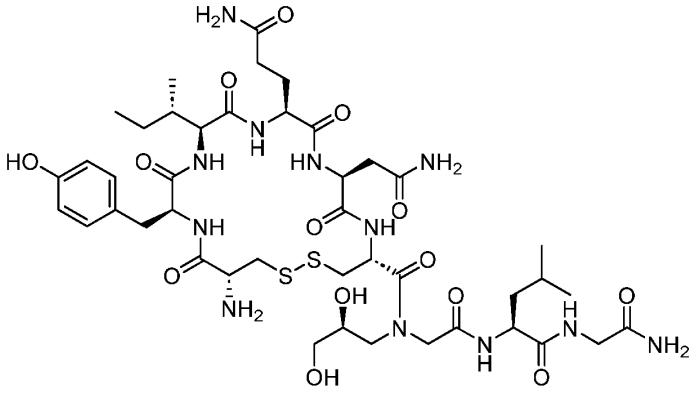
[0056] In certain embodiments, the compound is selected from a group consisting of:

Compound 20

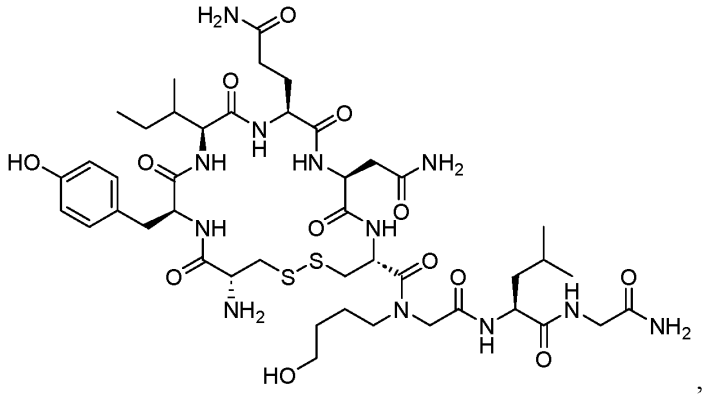


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Compound 21

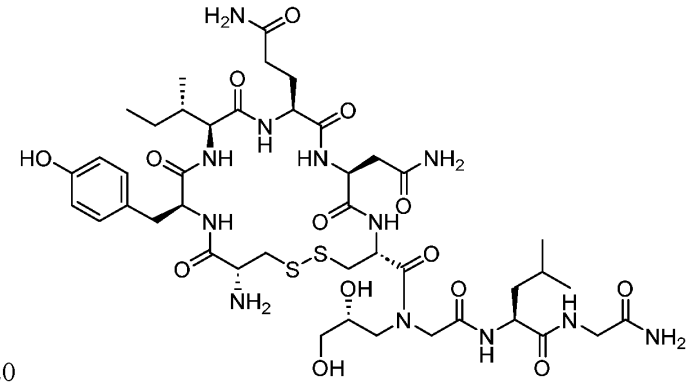


, and

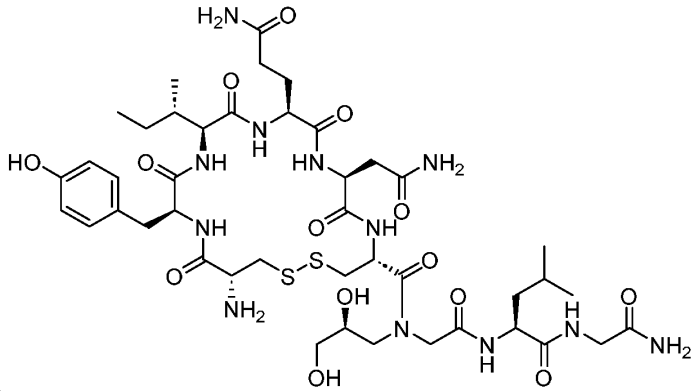
Compound 23 ,

or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

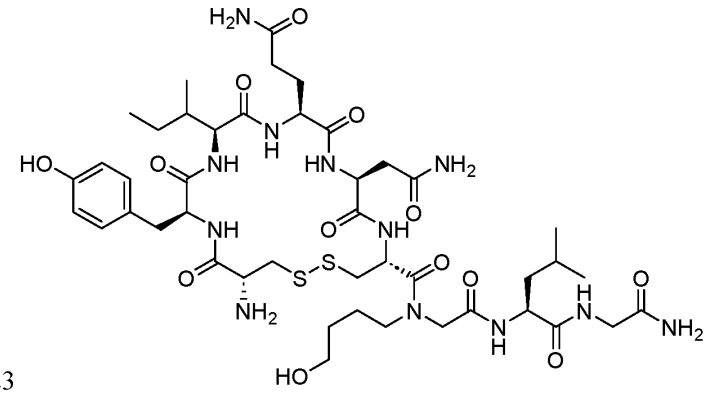
[0057] In certain embodiments, the compound is:

Compound 20 , or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

[0058] In certain embodiments, the compound is:

Compound 21 , or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

[0059] In certain embodiments, the compound is:



Compound 23 , or enantiomers, racemates, tautomers, hydrates, or solvates thereof, or a pharmaceutically acceptable salt of any of them.

[0060] In certain embodiments, the compound is in the form of a free base. In certain embodiments, the compound is in the form of a pharmaceutically acceptable salt. In certain embodiments, the compound is in the form of an enantiomer. In certain embodiments, the compound is in the form of a racemate. In certain embodiments, the compound is in the form of a hydrate. In certain embodiments, the compound is in the form of a solvate.

Methods of Treatment

[0061] The present disclosure relates to methods of treatment of various medical conditions associated with inadequate OTR activity using an oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, or pharmaceutically acceptable compositions of any of them. These methods comprise administration of an oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, or pharmaceutically acceptable compositions thereof to a subject in need thereof.

[0062] In one aspect, this disclosure provides a method for treating a medical condition such as a headache disorder associated with inadequate OTR activity comprising administering to a subject in need thereof a therapeutically effective amount of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, or a pharmaceutically composition of any of them, wherein the therapeutically effective amount is delivered via parenteral, via craniofacial mucosal, or via intranasal administration. In some embodiments the headache disorder is a migraine, tension headache, cluster headache, trigeminal neuralgia, or secondary headache. In some embodiments, the headache

disorder is a migraine. In some embodiments, the headache disorder is a tension headache. In some embodiments, the headache disorder is a cluster headache. In some embodiments, the headache disorder is a trigeminal neuralgia. In some embodiments, the headache disorder is a secondary headache. In some embodiments, the administration is craniofacial mucosal administration. In some embodiments the administration is intranasal administration. In some embodiments, the administration is parenteral administration.

[0063] In one aspect, this disclosure provides a method for treating an insulin resistant disorder associated with inadequate OTR activity comprising administering to a subject in need thereof a therapeutically effective amount of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, or a pharmaceutically acceptable composition of any of them, wherein the therapeutically effective amount is delivered via parenteral or via enteral administration. In some embodiments, the administration is parenteral administration. In some embodiments, the administration is enteral administration. In some embodiments, the insulin resistant disorder is type II diabetes. In some embodiments, the insulin resistant disorder is obesity.

[0064] In one aspect, this disclosure provides a method for treating a feeding disorder with onset during neonate development associated with inadequate OTR activity, comprising administering to a subject in need thereof a therapeutically effective amount of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, or a pharmaceutically acceptable composition of any of them, wherein the therapeutically effective amount is delivered via parenteral, via enteral, via craniofacial mucosal, or via intranasal administration. In some embodiments, the administration is craniofacial mucosal administration. In some embodiments the administration is intranasal administration. In some embodiments, the administration is parenteral administration. In some embodiments, the administration is enteral administration. In some embodiments, the feeding disorder is Prader-Willi syndrome. In some embodiments, the feeding disorder is NOFITT.

[0065] In yet another aspect, the present disclosure provides a use of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for the manufacture of a medicament for the treatment or prevention of one of the aforementioned conditions and diseases.

[0066] In yet another aspect, the present disclosure provides an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, for use in the treatment or prevention of one of the aforementioned conditions and diseases.

[0067] In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, or a pharmaceutically acceptable composition of any of them, is delivered as part of a formulation or composition designed for craniofacial mucosal, parenteral or enteral administration. In some embodiments, the administration is sublingual administration. In some embodiments, craniofacial administration is intranasal administration. In some embodiments, the parenteral administration is intravenous administration. In some embodiments, the parenteral administration is intramuscular administration. In some embodiments, the parenteral administration is subcutaneous administration. In some embodiments, the enteral administration is oral administration. In some embodiments, the enteral administration is rectal administration.

[0068] In some embodiments, the formulation is a liquid formulation. In some embodiments, the liquid formulation is an aqueous solution. In some embodiments, the formulation is administered in a volume that is between about 5 μL and about 1000 μL, which volume comprises the amounts of the oxytocin peptide analog of this disclosure, racemate, enantiomer, or a tautomer thereof. In some embodiments, the formulation is administered in a volume that is about 50 μL. In some embodiments, the formulation is administered in a volume that is about 100 μL. In some embodiments, the formulation is administered in a volume that is about 200 μL. In some embodiments, the formulation is administered in a volume that is about 1000 μL. In some embodiments, the formulation is administered in a volume that is 50 μL. In some embodiments, the formulation is administered in a volume that is 100 μL. In some embodiments, the formulation is administered in a volume that is 200 μL. In some embodiments, the formulation is administered in a volume that is 1000 μL.

[0069] In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, is administered intranasally to the mucosa tissue within the nasal cavity using a suitable device

for intranasal delivery, such as a nasal delivery device of this disclosure. Suitable regions within the nasal cavity include, but are not limited to, the inferior two-thirds of the nasal cavity, or the upper third, or the entire nasal passage. In some embodiments, the oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, is administered to the upper third of the nasal cavity. In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, is administered to the lower two thirds of the nasal cavity. In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition of any of them, is administered specifically to reach both the lower two thirds and the upper third of the nasal cavity.

[0070] In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or enantiomer, racemate, tautomer of any of them, is a daily dose of about 0.5 μg to about 2000 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or enantiomer, racemate, tautomer of any of them, is a daily dose of about 0.5 μg to about 1000 μg, about 1 μg to about 1000 μg or about 1 μg to about 2000 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, enantiomer, racemate, tautomer thereof, is a daily dose of about 4 μg to about 1000 μg, about 8 μg to about 1000 μg, about 8 μg to about 800 μg, about 8 μg to about 500 μg, about 8 μg to about 400 μg, about 8 μg to about 300 μg, about 8 μg to about 200 μg, 8 μg to about 120 μg, about 8 μg to about 100 μg, about 8 μg to about 80 μg, about 8 μg to about 50 μg, about 8 μg to about 32 μg, about 10 μg to about 1000 μg, about 10 μg to about 500 μg, about 10 μg to about 200 μg, about 10 μg to about 100 μg, about 15 μg to about 1000 μg, about 15 μg to about 800 μg, about 15 μg to about 500 μg, about 15 μg to about 400 μg, about 15 μg to about 300 μg, about 15 μg to about 200 μg, about 15 μg to about 120 μg, about 15 μg to about 100 μg, about 15 μg to about 80 μg, about 15 μg to about 50 μg, about 15 μg to about 32 μg, about 16 μg to about 1000 μg, about 16 μg to about 800 μg, about 16 μg to about 500 μg, about 16 μg to about 400 μg, about 16 μg to about 200 μg, about 16 μg to about 160 μg, about 16 μg to about 120 μg, about 16 μg to about 80 μg, about 16 μg to about 32 μg, about 20 μg

to about 1000 μg, about 20 μg to about 800 μg, about 20 μg to about 500 μg, about 20 μg to about 200 μg, about 20 μg to about 100 μg, about 20 μg to about 80 μg, about 20 μg to about 50 μg, about 20 μg to about 32 μg, about 30 μg to about 1000 μg, about 30 μg to about 500 μg, about 30 μg to about 300 μg, about 30 μg to about 120 μg, about 30 μg to about 80 μg, about 30 μg to about 50 μg, about 50 μg to about 1000 μg, about 50 μg to about 500 μg, about 50 μg to about 250 μg, about 50 μg to about 100 μg, or about 50 μg to about 80 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of about 8 μg, about 16 μg, about 32 μg, about 48 μg, about 64 μg, about 80 μg, about 96 μg, about 128 μg, about 256 μg, about 10 μg, about 20 μg, about 30 μg, about 40 μg, about 50 μg, about 60 μg, about 70 μg, about 80 μg, about 90 μg, about 100 μg, about 120 μg, about 150 μg, about 200 μg, about 400 μg, about 600 μg, about 800 μg, or about 100 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of about 8 μg to about 120 μg, about 15 μg to about 120 μg, about 30 μg to about 120 μg.

[0071] In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 0.5 μg to 2000 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 0.5 μg to 1000 μg, 1 μg to 1000 μg or 1 μg to 2000 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, of this disclosure is a daily dose of 4 μg to 1000 μg, 8 μg to 1000 μg, 8 μg to 800 μg, 8 μg to about 500 μg, 8 μg to 400 μg, 8 μg to 300 μg, 8 μg to 200 μg, 8 μg to 120 μg, 8 μg to 100 μg, 8 μg to 80 μg, 8 μg to 50 μg, 8 μg to 32 μg, 10 μg to 1000 μg, 10 μg to 500 μg, 10 μg to 200 μg, 10 μg to 100 μg, 15 μg to 1000 μg, 15 μg to 800 μg, 15 μg to 500 μg, 15 μg to 400 μg, 15 μg to 300 μg, 15 μg to 200 μg, 15 μg to 120 μg, 15 μg to 100 μg, 15 μg to 80 μg, 15 μg to 50 μg, 15 μg to 32 μg, 16 μg to 1000 μg, 16 μg to 800 μg, 16 μg to 500 μg, 16 μg to 400 μg, 16 μg to 200 μg, 16 μg to 160 μg, 16 μg to 120 μg, 16 μg to 80 μg, 16 μg to 32 μg, 20 μg to 1000 μg, 20 μg to 800 μg, 20 μg to 500 μg, 20 μg to 200 μg, 20 μg to 100 μg, 20 μg to 80 μg, 20 μg to 50 μg, 20 μg to 32 μg, 30 μg to 1000 μg, 30 μg to 500 μg, 30 μg to 300 μg, 30 μg to 120 μg, 30 μg to 80 μg, 30 μg to 50 μg, 50 μg to 1000 μg, 50 μg to 500 μg, 50 μg to 250 μg, 50 μg to

100 μg, or 50 μg to 80 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, is a daily dose of 8 μg, 16 μg, 32 μg, 48 μg, 64 μg, 80 μg, 96 μg, 128 μg, 256 μg, 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg, 120 μg, 150 μg, 200 μg, 400 μg, 600 μg, 800 μg, or 100 μg. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, is a daily dose of 8 μg to 120 μg, 15 μg to 120 μg, 30 μg to 120 μg.

[0072] In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, is about 0.25 IU to about 1000 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, is a daily dose of about 0.25 IU to about 500 IU, about 0.5 IU to about 500 IU or about 0.5 IU to about 1000 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof, is a daily dose of about 2 IU to about 500 IU, about 4 IU to about 500 IU, about 4 IU to about 400 IU, about 4 IU to about 250 IU, about 4 IU to about 200 IU, about 4 IU to about 150 IU, about 4 IU to about 100 IU, about 4 IU to about 50 IU, about 4 IU to about 40 IU, about 4 IU to about 25 IU, about 5 IU to about 500 IU, about 5 IU to about 250 IU, about 5 IU to about 100 IU, about 5 IU to about 50 IU, about 8 IU to about 500 IU, about 8 IU to about 400 IU, about 8 IU to about 250 IU, about 8 IU to about 200 IU, about 8 IU to about 100 IU, about 8 IU to about 80 IU, about 8 IU to about 60 IU, about 8 IU to about 40 IU, about 10 IU to about 500 IU, about 10 IU to about 400 IU, about 10 IU to about 250 IU, about 10 IU to about 100 IU, about 10 IU to about 50 IU, about 15 IU to about 500 IU, about 15 IU to about 250 IU, about 15 IU to about 150 IU, about 15 IU to about 60 IU, about 15 IU to about 45 IU, about 25 IU to about 500 IU, about 25 IU to about 250 IU, about 25 IU to about 125 IU, about 25 IU to about 50 IU, or about 25 IU to about 40 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of about 4 IU, about 8 IU, about 16 IU, about 24 IU, about 32 IU, about 40 IU, about 48 IU, about 64 IU, about 128 IU, about 5 IU, about 10 IU, about 15 IU, about 20 IU, about 25 IU, about 30 IU, about 35 IU, about 40 IU, about 45 IU, about 50 IU, about 60 IU, about 75 IU, about 100 IU, about 200 IU, about 300 IU, about 400 IU or about 50 IU. In some

embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of about 4 IU to about 60 IU, about 7.5 IU to about 60 IU, about 15 IU to about 60 IU, or about 30 IU.

[0073] In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 0.25 IU to 1000 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 0.25 IU to 500 IU, 0.5 IU to 500 IU or 0.5 IU to 1000 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 2 IU to 500 IU, 4 IU to 500 IU, 4 IU to 400 IU, 4 IU to 250 IU, 4 IU to 200 IU, 4 IU to 150 IU, 4 IU to 100 IU, 4 IU to 50 IU, 4 IU to 40 IU, 4 IU to 25 IU, 5 IU to 500 IU, 5 IU to 250 IU, 5 IU to 100 IU, 5 IU to 50 IU, 8 IU to 500 IU, 8 IU to 400 IU, 8 IU to 250 IU, 8 IU to 200 IU, 8 IU to 100 IU, 8 IU to 80 IU, 8 IU to 60 IU, 8 IU to 40 IU, 10 IU to 500 IU, 10 IU to 400 IU, 10 IU to 250 IU, 10 IU to 100 IU, 10 IU to 50 IU, 15 IU to 500 IU, 15 IU to 250 IU, 15 IU to 150 IU, 15 IU to 60 IU, 15 IU to 45 IU, 25 IU to 500 IU, 25 IU to 250 IU, 25 IU to 125 IU, 25 IU to 50 IU, or 25 IU to 40 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 4 IU, 8 IU, 16 IU, 24 IU, 32 IU, 40 IU, 48 IU, 64 IU, 128 IU, 5 IU, 10 IU, 15 IU, 20 IU, 25 IU, 30 IU, 35 IU, 40 IU, 45 IU, 50 IU, 60 IU, 75 IU, 100 IU, 200 IU, 300 IU, 400 IU or 50 IU. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, is a daily dose of 4 IU to 60 IU, 7.5 IU to 60 IU, 15 IU to 60 IU, or 30 IU.

Oxytocin Peptide Analog Formulations

[0074] In some embodiments, the methods for the treatment and uses of this disclosure comprise administering to a subject having a condition associated with inadequate OTR activity in need thereof a therapeutically effective dose of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, in a pharmaceutically acceptable composition or formulation. In some embodiments, the pharmaceutically acceptable

formulation or composition is a liquid formulation. In some embodiments, the liquid formulation is an aqueous solution.

[0075] In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, is administered in a liquid formulation in a volume that is between about 5 μL and about 1000 μL. In some embodiments, the volume of the liquid formulation of this disclosure administered is between about 5 μL and about 500 μL, between about 5 μL and about 250 μL, between about 5 μL and about 100 μL, between about 5 μL and about 50 μL, between about 10 μL and about 1000 μL, between about 10 μL and about 500 μL, between about 10 μL and about 250 μL, between about 10 μL and about 100 μL, between about 25 μL and about 1000 μL, between about 25 μL and about 500 μL, between about 25 μL and about 250 μL, between about 25 μL and about 100 μL, between about 50 μL and about 1000 μL, between about 50 μL and about 750 μL, between about 50 μL and about 500 μL, between about 50 μL and about 450 μL, between about 50 μL and about 400 μL, between about 50 μL and about 350 μL, between about 50 μL and about 300 μL, between about 50 μL and about 250 μL, between about 50 μL and about 200 μL, between about 50 μL and about 150 μL, between about 100 μL and about 500 μL, between about 100 μL and about 400 μL, between about 100 μL and about 300 μL, or between about 100 μL and about 200 μL.

[0076] In some embodiments, the volume of the liquid formulation of this disclosure administered is between 5 μL and 500 μL, between 5 μL and 250 μL, between 5 μL and 100 μL, between 5 μL and 50 μL, between 10 μL and 1000 μL, between 10 μL and 500 μL, between 10 μL and 250 μL, between 10 μL and 100 μL, between 25 μL and 1000 μL, between 25 μL and 500 μL, between 25 μL and 250 μL, between 25 μL and 100 μL, between 50 μL and 1000 μL, between 50 μL and 750 μL, between 50 μL and 500 μL, between 50 μL and 450 μL, between 50 μL and 400 μL, between 50 μL and about 350 μL, between 50 μL and 300 μL, between 50 μL and 250 μL, between 50 μL and 200 μL, between 50 μL and 150 μL, between 100 μL and 500 μL, between 100 μL and 400 μL, between 100 μL and 300 μL, or between 100 μL and 200 μL. In some embodiments, the volume of the liquid formulation administered is about 50 μL, about 100 μL, about 150 μL, about 200 μL, about 250 μL, about 300 μL, about 350 μL, about 400 μL, about 450 μL, or about 500 μL. In some embodiments, the volume of the liquid formulation administered is 50 μL, 100 μL, 150 μL, 200 μL, 250 μL, 300 μL, 350 μL, 400 μL, 450 μL, or 500 μL. In some embodiments, the volume

administered is about 50 μL, or about 100 μL. In some embodiments, the volume administered is about 50 μL. In some embodiments, the volume administered is 50 μL. In some embodiments, the volume administered is about 100 μL. In some embodiments, the volume administered is 100 μL. In some embodiments, the volume administered is about 50 μL, about 100 μL. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, is administered via craniofacial mucosal administration. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, is administered in a formulation or composition intranasally. In some embodiments, therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, is administered intranasally in a liquid formulation contained in a nasal device of this disclosure. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, in the composition or formulation is administered parenterally. In some embodiments, the therapeutically effective dose of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, in the composition or formulation is administered enterally.

[0077] In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure comprise between about 0.01 mg/mL and about 16 mg/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them. In some embodiments, the amount of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, in the formulation or composition of this disclosure is in the range of from about 0.01 to 16 mg/mL. In some embodiments, the formulation or composition of this disclosure comprises between about 0.01 mg/mL and about 12 mg/mL, between about 0.05 mg/mL and about 16 mg/mL, between about 0.1 mg/mL and about 12 mg/mL, between about 0.1 mg/mL and about 8 mg/mL, between about 0.1 mg/mL and about 4 mg/mL, between about 0.1 mg/mL and about

2 mg/mL, between about 0.1 mg/mL and about 1.6 mg/mL, between about 0.1 mg/mL and about 1.2 mg/mL, between about 0.1 mg/mL and about 1 mg/mL, between about 0.1 mg/mL and about 0.8 mg/mL, between about 0.1 mg/mL and about 0.4 mg/mL, between about 0.1 mg/mL and about 0.3 mg/mL, between about 0.2 mg/mL and about 16 mg/mL, between about 0.2 mg/mL and about 12 mg/mL, between about 0.2 mg/mL and about 10 mg/mL, between about 0.2 mg/mL and about 8 mg/mL, between about 0.2 mg/mL and about 6 mg/mL, between about 0.2 mg/mL and about 4 mg/mL, between about 0.2 mg/mL and about 2 mg/mL, between about 0.2 mg/mL and about 1.6 mg/mL, between about 0.2 mg/mL and about 1.2 mg/mL, between about 0.2 mg/mL and about 1 mg/mL, between about 0.2 mg/mL and about 0.8 mg/mL, between about 0.2 mg/mL and about 0.6 mg/mL, between about 0.2 mg/mL and about 0.4 mg/mL, between about 0.2 mg/mL and about 0.3 mg/mL, between about 0.3 mg/mL and about 16 mg/mL, between about 0.3 mg/mL and about 12 mg/mL, between about 0.3 mg/mL and about 10 mg/mL, between about 0.3 mg/mL and about 8 mg/mL, between about 0.3 mg/mL and about 4 mg/mL, between about 0.3 mg/mL and about 3 mg/mL, between about 0.3 mg/mL and about 1 mg/mL, between about 0.3 mg/mL and about 0.5 mg/mL, between about 0.5 mg/mL and about 16 mg/mL, between about 0.5 mg/mL and about 10 mg/mL, between about 0.5 mg/mL and about 5 mg/mL, between about 0.5 mg/mL and about 1 mg/mL, between about 1 mg/mL and about 16 mg/mL, between about 1 mg/mL and about 10 mg/mL, or between about 1 mg/mL and about 5 mg/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them. In some embodiments, the formulation or composition of this disclosure comprises between about 0.1 mg/mL and about 2 mg/mL, between about 0.15 mg/mL and about 1.5 mg/mL, or between about 0.2 mg/mL and about 1.2 mg/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them.

[0078] In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure comprise between 0.01 mg/mL and 16 mg/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer thereof. In some embodiments, the amount of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them, in the formulation or pharmaceutically acceptable composition of this disclosure is in the range of from 0.01 to 16 mg/mL. In some embodiments, the formulation or pharmaceutically acceptable composition of this disclosure comprises between 0.01 mg/mL and 12 mg/mL, between 0.05 mg/mL and 16 mg/mL, between 0.1 mg/mL and 12 mg/mL, between 0.1 mg/mL and 8 mg/mL, between

0.1 mg/mL and 4 mg/mL, between 0.1 mg/mL and 2 mg/mL, between 0.1 mg/mL and 1.6 mg/mL, between 0.1 mg/mL and 1.2 mg/mL, between 0.1 mg/mL and 1 mg/mL, between 0.1 mg/mL and 0.8 mg/mL, between 0.1 mg/mL and 0.4 mg/mL, between 0.1 mg/mL and 0.3 mg/mL, between0.2 mg/mL and 16 mg/mL, between 0.2 mg/mL and 12 mg/mL, between 0.2 mg/mL and 10 mg/mL, between 0.2 mg/mL and 8 mg/mL, between 0.2 mg/mL and 6 mg/mL, between 0.2 mg/mL and 4 mg/mL, between 0.2 mg/mL and 2 mg/mL, between 0.2 mg/mL and 1.6 mg/mL, between 0.2 mg/mL and 1.2 mg/mL, between 0.2 mg/mL and 1 mg/mL, between 0.2 mg/mL and 0.8 mg/mL, between 0.2 mg/mL and 0.6 mg/mL, between 0.2 mg/mL and 0.4 mg/mL, between 0.2 mg/mL and 0.3 mg/mL, between 0.3 mg/mL and 16 mg/mL, between 0.3 mg/mL and 12 mg/mL, between 0.3 mg/mL and 10 mg/mL, between 0.3 mg/mL and 8 mg/mL, between 0.3 mg/mL and 4 mg/mL, between 0.3 mg/mL and 3 mg/mL, between 0.3 mg/mL and 1 mg/mL, between 0.3 mg/mL and 0.5 mg/mL, between 0.5 mg/mL and 16 mg/mL, between 0.5 mg/mL and 10 mg/mL, between 0.5 mg/mL and 5 mg/mL, between 0.5 mg/mL and 1 mg/mL, between 1 mg/mL and 16 mg/mL, between 1 mg/mL and 10 mg/mL, or between 1 mg/mL and 5 mg/mL of the oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer thereof. In some embodiments, the formulation or pharmaceutically acceptable composition of this disclosure comprises between 0.1 mg/mL and 2 mg/mL, between 0.15 mg/mL and 1.5 mg/mL, or between 0.2 mg/mL and 1.2 mg/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them.

[0079] In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure comprise between about 5 IU/mL and about 8000 IU/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them. In some embodiments, the oxytocin peptide analog or Compound B formulation or pharmaceutically acceptable composition of this disclosure comprises between about 5 to 8000 IU/mL of the oxytocin peptide analog, or an enantiomer, racemate, tautomer thereof. In some embodiments, the oxytocin peptide analog, or Compound B formulation or pharmaceutically acceptable composition of this disclosure comprises between about 500 IU/mL and about 6000 IU/mL, between about 25 IU/mL and about 8000 IU/mL, between about 50 IU/mL and about 6000 IU/mL, between about 50 IU/mL and about 4000 IU/mL, between about 50 IU/mL and about 2000 IU/mL, between about 50 IU/mL and about 1000 IU/mL, between about 50 IU/mL and about 800 IU/mL, between about 50 IU/mL and about 600 IU/mL, between about 50 IU/mL and about 500 IU/mL, between about 50 IU/mL and

about 400 IU/mL, between about 50 IU/mL and about 200 IU/mL, between about 50 IU/mL and about 150 IU/mL, between about 100 IU/mL and about 8000 IU/mL, between about 100 IU/mL and about 6000 IU/mL, between about 100 IU/mL and about 5000 IU/mL, between about 100 IU/mL and about 4000 IU/mL, between about 100 IU/mL and about 3000 IU/mL, between about 100 IU/mL and about 2000 IU/mL, between about 100 IU/mL and about 1000 IU/mL, between about 100 IU/mL and about 800 IU/mL, between about 100 IU/mL and about 600 IU/mL, between about 100 IU/mL and about 500 IU/mL, between about 100 IU/mL and about 400 IU/mL, between about 100 IU/mL and about 300 IU/mL, between about 100 IU/mL and about 200 IU/mL, between about 100 IU/mL and about 150 IU/mL, between about 150 IU/mL and about 8000 IU/mL, between about 150 IU/mL and about 6000 IU/mL, between about 150 IU/mL and about 5000 IU/mL, between about 150 IU/mL and about 4000 IU/mL, between about 150 IU/mL and about 2000 IU/mL, between about 150 IU/mL and about 1500 IU/mL, between about 150 IU/mL and about 500 IU/mL, between about 150 IU/mL and about 250 IU/mL, between about 250 IU/mL and about 8000 IU/mL, between about 250 IU/mL and about 5000 IU/mL, between about 250 IU/mL and about 2500 IU/mL, between about 250 IU/mL and about 500 IU/mL, between about 500 IU/mL and about 8000 IU/mL, between about 500 IU/mL and about 5000 IU/mL, or between about 500 IU/mL and about 2500 IU/mL of the oxytocin peptide analog or of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them. In some embodiments, the oxytocin peptide analog formulation or composition of this disclosure comprises between about 50 IU/mL and about 1000 IU/mL, between about 75 IU/mL and about 750 IU/mL, or between about 100 IU/mL and about 600 IU/mL of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them.

[0080] In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure further comprise one or more pharmaceutically acceptable carriers and optionally other ingredients, such as excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Exemplary excipients include solubilizers, surfactants and chelators. For example, formulations and compositions of this disclosure may include, methyl-f3-cyclodextrin (Me-f3-CD), edetate disodium, arginine, sorbitol, NaCl, methylparaben sodium (MP), propylparaben sodium (PP), chlorobutanol

(CB), benzyl alcohol, zinc chloride, ethyl alcohol, didecanoyl L-α-phosphatidylcholine (DDPC), polysorbate, lactose, citrate, tartrate, acetate, and/or phosphate.

[0081] Pharmaceutically acceptable liquid carriers include, but are not limited to, water, saline, aqueous dextrose, and glycols particularly (when isotonic) for solutions. The carrier can also be selected from various oils, including those of petroleum, animal, vegetable or synthetic origin (*e.g.*, peanut oil, olive oil, soybean oil, mineral oil, sesame oil, and the like). Suitable pharmaceutical excipients include, but are not limited to, starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions and formulations of this disclosure can be subjected to conventional pharmaceutical processes, such as sterilization, and can contain conventional pharmaceutical additives, such as preservatives, stabilizing agents, reducing agents, anti-oxidants, chelating agents, wetting agents, emulsifying agents, dispersing agents, jelling agents, salts for adjusting osmotic pressure, buffers, and the like. A pharmaceutically acceptable liquid carrier may be hypotonic or isotonic with body fluids and may have a pH within the range of 3.5-8.5. The use of additives in the preparation of peptide and/or protein-based compositions, particularly pharmaceutical compositions, is well known in the art. In some embodiments, the oxytocin peptide analog or Compound B formulation or composition of this disclosure has a pH of about 2 to about 7. In some embodiments, the formulation has a pH of about 4 to about 7. In some embodiments, the pH of the formulation or composition is about 4.5.

[0082] In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure further comprise one or more solvent or excipient selected from the group consisting of chlorobutanol, benzalkonium, methyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, acetic acid, citric acid, glycerol, sodium chloride, sodium monohydrogen phosphate, sorbitol and water. In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure further comprise chlorobutanol, acetic acid and water. In some embodiments, the oxytocin peptide analog formulation or pharmaceutically acceptable composition of this disclosure further comprises a citrate salt, a succinate salt or a pyrophosphate salt. In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure further comprise a surface-active agent, such as a nonionic surfactant (e.g., polysorbate-80), and one or more buffers, stabilizers, or tonicifiers.

[0083] To enhance delivery into or across a mucosal surface and/or absorption of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, solvate of any of them, or pharmaceutically acceptable salt of any of them, in some embodiments, an absorption-enhancing agent is optionally included in the formulation or composition of this disclosure. These agents enhance one or more of the release or solubility (e.g., from a formulation delivery vehicle), diffusion rate, penetration capacity and timing, uptake, residence time, stability, effective half-life, peak or sustained concentration levels, clearance or other desired mucosal delivery characteristics (e.g., as measured at the site of delivery) of the oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, solvate or pharmaceutically acceptable salt thereof.. Enhancement of mucosal delivery can thus occur by any of a variety of mechanisms, for example by increasing the diffusion, transport, persistence or stability of the oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, solvate or pharmaceutically acceptable salt thereof, increasing membrane fluidity, modulating the availability or action of calcium and other ions that regulate intracellular or paracellular permeation, solubilizing mucosal membrane components (e.g., lipids), changing non-protein and protein sulfhydryl levels in mucosal tissues, increasing water flux across the mucosal surface, modulating epithelial junctional physiology, reducing the viscosity of mucus overlying the mucosal epithelium, reducing mucociliary clearance rates, and other mechanisms. In some embodiments, the formulations or pharmaceutically acceptable compositions of this disclosure may further comprise one or more mucosal delivery-enhancing agents. For example, mucosal delivery-enhancing agents may be selected from (A)-(K): (A) solubilization agents; (B) charge modifying agents; (C) pH control agents; (D) degradative enzyme inhibitors; (E) mucolytic or mucus clearing agents; (F) ciliostatic agents; (G) membrane penetration-enhancing agents; (H) modulatory agents of epithelial junction physiology, such as nitric oxide (NO) stimulators, chitosan, and chitosan derivatives; (I) vasodilator agents; (J) selective transport-enhancing agents; and (K) stabilizing delivery vehicles, carriers, supports or complex-forming species with which the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, solvate of any of them, or pharmaceutically acceptable salt of any of them, is effectively combined, associated, contained, encapsulated or bound to stabilize the active agent for enhanced mucosal delivery. Membrane penetration-enhancing agents in Group (G) may be (i) a surfactant, (ii) a bile salt, (iii) a phospholipid or fatty acid additive, mixed micelle,

liposome, or carrier, (iv) an alcohol, (v) an enamine, (iv) an NO donor compound, (vii) a long-chain amphipathic molecule, (viii) a small hydrophobic penetration enhancer; (ix) sodium or a salicylic acid derivative; (x) a glycerol ester of acetoacetic acid, (xi) a cyclodextrin or beta-cyclodextrin derivative, (xii) a medium-chain fatty acid, (xiii) a chelating agent, (xiv) an amino acid or salt thereof, (xv) an N-acetylamino acid or salt thereof, (xvi) an enzyme degradative to a selected membrane component, (xvii) an inhibitor of fatty acid synthesis, (xviii) an inhibitor of cholesterol synthesis; or (xiv) any combination of the membrane penetration enhancing agents of (i)-(xviii). In various embodiments of this disclosure, an oxytocin peptide analog of this disclosure, or an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salt thereof, may be combined with one, two, three, four or more of the mucosal delivery-enhancing agents recited in (A)-(K). These mucosal delivery-enhancing agents, in some embodiments are admixed, alone or together with the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, solvate of any of them, or pharmaceutically acceptable salt of any of them, or otherwise combined therewith in a pharmaceutically acceptable formulation or delivery vehicle. The formulations or pharmaceutically acceptable compositions of this disclosure in some embodiments provide increased bioavailability of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate of solvate of any of them, following delivery thereof to a mucosal surface (e.g., in the nasal cavities) of a mammalian subject.

[0084] Mucosal absorption enhancing compounds may also be included in the formulations or pharmaceutically acceptable compositions of this disclosure. Useful absorption compounds include, but are not limited to, gelling systems, surfactants, bile salts, dihydrofusidates, bioadhesive/mucoadhesive agents, phospholipid additives, mixed micelles, liposomes, or carriers, alcohols, enamines, cationic polymers, NO donor compounds, long-chain amphipathic molecules, small hydrophobic penetration enhancers; sodium or a salicylic acid derivatives, glycerol esters of acetoacetic acid, cyclodextrin or beta-cyclodextrin derivatives, medium-chain fatty acids, chelating agents, amino acids or salts thereof, N-acetylamino acids or salts thereof, mucolytic agents, enzymes specifically targeted to a selected membrane component, inhibitors of fatty acid synthesis and inhibitors of cholesterol synthesis.

[0085] Gelling systems useful in the formulations and compositions of this disclosures may include any known gelling system, such as a chemically reactive pectin-based gelling system

(e.g., PecSys™, Archimedes Pharma) and a thermoreactive polymer gelling system (e.g., Pluronic® F127, BASF). PecSys™ is a low viscosity aqueous pectin-based solution, delivered as a fine mist in which each droplet gels on contact with calcium ions in the nasal mucosa. Other low methoxy pectin could also be employed, e.g., at about 1% concentration. Pluronic® F127 contains ethylene oxide/propylene oxide block copolymers. The gelling temperatures vary depending on the ratios of components and the amount of co-polymer employed in the final formulation. Gelling in the human nasal cavity has been demonstrated for Pluronic® F127 at approximately 18-20 % wt/vol, for examples, as used in a vitamin B12 gel supplement (EnerB, Nature's Bounty, NY) and in a gelling sumatriptan, which contains 18% wt/vol Pluronic® F127 and 0.3% wt/vol Carbopol (anionic bioadhesive polymer C934P). The monomer ratios and concentrations may be adjusted for the intended oxytocin peptide analog or Compound B formulations to ensure gelling at 25-37 °C, around the typical temperature of 34 °C in nasal cavity. If the gelation temperature is lower than 25 °C, the formulation could gel at room temperature; if the gelation temperature is above 37 °C, the formulation would not fully gel on contact with the nasal mucosa. Addition of a mucoadhesive, e.g., addition of up to 0.5 % Carbopol, may further lower the gelation temperature. In some embodiments, the formulations or compositions of this disclosure further comprise one or more gelling agents, such that the formulation or composition forms a gel in the nasal cavity, thus enhancing nasal absorption of the oxytocin peptide. In some embodiments, the formulations or compositions of this disclosure further comprise a mucoadhesive agent such as Carbopol.

[0086] In some embodiments, thiolated chitosans (*e.g.*, chitosan covalently modified with 2-iminothiolane) are used as mucosal absorption enhancing excipient in the formulations or compositions of this disclosure. In some embodiments, chitosan co-polymer nanoparticles may be used, such as nanoparticles containing chitosan glutamate and a negatively charged polymer (e.g., tripolyphosphate pentasodium). In some embodiments, the formulations or compositions of this disclosure further comprise about 1% of the chitosan-containing excipient. In some embodiments, the formulations or compositions of this disclosure further comprise a chitosan-containing excipient such as ChiSys®, http://www.archimedespharma.com/productArchiDevChiSys.html.

[0087] The lists of carriers and additives discussed herein are not limiting and a worker skilled in the art can choose carriers and excipients from the GRAS (generally regarded as safe) list of chemicals used in pharmaceutical preparations and those that are currently

allowed by the U.S. Food and Drug Administration in topical and parenteral formulations, and those that become allowed in the future. (See also Wang et al., (1980) *J. Parent. Drug Assn.*, 34:452-462; Wang et al., (1988) *J. Parent. Sci. and Tech.*, 42: S4-S26.)

Delivery Systems

[0088] In some embodiments, the oxytocin peptide analogs of this disclosure, Compound B, or an enantiomers, racemates, tautomers, hydrates, or solvates of any of them, and pharmaceutically acceptable salts of any of them, and the formulations and compositions of this disclosure are adapted for intranasal administration. In some embodiments, the formulation or pharmaceutically acceptable composition is adapted for intranasal administration and further comprises a device for intranasal administration, such as a dropper, pump spray, squeeze bottle, airless and preservative-free sprays, or a nasal pump apparatus, e.g., a nasal pump apparatus comprising a reservoir bottle attached to an aerosolizer. In some embodiments the device is prepackaged with the analog, Compound B, enantiomers, racemates, tautomers, hydrates, solvates or salts of any of them, composition or formulation. In some embodiments, the subject or health care provider adds the analog, Compound B, enantiomers, racemates, tautomers, hydrates, solvates or salts of any of them, formulation or composition to the device.

[0089] In some embodiments the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them or a pharmaceutically acceptable salt of any of them, and their formulations and compositions are administered intranasally, and in some embodiments the formulation or composition is prepared as a liquid aerosol formulation combined with a dispersing agent and/or a physiologically acceptable diluent. Alternatively, dry powder aerosol formulations or compositions may be used, and may contain a finely divided solid form of an oxytocin peptide analog, Compound B, enantiomer, racemate, tautomer, hydrate, solvate, or pharmaceutically acceptable salts of any of them, of this disclosure and a dispersing agent allowing for the ready dispersal of the dry powder particles. With either liquid or dry powder aerosol formulations, in some embodiments the formulation is aerosolized into small, liquid or solid particles in order to ensure that the aerosolized dose reaches the mucous membranes of the nasal passages or the lung. The term “aerosol particle” is used herein to describe a liquid or solid particle suitable of a sufficiently small particle diameter for nasal (in a range of from about 10 microns) distribution to targeted mucous or alveolar membranes. Other considerations include the construction of the delivery device, additional components in the

formulation, and particle characteristics. These aspects of nasal administration of drugs are well known in the art, a manipulation of formulations, aerosolization means, and construction of delivery devices, is also well known to the ordinary skill in the art.

[0090] In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them or a pharmaceutically acceptable salt of any of them, or the formulations or compositions of this disclosure are administered using a device for intranasal delivery. The device may be any device suitable for intranasal administration of any form of the oxytocin peptide analog of this disclosure, Compound B, an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salts of any of them, or the formulations or the compositions of this disclosure. In some embodiments, the device is suitable for delivery of the oxytocin peptide analog, of this disclosure, Compound B, an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salts of any of them, to specific region within the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide analog of this disclosure, Compound B, an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salts of any of them, to the inferior two-thirds of the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide analog of this disclosure, Compound B, an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salts of any of them, to the upper third of the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide analog of this disclosure, Compound B, an enantiomer, racemate, tautomer, hydrate, of solvate, or pharmaceutically acceptable salts of any of them to the entire nasal passage.

[0091] In some embodiments, the device for intranasal delivery is a nasal pump apparatus. In some embodiments, the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator. In some embodiments, the pump actuator is metered to deliver a specified volume (*e.g.*, about 5 to about 1000 µL, about 50 to about 150 µL, about 50 µL, or about 100 µL) per spray or squirt into each nostril in a specified distribution of droplet sizes. In some embodiments, the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer, *e.g.*, an Equadel® pump marketed by Aptar Pharma. In some embodiments, the device for intranasal administration functions irrespective of the pressure applied to the pump once a threshold value is reached. In some embodiments, the device for nasal administration is a mucosal atomization device (e.g., LMA® MAD NASAL™) that can be added to a syringe. For administration in large mammals, the nasal pump apparatus may comprise a

reservoir bottle attached to a pump actuator that is metered to deliver larger volumes (e.g., about 100 μL to about 600 μL, or higher).

[0092] In some embodiments, the device for intranasal delivery is designed for delivery of multiple doses of the drug formulations. For example, a nasal pump apparatus may comprise a reservoir bottle attached to a pump actuator where the reservoir bottle holds multiple doses of the liquid formulation of one of the forms of the oxytocin peptide analogs of this disclosure and the pump actuator is metered to deliver a specified volume that is a fraction of the liquid formulation held in the reservoir bottle. In some embodiments, the pump actuator is metered to deliver about 50 μL of the liquid formulation per spray into each nostril. In some embodiments, the pump actuator is metered to deliver about 100 μL of the liquid formulation per spray into each nostril. In some embodiments, the pump actuator is metered to deliver about 200 μL of the liquid formulation per spray into each nostril. The nasal pump apparatus may comprise a filter for preventing back flow in order to reduce contaminant (*e.g.*, bacterial) ingress into the reservoir bottle. In some embodiments, the nasal pump apparatus comprises a metal-free path for delivery of the liquid formulation (e.g., a plastic path). In some embodiments, the pump apparatus uses plastic material that is stable to gamma radiation (used for sterilizing the nasal apparatus). In some embodiments, the device for intranasal delivery is equipped with a multi-dose pump comprising a microbial filter and an auto-blocking mechanism in the pump actuator, for example, a spray device described in US Patent No. 5,988,449, which is incorporated by reference in its entirety.

[0093] In some embodiments, the device for intranasal delivery is a breath-actuated nasal delivery device, such as the devices described in US Patents No. 7,784,460 and 7,854,227. Such devices may improve delivery to a target site deep into the nasal cavity. In some embodiments, a standard metered dose spray device is incorporated into a housing that allows the patient to blow into a mouthpiece to actuate the device. In some embodiments, the device is comprised of a conical sealing nosepiece and a mouthpiece that incorporate a traditional mechanical spray pump (*e.g.*, an Equadel® pump marketed by Aptar Pharma), a chargeable spring and a breath actuation mechanism. The system can be used for single or multi-dose delivery. One example of such a liquid delivery device is the OptiMist™ device. When in use, the nasal piece of the device is inserted into the nostril and the mouth piece is blown into. This closes the soft palate, transfers pressure to the nostril, opens passages providing airflow behind the nasal septum and allows air to exit the other nostril (bidirectional flow). Since the

device is breath actuated, small particles cannot enter the lungs. Modifications to flow rate and particle size allows for targeting of specific nasal regions.

[0094] Drop size, plume volume and flow rate can be modified to target specific nasal regions. The liquid spray may provide droplet size between 5 and 50 microns in order to target olfactory and/or respiratory epithelium. Larger droplets primarily travel down the nasopharynx and are swallowed, while smaller droplets are targeted to the pulmonary tissue. The Mass Median Equivalent Aerodynamic Diameter (MMAD) is used to specify the drop size. In some embodiments, the pH of the nasal spray is adjusted to deliver charged peptide in mostly an unionized state. The nose will generally tolerate solutions having a pH of about 3-8. The nasal mucosa can generally absorb volumes of approximately 100 µL before saturation occurs and liquid begins to drip out of the nose. Therefore, plume volume may be up to (and including) 100 µL. For use in large mammals, plume volume may be up to (and including) 150 µL or higher (*e.g.*, 600 µL or higher). For infant and pediatric use, or for veterinary use in smaller animals (*e.g.*, rodents, cats), smaller plume volumes (5-50 µL) could be used. In some embodiments, the device for intranasal delivery is a unit-dose metering spray device suited for single administration of the oxytocin peptide analog formulation. In some embodiments, the device for intranasal delivery is a multi-dose metering spray pump apparatus suited for repeated administrations of an oxytocin peptide analog.

[0095] In some embodiments, the device for intranasal delivery is an ergonomically designed to facilitate patient compliance, such as a pump apparatus with a side-actuation triggering mechanism. In some embodiments, the device for intranasal delivery comprises a metering spray pump working as a closed system, which does not allow air to enter into the pump apparatus thus preventing contamination from airborne germs. In some embodiment, the device for intranasal delivery comprises a metering spray pump working with a filter. The venting air is sucked through a filter assembled inside the pump, keeping airborne germs out of the pump apparatus. In some embodiments, the intranasal delivery device comprising a nasal pump apparatus may further comprise micro-electronic devices that may facilitate data transmission and treatment monitoring.

[0096] In some embodiments, the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them or a pharmaceutically acceptable salt of any of them, or the formulations or pharmaceutically acceptable compositions of this disclosure comprise an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of

them, or a pharmaceutically acceptable salt of any of them, contained in any one of the devices for intranasal delivery described herein, and wherein the concentration of the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them or a pharmaceutically acceptable salt of any of them , is within any of the concentration or dosage ranges described herein, as if each and every combination of device and concentration is described individually.

Kits

[0097] Provided herein are kits for carrying out any of the methods or uses described herein. Kits are provided for use in treatment of a headache disorder, an insulin resistance disorder, or a feeding disorder in a newborn. In some embodiments, the kit comprises any of the analogs, formulations and compositions described in various embodiments above, comprising a therapeutically effective amount of an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer of any of them Other kits may further comprise instructions providing information to the user and/or health care provider for carrying out any one of the methods and uses described herein. Kits may further comprise instructions for predicting efficacy of the oxytocin peptide analog.

[0098] Also provided is a kit comprising an oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, or a pharmaceutically acceptable composition or a formulation of this disclosure contained in a device for intranasal administration (such as a nasal pump apparatus) and suitable packaging. The kit may further comprise instructions for administering the oxytocin peptide analog of this disclosure, Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them, or a pharmaceutically acceptable formulation or a formulation of this disclosure, to a subject suffering from a condition associated with inadequate OTR activity in need thereof.

[0099] The instructions relating to the use of the kit for carrying out the methods of this disclosure generally describe how the contents of the kit are used to carry out the methods of the disclosure. Instructions supplied in the kits for carrying out the methods and uses of this disclosure are typically provided on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

Incorporation by Reference

[0100] All references cited herein, including patents, patent applications, papers, textbooks, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

EXAMPLES

[0101] In order that the disclosures described herein may be more fully understood, the following examples are set forth. The synthetic examples described in this application are offered to illustrate the compounds, pharmaceutical compositions and methods provided herein and are not to be construed in any way as limiting their scope.

Materials and Methods

[0102] The oxytocin peptide analogs and their various forms provided herein may be prepared from known or commercially available starting materials and reagents by one skilled in the art of organic synthesis. The oxytocin peptide analogs provided herein can be prepared from readily available starting materials using general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine and conventional experimentation.

[0103] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and P. G. M. Wuts, Protecting Groups in Organic Synthesis, Second Edition, Wiley, New York, 1991, and references cited therein.

[0104] The oxytocin peptide analogs provided herein may be isolated and purified by known standard procedures. Such procedures include (but are not limited to) recrystallization, column chromatography, or HPLC. Exemplary chiral columns available for use in the separation/purification of the enantiomers/diastereomers provided herein include,

but are not limited to, CHIRALPAK® AD-10, CHIRALCEL® OB, CHIRALCEL® OB-H, CHIRALCEL® OD, CHIRALCEL® OD-H, CHIRALCEL® OF, CHIRALCEL® OG, CHIRALCEL® OJ and CHIRALCEL® OK.

[0105] *Abbreviation List*

SPPS = Solid-phase peptide synthesis,

CTC-Rink-linker resin = 4-[(2,4-Dimethoxyphenyl)Fmoc- aminomethyl]phenoxyacetoxy (3-chlorophenyl)-diphenylmethyl resin from Matrix Innovation

Fmoc = 9-Fluorenylmethoxycarbonyl

Gly = Glycine

Leu = Leucine

Asn(Trt) = Trityl-protected asparagine

Gln(Trt) = Trityl-protected glutamine

lle = Isoleucine

Tyr(tBu) = tert Butyl-protected tyrosine

Pro = Proline

DMF = N,N-Dimethylformamide

HAt = 1-Hydroxyazabenzotriazole

HOBt = 1-Hydroxybenzotriazole

DIC = N,N'-Diisopropylcarbodiimide

NEP = N-Ethylpyrrolidone

DIPEA = Diisopropylethyl amine

MeOH = Methanol

CD_3OD = deuterated methanol

$CDCl_3$ = deuterated chloroform

DCM/CH_2C1_2 = Dichloromethane

MTBE = Methyl tert-butyl ether

MeTHF = 2-Methyltetrahydrofuran

TFA = Trifluoroacetic acid

MeCN =Acetonitrile

HATU = l-[Bis(dimethylamino) methylene]-lH-l,2,3-triazolo[4,5- b]pyridinium 3-oxid hexafluorophosphate

HFIP = Hexafluoro-2-propanol

TIS = Triisopropylsilane

DCE – Dichloroethane

KTOMS = Potassium trimethylsilanolate

DBAD = Di-tert-butyl Azodicarboxylate

DIC = N,N'-Diisopropylcarbodiimide

Bn = Benzyl

IPA = Isopropyl alcohol

NMP = l-Methyl-2- pyrrolidinone

TBDMS/TBS = tert-Butyldimethylsilyl

DMSO = Dimethyl sulfoxide

DMI = l,3-Dimethyl-2-imidazolidinone

DMPU = l,3-Dimethyl-3,4,5,6-tetrahydro-2(lH)-pyrimidinone

NMM = N-Methylmorpholine

DMAP = N,N-Dimethyl-4-aminopyridine

DAG: Diacylglycerol

DPBS: Dulbecco's Phosphate Buffered Saline

FBS: Fetal Bovine Serum

MOR: Mu Opioid Receptor

NFU: Normalized fluorescence units

General Methods

[0106] All temperatures are in degrees Celsius (°C) and are uncorrected. Reagent grade chemicals and anhydrous solvent were purchased from commercial sources and unless otherwise mentioned, were used without further purification. The names of the products were determined using the naming software included in Biovia electronic lab notebook. Silica gel chromatography was performed on Teledyne Isco instruments using pre-packaged disposable SiO_2 stationary phase columns with eluent flow rate range of 15 to 200 mL/min, UV detection (200-300, 254 and 280 nm). Reverse phase preparative HPLC was carried out using C18 columns, UV detection (214 and 254 nm) eluting with gradients of MeCN in H_2O (0.03% $(NH_4)_2CO_3$/ 0.375% NH_4OH) (high pH), MeCN in H_2O (0.1% HCOOH) (low pH) or MeCN in H_2O (neutral pH). The analytical HPLC chromatograms were performed using an Agilent 1100 series instrument with DAD detector (190 nm to 300 nm). The mass spectra were recorded with a Waters Micromass ZQ detector at 130 ºC. The mass spectrometer was equipped with an electrospray ion source (ESI) operated in a positive ion mode and was set to scan between *m/z* 150-750 with a scan time of 0.3 s. Products and intermediates were

analyzed by HPLC/MS on a Gemini-NX (5 uM, 2.0 × 30 mm) using a high pH buffer gradient of 5% to 100% of MeCN in H_2O (0.03% $(NH_4)_2CO_3$/ 0.375% NH_4OH) over 10 min at 1.8 mL/min for a 10 min run (B05), EVO C18 (5 uM, 3.0 × 50 mm) using a low pH buffer gradient of 5% to 100% of MeCN in H_2O (0.1% HCOOH) over 2.5 min at 2.2 mL/min for a 3.5 min run (A05). The ^1H NMR spectra were recorded on a Bruker UltraShield 500 MHz/54 mm instrument (BZH 43/500/70B, D221/54-3209) or on a 300 MHz instrument. The chemical shifts are referenced to solvent peaks, which in ^1H NMR appears at 7.26 ppm for $CDCl_3$, 2.50 for DMSO-d_6, 4.79 for D_2O, and 3.31 ppm for CD_3OD.

Analytical QA/QC Methods

[0107] The analytical QA/QC chromatograms were performed using a Waters Acquity UPLC instrument with DAD detector (200 nm to 320 nm) and a thermostatted column compartment. The mass spectra were recorded with a Waters SQD detector with the desolvation temperature set to 450 °C, the source temperature set to 150 °C, and the desolvation gas flow set to 1000 L/h. The mass spectrometer was equipped with an electrospray ion source (ESI) operated in a positive, or negative, ion mode and was set to scan between m/z 100-2000 with a scan time of 0.2s. Products were analyzed by UPLC/MS using one of the following methods:

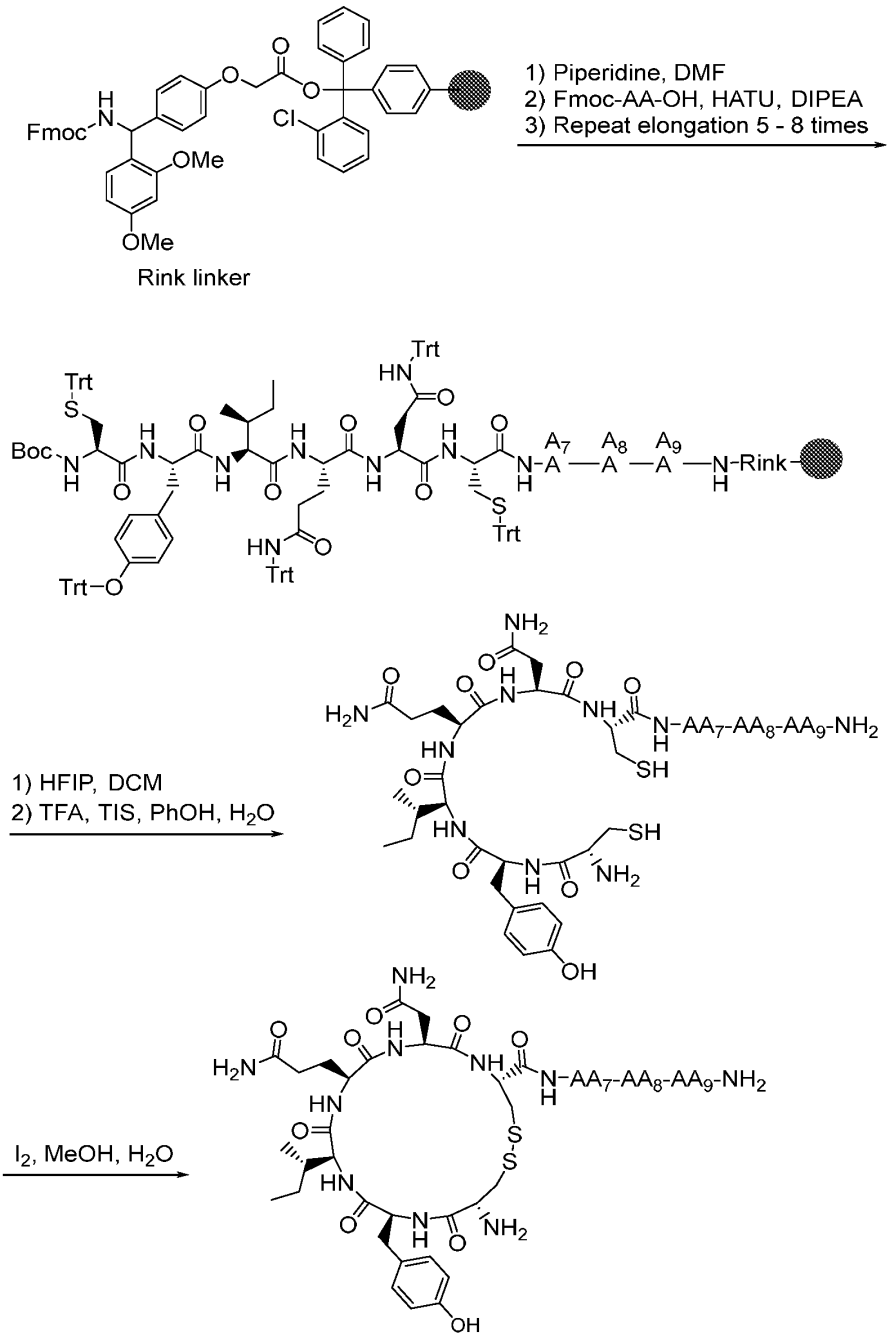
(XCB/XCM/XCW) ACN_TFA_QC_10min_V1 is a 10.00 min method using an Acquity UPLC BEH C18 (1.7 µM, 2.1 x 50mm) column heated to 35 °C, a flow rate of 0.700 mL/min, a buffer consisting of 0.1 % TFA in water, and MeCN as the organic solvent. The percentage MeCN increases from 5 % to 100 % from 0.00-9.00 min and then kept stable at 100 % from 9.00-10.00 min.

(XCB/XCM/XCW) ACN_AmBicarb_QC_10min_V1 is a 10.00 min method using an Acquity UPLC BEH C18 (1.7 µM, 2.1 x 50mm) column heated to 35 °C, a flow rate of 0.700 mL/min, a buffer consisting of 10 mM NH_4HCO_3 in H_2O adjusted to pH 10 with NH_4OH, and MeCN as the organic solvent. The percentage MeCN increases from 5 % to 100% from 0.00-9.00 min and then kept stable at 100% from 9.00-10.00 min.

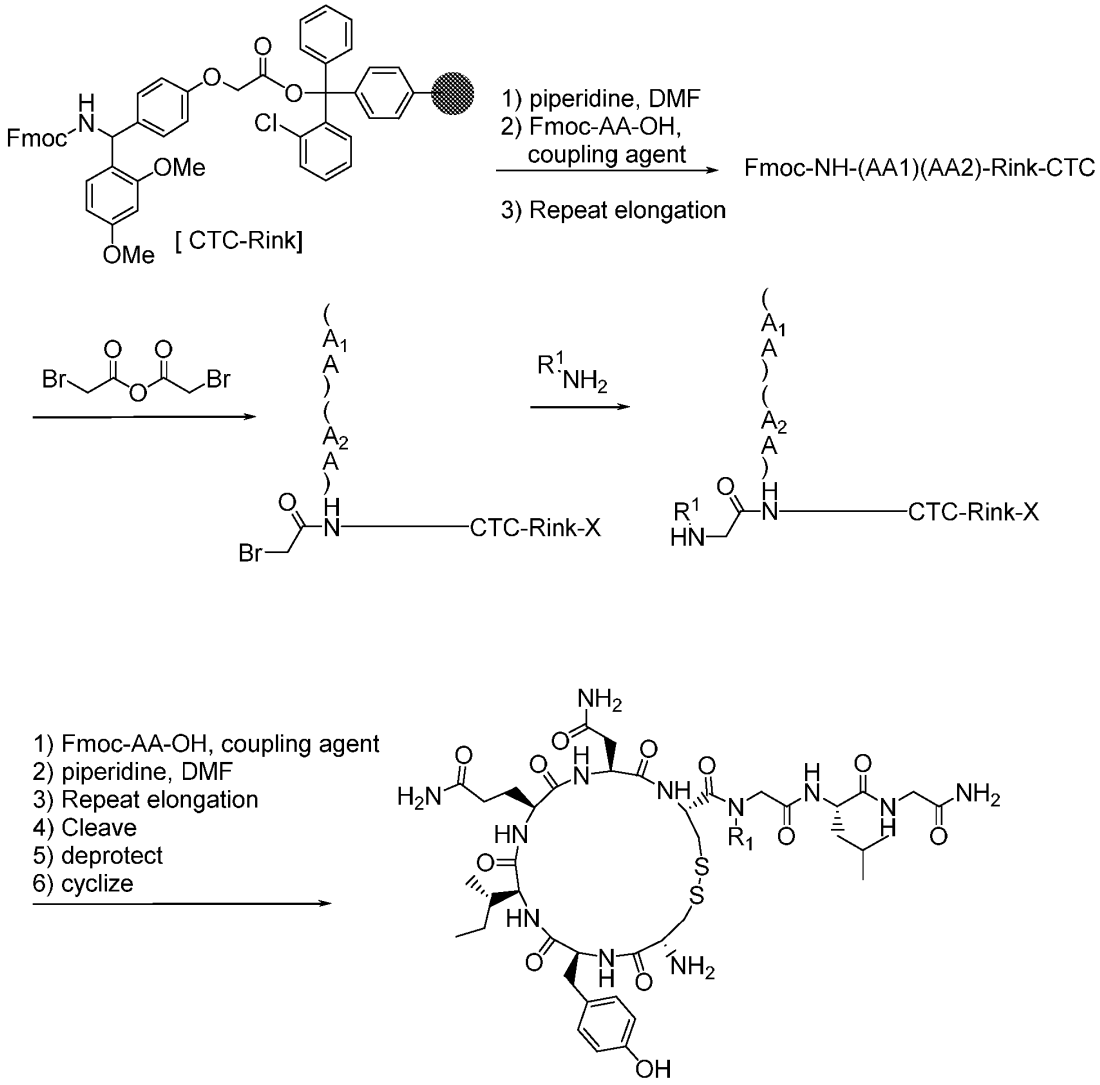
Synthetic Methods

[0108] Oxytocin peptide analogs disclosed herein are synthesized using general synthetic methods as depicted Schemes A-N.

[0109] **Scheme A**

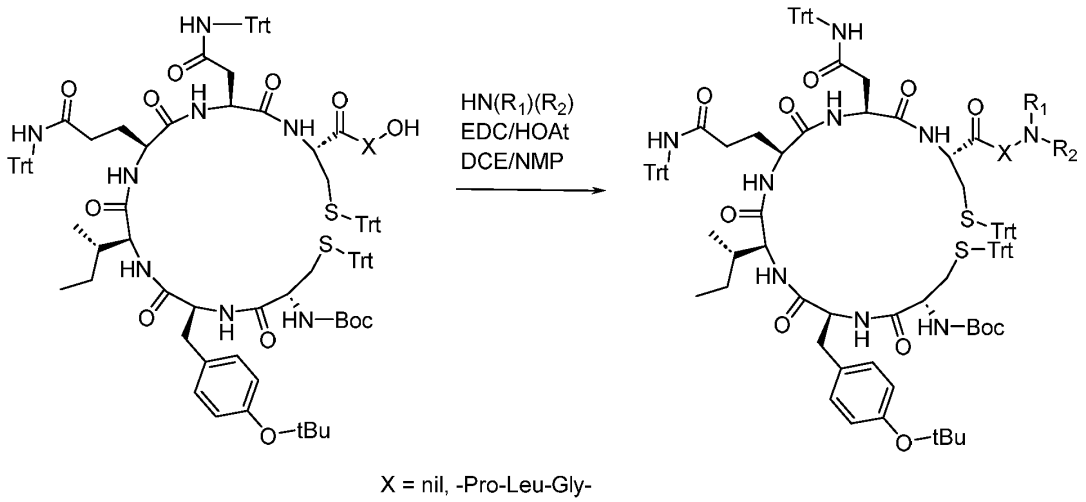


[0110] **Scheme B**

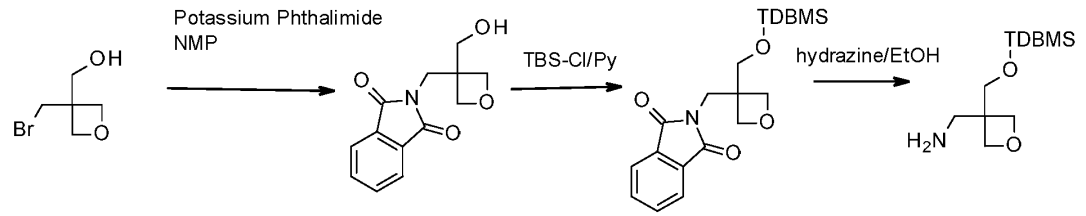


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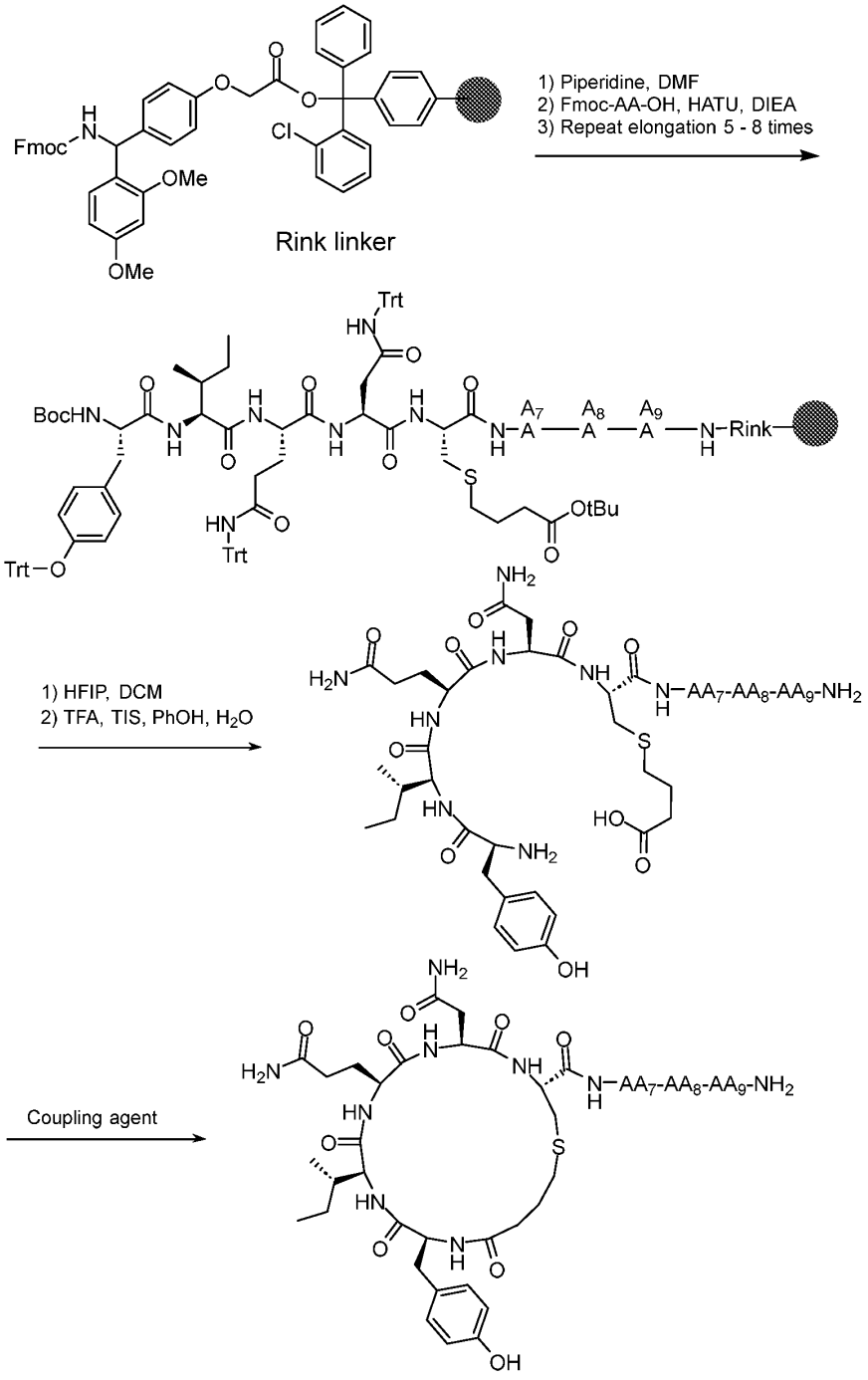
[0111] **Scheme C**



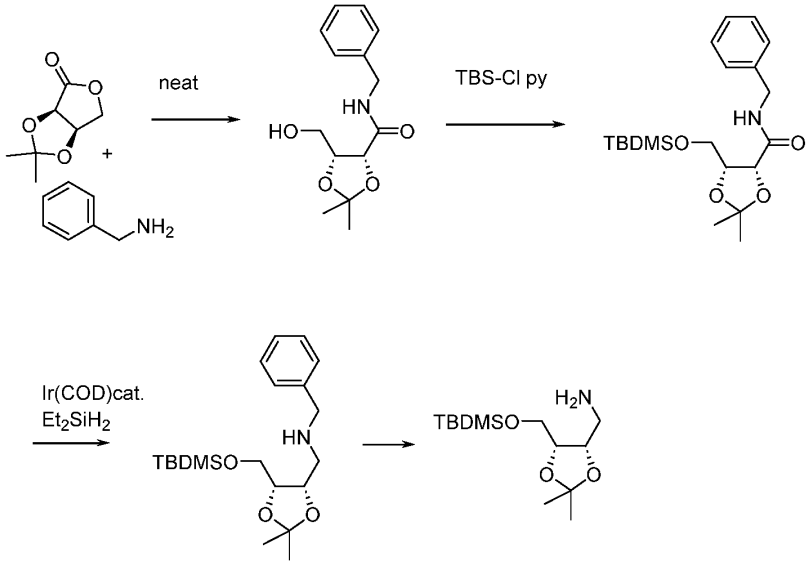
[0112] **Scheme D**



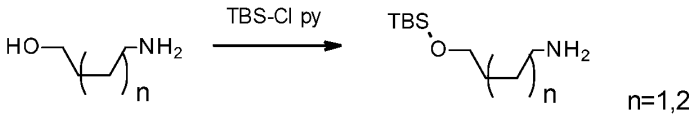
[0113] **Scheme E**



[0114] **Scheme F**

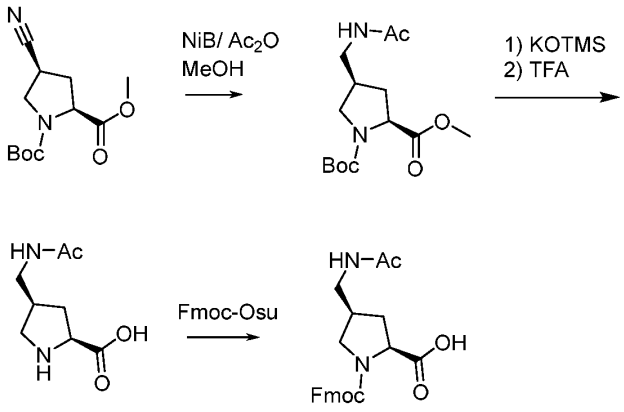


[0115] **Scheme G**

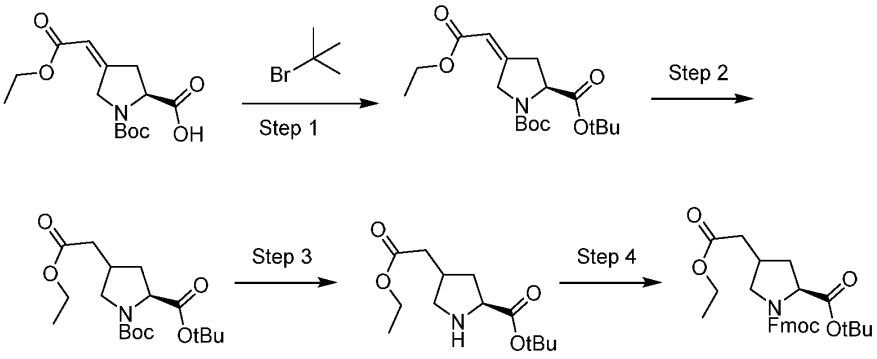


n=1,2

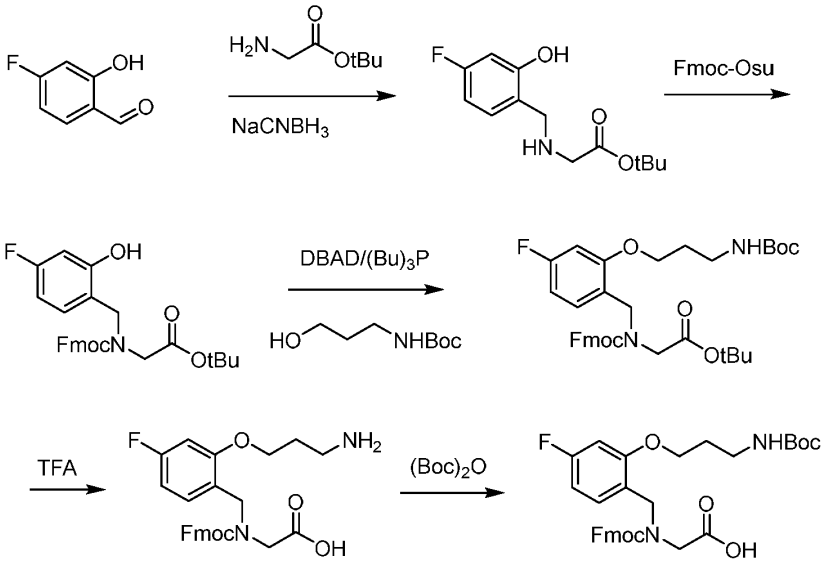
[0116] **Scheme J**



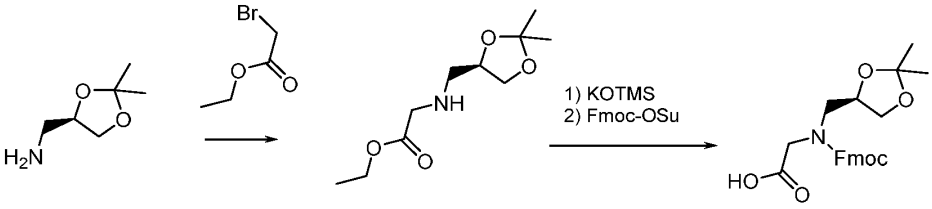
[0117] **Scheme K**



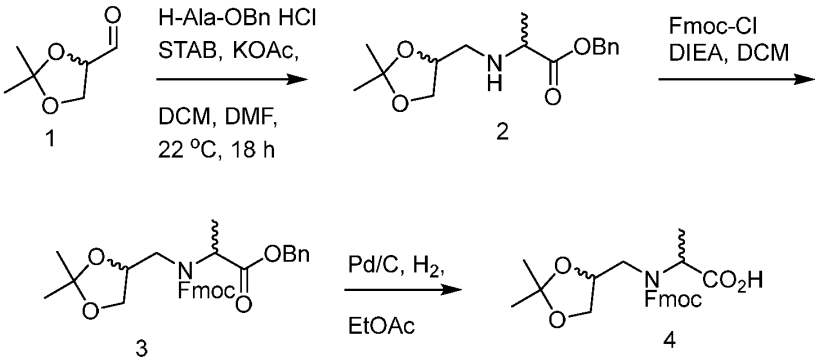
[0118] **Scheme L**



[0119] **Scheme M**

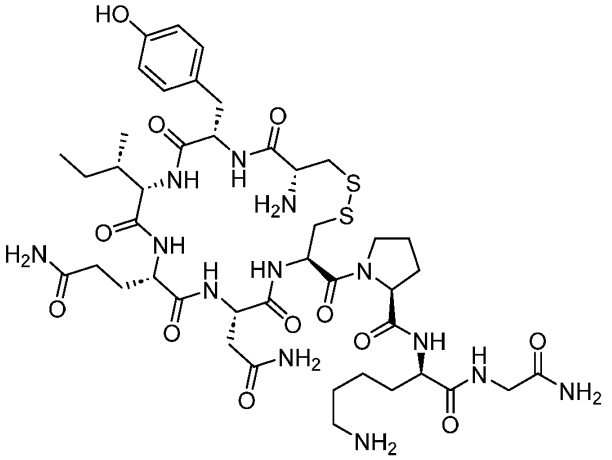


[0120] **Scheme N**



[0121] **Example 1**

General Procedure A:



(2R)-6-amino-2-{[(2S)-1-[(4R,7S,10S,13S,16S,19R)-19-amino-13-[(2S)-butan-2-yl]-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosane-4-carbonyl]pyrrolidin-2-yl]formamido}-N-(carbamoylmethyl)hexanamide

Step A: Fmoc SPPS and resin cleavage using General Procedure A.

DMF (10.0 mL) was added to 2-[4-[(2,4-dimethoxyphenyl)-(9H-fluoren-9-ethoxycarbonylamino) methyl]phenoxy] acetic acid (on chlorotrityl chloride resin, 0.500 mmol/g loading, 0.400 g, 0.220 mmol), and the mixture was agitated for 0.5 h. The mixture was filtered, and the resin was washed with DMF (3 x 10.0 mL), DCM (3 x 10.0 mL), IPA (3 x 10.0 mL) and DMF (10.0) mL. The resin was mixed with piperidine (20% in DMF, 20.0 mL, 64.0 mmol), filtered, washed with DCM and DMF (1:1, 5 x 10.0 mL) and the process was repeated once.

HATU (0.255 mg, 0.700 mmol) was added to a mixture of Fmoc-Gly-OH (0.20 g, 0.623 mmol) and 2,4,6-collidine (0.100 mL, 0.900 mmol) in DMF (20.0 mL) at 22 °C under nitrogen and stirred for 0.5 h. A portion of the mixture (10.0 mL) was added to the resin, and after 1.5 h, the mixture was filtered. Additional mixture (10.0 mL) was added to the resin, and after 0.5 h, the mixture was filtered. The resin was washed with DMF (2 x 10.0 mL), DCM (2 x 10.0 mL) and IPA (10.0 ml).

The deprotection and coupling cycle was repeated with: D-Fmoc-Lys(Boc)OH (412 mg, 0.880 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Pro-OH (223 mg, 0.660 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Cys(trt)OH (387 mg, 0.660 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Asn(trt)OH (382 mg, 0.660 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Gln(trt)OH (394 mg, 0.660 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Ileu-OH (213 mg, 0.660 mmoL), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Fmoc-Tyr(tBu)OH (303 mg, 0.660 mmol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol); L-Boc-Cys(Trt)OH (250 mg, 0.660 mol), HATU (255 mg, 0.700 mmol) and 2,4,6-collidine (0.100 mL, 1.00 mmol). The resin was filtered and washed with DCM (40.0 mL), IPA (40.0 mL) and MTBE (40.0 mL), and dried.

Hexafluoropropanol (20% in DCM, 25 mL) was added to the CTC-resin and the mixture was agitated for 2 h, filtered and the filtrate was evaporated to provide the title compound as a solid (377mg, 67.2%). m/z (ES+) [M+2H]2+ = 1275; HPLC (B05) tR = 3.03min.

Step B: Global Deprotection.

Boc-Cys(trt)-Tyr(tBu)-Ile-Gln(trt)-Asn(trt)-Cys(trt)-Pro-D-Lys(Boc)-Gly-N-Rink spacer (100 mg, 0.0408 mmol) was added to a solution of TFA/H_2O/TIS/phenol (10.0 mL/100 uL/100 uL/25 mg) and stirred at 0 °C for 4 h. After removal of TFA under a stream of N_2, the residue was washed by ice-cold ether, and vacuum-dried to provide the title compound as a solid. (39 mg crude, 23.1%). The crude peptide was purified by prep HPLC (BEH C18 30x150 ACN/TFA 10-20%) to provide the linear peptide as a solid (13.6 mg). m/z (ES+) [M+H]+ = 1024; HPLC (A05) tR = 1.56 min.

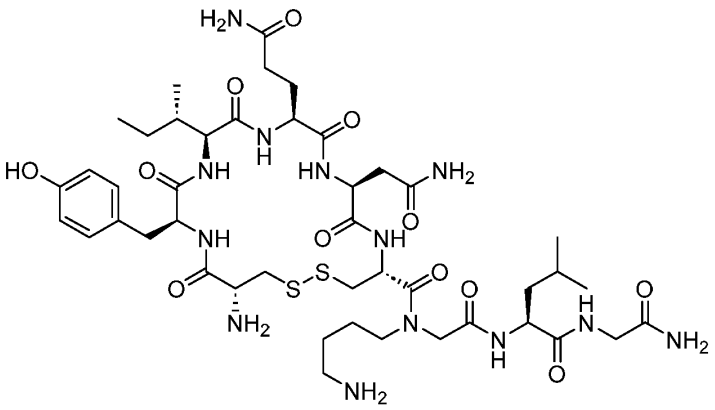
Step C: Cyclization.

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-(D-Lys)-Gly-NH_2 (12.0 mg, 0.0116 mmol) was added to MeCN (15.0 mL) and water (15.0 mL). Iodine (9.00 mg, 0.0390 mmol) in MeOH (1.00 mL)

was added dropwise and the mixture was stirred at 22 °C for 4 h. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (10.0 mg, 5 % overall yield). ^{1}H NMR (300 MHz, D_2O) δ 7.06 (d, J = 8.5 Hz, 2H), 6.77 – 6.67 (m, 2H), 4.35 – 4.25 (m, 1H), 4.8-4.5 (m, 3H), 4.13 (q, J = 6.5 Hz, 2H), 3.95 (t, J = 7.0 Hz, 1H), 3.81 (d, J = 6.5 Hz, 1H), 3.75 (d, J = 4.8 Hz, 2H), 3.67 (q, J = 7.2 Hz, 1H), 3.61 – 3.48 (m, 1H), 3.33 (dd, J = 15.1, 5.5 Hz, 1H), 3.13 (d, J = 4.9 Hz, 1H), 3.07 (dd, J = 7.6, 4.2 Hz, 1H), 3.02 (d, J = 7.2 Hz, 1H), 2.97 (d, J = 7.1 Hz, 1H), 2.83 (td, J = 13.8, 6.1 Hz, 4H), 2.72 (d, J = 6.9 Hz, 2H), 2.35 – 2.22 (m, 2H), 2.15 (dt, J = 13.8, 7.3 Hz, 1H), 1.91 (d, J = 7.3 Hz, 3H), 1.84 – 1.69 (m, 2H), 1.66 – 1.57 (m, 1H), 1.57 – 1.45 (m, 2H), 1.39 – 1.22 (m, 2H), 1.12 (t, J = 7.3 Hz, 1H), 1.00-0.75 (m, 2H), (q, J = 7.2 Hz, 6H), 18 protons not observed; m/z (ES+) [M+H]+ = 1022.8; UPLC (XCM_ACN_TFA_QC_10min_V1), tR = 2.37 min.

[0122] **Example 2**

General Procedure B



(2S)-2-(2-{1-[(4R,7S,10S,13S,16S,19R)-19-amino-13-[(2S)-butan-2-yl]-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosan-4-yl]-N-(4-aminobutyl)formamido}acetamido)-N-(carbamoylmethyl)-4-methylpentanamide

Step A: Fmoc SPPS and resin cleavage using general procedure B

2-[4-[(2,4-dimethoxyphenyl)-(9H-fluoren-9-ylmethoxycarbonylamino)methyl]phenoxy]acetic acid CTC resin, (0.750 mmol/g loading, 0.50 g, 0.375 mmol) in DMF (20.0 mL) was agitated for 0.5 h. The mixture was filtered and the resin was washed with DMF (3 x 10.0 mL), DCM (3 x 10.0 mL), IPA (3 x 5.00 mL) and DMF (10.0 mL). The resin was treated with piperidine (20% in DMF, 10.0 mL, 32.0 mmol)

for 30 min and washed with DCM and DMF (1:1, 5 x 10.0 mL), and the process was repeated once. HATU (0.420 g, 1.11 mmol) was added to a mixture of Fmoc-Gly-OH (0.335 g, 1.13 mmol) and 2,4,6-collidine (0.20 mL, 1.90 mmol) in DMF (20.0 mL) at 22 °C under nitrogen and the mixture was stirred for 0.5 h. A portion of the mixture (10.0 mL) was added to the resin, and after 1.5 h, the mixture was filtered. Additional mixture (10.0 mL) was added to the resin, and after 0.5 h, the mixture was filtered. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml).

The deprotection and coupling cycle was repeated with: L-Fmoc-Leu-OH (0.398 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.90 mmol).

Bromoacetic acid (417 mg, 3.00 mmol), DIC (189 mg, 1.50 mmol), HOAt (0.75 mL, 1M solution in DMA) and 2,4,6-collidine (0.400 mL, 3.90 mmol) in DCM. The mixture was agitated for 1h at at 22 °C under nitrogen. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and N-Boc-1,4-diaminobutane (706 mg, 3.75 mmol) in DMF (6.00 mL) was added to the resin and the mixture was agitated for 1 h at 22 °C under nitrogen. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml).

The deprotection and coupling cycle was repeated with: L-Fmoc-Cys(trt)OH (0.665 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol); L-Fmoc-Asn(trt)OH (0.672 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol); L-Fmoc-Gln(trt)OH (0.687 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol). This coupling reaction was repeated after 1h at R.T. L-Fmoc-Ile-OH (0.398 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol); L-Fmoc-Tyr(tBu)OH (0.522 g, 1.12 mmol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol); L-Boc-Cys(Trt)OH (1.04 g, 2.23 mol), HATU (0.420 g, 1.11 mmol) and 2,4,6-collidine (0.400 mL, 3.09 mmol).

The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml) and MTBE (40.0 mL), and dried in a vacuum oven at RT for 18 h. Hexafluoropropanol (20% in DCM, 25.0 mL) was added to the CTC-resin and the mixture was agitated for 2 h, filtered and the filtrate was evaporated to provide the linear protected crude peptide as a solid (474 mg, 49 %). m/z (ES+): [M+2H]2+ = 1283 (B50) tR = 2.24 min.

Step B: Deprotection with TFA

Boc-Cys(trt)-Tyr(tBu)-Ile-Gln(trt)-Asn(trt)-Cys(trt)-(N-butanediamine(Boc)Gly)-Leu-Gly-N-Rink spacer (180 mg, 0.00631 mmol) was added to a solution of TFA/H_2O/TIS/phenol (4.00 mL/400 uL/400 uL/80.1 mg) and the mixture was stirred at 0 °C for 4h. After removal of

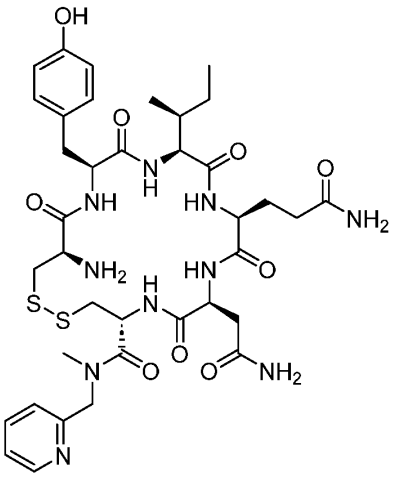
TFA under a stream of N_2, the residue was washed by ice-cold ether, and vacuum-dried to provide the title compound as a solid. (85 % pure, 68.0 mg, 88 %). m/z (ES+) [M+H]+ = 1040; LCMS (A05); tR = 1.64 min.

Step C: Cyclization and purification

H-Cys-Tyr-Ile-Gln-Asn-Cys-)-(N-butanediamineGly)-Leu-Gly-NH_2 (68.0 mg, 0.0576 mmol) was added to MeCN (15.0 mL) and water (15.0 mL). Iodine (19.00 mg, 0.0890 mmol) in MeOH (1.00 mL) was added dropwise and the mixture was stirred at 22 °C for 4 h. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21%) to provide the title compound as a solid (11.8 mg, 6% overall yield). ^{1}H NMR (300 MHz, D_2O) δ 7.06 (dd, J = 8.6, 3.2 Hz, 2H), 6.73 (d, J = 8.1 Hz, 2H), 5.01 (s, 1H), 4.31 – 4.02 (m, 3H), 3.98 (t, J = 7.0 Hz, 1H), 3.89 – 3.69 (m, 4H), 3.53 – 2.95 (m, 5H), 2.84 (dt, J = 14.8, 7.4 Hz, 3H), 2.71 (t, J = 7.9 Hz, 2H), 2.25 (hept, J = 7.7 Hz, 3H), 2.01 – 1.85 (m, 2H), 1.78 (s, 1H), 1.63 – 1.39 (m, 9H), 1.02 (s, 2H), 0.74 (td, J = 15.4, 7.9 Hz, 16H), 15 protons not observed. m/z (ES+) [M+H]+ = 1038; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.10 min.

[0123] **Example 3**

General Procedure C



(4R,7S,10S,13S,16S,19R)-19-amino-13-[(2S)-butan-2-yl]-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(4-hydroxyphenyl)methyl]-N-methyl-6,9,12,15,18-pentaoxo-N-[(pyridin-2-yl)methyl]-1,2-dithia-5,8,11,14,17-pentaazacycloicosane-4-carboxamide

Step A: Preparation of the globally protected pro-Tocinoic acid hexamer.

H-L-Cys(trt)-CTC resin (0.460 mmol/g loading, 1.00 g, 0.460 mmol) was added to DMF (10.0 mL) and the mixture was agitated for 0.5 h. The mixture was filtered, and the resin was

washed with DMF (3 x 10.0 mL), DCM (3 x 10.0 mL), IPA (3 x 10.0 mL) and DMF (1 x 10.0 mL). The resin was mixed with piperidine (20% in DMF, 20.0 mL, 32.0 mmol) for 30 min and washed with DCM and DMF (1:1, 5 x 10.0 mL), and the process was repeated once. HATU (550 mg, 1.50 mmol) was added to a mixture of L-Fmoc-Asn(trt)-OH (820 mg, 1.40 mmol) and 2,4,6-collidine (0.350 mL, 3.00 mmol) in DMF (20.0 mL) at 22 °C under nitrogen and the mixture was stirred for 1.5 h. The mixture was added to the resin, and after 3 h, the mixture was filtered. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml).

The deprotection and coupling cycle was repeated with: L-Fmoc-Gln(trt)OH (0.897 mg, 1.40 mmol), HATU (550 mg, 1.50 mmol) and 2,4,6-collidine (0.350 mL, 3.00 mmol); L-Fmoc-Ile-OH (500 mg, 1.40 mmoL), HATU (550 mg, 1.50 mmol) and 2,4,6-collidine (0.350 mL, 3.00 mmol); L-Fmoc-Tyr(tBu)OH (655 mg, 1.40 mmol), HATU (550 mg, 1.50 mmol) and 2,4,6-collidine (0.350 mL, 3.00 mmol); L-Boc-Cys(Trt)OH (850 mg, 1.80 mol), HOBt (246 mg, 1.80 mmol) and DCC (0.360 g, 1.8 mmol) were mixed in DCM (20.0 mL) at 0 °C under nitrogen. The mixture was stirred for 20 min and filtered through celite. A portion of the filtrate solution (15.0 mL) was added to the resin. After 30 min, 2,4,6-collidine (0.350 mL mL, 3.00 mmol) was added to the resin, and after 30 min, the mixture was filtered. The coupling process was repeated with the remaining portion of the filtrate (15.0 mL). The resin was then filtered, washed with DCM (20.0 mL), IPA (20.0 mL) and MTBE (20.0 mL), and dried. Hexafluoropropanol (20% in DCM, 25.0 mL) was added to the CTC-resin and the mixture was agitated for 2 h, filtered and the filtrate was concentrated and dried to provide the title compound as a solid (0.865g 98% yield). m/z (ES+) [M+H]+ = 1870; HPLC (B05), tR = 2.90 min.

Step B: Coupling to the globally protected hexamer.

N-methyl-1-(2-pyridyl)methanamine (40.0 mg, 0.327 mmol), EDC·HCl (31.0 mg, 0.161 mmol) and HOAt (80.0 µL, 0.0800 mmol) were added to a mixture of 2-[[2-[[2-[[2-[[2-[[2-(tert-butoxycarbonylamino)-3-tritylsulfanyl-propanoyl]amino]-3-(4-tert-butoxyphenyl) propanoyl] amino]-3-methyl-pentanoyl]amino]-5-oxo-5-(tritylamino)pentanoyl]amino]-4-oxo-4-(tritylamino)butanoyl]amino]-3-tritylsulfanyl-propanoic acid (50.0 mg, 0.0268 mmol) and DIPEA (0.0795 mL, 0.464 mmol) in DMF (4.00 mL) under nitrogen. The mixture was stirred at 55 °C for 4 h and diluted with citric acid (5 % in water, 10.0 mL). The aqueous phase was extracted with EtOAc (2 x 20.0 mL). The combined organic phases were washed

with sat. aq. $NaHCO_3$ (20.0 mL) and brine (20.0 mL), dried ($MgSO_4$), filtered, and concentrated. LCMS (A05); tR = 1.74 min.

Step C: Deprotection.

The above product was added to a solution of TFA/H_2O/TIS/phenol (4.00 mL/400 uL/400 uL/80.1 mg) and stirred at 0 °C for 4 h. After removal of TFA under a stream of nitrogen, the residue was washed by ice-cold ether, and vacuum dried to provide the title compound as a solid. (85% pure, 68.0 mg, 88%). m/z (ES+) [M+H]+ = 847; LCMS (A05, 3.5 min) tR = 1.64 min

Step D: Cyclization.

Iodine (9.00 mg, 0.0390 mmol) in MeOH (1.00 mL) was added dropwise to a mixture of H-Cys-Tyr-Ile-Gln-Asn-Cys-N-methyl-1-(2-pyridyl)methanamide (21.0 mg, 0.0223 mmol) in MeCN (15.0 mL) and water (15.0 mL), and the mixture was stirred at 22 °C for 4 h. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21 %) to provide the title compound as a solid (10.0 mg, 9 % overall yield). 1H NMR (300 MHz, D_2O) δ 8.55 (d, J = 6.0 Hz, 1H), 8.41 (t, J = 8.0 Hz, 1H), 7.82 (t, J = 6.9 Hz, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.04 (d, J = 8.3 Hz, 2H), 6.71 (d, J = 8.4 Hz, 2H), 4.97 – 4.86 (m, 2H), 4.70 (d, J = 5.0 Hz, 1H), 4.58 – 4.49 (m, 2H), 4.11 (q, J = 6.6 Hz, 1H), 3.94 (t, J = 7.0 Hz, 1H), 3.85 (d, J = 7.0 Hz, 1H), 3.33 (dd, J = 15.1, 5.6 Hz, 1H), 3.17 (s, 3H), 3.10 (dd, J = 9.8, 4.7 Hz, 1H), 3.00 (dd, J = 14.0, 7.1 Hz, 1H), 2.86 (dt, J = 14.0, 8.8 Hz, 2H), 2.73 (d, J = 6.9 Hz, 2H), 2.24 (q, J = 7.1 Hz, 2H), 1.92 (d, J = 11.4 Hz, 2H), 1.73 (d, J = 8.8 Hz, 2H), 1.07 (s, 1H), 0.85 (dd, J = 14.6, 7.5 Hz, 1H), 0.69 (q, J = 7.0 Hz, 6H), 12 protons not observed; m/z (ES+) [M+H]+ = 845; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 1.79 min.

[0124] **Example 4 – Compound 1**

N-{(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutyl}-(2S,4R)-1-({(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-4-(acetylaminomethyl)-2-pyrrolidinecarboxamide

Step A: O-1-tert-butyl O2-methyl 4-(acetamidomethyl)pyrrolidine-1,2-dicarboxylate (**Scheme J**).

Nickelous;dichloride;hexahydrate (93.5 mg, 0.393 mmol) was added to a mixture of O1-tert-butyl O2-methyl 4-cyanopyrrolidine-1,2-dicarboxylate (500 mg, 1.97 mmol) and acetyl

acetate (743 µL, 7.87 mmol) in MeOH (15.0 mL) at 22 °C under nitrogen. The mixture was stirred at 22 °C for 30 min and cooled to 0 °C. Sodium;boranide (26.1 mg, 0.690 mmol) was added in portions over 5 mins at 0 °C. The mixture was stirred at 22 °C for 8 h and diluted with citric acid (10.0 mL). The aqueous phase was extracted with MTBE (2 x 20.0 mL), combined organic layers were washed with brine (20.0 mL), dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (80 g cartridge) with DCM and MeOH (0-20 %) to provide the title compound as an oil (410 mg, 66%). (ES+) [M+H+Na]+ = 323.2; LCMS (A05); tR = 1.86 min.

Step B: 4-(acetamidomethyl)-1-(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carboxylic acid.

TFA (10.0 mL) was added to a mixture of O1-tert-butyl O2-methyl 4-(acetamidomethyl)pyrrolidine-1,2-dicarboxylate (95 % pure, 410 mg, 1.30 mmol) in DCM (2.00 mL) at 0 °C. The mixture was stirred for 30 min at 22 °C and concentrated. The residue was dissolved in NaOH (2 M, 6.48 mL) and stirred for 1 h at 22 °C and (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (437 mg, 1.30 mmol) in ACN (7.00 mL) was added to the mixture. The stirred 30 min at 22 °C and ACN was removed by evaporation. The mixture was diluted with MTBE (40.0 mL) and the aqueous phase was extracted with MTBE (3 x 40.0 mL), combined organic layers were washed with brine (50.0 mL), dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (24 g cartridge) with DCM and MeOH (0-10 %) to provide the title compound as a solid. m/z: ES+ [M+H]+ = 409.2; LCMS (A05); tR = 1.98 min. 1H NMR (300 MHz, cdcl3) δ 7.75 (s, 2H), 7.55 (s, 2H), 7.44 – 7.28 (m, 4H), 6.55 – 5.97 (m, 1H), 5.30 (d, J = 0.8 Hz, 1H), 4.62 – 4.12 (m, 4H), 3.78 – 3.52 (m, 1H), 3.45 – 2.77 (m, 3H), 2.63 – 2.36 (m, 2H), 2.23 (s, 1H), 1.98 (d, J = 14.4 Hz, 3H).

Step C:

In a manner analogous to Example 1, **Compound 1** was prepared using the amino acids Fmoc-Gly, Fmoc-leu, Fmoc-(cis-4-CH2-N-Acetamide)Pro, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure A on an O-chlorotrityl resin (0.50 mmol/g) on a 0.150 mmol scale. The final compound was purified by preparative RP-LC (BEH, C18, 30g cartridge) with water [1% TFA] and MeOH (5-50 %) to provide the title compound as a solid (33 mg, 19 % overall). m/z (ES+) [M+H]+ = 1078.8, UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.11 and 2.17 min. 1H NMR (300 MHz, D_2O) δ 7.07 (d, J = 8.2 Hz, 2H), 6.73 (d, J = 8.1 Hz, 2H), 4.80 – 4.71 (m, 1H), 4.30 (t, J

= 8.4 Hz, 1H), 4.16 (t, J = 5.7 Hz, 2H), 4.06 – 3.62 (m, 4H), 3.39 – 2.60 (m, 10H), 2.55 – 2.09 (m, 3H), 1.91 (d, J = 6.3 Hz, 2H), 1.85 (s, 3H), 1.64 – 1.35 (m, 4H), 0.75 (ddd, J = 18.0, 12.4, 6.6 Hz, 14H) (23 unobserved exchangeable protons).

[0125] **Example 5 – Compound 2**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-2-cyclopropylethylcarbamoyl}methyl)-N-2,3-dihydroxypropyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 2** was prepared using (rac)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, (2S)-3-cyclopropyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21 %) to provide the title compound as a solid (10 mg, 9 %). m/z (ES+) [M+H]+ = 1039.3, UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.06 min. 1H NMR (300 MHz, D_2O) δ 7.17 – 6.96 (m, 2H), 6.71 (d, J = 8.0 Hz, 2H), 5.27 – 4.74 (m, 1H), 4.34 – 2.40 (m, 16H), 2.40 – 1.82 (m, 5H), 1.82 – 1.33 (m, 2H), 0.70 (q, J = 6.7 Hz, 8H), 0.32 (d, J = 8.5 Hz, 2H), 0.08 – -0.20 (m, 2H) (26 unobserved exchangeable protons).

[0126] **Example 6 – Compound 3**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]butylcarbamoyl}methyl)-N-2,3-dihydroxypropyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 3** was prepared using (rac)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, Fmoc-L-norValine, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21 %) to provide the title compound as a solid (10 mg, 6% overall yield). m/z (ES+) [M+H]+ = 1027.8, UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.03 min. 1H NMR (300 MHz, D_2O) δ 7.05 (d, J = 8.0 Hz, 2H), 6.71 (d, J = 8.2 Hz, 2H), 5.14 – 4.93 (m, 1H), 4.70 (s, 1H), 4.60 – 4.44 (m, 2H), 4.27 – 3.60 (m, 9H), 3.60 – 2.54 (m, 9H), 2.25 (s, 3H), 1.91 (d, J = 7.1 Hz, 1H), 1.22 (d, J = 7.6 Hz, 1H), 0.73 (dtd, J = 14.4, 7.3, 3.8 Hz, 13H) (18 unobserved exchangeable protons).

[0127] **Example 7 – Compound 4**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]pentyl]amino]-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-(2,3-dihydroxypropyl)-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

In a manner analogous to Example 2, **Compound 4** was prepared using (rac)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, Fmoc-L-norLeucine, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21%) to provide the title compound as a solid (10 mg, 5% overall yield). m/z (ES+) [M+H]+ = 1041.5, HPLC (TFA, 10 min) tR = 2.24 min. ^{1}H NMR (300 MHz, D_2O) δ 7.06 (d, J = 7.9 Hz, 2H), 6.72 (d, J = 8.3 Hz, 2H), 5.16 – 4.94 (m, 1H), 4.63 – 4.39 (m, 8H), 4.28 – 3.60 (m, 7H), 3.60 – 2.43 (m, 7H), 2.38 – 2.12 (m, 2H), 2.08 – 1.37 (m, 6H), 1.17 (s, 6H), 0.85 – 0.39 (m, 10H), (18 unobserved exchangeable protons).

[0128] **Example 9 – Compound 5**

Ethyl [(3R,5S)-5-{N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}-1-({(4S,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-3-pyrrolidinyl]acetate

Step A: Di-t-butyl (2S,4Z)-4-(2-ethoxy-2-oxo-ethylidene)pyrrolidine-1,2-dicarboxylate.

A mixture of (2S,4Z)-1-tert-butoxycarbonyl-4-(2-ethoxy-2-oxo-ethylidene) pyrrolidine-2-carboxylic acid (95% pure, 0.6 g, 1.95 mmol), BTBC (594.0 mg, 1.90 mmol), K_2CO_3 (2.10 g, 15.2 mmol) and (2-bromo-2-methylpropane), 3914 mg, 28.6 mmol) in DMA (8.00 mL) was stirred at 50 °C for 18 h. The mixture was diluted with water (10.0 mL). The aqueous phase was extracted with EtOAc (3 x 20.0 mL), and the combined organic phases were washed with water (20.0 mL), brine (20.0 mL), dried with Na_2SO_4, filtered, and concentrated. The residue was purified on a short column of Basic Alumina (10.0 g) with Hexane and EtOAc (9:1) to provide the title compound as an oil (550 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.00 – 5.40 (m, 1H), 4.21 – 4.07 (m, 3H), 3.36 – 2.64 (m, 3H), 1.54 (dd, J = 9.2, 0.8 Hz, 1H), 1.50 – 1.34 (m, 18H), 1.32 – 1.21 (m, 3H). m/z (ES+) [M+Na] + = 378.17; HPLC (A05) tR = 3.11 min.

Step B: Di-t-butyl 4-(2-ethoxy-2-oxo-ethyl)pyrrolidine-1,2-dicarboxylate.

A mixture of ditert-butyl (4E)-4-(2-ethoxy-2-oxo-ethylidene)pyrrolidine-1,2-dicarboxylate (1.00 g, 2.81 mmol) and $Pd(OH)_2$ (20 wt %, 1.98 g, 2.81 mmol) in THF (28.7 mL) was placed under an atmosphere of H_2 (1 atm) at 22 °C. The mixture was stirred at 22 °C for 2 h and diluted with THF (20.0 mL). The mixture was filtered through Celite washing with THF (40.0 mL) and the filtrate was concentrated to provide the title compound as a solid (95% pure, 986 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$-d6) δ 4.12 (ddt, J = 10.2, 7.3, 3.4 Hz, 2H), 3.87 – 3.69 (m, 1H), 3.14 – 2.99 (m, 1H), 2.62 – 2.31 (m, 3H), 1.64 – 1.33 (m, 20H), 1.25 (td, J = 7.2, 2.2 Hz, 3H) (one proton not observed).

Step C: 4-(2-ethoxy-2-oxo-ethyl)pyrrolidine-2-carboxylic acid.

TFA (10.0 mL) was added dropwise to di-t-butyl 4-(2-ethoxy-2-oxo-ethyl)pyrrolidine-1,2-dicarboxylate (95% pure, 210 mg, 0.558 mmol) in DCM (1.00 mL) at 22 °C. The mixture was stirred for 2 h and concentrated to provide the title compound as a solid (95 % pure, 95.0 mg, 80 %). ^{1}H NMR (300 MHz, $CDCl_3$-d6) δ 9.47 (s, 1H), 8.14 – 7.85 (m, 1H), 5.30 (s, 1H), 4.43 (s, 1H), 4.14 (q, J = 7.1 Hz, 2H), 3.72 (s, 1H), 3.15 (d, J = 12.6 Hz, 1H), 2.89 – 2.62 (m, 2H), 2.52 (s, 2H), 1.26 (t, J = 7.1 Hz, 3H); m/z (ES+) [M+H]+ = 202.3; LCMS (A05); tR = 0.31 min.

Step D:

(2S)-4-(2-ethoxy-2-oxo-ethyl)-1-(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carboxylic acid Fmoc-OSu (0.896 g, 2.66 mmol) was added to a mixture of 4-(2-ethoxy-2-oxo-ethyl)pyrrolidine-2-carboxylic acid (475 mg, 2.12 mmol) and K_2CO_3 (2 M, 1.06 mL, 2.12 mmol) in acetonitrile (10.0 mL) at 22 °C. The mixture was stirred at 22 °C for 1 h. The aqueous phase was extracted with EtOAc (3 x 5.00 mL), and the combined organic phases were dried (Na_2SO_4), filtered, and concentrated to provide the title compound as a solid (90 % pure, 225 mg, 23 % yield). ^{1}H NMR (300 MHz, $CDCl_3$-d6) δ 7.77 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 7.3 Hz, 2H), 7.46 – 7.28 (m, 4H), 4.60 – 4.35 (m, 2H), 4.28 (d, J = 7.2 Hz, 1H), 4.15 (dq, J = 14.1, 7.0 Hz, 2H), 3.93 – 3.81 (m, 1H), 3.13 (dd, J = 10.7, 7.8 Hz, 1H), 2.83 (s, 1H), 2.72 – 2.34 (m, 4H), 2.04 (s, 1H), 1.32 – 1.22 (m, 3H), (one proton not observed). m/z (ES+) [M+H]+ = 424.3; LCMS (A05); tR = 2.55 min.

Step E: Compound 5

In a manner analogous to Example 1, **Compound 5** was prepared using the amino acids Fmoc-Gly-OH, Fmoc-Leu, (2S)-4-(2-ethoxy-2-oxo-ethyl)-1-(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carboxylic acid, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure A on a 0.35

mmol scale. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (28.0 mg, 49 %), m/z (ES+) [M+H]+ = 1052.0; HPLC (TFA, 10 min); tR = 2.30 min. ^{1}H NMR (300 MHz, D_2O) δ 7.19 – 6.89 (m, 6H), 6.79 – 6.55 (m, 2H), 5.00 – 4.71 (m, 2H), 4.50 – 3.77 (m, 4H), 3.43 – 3.11 (m, 4H), 3.11 – 2.50 (m, 6H), 2.33 – 2.15 (m, 3H), 2.08 (dd, J = 8.5, 6.1 Hz, 2H), 1.94 (s, 3H), 1.39 (s, 3H), 1.23 – 0.87 (m, 5H), 0.87 – 0.52 (m, 7H) (Twenty-three protons not observed).

[0129] **Example 10 – Compound 6**

N-({N-(R)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[(R)-2,3-dihydroxypropyl]-(6S,9S,12S,15S)-12-(sec-butyl)-9-(2-carbamoylethyl)-6-(carbamoylmethyl)-15-[(p-hydroxyphenyl)methyl]-5,8,11,14,17-pentaoxo-1-thia-4,7,10,13,16-pentaaza-3-cycloicosanecarboxamide

General Method E

Step A:

2-[4-[(2,4-dimethoxyphenyl)-(9H-fluoren-9-ylmethoxycarbonylamino) methyl] phenoxy] acetic acid CTC resin, (0.50 mmol/g loading, 0.33 g, 0.150 mmol) in DMF (5.0 mL) was agitated for 0.5 h. The mixture was filtered, and the resin was washed with DMF (3 x10.0 mL), (DCM 3 x 10.0 mL) and DMF (10.0 mL). The resin was treated with piperidine (20 % in DMF, 10.0 mL 32.0 mmol)) for 30 min and washed with DCM and DMF (1:1, 5 x 10.0 mL) and the process was repeated once. HAt (0.033 mL, 0.0.33 mmol) was added to a mixture of L-Fmoc-Gly-OH (0.099 g, 0.334 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol) in DMF (6.0 mL) at 22 °C under nitrogen and stirred for 0.5 h. A portion of the mixture (10.0 mL) was added to the resin, and after 1.5 h, the mixture was filtered. Additional mixture (20.0 mL) was added to the resin, and after 0.5 h, the mixture was filtered. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml).

The deprotection and coupling cycle was repeated with:

L-Fmoc-Leu-OH (0.118 g, 0.33 mmol), HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol), Bromoacetic acid (417 mg, 3.00 mmol), DIC (189 mg, 1.5 mmol), HAT (1.50 mL, 1M solution) in DCM. The mixture was reacted for 1h at 22 °C under nitrogen. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and (R)-(2,2-dimethyl-1-3-dioxolan-4yl)-methanamine (305 mg, 1.50 mmol) in

DMF (6.00 mL) was added to the resin and reacted for 1h at 22 °C under nitrogen. The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml). (2R)-3-(4-tert-butoxy-4-oxo-butyl) sulfanyl-2-(9H-fluoren-9-ylmethoxycarbonylamino) propanoic acid 0.162g 0.334 mmol), HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol), L-Fmoc-Asn(trt)OH (0.199g, 0.33 mmol), HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol). L-Fmoc-Gln(trt)OH (0.203 g, 0.33 mmol), HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol). This coupling reaction was repeated after 1h at rt.

L-Fmoc-Ileu-OH (0.118 g, 0.33 mmol), HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol); L-Boc-Tyr(BOC)OH, (0.153 g, 0.33 mmol) HAt (0.033 mL, 0.0.33 mmol), DIC (0.052 mL, 0.334 mmol) and DIPEA (0.030 mL, 0.33 mmol).

(The resin was washed with DMF (2 x 20.0 mL), DCM (2 x 20.0 mL) and IPA (20.0 ml) and MTBE (40.00 mL,) dried in a vacuum oven at rt for 18h. Hexafluoropropanol (20 % in DCM, 25.0 mL) was added to the CTC-resin and the mixture was agitated for 2 h, filtered and the filtrate was evaporated to provide the title compound as a solid (90 % pure, 351.0 mg, 99%). m/z (ES+): ([M-2H]2- = 1052.3, LCMS (B50, 3.5 min); tR = 2.04 min.

Step B:

2-[4-[[[2-[[(2S)-2-[[2-[[(2R)-2-[[(2S)-2-[[(2S)-2-[[(2S,3S)-2-[[(2S)-2-(tert-butoxycarbonylamino)-3-(4-tert-butoxycarbonyloxyphenyl)propanoyl]amino]-3-methyl-pentanoyl]amino]-5-oxo-5-(tritylamino)pentanoyl]amino]-4-oxo-4-tritylamino)butanoyl]amino]-3-(4-tert-butoxy-4-oxo-butyl)sulfanyl-propanoyl]-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]amino]acetyl]amino]-4-methyl-entanoyl]amino]acetyl]amino]-(2,4-dimethoxyphenyl)methyl] phenoxy]acetic acid (351.0 mg, 0.000167 mol) was added to a solution of TFA/H_2O/TIS/phenol (4.00 mL/400 µL/400 µL/200 mg) and stirred at 0 °C for 4 h. After removal of TFA by N_2 stream, the residue was washed with ice-cold ether (20.0 mL), filtered and dried to provide the title compound as a solid. (95 % pure, 138.9 mg, 77 % yield). m/z (ES+) [M+H]+ = 1026.4; LCMS (A05, 3.5 min) tR = 2.55 min.

Step C:

4-[(2R)-2-[[(2S)-4-amino-2-[[(2S)-5-amino-2-[[(2S,3S)-2-[[(2S)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino]-3-methyl-pentanoyl]amino]-5-oxo-pentanoyl]amino]-4-

oxo-butanoyl]amino]-3-[[2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-2-oxo-ethyl]-[(2R)-2,3-dihydroxypropyl]amino]-3-oxo-propyl]sulfanylbutanoic acid (0.135 g, 0.132 mmol) in DMF (70.0 ml) was added in 3 portions (3 x 23.0 ml) to a vigorously stirred solution of DIPEA (0.45 ml) in DMF (27.0 ml). HATU (50.0 mg, 0.132 mmol) in DMF (3.00 ml) was added to the reaction mixture after each portion (23.0 mL) of addition. The reaction solution was stirred at 55 °C for 5 h, concentrated in vacuo, and the residue diluted with EtOAc (10.0 mL), citric acid 5 % (5.0 mL). The organic phase was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (7.7 mg, 6%). m/z (ES+) [M+H]+ = 1008.6; HPLC (B05, 10 min); tR = 2.35 min. ^{1}H NMR (300 MHz, D_2O) δ 7.06 (d, J = 8.2 Hz, 2H), 6.74 (d, J = 8.1 Hz, 2H), 4.97 – 4.69 (m, 2H), 4.62 – 4.37 (m, 2H), 4.33 – 3.90 (m, 4H), 3.90 – 3.65 (m, 3H), 3.61 – 3.31 (m, 3H), 2.81 – 2.69 (m, 4H), 2.57 – 1.61 (m, 9H), 1.52 (s, 3H), 1.27 (s, 1H), 0.88 – 0.64 (m, 12H) (6 protons were not unobserved).

[0130] **Example 11 – Compound 7**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-3,4-dihydroxybutyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 8** was prepared using (rac)- 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanamine, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (18.0 mg, 16 %). m/z (ES+) [M+H]+ = 1055.6; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.20 min. ^{1}H NMR (300 MHz, D_2O) δ 7.08 (d, J = 8.1 Hz, 2H), 6.74 (d, J = 8.0 Hz, 2H), 5.04 (s, 1H), 4.33 – 3.71 (m, 5H), 3.71 – 2.95 (m, 6H), 2.95 – 2.56 (m, 3H), 2.28 (s, 2H), 1.95 (s, 2H), 1.79 (s, 2H), 1.51 (s, 4H), 1.02 (s, 1H), 0.75 (td, J = 13.8, 6.1 Hz, 12H) (28 protons were not observed).

[0131] **Example 12 – Compound 8**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-5-hydroxypentyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-

carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

Step A: 5-[tert-butyl(dimethyl)silyl]oxypentan-1-amine (**Scheme G**).

4-amino pentan-1-ol (1.03 g, 0.010 mol) and TBDMS-Cl (1.66 g, 0.011 mol) were dissolved in pyridine (2.00 ml) and stirred at 0 °C 0.5h then stirred at rt for 18 h and diluted with 10% K_2CO_3 40 mL and MeOH (10.0 mL). The aqueous phase was extracted with MTBE (3x 20.0 mL), dried (Na_2SO_4), filtered over basic alumina (2.00 g) washing with MTBE (10.0 mL) and concentrated to provide the title compound (2.05 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (td, J = 6.5, 1.2 Hz, 2H), 2.69 (td, J = 6.9, 1.2 Hz, 2H), 1.57 – 1.28 (m, 6H), 0.88 (d, J = 1.2 Hz, 9H), 0.04 (d, J = 1.3 Hz, 6H).

Step B:

In a manner analogous to Example 2, **Compound 8** was prepared using, 5-[tert-butyl(dimethyl)silyl]oxypentan-1-amine and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [10 mM $(NH_4)(HCO_3)$] and MeCN (16-26 %) to provide the title compound as a solid (2.00 mg, 4 %), m/z (ES+) [M+H]+ = 1053.0; HPLC (B05, 10 min); tR = 2.54 min. ^{1}H NMR (300 MHz, D_2O) δ 7.22 (d, J = 8.1 Hz, 2H), 6.88 (d, J = 7.9 Hz, 2H), 5.01 (d, J = 9.8 Hz, 1H), 4.47 – 3.80 (m, 11H), 3.70 – 3.43 (m, 6H), 3.40 – 3.10 (m, 4H), 3.10 – 2.74 (m, 8H), 2.39 (d, J = 7.8 Hz, 2H), 2.23 (s, 1H), 2.09 (s, 4H), 1.79 – 1.01 (m, 16H), 0.92 (d, J = 8.5 Hz, 14H) (one proton was not observed).

[0132] **Example 13 – Compound 9**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[m-(hydroxymethyl)phenyl]methyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 9** was prepared using, 3-[(tertbutoxy)methyl)phenyl)methamine and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21%) to provide the title compound as a solid (11.3 mg, 13 %). m/z (ES+) [M+H] + = 1088.0; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.47 min. ^{1}H NMR (300 MHz, D_2O) δ: 7.55 – 7.16

(m, 6H), 6.91 – 6.83 (m, 2H), 5.17 – 4.91 (m, 1H), 4.83 (s, 2H), 4.38 – 3.74 (m, 8H), 3.53 – 2.56 (m, 6H), 2.40 (t, J = 7.6 Hz, 2H), 2.16 – 1.80 (m, 5H), 1.74 – 1.43 (m, 2H), 1.07 – 0.70 (m, 16H) (20 exchangeable protons unobserved).

[0133] **Example 14 – Compound 10**

N-{(R)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutyl}-(2S,4S)-1-({(4R,7S,10S,13S,16S,19R)-13-[(S)-1-methylpropyl]-19-amino-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-4-cyano-2-pyrrolidinecarboxamide

In a manner analogous to Example 1, **Compound 10** was prepared using the amino acids Fmoc-Gly, Fmoc-leu, Fmoc-(cis-4 Cyano)Pro, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure A on an O-chlorotrityl resin (0.50 mmol/g) on a 0.150 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21%) to provide the title compound as a solid (5.7 mg, 13% overall yield). m/z (ES+) [M+H]+ = 1032; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.21 min. ^{1}H NMR (300 MHz, D_2O) δ 7.22 (dd, J = 8.2, 6.2 Hz, 2H), 6.99 – 6.81 (m, 2H), 4.80 (tt, J = 16.5, 7.6 Hz, 2H), 4.45 – 3.79 (m, 7H), 3.67 – 3.07 (m, 5H), 3.08 – 2.79 (m, 3H), 2.40 (q, J = 8.3 Hz, 2H), 2.07 (dtd, J = 4.7, 2.8, 1.6 Hz, 3H), 1.67 (s, 3H), 1.26 (s, 1H), 0.93 (td, J = 11.4, 5.9 Hz, 13H) (22 exchangeable protons were not observed).

[0134] **Example 15 – Compound 11**

N-[(S)-1-{[(N-2,3-dihydroxypropylcarbamoyl)methyl]carbamoyl}-3-methylbutyl]-(S)-1-({(4R,7S,10S,13S,16S,19R)-13-[(S)-1-methylpropyl]-19-amino-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-2-pyrrolidinecarboxamide

In a manner analogous to Example 3, **Compound 11** was prepared using the precursors (2,2-Dimethyl-1,3-dioxolan-4-yl)methanamine (19.1 µL, 147 µmol), [(S)-2-[(S)-1-({(4R,7S,10S,13S,16S,19R)-13-[(S)-1-methylpropyl]-19-amino-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-2-pyrrolidinylcarbonylamino]-4-methylvalerylamino]acetic acid (105 mg, 49.2 µmol) and General procedure C on a 49.2 µmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1% TFA) and MeOH (20 – 60 %), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeCN (10 -

60%). The residue was diluted with H2O (3.00 mL), MeCN (1.00 mL), HCl (3 M in H_2O, 150 µL, 450 µmol), and concentrated to provide the title compound as a solid (4.43 mg, 8% overall). ^{1}H NMR (500 MHz, D_2O) δ 7.33 – 7.13 (m, 2H), 6.95 – 6.81 (m, 2H), 5.00 – 4.72 (m, 2H), 4.68 – 4.26 (m, 3H), 4.22 – 3.68 (m, 7H), 3.66 – 2.94 (m, 10H), 2.92 – 2.71 (m, 2H), 2.58 – 2.27 (m, 3H), 2.23 – 1.78 (m, 7H), 1.75 – 1.47 (m, 3H), 1.37 – 1.15 (m, 1H), 1.12 – 0.68 (m, 13H) (18 exchangeable protons were not observed). m/z (ES+) [M+H]+ = 1081.5; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.36 [2.42] min.

[0135] **Example 16 – Compound 12**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[(2S,3R)-2,3,4-trihydroxybutyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

Step A: N-benzyl-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane-4-carboxamide (**Scheme F**).

2,2-dimethyl-6,6a-dihydro-3aH-furo[3,4-d][1,3]dioxol-4-one (0.400 g, 2.53 mmol) and benzyl amine (0.500 g, 4.67 mmol) were dissolved in dioxane (10.00 mL) and stirred at 22 °C for 3h. The mixture poured on ice and diluted with 10% citric acid and extracted with EtOAc (2x 20.00 mL). The organic fractions were combined and concentrated to provide the title compound as a solid (0.65 g, 93 %). m/z: ES+ [M+H]+ = 266.2; LCMS (A05); tR = 1.95 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.23 (m, 5H), 7.06 (s, 1H), 4.69 (d, J = 7.6 Hz, 1H), 4.64 – 4.39 (m, 3H), 3.82 (dd, J = 11.9, 4.6 Hz, 1H), 3.73 – 3.57 (m, 1H), 1.49 (s, 3H), 1.39 (s, 3H) (one exchangeable proton not observed).

Step B: N-benzyl-5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolane-4-carboxamide.

N-benzyl-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane-4-carboxamide (1.33 g, 5.01 mmol mmol) was added to tert-butyl-chloro-dimethyl-silane (3.02 g, 20.1 mmol) in pyridine (6.00 mL) and was stirred for 18 h at 20°C. The mixture was diluted with EtOAc (50.0 mL) and extracted with 10 % citric acid (2 x 50.0 ml), 10 % K_2CO_3 (2 x 50.0 ml). The organic phase was washed with water (2 x 25.0 mL) and brine (40.0 mL) dried and concentrated. The residue was dissolved in EtOAc (1.00 mL), filtered on Silica (100 g, 10cm plug) washing with 1:1 EtOAc: Hexanes (400.0 mL), the fractions pooled and concentrated to provide the title compound (1.42 g, 73 %). m/z: ES+ [M+H]+ = 380.2; LCMS (A05); tR = 2.70 min.

Step C: N-[[5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]-1-phenyl-methanamine.

(1,5-Cyclooctadiene)(methoxy)Iridium(I) Dimer 0.025 g, 37.5 µmol) was added to diethyl-silane (0.331g, 3.75 mmol) in toluene (3.00 mL) and was stirred for 1h at 20°C. N-benzyl-5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolane-4-carboxamide (0.285g, 0.75 mmol) was added to the mixture and was stirred for 27 h at 20°C. The mixture was diluted with MTBE (20.0 mL), cooled to 0 °C and HCl added (4M in dioxane, 0.40 ml, 1.6mmol). The precipitate was filtered, washing with MTBE (2 x 10.0 ml), and dried to provide the title compound (0.275 g, 98%). m/z: ES+ [M+H]+ = 366.7 ; LCMS (A05); tR = 2.60 min. ^{1}H NMR (300 MHz, CD_3OD) δ 7.67 – 7.25 (m, 5H), 4.61 – 4.12 (m, 4H), 3.75 – 3.62 (m, 2H), 3.27 – 3.11 (m, 2H), 1.52 – 1.26 (m, 6H), 0.85 (s, 9H), 0.04 (d, J = 5.4 Hz,6H).

Step D:

[5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methanamine N-[[5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]-1-phenyl-methanamine (0.274 g, 0.750 mmol) was added to a mixture of Pd/C (10% wt., 80.0 mg, 75.0 µmol) in ethyl acetate (25.0 mL)and was stirred for 27 h at 20 °C under hydrogen. The mixture was filtered on Celite, washing with EtOAc (2 x 10.0 ml). The combined filtrates were concentrated and dried to provide the title compound (0.169 g, 80%). m/z: ES+ [M+H]+ = 276.2; LCMS (A05); tR = 2.07 min. 1H NMR (300 MHz, CD_3OD) δ 4.36 – 3.99 (m, 2H), 3.90 – 2.57 (m, 4H), 1.51 – 1.24 (m, 6H), 0.91 (s 9H), 0.23 – 0.20 (m, 6H) (two exchangeable protons were not observed).

Step E:

In a manner analoguous to Example 2, **Compound 12** was prepared using [5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methanamine, and the amino acids Fmoc-Gly-OH, Fmoc-L-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by RP-LC ((C18, 30 g cartridge) with water [0.1% TFA] and MeOH (20-60%) to provide the title compound as a solid (9.00 mg, 13 %). m/z (ES+) [M+H]+ = 1071; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.42 min. ^{1}H NMR (300 MHz, CD_3OD) δ 7.22 (d, J = 8.1 Hz, 2H), 6.74 (d, J = 7.9 Hz, 2H), 5.56 – 4.93 (m, 1H), 4.77 – 4.24 (m, 3H), 4.24 – 3.45 (m, 10H), 3.43 – 3.33 (m, 1H), 3.26 (m, 2H), 3.12 – 2.63 (m, 4H), 2.37 (m, 2H), 2.26 – 1.51 (m, 7H), 1.29 (m, 2H), 0.93 (m, 14H) (20 unobserved exchangeable protons).

[0136] **Example 17 – Compound 13**

(4R,7S,10S,13S,16S)-7-(2-amino-2-oxo-ethyl)-N-[2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[[2-(3-aminopropoxy)-4-fluoro-phenyl]methyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

In a manner analogous to Example 1, **Compound 13** was prepared using the amino acids Fmoc -Gly-OH, Fmoc-Leu-OH, 2-[[2-[3-(tert-butoxycarbonylamino)propoxy]-4-fluoro-phenyl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]acetic acid, Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH and Boc-Mpa(Trt)-OH using General Procedure A on a 0.200 mmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 - 70%) to provide the title compound as a solid (17.3 mg, 40%). ^{1}H NMR (500 MHz, D_2O) δ 8.38 (s, 1H), 7.22 – 7.12 (m, 1H), 7.10 – 7.01 (m, 2H), 6.85 – 6.60 (m, 4H), 5.00 – 4.68 (m, 1H), 4.67 – 3.99 (m, 10H), 3.90 – 3.67 (m, 2H), 3.24 – 2.63 (m, 10H), 2.55 – 2.39 (m, 2H), 2.38 – 2.25 (m, 2H), 2.16 – 1.83 (m, 5H), 1.56 – 1.17 (m, 4H), 1.05 – 0.92 (m, 1H), 0.91 – 0.63 (m, 12H) (17 exchangeable protons not observed). ^{19}F NMR (282 MHz, D_2O) δ -110.6 [-111.7]; ^{19}F NMR (282 MHz, D_2O, 90 °C) δ -110.7 [-111.1]. m/z (ES+) [M+H]+ = 1133.4; HPLC (XCM_ACN_AmBicarb, 10 min); tR = 3.24 min.

[0137] **Example 18 – Compound 14**

N-{(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutyl}-(2S,4S)-1-({(4R,7S,10S,13S,16S,19R)-13-[(S)-1-methylpropyl]-19-amino-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-4-(allyloxy)-2-pyrrolidinecarboxamide

Step A: (2S,4S)-4-allyloxy-1-tert-butoxycarbonyl-pyrrolidine-2-carboxylic acid. (2S,4S)-1-tert-butoxycarbonyl-4-hydroxy-pyrrolidine-2-carboxylic acid (0.693 g, 0.00299 mol) was dissolved in THF (25.00 ml), KHMDS (18.0 mL, 9.00 mmol) was added portion-wise and stirred at -10 °C for 0.5 h. Allyl bromide (0.725 g, 0.00599 mol) was added dropwise over 10 minutes. The mixture was stirred at rt for 14h and diluted with 10% K_2CO_3. The aqueous phase was extracted with Et_2O (2 x 25.0 mL), acidified to pH 2 (6N HCl, 3 mL), extracted with MTBE (2 x 25 mL) and the combined organic phases dried ($MgSO_4$), filtered and concentrated to provide the title compound (0.628 g, 77 %). m/z (ES+) [M+Na]+ =

294.2

LCMS (A05); tR= 1.70 min.

Step B: (2S,4S)-4-allyloxy-1-(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carboxylic acid.

((2S,4S)-4-allyloxy-1-tert-butoxycarbonyl-pyrrolidine-2-carboxylic acid (544 mg, 2.00 mmol), was dissolved in 6 mL TFA at 0 °C and stirred for 2h. The mixture was concentrated, and the residue dissolved in 10% K_2CO_3 (50 mL). (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (0.676 g, 2.00 mmol) in MeCN (30.0 mL) was added portion-wise was stirred for 1h at 10 °C. The mixture was concentrated to 30.0 ml, acidified to pH 4 (citric acid) and the precipitate filtered, washing with ice water (30.0 mL). The solid residue was dried to provide the title compound (0.649 g, 81%). m/z: ES+ [M+H]+ = 394.2; LCMS (A05); tR = 1.951 min. ^{1}H NMR (300 MHz, CDC_3OD) δ 7.79 (t, J = 6.7 Hz, 2H), 7.61 (d, J = 7.0 Hz, 2H), 7.43 – 7.21 (m, 4H), 5.85 (dddd, J = 15.7, 10.5, 5.2, 3.2 Hz, 1H), 5.26 (ddd, J = 17.3, 3.6, 1.8 Hz, 1H), 5.17 – 5.05 (m, 1H), 4.45 – 4.32 (m, 4H), 4.32 – 4.15 (m, 1H), 4.12 (s, 1H), 3.95 (dt, J = 5.3, 1.5 Hz, 2H), 3.68 – 3.38 (m, 2H), 2.44 – 2.21 (m, 2H).

Step C:

In a manner analogous to Example 1, **Compound 14** was prepared using the amino acids Fmoc-Gly, Fmoc-leu, (2S,4S)-4-allyloxy-1-(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carboxylic acid, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure A on an O-chlorotrityl resin (0.50 mmol/g) on a 0.150 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (24 mg, 14% overall yield). m/z (ES+) [M+H]+ = 1062; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.88 min. ^{1}H NMR (300 MHz, D_2O) δ 7.05 (d, J = 8.4 Hz, 2H), 6.71 (d, J = 8.3 Hz, 2H), 5.75 (ddt, J = 16.5, 11.2, 5.8 Hz, 1H), 5.25 – 5.03 (m, 2H), 4.75 (q, J = 4.4 Hz, 1H), 4.58 (d, J = 7.6 Hz, 2H), 4.40 (dd, J = 9.7, 3.6 Hz, 1H), 4.26 – 3.41 (m, 12H), 3.38 – 2.59 (m, 8H), 2.40 – 2.14 (m, 1H), 2.05 (d, J = 14.0 Hz, 1H), 1.97 – 1.83 (m, 2H), 1.84 – 1.33 (m, 5H), 1.08 (s, 1H), 0.98 – 0.58 (m, 15H) (14 unobserved exchangeable protons).

[0138] **Example 20 – Compound 15**

(2S)-1-[(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-10-(3-amino-3-oxo-propyl)-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carbonyl]-N-[(1S)-1-[[2-[[3-hydroxy-2-

(hydroxymethyl)propyl]amino]-2-oxo-ethyl]carbamoyl]-3-methyl-butyl]pyrrolidine-2-carboxamide

In a manner analoguous to Example 3, **Compound 15** was prepared using the precursors oxetan-3-ylmethanamine (36.2 µL, 421 µmol), [(S)-2-[(S)-1-({(4R,7S,10S,13S,16S,19R)-13-[(S)-1-methylpropyl]-19-amino-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosyl}carbonyl)-2-pyrrolidinylcarbonylamino]-4-methylvalerylamino]acetic acid (300 mg, 140 µmol) and General procedure C on a 140 µmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1 % TFA) and MeOH (20 – 60 %), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 – 100 %). The residue was diluted with HCl (3.0 M in H_2O, 1.00 mL, 3.00 mmol) and concentrated to provide the title compound as a solid (30.4 mg, 19 % yield overall). ^{1}H NMR (500 MHz, D_2O) δ 7.24 – 7.05 (m, 2H), 6.82 (d, J = 7.4 Hz, 2H), 4.86 – 4.77 (m, 1H), 4.67 – 4.56 (m, 1H), 4.51 – 4.14 (m, 4H), 4.12 – 3.35 (m, 11H), 3.31 – 2.87 (m, 7H), 2.84 – 2.72 (m, 2H), 2.54 – 2.18 (m, 4H), 2.14 – 1.72 (m, 7H), 1.68 – 1.41 (m, 3H), 1.29 – 1.05 (m, 1H), 1.01 – 0.55 (m, 12H) (18 exchangeable protons were not oberved). m/z (ES+) [M+H]+ = 1095.6; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = [2.34] 2.37 min.

[0139] **Example 21 – Compound 16**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3,3-dimethylbutylcarbamoyl}methyl)-N-[(R)-2,3-dihydroxypropyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 16** was prepared using (R)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4,4-dimethyl-pentanoic acid, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by RPLC (C18, 30 g cartridge) with water (0.1 % TFA) and MeOH (20-60 %) to provide the title compound as a solid (16.0 mg, 10 %). m/z (ES+) [M+H]+ = 1055.3, UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.36 min. ^{1}H NMR (300 MHz, D_2O) δ 6.75 (d, J = 7.9 Hz, 2H), 6.41 (d, J = 8.3 Hz, 2H), 4.91 – 4.35 (m, 4H), 4.02 – 3.75 (m, 3H), 3.67 (m 2H), 3.58 – 2.91 (m, 8H), 2.89 – 2.18 (m, 5H),

1.95 (m 3H), 1.79 – 1.34 (m, 2H), 1.28 (m, 1H), 0.93 – 0.25 (m, 18H), (18 unobserved exchangeable protons).

[0140] **Example 23 – Compound 17**

(S)-N-((S)-1-((2-amino-2-oxoethyl)(2-guanidinoethyl)amino)-4-methyl-1-oxopentan-2-yl)-1-((4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13-((S)-sec-butyl)-16-(4-hydroxybenzyl)-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosane-4-carbonyl)pyrrolidine-2-carboxamide

In a manner analogous to Example 2, **Compound 17** was prepared using tert-butyl (NZ)-N-[(2-aminoethylamino)-(tert-butoxycarbonylamino)methylene]carbamate, and the amino acids Fmoc-Leu, Fmoc-Pro-OH, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.35 mmol scale. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21 %) to provide the title compound as a solid (90% pure, 5.7 mg, 20 %). m/z (ES+) [M+H]+ = 1093; HPLC (TFA, 10 min); tR = 2.07 min.

[0141] **Example 24 – Compound 18**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[2-(2-hydroxyethoxy)ethyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

Step A: 2-[2-[tert-butyl(dimethyl)silyl]oxyethoxy]ethanamine (**Scheme G**).

2-(2-aminoethoxy)ethanol (2.00 g, 0.0190 mol) was added to a mixture of TBDMS-Cl (3.15 g, 0.0209 mol) in pyridine (3.81 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C, then stirred at 22 °C for 16 h and diluted with 10 % K_2CO_3 (40.0 mL) and MeOH (10.0 mL). The aqueous phase was extracted with MTBE (3 x 30.0 mL) and the combined organic phases were dried (Na_2SO_4), filtered over basic alumina (2.00 g) washing with MTBE (60.0 mL) and concentrated to provide the title compound as an oil (1.90 g, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 – 3.70 (m, 2H), 3.59 – 3.48 (m, 5H), 2.93 – 2.85 (m, 2H), 0.89 (d, J = 1.1 Hz, 10H), 0.07 (d, J = 1.2 Hz, 6H).

Step B:

In a manner analogous to Example 2, **Compound 18** was prepared using 2-[2-[tert-butyl(dimethyl)silyl]oxyethoxy]ethanamine and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by

preparative HPLC (BEH, C18, 30x150 mm) with [10 mM $(NH_4)(HCO_3)$] and MeCN (12-22 %) to provide the title compound as a solid (5.00 mg, 4 %). m/z (ES+) [M+H]+ = 1052.0; HPLC (B05, 10 min); tR = 2.30 min. ^{1}H NMR (300 MHz, D_2O) δ 7.31 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 8.1 Hz, 2H), 5.27 (dd, J = 8.8, 4.1 Hz, 1H), 4.52 – 3.62 (m, 17H), 3.49 – 2.85 (m, 9H), 2.57 – 2.42 (m, 2H), 2.32 – 1.97 (m, 3H), 1.73 (d, J = 6.5 Hz, 3H), 1.28 (d, J = 9.3 Hz, 1H), 1.12 – 0.80 (m, 13H) (17 exchangeable protons not observed).

[0142] **Example 25 – Compound 19**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[(S)-2,3-dihydroxypropyl]-(4R,7S,10S,13S,16S)-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 19** was prepared using (S)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and 3-tritylsulfanylpropanoic acid (Mpa(trt)) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (7.7 mg, 9 %). m/z (ES+) [M+H]+ = 1027; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.33 min. ^{1}H NMR (300 MHz, D_2O) δ 7.20 – 7.05 (m, 2H), 6.80 (d, J = 7.9 Hz, 2H), 5.01 (ddd, J = 14.4, 8.9, 4.5 Hz, 1H), 4.45 – 3.67 (m, 6H), 3.64 – 3.29 (m, 3H), 3.31 – 2.65 (m, 7H), 2.55 (d, J = 5.6 Hz, 2H), 2.33 (t, J = 7.5 Hz, 2H), 2.18 – 1.84 (m, 5H), 1.59 (s, 3H), 1.29 (s, 1H), 1.04 (s, 1H), 0.86 (dd, J = 13.5, 6.1 Hz, 12H) (18 exchangeable and 4 protons beneath solvent peak unobserved).

[0143] **Example 26 – Compound 20**

(2S)-2-(2-{1-[(4R,7S,10S,13S,16S,19R)-19-amino-13-[(2S)-butan-2-yl]-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosan-4-yl]-N-(2,3-dihydroxypropyl)formamido}acetamido)-N-(carbamoylmethyl)-4-methylpentanamide

In a manner analogous to Example 2, **Compound 20** was prepared using (R)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.35 mmol scale. The crude product was concentrated and added to cold MTBE (25.0 mL) to provide the title compound as a solid (85 % pure, 348.0 mg, 97 %). 170 mg of the residue was purified by reverse-phase chromatography (C18, 30 g cartridge) with

water (0.1% TFA) and MeOH (5-50 %), and by reverse phase chromatography (C18, 30 g cartridge) with water (0.1% FA) and MeOH (5-50 %) to provide title compound as a solid (41 mg). The residue was then dissolved in water (4.00 mL) and added to ion-exchange resin Amberlite IRN78 (2.00 g, pre-equilibrated with aqueous formic acid 2 % (5 x 5.00 mL)), stirred for 1h, then filtered, washing with water (5 x 4.00 mL). The fractions were pooled and lyophilized 48 h to provide the title compound as a solid (>95 % pure, 27.0 mg, 66 % recovery, 8 % overall yield). m/z (ES+) [M+H]+ = 1041; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.23 min. ^{1}H NMR (300 MHz, D_2O) δ 8.11 (s, 2H), 7.05 (d, J = 8.1 Hz, 2H), 6.71 (d, J = 8.2 Hz, 2H), 4.17 (t, J = 9.0 Hz, 3H), 3.99 (s, 2H), 3.87 – 3.68 (m, 3H), 3.56 – 3.37 (m, 2H), 3.19 – 2.93 (m, 3H), 2.69 (d, J = 6.6 Hz, 2H), 2.25 (s, 3H), 1.51 – 1.44 (m, 4H), 0.98 (s, 1H), 0.80 – 0.63 (m, 12H). (9 unobserved protons).

[0144] **Example 27 – Compound 21**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[(S)-2,3-dihydroxypropyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 21** was prepared using (S)-2,2-dimethyl-1,3-dioxolane-4-methamine, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (29.2 mg, 14 %). m/z (ES+) [M+H]+ = 1041; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.22 min. ^{1}H NMR (300 MHz, D_2O) δ 7.08 (d, J = 8.1 Hz, 2H), 6.74 (d, J = 8.1 Hz, 2H), 5.04 (s, 1H), 4.36 – 3.59 (m, 9H), 3.59 – 2.63 (m, 9H), 2.27 (s, 2H), 1.93 (d, J = 7.9 Hz, 2H), 1.51 (s, 3H) 0.64-1-11 (m, 13H) (22 unobserved protons).

[0145] **Example 28 – Compound 22**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-[3-hydroxy-2-(hydroxymethyl)propyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

In a manner analogous to Example 2, **Compound 22** was prepared using, oxetan-3-ylmethanamine and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-

Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % FA] and MeCN (05-40 %) to provide the title compound as a solid (14.1 mg, 10 % overall yield). m/z (ES+) [M+H]+ = 1055.5; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.21 min. ^{1}H NMR (300 MHz, D_2O) δ 7.08 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.2 Hz, 2H), 5.04 (d, J = 5.8 Hz, 1H), 4.61 – 4.48 (m, 1H), 4.18 (dd, J = 10.3, 5.4 Hz, 2H), 4.13 – 3.98 (m, 2H), 3.98 – 3.83 (m, 2H), 3.83 – 3.65 (m, 2H), 3.57 – 3.33 (m, 4H), 3.29 – 2.95 (m, 4H), 2.82 (s, 1H), 2.83 – 2.68 (m, 2H), 2.36 – 2.19 (m, 1H), 1.92 (dt, J = 14.2, 6.8 Hz, 2H), 1.81 (s, 1H), 1.51 (d, J = 5.4 Hz, 4H), 1.02 (s, 1H), 0.76 (td, J = 13.5, 5.9 Hz, 15H) (21 protons not observed).

[0146] **Example 29 – Compound 23**

N-({N-(S)-1-[(carbamoylmethyl)carbamoyl]-3-methylbutylcarbamoyl}methyl)-N-4-hydroxybutyl-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide

Step A: [tert-butyl(dimethyl)silyl]oxybutan-1-amine (**Scheme G**).

4-amino butan-1-ol (891 mg, 0.010 mol) and TBDMS-Cl (1.66g, 0.011 mol) were dissolved in pyridine (2.00 ml) and stirred at 0 °C 0.5 h then stirred at rt for 18 h and diluted with 10 % K_2CO_3 (40 mL) and MeOH (10.0 mL). The aqueous phase was extracted with methyl tert-butyl ether (MTBE) (3 x 20.0 mL), and the combined organic phases dried with Na_2SO_4, filtered over basic alumina (2.00 g) washing with MTBE and concentrated to provide the title compound (1.99 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (td, J = 6.2, 1.2 Hz, 2H), 2.71 (td, J = 6.8, 1.2 Hz, 2H), 1.62 – 1.41 (m, 4H), 0.89 (dd, J = 5.3, 1.2 Hz, 9H), 0.06 (dd, J = 11.7, 1.2 Hz, 6H).

Step B:

In a manner analogous to Example 2, **Compound 23** was prepared using, [tert-butyl(dimethyl)silyl]oxybutan-1-amine and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.15 mmol scale. The final compound was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (6.0 mg, 3 %). m/z (ES+) [M+H]+ = 1039; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.32 min. ^{1}H NMR (300 MHz, D_2O) δ 7.34 (d, J = 8.2 Hz, 2H), 6.99 (d, J = 8.1 Hz, 2H), 5.31 – 5.06 (m, 1H), 5.06 – 4.76 (m, 1H), 4.53 – 3.80

(m, 7H), 3.80 – 2.84 (m, 10H), 2.52 (t, J = 7.3 Hz, 2H), 2.35 – 1.93 (m, 5H), 1.93 – 1.55 (m, 6H), 1.34 (s, 1H), 1.03 (tt, J = 13.8, 6.5 Hz, 13H) (20 unobserved protons).

[0147] **Example 30 – Compound 24**

(2S)-2-(2-{1-[(4R,7S,10S,13S,16S,19R)-19-amino-13-[(2S)-butan-2-yl]-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosan-4-yl]-N-{[(3S)-oxolan-3-yl]methyl}formamido}acetamido)-N-(carbamoylmethyl)-4-methylpentanamide

In a manner analogous to Example 2, **Compound 24** was prepared using (S)-tetrahydrofuran-3-ylmethanamine, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure B on a 0.375 mmol scale. The mixture was concentrated, lyophilized, and purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1% TFA] and MeCN (11-21 %) to provide the title compound as a solid (28.0 mg, 7% overall yield). ^{1}H NMR (300 MHz, D_2O) δ 7.19 – 6.89 (m, 6H), 6.79 – 6.55 (m, 2H), 5.00 – 4.71 (m, 2H), 4.50 – 3.77 (m, 4H), 3.43 – 3.11 (m, 4H), 3.11 – 2.50 (m, 6H), 2.33 – 2.15 (m, 3H), 2.08 (dd, J = 8.5, 6.1 Hz, 2H), 1.94 (s, 3H), 1.39 (s, 3H), 1.23 – 0.87 (m, 5H), 0.87 – 0.52 (m, 7H) (23 protons not observed). m/z (ES+) [M+H]+ = 1052.0; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.30 min.

[0148] **Example 31 – Compound 25**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-N-(thiazol-2-ylmethyl)-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

In a manner analogous to Example 2, **Compound 25** was prepared using 2-aminomethylthiazole, and the amino acids Fmoc-Gly-OH, Fmoc-Leu, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General procedure B on a 0.35 mmol scale. The final product was purified by preparative HPLC (BEH, C18, 30x150 mm) with water [1 % TFA] and MeCN (11-21 %) to provide the title compound as a solid (28.0 mg, 35 % overall yield). ^{1}H NMR (300 MHz, D_2O) δ 7.47 (dd, J = 16.8, 3.3 Hz, 1H), 7.33 (dd, J = 8.4, 3.5 Hz, 1H), 6.86 (d, J = 7.4 Hz, 2H), 6.52 (d, J = 7.9 Hz, 2H), 4.53 – 4.28 (m, 2H), 4.08 – 3.72 (m, 5H), 3.69 – 3.45 (m, 3H), 3.09 – 2.38 (m, 7H), 2.08 (s, 3H), 1.41 – 1.17 (m, 5H), 0.68 – 0.39 (m, 16H) (18 protons not observed). m/z (ES+) [M+H]+ = 1064; UPLC (XCM_ACN_TFA_QC_10min_V1); tR = 2.40 min.

[0149] **Example 32 – Compound 26**

N-({N-1-[(carbamoylmethyl)carbamoyl]cyclopentylcarbamoyl}methyl)-N-[(R)-2,3-dihydroxypropyl]-(4R,7S,10S,13S,16S,19R)-19-amino-13-(sec-butyl)-10-(2-carbamoylethyl)-7-(carbamoylmethyl)-16-[(p-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaaza-4-cycloicosanecarboxamide—trifluoroacetic acid (1/1)

Step A: ethyl 2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methylamino]acetate (**Scheme K**). Ethyl bromoacetate (334 mg 2.00 mmol), and (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (394 mg, 3.00 mmol) were dissolved in MTBE (20.00 mL) and stirred at 20 °C for 23 h. The slurry was filtered and the filtrate was concentrated to provide the title compound as a semi-solid 422 mg, 97 %). ^{1}H NMR (300 MHz, CDCl3) δ 4.36 – 3.98 (m, 4H), 3.87 – 3.43 (m, 4H), 3.11 – 2.68 (m, 1H), 1.53 – 1.11 (m, 9H) (One exchangeable proton unobserved).

Step B: 2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]acetic acid.

Potassium trimethylsilanoate (0.561 g, 3.88 mmol) was added to ethyl 2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methylamino]acetate (422 mg, 1.94 mmol), dissolved in MTBE (40.00 mL) and stirred at 20 °C for 2h. The slurry was diluted with water (14.00 mL) and the organic phase removed. The aqueous phase was buffered with 10% K_2CO_3 (14.00 mL) and (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (0.665 g, 1.94 mmol) in MeCN (14.00 mL) was added, while stirring at 20 °C for 2 h. The solution was extracted with MTBE (2 x 30.0 mL). The aqueous phase was acidified with 10% citric acid, extracted with MTBE (2 x 30.0 mL), the organic phases were pooled, dried over Na_2SO_4, filtered and the filtrate was concentrated to provide the title compound as a solid (590 mg, 74 %). m/z: ES+ [M+H]+ = 412.3; LCMS (A05); tR = 2.03 min.

Step C:

In a manner analogous to Example 1, **Compound 26** was prepared using the amino acids Fmoc-Gly, 1-(9H-fluoren-9-ylmethoxycarbonylamino) cyclopentanecarboxylic acid, 2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl) amino]acetic acid, Fmoc-Cys(trt), Fmoc-Asn(trt), Fmoc-Gln(trt), Fmoc-Ile, Fmoc-Tyr(OtBu) and Boc-Cys(trt) using General Procedure A on an O-chlorotrityl resin (0.50 mmol/g) on a 0.150 mmol scale. The final compound was purified by RPLC (C18, 30 g cartridge) with water (0.1 % TFA) and MeOH (20-60 %) to provide the title compound as a solid (40.0 mg, 24 % yield overall). m/z (ES+) [M+H]+ = 1039.9, UPLC

(XCM_ACN_TFA_QC_10min_V1) tR = 1.90 min. ^{1}H NMR (500 MHz, MeOD) δ 7.32 – 6.66 (m, 4H), 5.37 (m, 1H), 4.74 (m, 1H), 4.42 – 3.42 (m, 14H), 3.29 – 2.64 (m, 7H), 2.49 – 1.70 (m, 13H), 1.42 (s, 1H), 1.17 – 0.74 (m, 7H) (18 unobserved exchangeable protons).

[0150] **Example 33 – Compound 27**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1S)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2S)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

Step A (Procedure N): (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (Scheme N)

Step N-1: benzyl (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methylamino]propanoate. STAB (2.76 g, 13 mmol) in dry DMF (15.0 mL) was added to a mixture of H-Ala-OBn hydrochloride (2.16 g, 10.0 mmol), (4R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (50 % in DCM, 2.79 g, 10.7 mmol) and KOAc (981 mg, 10.0 mmol) in DMF (30 mL) at 0 °C. The mixture was stirred at 22 °C for 18 h and diluted with K_2CO_3 (1 M in H_2O, 60.0 mL) and H_2O (40.0 mL). The aqueous phase was extracted with MTBE (4 x 25.0 mL), and the combined organic extracts were washed with NH_4Cl (sat. in H_2O, 100 mL), brine (100 mL), and concentrated (100 Torr, 40 °C) to provide the title compound as an oil (2.99 g, > 98%). m/z (ES+) [M+H]+ = 294.3; HPLC (A05, 3.5 min) tR = 2.01 min.

Step N-2: benzyl (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoate.

Fmoc-Cl (2.76 g, 10.6 mmol) was added to a mixture of benzyl (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methylamino]propanoate (2.84 g, 9.68 mmol) and DIEA (3.31 mL, 19.4 mmol) in DCM (30.0 mL) at 22 °C. The mixture was stirred at 22 °C for 2 h and diluted with NH4Cl (33% sat. in H_2O, 30.0 mL). The aqueous phase was extracted with DCM (2 x 10.0 mL) and the combined organic phases were concentrated. The residue was purified by silica gel chromatography (80 g cartridge) using a gradient of hexanes in EtOAc (10-60 %) to provide the title compound as an oil (4.58 g, 92%). m/z (ES+) [M+H]+ = 516.4; HPLC (A05, 3.5 min) tR = 2.84 min.

Step N-3: (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid.

EtOAc (40.0 mL) was added to a mixture of benzyl (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoate (52.0 mg, 101 µmol) and 20 % $Pd(OH)_2$/C/50 % H_2O (499 mg, 355 µmol) at 22 °C under nitrogen, and the atmosphere was replaced with hydrogen (1 atm). The mixture was stirred at 22 °C for 2 h, filtered through celite (15 g) washing with DCM (300 mL), and the filtrate was concentrated. The residue was by silica gel chromatography (40 g cartridge) using a gradient of hexanes in DCM (0-100%), then DCM in EtOAc (1-100%) to provide the title compound as a solid (1.02 g, 27%). ^{1}H NMR (500 MHz, DMSO-d6) δ 12.50 (s, 1H), 7.95 – 7.81 (m, 2H), 7.72 – 7.56 (m, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.36 – 7.25 (m, 2H), 4.61 – 4.49 (m, 1H), 4.47 – 3.84 (m, 4H), 3.80 – 3.38 (m, 2H), 3.27 – 2.90 (m, 2H), 1.32 – 1.14 (m, 9H). m/z (ES+) [M+H]+ = 426.3; HPLC (A05, 3.5 min) tR = 2.35 min.

Step B (Procedure A): 2-[4-[[[2-[[(2S)-2-[[(2S)-2-[[(2R)-2-[[(2S)-2-[[(2S)-2-[[(2S,3S)-2-[[(2S)-2-[[(2R)-2-(tert-butoxycarbonylamino)-3-tritylsulfanyl-propanoyl]amino]-3-(4-tert-butoxyphenyl)propanoyl]amino]-3-methyl-pentanoyl]amino]-5-oxo-5-(tritylamino)pentanoyl]amino]-4-oxo-4-(tritylamino)butanoyl]amino]-3-tritylsulfanyl-propanoyl]-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl]amino]propanoyl]amino]-4-methyl-pentanoyl]amino]acetyl]amino]-(2,4-dimethoxyphenyl)methyl]phenoxy]acetic acid. In a manner analoguous to Example 1, **Compound 27** was prepared using the amino acids Fmoc-Gly-OH, Fmoc-Leu-OH, (2S)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid, Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH and Boc-Cys(Trt)-OH and General procedure A on a 0.230 mmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1 % TFA) and MeOH (20 – 60 %), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 – 100 %). The residue was diluted with HCl (3.0 M in H_2O, 1.00 mL, 3.00 mmol) and concentrated to provide the title compound as a solid (4.47 mg, 1.8 % overall). m/z (ES+) [M+H]+ = 1055.9; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.13 min.

[0151] **Example 34 – Compound 28**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1R)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2R)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-

[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

Step A (Procedure N): (2R)-2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*R*,4'*R*-4).

In a manner analogous to Example 33, amino acid 2*R*,4'*R*-4 was prepared using H-D-Ala-OBn tosylic acid and (4S)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde and General Procedure N on a 4.00 mmol scale. The product was concentrated to provide the title compound as a solid (319 mg, 19% overall). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.95 – 7.80 (m, 2H), 7.71 – 7.53 (m, 2H), 7.45 – 7.27 (m, 4H), 4.63 – 4.47 (m, 1H), 4.45 – 3.80 (m, 4H), 3.78 – 3.34 (m, 2H), 3.27 – 2.89 (m, 2H), 1.32 – 1.15 (m, 9H) (1 exchangeable proton not observed). m/z (ES+) [M+H]+ = 426.3; HPLC (A05, 3.5 min) tR = 2.34 min.

Step B (Procedure A): (4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1R)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2R)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide.

In a manner analogous to Example 1, **Compound 28** was prepared using the amino acids Fmoc-Gly-OH, Fmoc-Leu-OH, (2R)-2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*R*,4'*R*-4), Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH and Boc-Cys(Trt)-OH and General procedure A on a 0.230 mmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1% TFA) and MeOH (20 - 60%), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 - 60%). The residue was diluted with HCl (3.0 M in H_2O, 1.00 mL, 3.00 mmol) and concentrated to provide the title compound as a solid (4.38 mg, 0.7% overall). m/z (ES+) [M+H]+ = 1055.6; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.34 min.

[0152] **Example 35 – Compound 29**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1S)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2R)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

Step A (Procedure N): (2S)-2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*S*,4'*R*-4)

In a manner analogous to Example 33, amino acid 2*S*,4'*R*-4 was prepared using H-Ala-OBn hydrochloride and (4S)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde and General Procedure N on a 4.00 mmol scale. The product was concentrated to provide the title compound as an oil (356 mg, 21% overall). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.40 (s, 1H), 7.98 – 7.80 (m, 2H), 7.74 – 7.55 (m, 2H), 7.49 – 7.25 (m, 4H), 4.74 – 3.79 (m, 5H), 3.76 – 3.34 (m, 2H), 3.27 – 2.74 (m, 2H), 1.32 – 1.15 (m, 9H). m/z (ES+) [M+H]+ = 426.3; HPLC (A05, 3.5 min) tR = 2.36 min.

Step B (Procedure A): (4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1S)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2R)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

In a manner analogous to Example 1, **Compound 29** was prepared using the amino acids Fmoc-Gly-OH, Fmoc-Leu-OH, (2S)-2-[[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*S*,4'*R*-4), Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH and Boc-Cys(Trt)-OH and General procedure A on a 0.230 mmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1% TFA) and MeOH (20 – 60 %), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 – 60 %). The residue was diluted with HCl (3.0 M in H_2O, 1.00 mL, 3.00 mmol) and concentrated to provide the title compound as a solid (1.14 mg, 0.5 % overall). m/z (ES+) [M+H]+ = 1055.7; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.29 [2.36] min.

[0153] **Example 36 – Compound 30**

(4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1R)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2S)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide

Step A (Procedure N): (2R)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*R*,4'*S*-4).

In a manner analogous to Example 33, amino acid 2*R*,4'*S*-4 was prepared using H-D-Ala-OBn tosylic acid and (4R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde and General Procedure N on a 20.0 mmol scale. The crude product was purified by silica gel chromatography (12 g cartridge) using a gradient of hexanes in EtOAc (0-100 %) to provide the title compound as an oil (654 mg, 7.7 % overall). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.46 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.71 – 7.59 (m, 2H), 7.47 – 7.38 (m, 2H), 7.38 – 7.27 (m, 2H), 4.59 – 4.07 (m, 4H), 4.00 – 3.33 (m, 3H), 3.29 – 2.92 (m, 2H), 1.34 – 1.19 (m, 9H). m/z (ES+) [M+H]+ = 426.4; HPLC (A05, 3.5 min) tR = 2.39 min.

Step B (Procedure A): (4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxo-ethyl)-N-[(1R)-2-[[(1S)-1-[(2-amino-2-oxo-ethyl)carbamoyl]-3-methyl-butyl]amino]-1-methyl-2-oxo-ethyl]-10-(3-amino-3-oxo-propyl)-N-[(2S)-2,3-dihydroxypropyl]-16-[(4-hydroxyphenyl)methyl]-13-[(1S)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carboxamide.

In a manner analogous to Example 1, **Compound 30** was prepared using the amino acids Fmoc-Gly-OH, Fmoc-Leu-OH, (2R)-2-[[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl-(9H-fluoren-9-ylmethoxycarbonyl)amino]propanoic acid (2*R*,4'*S*-4), Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH and Boc-Cys(Trt)-OH and General Procedure A on a 0.230 mmol scale. The crude product was purified by reverse phase chromatography (C18, 30 g cartridge) with water (0.1% TFA) and MeOH (20 – 60 %), and by reverse phase chromatography (C18, 30 g cartridge) with water [10 mM $(NH_4)(HCO_3)$] and MeOH (20 – 60 %). The residue was diluted with HCl (3.0 M in H_2O, 1.00 mL, 3.00 mmol) and concentrated to provide the title compound as a solid (1.98 mg, 0.8 % overall). m/z (ES+) [M+H]+ = 1055.8; UPLC (XCM_ACN_TFA_QC_10min_V1) tR = 2.44 min.

[0154] **Example 37 – Compound B**

(2S,4S)-N-((S)-1-((2-amino-2-oxoethyl)amino)-4-methyl-1-oxopentan-2-yl)-1-((4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13-((S)-sec-butyl)-16-(4-hydroxybenzyl)-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosane-4-carbonyl)-4-hydroxypyrrolidine-2-carboxamide

Procedure to be inserted

[0155] **Example 38** - Gq activity data

Step A: Transduction.

500 mL of complete growth medium was prepared or growth medium stored at 4 °C was used that is free of contamination and no more than 30 days old by combining 450 mL of Eagle's Minimum Essential Medium (EMEM) with 1.5 g/L sodium bicarbonate and supplemented with non-essential amino acids, L-Glutamine and sodium pyruvate (Corning 10-009-CV), 50 ml of FBS, certified, United States (Gibco 16000044), 5 ml of Penicillin-Streptomycin 10,000 U/ml (Thermo Fisher 15140122). HEK293T cells were passaged according to HEK293T standard operating procedure and diluted cell suspension to 500,000 cells/mL. OXTR transduction mix was prepared using BacMam virus encoding upward DAG biosensor (Montana Molecular U0300G) with titer standardized to 2 x 10^{10} VG/mL: 2 µL/well; BacMam virus encoding human Oxytocin Receptor (Gene ID: OXTR) (Montana Molecular Z0900N) with titer standardized to 2 x 10^{10} VG/mL: 1 µL/well. To the OXTR transduction mix was added 0.12 µL/well of sodium butyrate solution at 500 mM, 6.88 µL/well of complete growth medium and mixed several times by pipetting up and down.

Gq positive control transduction mix was prepared with BacMam virus encoding upward DAG biosensor (Montana Molecular U0300G) with titer standardized to 2 x 1010 VG/mL: 2 µl/well; and BacMam virus encoding human M1 Muscarinic Receptor (Gene ID: CHRM1) (Montana Molecular Z0200R) with titer standardized to 2 x 1010 VG/ml: 1 µl/well. 0.12 µl/well of sodium butyrate solution at 500 mM and 6.88 µl/well of complete growth medium was added and mixed several times by pipetting up and down. 20 µl/well of diluted cell suspension was added to each transduction mix, pipetting up and down several times to mix. 30 µl/well in a black-walled clear bottom 384-well plate pre-coated with Poly-D-Lysine (Corning 356697) was seeded. (Note: Final concentration of sodium butyrate is 2 mM. Total amount of cells is 10,000 cells/well). Plate was incubated at room temperature in the dark for 1 hour and place in 37°C incubator with 5% CO_2 for 24 hours.

Step B: Assay Buffer

DPBS (without magnesium) was prepared using potassium chloride (KCl): 0.2 g/L (2.67 mM) (Sigma P5405), Potassium Phosphate monobasic (KH_2PO_4): 0.2 g/L (1.47 mM) (Sigma P5655), Sodium Chloride (NaCl): 8 g/L (137.9 mM) (Sigma 793566), Sodium Phosphate dibasic (Na_2HPO_4-$7H_2O$): 2.16 g/L (8 mM) (Sigma S5136). Distilled water (to 1 L) was added, stirred until completely dissolved, autoclaved and allowed to cool to room temperature. pH was adjusted to 7.0 to 7.25 with 1 N HCl. DPBS was supplemented with 1 mM calcium chloride by adding 1 mL of high purity 1 M Calcium Chloride solution (Sigma 21115) per 1 L of DPBS dropwise with gentle shaking. DPBS was supplemented with

magnesium by adding 1.75 mM magnesium (41.7 mg of magnesium chloride (Sigma M4880) dissolved in 50 mL of DPBS. This procedure prepared a solution of magnesium at 8.75 mM). When mixed with compounds in DPBS without magnesium (12 µL of compound + 48 µL of 8.75 mM magnesium), concentration of magnesium was 7 mM. When further diluted in assay plate during treatment (10 µL added to 30 uL of DPBS), magnesium concentration was 1.75 mM).

Treatment with 0.5 mM magnesium: diluted 10 mL of magnesium solution at 8.75 mM with 25 mL of DPBS without magnesium. (Note: This procedure prepared a solution of magnesium at 2.5 mM. When mixed with compounds in DPBS without magnesium (12 µL of compound + 48 µL of 2.5 mM magnesium), concentration of magnesium was 2 mM. When further diluted in assay plate during treatment (10 µL added to 30 µL of DPBS), magnesium concentration was 0.5 mM.) 100 µL/well of DPBS was loaded with different magnesium concentrations (0, 2.5 or 8.75 mM) in a 384-well compound plate according to plate map.

Step C: Compound Dilutions

Compounds in DMSO were diluted to 333.33 x the final desired concentration for testing. If 10 µM was the highest concentration in the assay, compounds were diluted to 3.33 mM. serial dilution in DMSO were performed in low binding 384-well compound plates (Eppendorf according to dilution factor: (a) 1-log: 10 µL transfer into 90 µL DMSO; (b) 0.67-log: 12.9 µl transfer into 47.1 µl DMSO; (c) 0.5-log: 19 µL transfer into 41 µL DMSO. 12 µL of serially diluted compounds were transferred to 188 µL of DPBS without magnesium. 12 µL of compounds in DPBS were transferred to a new 384-well compound plate in duplicate. (Note: The previous steps in procedure 5.3.1 are performed by the Eppendorf EpMotion 5070 using validated liquid handling protocol.) 48 µL of DPBS was with different concentrations of Magnesium. (Note: This procedure prepares compounds at 4x the final desired concentration for treatment. Note: This procedure is performed on Integra Viaflo 384 using custom program: DILUTE48µL_MIX).

Positive control agonist was prepared using Carbachol 50 µM treatment: 8 µl of 25 mM carbachol in water (Sigma C4382) + 992 µl of DPBS without magnesium. 60 µL/well of carbachol or DPBS was loaded in compound plate according to plate map.

Step D: Plate Assay.

At 24 hours post-transduction, cell culture media was washed with 1x DPBS without Magnesium: 5 wash cycles with BioTek 405TS microplate washer/ leaving 30 µL of DPBS in the well (protocol: 384cw Corning 30ul final), and checked under the microscope for

fluorescence, background and cell health. Cells were equilibrated in DPBS for 20-30 min at room temperature in the dark. The plate was placed in the Hamamatsu FDSS/uCell, exposure (0.6 to 1 s) and gain (30 to 35) were adjusted until wells appear blue/green (no yellow), and baseline fluorescence was measured for 1 minute at 2 s intervals. 10 µl of 4x compounds was added using on-board liquid handling in instrument and the fluorescence was measured for 10 minutes at 2 s intervals.

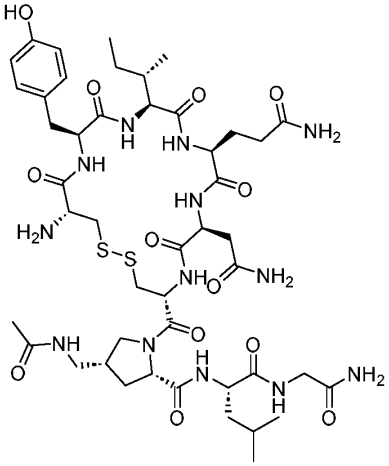
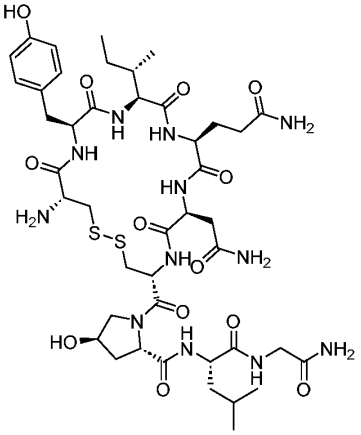
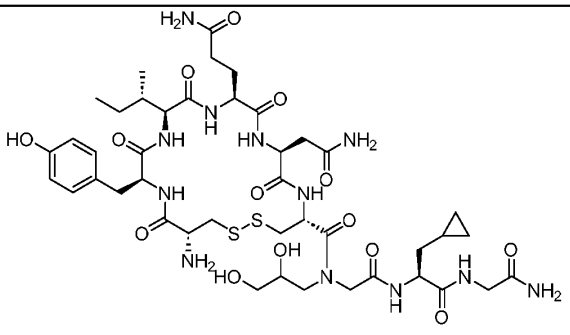
Step E: Data generation

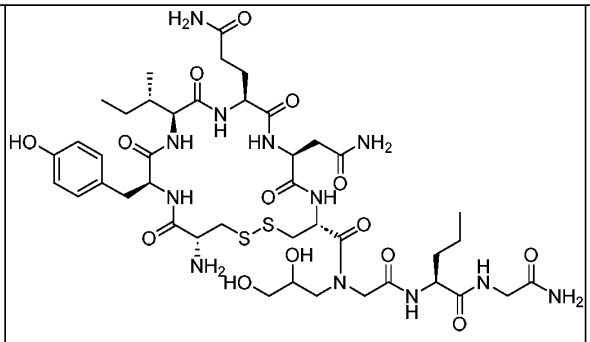
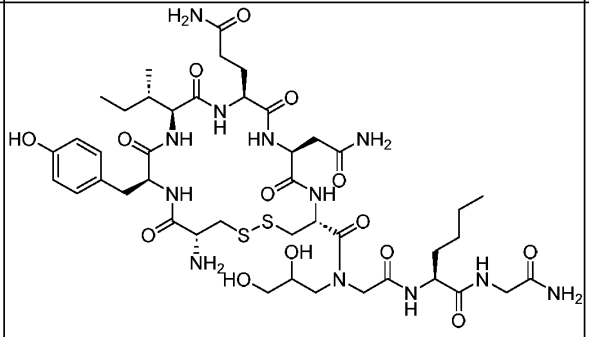
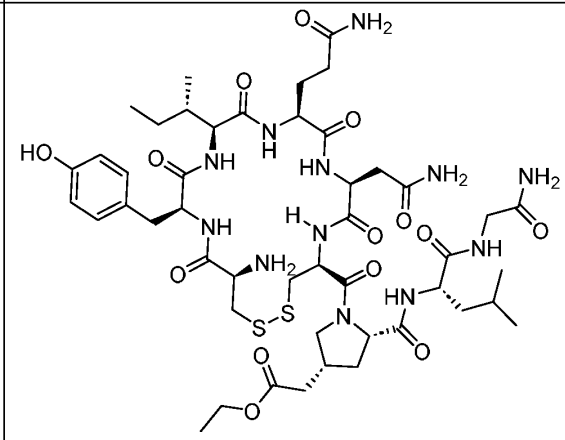
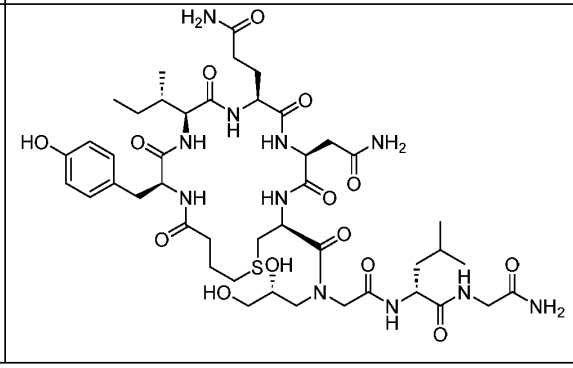
The average relative fluorescence units measured during the baseline period was calculate. Fluorescence change ratio was calculated using the formula:

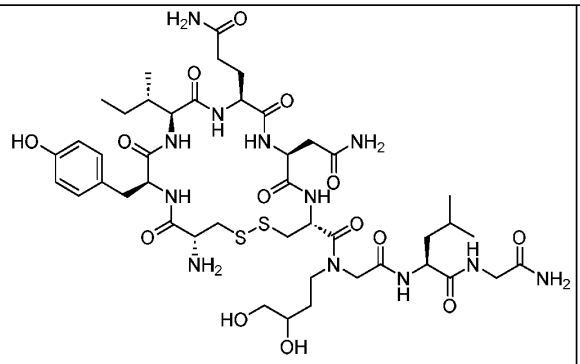
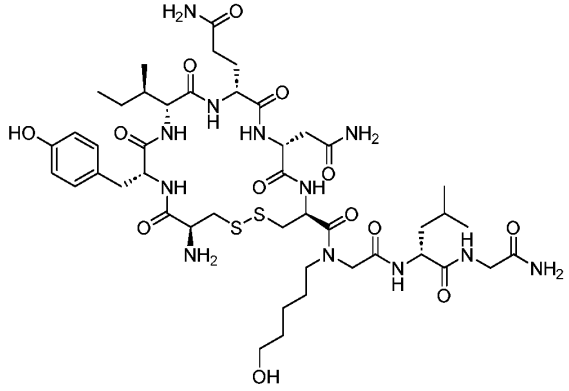
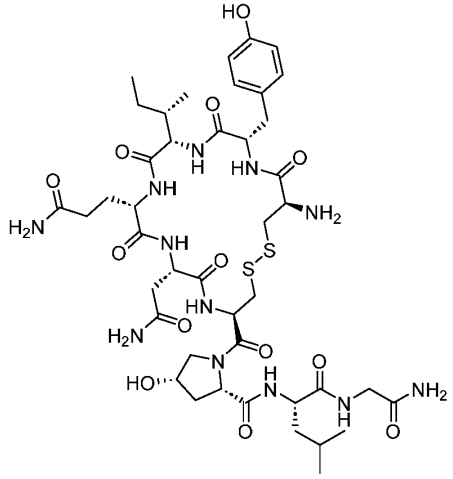
$$Normalized\ fluorescence\ units = \frac{Relative\ Fluorescence\ Units}{Average\ Fluorescence\ at\ baseline}$$

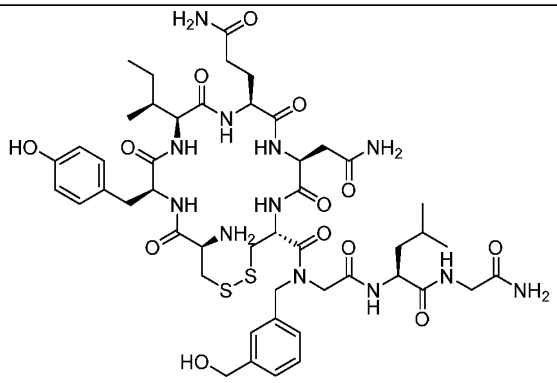
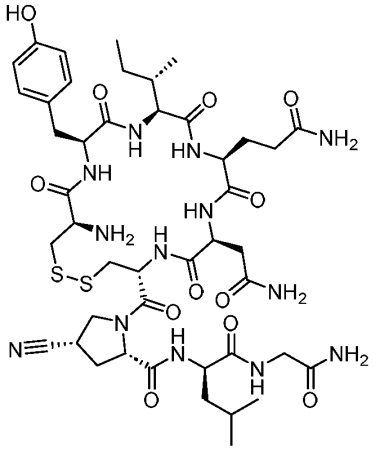
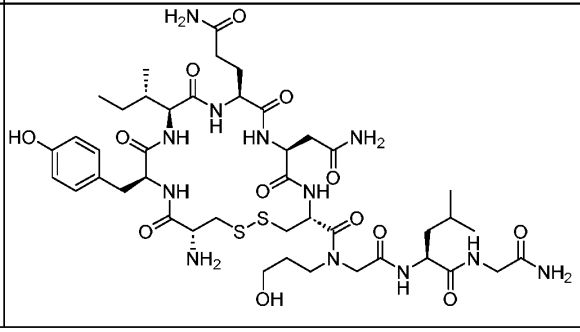
Time-matched normalized fluorescence for vehicle-only treated cells was subtracted from compound treatment data and calculate peak DAG and initial rate by curve fitting (Table #).

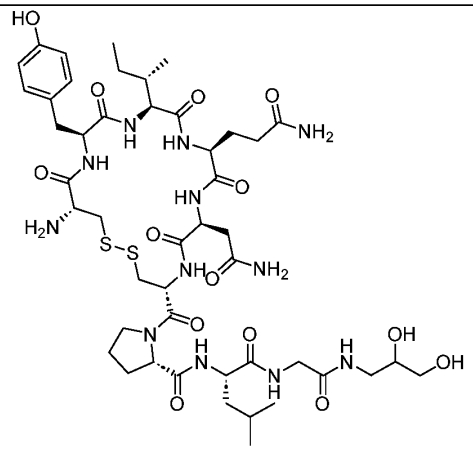
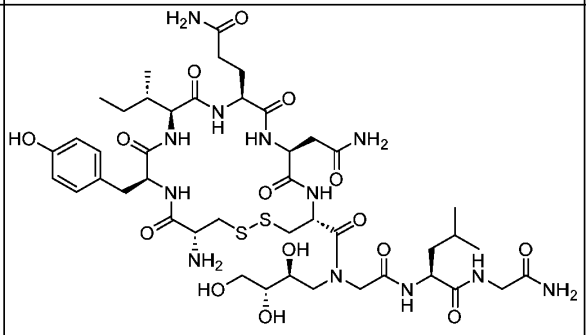
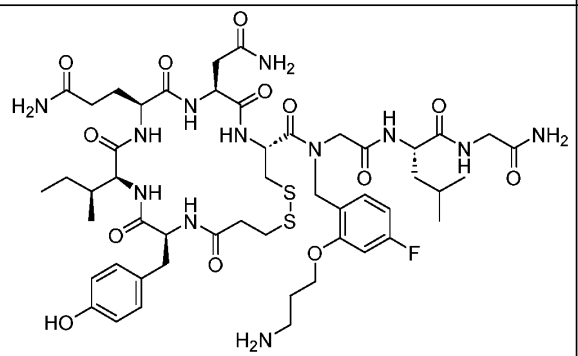
Table 1 - DAG Gq assay for Oxytocin Receptor

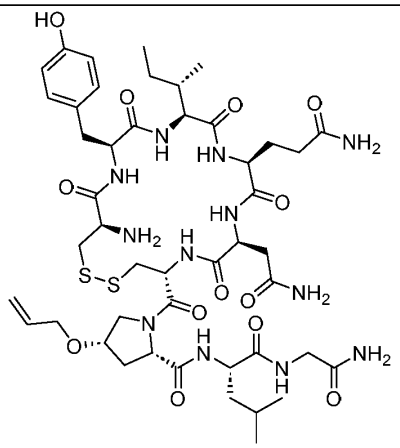
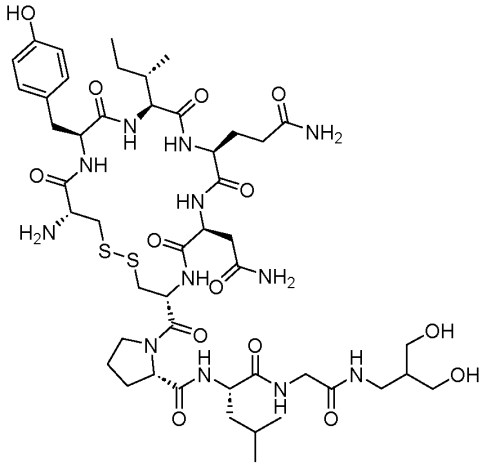
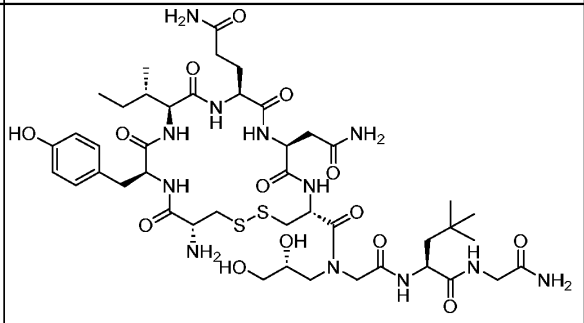
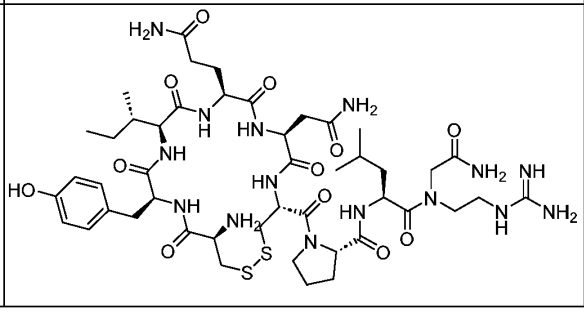
Cpd. No.	Structure	0.5 mM $MgCl_2$		1.75 mM $MgCl_2$	
		EC50 (nM)	Emax (%)	EC50 (nM	Emax (%)
1		250	35.1	287	48.8
A		11.8	104.7	9.69	100.5
2		14.2	26.0	15.2	47.6

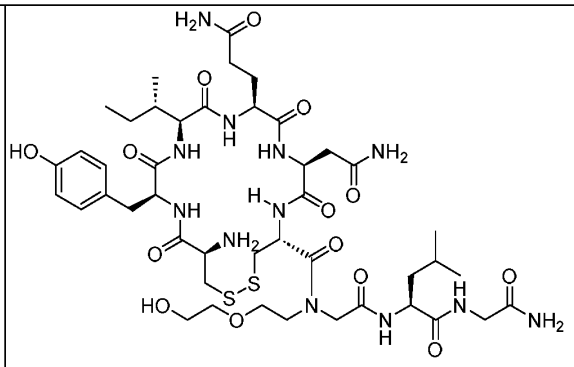
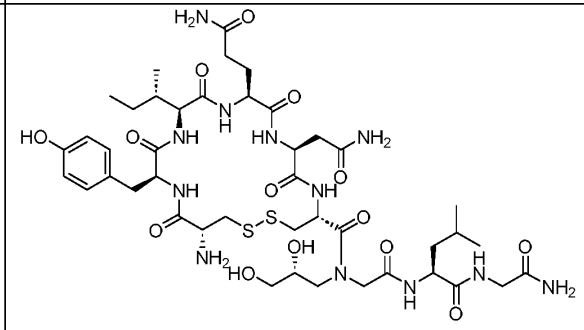
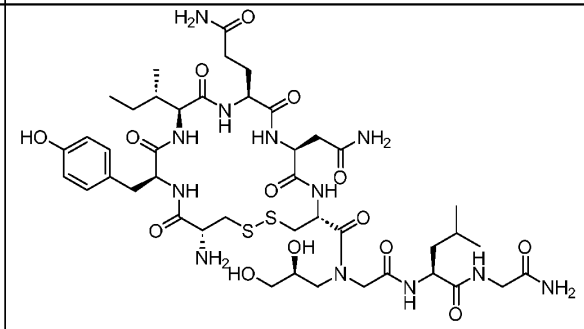
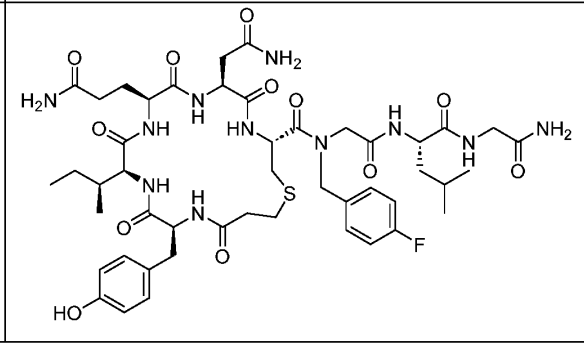
3		21.2	68.2	11.3	83.6
4		25.7	62.9	13.6	75.0
5		93.2	48.2	67.8	70.1
6		14.5	88.9	12.5	96.7

7		18.4	91.8	11.7	93.8
8		24.5	77.2	18.7	87.1
B		14.9	95.9	10.4	91.6

9		17.2	59.4	15.8	74.3
10		9.13	80.0	11.0	86.9
C		12.7	78.7	8.84	90.8

11		199.7	79.5	121.9	78.9
12		71.6	60.9	44.6	81.5
13		29.2	39.2	38.0	55.8

14		135.8	30.8	100.0	45.1
15		280.5	11.1	137.2	14.8
16		38.0	17.4	28.4	43.0
17		274.2	50.6	346.1	57.1

18		18.7	87.1	14.4	96.5
20		32.6	37.6	31.3	66.2
21		24	73.0	15	88.9
D		5.88	86.6	5.65	87.8

[0156] **Example 39** - Screening of efficacy and potency of oxytocin analogs using rat TG electrophysiology.

Protocol: The experiments were done on trigeminal ganglia (TG) neurons prepared from male rats (350-430 g) injected with CFA (50 µl) into left TMJ. The recordings were

performed on external solution with either 0.5 mM or 1.75 mM $MgCl_2$. Bath solution was composed of NaCl (130 mM), HEPES-Na (10), KCl (5), $CaCl_2$ (1) and Glucose (10), and pH was adjusted to 7.3-7.4 using HCl and to the desired $MgCl_2$ concentration. Osmolarity was adjusted to 299 mOsm/kg with sucrose. Electrode internal solution was composed of KF (120 mM), HEPES (10 mM), EGTA (11 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), KCl (10 mM), and KOH (11 mM), and pH was adjusted to 7.4 using KOH. Osmolarity was adjusted to 293 mOsm/kg with sucrose. Glass pipettes were filled with this intracellular saline with a resistance of 3-5MΩ.

Results: The clinically relevant concentration range for electrophysiology on TG is between 8 x 10^{-9} M to 2.5 x 10^{-8} M. At 1 x 10^{-8}M, in the presence of 1.75 mM $MgCl_2$ **Compounds 20**, **21**, and **23** show similar potency, independent of $MgCl_2$ concentration (**Figures 1-4**).

[0157] **Example 40** - Analgesic Dose/Response of intranasal oxytocin analog **Compound 20** in rat thermal craniofacial pain model

Protocol: Four concentrations of oxytocin analog in saline vehicle were combined with 0.5 and 1.75 mM $MgCl_2$. Each solution was coded blind to the experiment and the experiment was performed in strictly blinded manner including drug injection and data analysis. Rats were individually habituated in tip-less decapicone for 2 days, 5-10 minutes/ rat prior to Day 0. On Day 0, male rat's left cheek was depilated and was individually habituated in a tip-less decapicone for at least 15 minutes. On Day 1, baseline heat tolerance response was assessed and electrocutaneous stimulation was performed (ES, 1 hour stimulation with 0.4 mA current, 10 ms pulse at 1s inter-pulse interval for each rat) x 12 rats/day. On Day 2 (24 hrs after stimulation), stimulation response was assessed by heat tolerance to make sure electrocutaneous stimulation was successful. Once validated that electrocutaneous stimulation was successful, nasal application of the coded drugs was performed. Thermal withdrawal latencies measured for the following post-IN dosing timepoints: 15, 45, 60, 120 min. Thermal thresholds were assessed using Thermal Testing Lamp, which was fixed ~ 5 cm above depilated left cheek of rat. Voltage regulator was set to 90V for A-delta fiber testing. Cutoff was set at 6 seconds.

Results:

A-delta Fiber

Compound 20 had an analgesic effect in dose dependent manner for A-delta fiber. At high concentrations of Compound 20, the concentration on Mg^{2+} (high or low) had no effect on the analgesic effect. At low concentrations of Compound 20, the concentration of Mg^{2+} (high or

low) had a slight effect on the analgesic effect. Overall, Compound 20 (regardless of the Mg^{2+} concentration) showed similar analgesic effect (See **Table 2** and **Figures 5** and **6**).

Table 2 – A-delta Fiber: Comparison of low and high $MgCl_2$ concentrations

Concentration of **Compound 20**	Withdrawal Latency (s) 0.5 mM $MgCl_2$	Withdrawal Latency (s) 1.75 mM $MgCl_2$
4 µg	1.725	1.175
10 µg	1.55	1.325
32 µg	2.6	2.275
128 µg	2.3125	2.6875

C Fiber

Compound 20 had an analgesic effect in dose dependent manner for C fiber. At a 4 µg dose of Compound 20, the concentration of Mg^{2+} (high or low) had a slight effect on the analgesic effect. Overall, Compound 20 (regardless of the Mg^{2+} concentration) showed similar analgesic effect (See **Figure 7**).

[0158] **Example 41** – Magnesium Dependence of Hyperpolarization in Rat Ex-Vivo Trigeminal Ganglia Electrophysiology

Compound 20 and oxytocin were tested using the protocol described by Bharadwaj, V.N. and Yeomans, D.C., et al., in "Impact of Magnesium on Oxytocin Receptor Function" Pharmaceutics 2022, 14, 1105.

Sample Preparation

OT and Compound 20 were dissolved in distilled water as 1 mM stock and diluted for external solutions. Different concentrations of OT and Compound 20 (0.1, 1.0, 10, 100, 300, and 1000 nM) were applied from the reservoir using gravity feeding through a 27G tip-blunted needle with an opening placed about 200 µm away from the recorded cell. The stream of solution covered the cell well when the switch was turned on. The cells were physically stable during perfusion.

Induction of Inflammation

Rats (male, 250–330 g, Envigo, n = 10) were placed in an anesthesia chamber and anesthetized with 2.5% isoflurane. Prior to TMJ injection, the rat's mouths were propped open to palpate the target area. In this position, an oval-shaped groove located in the center of the cheek and above the mandible can be distinctly felt. With the syringe positioned at a 30-degree angle from the rat's cheek, the tip of the needle was inserted just under the articular disc (approximately 1.5 mm in diameter and 1.0 mm deep). Thereafter, 50 µL of CFA

(DIFCO; Sigma Aldrich, St. Louis, MO, USA) was injected (1 mL syringe with a 25G 5/8-inch needle) into the left TMJ to produce robust and prolonged orofacial inflammation. After CFA injection, rats were returned to home cages. Approximately 24 h later, rats were euthanized by decapitation after induction of deep anesthesia with isoflurane.

Tissue Processing

The rat's TG were carefully dissected from the surrounding connective tissues and minced into small pieces with an iris scissor. The TG were digested in 0.5 mL of mixed enzyme solution: (w/v, final concentration) 0.1% trypsin (Sigma, T9201), 0.1% collagenase Sigma, C1764), and 0.01% DNase (Sigma, D5025) diluted in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (Sigma, St. Louis, MO, USA). The tissue pieces were then incubated at 32 °C with a water bath for 55 min. Following digestion, tissue fragments were mechanically dissociated using a series of glass Pasteur pipettes with decreasing internal diameter. Dissociated cells were centrifuged at 180 g for 3 min, the supernatant was removed, and the cells were gently re-suspended in an external recording solution. Cells were then plated onto poly-L-lysine (Sigma, St. Louis, MO, USA) coated cover slips (Chemglass Life Sciences Vineland, NJ, USA. *Current Clamp Recording*

Whole-cell voltage-clamp recordings were performed using the MultiClamp 700B amplifier (Molecular Devices, San Jose, CA, USA) and analyzed offline with pCLAMP10.4 software (Molecular Devices, San Jose CA, USA). The external solution was composed of (in mM) NaCl (130), N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid (HEPES)-Na (10), KCl (5), $CaCl_2$ (1), and Glucose (10), pH adjusted to 7.3–7.4 using HCl, with or without 1.75 mM $MgCl_2$. The electrode internal solution was composed of (in mM) KF (120), HEPES (10), ethylene glycol-bis(-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (11), $CaCl_2$ (1), $MgCl_2$ (1), KCl (10), and KOH (11), pH adjusted to 7.3–7.4 using KOH. Patch-pipettes were fabricated from 1.5 mm outside diameter (OD) borosilicate capillary glass (Warner Instruments, Hamden, CT, USA) using a micropipette puller (Model P-87, Sutter Instrument, Novato, CA, USA). NaCl, HEPES-Na, KCl, $CaCl_2$, Glucose, HCl, $MgCl_2$, KF, HEPES, EGTA, KOH were purchased from Sigma (St. Louis, MO, USA). Glass pipettes filled this intracellular saline with a resistance of 3–5 MW. Whole-cell patch recordings had series resistances of <25 MW after whole-cell configuration and were periodically checked with the seal test voltage step (10 mV, 10 ms) to monitor series resistances throughout the recordings. Hyperpolarizing current pulses (about -0.3 nA, 500 ms) were delivered every

5 s throughout the experiment, unless otherwise specified, in order to monitor membrane input resistance and stabilize membrane potential in control external solution.

Measurement of change in membrane potential: After successful current clamp recording, the effect of the vehicle external solution application to cells on membrane potential was measured. After the membrane potential had stabilized for at least 10 s, a solution containing OT plus or minus 0.5 mM and 1.75 mM Mg^{2+} or Compound 20 plus or minus 0.5 mM and 1.75 mM Mg^{2+} was applied for 2–5 min until the membrane potential stabilized further (on a new level) for at least 10 s.

For OT, the Mg^{2+} dependency was calculated by subtracting hyperpolarization measured in mV for OT with 0.5 mM of Mg^{2+} from the hyperpolarization measured in mV for OT with 1.75 mM of Mg^{2+}. For Compound 20, the Mg^{2+} dependency was calculated by subtracting hyperpolarization measured in mV for Compound 20 with 0.5 mM of Mg^{2+} from the hyperpolarization measured in mV Compound 20 with 1.75 mM of Mg^{2+}. Results show that difference in hyperpolarization in the presence of OT and Mg^{2+} increased with OT concentration, showing dependency to Mg^{2+}. However, no such increase in Mg^{2+} dependency with Compound 20 concentration was observed for Compound 20 (*see* **Figure 8**).

[0159] **Example 42** – Magnesium Dependence of Antinociception in the C-Fiber Test of Rat Craniofacial Pain

Sample Preparation

Compound 20 and oxytocin were tested using the protocol described by Bharadwaj, et al. The appropriate amount of test compound was accurately weighed out using a calibrated electrical balance and placed into microcentrifuge tubes. Solutions were prepared for an administration volume of 50 µL containing vehicle or one solution for one of five doses of OT (0.5, 1.0, 4.0, 8.0, or 32.0 µg) with or without 300 mM magnesium citrate. Solutions of Compound 20 were prepared for an administration volume of 50 µL containing vehicle or a solution for one of four doses (4.0, 10.0, 32.0, or 128 µg) with 0.5 mM or 1.75 mM Mg^{2+} []. Ten groups of 10 rats each were randomly assigned to receive a vehicle or one of the five doses of OT or OT plus 300 mM Mg^{2+}, or the Compound 20 doses with either 0.5 or 1.75 mM Mg^{2+}. Each solution was coded and the experiment was performed in a strictly blinded manner, including drug administration and data analysis.

Withdrawal Latency

Rats (male, 250–330 g, Envigo, n = 10 per group) were used and treated with CFA injection into the TMJ as described above in order to produce a robust inflammation of trigeminally innervated tissue. Approximately 24 h after CFA injection, withdrawal latencies in response to noxious heat applied to the depilated (NAIR® hair removal cream; Church & Dwight Co., Ewing, NJ, USA) and blackened (with India ink (Chartpak Inc., Leeds, MA, USA)) cheek were determined. Latency to withdrawal response was used as an indicator of nociceptive responsiveness. Low intensity (slow ramp) skin heating evokes withdrawal responses mediated by the activation of C- (unmyelinated) nociceptive fibers; higher intensity skin heating (rapid ramp) selectively elicits responses mediated by A-delta (myelinated) thermonociceptors. Briefly, to assess C fiber mediated responses, heat intensity was adjusted by altering the supply voltage (35–55 V) of the focused lamp until a withdrawal response was observed to occur with latency between 7.5 and 8.5 s. In order to reduce the potential for tissue damage, a cut-off latency of 15 s was implemented after which the stimulus was terminated. Rats not responding (within 10 s) to a supply voltage of 55 V during baseline testing were excluded from the study. The intensity applied to achieve such latencies was noted for each animal and used to assess withdrawal latencies prior to and following nasal administration of the test agent. Rats not responding (within 3.5 s) to a supply voltage of 85 V were excluded from the study. Baseline withdrawal latencies were determined for each fiber type prior to nasal application of the test agent.

To deliver intranasal OT or vehicle the rats were anesthetized in a chamber using isoflurane (2%). They were then placed on a heating pad in a supine position as the anesthesia was continued with a nose cone. This horizontal position of the head was maintained throughout the procedure preventing drainage of the drug solution to the trachea and esophagus. The total volume of 50 µL solutions was administered by pipette in 6–7 µL drops in alternating naris every two min, over a total of 14 min. The drop was placed at the naris opening while occluding the opposite naris allowing the animal to snort the drop into the nasal cavity. The rats were allowed to wake up in a separate cage on a heating pad. Rats were then returned to their home cage. Withdrawal latencies in response to C fiber cheek stimulation were then remeasured at 60 min following dosing. At the end of the testing session, rats were euthanized by CO_2 inhalation.

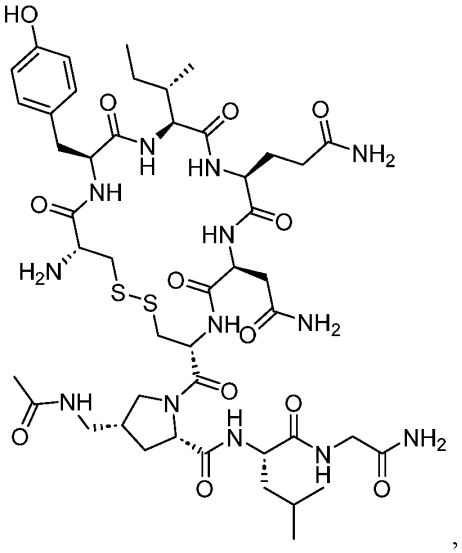
For OT, the Mg^{2+} dependency was calculated by subtracting latency of withdrawal measured in seconds for the rats receiving OT with 0 mM of Mg^{2+} from the latency of withdrawal measured in seconds for the rats receiving OT with 300 mM of Mg^{2+}. For Compound 20, the

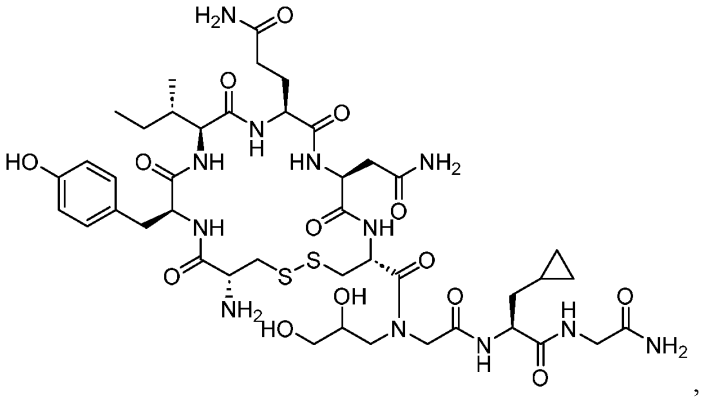
Mg^{2+} dependency was calculated by subtracting latency of withdrawal measured in seconds for the rats receiving Compound 20 with 0.5 mM of Mg^{2+} from the latency of withdrawal measured in seconds for the rats receiving Compound 20 with 1.75 mM of Mg^{2+}. Results show a difference in latency of withdrawal in the presence of OT and Mg^{2+} increased with OT concentration, showing dependency to Mg^{2+}. However, no such Mg^{2+} dependency was observed for Compound 20 (*see* **Figure 9**).

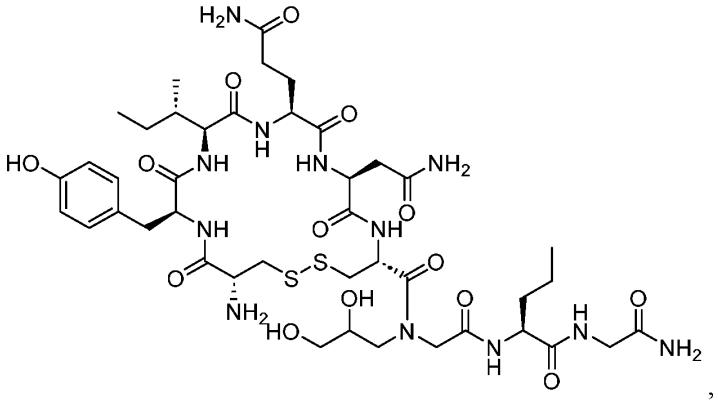
[0160] Although the foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practiced without departing from the disclosure. Therefore, the descriptions and examples should not be construed as limiting the scope of the disclosure.

Embodiments

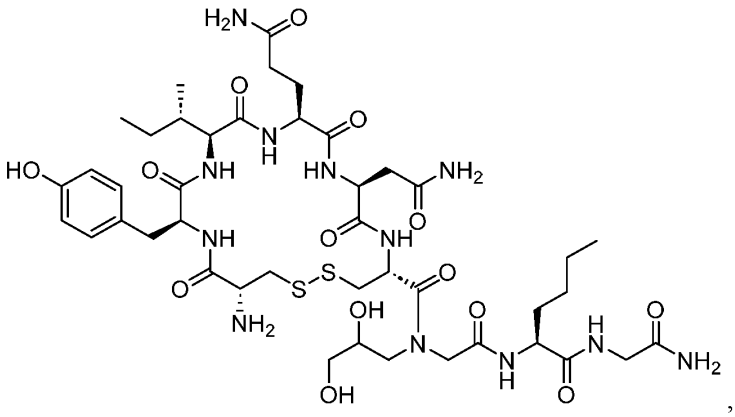
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Compound 2 ,

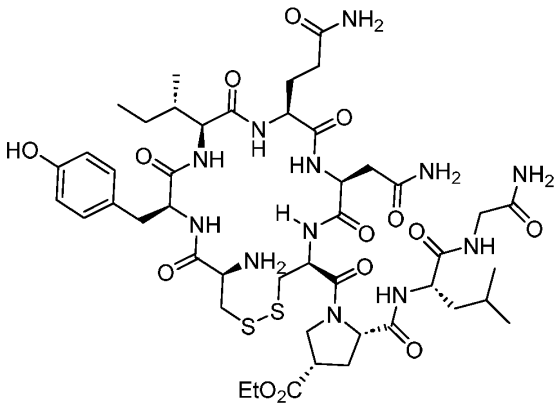
Compound 3 ,

Compound 4



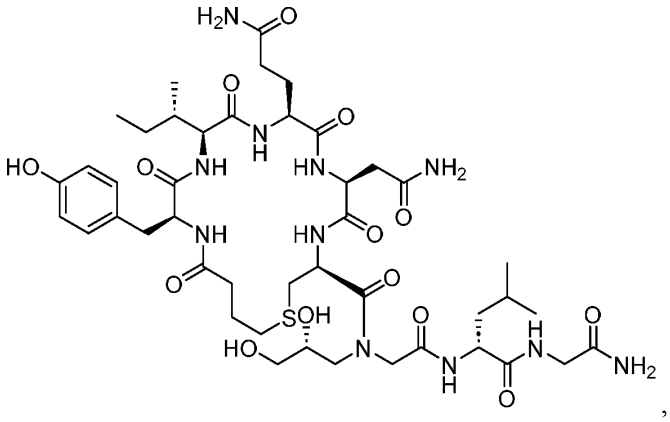
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Compound 5



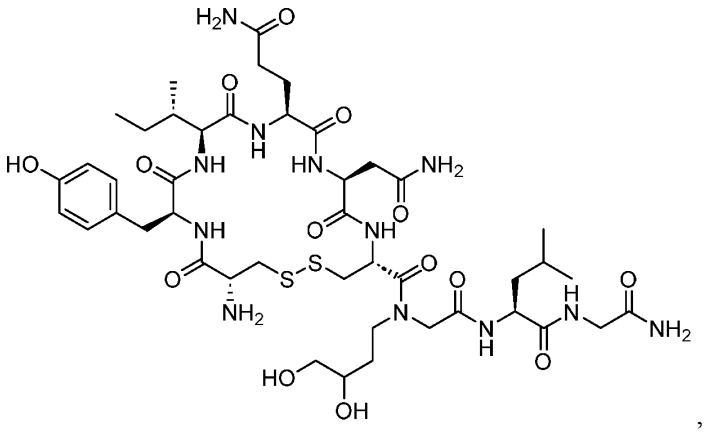
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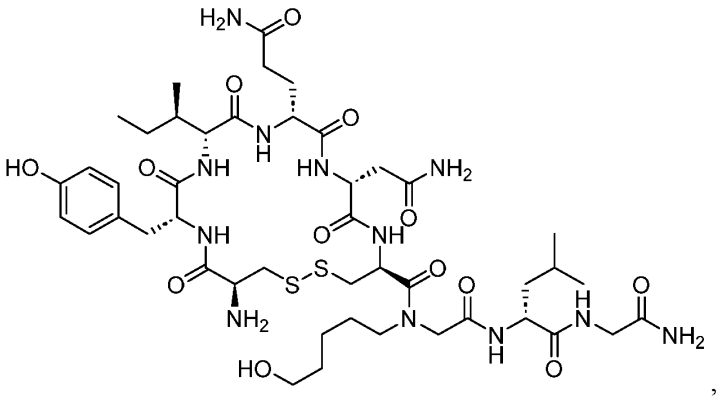
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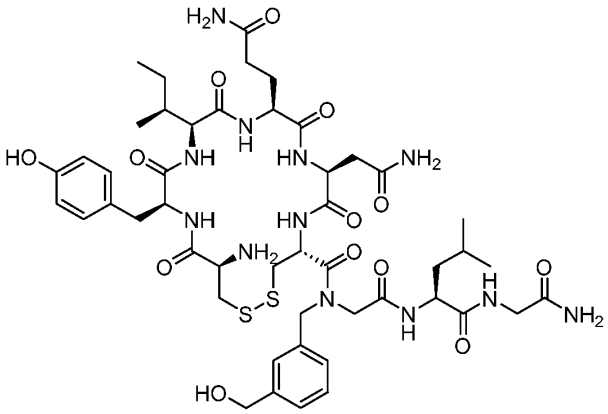
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Compound 8

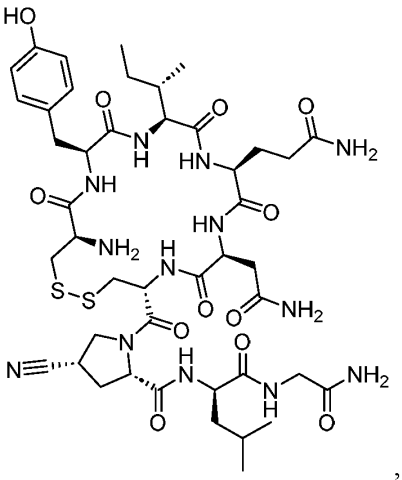


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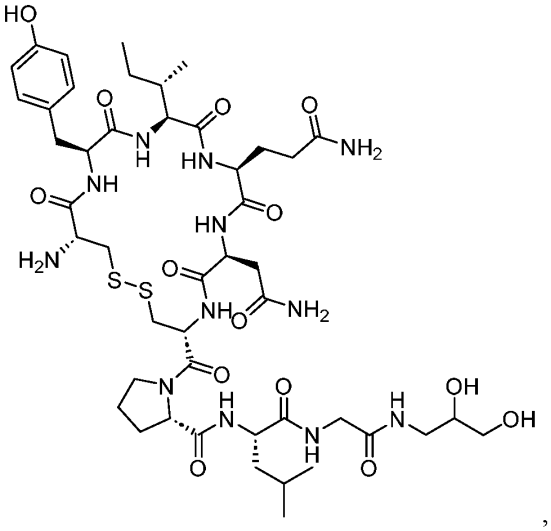
Compound 9



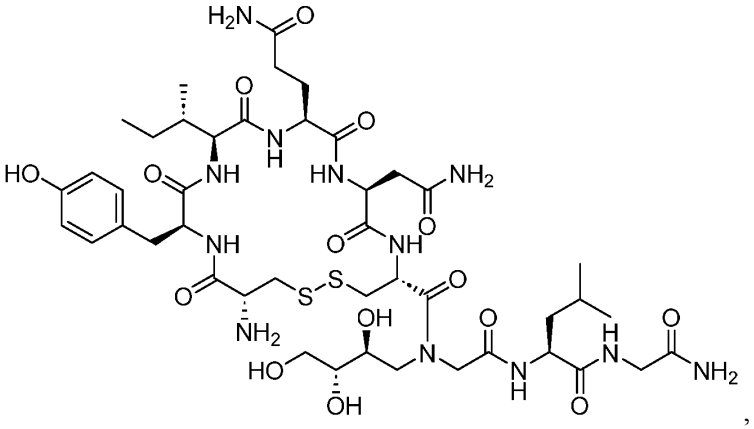
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Compound 10 

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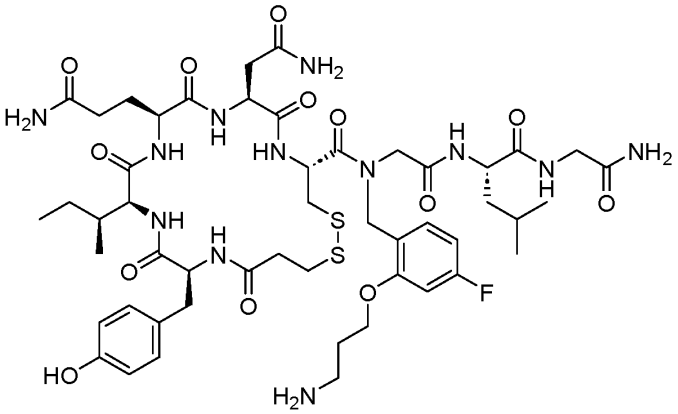
Compound 11 

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Compound 12 

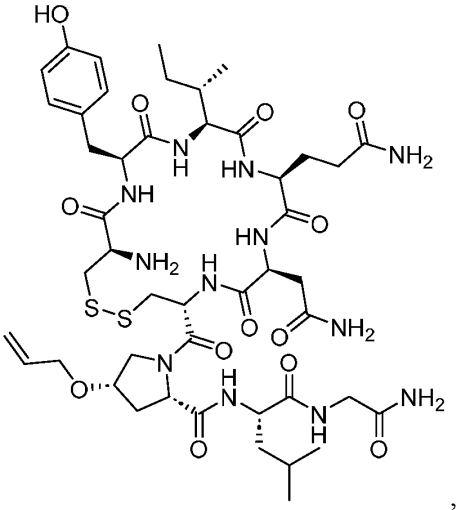
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Compound 13



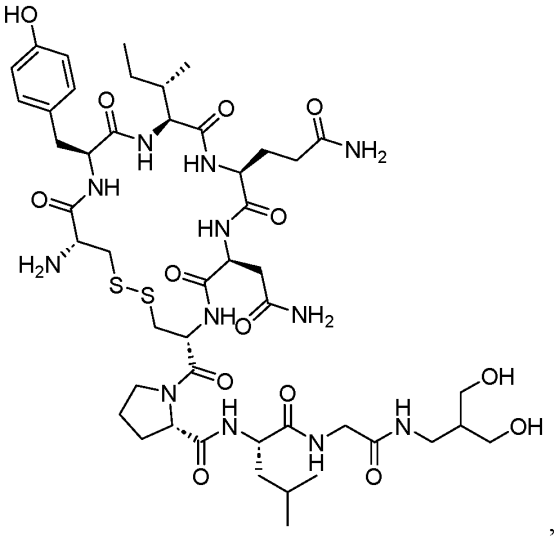
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Compound 14



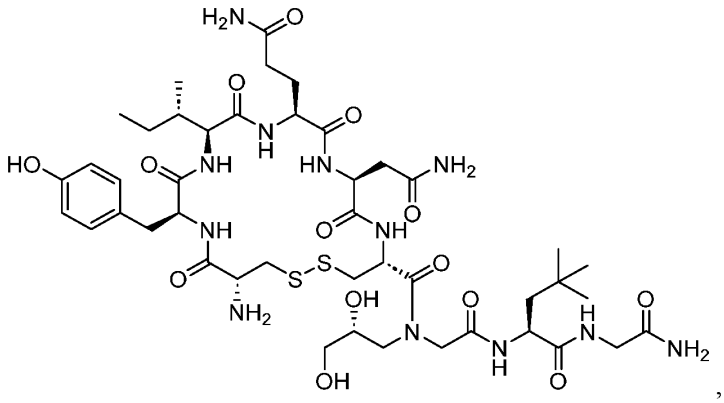
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Compound 15



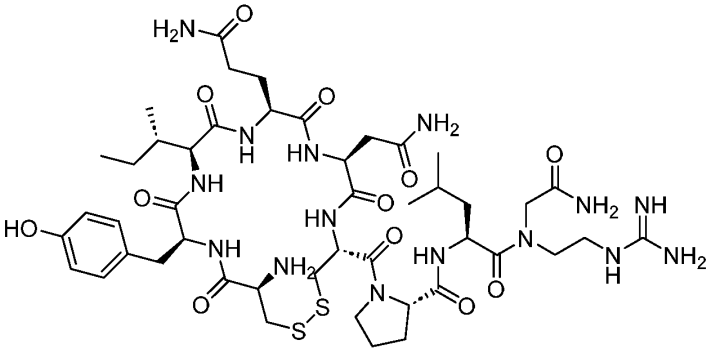
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Compound 16



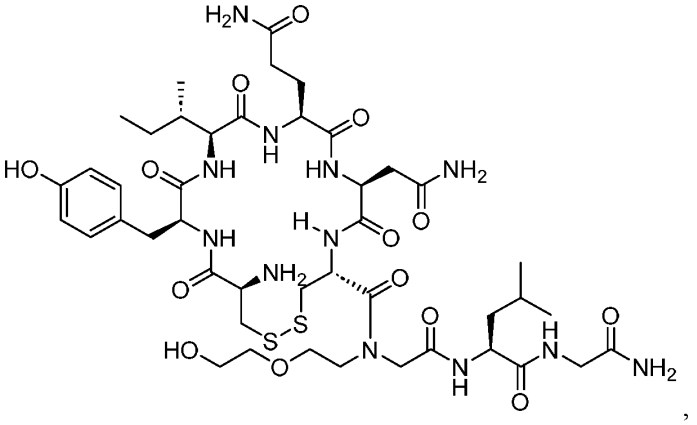
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Compound 17



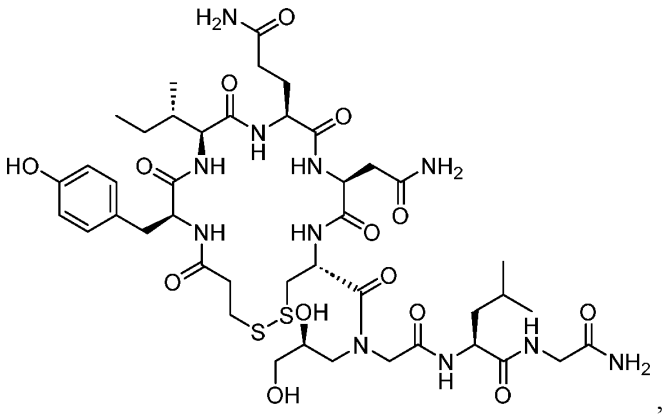
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Compound 18



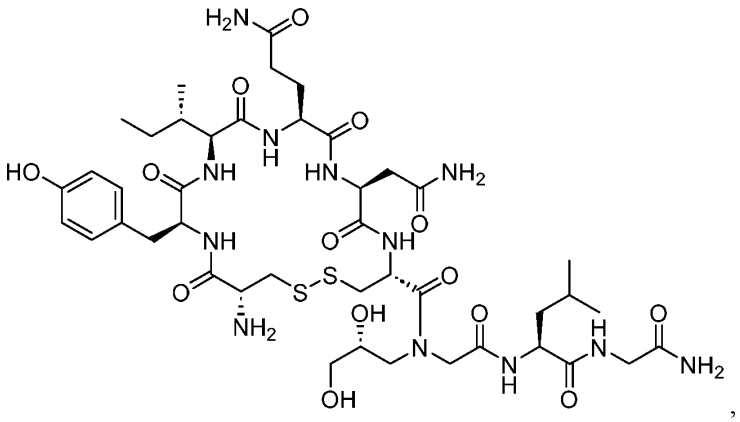
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Compound 19



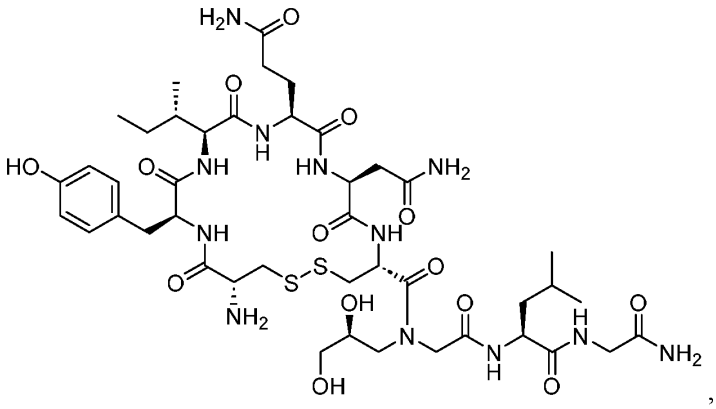
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Compound 20



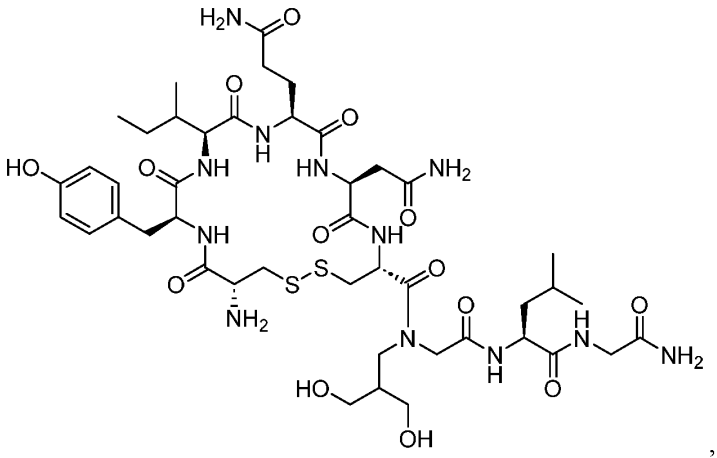
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Compound 21



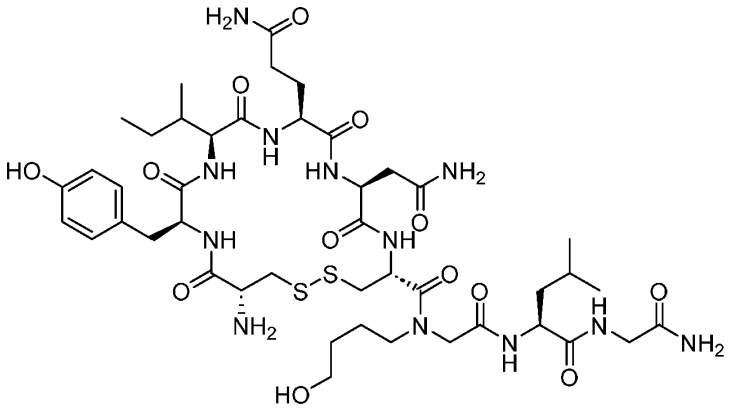
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Compound 22



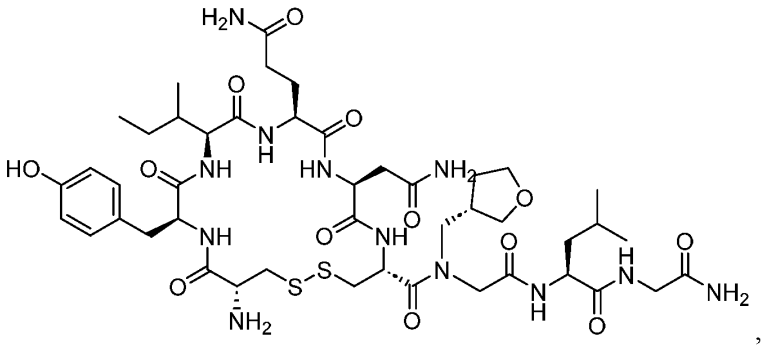
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Compound 23



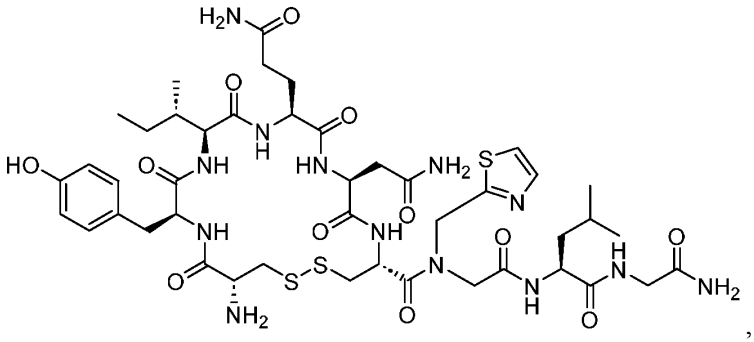
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Compound 24



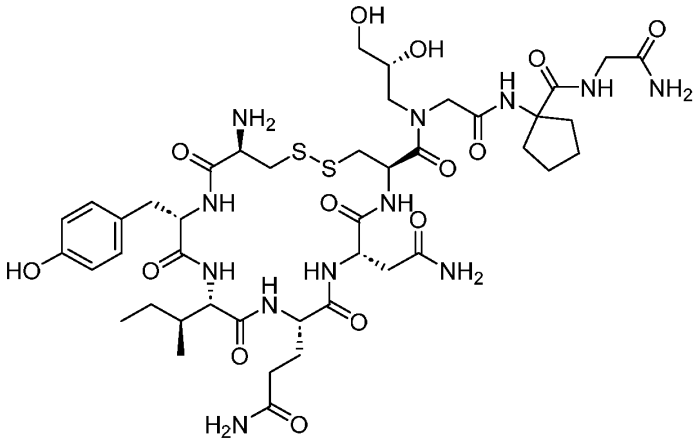
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Compound 25



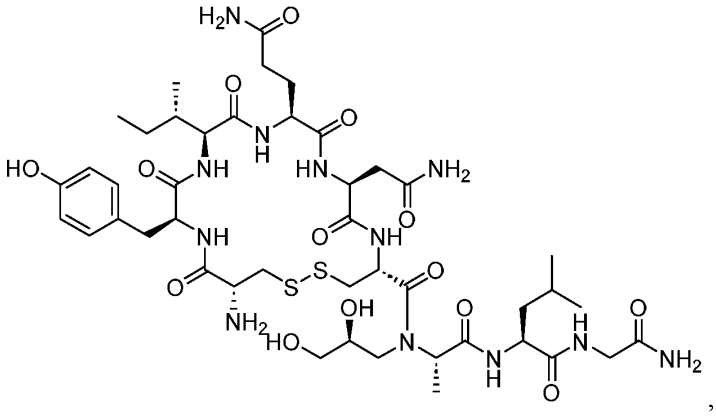
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Compound 26



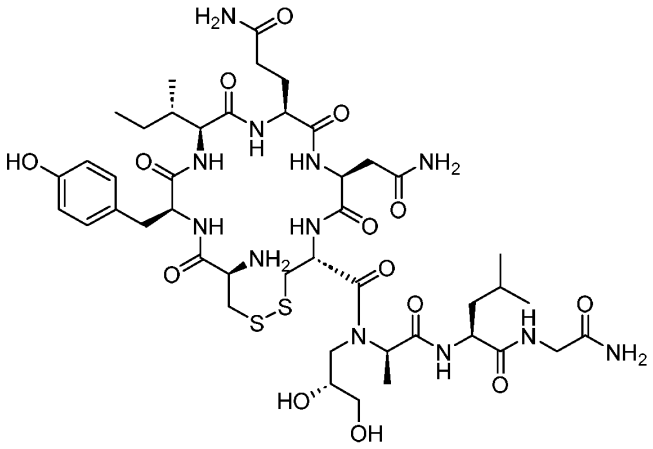
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Compound 27

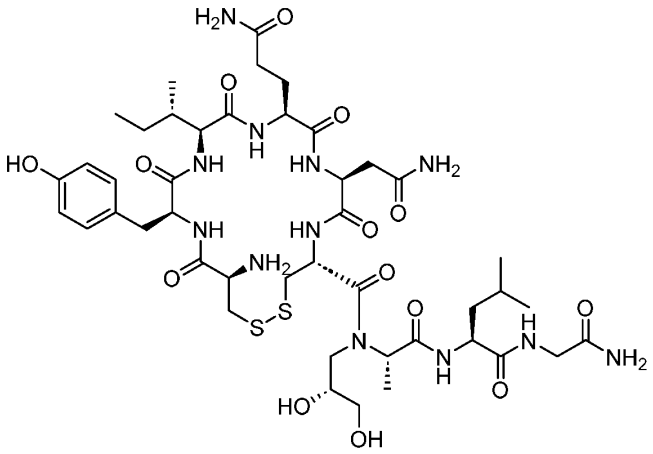


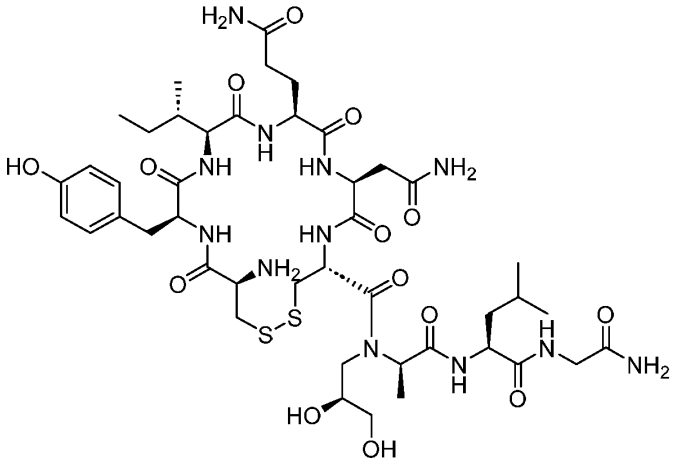
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Compound 28



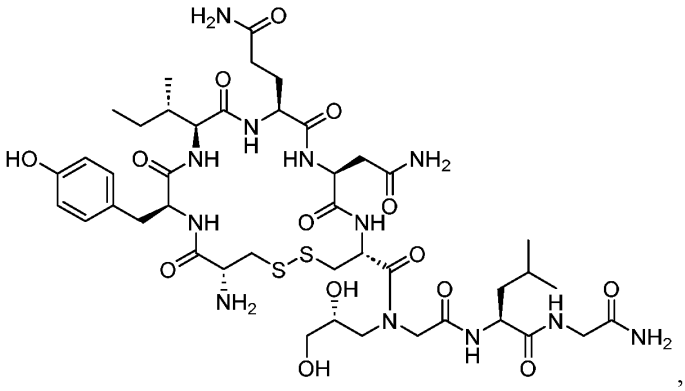
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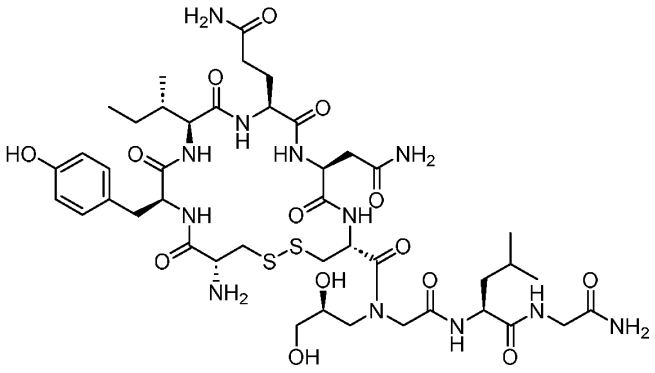
Compound 29 , and

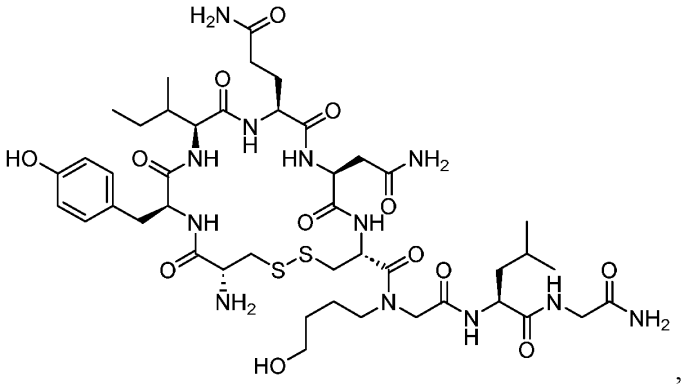
Compound 30 ,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

2. The compound of Embodiment 1, wherein the compound is selected from the group consisting of:

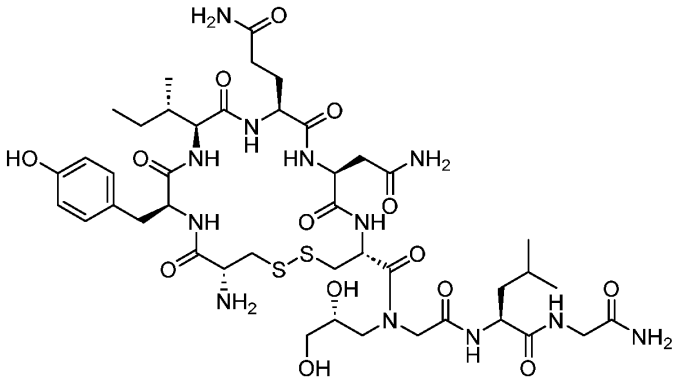
Compound 20 ,

Compound 21 , and

Compound 23 ,

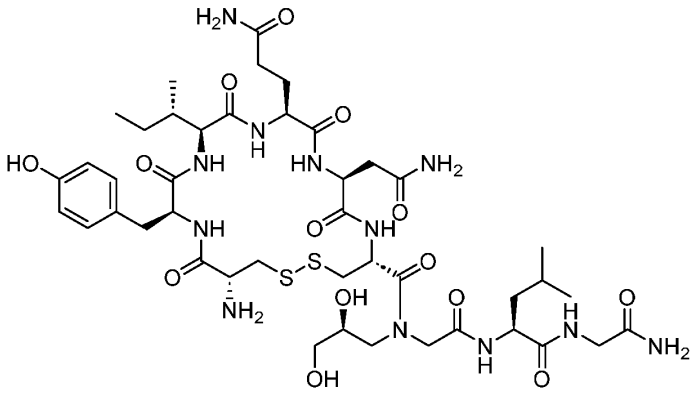
or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

3. The compound of Embodiment 2, wherein the compound is Compound 20:

 Compound 20,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

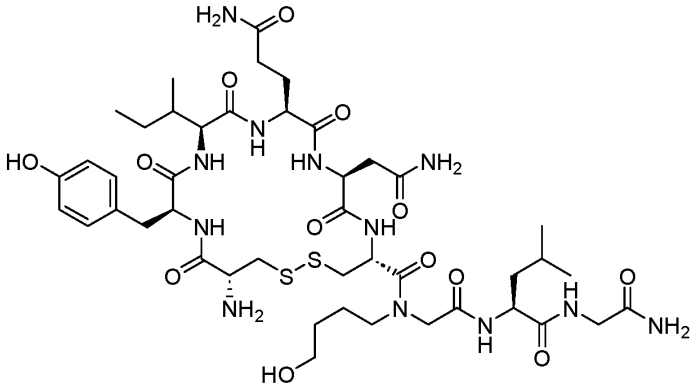
4. The compound of Embodiment 2, wherein the compound is Compound 21:



Compound 21,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

5. The compound of Embodiment 2, wherein the compound is Compound 23:



Compound 23,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

6. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to any one of the Embodiments 1-5 and a pharmaceutically acceptable carrier.

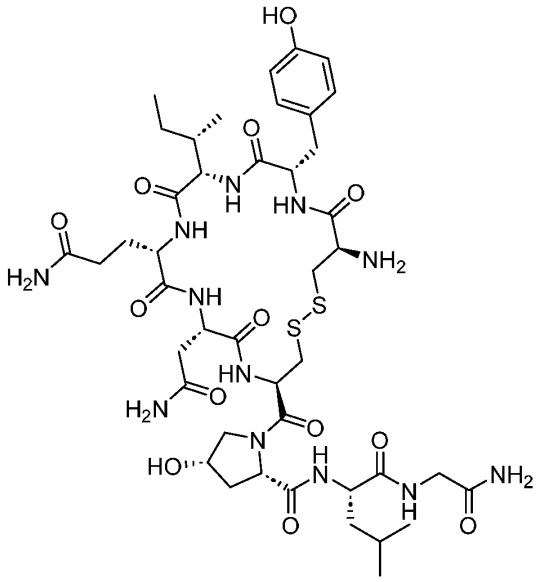
7. The pharmaceutical composition of Embodiment 6, wherein the pharmaceutical composition is a liquid formulation.

8. The pharmaceutical composition of Embodiment 7, wherein the liquid formulation is an aqueous solution.

9. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt

of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.

10. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B,



Compound B,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

11. The method of Embodiment 9 or 10, wherein the administration is via craniofacial mucosal administration.

12. The method of Embodiment 11, wherein the craniofacial mucosal administration is intranasal administration.

13. The method of Embodiment 9 or 10, wherein the administration is via parenteral administration.

14. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 1-6, or a pharmaceutical composition of any one of Embodiments 7-9, wherein the

volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

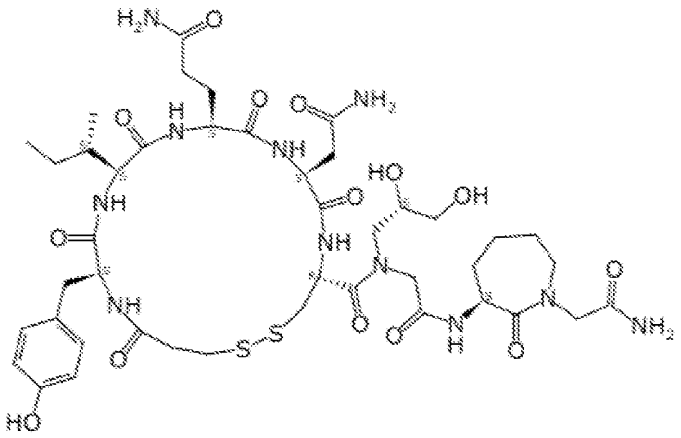
15. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
16. The method of either Embodiment 9, 10, 14 or 15, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.
17. The method of Embodiment 16, wherein the headache disorder is a migraine.
18. The method of Embodiment 16, wherein the headache disorder is a tension headache.
19. The method of Embodiment 16, wherein the headache disorder is a cluster headache.
20. The method of Embodiment 16, wherein the headache disorder is trigeminal neuralgia.
21. The method of Embodiment 16, wherein the headache disorder is a secondary headache.
22. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.
23. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.
24. The method of Embodiment 22 or 23, wherein compound or the pharmaceutical composition is administered via craniofacial mucosal administration.
25. The method of Embodiment 24, wherein the craniofacial mucosal administration is intranasal administration.

26. The method of Embodiment 22 or 23, wherein compound or the pharmaceutical composition is administered via parenteral administration.
27. The method of Embodiment 22 or 23, wherein the administration is via enteral administration.
28. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
29. A method for treating an insulin resistant disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
30. The method of either Embodiment 22, 23, 28 or 28, wherein the insulin resistant disorder is type II diabetes.
31. The method of either Embodiment 22, 23, 28 or 28, wherein the insulin resistant disorder is obesity.
32. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8.
33. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

34. The method of Embodiment 32 or 33, wherein the administration is via craniofacial mucosal administration.
35. The method of Embodiment 34, wherein the craniofacial mucosal administration is intranasal administration.
36. The method of Embodiment 32 or 33, wherein the administration is via parenteral administration.
37. The method of Embodiment 32 or 33, wherein the administration is via enteral administration.
38. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-6, or a pharmaceutical composition of any of Embodiments 7-9, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
39. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
40. The method of either Embodiment 32, 33, 38 or 39, wherein the feeding disorder is Prader-Willi syndrome.
41. The method of either Embodiment 32, 33, 38 or 39, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).
42. The method of any of the Embodiments 10-41, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.
43. The method of any of the Embodiments 14, 15, 28, 29, 38 or 39, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.
44. The method of any of the Embodiments 14, 15, 28, 29, 38 or 39, wherein the liquid formulation is contained in a device for intranasal administration.

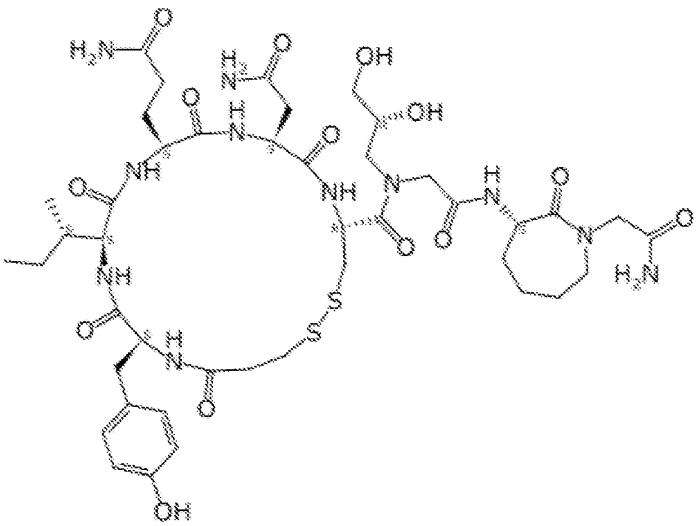
45. The method of Embodiment 44, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.
46. The method of Embodiment 44, wherein the device for intranasal administration is a nasal pump apparatus.
47. The method of Embodiment 46, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.
48. The method of Embodiment 47, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.
49. The method of Embodiment 46, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.
50. The method of any one of Embodiments 46-49, wherein the nasal pump apparatus comprises one of more of the following:
    i) a filter for preventing back flow,
    ii) a metal-tree fluid path, and
    iii) a plastic material stable to gamma-radiation.
51. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, wherein the compound is selected from the group consisting of:

Compound 31



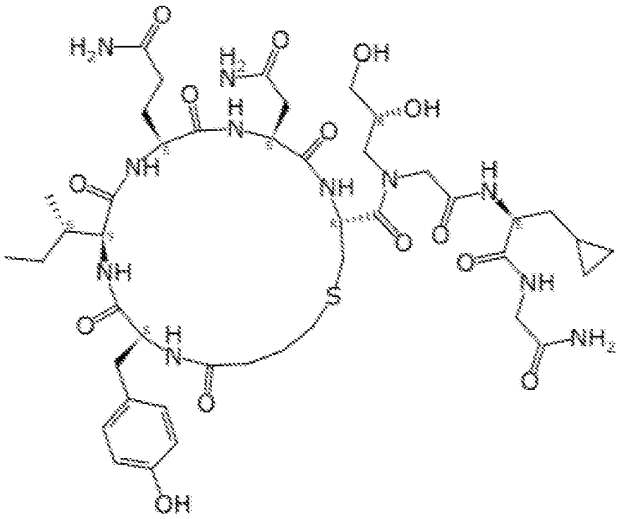
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Compound 32



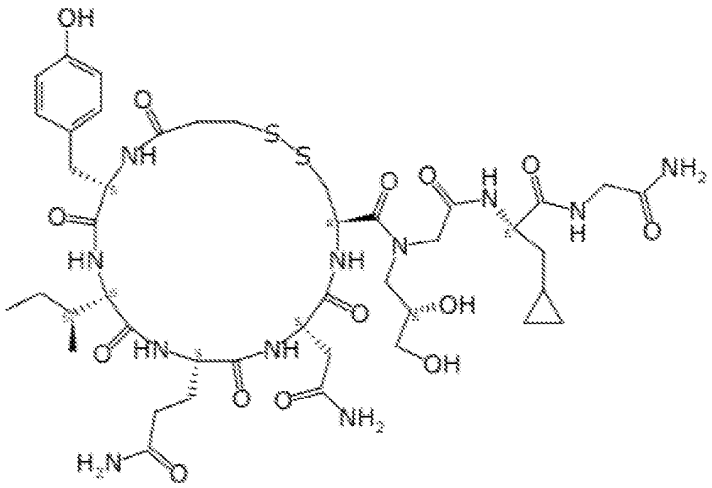
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Compound 33



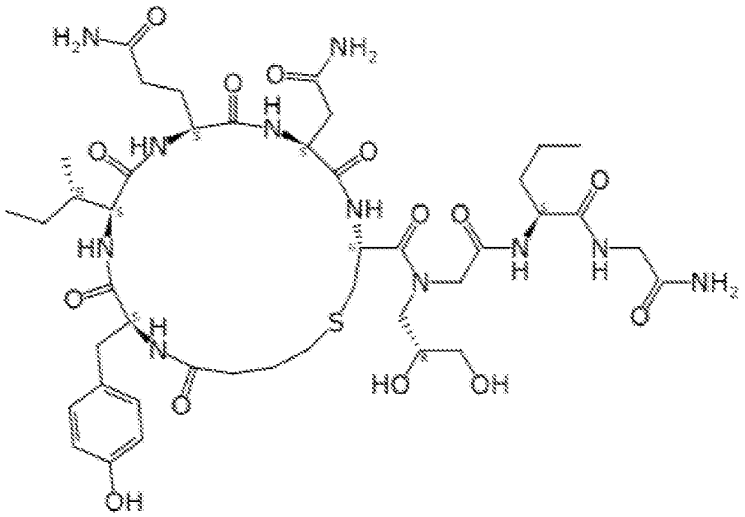
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Compound 34



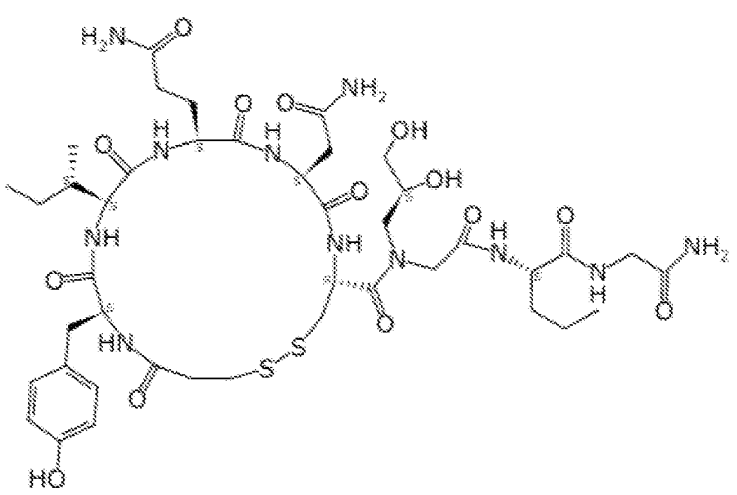
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Compound 35



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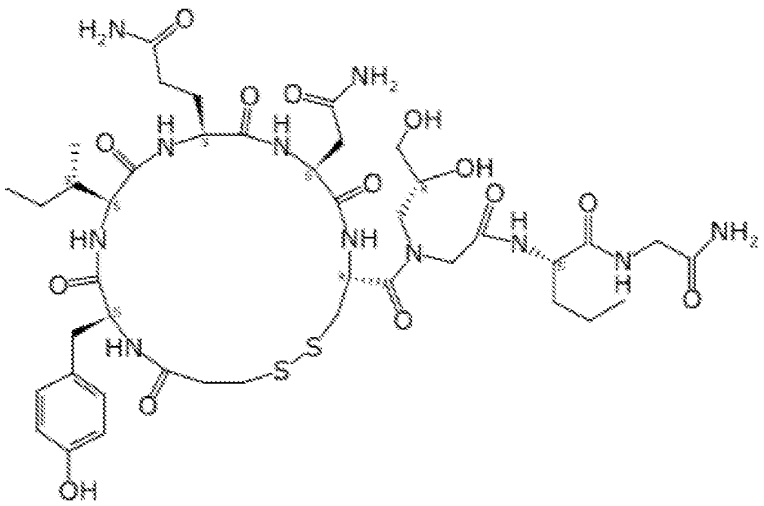
Compound 36



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and

Compound 37



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52. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to Embodiment 51 and a pharmaceutically acceptable carrier.
53. The pharmaceutical composition of Embodiment 52, wherein the pharmaceutical composition is a liquid formulation.
54. The pharmaceutical composition of Embodiment 53, wherein the liquid formulation is an aqueous solution.
55. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer,

racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54.

56. The method of Embodiment 55, wherein the compound or the pharmaceutical composition is administered via craniofacial mucosal administration.

57. The method of Embodiment 56, wherein the craniofacial mucosal administration is intranasal administration.

58. The method of Embodiment 56, wherein the compound, or the pharmaceutical composition is administered via parenteral administration.

59. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

60. The method of either Embodiment 54 or 55, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

61. The method of Embodiment 60, wherein the headache disorder is a migraine.

62. The method of Embodiment 60, wherein the headache disorder is a tension headache.

63. The method of Embodiment 60, wherein the headache disorder is a cluster headache.

64. The method of Embodiment 60, wherein the headache disorder is trigeminal neuralgia.

65. The method of Embodiment 60, wherein the headache disorder is a secondary headache.

66. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiments 51, or a pharmaceutical composition of any one of Embodiments 52-54.

67. The method of Embodiment 66, wherein compound or the pharmaceutical composition is administered via craniofacial mucosal administration.

68. The method of Embodiment 67, wherein the craniofacial mucosal administration is intranasal administration.
69. The method of Embodiment 66, wherein compound or the pharmaceutical composition is administered via parenteral administration.
70. The method of Embodiment 66, wherein the compound or the pharmaceutical composition is administered via enteral administration.
71. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.
72. The method of either Embodiment 66 or 71, wherein the insulin resistant disorder is type II diabetes.
73. The method of either Embodiment 66 or 71, wherein the insulin resistant disorder is obesity.
74. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54.
75. The method of Embodiment 74, wherein the administration is via craniofacial mucosal administration.
76. The method of Embodiment 75, wherein the craniofacial mucosal administration is intranasal administration.
77. The method of Embodiment 74, wherein the administration is via parenteral administration.
78. The method of Embodiment 74, wherein the administration is via enteral administration.
79. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer,

racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiments 51, or a pharmaceutical composition of any of Embodiments 52-54, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

80. The method of either Embodiment 74 or 79, wherein the feeding disorder is Prader-Willi syndrome.

81. The method of either Embodiment 74 or 79, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

82. The method of any of the Embodiments 55-81, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

83. The method of any of the Embodiments 59, 71, or 79, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.

84. The method of any of the Embodiments 59, 71 or 79, wherein the liquid formulation is contained in a device for intranasal administration.

85. The method of Embodiment 84, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

86. The method of Embodiment 84, wherein the device for intranasal administration is a nasal pump apparatus.

87. The method of Embodiment 86, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

88. The method of Embodiment 87, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

89. The method of Embodiment 88, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

90. The method of any one of Embodiments 87-90, wherein the nasal pump apparatus comprises one of more of the following:

i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

91. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5,

or a pharmaceutical composition of any one of Embodiments 6-8 for the manufacture of a medicament for treating a headache disorder in a subject.

92. The use of Embodiment 91, wherein the medicament is formulated for craniofacial mucosal administration.

93. The use of Embodiment 92, wherein the craniofacial mucosal administration is intranasal administration.

94. The use of Embodiment 91, wherein the medicament is formulated for parenteral administration.

95. The use of Embodiment 96, wherein the medicament is formulated as a liquid formulation.

96. The use of Embodiment 90, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

97. The use of Embodiment 91, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

98. The use of Embodiment 97, wherein the headache disorder is a migraine.

99. The use of Embodiment 97, wherein the headache disorder is a tension headache.

100. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8 for the manufacture of a medicament for treating an insulin resistant disorder in a subject.

101. The use of Embodiment 100, wherein the medicament is formulated for craniofacial mucosal administration.

102. The use of Embodiment 101, wherein the craniofacial mucosal administration is intranasal administration.

103. The use of Embodiment 100, wherein the medicament is formulated for parenteral administration.

104. The use of Embodiment 100, wherein the medicament is formulated as a liquid formulation.

105. The use of Embodiment 104, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

106. The use of Embodiment 100, wherein the insulin resistant disorder is type II diabetes.

107. The use of Embodiment 100, wherein the insulin resistant disorder is obesity.

108. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, or a pharmaceutical composition of any one of Embodiments 6-8 for the manufacture of a medicament for treating a feeding disorder with onset during neonate development in a subject.

109. The use of Embodiment 108, wherein the medicament is formulated for craniofacial mucosal administration.

110. The use of Embodiment 109, wherein the craniofacial mucosal administration is intranasal administration.

111. The use of Embodiment 108, wherein the medicament is formulated for parenteral administration.

112. The use of Embodiment 108, wherein the medicament is formulated as a liquid formulation.

113. The use of Embodiment 112, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

114. The use of Embodiment 108, wherein the feeding disorder is Prader-Willi syndrome.

115. The use of Embodiment 108, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

116. The use of any of the Embodiments 96, 105, or 113, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

117. The use of any of the Embodiments 87, 96, or 104, wherein the liquid formulation is contained in a device for intranasal administration.

118. The use of Embodiment 117, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

119. The use of Embodiment 117, wherein the device for intranasal administration is a nasal pump apparatus.

120. The use of Embodiment 119, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

121. The use of Embodiment 120, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

122. The use of Embodiment 121, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

123. The use of any one of Embodiments 121 to 122, wherein the nasal pump apparatus comprises one of more of the following:

i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

124. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 6-8 for the manufacture of a medicament for treating a headache disorder in a subject.

125. The use of Embodiment 124, wherein the medicament is formulated for craniofacial mucosal administration.

126. The use of Embodiment 125, wherein the craniofacial mucosal administration is intranasal administration.

127. The use of Embodiment 126, wherein the medicament is formulated for parenteral administration.

128. The use of Embodiment 124, wherein the medicament is formulated as a liquid formulation.

129. The use of Embodiment 128, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

130. The use of Embodiment 124, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

131. The use of Embodiment 124, wherein the headache disorder is a migraine.

132. The use of Embodiment 124, wherein the headache disorder is a tension headache.

133. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54 for the manufacture of a medicament for treating an insulin resistant disorder in a subject.

134. The use of Embodiment 133, wherein the medicament is formulated for craniofacial mucosal administration.

135. The use of Embodiment 134, wherein the craniofacial mucosal administration is intranasal administration.

136. The use of Embodiment 133, wherein the medicament is formulated for parenteral administration.

137. The use of Embodiment 133, wherein the medicament is formulated as a liquid formulation.

138. The use of Embodiment 137, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

139. The use of Embodiment 133, wherein the insulin resistant disorder is type II diabetes.

140. The use of Embodiment 133, wherein the insulin resistant disorder is obesity.

141. Use of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or a pharmaceutical composition of any one of Embodiments 52-54 for the manufacture of a medicament for treating a feeding disorder with onset during neonate development in a subject.

142. The use of Embodiment 141, wherein the medicament is formulated for craniofacial mucosal administration.

143. The use of Embodiment 143, wherein the craniofacial mucosal administration is intranasal administration.

144. The use of Embodiment 141, wherein the medicament is formulated for parenteral administration.

145. The use of Embodiment 141, wherein the medicament is formulated as a liquid formulation.

146. The use of Embodiment 145, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

147. The use of Embodiment 141, wherein the feeding disorder is Prader-Willi syndrome.

148. The use of Embodiment 141, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

149. The use of any of the Embodiments 129, 138, or 146, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

150. The use of any of the Embodiments 129, 138, or 146, wherein the liquid formulation is contained in a device for intranasal administration.

151. The use of Embodiment 150, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

152. The use of Embodiment 150, wherein the device for intranasal administration is a nasal pump apparatus.

153. The use of Embodiment 152, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

154. The use of Embodiment 153, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

155. The use of Embodiment 154, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

156. The use of any one of Embodiments 152 to 155, wherein the nasal pump apparatus comprises one of more of the following:

i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

157. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5 for use in treating a headache disorder in a subject.

158. The compound for use of Embodiment 157, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

159. The compound for use of Embodiment 158, wherein the craniofacial mucosal administration is intranasal administration.

160. The compound for use of Embodiment 157, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

161. The compound for use of Embodiment 158, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

162. The compound for use of Embodiment 161, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

163. The compound for use of Embodiment 157, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

164. The compound for use of Embodiment 163, wherein the headache disorder is a migraine.

165. The compound for use of Embodiment 163, wherein the headache disorder is a tension headache.

166. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, for use in treating an insulin resistant disorder in a subject.

167. The compound for use of Embodiment 166, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

168. The compound for use of Embodiment 167, wherein the craniofacial mucosal administration is intranasal administration.

169. The compound for use of Embodiment 166, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

170. The compound for use of Embodiment 166, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

171. The compound for use of Embodiment 170, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

172. The compound for use of Embodiment 166, wherein the insulin resistant disorder is type II diabetes.

173. The compound for use of Embodiment 166, wherein the insulin resistant disorder is obesity.

174. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5, for use in treating a feeding disorder with onset during neonate development in a subject.

175. The compound for use of Embodiment 174, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

176. The compound for use of Embodiment 175, wherein the craniofacial mucosal administration is intranasal administration.

177. The compound for use of Embodiment 174, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

178. The compound for use of Embodiment 176, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

179. The compound for use of Embodiment 178, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

180. The compound for use of Embodiment 174, wherein the feeding disorder is Prader-Willi syndrome.

181. The compound for use of Embodiment 174, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

182. The compound for use of any of the Embodiments 162, 171, or 179, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

183. The compound for use of any of the Embodiments 162, 171, or 179, wherein the liquid formulation is contained in a device for intranasal administration.

184. The compound for use of Embodiment 183, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

185. The compound for use of Embodiment 183, wherein the device for intranasal administration is a nasal pump apparatus.

186. The compound for use of Embodiment 185, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

187. The use of Embodiment 186, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

188. The use of Embodiment 185, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

189. The use of any one of Embodiments 185 to 188, wherein the nasal pump apparatus comprises one of more of the following:

i. a filter for preventing back flow,

ii. a metal-tree fluid path, and

iii. a plastic material stable to gamma-radiation.

190. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51 for use in treating a headache disorder in a subject.

191. The compound for use of Embodiment 190, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

192. The compound for use of Embodiment 191, wherein the craniofacial mucosal administration is intranasal administration.

193. The compound for use of Embodiment 190, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

194. The compound for use of Embodiment 193, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

195. The compound for use of Embodiment 194, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

196. The compound for use of Embodiment 190, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

197. The compound for use of Embodiment 196, wherein the headache disorder is a migraine.

198. The compound for use of Embodiment 196, wherein the headache disorder is a tension headache.

199. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, for use in treating an insulin resistant disorder in a subject.

200. The compound for use of Embodiment 199, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

201. The compound for use of Embodiment 200, wherein the craniofacial mucosal administration is intranasal administration.

202. The compound for use of Embodiment 199, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

203. The compound for use of Embodiment 200, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

204. The compound for use of Embodiment 201, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

205. The compound for use of Embodiment 199, wherein the insulin resistant disorder is type II diabetes.

206. The compound for use of Embodiment 199, wherein the insulin resistant disorder is obesity.

207. A compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, for use in treating a feeding disorder with onset during neonate development in a subject.

208. The compound for use of Embodiment 207, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

209. The compound for use of Embodiment 208, wherein the craniofacial mucosal administration is intranasal administration.

210. The compound for use of Embodiment 207, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

211. The compound for use of Embodiment 200, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

212. The compound for use of Embodiment 211, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

213. The compound for use of Embodiment 207, wherein the feeding disorder is Prader-Willi syndrome.

214. The compound for use of Embodiment 207, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

215. The compound for use of any of the Embodiments 195, 204, or 212, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

216. The compound for use of any of the Embodiments 195, 204, or 212, wherein the liquid formulation is contained in a device for intranasal administration.

217. The compound for use of Embodiment 216, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

218. The compound for use of Embodiment 216, wherein the device for intranasal administration is a nasal pump apparatus.

219. The compound for use of Embodiment 218, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

220. The use of Embodiment 219, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

221. The use of Embodiment 220, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

222. The use of any one of Embodiments 218 to 221, wherein the nasal pump apparatus comprises one of more of the following:

i. a filter for preventing back flow,

ii. a metal-tree fluid path, and

iii. a plastic material stable to gamma-radiation.

223. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate

thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

224. The method of Embodiment 223, wherein the administration is via craniofacial mucosal administration.

225. The method of Embodiment 224, wherein the craniofacial mucosal administration is intranasal administration.

226. The method of Embodiment 223, wherein the administration is via parenteral administration.

227. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

228. The method of either Embodiment 223 or 227, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

229. The method of Embodiment 228, wherein the headache disorder is a migraine.

230. The method of Embodiment 228, wherein the headache disorder is a tension headache.

231. The method of Embodiment 228, wherein the headache disorder is a cluster headache.

232. The method of Embodiment 228, wherein the headache disorder is trigeminal neuralgia.

233. The method of Embodiment 228, wherein the headache disorder is a secondary headache.

234. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

235. The method of Embodiment 234, wherein administration is via craniofacial mucosal administration.

236. The method of Embodiment 235, wherein the craniofacial mucosal administration is intranasal administration.

237. The method of Embodiment 234, wherein the administration is via parenteral administration.

238. The method of Embodiment 234, wherein the administration is via enteral administration.

239. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

240. The method of either Embodiment 234 or 239, wherein the insulin resistant disorder is type II diabetes.

241. The method of either Embodiment 234 or 239, wherein the insulin resistant disorder is obesity.

242. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them or a pharmaceutical composition comprising any of them.

243. The method of Embodiment 242, wherein the administration is via craniofacial mucosal administration.

244. The method of Embodiment 243, wherein the craniofacial mucosal administration is intranasal administration.

245. The method of Embodiment 242, wherein the administration is via parenteral administration.

246. The method of Embodiment 243, wherein the administration is via enteral administration.

247. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

248. The method of either Embodiment 242 or 247, wherein the feeding disorder is Prader-Willi syndrome.

249. The method of either Embodiment 242 or 247, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

250. The method of any of the Embodiments 223 to 249, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

251. The method of any of the Embodiments 227, 239, or 247, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.

252. The method of any of the Embodiments 227, 239, or 247, wherein the liquid formulation is contained in a device for intranasal administration.

253. The method of Embodiment 252, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

254. The method of Embodiment 253, wherein the device for intranasal administration is a nasal pump apparatus.

255. The method of Embodiment 254, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

256. The method of Embodiment 255, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

257. The method of Embodiment 252, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

258. The method of any one of Embodiments 254-257, wherein the nasal pump apparatus comprises one of more of the following:

i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

259. Use of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for the manufacture of a medicament for treating a headache disorder in a subject.

260. The use of Embodiment 259, wherein the medicament is formulated for craniofacial mucosal administration.

261. The use of Embodiment 260, wherein the craniofacial mucosal administration is intranasal administration.

262. The use of Embodiment 259, wherein the medicament is formulated for parenteral administration.

263. The use of Embodiment 259, wherein the medicament is formulated as a liquid formulation.

264. The use of Embodiment 263, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

265. The use of Embodiment 259, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

266. The use of Embodiment 259, wherein the headache disorder is a migraine.

267. The use of Embodiment 259, wherein the headache disorder is a tension headache.

268. Use of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for the manufacture of a medicament for treating an insulin resistant disorder in a subject.

269. The use of Embodiment 268, wherein the medicament is formulated for craniofacial mucosal administration.

270. The use of Embodiment 269, wherein the craniofacial mucosal administration is intranasal administration.

271. The use of Embodiment 268, wherein the medicament is formulated for parenteral administration.

272. The use of Embodiment 268, wherein the medicament is formulated as a liquid formulation.

273. The use of Embodiment 272, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

274. The use of Embodiment 268, wherein the insulin resistant disorder is type II diabetes.

275. The use of Embodiment 268, wherein the insulin resistant disorder is obesity.

276. Use of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for the manufacture of a medicament for treating a feeding disorder with onset during neonate development in a subject.

277. The use of Embodiment 276, wherein the medicament is formulated for craniofacial mucosal administration.

278. The use of Embodiment 277, wherein the craniofacial mucosal administration is intranasal administration.

279. The use of Embodiment 276, wherein the medicament is formulated for parenteral administration.

280. The use of Embodiment 276, wherein the medicament is formulated as a liquid formulation.

281. The use of Embodiment 280, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

282. The use of Embodiment 276, wherein the feeding disorder is Prader-Willi syndrome.

283. The use of Embodiment 276, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

284. The use of any of the Embodiments 268, 273, or 281, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

285. The use of any of the Embodiments 268, 273, or 281, wherein the liquid formulation is contained in a device for intranasal administration.

286. The use of Embodiment 285, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

287. The use of Embodiment 285, wherein the device for intranasal administration is a nasal pump apparatus.

288. The use of Embodiment 287, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

289. The use of Embodiment 288, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

290. The use of Embodiment 287, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

291. The use of any one of Embodiments 287 to 290, wherein the nasal pump apparatus comprises one of more of the following:

i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

292. A compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for use in treating a headache disorder in a subject.

293. The compound for use of Embodiment 292, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

294. The compound for use of Embodiment 293, wherein the craniofacial mucosal administration is intranasal administration.

295. The compound for use of Embodiment 292, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

296. The compound for use of Embodiment 292, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

297. The compound for use of Embodiment 296, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

298. The compound for use of Embodiment 292, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

299. The compound for use of Embodiment 298, wherein the headache disorder is a migraine.

300. The compound for use of Embodiment 298, wherein the headache disorder is a tension headache.

301. A compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for use in treating an insulin resistant disorder in a subject.

302. The compound for use of Embodiment 301, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

303. The compound for use of Embodiment 302, wherein the craniofacial mucosal administration is intranasal administration.

304. The compound for use of Embodiment 301, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

305. The compound for use of Embodiment 301, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

306. The compound for use of Embodiment 302, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

307. The compound for use of Embodiment 301, wherein the insulin resistant disorder is type II diabetes.

308. The compound for use of Embodiment 301, wherein the insulin resistant disorder is obesity.

309. A compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, for use in treating a feeding disorder with onset during neonate development in a subject.

310. The compound for use of Embodiment 309, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for craniofacial mucosal administration.

311. The compound for use of Embodiment 310, wherein the craniofacial mucosal administration is intranasal administration.

312. The compound for use of Embodiment 309, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated for parenteral administration.

313. The compound for use of Embodiment 309, wherein the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, is formulated as a liquid formulation.

314. The compound for use of Embodiment 313, wherein the volume of the liquid formulation is between about 5 μL and about 1000 μL.

315. The compound for use of Embodiment 309, wherein the feeding disorder is Prader-Willi syndrome.

316. The compound for use of Embodiment 309, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

317. The compound for use of any of the Embodiments 297, 306, or 314, wherein the volume of the liquid formulation is between about 50 μL and about 200 μL.

318. The compound for use of any of the Embodiments 297, 306, or 314, wherein the liquid formulation is contained in a device for intranasal administration.

319. The compound for use of Embodiment 318, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

320. The compound for use of Embodiment 318, wherein the device for intranasal administration is a nasal pump apparatus.

321. The compound for use of Embodiment 320, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

322. The compound for use of Embodiment 321, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

323. The compound for use of Embodiment 322, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

324. The compound for use of any one of Embodiments 320 to 323, wherein the nasal pump apparatus comprises one of more of the following:

i. a filter for preventing back flow,

ii. a metal-tree fluid path, and

iii. a plastic material stable to gamma-radiation.

325. The use of any one of Embodiments 91 to 115, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

326. The use of any one of Embodiments 124 to 148, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

327. The use of any one of Embodiments 259 to 283, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

328. A compound for the use of any one of Embodiments 157 to 181, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

329. A compound for the use of any one of Embodiments 190 to 214, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

330. A compound for the use of any one of Embodiments 292 to 316, wherein the therapeutically effective dose of the compound, or an enantiomer, racemate, or tautomer hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

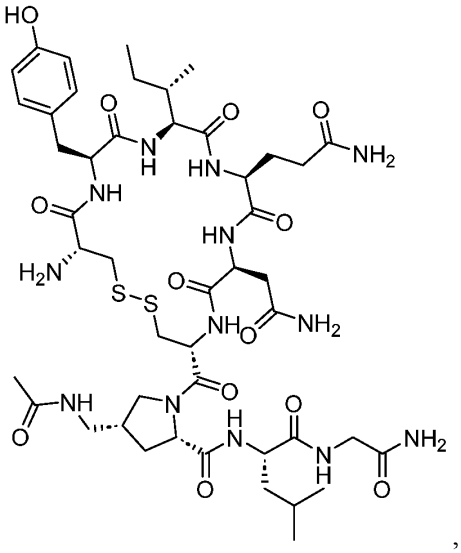
331. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, of any one of Embodiments 1-5 or the pharmaceutical composition of any one of Embodiments 6-8.

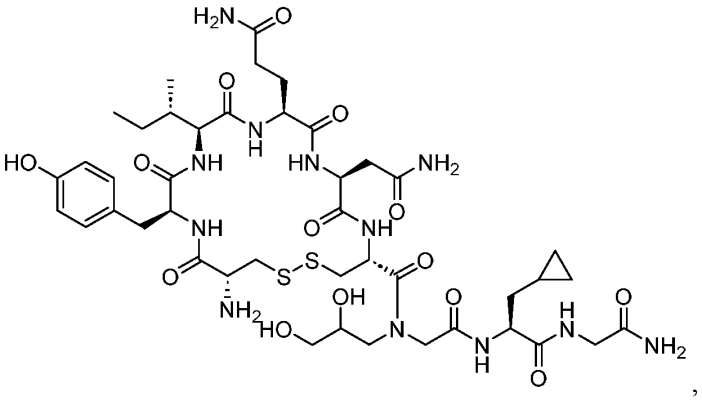
332. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

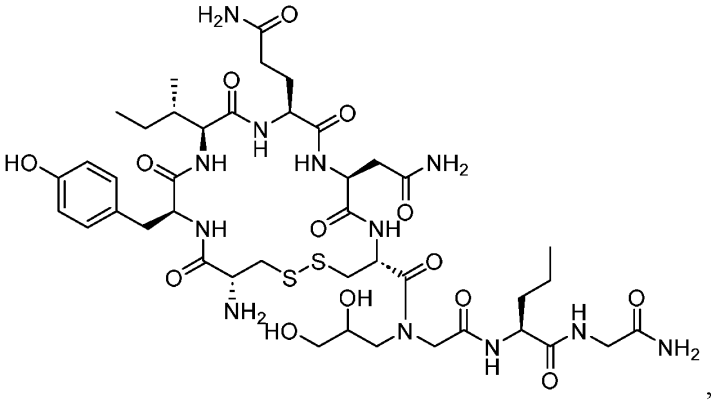
333. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of Embodiment 51, or the pharmaceutical composition of any one of Embodiments 52-54.

CLAIMS

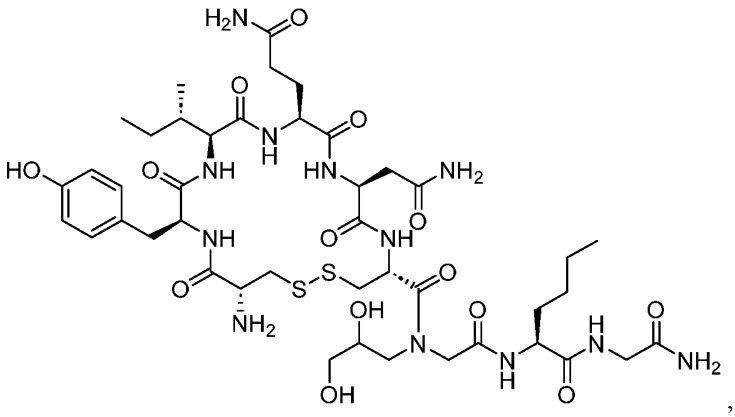
1. A compound selected from the group consisting of:

Compound 1 ,

Compound 2 ,

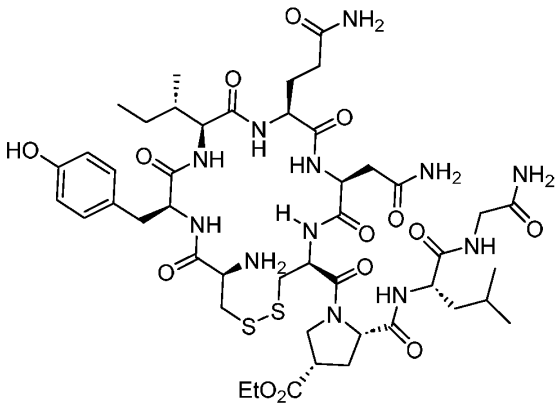
Compound 3 ,

Compound 4



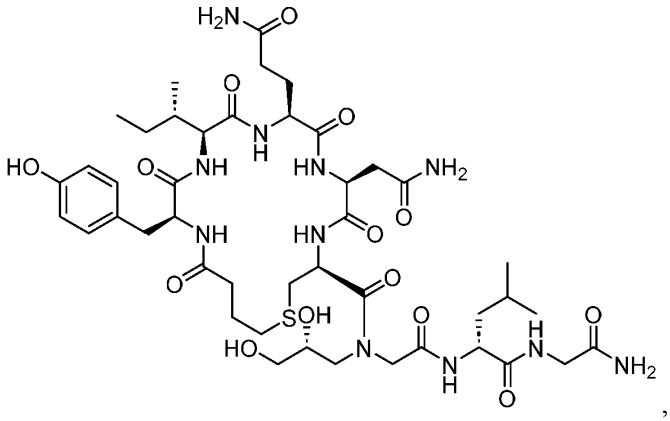
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Compound 5



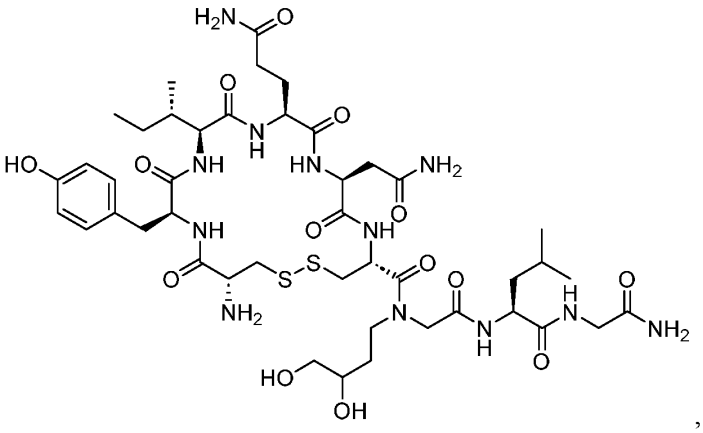
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Compound 6



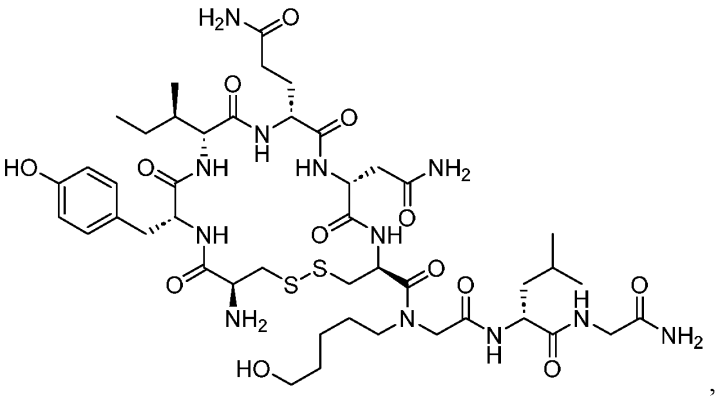
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Compound 7



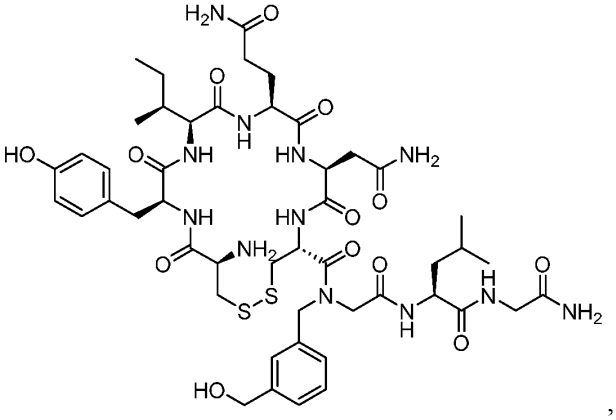
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Compound 8

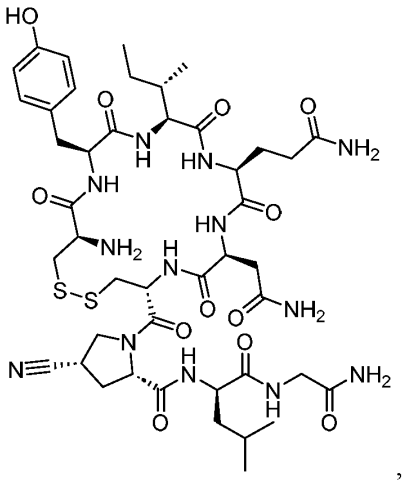


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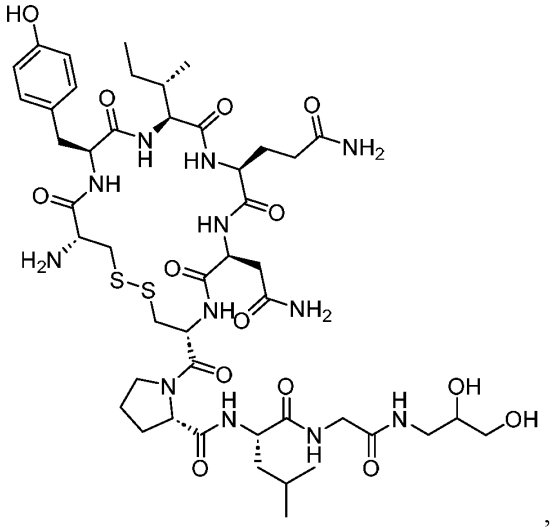
Compound 9



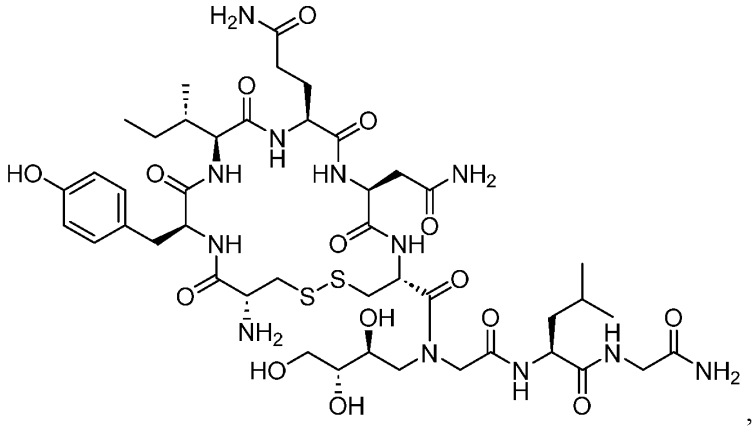
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Compound 10 

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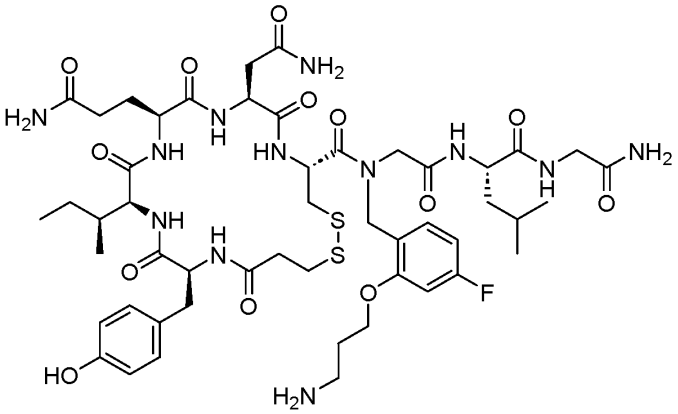
Compound 11 

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Compound 12 

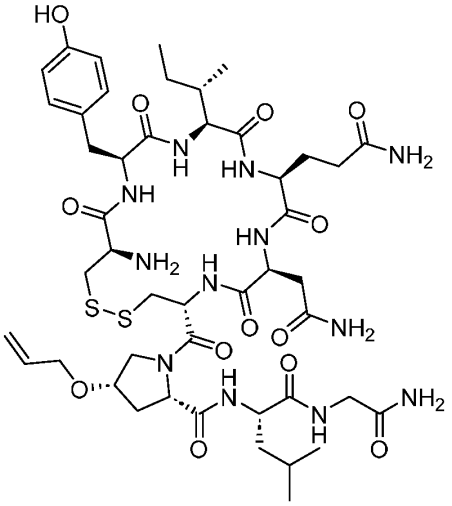
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Compound 13



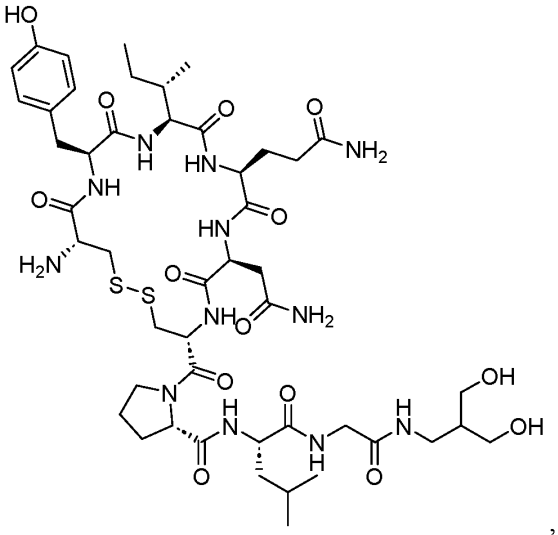
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Compound 14



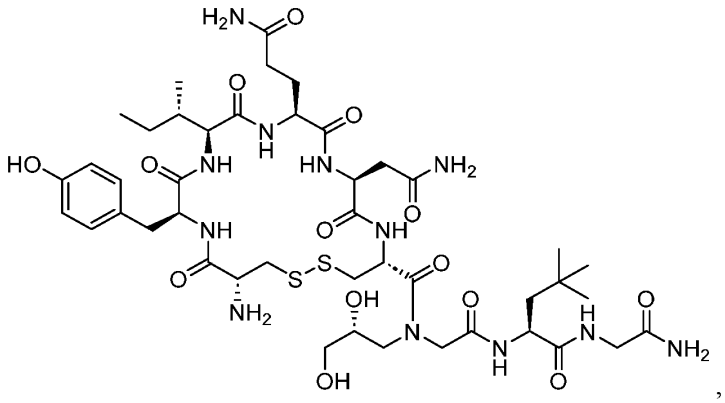
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Compound 15



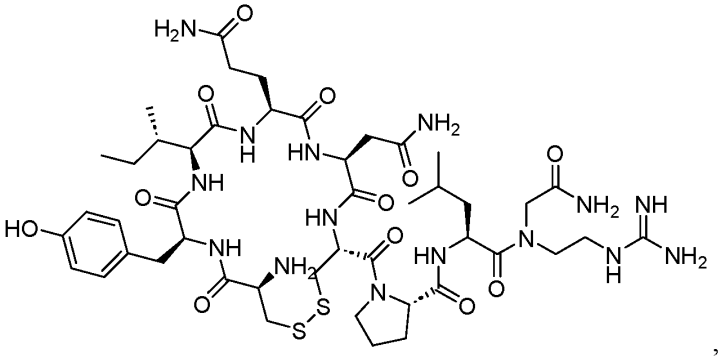
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Compound 16



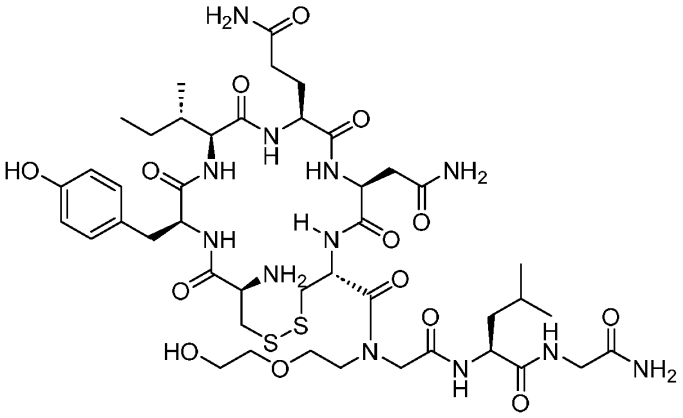
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Compound 17

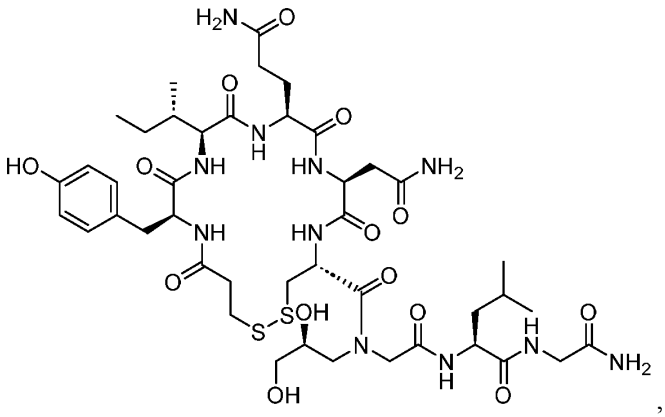


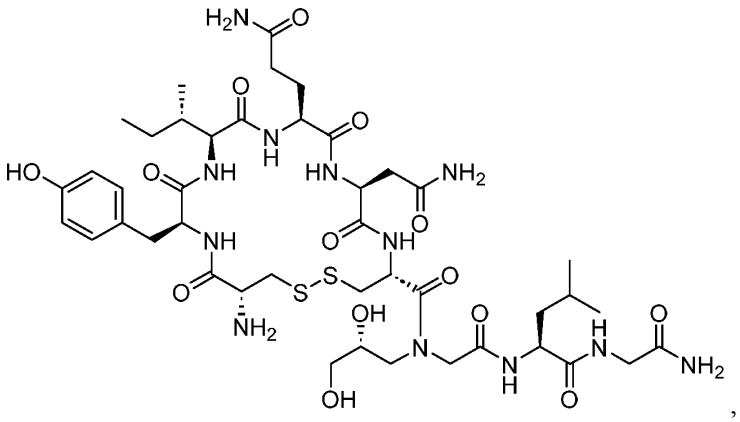
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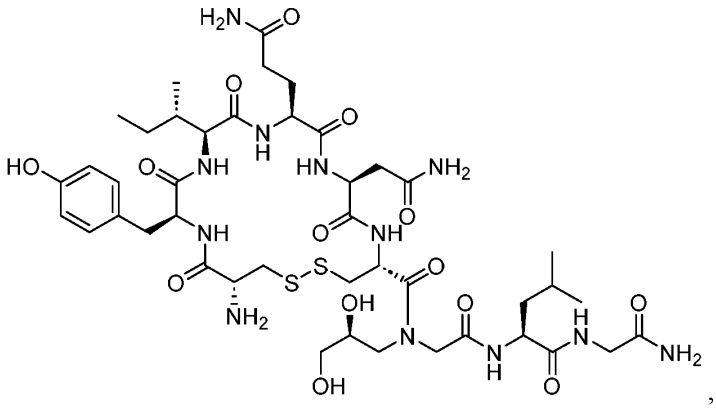
Compound 18



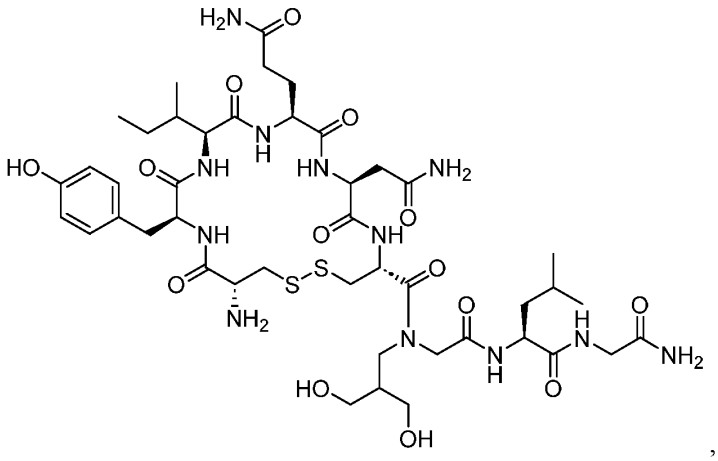
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Compound 19 ,

Compound 20 ,

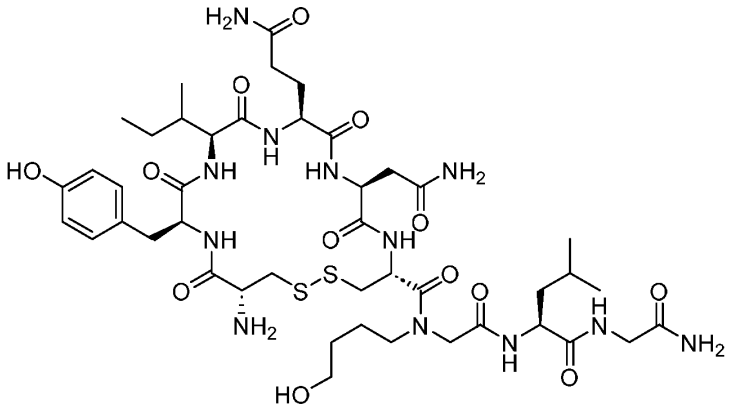
Compound 21 ,

Compound 22



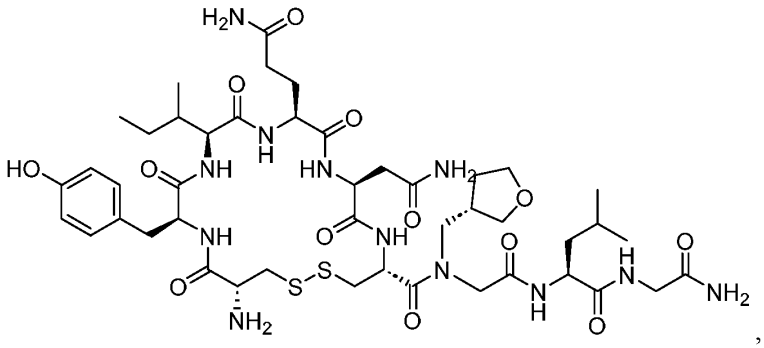
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Compound 23



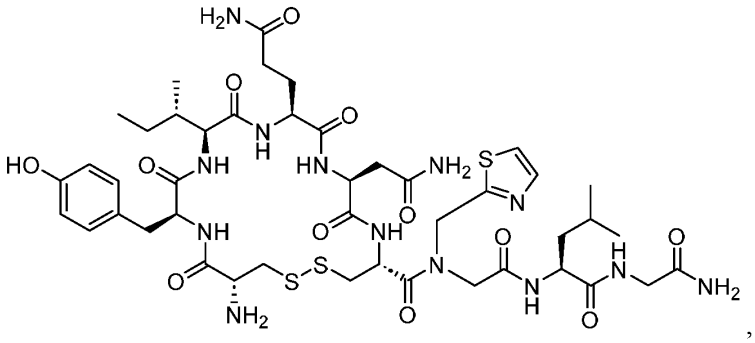
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Compound 24



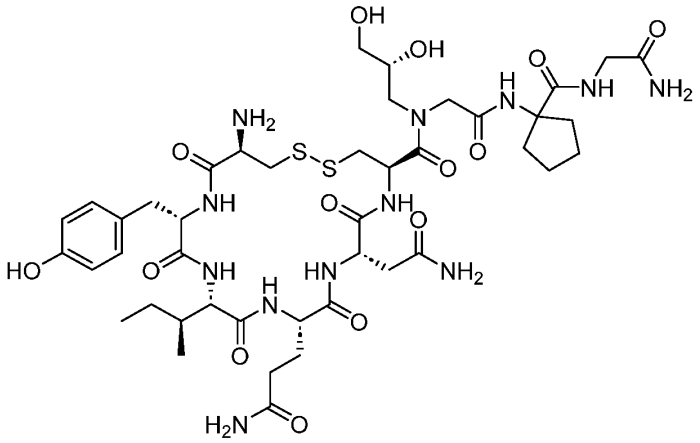
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Compound 25



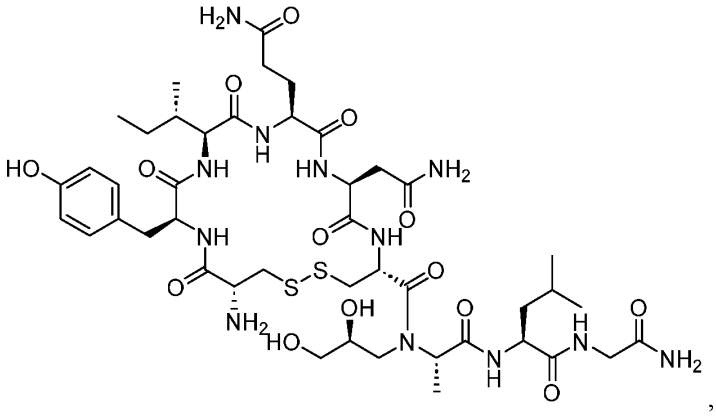
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Compound 26



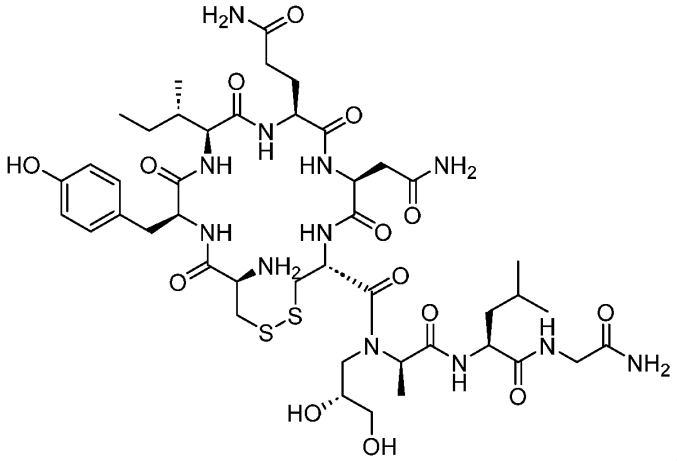
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Compound 27



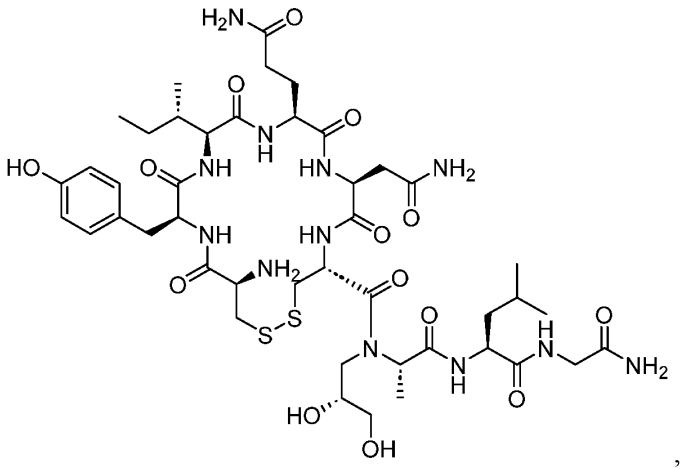
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Compound 28



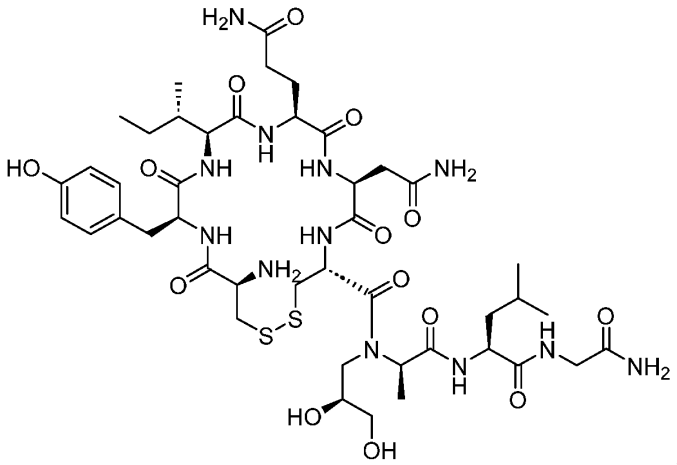
,

Compound 29



, and

Compound 30

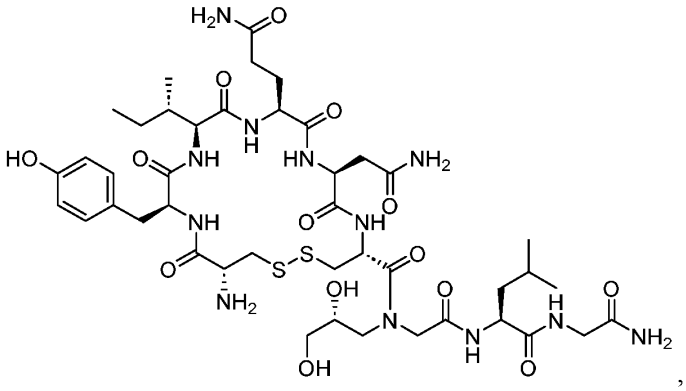


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or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them.

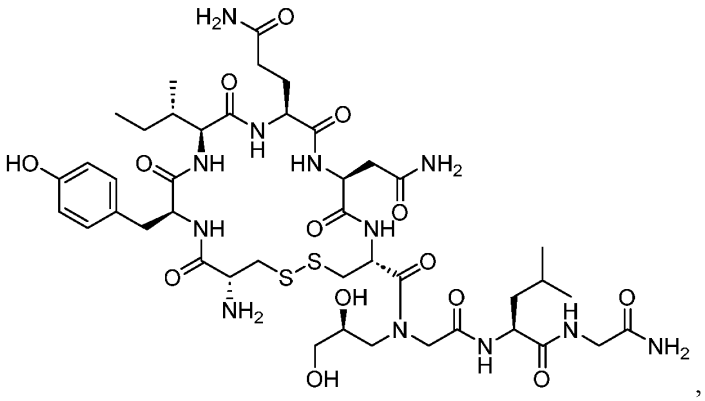
2. The compound of claim 1, wherein the compound is selected from the group consisting of:

Compound 20



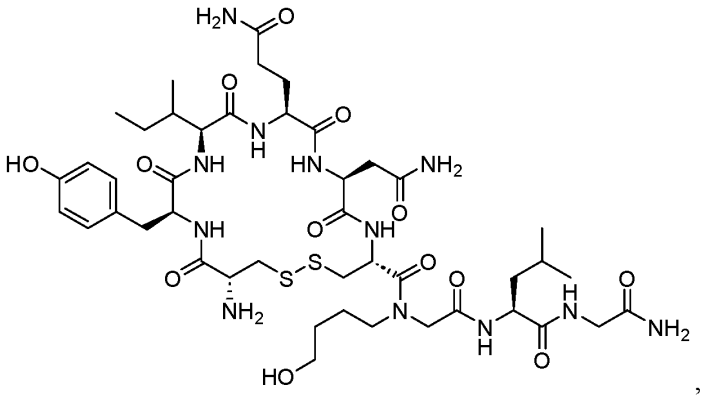
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Compound 21



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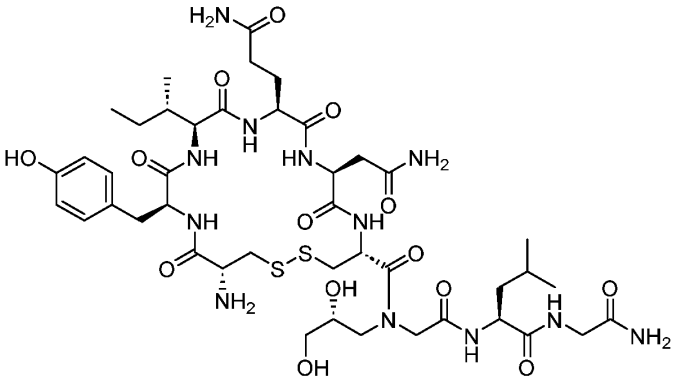
Compound 23



,

or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them.

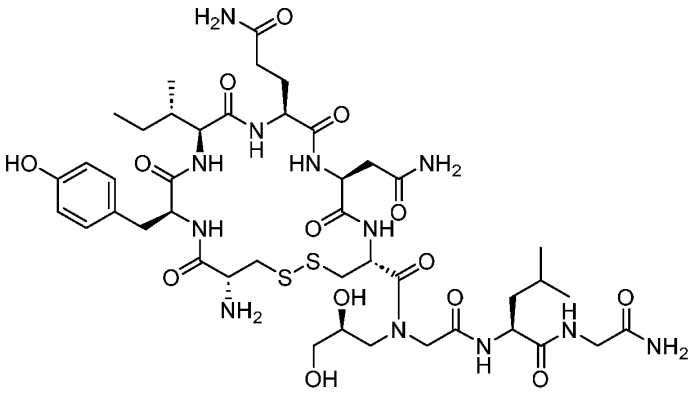
3. The compound of claim 1, wherein the compound is Compound 20:



Compound 20,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

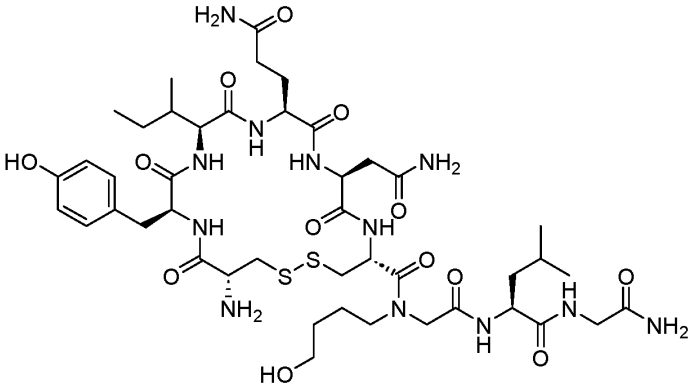
4. The compound of claim 1, wherein the compound is Compound 21:



Compound 21,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

5. The compound of claim 1, wherein the compound is Compound 23:



Compound 23,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them.

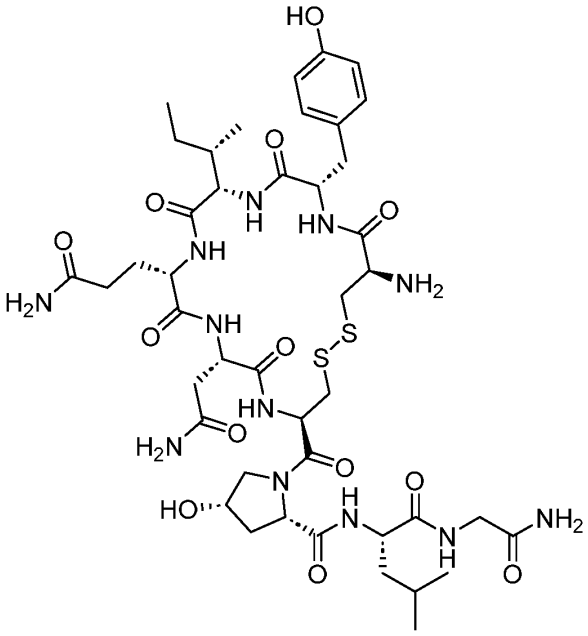
6. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to any one of the claims 1-5 and a pharmaceutically acceptable carrier.

7. The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is a liquid formulation.

8. The pharmaceutical composition of claim 7, wherein the liquid formulation is an aqueous solution.

9. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of claims 1-5, or the pharmaceutical composition of any one of claims 6-8.

10. A method for treating a headache disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B,



Compound B,

or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

11. The method of claim 9 or 10, wherein the administration is via craniofacial mucosal administration.

12. The method of claim 11, wherein the craniofacial mucosal administration is intranasal administration.

13. The method of claim 9 or 10, wherein administration is via parenteral administration.

14. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of claims 1-5, or a pharmaceutical composition of any one of claims 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

15. A method for treating a headache disorder comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a

pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

16. The method of either claim 9, 10, 14 or 15, wherein the headache disorder is selected from the group consisting of a migraine, tension headache, cluster headache, trigeminal neuralgia, and secondary headache.

17. The method of claim 16, wherein the headache disorder is a migraine.

18. The method of claim 16, wherein the headache disorder is a tension headache.

19. The method of claim 16, wherein the headache disorder is a cluster headache.

20. The method of claim 16, wherein the headache disorder is trigeminal neuralgia.

21. The method of claim 16, wherein the headache disorder is a secondary headache.

22. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of claims 1-5, or a pharmaceutical composition of any one of claims 6-8.

23. A method for treating an insulin resistant disorder comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

24. The method of claim 22 or 23, wherein the administration is via craniofacial mucosal administration.

25. The method of claim 24, wherein the craniofacial mucosal administration is intranasal administration.

26. The method of claim 22 or 23, wherein compound or the pharmaceutical composition is administered via parenteral administration.

27. The method of claim 22 or 23, wherein the administration is via enteral administration.

28. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of claims 1-5, or a pharmaceutical composition of any one of claim 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

29. A method for treating an insulin resistant disorder comprising administering daily, parenterally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

30. The method of either claim 22, 23, 28 or 29, wherein the insulin resistant disorder is type II diabetes.

31. The method of either claim 22, 23, 28 or 29, wherein the insulin resistant disorder is obesity.

32. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of any one of claims 1-5, or a pharmaceutical composition of any one of claims 6-8.

33. A method for treating a feeding disorder with onset during neonate development comprising administering to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

34. The method of claim 32 or 33, wherein the administration is via craniofacial mucosal administration.

35. The method of claim 340, wherein the craniofacial mucosal administration is intranasal administration.

36. The method of claim 32 or 33, wherein the administration is via parenteral administration.

37. The method of claim 32 or 33, wherein the administration is via enteral administration.

38. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, of

any one of claims 1-5, or a pharmaceutical composition of any of claims 6-8, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

39. A method for treating a feeding disorder with onset during neonate development comprising administering daily, intranasally in a liquid formulation, to a subject in need thereof, a therapeutically effective dose of a compound, wherein the compound is Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them, wherein the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

40. The method of either claim 32, 33, 38 or 39, wherein the feeding disorder is Prader-Willi syndrome.

41. The method of either claim 32, 33, 38 or 39, wherein the feeding disorder is Non-Organic Failure To Thrive (NOFITT).

42. The method of any of the claims 9-41, wherein the therapeutically effective dose of the compound, an enantiomer, racemate, tautomer, hydrate, or solvate thereof, is about 0.5 μg to about 2000 μg.

43. The method of any of the claims 14, 15, 28, 29, 38 or 39, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.

44. The method of any of the claims 14, 15, 28, 29, 38 or 39, wherein the liquid formulation is contained in a device for intranasal administration.

45. The method of claim 44, wherein the device for intranasal administration is capable of administering the liquid formulation in 1 to 4 units of about 50 μL per unit.

46. The method of claim 44, wherein the device for intranasal administration is a nasal pump apparatus.

47. The method of claim 46, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

48. The method of claim 47, wherein the pump actuator is metered to deliver a specified volume of about 50 μL.

49. The method of claim 48, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

50. The method of any one of claims 46-49, wherein the nasal pump apparatus comprises one of more of the following:

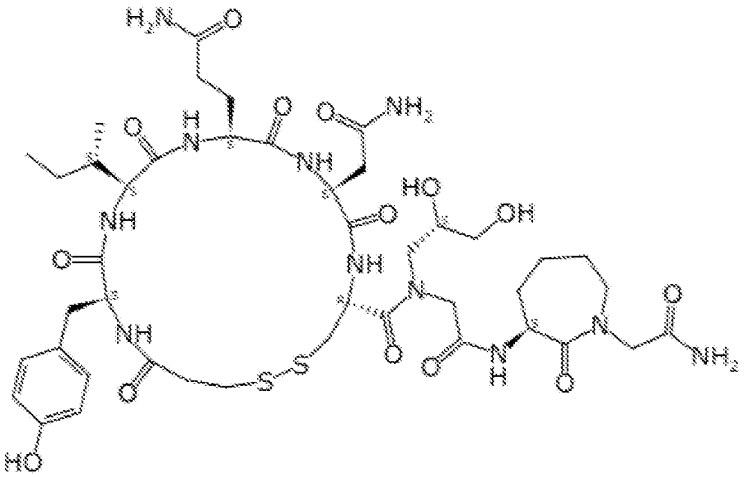
i) a filter for preventing back flow,

ii) a metal-tree fluid path, and

iii) a plastic material stable to gamma-radiation.

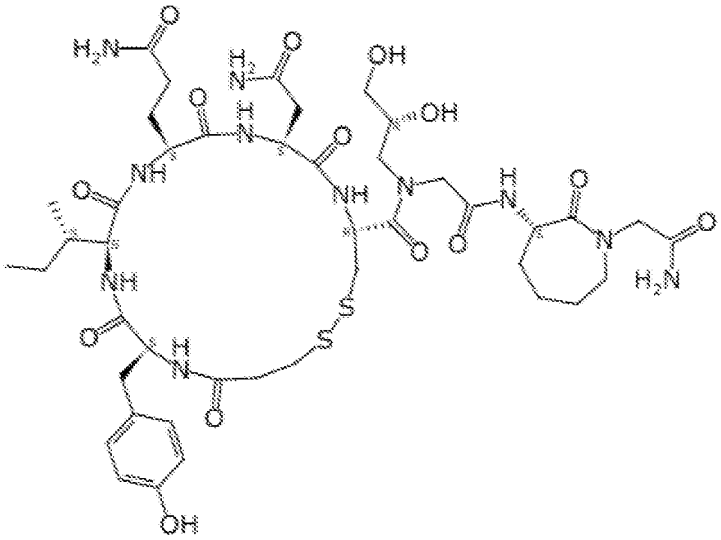
51. A compound selected from the group consisting of:

Compound 31



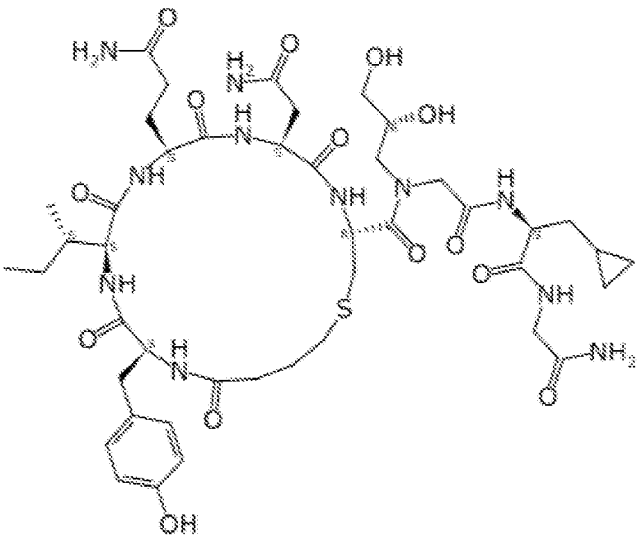
,

Compound 32



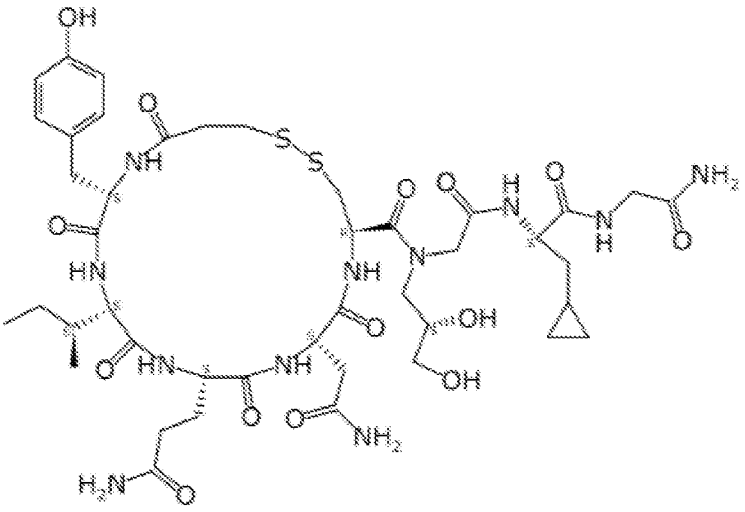
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Compound 33



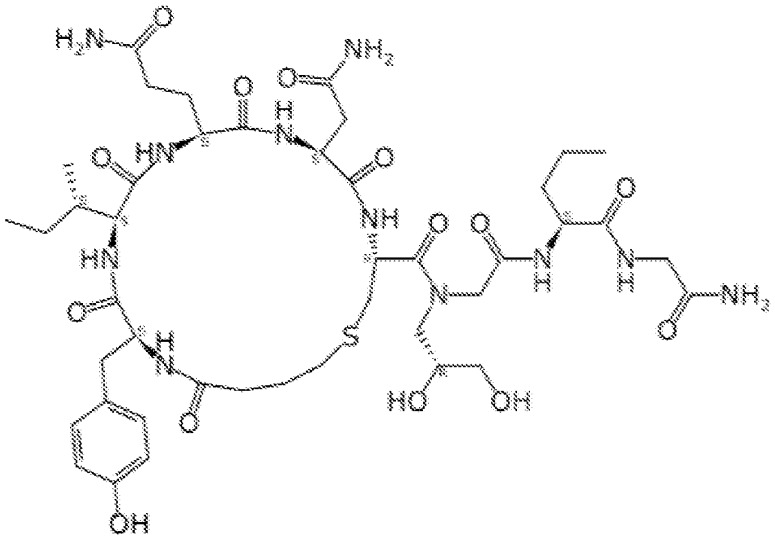
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Compound 34



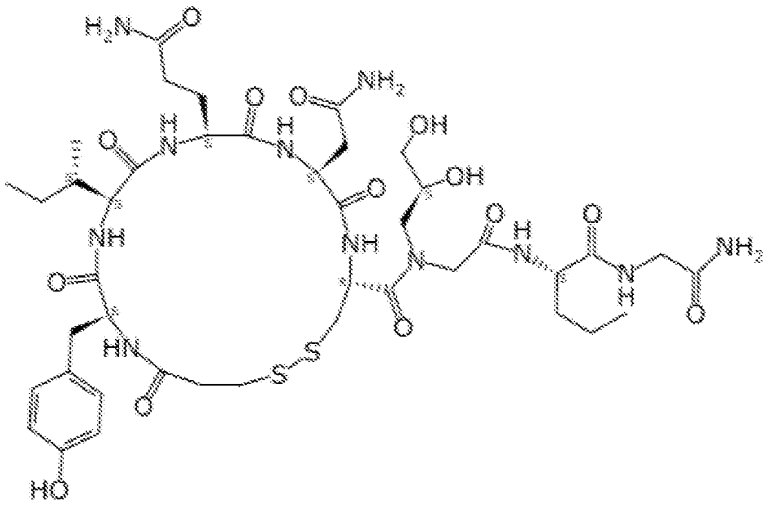
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Compound 35



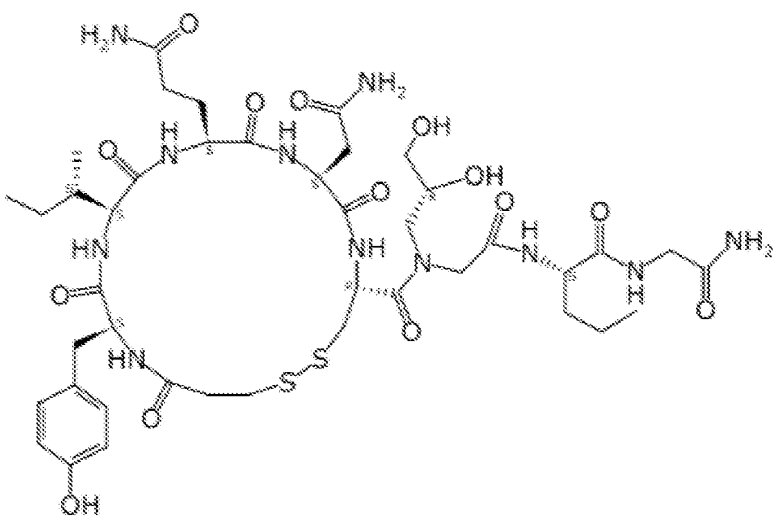
,

Compound 36



,

Compound 37



,

or an enantiomer, racemate, tautomer, hydrate, or solvate of any of them, or a pharmaceutically acceptable salt of any of them.

52. A pharmaceutical composition comprising a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, or a pharmaceutically acceptable salt of any of them, according to claim 51 and a pharmaceutically acceptable carrier.

53. The pharmaceutical composition of claim 52, wherein the pharmaceutical composition is a liquid formulation.

54. The pharmaceutical composition of claim 53, wherein the liquid formulation is an aqueous solution.

55. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of the compound, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, of any one of claims 1-5 or the pharmaceutical composition of any one of claims 6-8.

56. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a Compound B, or an enantiomer, racemate, tautomer, hydrate, or solvate thereof, a pharmaceutically acceptable salt of any of them, or a pharmaceutical composition comprising any of them.

57. A method for reducing the impact of magnesium ions (Mg^{+2}) concentration on the oxytocin-oxytocin receptor binding efficacy comprising administering to a subject an effective amount of a compound, or an enantiomer, racemate, tautomer, hydrate, or solvate

thereof, or a pharmaceutically acceptable salt of any of them, of claim 51, or the pharmaceutical composition of any one of claims 52-54.