

CONOTOXIN PEPTIDE ANALOGS AND USES FOR THE TREATMENT OF PAIN AND INFLAMMATORY CONDITIONS

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] This application incorporates by reference a Sequence Listing submitted with this application as a text file entitled “SEQ_LISTING_14520-002-228.txt” created on January 3, 2019 and having a size of 66,707 bytes.

1. FIELD

[0002] Provided herein are alpha-conotoxin peptide analogs, including alpha-conotoxin peptide analogs that are covalently attached to polyethylene glycol (PEG), and pharmaceutical compositions of such alpha-conotoxin peptide analogs. Also provided herein are methods of treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) (e.g., the $\alpha 9\alpha 10$ subtype of the nAChR) in a subject.

2. BACKGROUND

[0003] Predatory marine snails in the genus *Conus* have venoms that are rich in neuropharmacologically active peptides (conotoxin peptides or conotoxins) (Olivera et al., 1990, *Science* 249:257-263). There are approximately 500 species in *Conus*, and among those that have been examined so far, a conserved feature is the presence of alpha-conotoxin peptides in their venom. Native alpha-conotoxin peptides are highly disulfide cross-linked peptides with a C1-C3 and C2-C4 disulfide bridge pattern (Azam and McIntosh, 2009, *Acta Pharmacol. Sin.* 30:771-783).

[0004] Alpha-conotoxin peptides have generally been shown to be nicotinic acetylcholine receptor (nAChR) antagonists (Nicke et al., 2004, *Eur. J. Biochem.* 271:2305-2319). nAChRs are pentameric ligand-gated ion channels assembled from one or more α subunits ($\alpha 1$ - $\alpha 10$) either alone or together with one or more non- α subunits ($\beta 1$ - $\beta 4$) (Sine and Engel, 2006, *Nature* 440:448-455).

[0005] The $\alpha 9\alpha 10$ nAChR subunits are expressed in diverse tissues. In the inner ear, $\alpha 9\alpha 10$ nAChRs mediate synaptic transmission between efferent olivocochlear fibers and cochlear hair

cells (Vetter et al., 1999, Neuron 23:93-103). The $\alpha 9\alpha 10$ subunits are also found in dorsal root ganglion neurons (Lips et al., 2002, Neuroscience 115:1-5), lymphocytes (Peng et al., 2004, Life. Sci. 76:263-280), skin keratinocytes (Nguyen et al., 2000, Am. J. Pathol. 157:1377-1391), and the pars tuberalis of the pituitary (Elgoyhen et al., 1994, Cell 79:705-715; Zuo et al., 1999, Proc. Natl. Acad. Sci. USA 96:14100-14105).

[0006] Compounds that have a pharmacological profile that includes $\alpha 9\alpha 10$ antagonist activity prevent or attenuate the expression of pain in several rodent models, including neuropathic pain induced by chemotherapy, traumatic nerve injury, and diabetes (see Hone and McIntosh, 2018, FEBS Lett. 592:1045-1062).

[0007] Native conotoxin peptide RgIA has the amino acid sequence Gly-Cys-Cys-Ser-Asp-Pro-Arg-Cys-Arg-Tyr-Arg-Cys-Arg (SEQ ID NO:1) (Ellison et al., 2008, J. Mol. Biol. 377:1216-1227). Alpha-conotoxin peptide RgIA has been shown to block $\alpha 9\alpha 10$ nAChR activity (Romero et al., 2017, Proc. Natl. Acad. Sci. USA 14:E1825-E1832).

[0008] RgIA belongs to the α -4/3 family of α -conotoxins. The native structure of RgIA is characterized by two compact intra-cysteine loops defined by two disulfide bonds formed between Cys2-Cys8 (sometimes referred CysI-CysIII) and Cys3-Cys12 (sometimes referred to CysII-CysIV) (Ellison et al, 2008, J. Mol. Biol. 377:1216-1227; Armishaw, 2010, Toxins 2:1471-1499). In the α -conotoxins, the first and second cysteine residues are always adjacent, but the number of amino acid residues between the second and third cysteine, and between the third and fourth cysteine residues can vary. This gives rise to two loops of intervening amino acids denoted loop 1 or the m-loop and loop 2 or the n-loop. In RgIA, loop 1 contains 4 amino acids and loop 2 contains 3 amino acids. The disulfide bonds and therefore the secondary structure of α -conotoxins are unstable and subject to rearrangement (Armishaw, 2010, Toxins 2:1471-1499).

[0009] The two disulfide bonds of the α -cono peptide are subject to rearrangement and can form alternative three-dimensional structures including a ribbon form (disulfide bonds Cys-2-Cys12 and Cys3-Cys8) and a bead form (disulfide bonds Cys2-Cys3 and Cys8-Cys12). While the native or globular form of the peptide is active on the $\alpha 9\alpha 10$ -nAChR, neither the ribbon or bead form are thought to be active (Dekan et al., 2011, J. Am. Chem. Soc. 133:15866-15869;

Armishaw et al., 2006, J. Bio. Chem. 281:14136-14143).

[0010] Hargittai et al. evaluated four lactam-bridged derivatives of the alpha-conotoxin SI, and only one of the four lactam-bridged derivatives did not lose significant activity in binding to BC3H1 cells (Hargittai et al., 2000, J. Med. Chem. 43:4787-4792). Bondebjerg et al. evaluated synthesized thioether mimetics of the alpha-conotoxin GI and found that the two isomers tested had significantly less pharmacologic activity than the native peptide (Bondebjerg et al., 2003, ChemBioChem 4:186-194). Non-reducible dicarba analogs of alpha-ImI were evaluated and in both instances found to have an approximately 10-fold reduction in pharmacological activity (MacRaidl et al., 2009, J. Med. Chem. 52:755-762). Certain specific selenocysteine and cystathionine derivatives of ImI retained activity in blocking the rat $\alpha 7$ nAChR (Dekan et al., 2011, J. Am. Chem. Soc. 133:15866-15869; Armishaw et al., 2006, J. Bio. Chem. 281:14136-14143).

[0011] van Lierop evaluated dicarba substitutions of the disulfide bonds of alpha-conotoxin Vc1.1 and found that the [2,8]-cis and [2,8]-trans dicarba analogs had no activity on the rat $\alpha 9\alpha 10$ nAChR, whereas the [3,16]-cis and [3,16]-trans isomers of Vc1.1 had greatly reduced (10-100-fold) activity on rat $\alpha 9\alpha 10$ nAChR (van Lierop et al., 2013, ACS Chemical Biology 8:1815-1821). Yu et al created N-terminal to C-terminal peptide-linked derivatives of Vc1.1 that had one or more cysteine residues replaced with His or Phe. These derivatives had significantly reduced potency (20 to 40-fold lower) on the human $\alpha 9\alpha 10$ nAChR relative to native Vc1.1 (Yu et al. 2015, Sci. Rep. 5:13264.). In a separate study by the same group, substitution of Cys2 to His and Cys8 to Phe in Vc1.1 in the absence of N- to C-terminal cyclization also resulted in loss of activity on the human $\alpha 9\alpha 10$ nAChR, despite analysis that demonstrated conservation of secondary structure (Tabassum et al. 2017, ACS Omega 2:4621-4631).

[0012] Similar efforts have been explored to replace the disulfide bonds in RgIA with dicarba linkages (Chhabra et al., 2014, J. Med. Chem. 57:993-9944). While this study found that dicarba analogs of RgIA were synthetically feasible, NMR-based structural comparisons of native RgIA to the dicarba analogs demonstrated significant structural differences in the second loop of the peptides. Consistent with these structural differences and observations from the related Vc1.1, the [2,8]-cis and [2,8]-trans dicarba analogs of RgIA had no activity on the rat

$\alpha 9\alpha 10$ nAChR, whereas the [3,12]-cis and [3,12]-trans isomers both had >100-fold reductions in inhibition of acetylcholine-evoked $\alpha 9\alpha 10$ currents. Chhabra et al. did not evaluate the activities of dicarba-bridged RgIA analogs on the human $\alpha 9\alpha 10$ nAChR.

[0013] Knuhtsen et al. (Knuhtsen et al., Chemical Science, 2019, Advance Article) studied analogs of the alpha-conotoxin GI, in which the Cys2-Cys7 or Cys3-Cys13 disulfide bonds were replaced with 1,5-disubstituted 1,2,3-triazole bridges.

[0014] Native RgIA has approximately 300-fold lower affinity for the human than the rat $\alpha 9\alpha 10$ nAChR, and this difference in affinity has been mapped to variation in the amino acid sequence of the alpha 9 subunit within the RgIA binding pocket (Azam et al., 2015, Mol. Pharmacol. 87:855-864).

[0015] Conotoxin peptide analogs of RgIA have been reported (WO 2008/011006; Romero et al., 2017, Proc. Natl. Acad. Sci. USA 14:E1825-E1832; WO 2016/073949).

[0016] Studies have demonstrated a greater degree of structural flexibility in loop 2 of RgIA, especially around the carboxy-terminus, and the importance of loop 2 residues in human receptor binding interactions (Clark et al., 2008, FEBS Lett. 582(5):597-602). NMR spectroscopy also indicates that Cys12 of RgIA may interact with Tyr10 in the wild type or native peptide (Ellison et al, 2008, J. Mol. Biol. 377:1216-1227; Armishaw, 2010, Toxins 2:1471-1499).

[0017] Conotoxin peptides and their analogs are competitive inhibitors of the $\alpha 9\alpha 10$ nAChR and they bind at the ACh binding site, *i.e.*, at the interface between adjacent subunits. The subunit interfaces buries to a 1.4 Å probe radius, a surface area of ~1300 Å², where these conotoxins interact with residues between subunits encompassing distances less than 5 Å (Hansen et al., 2005, EMBO J., 24(20):3635-3646).

[0018] Amino acid comparison with other known nAChR subunits indicates that $\alpha 9\alpha 10$ nAChRs are closely related to $\alpha 7$ nAChR (Elgoyhen et al., 1994, Cell 79(4):705-715; Lustig et al., 2001, Genomics, 73(3):272-283; Sgard et al., 2002, Mol. Pharmacol. 61(1):150-159). The $\alpha 7$ nAChR has pleiotropic effects in the nervous system (Ellison et al., 2008, J. Mol. Biol. 377:1216-1227; Armishaw, 2010, Toxins 2:1471-1499).

[0019] Non-covalent or covalent attachment to polyethylene glycol polymer(s) (PEGylation) can change the physical and chemical properties of a biomolecule such as a peptide, such as its

conformation, electrostatic binding, and hydrophobicity (Veronese and Mero, 2008, BioDrugs, 22:315-329; see also Harris et al., 2001, Clin. Pharmacokinet. 40:539-551).

[0020] PEGylation of a therapeutic protein often leads to a loss in its binding affinity due to the steric hindrance of the PEG polymer to the drug-target binding interaction (Fishburn, 2008, J. Pharm. Sci. 97:4167-4183). PEGylated therapeutic proteins can lose biological activity or potency, either through steric effects or through hydrophobic-hydrophobic interactions between the PEG and hydrophobic domains within the protein (Parrott and DeSimone, 2011, Nat. Chem. 4:13-14).

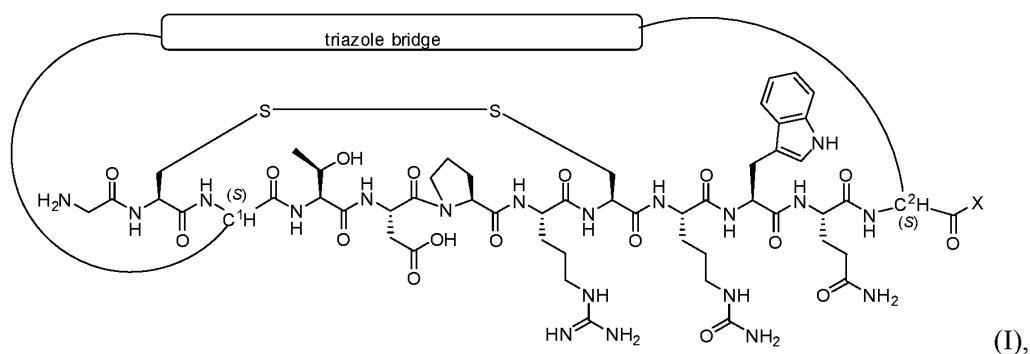
[0021] Studies have indicated that antagonists of $\alpha 9$ -containing nAChRs exhibit analgesic activity in animal models of neuropathic pain (Hone, et al., 2018, British Journal of Pharmacology, 175:1915-1927). Studies have also shown that inhibition of the $\alpha 9\alpha 10$ nAChR in rodents prevents chemotherapy-induced neuropathic pain (Romero et al., 2018, Proc. Natl. Acad. Sci. USA 114(10):E1825-E1832). See also, Mohammadi and Christie, 2014, Molecular Pain, 10:64-72; Simard et al., 2013, Immunology and Cell Biology, 91:195-200.

[0022] There is an unmet medical need for conotoxin peptide analogs with enhanced metabolic stability while maintaining sufficient antagonist activity on $\alpha 9$ -containing nAChRs. The conotoxin peptide analogs of the present invention meet these needs.

[0023] Citation of a reference herein shall not be construed as an admission that such is prior art to the present disclosure.

3. SUMMARY OF THE INVENTION

[0024] The present disclosure provides conotoxin peptide analogs of Formula (I) (SEQ ID NO:93):



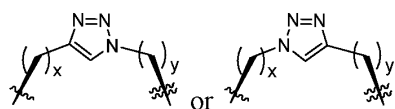
or a pharmaceutically acceptable salt thereof,

wherein

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr;

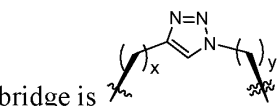
wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[0025] In a specific embodiment, the triazole bridge is

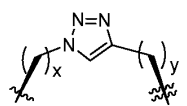


, wherein the single wavy line (~~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (=====) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog; and wherein x is 1, 2, 3, or 4; and y is 2, 3 or 4.

[0026] In a specific embodiment, wherein the triazole bridge is



[0027] In a specific embodiment, the triazole bridge is



[0028] In a specific embodiment, x is 1, 2, or 3.

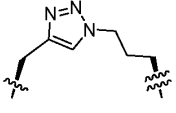
[0029] In a specific embodiment, x is 1.

[0030] In a specific embodiment, y is 2 or 3.

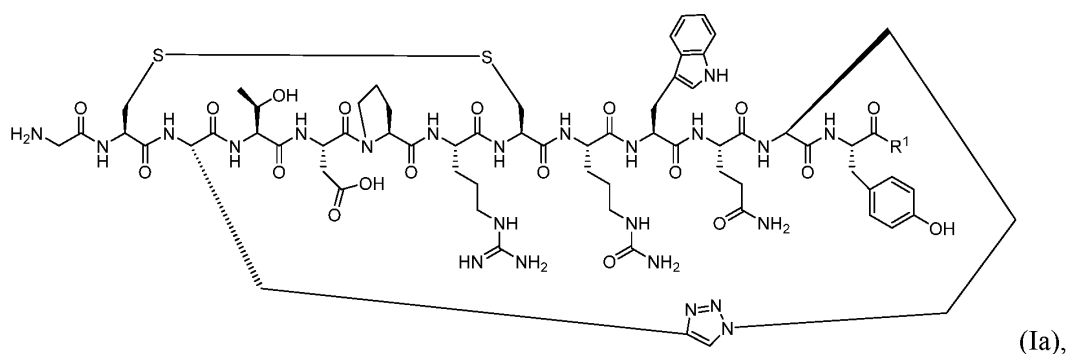
[0031] In a specific embodiment, y is 3.

[0032] In a specific embodiment, x is 1, 2, or 3, and y is 2 or 3.

- [0033] In a specific embodiment, x is 1 and y is 3.
- [0034] In a specific embodiment, x is 2, and y is 3.
- [0035] In a specific embodiment, x is 2, and y is 2.
- [0036] In a specific embodiment, x is 1, and y is 3.
- [0037] In a specific embodiment, x is 2, and y is 2.

[0038] In a specific embodiment, the triazole bridge is , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

- [0039] In a specific embodiment, X is X_{AA}¹.
- [0040] In a specific embodiment, X is X_{AA}¹X_{AA}².
- [0041] In a specific embodiment, X_{AA}¹ is selected from the group consisting of Tyr, D-Tyr and Phe.
- [0042] In a specific embodiment, X is Tyr.
- [0043] In a specific embodiment, the C-terminus of the conotoxin peptide analog is OH.
- [0044] In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH₂.
- [0045] In a specific embodiment, the conotoxin peptide analog is of Formula (Ia) (SEQ ID NO:94)

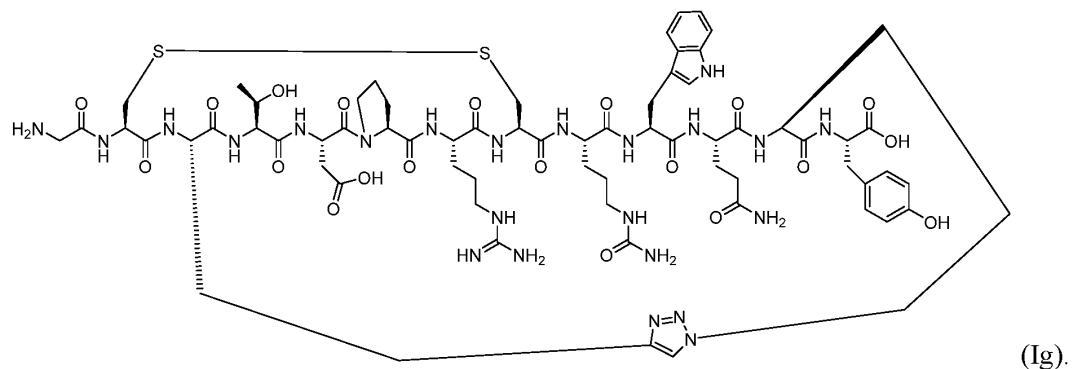


wherein R¹ is OH or NH₂.

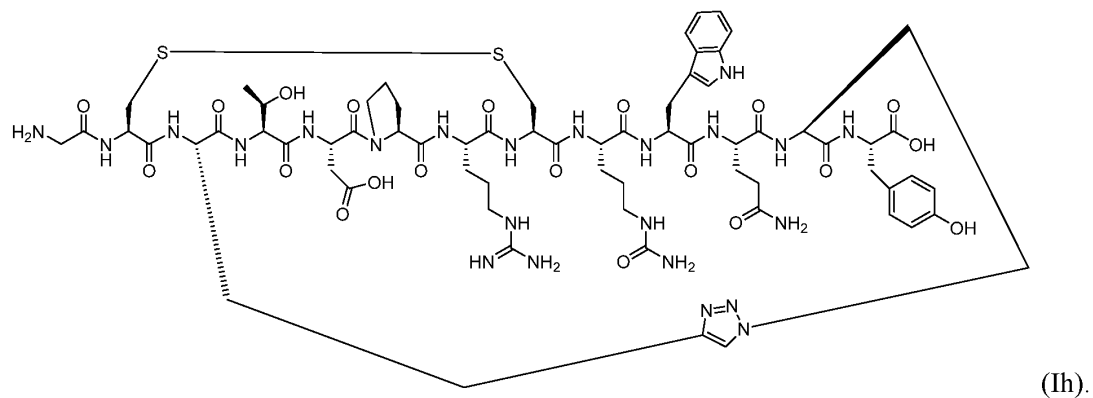
- [0046] In a specific embodiment, R¹ is OH.

[0047] In a specific embodiment, R¹ is NH₂.

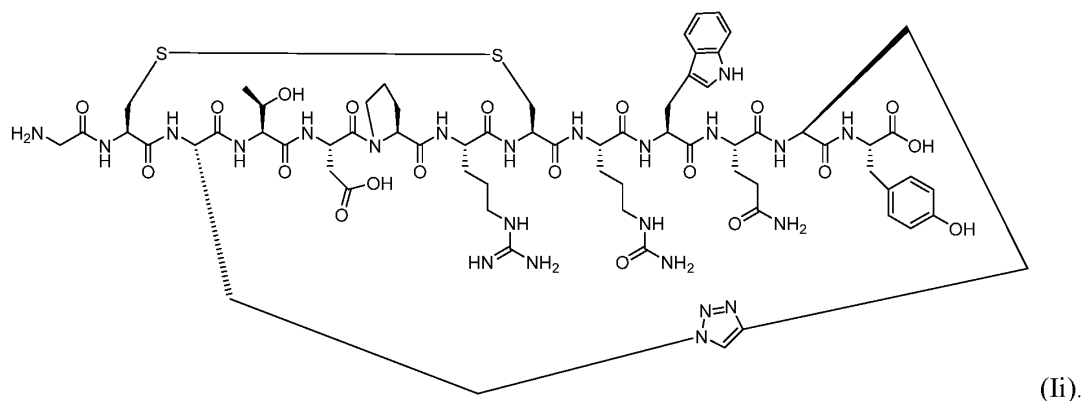
[0048] In a specific embodiment, the conotoxin peptide analog is of Formula (Ig) (SEQ ID NO:30):



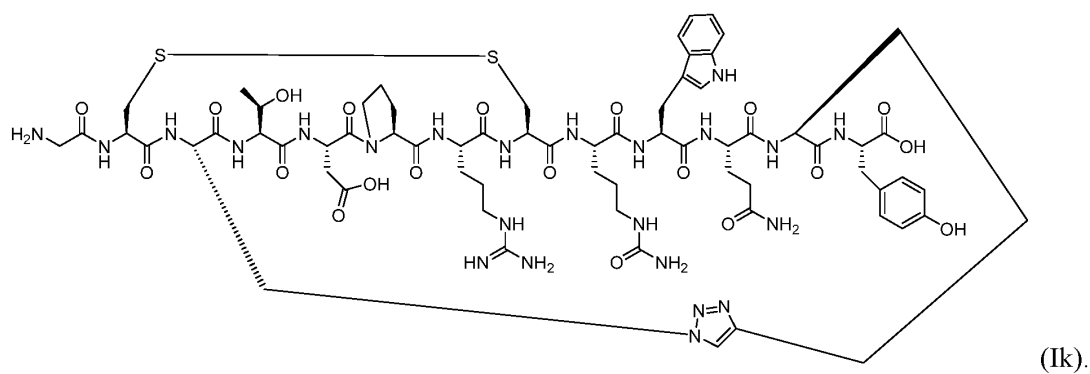
[0049] In a specific embodiment, the conotoxin peptide analog is of Formula (Ih) (SEQ ID NO:33):



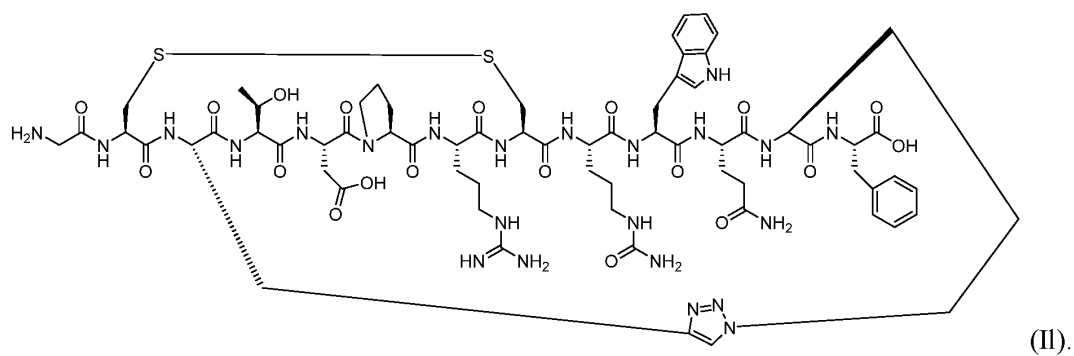
[0050] In a specific embodiment, the conotoxin peptide analog is of Formula (Ii) (SEQ ID NO:36):



[0051] In a specific embodiment, the conotoxin peptide analog is of Formula (Ik) (SEQ ID NO:42):

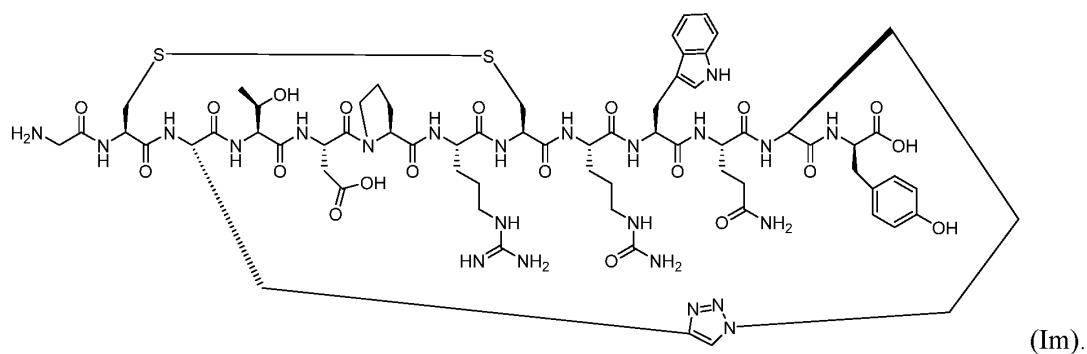


[0052] In a specific embodiment, the conotoxin peptide analog is of Formula (II) (SEQ ID NO:45):

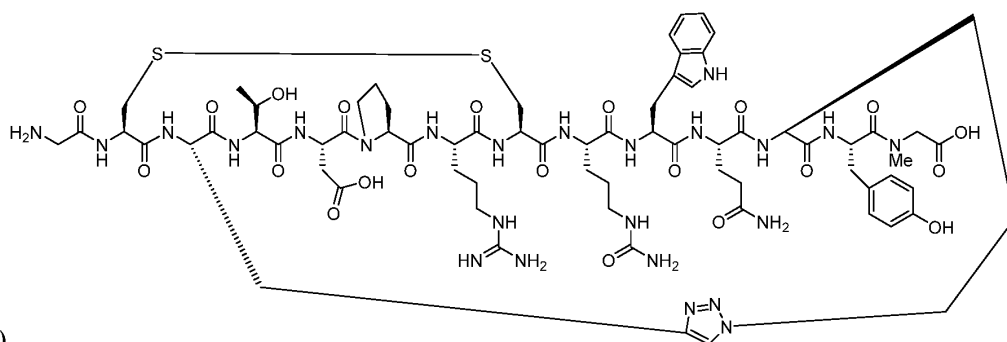


[0053] In a specific embodiment, the conotoxin peptide analog is of Formula (Im) (SEQ ID

NO:48)



[0054] In a specific embodiment, the conotoxin peptide analog is of Formula (In) (SEQ ID

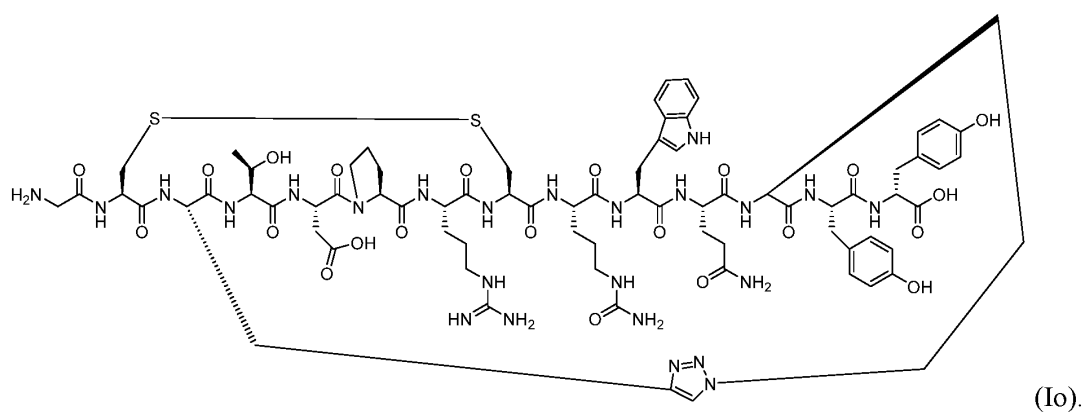


NO:51)

(In).

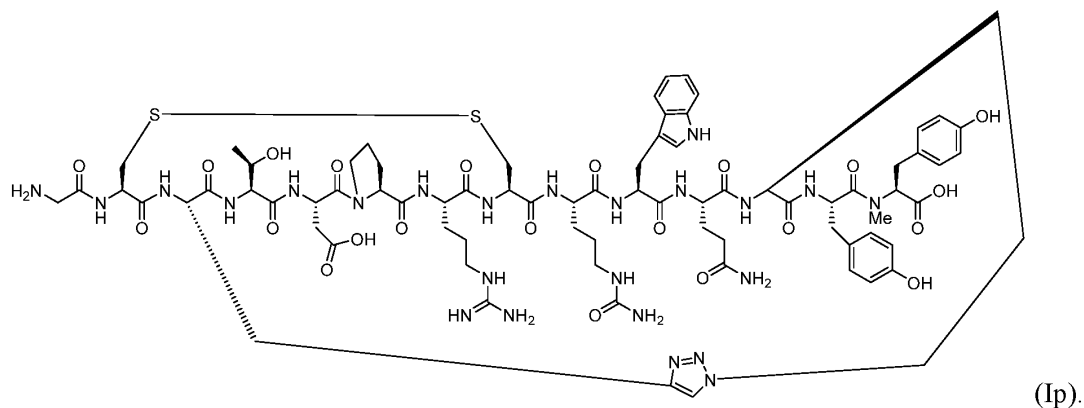
[0055] In a specific embodiment, the conotoxin peptide analog is of Formula (Io) (SEQ ID

NO:54)

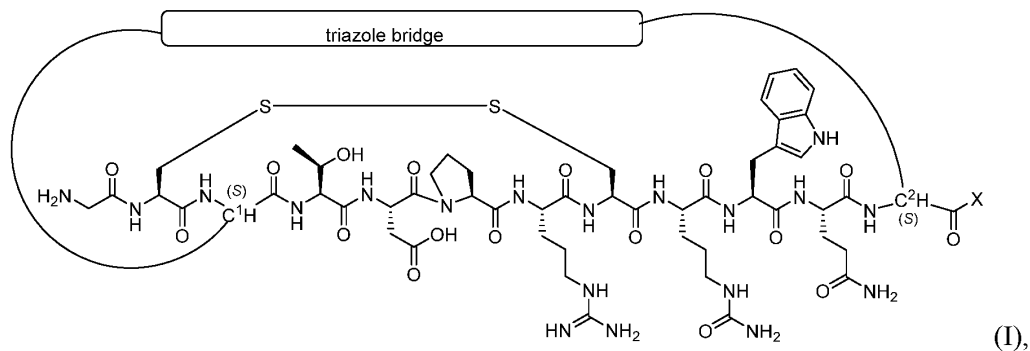


[0056] In a specific embodiment, the conotoxin peptide analog is of Formula (Ip) (SEQ ID

NO:57)



[0057] Also provided herein are PEGylated conotoxin peptide analogs or pharmaceutically acceptable salt thereof, wherein the conotoxin peptide analog is of Formula (I) (SEQ ID NO:93):



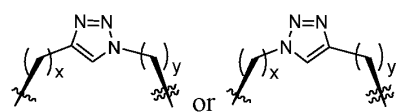
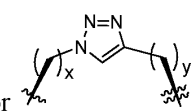
wherein

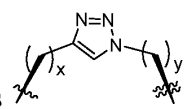
X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr;

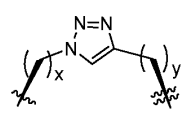
wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group; and

wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one or more polyethylene glycol (PEG) polymers.

[0058] In a specific embodiment, the triazole bridge is


 or
 
 , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog; and wherein x is 1, 2, 3, or 4; and y is 2, 3 or 4.

[0059] In a specific embodiment, wherein the triazole bridge is
 
 .

[0060] In a specific embodiment, the triazole bridge is
 
 .

[0061] In a specific embodiment, x is 1, 2, or 3.

[0062] In a specific embodiment, x is 1.

[0063] In a specific embodiment, y is 2 or 3.

[0064] In a specific embodiment, y is 3.

[0065] In a specific embodiment, x is 1, 2, or 3, and y is 2 or 3.

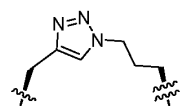
[0066] In a specific embodiment, x is 1 and y is 3.

[0067] In a specific embodiment, x is 2 and y is 3.

[0068] In a specific embodiment, x is 2 and y is 2.

[0069] In a specific embodiment, x is 1 and y is 3.

[0070] In a specific embodiment, x is 2 and y is 2.

[0071] In a specific embodiment, the triazole bridge is
 
 , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

[0072] In a specific embodiment, X is X_{AA}¹.

[0073] In a specific embodiment, X is X_{AA}¹X_{AA}².

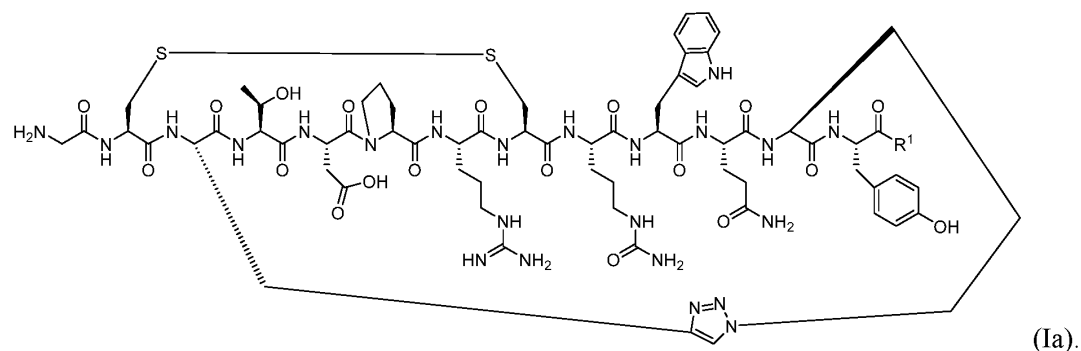
[0074] In a specific embodiment, X_{AA}^1 is selected from the group consisting of Tyr, D-Tyr and Phe.

[0075] In a specific embodiment, X is Tyr.

[0076] In a specific embodiment, the C-terminus of the conotoxin peptide analog is OH.

[0077] In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH_2 .

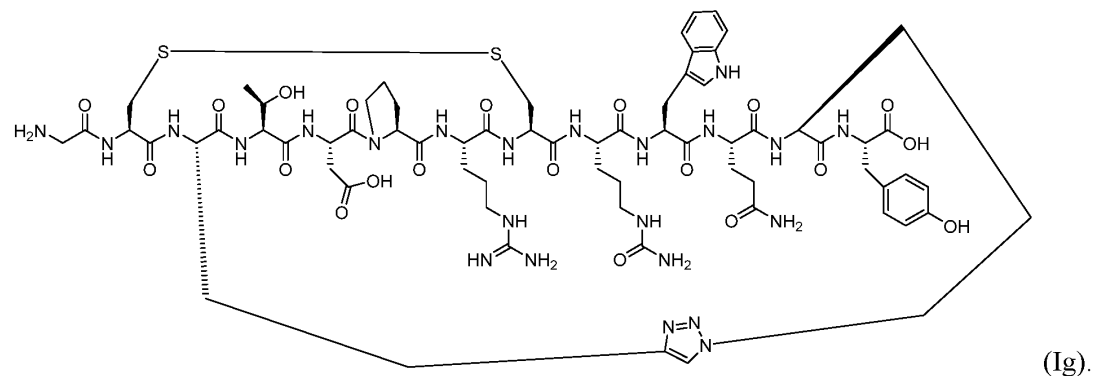
[0078] In a specific embodiment, the conotoxin peptide analog is of Formula (Ia) (SEQ ID NO:94)



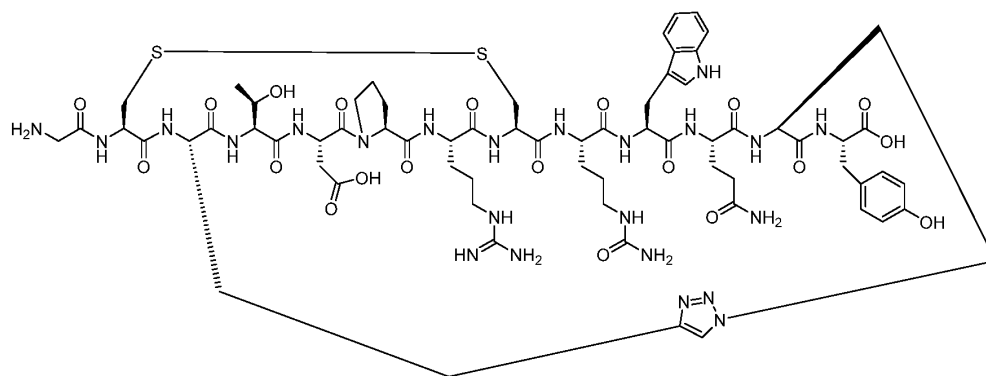
[0079] In a specific embodiment, R^1 is OH.

[0080] In a specific embodiment, R^1 is NH_2 .

[0081] In a specific embodiment, the conotoxin peptide analog is of Formula (Ig) (SEQ ID NO:30):

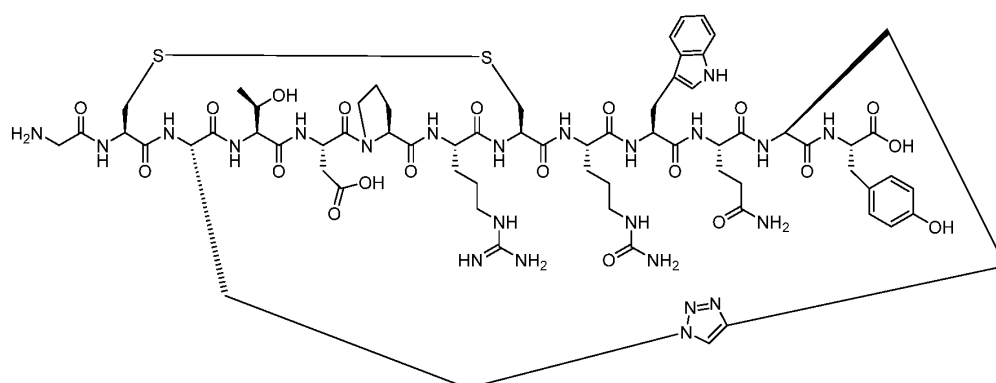


[0082] In a specific embodiment, the conotoxin peptide analog is of Formula (Ih) (SEQ ID NO:33):



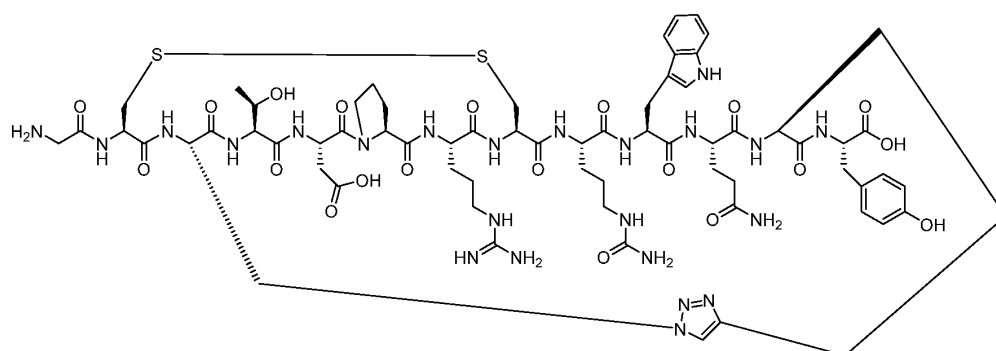
(Ih).

[0083] In a specific embodiment, the conotoxin peptide analog is of Formula (Ii) (SEQ ID NO:36):



(Ii).

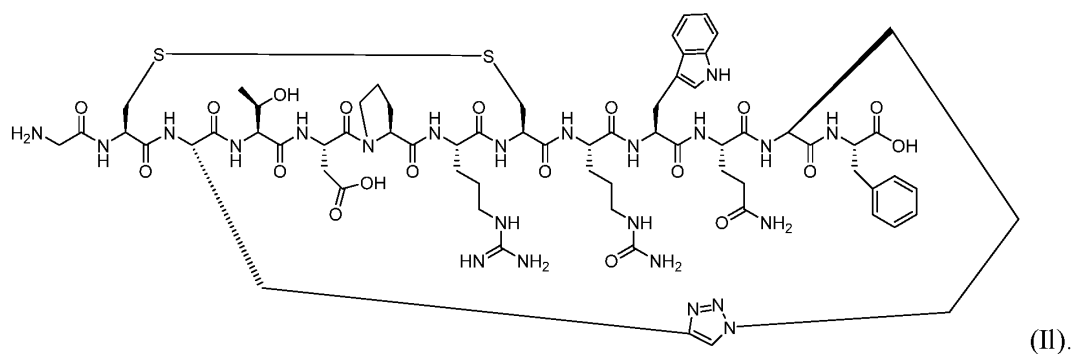
[0084] In a specific embodiment, the conotoxin peptide analog is of Formula (Ik) (SEQ ID NO:42):



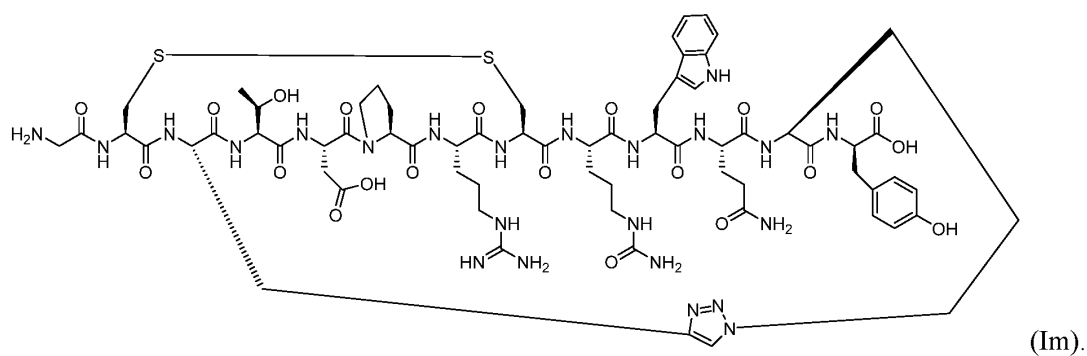
(Ik).

[0085] In a specific embodiment, the conotoxin peptide analog is of Formula (II) (SEQ ID

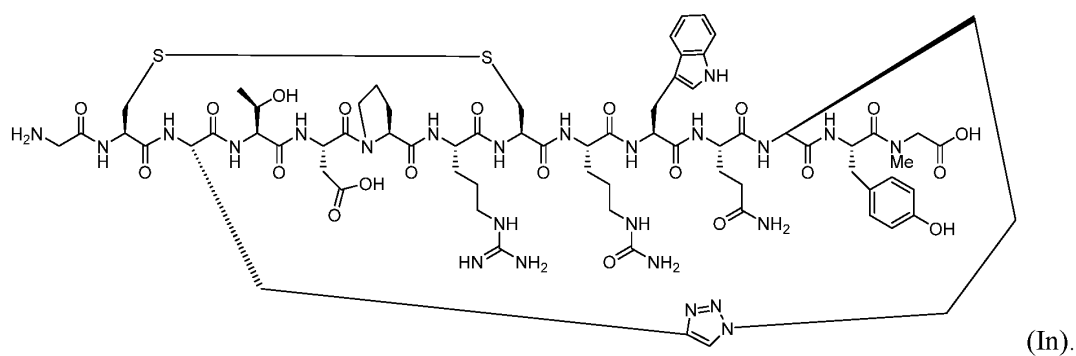
NO:45):



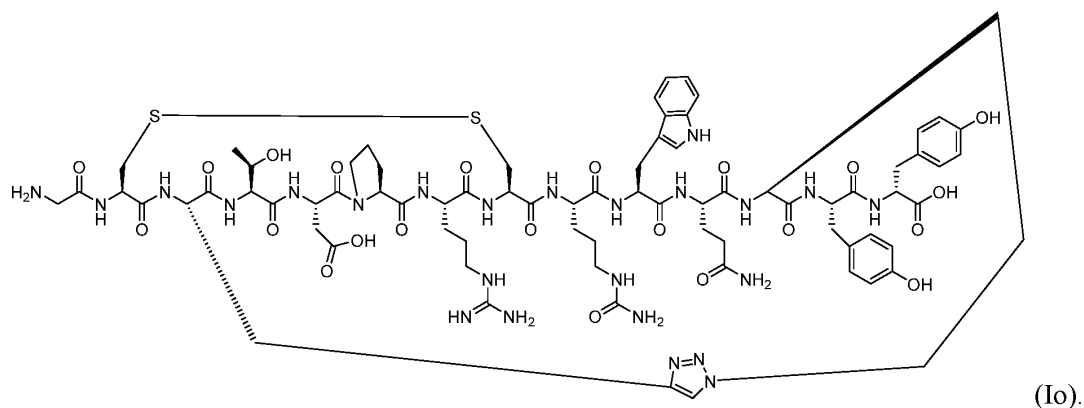
[0086] In a specific embodiment, the conotoxin peptide analog is of Formula (Im) (SEQ ID NO:48)



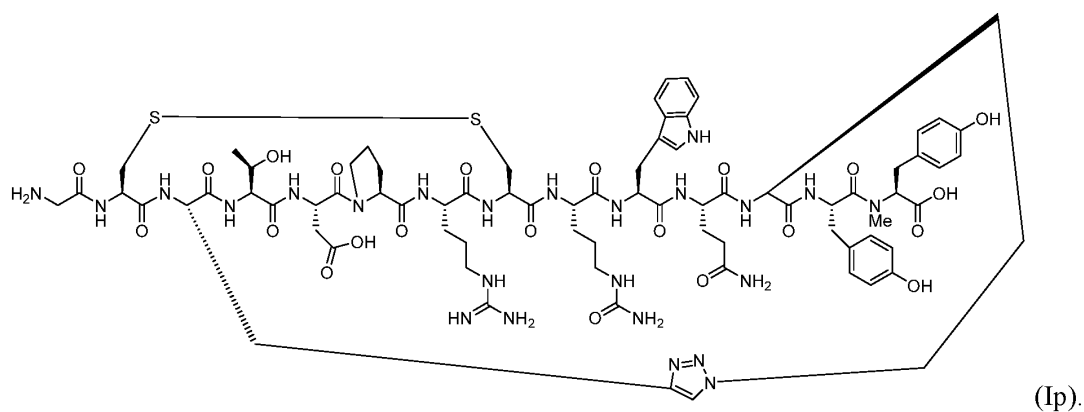
[0087] In a specific embodiment, the conotoxin peptide analog is of Formula (In) (SEQ ID NO:51)



[0088] In a specific embodiment, the conotoxin peptide analog is of Formula (Io) (SEQ ID NO:54)



[0089] In a specific embodiment, the conotoxin peptide analog is of Formula (Ip) (SEQ ID NO:57)



[0090] In a specific embodiment, the conotoxin peptide analog is covalently attached to one PEG polymer.

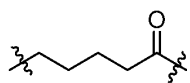
[0091] In a specific embodiment, the PEG polymer is covalently attached to the N-terminus of the conotoxin peptide analog.

[0092] In a specific embodiment, the PEG polymer is covalently attached to the C-terminus of the conotoxin peptide analog.

[0093] In a specific embodiment, the PEG polymer is covalently attached to an amino acid residue position that is not the N-terminus or the C-terminus of the conotoxin peptide analog.

[0094] In a specific embodiment, the PEG polymer is covalently attached to the conotoxin peptide analog via a linking group.

[0095] In a specific embodiment, the linking group is a valerate linker having a formula of



[0096] In a specific embodiment, the linking group is a butylene.

[0097] In a specific embodiment, the linking group is a carbonyl.

[0098] In a specific embodiment, the PEG polymer is a linear or branched PEG polymer.

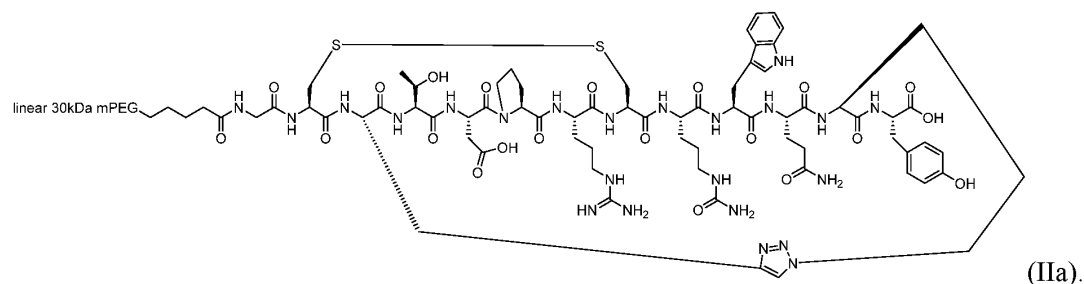
[0099] In a specific embodiment, the PEG polymer is a linear PEG polymer.

[00100] In a specific embodiment, the PEG polymer has molecular weight in the range of 10 kDa and 40 kDa.

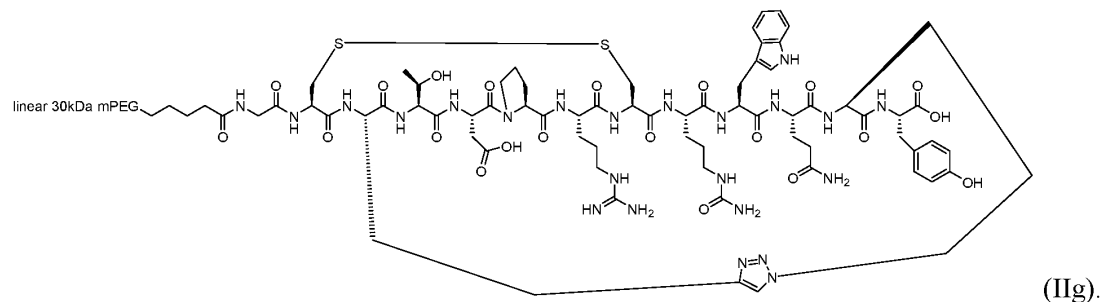
[00101] In a specific embodiment, the PEG polymer is a linear 30 kDa PEG polymer.

[00102] In a specific embodiment, the PEG polymer is a linear 30 kDa mPEG polymer.

[00103] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIa) (SEQ ID NO:83):

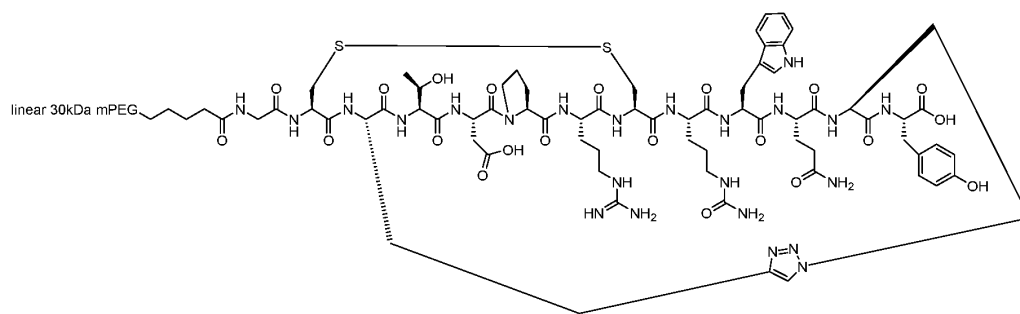


[00104] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIg) (SEQ ID NO:95):



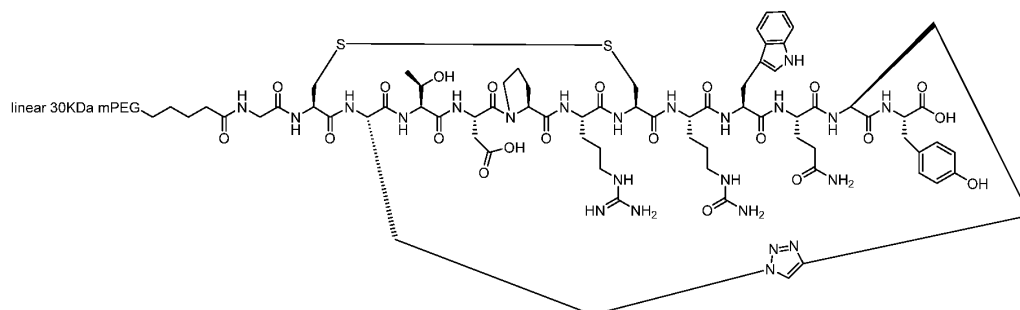
[00105] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula

(IIh) (SEQ ID NO:96):



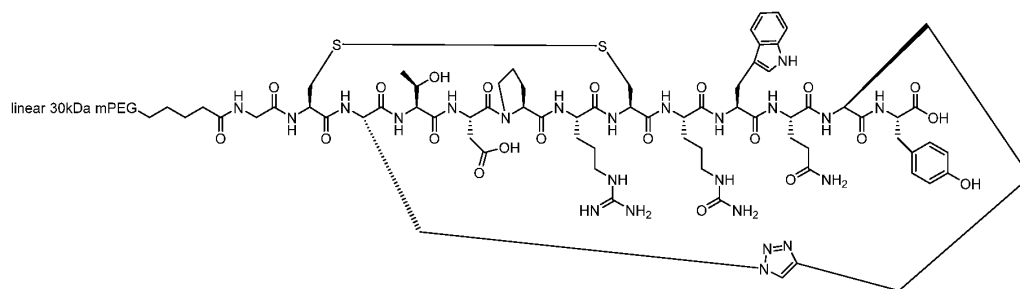
(IIh).

[00106] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIi) (SEQ ID NO:97):



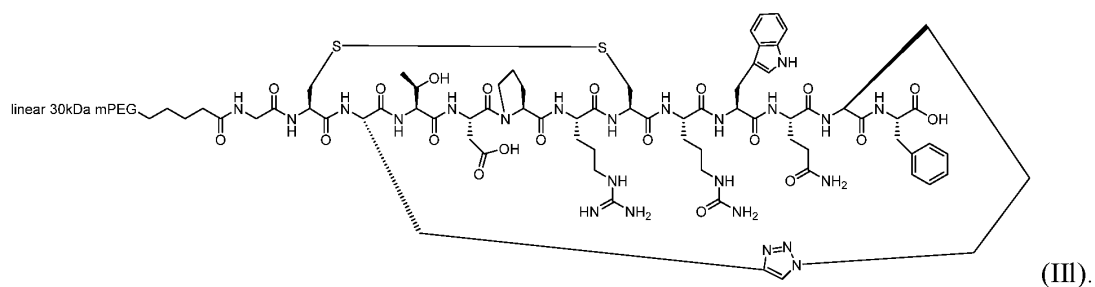
(IIi).

[00107] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIk) (SEQ ID NO:98):

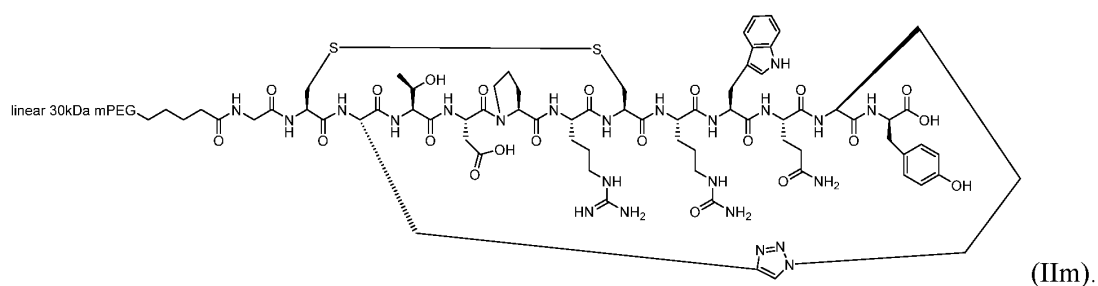


(IIk).

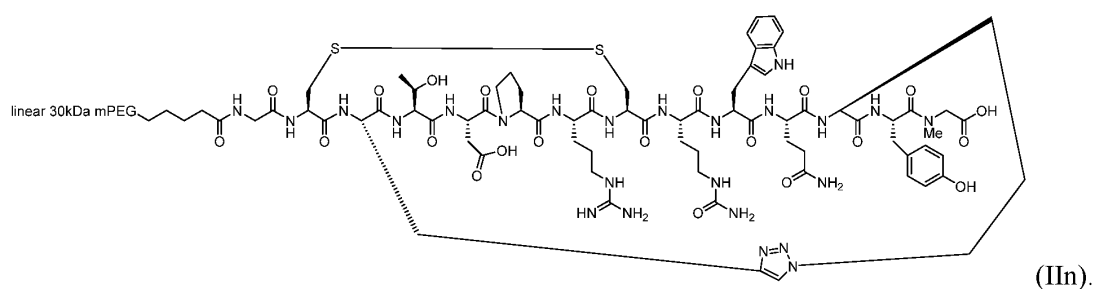
[00108] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (III) (SEQ ID NO:99):



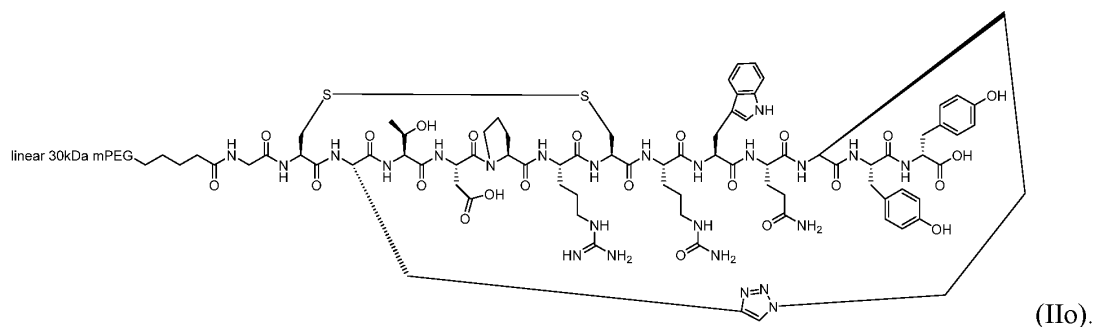
[00109] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIm) (SEQ ID NO:100):



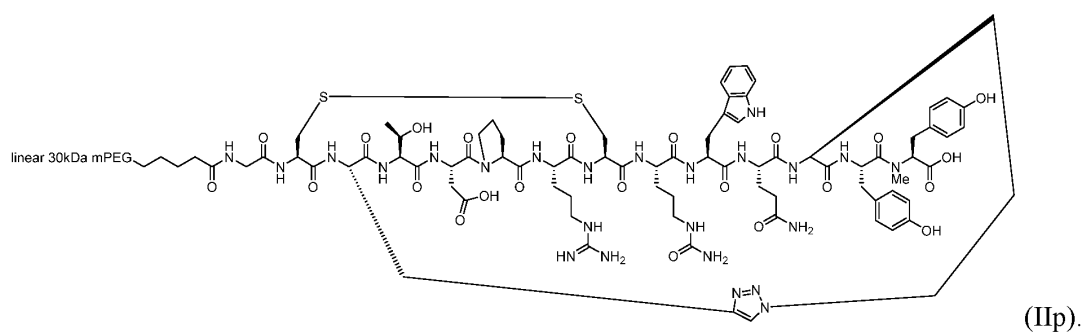
[00110] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIn) (SEQ ID NO:101):



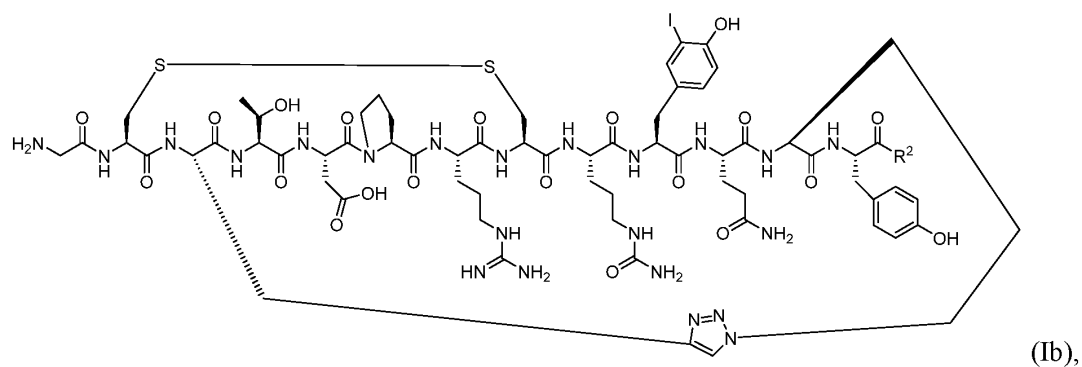
[00111] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIo) (SEQ ID NO:102):



[00112] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIp) (SEQ ID NO:103):



[00113] Also provided herein are conotoxin peptide analogs of Formula (Ib) (SEQ ID NO:104):

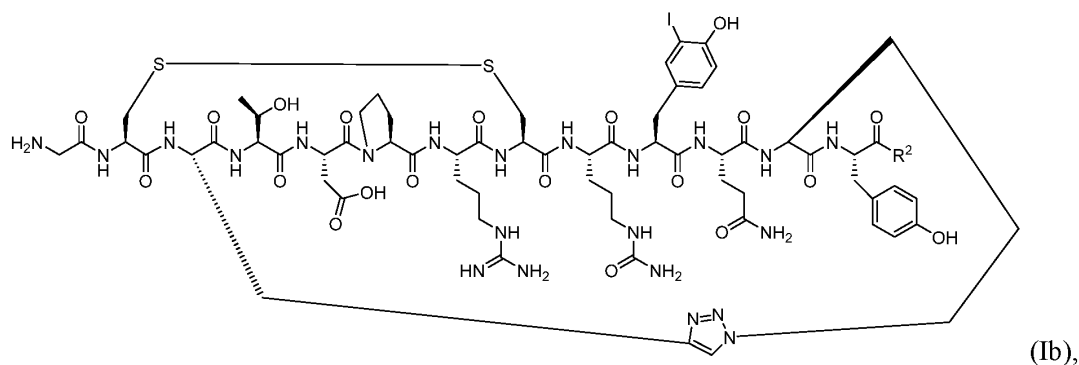


or a pharmaceutically acceptable salt thereof,
wherein R^2 is OH or NH_2 .

[00114] In a specific embodiment, R^2 is OH.

[00115] In a specific embodiment, R^2 is NH_2 .

[00116] Also provided herein are PEGylated conotoxin peptide analogs or a pharmaceutically acceptable salt thereof, wherein the conotoxin peptide analog is of Formula (Ib) (SEQ ID NO:104):



wherein R^2 is OH or NH_2 ; and

wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one or more polyethylene glycol (PEG) polymers.

[00117] In a specific embodiment, R^2 is OH.

[00118] In a specific embodiment, R^2 is NH_2 .

[00119] In a specific embodiment, the conotoxin peptide analog is covalently attached to one PEG polymer.

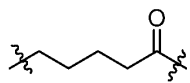
[00120] In a specific embodiment, the PEG polymer is covalently attached to the N-terminus of the conotoxin peptide analog.

[00121] In a specific embodiment, the PEG polymer is covalently attached to the C-terminus of the conotoxin peptide analog.

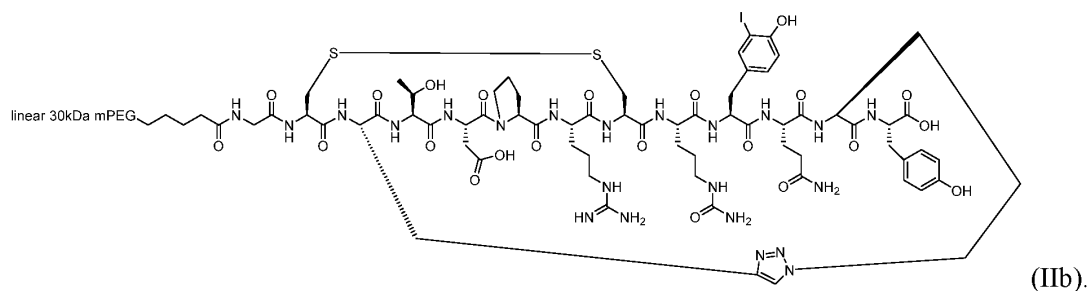
[00122] In a specific embodiment, the PEG polymer is covalently attached to an amino acid residue position that is not the N-terminus or the C-terminus of the conotoxin peptide analog.

[00123] In a specific embodiment, the PEG polymer is covalently attached to the conotoxin peptide analog via a linking group.

[00124] In a specific embodiment, the linking group is a valerate linker having a formula of



- [00125] In a specific embodiment, the linking group is a butylene.
- [00126] In a specific embodiment, the linking group is a carbonyl.
- [00127] In a specific embodiment, the PEG polymer is a linear or branched PEG polymer.
- [00128] In a specific embodiment, the PEG polymer is a linear PEG polymer.
- [00129] In a specific embodiment, the PEG polymer has molecular weight in the range of 10 kDa and 40 kDa.
- [00130] In a specific embodiment, the PEG polymer is a linear 30 kDa PEG polymer.
- [00131] In a specific embodiment, the PEG polymer is a linear 30 kDa mPEG polymer.
- [00132] In a specific embodiment, the PEGylated conotoxin peptide analog is of Formula (IIb) (SEQ ID NO:105):



- [00133] Also provided herein are conotoxin peptide analogs selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, **Ib'**, **Ic**, **Id**, **Ie**, **If**, **Ig**, **Ih**, **Ii**, **Ij**, **Ik**, **Il**, **Im**, **In**, **Io**, **Ip**, **Iq**, **Ir**, **Is**, **It**, **Iu**, and **Iv**, or a pharmaceutically acceptable salt thereof.
- [00134] In a specific embodiment, the conotoxin peptide analog is selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, **Ib'**, **Ig**, **Ih**, **Ii**, **Ik**, **Il**, **Im**, **In**, **Io**, and **Ip**.
- [00135] In a specific embodiment, the conotoxin peptide analog is selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, and **Ib'**.
- [00136] Also provided herein are pharmaceutical compositions comprising a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof described herein, and optionally a pharmaceutically acceptable carrier.

[00137] Also provided herein are methods of treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, or pharmaceutical composition described herein.

[00138] Also provided herein are methods of treating or preventing a condition associated with activation of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, or pharmaceutical composition described herein.

[00139] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of the $\alpha 9$ -containing nAChR is pain or inflammation.

[00140] In a specific embodiment, the condition is pain.

[00141] In a specific embodiment, the pain is selected from the group consisting of general pain, chronic pain, neuropathic pain, nociceptive pain, inflammatory pain, visceral pain, somatic pain, pain induced by peripheral nerve damage, pain induced by an inflammatory disorder, pain induced by a metabolic disorder, pain induced by cancer, pain induced by chemotherapy, pain induced by a surgical procedure, and pain induced by a burn.

[00142] In a specific embodiment, the pain is cancer-related chronic pain.

[00143] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of the $\alpha 9$ -containing nAChR is an inflammatory condition.

[00144] In a specific embodiment, the inflammatory condition is selected from the group consisting of inflammation, chronic inflammation, a rheumatic disease, sepsis, fibromyalgia, inflammatory bowel disease, sarcoidosis, endometriosis, uterine fibroids, an inflammatory skin disease, an inflammatory condition of the lungs, a disease associated with inflammation of the nervous system, periodontal disease, and cardiovascular disease.

[00145] In a specific embodiment, the inflammatory condition is mediated by immune cells.

[00146] In a specific embodiment, the inflammatory condition is long-term inflammation

and/or peripheral neuropathy following injury.

[00147] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of the $\alpha 9$ -containing nAChR is pain and inflammation.

[00148] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of the $\alpha 9$ -containing nAChR is inflammation and neuropathy.

[00149] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) is a condition conducive to treatment or prevention by inhibition of an $\alpha 9\alpha 10$ subtype of nAChR.

[00150] In a specific embodiment, the subject is a human.

[00151] Also provided herein are methods of treating or preventing pain or inflammation in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, or pharmaceutical composition described herein.

[00152] Also provided herein are conotoxin peptide analogs or pharmaceutically acceptable salt thereof, or PEGylated conotoxin peptide analogs or pharmaceutically acceptable salt thereof, or pharmaceutical compositions described herein, for use in treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) in a subject.

[00153] Also provided herein are pharmaceutical compositions comprising a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, for use in treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) in a subject.

[00154] Also provided herein are uses of a conotoxin peptide analog or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, or pharmaceutical composition described herein, in the preparation of a medicament for treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) in a subject.

[00155] Also provided herein are conotoxin peptide analogs or a salt thereof, wherein the amino acid sequence of the conotoxin peptide analog is

Gly-Cys-X_{AA}³-Thr-Asp-Pro-Arg-Cys-X_{AA}⁹-Trp-Gln-X_{AA}¹²-X (SEQ ID NO:106),

wherein

X_{AA}³ is selected from the group consisting of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline and (S)-bishomopropargyl glycine;

X_{AA}⁹ is Citrulline;

X_{AA}¹² is selected from the group consisting of (S)-azidohomoalanine, (S)-homopropargyl glycine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine;

wherein when X_{AA}³ is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}¹² is (S)-azidohomoalanine or (S)-azidonorvaline; and when X_{AA}³ is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}¹² is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;

X is X_{AA}¹ or X_{AA}¹X_{AA}²; wherein X_{AA}¹ is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}² is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00156] In a specific embodiment, X_{AA}³ is (S)-propargyl glycine or (S)-azidoalanine.

[00157] In a specific embodiment, X_{AA}¹² is (S)-azidonorvaline or (S)-bishomopropargyl glycine.

[00158] In a specific embodiment, X_{AA}³ is (S)-propargyl glycine and X_{AA}¹² is (S)-azidonorvaline.

[00159] In a specific embodiment, X_{AA}³ is (S)-homopropargyl glycine and X_{AA}¹² is (S)-azidonorvaline.

[00160] In a specific embodiment, X_{AA}³ is (S)-homopropargyl glycine and X_{AA}¹² is (S)-azidohomoalanine.

[00161] In a specific embodiment, X_{AA}³ is (S)-azidohomoalanine and X_{AA}¹² is (S)-homopropargyl glycine.

- [00162]** In a specific embodiment, X_{AA}^3 is (S)-azidoalanine and X_{AA}^{12} is (S)-bishomopropargyl glycine.
- [00163]** In a specific embodiment, X is X_{AA}^1 .
- [00164]** In a specific embodiment, X is $X_{AA}^1X_{AA}^2$.
- [00165]** In a specific embodiment, X_{AA}^1 is selected from the group consisting of Tyr, D-Tyr and Phe.
- [00166]** In a specific embodiment, X is Tyr.
- [00167]** In a specific embodiment, the C-terminus of the conotoxin peptide analog is OH.
- [00168]** In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH_2 .
- [00169]** In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr.
- [00170]** In a specific embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid group.
- [00171]** In a specific embodiment, the C-terminus of the conotoxin peptide analog is an amide group.
- [00172]** In a specific embodiment, X_{AA}^3 is (S)-homopropargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.
- [00173]** In a specific embodiment, X_{AA}^3 is (S)-homopropargyl glycine, X_{AA}^{12} is (S)-azidohomoalanine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.
- [00174]** In a specific embodiment, X_{AA}^3 is (S)-azidohomoalanine, X_{AA}^{12} is (S)-homopropargyl glycine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.
- [00175]** In a specific embodiment, X_{AA}^3 is (S)-azidoalanine, X_{AA}^{12} is (S)-bishomopropargyl glycine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.
- [00176]** In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Phe; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00177] In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is D-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00178] In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-N-Me-Gly; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00179] In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-D-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00180] In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-N-Me-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00181] Also provided herein are conotoxin peptide analogs or a salt thereof, wherein the amino acid sequence of the conotoxin peptide analog is

Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 - X_{AA}^{10} -Gln- X_{AA}^{12} -Tyr (SEQ ID NO:107),

wherein

X_{AA}^3 is (S)-propargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{10} is 3-iodo-Tyr;

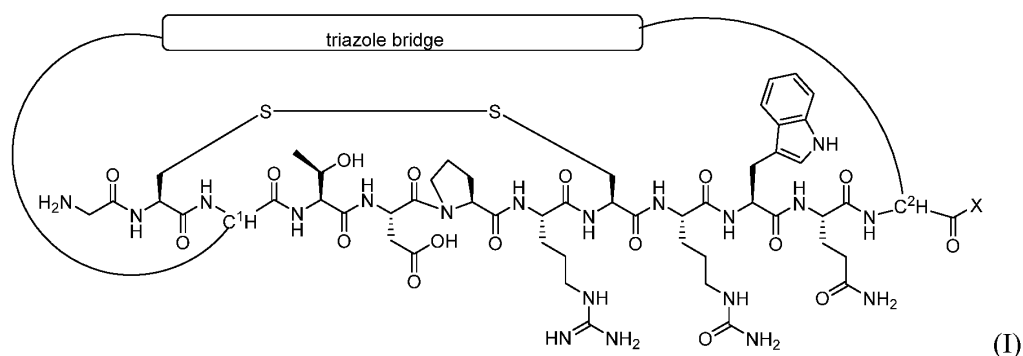
X_{AA}^{12} is (S)-azidonorvaline;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00182] In a specific embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00183] In a specific embodiment, the C-terminus of the conotoxin peptide analog is an amide group.

[00184] Also provided herein are methods of making a conotoxin peptide analog of Formula (I) (SEQ ID NO:93) or a pharmaceutically acceptable salt thereof,



wherein

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the conotoxin peptide analog of Formula (I) is a carboxylic acid or an amide group;

comprising subjecting an intermediate conotoxin peptide analog or a salt thereof to triazole formation conditions, wherein the amino acid sequence of the intermediate conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 -Trp-Gln- X_{AA}^{12} -X (SEQ ID NO:106), wherein

X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline and (S)-bishomopropargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{12} is selected from the group consisting of (S)-azidohomoalanine, (S)-homopropargyl glycine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine;

wherein when X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}^{12} is (S)-azidohomoalanine or (S)-azidonorvaline; when X_{AA}^3 is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}^{12} is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;

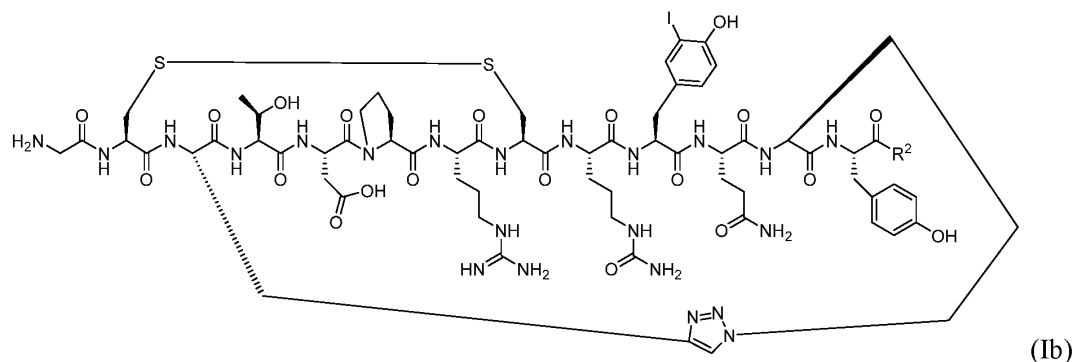
X is as defined above for the conotoxin peptide analog of Formula (I); and

wherein the C-terminus of the intermediate conotoxin peptide analog is as defined above for the

conotoxin peptide analog of Formula (I); and

wherein under said triazole formation conditions X_{AA}^3 reacts with X_{AA}^{12} to form the triazole bridge in the conotoxin peptide analog of Formula (I).

[00185] Also provided herein are methods of making a conotoxin peptide analog of Formula (Ib) (SEQ ID NO:104) or a pharmaceutically acceptable salt thereof,



wherein the C-terminus of the conotoxin peptide analog of Formula (Ib) is a carboxylic acid or an amide group,

comprising subjecting an intermediate conotoxin peptide analog or a salt thereof to triazole formation conditions, wherein the amino acid sequence of the intermediate conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 - X_{AA}^{10} -Gln- X_{AA}^{12} -Tyr (SEQ ID NO:107), wherein

X_{AA}^3 is (S)-propargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{10} is 3-iodo-Tyr;

X_{AA}^{12} is (S)-azidonorvaline; and

wherein the C-terminus of the intermediate conotoxin peptide analog is as defined above for the conotoxin peptide analog of Formula (Ib); and

wherein under said triazole formation conditions X_{AA}^3 reacts with X_{AA}^{12} to form a triazole bridge as depicted in the conotoxin peptide analog of Formula (Ib).

[00186] Also provided herein are methods of making a PEGylated conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a

conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent bond with the conotoxin peptide analog whereby the conotoxin peptide analog is covalently attached directly or via a linking group to the one or more PEG polymers, wherein the amino acid sequence of the conotoxin peptide analog is Gly-Cys-X_{AA}³-Thr-Asp-Pro-Arg-Cys-X_{AA}⁹-Trp-Gln-X_{AA}¹²-X (SEQ ID NO106), wherein

X_{AA}³ is selected from the group consisting of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline and (S)-bishomopropargyl glycine;

X_{AA}⁹ is Citrulline;

X_{AA}¹² is selected from the group consisting of (S)-azidohomoalanine, (S)-homopropargyl glycine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine;

wherein when X_{AA}³ is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}¹² is (S)-azidohomoalanine or (S)-azidonorvaline; when X_{AA}³ is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}¹² is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;

X is X_{AA}¹ or X_{AA}¹X_{AA}²; wherein X_{AA}¹ is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}² is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00187] Also provided herein are methods of making a PEGylated conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent

bond with the conotoxin peptide analog whereby the conotoxin peptide analog is covalently attached directly or via a linking group to the one or more PEG polymers, wherein the amino acid sequence of the conotoxin peptide analog is Gly-Cys-X_{AA}³-Thr-Asp-Pro-Arg-Cys-X_{AA}⁹-X_{AA}¹⁰-Gln-X_{AA}¹²-Tyr (SEQ ID NO107), wherein

X_{AA}³ is (S)-propargyl glycine;

X_{AA}⁹ is Citrulline;

X_{AA}¹⁰ is 3-iodo-Tyr;

X_{AA}¹² is (S)-azidonorvaline;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

4. BRIEF DESCRIPTION OF FIGURES

[00188] FIG. 1A-FIG. 1D: Disulfide shuffling of **CSP-4-NH2** in human serum. **FIG. 1A:** **CSP-4-NH2** was spiked into human serum and incubated at 37 °C for the specified period, after which samples were frozen, purified by precipitation and filtration and analyzed by RP-HPLC. A time-dependent conversion of main peak to secondary peak with faster retention time was observed. **FIG. 1B:** Individual injections of synthetically constructed **CSP-4-NH2** ribbon, bead and native isomers together with a sample of **CSP-4-NH2** isolated from human serum were analyzed. Comigration of serum-derived peaks indicated conversion of the native form to the ribbon isomer. **FIG. 1C:** Isolation of serum-derived compound **CSP-4-NH2** followed by lyophilization and mass spectrometry analysis indicated that both the native and ribbon isomer have the same mass-to-charge ratio. **FIG. 1D:** Conversion of the globular to ribbon form by glutathione reduction of **CSP-4-NH2** indicated that disulfide shuffling underlies isomerization.

[00189] FIG. 2A-FIG. 2B: Reverse phase HPLC analyses of conotoxin peptide analog **Ia** and conotoxin peptide analog **CSP-4-NH2** in rat plasma. **FIG. 2A:** reverse phase HPLC analyses of conotoxin peptide analog **Ia** at 0 h, 8 h and 24 h (black line: 0 h; dark grey line: 8 h; light grey line: 24 h). **FIG. 2B:** reverse phase HPLC analyses of conotoxin peptide analog **CSP-4-NH2** (“native” form, consisting of two disulfide bonds, one between Cys2 and Cys8, and a second between Cys3 and Cys12) in rat plasma at 0 h, 8 h and 24 h (black line: 0 h; dark grey

line: 8 h; light grey line: 24 h).

[00190] FIG. 3A-FIG. 3B: Concentration-response curves of conotoxin peptide analogs for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR. **FIG. 3A:** Concentration-response curves of conotoxin peptide analogs **Ia**, **Ia'** and **Ib'** for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR (●: **Ia**; ▲: **Ia'**; □: **Ib'**). **FIG. 3B:** Concentration-response curves of conotoxin peptide analogs **CSP-4-NH2** and **CSP-4-OH** for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR (●: **CSP-4-NH2**; ▲: **CSP-4-OH**).

[00191] FIG. 4: Comparison of the amino acid sequences of RgIA (SEQ ID NO:1), ImI (SEQ ID NO:2) and Vc1.1 (SEQ ID NO:3).

[00192] FIG. 5A-FIG. 5B: Concentration-response curves of conotoxin peptide analogs for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR. **FIG. 5A:** Concentration-response curves of conotoxin peptide analogs **CSP-4-OH** and **CSP-4-desTyr-OH** for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR (▲: **CSP-4-OH**; ■: **CSP-4-desTyr-OH**).

FIG. 5B: Concentration-response curves of conotoxin peptide analogs **1a** and **1q** for inhibition of ACh-gated currents in the human $\alpha 9\alpha 10$ nAChR (●: **1a**; ■: **1q**).

[00193] FIG. 6A-FIG. 6B: Representative traces from an oocyte injected with either the human (left) or rat (right) $\alpha 9\alpha 10$ nAChR. ACh-gated currents before and after exposure to compound PEGylated conotoxin peptide analog **IIa**. **FIG. 6A:** Response to ACh of the human $\alpha 9\alpha 10$ nAChR. **FIG. 6B:** Response to ACh of the rat $\alpha 9\alpha 10$ nAChR.

[00194] FIG. 7A-FIG. 7B: Pharmacokinetic profile of PEGylated conotoxin peptide analogs in rats. Mean concentration vs. time profiles of PEGylated conotoxin peptide analog **IIa** and conotoxin peptide analog **Ia** administered intravenously and subcutaneously to rats at 1 mg/kg (n=3). **FIG. 7A:** Pharmacokinetic profile of PEGylated conotoxin peptide analog **IIa** (●: 1 mg/kg IV administration; ■: 1 mg/kg SC administration). **FIG. 7B:** Pharmacokinetic profile of conotoxin peptide analog **Ia** (●: 1 mg/kg IV administration; ■: 1 mg/kg SC administration).

[00195] FIG. 8: Pharmacokinetic profile of PEGylated conotoxin peptide analog **IIa** in monkeys. Mean concentration vs time profile of compound 21 administered intravenously and subcutaneously to cynomolgus macaque monkeys at 1 mg/kg (n=3) (●: 1 mg/kg IV

administration; ■: 1 mg/kg SC administration).

[00196] **FIG. 9:** Analgesic efficacy and duration of effect of conotoxin peptide analog **Ib'** in a rat chronic constriction injury (CCI) model (■: 0.1 mg/kg/day of conotoxin peptide analog **Ib'**; ●: vehicle).

[00197] **FIG. 10A-FIG. 10C:** Analgesic efficacy and duration of effect of conotoxin peptide analog **CSP-4-NH2** and PEGylated derivatives of **CSP-4-NH2** in rat CIPN model. **FIG. 10A:** Mechanical hyperalgesia was measured by Randall-Selitto testing, demonstrating analgesic efficacy (normalized response) of **CSP-4-NH2** and PEGylated conotoxin peptides **VIII** and **X** in the rat CIPN model over 96 h post dose. **FIG. 10B:** Mechanical hyperalgesia was measured by Randall-Selitto testing, demonstrating analgesic efficacy (normalized response) of **CSP-4-NH2** and PEGylated conotoxin peptides **VII**, **IX**, **XI** and **XII** in the rat CIPN model over 96 h post dose. **FIG. 10C:** Summary plot of duration of efficacy of **CSP-4-NH2** and PEGylated derivatives of **CSP-4-NH2** showed that duration of efficacy correlates with both PEGylation conjugation chemistry (branch vs. linear) and conjugated PEG polymer size.

[00198] **FIG. 11A-FIG. 11C:** Analgesic efficacy and duration of effect of conotoxin peptide analogs **Ia'**, **Ia**, and PEGylated conotoxin peptide analog **IIa** in a rat chemotherapy induced peripheral neuropathy (CIPN) model. **FIG. 11A:** Administration of a single 0.5 mg/kg dose of conotoxin peptide analog **Ia'** to rats having oxaliplatin-induced peripheral neuropathy, 14 days following induction of neuropathy, resulted in a statistically significant reduction in mechanical hyperalgesia compared to vehicle treated control animals for up to 24 h. (■: 0.5 mg/kg of conotoxin peptide analog **Ia'**; ●: vehicle). **FIG. 11B:** Administration of a single 0.5 mg/kg dose of **Ia'** to rats with spared nerve injury on day 14 following surgical induction of the model resulted in a statistically significant reduction in mechanical hyperalgesia compared to vehicle treated control animals for up to 24 h (■: 0.5 mg/kg of conotoxin peptide analog **Ia'**; ●: vehicle). **FIG. 11C:** Administration of a single 0.5 mg/kg dose of conotoxin peptide analog **Ia** or PEGylated conotoxin peptide analog **IIa** to rats with oxaliplatin-induced peripheral neuropathy, 14 days following induction of neuropathy, resulted in a significant reduction in mechanical hyperalgesia compared to vehicle treated control animals for up to 4 h (conotoxin

peptide analog **Ia**) or 72 h (PEGylated conotoxin peptide analog **IIa**) post dose (◊ : vehicle; ◆ : conotoxin peptide analog **Ia**; ■ : PEGylated conotoxin peptide analog **IIa**). Mechanical hyperalgesia was measured by Randall Selitto paw withdrawal threshold in grams. The endpoint for all studies was the Randall Siletto paw withdrawal threshold in grams. Two-way ANOVA, *p<0.05, **p<0.01, ***p<0.001.

5. DETAILED DESCRIPTION

[00199] It is to be understood that the disclosure is not limited to the particular embodiments set forth herein, and it is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[00200] Provided herein are alpha-conotoxin peptide analogs, including alpha-conotoxin peptide analogs that are covalently attached to polyethylene glycol (PEG), and pharmaceutical compositions of such alpha-conotoxin peptide analogs. Also provided herein are methods of treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) (e.g., the $\alpha 9\alpha 10$ subtype of the nAChR) in a subject.

5.1. Abbreviations used herein

Abbreviation	Meaning
AcOH	Acetic acid
(Boc) ₂ O	Di-tert-butyl dicarbonate
<i>t</i> Bu	<i>tert</i> -Butyl
CH ₂ Cl ₂	Dichloromethane
CH ₃ CN	Acetonitrile
CuSO ₄	Copper(II) Sulfate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIPEA	N,N-diisopropylethylamine
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
EDT	1,2-Ethanedithiol
Fmoc	Fluorenylmethyloxycarbonyl
Et ₂ O	Diethyl Ether
HOAt	1-Hydroxy-7-azabenzotriazole
h	Hour(s)
ⁱ PrOH	Iso-propanol
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3 oxid hexafluorophosphate
min	Minute(s)
MeOH	Methanol
PBS	Phosphate-buffered saline
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PEG	Polyethylene glycol
PPTS	Pyridinium p-toluenesulfonate
RT	Retention Time
TBTU	O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
TEA	Triethylamine
TFA	Trifluoroacetic acid
Trt	Triphenylmethyl

[00201] It will be appreciated that throughout this disclosure reference is made to amino acids according to the single letter or three letter codes. The amino acids forming all or a part of a peptide may be from among the known 21 naturally occurring amino acids, which are referred to by both their single letter abbreviations and their common three-letter abbreviation. In the peptide sequences provided herein, conventional amino acid residues have their conventional meaning. Thus, “Leu” is leucine, “Ile” is isoleucine, “Nle” is norleucine, and so on. To assist the reader, conventional amino acids and their corresponding three letter and single letter abbreviations are as follows:

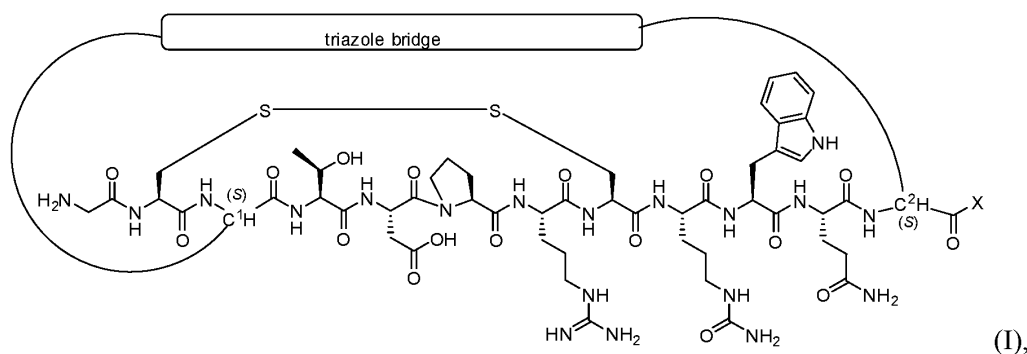
alanine	Ala	(A)
arginine	Arg	(R)
asparagine	Asn	(N)
aspartic acid	Asp	(D)
cysteine	Cys	(C)
glutamic acid	Glu	(E)
glutamine	Gln	(Q)
glycine	Gly	(G)
histidine	His	(H)
isoleucine	Ile	(I)
leucine	Leu	(L)
lysine	Lys	(K)
methionine	Met	(M)
phenylalanine	Phe	(F)
proline	Pro	(P)
serine	Ser	(S)
threonine	Thr	(T)
tryptophan	Trp	(W)
tyrosine	Tyr	(Y)
valine	Val	(V)

[00202] In the peptide sequences provided herein, other amino acids and their corresponding three letter abbreviations are as follows:

(S)-5-azidonorvaline	5-azidoNVa
(S)-propargyl glycine	Pra
(S)-3-azido-alanine	3-azidoAla
(S)-homopropargyl glycine	homoPra
(S)- γ -azido-homoalanine	azidoHomoAla
(S)-bis-homopropargyl glycine	bishomoPra
Citrulline	Cit

5.2. Conotoxin Peptide RgIA Analogs

[00203] In one aspect, provided herein are conotoxin peptide analogs of Formula (I) (SEQ ID NO:93):



or a pharmaceutically acceptable salt thereof,

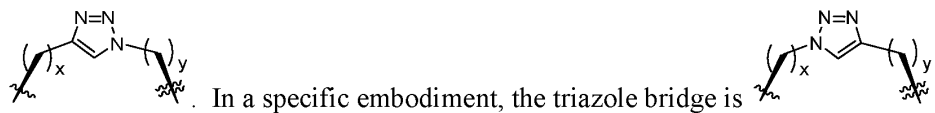
wherein

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00204] The “(S)” notations near C¹ and C² carbon in Formula (I) indicate the absolute configuration of C¹ and C² carbon.

[00205] In various embodiments, the triazole bridge is or , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog; and wherein x is 1, 2, 3, or 4; y is 2, 3 or 4. In a specific embodiment, the triazole bridge is

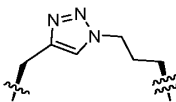


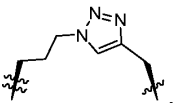
[00206] In a specific embodiment, x is 1, 2, 3, or 4. In one preferred embodiment, x is 1, 2, or 3. In a specific embodiment, x is 1 or 2. In one embodiment, x is 1. In another embodiment, x

is 2.

[00207] In a specific embodiment, y is 2, 3, or 4. In a specific embodiment, y is 2 or 3. In one embodiment, y is 3. In another embodiment, y is 2.

[00208] In a specific embodiment, x is 1, 2 or 3, and y is 2 or 3. In a specific embodiment, x is 1, 2, or 3, and y is 2. In a specific embodiment, x is 1, 2, or 3, and y is 3. In a specific embodiment, x is 1 or 2, and y is 2 or 3. In a specific embodiment, x is 1 or 3, and y is 2 or 3. In a specific embodiment, x is 2 or 3, and y is 2 or 3. In a specific embodiment, x is 1, and y is 2 or 3. In a specific embodiment, x is 2, and y is 2 or 3. In a specific embodiment, x is 3, and y is 2 or 3. In one preferred embodiment, x is 1 and y is 3. In one preferred embodiment, x is 2 and y is 3. In one preferred embodiment, x is 2 and y is 2.

[00209] In a specific embodiment, the triazole bridge is , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

[00210] In a specific embodiment, the triazole bridge is , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

[00211] In a specific embodiment, X is X_{AA}¹. In a specific embodiment, X is X_{AA}¹X_{AA}².

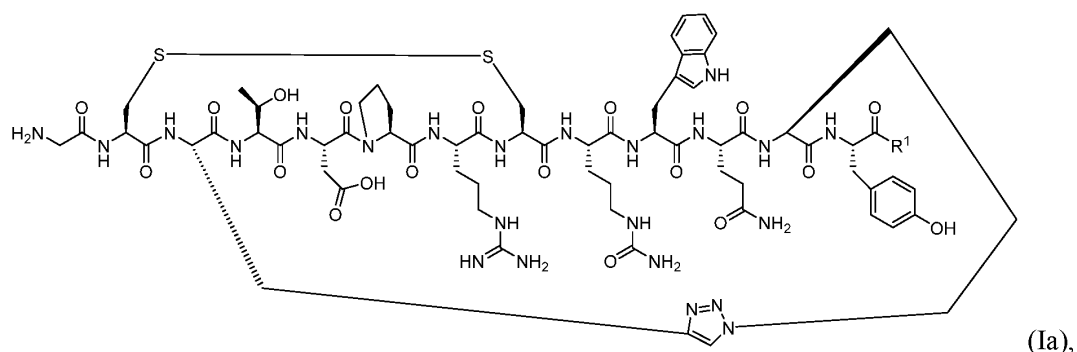
[00212] In a specific embodiment, X_{AA}¹ is Tyr, Phe, Trp, or a D- isomer of Tyr, Phe, or Trp. In a specific embodiment, X_{AA}¹ is Tyr, Phe, or Trp. In a specific embodiment, X_{AA}¹ is Tyr, D-Tyr, or Phe. In a specific embodiment, X_{AA}¹ is Tyr or D-Tyr. In a specific embodiment, X_{AA}¹ is Tyr or Phe. In a specific embodiment, X_{AA}¹ is D-Tyr or Phe. In a specific embodiment, X_{AA}¹ is Phe. In a specific embodiment, X_{AA}¹ is D-Phe. In a specific embodiment, X_{AA}¹ is Trp. In a specific embodiment, X_{AA}¹ is D-Trp. In a specific embodiment, X_{AA}¹ is D-Tyr. In one preferred embodiment, X_{AA}¹ is Tyr.

[00213] In a specific embodiment, X_{AA}² is N-Me-Gly, D-Tyr, or N-Me-Tyr. In a specific embodiment, X_{AA}² is N-Me-Gly or D-Tyr. In a specific embodiment, X_{AA}² is D-Tyr or N-Me-Tyr. In a specific embodiment, X_{AA}² is N-Me-Gly or N-Me-Tyr. In a specific embodiment, X_{AA}² is N-Me-Gly. In a specific embodiment, X_{AA}² is N-Me-Tyr. In a specific embodiment, X_{AA}² is D-Tyr. In a specific embodiment, X_{AA}² is optionally present. In a specific embodiment, X_{AA}² is present. In a specific embodiment, X_{AA}² is absent.

[00214] In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), (Tyr)-(N-Me-Tyr), N-Me-Tyr, D-Arg, N-Me-D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of Tyr, Phe, and D-Tyr. In a specific embodiment, X is selected from the group consisting of (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of N-Me-Tyr, D-Arg, N-Me-D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is Tyr. In a specific embodiment, X is Phe. In a specific embodiment, X is D-Tyr. In a specific embodiment, X is (Tyr)-(D-Tyr). In a specific embodiment, X is (Tyr)-(N-Me-Gly). In a specific embodiment, X is (Tyr)-(N-Me-Tyr). In a specific embodiment, X is N-Me-Tyr. In a specific embodiment, X is D-Arg. In a specific embodiment, X is N-Me-D-Tyr. In a specific embodiment, X is beta-Tyr. In a specific embodiment, X is N-Me-Arg.

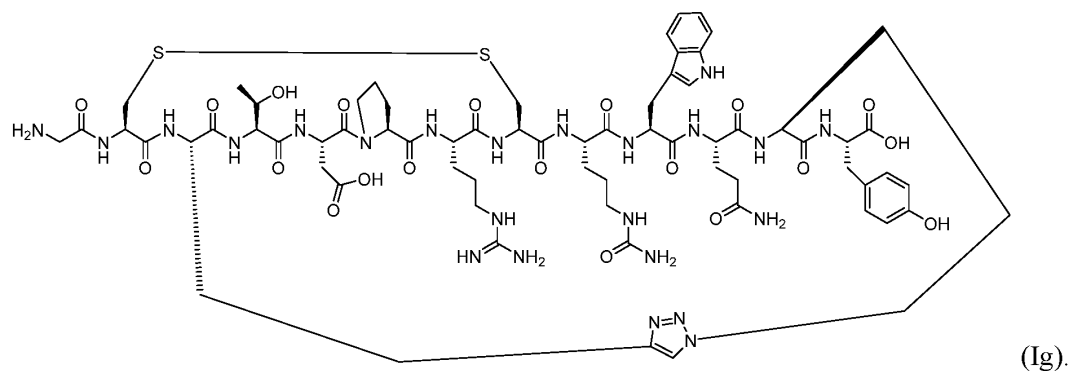
[00215] In a specific embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group. In one preferred embodiment, the C-terminus of the conotoxin peptide analog is OH. In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH₂.

[00216] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ia) (SEQ ID NO:94):

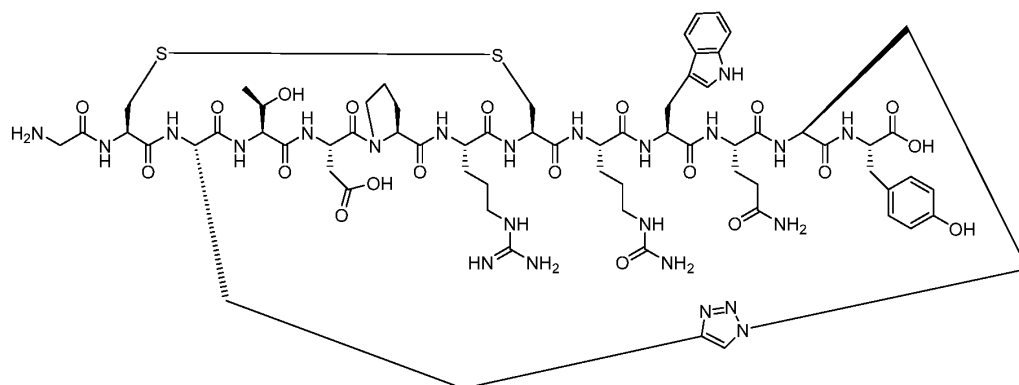


wherein R^1 is OH or NH_2 . In a specific embodiment, R^1 is OH. In a specific embodiment, R^1 is NH_2 .

[00217] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ig) (SEQ ID NO:30):

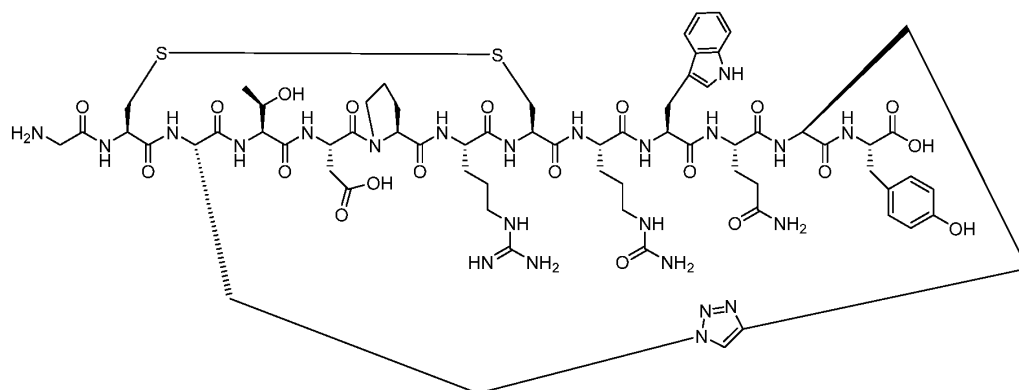


[00218] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ih) (SEQ ID NO:33):



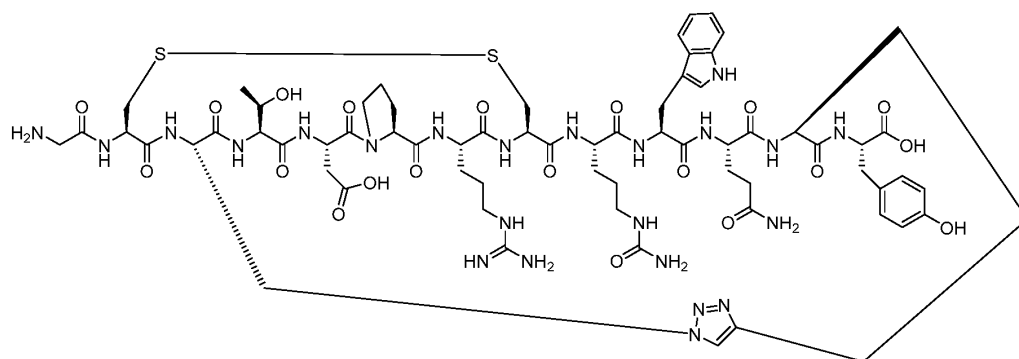
(Ih).

[00219] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ii) (SEQ ID NO:36):



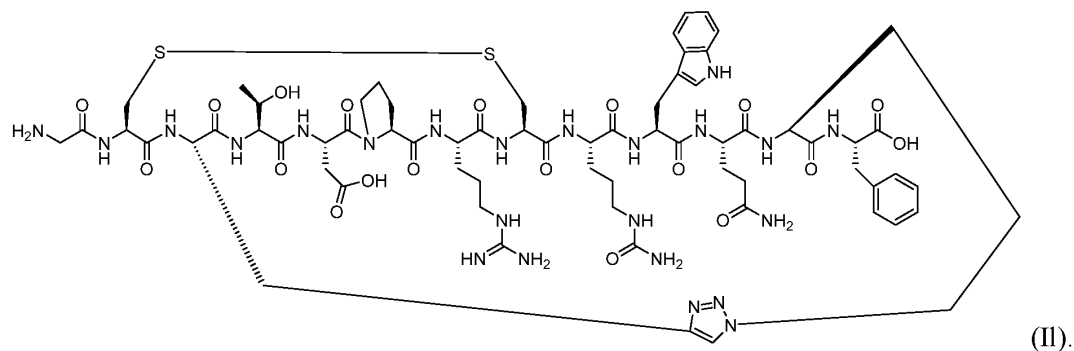
(Ii).

[00220] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ik) (SEQ ID NO:42):

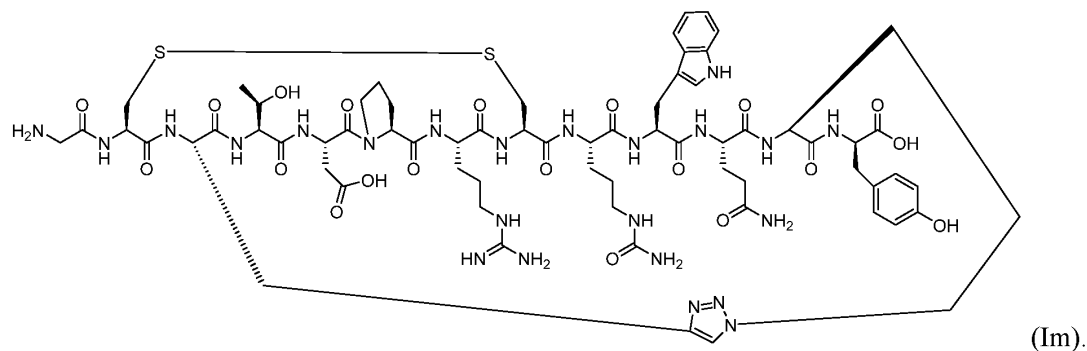


(Ik).

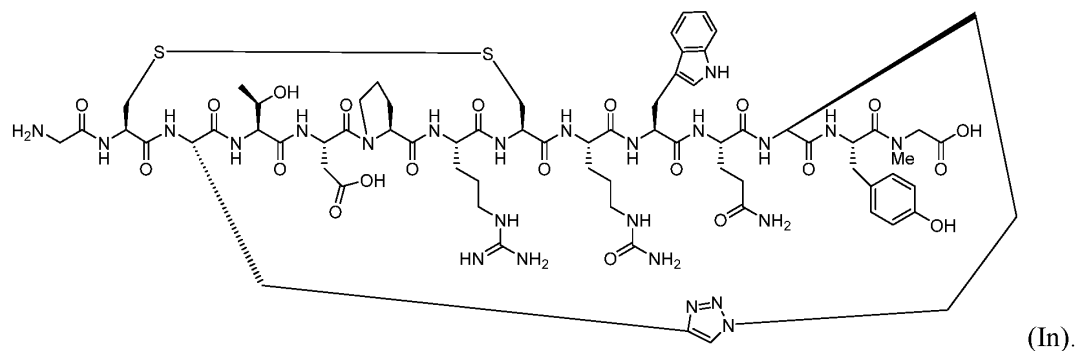
[00221] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (II) (SEQ ID NO:45):



[00222] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Im) (SEQ ID NO:48):

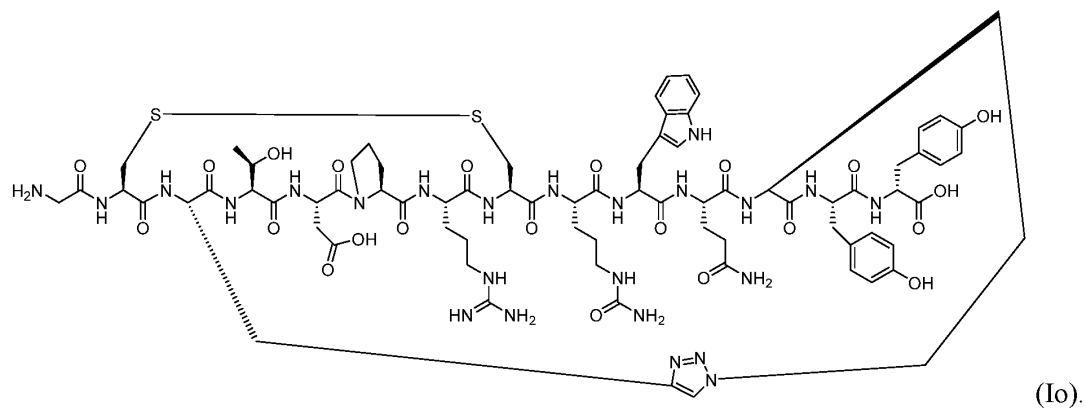


[00223] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (In) (SEQ ID NO:51):

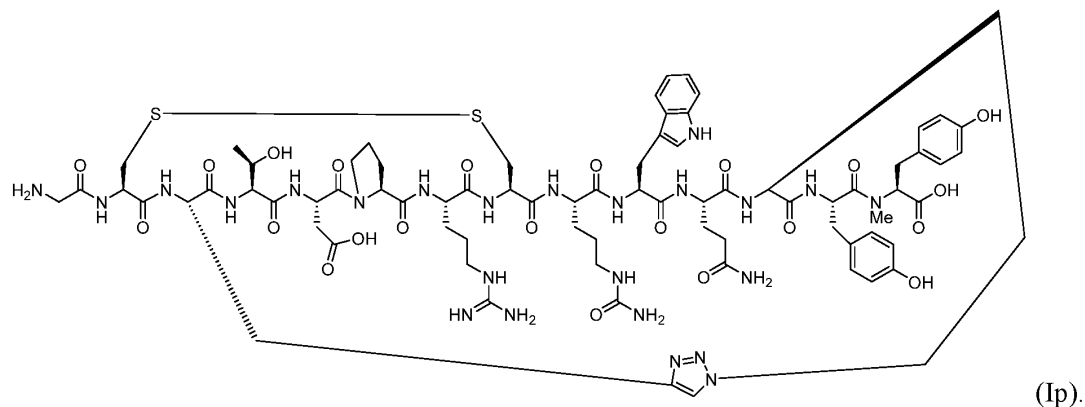


[00224] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (In) (SEQ ID NO:51):

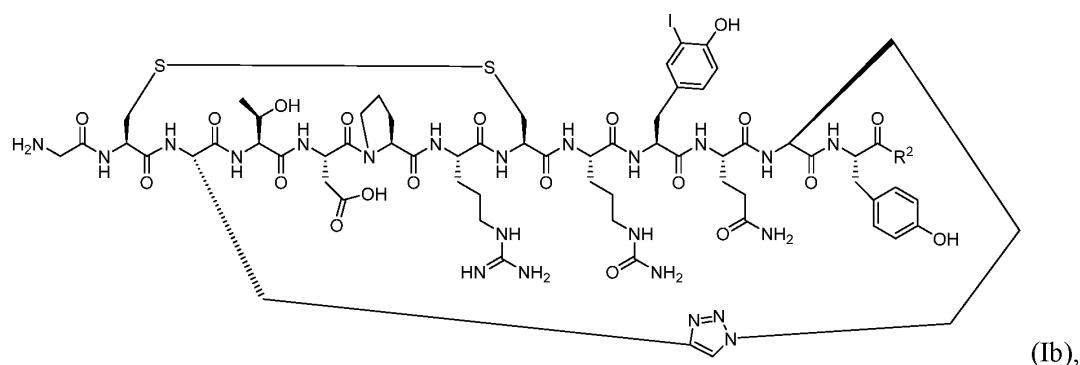
acceptable salt of Formula (I) is of Formula (Io) (SEQ ID NO:54):



[00225] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ip) (SEQ ID NO:57):



[00226] In another aspect, provided herein are conotoxin peptide analogs of Formula (Ib) (SEQ ID NO:104):



or a pharmaceutically acceptable salt thereof,

wherein R^2 is OH or NH_2 .

[00227] In a specific embodiment, R^2 is OH. In a specific embodiment, R^2 is NH_2 .

[00228] In another aspect, provided herein are conotoxin peptide analogs selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, **Ib'**, **Ic**, **Id**, **Ie**, **If**, **Ig**, **Ih**, **Ii**, **Ij**, **Ik**, **Il**, **Im**, **In**, **Io**, **Ip**, **Iq**, **Ir**, **Is**, **It**, **Iu**, and **Iv**, or a pharmaceutically acceptable salt thereof.

[00229] In a specific embodiment, the conotoxin peptide analog is selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, **Ib'**, **Ig**, **Ih**, **Ii**, **Ik**, **Il**, **Im**, **In**, **Io**, and **Ip**.

[00230] In a specific embodiment, the conotoxin peptide analog is selected from the group consisting of conotoxin peptide analogs **Ia**, **Ia'**, **Ib**, and **Ib'**. In one preferred embodiment, the conotoxin peptide analog is conotoxin peptide analog **Ia** or **Ia'**. In one preferred embodiment, the conotoxin peptide analog is conotoxin peptide analog **Ia**.

5.3. PEGylated Conotoxin Peptide RgIA Analogs

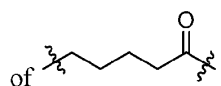
[00231] In one embodiment, the conotoxin peptide analogs of the invention are PEGylated, in particular, covalently attached to one or more PEG polymers.

[00232] In a specific embodiment, a conotoxin peptide analog is covalently attached to one PEG polymer. In a specific embodiment, a conotoxin peptide analog is covalently attached to more than one PEG polymers. In a specific embodiment, a conotoxin peptide analog is covalently attached to two PEG polymers. In a specific embodiment, a conotoxin peptide analog is covalently attached to three PEG polymers.

[00233] In a preferred embodiment, a PEG polymer is covalently attached to the N-terminus of a conotoxin peptide analog, most preferably one PEG polymer is attached only to the N-terminus. In a specific embodiment, a PEG polymer is covalently attached to the C-terminus of a conotoxin peptide analog. In a specific embodiment, a PEG polymer is covalently attached to an amino acid residue position that is not the N-terminus or the C-terminus of the conotoxin peptide analog.

[00234] In a specific embodiment, a PEG polymer is covalently attached to the conotoxin peptide analog via a linking group. In a specific embodiment, a PEG polymer is covalently attached to the conotoxin peptide analog directly.

[00235] In one preferred embodiment, the linking group is a valerate linker having a formula



of . In a specific embodiment, the linking group is a butylene. In a specific embodiment, the linking group is a carbonyl.

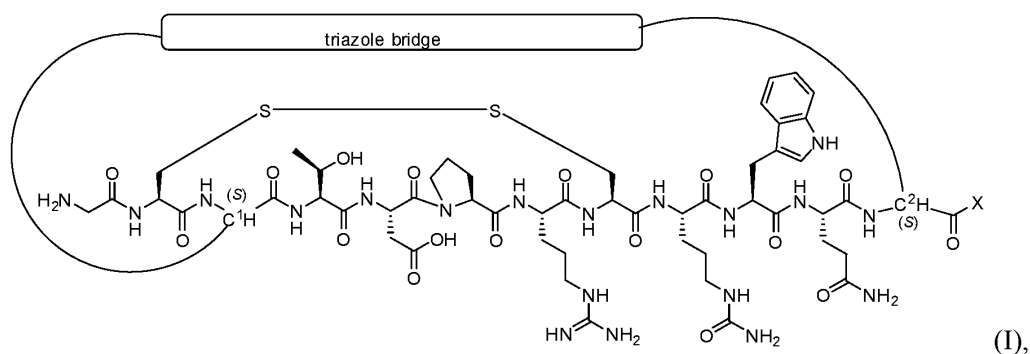
[00236] In a specific embodiment, the PEG polymer is a linear or branched PEG polymer. In a specific embodiment, the PEG polymer is a branched PEG polymer. In one preferred embodiment, the PEG polymer is a linear PEG polymer.

[00237] In a specific embodiment, the PEG polymer has a molecular weight in the range of 10 kDa and 40 kDa. In one preferred embodiment, the PEG polymer is a 30 kDa PEG polymer. In specific embodiments, the PEG polymer is a linear 30 kDa PEG polymer. In specific embodiments, the PEG polymer is a linear 30 kDa mPEG polymer.

[00238] In a preferred embodiment, one PEG polymer is attached at the amino terminus of a conotoxin peptide analog, and the PEG polymer is attached via a linking group to the conotoxin

peptide analog, and the linking group is a valerate linker having a formula of , and the PEG polymer is a linear 30 kDa mPEG polymer.

[00239] In a specific embodiment, provided herein is a PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, wherein the conotoxin peptide analog is of Formula (I) (SEQ ID NO:93):



wherein

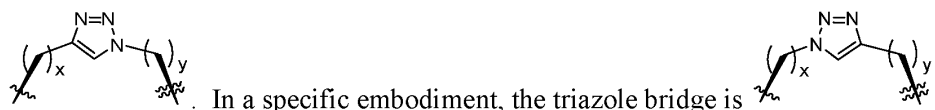
X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group;

and

wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one or more polyethylene glycol (PEG) polymers.

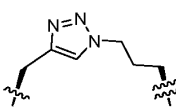
[00240] In various embodiments, the triazole bridge is or , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog; and wherein x is 1, 2, 3, or 4; y is 2, 3 or 4. In a specific embodiment, the triazole bridge is

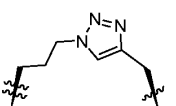


[00241] In a specific embodiment, x is 1, 2, 3, or 4. In one preferred embodiment, x is 1, 2, or 3. In a specific embodiment, x is 1 or 2. In one embodiment, x is 1. In another embodiment, x is 2.

[00242] In a specific embodiment, y is 2, 3, or 4. In a specific embodiment, y is 2 or 3. In one embodiment, y is 3. In another embodiment, y is 2.

[00243] In a specific embodiment, x is 1, 2 or 3, and y is 2 or 3. In a specific embodiment, x is 1, 2, or 3, and y is 2. In a specific embodiment, x is 1, 2, or 3, and y is 3. In a specific embodiment, x is 1 or 2, and y is 2 or 3. In a specific embodiment, x is 1 or 3, and y is 2 or 3. In a specific embodiment, x is 2 or 3, and y is 2 or 3. In a specific embodiment, x is 1, and y is 2 or 3. In a specific embodiment, x is 2, and y is 2 or 3. In a specific embodiment, x is 3, and y is 2 or 3. In one preferred embodiment, x is 1 and y is 3. In one preferred embodiment, x is 2 and y is 3. In one preferred embodiment, x is 2 and y is 2.

[00244] In a specific embodiment, the triazole bridge is , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

[00245] In a specific embodiment, the triazole bridge is , wherein the single wavy line (~~~~) indicates the point of attachment of the triazole bridge to the C¹ carbon of the conotoxin peptide analog, and the double wavy lines (~~~~) indicate the point of attachment of the triazole bridge to the C² carbon of the conotoxin peptide analog.

[00246] In a specific embodiment, X is X_{AA}¹. In a specific embodiment, X is X_{AA}¹X_{AA}².

[00247] In a specific embodiment, X_{AA}¹ is Tyr, Phe, Trp, or a D- isomer of Tyr, Phe, or Trp. In a specific embodiment, X_{AA}¹ is Tyr, Phe, or Trp. In a specific embodiment, X_{AA}¹ is Tyr, D-Tyr, or Phe. In a specific embodiment, X_{AA}¹ is Tyr or D-Tyr. In a specific embodiment, X_{AA}¹ is Tyr or Phe. In a specific embodiment, X_{AA}¹ is D-Tyr or Phe. In a specific embodiment, X_{AA}¹ is Phe. In a specific embodiment, X_{AA}¹ is D-Phe. In a specific embodiment, X_{AA}¹ is Trp. In a specific embodiment, X_{AA}¹ is D-Trp. In a specific embodiment, X_{AA}¹ is D-Tyr. In one preferred embodiment, X_{AA}¹ is Tyr.

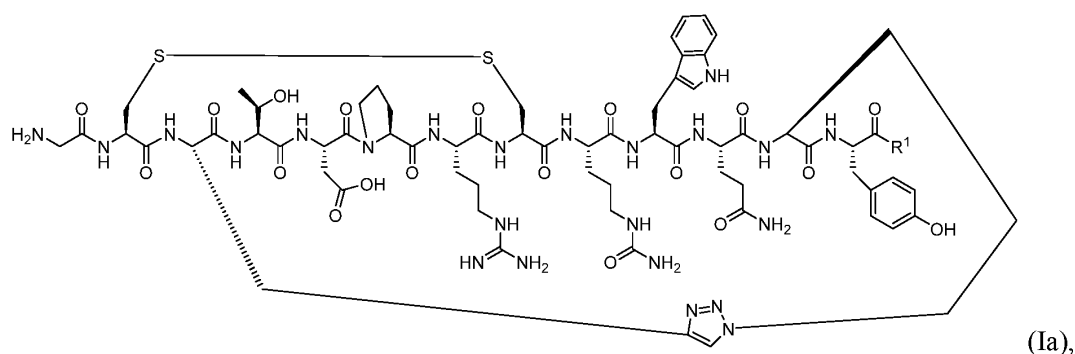
[00248] In a specific embodiment, X_{AA}² is N-Me-Gly, D-Tyr, or N-Me-Tyr. In a specific embodiment, X_{AA}² is N-Me-Gly or D-Tyr. In a specific embodiment, X_{AA}² is D-Tyr or N-Me-Tyr. In a specific embodiment, X_{AA}² is N-Me-Gly or N-Me-Tyr. In a specific embodiment,

X_{AA}² is N-Me-Gly. In a specific embodiment, X_{AA}² is N-Me-Tyr. In a specific embodiment, X_{AA}² is D-Tyr. In a specific embodiment, X_{AA}² is optionally present. In a specific embodiment, X_{AA}² is present. In a specific embodiment, X_{AA}² is absent.

[00249] In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), (Tyr)-(N-Me-Tyr), N-Me-Tyr, D-Arg, N-Me-D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of Tyr, Phe, and D-Tyr. In a specific embodiment, X is selected from the group consisting of (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of N-Me-Tyr, D-Arg, N-Me-D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is Tyr. In a specific embodiment, X is Phe. In a specific embodiment, X is D-Tyr. In a specific embodiment, X is (Tyr)-(D-Tyr). In a specific embodiment, X is (Tyr)-(N-Me-Gly). In a specific embodiment, X is (Tyr)-(N-Me-Tyr). In a specific embodiment, X is N-Me-Tyr. In a specific embodiment, X is D-Arg. In a specific embodiment, X is N-Me-D-Tyr. In a specific embodiment, X is beta-Tyr. In a specific embodiment, X is N-Me-Arg.

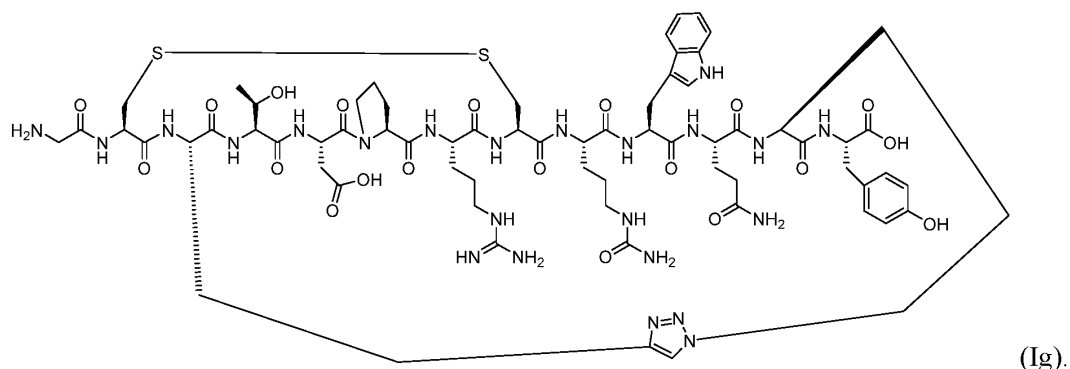
[00250] In a specific embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group. In one preferred embodiment, the C-terminus of the conotoxin peptide analog is OH. In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH₂.

[00251] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ia) (SEQ ID NO:94):

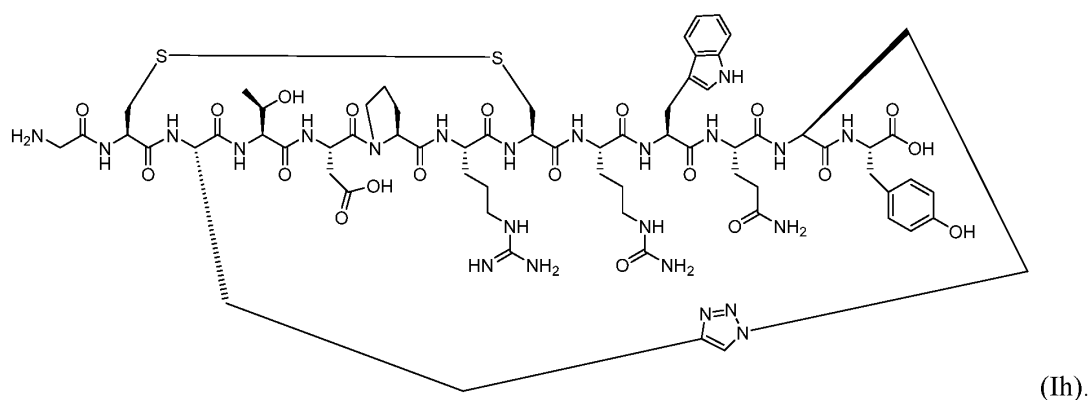


wherein R¹ is OH or NH₂. In a specific embodiment, R¹ is OH. In a specific embodiment, R¹ is NH₂.

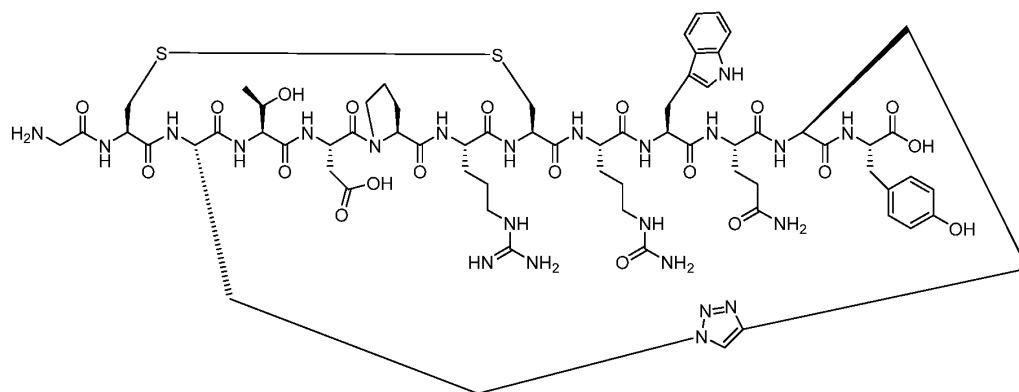
[00252] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ig) (SEQ ID NO:30):



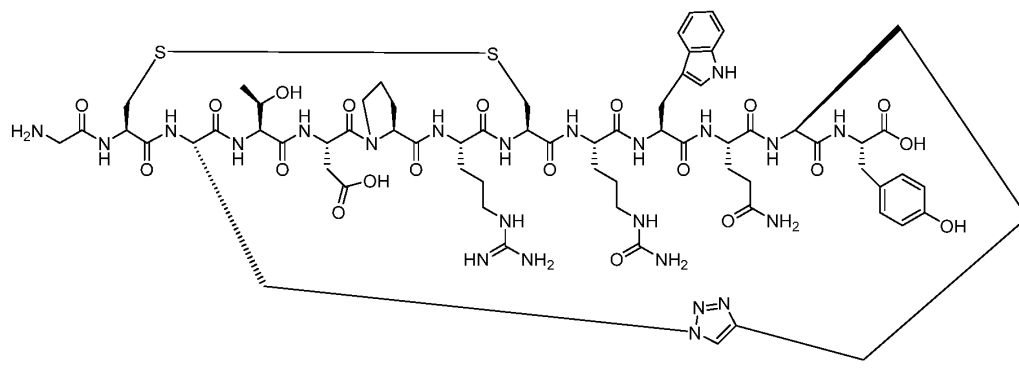
[00253] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ih) (SEQ ID NO:33):



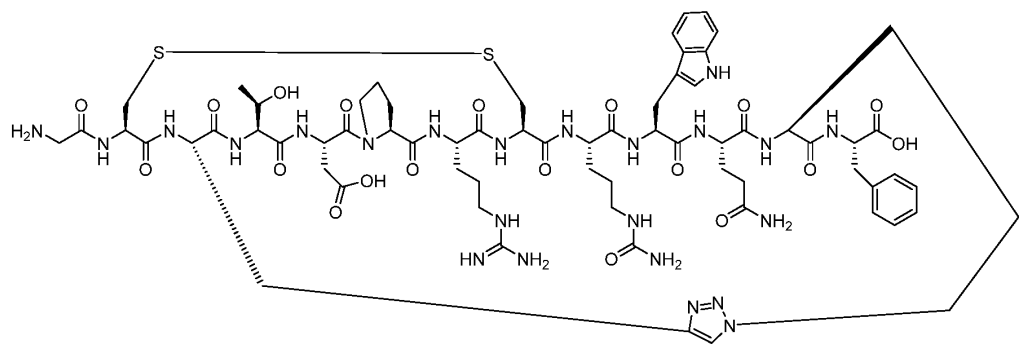
[00254] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ii) (SEQ ID NO:36):



[00255] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Ik) (SEQ ID NO:42):

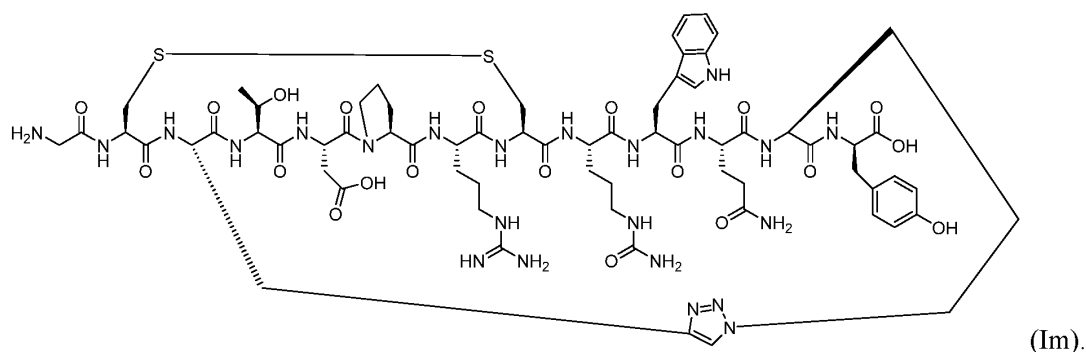


[00256] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (II) (SEQ ID NO:45):

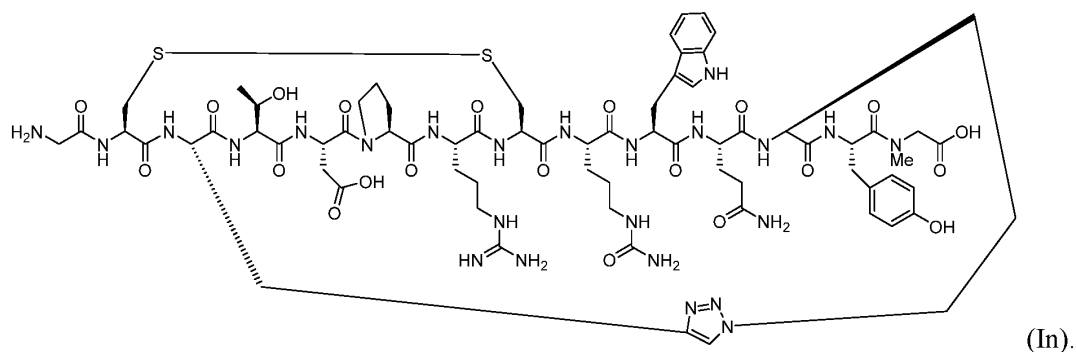


[00257] In specific embodiments, the conotoxin peptide analog or pharmaceutically

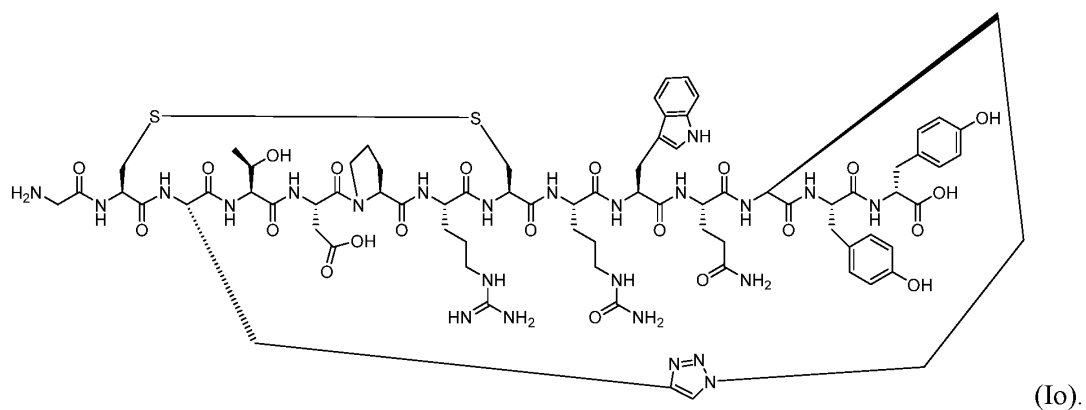
acceptable salt of Formula (I) is of Formula (Im) (SEQ ID NO:48):



[00258] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (In) (SEQ ID NO:51):

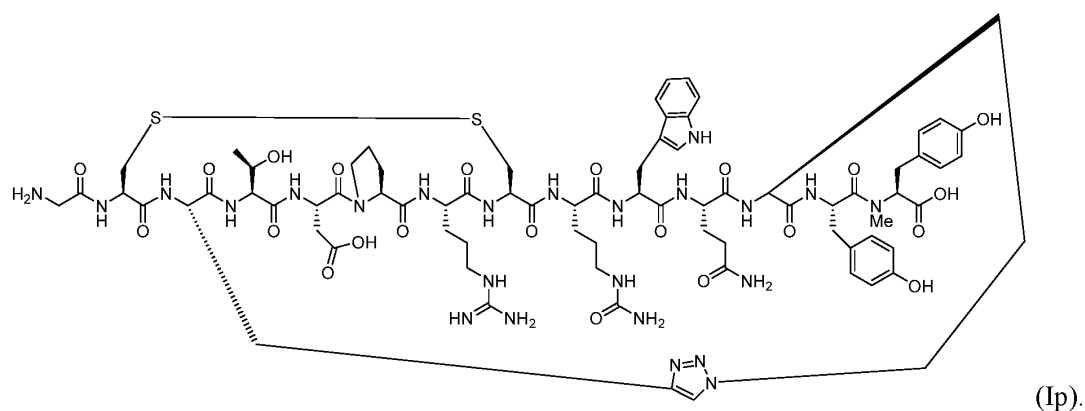


[00259] In specific embodiments, the conotoxin peptide analog or pharmaceutically acceptable salt of Formula (I) is of Formula (Io) (SEQ ID NO:54):

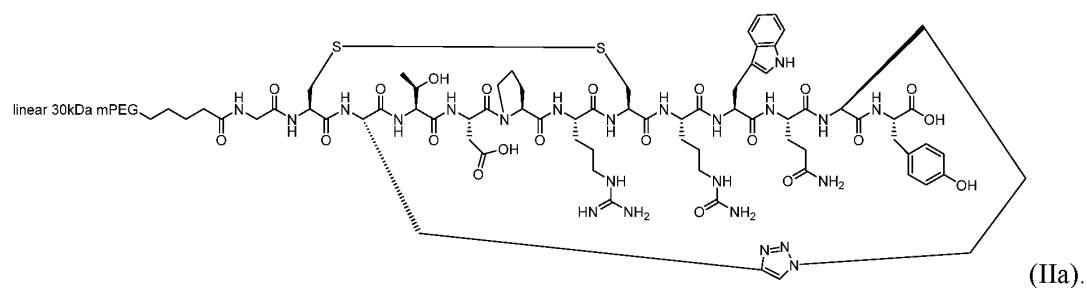


[00260] In specific embodiments, the conotoxin peptide analog or pharmaceutically

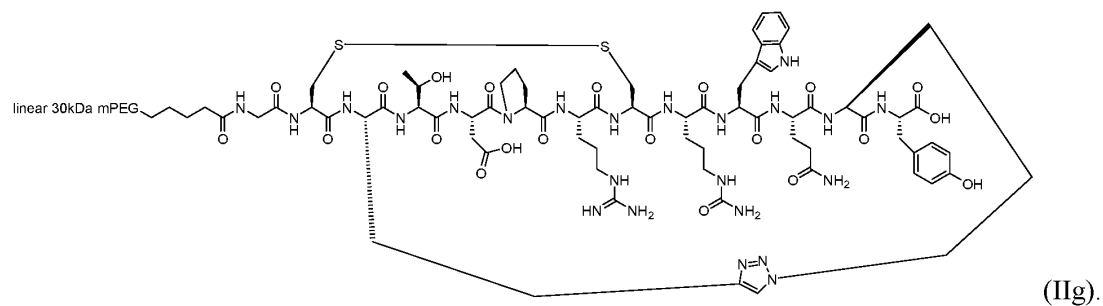
acceptable salt of Formula (I) is of Formula (Ip) (SEQ ID NO:57):



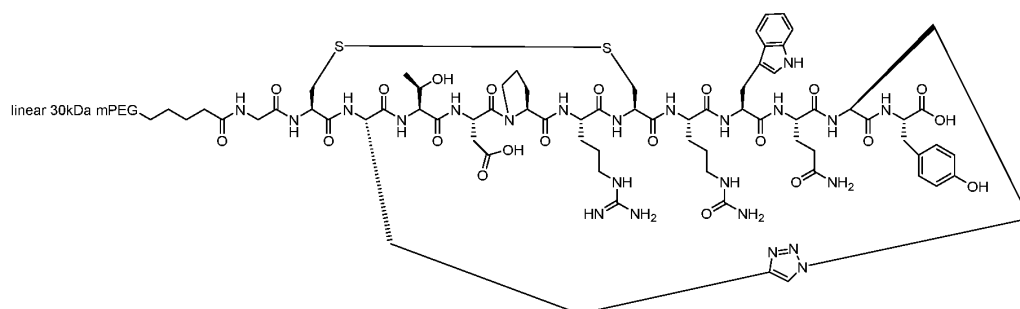
[00261] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIa) (SEQ ID NO:83):



[00262] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIg) (SEQ ID NO:95):

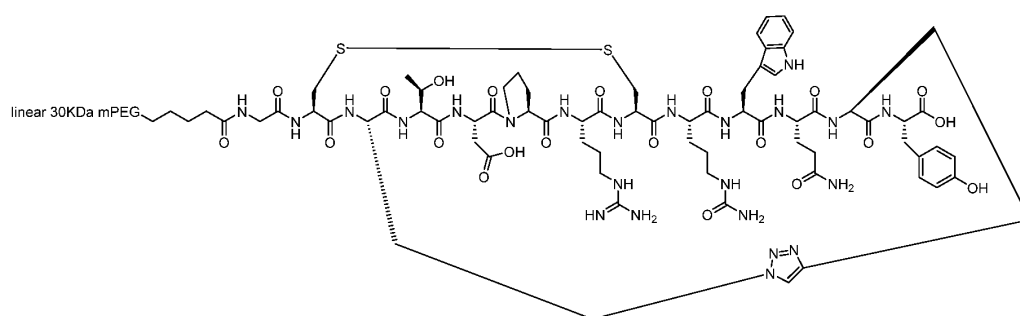


[00263] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIh) (SEQ ID NO:96):



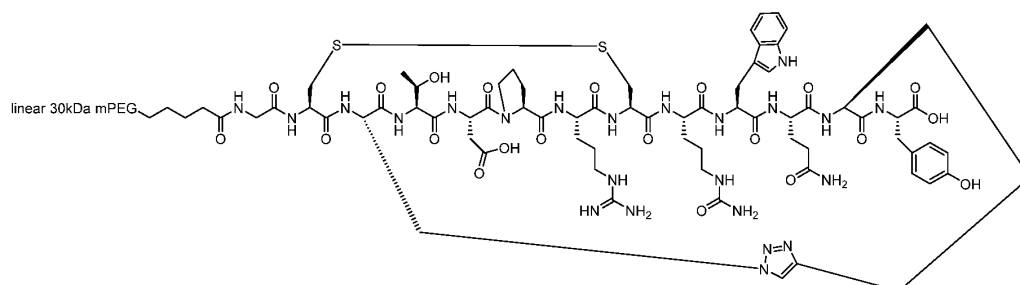
(IIh).

[00264] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIi) (SEQ ID NO:97):



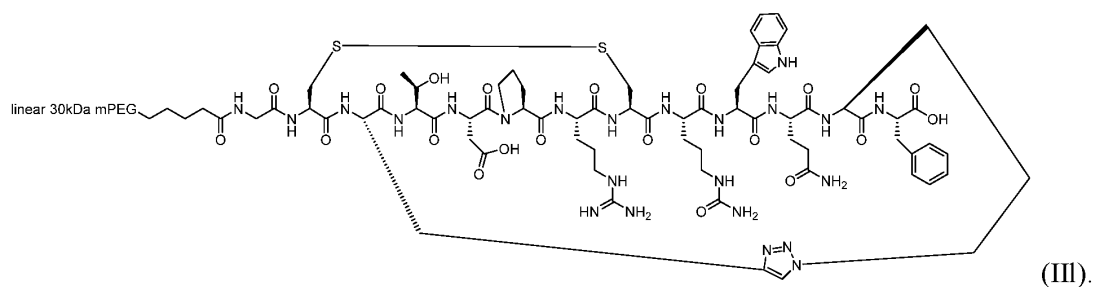
(IIi).

[00265] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIk) (SEQ ID NO:98):

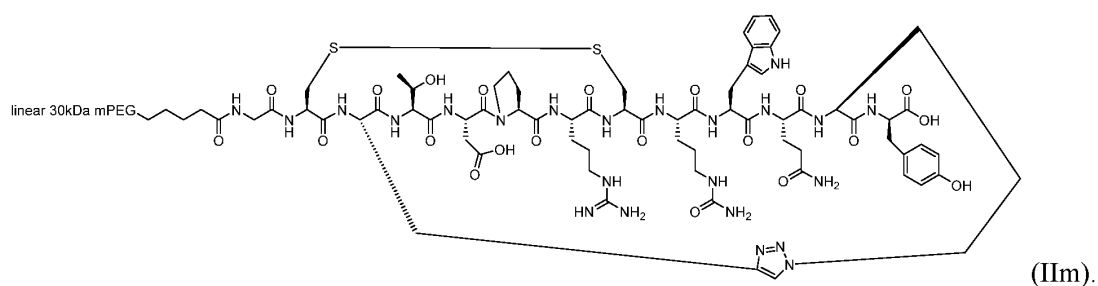


(IIk).

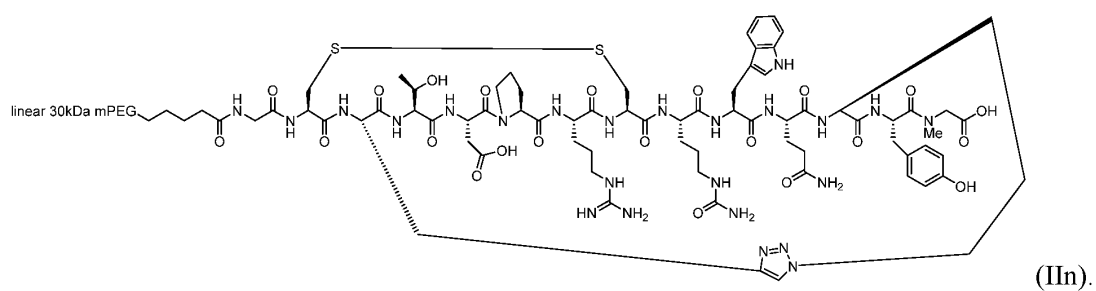
[00266] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (III) (SEQ ID NO:99):



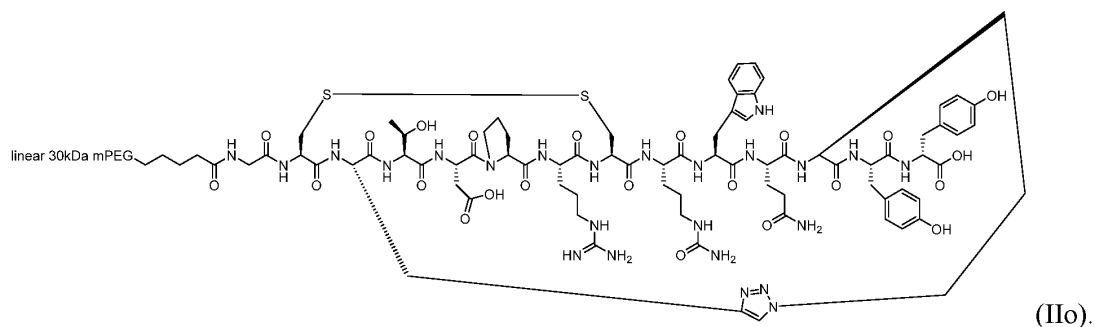
[00267] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIIm) (SEQ ID NO:100):



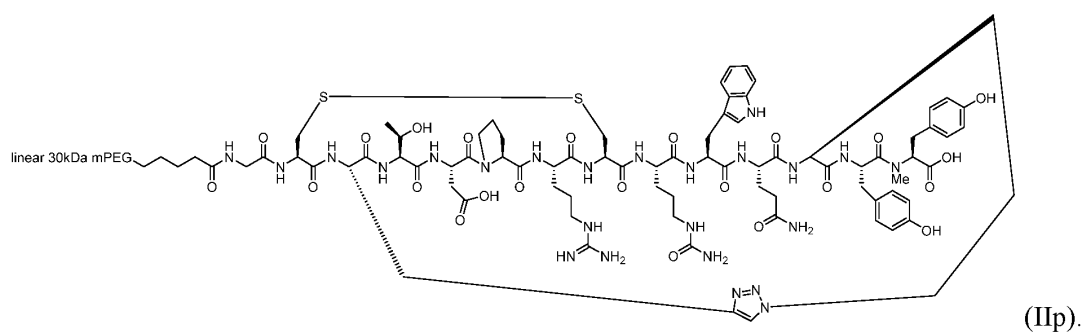
[00268] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIIn) (SEQ ID NO:101):



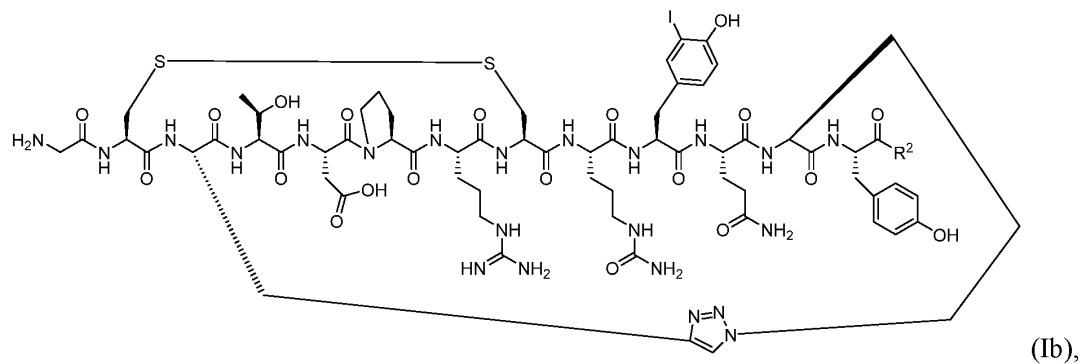
[00269] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIo) (SEQ ID NO:102):



[00270] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIp) (SEQ ID NO:103):



[00271] In another aspect, provided herein are PEGylated conotoxin peptide analogs or a pharmaceutically acceptable salt thereof, wherein the conotoxin peptide analog is of Formula (Ib) (SEQ ID NO:104):



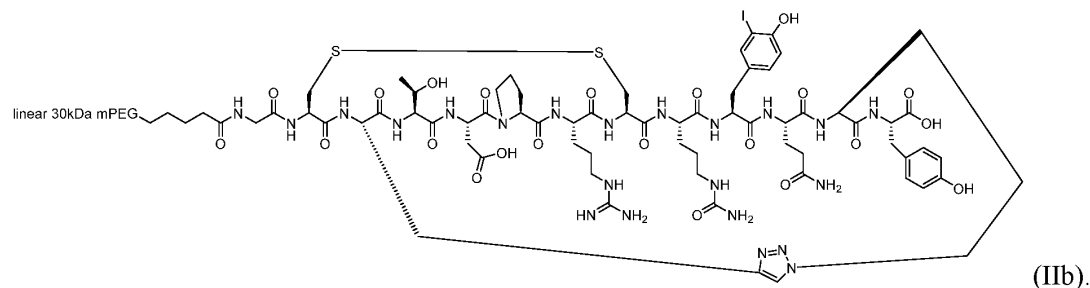
wherein R^2 is OH or NH_2 ; and

wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one

or more polyethylene glycol (PEG) polymers.

[00272] In a specific embodiment of Formula (Ib), R^2 is OH. In a specific embodiment of Formula (Ib), R^2 is NH_2 .

[00273] In specific embodiments, the PEGylated conotoxin peptide analog is of Formula (IIb) (SEQ ID NO:105):



5.4. Pharmaceutical Compositions

[00274] Also provided herein are “pharmaceutical compositions,” which comprise a conotoxin peptide analog provided herein or a PEGylated conotoxin peptide analog provided herein, and one or more pharmaceutically acceptable carriers. In a particular embodiment, a conotoxin peptide analog is present in a therapeutically effective amount. In a particular embodiment, a conotoxin peptide analog is present in a prophylactically effective amount. The pharmaceutical compositions can be used in accordance with the methods and uses provided herein. Thus, for example, the pharmaceutical compositions can be administered to a subject in order to practice the treatment or prevention methods and uses provided herein. Pharmaceutical compositions provided herein can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein.

[00275] Pharmaceutical compositions typically comprise a therapeutically effective amount of at least one of the conotoxin peptide analogs or PEGylated conotoxin peptide analogs provided herein, and a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, but are not limited to, antioxidants (*e.g.*, ascorbic acid), preservatives (*e.g.*, benzyl alcohol, methyl parabens, p-hydroxybenzoate), emulsifying agents, suspending agents, dispersing agents, solvents, buffers, lubricants, fillers, and/or diluents. For example, a suitable

vehicle may be physiological saline solution. Typical buffers that can be used include, but are not limited to pharmaceutically acceptable weak acids, weak bases, or mixtures thereof. Buffer components can also include water soluble reagents such as phosphoric acid, tartaric acids, succinic acid, citric acid, acetic acid, and salts thereof.

[00276] A vehicle may contain other pharmaceutically acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, or stability of the pharmaceutical composition. In a specific embodiment, the vehicle is an aqueous buffer. In a specific embodiment, a vehicle comprises, for example, sodium chloride.

[00277] Pharmaceutical compositions provided herein may contain still other pharmaceutically acceptable formulation agents for modifying or maintaining the rate of release of a conotoxin peptide analog described herein. Such formulation agents include, for example, those substances known to those skilled in the art in preparing sustained-release or controlled release formulations. Regarding pharmaceutically acceptable formulation agents, see, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712, and The Merck Index, 12th Ed. (1996, Merck Publishing Group, Whitehouse, NJ).

[00278] In a specific embodiment, a pharmaceutical composition is provided in a sterile vial as a solution, suspension, gel, emulsion, or dehydrated or lyophilized powder. Such compositions may, for example, be stored either in a ready to use form, a lyophilized form requiring reconstitution prior to use, or a liquid form requiring dilution prior to use. In specific embodiments, a pharmaceutical composition is provided in a single-use container (*e.g.*, a single-use vial, ampoule, syringe, or autoinjector). In a specific embodiment, a pharmaceutical composition is provided in a multi-use container (*e.g.*, a multi-use vial or cartridge). Any drug delivery apparatus may be used to deliver a conotoxin peptide analog or a PEGylated conotoxin peptide analog or pharmaceutical composition described herein, including implants (*e.g.*, implantable pumps) and catheters. In a specific embodiment, depot injections, can be utilized to release a conotoxin peptide analog or a PEGylated conotoxin peptide analog or pharmaceutical composition described herein over a defined period of time. Depot injections are generally administered subcutaneously or intramuscularly.

[00279] A pharmaceutical composition can be formulated to be compatible with its intended route of administration as described herein.

[00280] Pharmaceutical compositions may be in the form of a sterile injectable aqueous suspension. This suspension may be formulated using suitable dispersing or wetting agents and suspending agents known to those skilled in the art. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. In one embodiment, the sterile injectable solution can, for example, be a solution in 1,3-butane diol. Acceptable diluents, solvents or dispersion media that may be employed include water, Ringer's solution, saline, Cremophor EL™, phosphate buffered saline (PBS), ethanol, polyol (*e.g.*, glycerol or liquid polyethylene glycol), and mixtures thereof. Prolonged absorption of particular injectable formulations can be achieved by including an agent that delays absorption (*e.g.*, aluminum monostearate or gelatin).

[00281] Pharmaceutical compositions can also include carriers to protect the composition against degradation or elimination from the body. Various antibacterial and antifungal agents, for example, parabens, chlorobutanol, ascorbic acid, thimerosal, can be included in the pharmaceutical composition.

5.5. Methods of Treatment or Prevention

[00282] In one embodiment, provided herein is a method for treating or preventing a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) comprising administering to a subject a therapeutically effective amount of a conotoxin peptide analog as described herein or a pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a pharmaceutical composition as described herein.

[00283] In a preferred embodiment, the condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) is a condition conducive to treatment or prevention by inhibition of the $\alpha 9\alpha 10$ subtype of nAChR.

[00284] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nAChR, *e. g.*, the $\alpha 9\alpha 10$ subtype of nAChR, is pain or

inflammation.

[00285] In a preferred embodiment, the method provided herein is a method of treating a condition conducive to treatment by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) comprising administering to a subject a therapeutically effective amount of a conotoxin peptide analog as described herein or a pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a pharmaceutical composition as described herein.

[00286] In a preferred embodiment, the condition conducive to treatment by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) is a condition conducive to treatment by inhibition of the $\alpha 9\alpha 10$ subtype of nAChR.

[00287] In a specific embodiment, the condition conducive to treatment by inhibition of an $\alpha 9$ -containing nAChR, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, is pain or inflammation.

[00288] In a preferred embodiment, the method provided herein is a method of preventing a condition conducive to prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) comprising administering to a subject a therapeutically effective amount of a conotoxin peptide analog as described herein or a pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a pharmaceutical composition as described herein.

[00289] In a preferred embodiment, the condition conducive to prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR) is a condition conducive to treatment by inhibition of the $\alpha 9\alpha 10$ subtype of nAChR.

[00290] In a specific embodiment, the condition conducive to prevention by inhibition of an $\alpha 9$ -containing nAChR, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, is pain or inflammation.

[00291] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, is pain. In a specific embodiment, the pain is selected from the group consisting of general pain, chronic pain, neuropathic pain, nociceptive pain, inflammatory pain, pain induced by peripheral nerve damage, pain induced by an inflammatory disorder, pain induced by a metabolic disorder, pain induced by cancer, pain induced by chemotherapy, pain induced by a

surgical procedure, and pain induced by a burn. In a specific embodiment, the pain is cancer-related chronic pain. In a specific embodiment, the pain is pain associated with neuropathy, which, by way of example, can be drug (e.g., cancer chemotherapeutic)-induced neuropathy or infection-induced neuropathy. In a specific embodiment, the pain is neuralgia, including but not limited to postherpetic neuralgia, for example, postherpetic neuralgia due to shingles.

[00292] In a specific embodiment, the condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, is an inflammatory condition. In a specific embodiment, the inflammatory condition is selected from the group consisting of inflammation, chronic inflammation, a rheumatic disease, sepsis, fibromyalgia, inflammatory bowel disease, sarcoidosis, endometriosis, uterine fibroids, an inflammatory skin disease, an inflammatory condition of the lungs, a disease associated with inflammation of the nervous system, periodontal disease, and cardiovascular disease. In a specific embodiment, the inflammatory condition is mediated by immune cells. In a specific embodiment, the inflammatory condition is long-term inflammation and/or peripheral neuropathy following injury.

[00293] In a specific embodiment, the condition associated with the $\alpha 9$ -containing nicotinic acetylcholine receptor, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, is pain and inflammation. In a specific embodiment, the condition associated with the $\alpha 9\alpha 10$ subtype of nAChR condition is inflammation and neuropathy.

[00294] In another aspect, provided herein is a method of treating or preventing a condition associated with activation of an $\alpha 9$ -containing nAChR, e. g., the $\alpha 9\alpha 10$ subtype of nAChR, in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a pharmaceutical composition as described herein.

[00295] In another aspect, provided herein is a method of treating or preventing pain or inflammation in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog as described herein or pharmaceutically acceptable salt thereof, or a PEGylated conotoxin peptide analog as described herein or pharmaceutically

acceptable salt thereof, or pharmaceutical composition as described herein. In a specific embodiment of a method of treating or preventing pain, the pain is pain associated with neuropathy, which, by way of example, can be drug (e.g., cancer chemotherapeutic)-induced neuropathy or infection-induced neuropathy. In a specific embodiment, the pain is neuralgia, including but not limited to postherpetic neuralgia, for example, postherpetic neuralgia due to shingles.

[00296] In a specific embodiment, the method provided herein is a method of treating pain or inflammation. In a specific embodiment, the method provided herein is a method of treating pain. In a specific embodiment, the method provided herein is a method of treating inflammation.

[00297] In a specific embodiment, the method provided herein is a method of preventing pain or inflammation. In a specific embodiment, the method provided herein is a method of preventing pain. In a specific embodiment, the method provided herein is a method of preventing inflammation.

[00298] In a specific embodiment, the method of preventing pain is to prevent pain associated with neuropathy. In a specific embodiment of such method, the pain associated with neuropathy is neuralgia. In a specific embodiment, the neuralgia is postherpetic neuralgia. In a specific embodiment, the method of preventing pain is to prevent pain induced by cancer chemotherapy (and thus a conotoxin peptide analog or pharmaceutically acceptable salt thereof is administered to the patient prior to the patient being administered cancer chemotherapy). In a specific embodiment, the method of preventing pain is to prevent pain induced by a surgical procedure; in a specific embodiment of such method, a conotoxin peptide analog or pharmaceutically acceptable salt thereof is administered prior to and/or concurrently with the surgical procedure, in particular, where the surgical procedure is reasonably expected to result in pain and/or inflammation.

[00299] In another aspect, provided herein is a method of inhibiting an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR), e.g., the $\alpha 9\alpha 10$ subtype of nAChR, in a subject comprising administering to the subject a therapeutically effective amount of a conotoxin peptide analog as described herein or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition

as described herein.

[00300] In specific embodiments, “treat”, “treating”, “treatment” and the like refer to an action (such as administering a conotoxin peptide analog or pharmaceutically acceptable salt thereof or a pharmaceutical composition comprising a conotoxin peptide analog or pharmaceutically acceptable salt thereof) initiated after a disease, disorder or condition, or a symptom thereof, has been diagnosed, observed, and the like so as to eliminate, reduce, suppress, mitigate, or ameliorate, either temporarily or permanently, at least one of the underlying causes of a disease, disorder, or condition afflicting a subject, or at least one of the symptoms associated with a disease, disorder, condition afflicting a subject. Thus, treatment can include inhibiting (*i.e.*, arresting the development or further development of the disease, disorder or condition or clinical symptoms associated therewith) an active disease.

[00301] In specific embodiments of the invention in which a method of prevention is carried out, the prevention need not be complete; in a specific embodiment, the prevention is partial inhibition or partial prevention or a reduction in the disease, condition or disorder relative to that which would otherwise be expected to occur absent the administration of the conotoxin peptide analog or pharmaceutically acceptable salt thereof.

[00302] In specific embodiments, the administering can be by injecting or otherwise physically delivering a conotoxin peptide analog into a patient, such as by mucosal, intradermal, intravenous, intramuscular delivery and/or any other method of physical delivery described herein or known in the art. When a disease, disorder or condition, or a symptom thereof, is being treated, administration of the conotoxin peptide analog or pharmaceutically acceptable salt thereof typically occurs after the onset of disease, disorder or condition or symptom thereof. When a disease, disorder or condition, or a symptom thereof, is being prevented, administration of the conotoxin peptide analog or pharmaceutically acceptable salt thereof typically occurs before the onset of the disease, disorder or condition or symptom thereof. In a specific embodiment, a conotoxin peptide analog or pharmaceutically acceptable salt thereof is administered prior to and after the onset of pain and/or inflammation to both prevent and treat the pain and/or inflammation.

[00303] In various embodiments, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, as described herein, is used in any of the methods provided herein. For example, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, provided herein can be used in a method for treatment or prevention of a disease or disorder or condition described herein. Thus, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, provided herein can be for use as a medicament. A conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, provided herein can be for use in a method for the treatment or prevention of a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR), e.g. the $\alpha 9\alpha 10$ subtype of nAChR. A conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, provided herein can be for use in a method for the treatment or prevention of a condition conducive to treatment or prevention by inhibition of an $\alpha 9$ -containing nicotinic acetylcholine receptor (nAChR), e.g. the $\alpha 9\alpha 10$ subtype of nAChR. For example, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, provided herein can be for use in a method for the treatment or prevention of pain or inflammation.

5.6. Routes of Administration and Dosage

[00304] A conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, described herein may be administered to a patient by any of a variety of routes. These include, but are not limited to, parenteral, intra-articular, intranasal, intratracheal, oral, intradermal, topical, intramuscular, intraperitoneal, transdermal, intravenous, intratumoral, conjunctival, subcutaneous, and pulmonary routes. In a specific embodiment, a conotoxin peptide analog,

including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, described herein is administered via subcutaneous administration. In a specific embodiment, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, described herein is administered via intravenous administration. In a specific embodiment, a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, described herein is administered via intra-articular administration.

[00305] The amount of a conotoxin peptide analog, including but not limited to a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, to be administered to the patient will depend on the nature of the disease and the condition of the patient, and can be determined by standard clinical techniques and the knowledge of the physician.

[00306] The precise dose and regime to be employed in a composition will also depend on the route of administration, and the seriousness of the disease, and should be decided according to the judgment of the physician and each patient's circumstance. Determination of the proper dosage can be determined by one skilled in the medical arts. The total daily dosage may be divided and administered in portions throughout the day or by means providing continuous delivery.

[00307] In a specific embodiment, a conotoxin peptide analog or pharmaceutically acceptable salt thereof is administered to a human subject at a dosage of between 0.01 to about 50 mg/kg of body weight. In a specific embodiment, a conotoxin peptide analog, e.g., a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, is administered to a human subject at a dosage of about 0.5 mg/kg of body weight. In specific embodiments, the human dose is from 1 to 1000 mg/day. In specific embodiments, the human daily dose is from 1 to 750 mg/day; or from 10 to 500 mg/day.

[00308] A conotoxin peptide analog, e.g., a PEGylated conotoxin peptide analog, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, disclosed herein can be administered at once, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient

being treated, and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

5.7. Patients

[00309] The patient referred to in this disclosure, can be, but is not limited to, a human or non-human vertebrate such as a wild, domestic or farm animal. In a specific embodiment, the patient is a mammal, *e.g.*, a human, a cow, a dog, a cat, a goat, a horse, a sheep, a pig, a rabbit, a rat, or a mouse. In a preferred embodiment, the patient is a human patient.

[00310] In a specific embodiment, the human patient is an adult (at least age 16). In another specific embodiment, the human patient is an adolescent (age 12-15). In another specific embodiment, the patient is a child (under age 12).

5.8. Methods of Making Conotoxin Peptide Analogs

[00311] This disclosure provides methods of making conotoxin peptide analogs, in which intermediate conotoxin peptide analogs (prior to triazole bridge formation) are subjected to triazole formation conditions to form the conotoxin peptide analogs of the invention.

5.8.1. Intermediate Conotoxin Peptide Analogs

[00312] This disclosure provides syntheses of intermediate conotoxin peptide analogs for synthesizing conotoxin peptide analogs with a triazole bridge. An intermediate conotoxin peptide analog can, for example, be synthesized using solid phase peptide synthesis. When carrying out the solid phase peptide synthesis, necessary amino acids with appropriate reaction groups (for example, an azide group or an acetylene group) for forming a triazole bridge can be introduced into the peptide (*e.g.*, a conotoxin peptide of RgIA). The necessary amino acids can be introduced into the 3- and 12- position of the conotoxin peptide analog of RgIA.

[00313] Into one of the 3- and 12- positions is introduced an amino acid residue bearing an

acetylene group (e.g., (S)-propargyl glycine, (S)-homopropargyl glycine, or (S)-bishomopropargyl glycine), and into the other position is introduced an amino acid bearing an azide group (e.g., (S)-azidoalanine, (S)-azidohomoalanine, (S)-azidonorvaline), whereby the acetylene group and the azide group within the same intermediate conotoxin peptide can form a triazole ring when the intermediate conotoxin peptide is subjected to triazole formation conditions, thereby forming a triazole bridge in the resulting conotoxin peptide analog.

[00314] In a specific embodiment, the necessary amino acids for forming the triazole bridge are, for example, selected from the group of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine.

[00315] In another aspect, provided herein is an intermediate conotoxin peptide analog or a salt thereof, wherein the amino acid sequence of the conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 -Trp-Gln- X_{AA}^{12} -X (SEQ ID NO:106), wherein

X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline and (S)-bishomopropargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{12} is selected from the group consisting of (S)-azidohomoalanine, (S)-homopropargyl glycine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine;

wherein when X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}^{12} is (S)-azidohomoalanine or (S)-azidonorvaline; and when X_{AA}^3 is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}^{12} is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the intermediate conotoxin peptide analog is a carboxylic acid or an amide group.

[00316] In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine or (S)-azidoalanine. In a specific embodiment, wherein X_{AA}^{12} is (S)-azidonorvaline or (S)-bishomopropargyl glycine. In a specific embodiment, X_{AA}^3 is (S)-propargyl glycine and X_{AA}^{12} is (S)-azidonorvaline. In a specific embodiment, X_{AA}^3 is (S)-homopropargyl glycine and X_{AA}^{12} is (S)-azidonorvaline. In a specific embodiment, X_{AA}^3 is (S)-homopropargyl glycine and X_{AA}^{12} is (S)-azidohomoalanine. In a specific embodiment, X_{AA}^3 is (S)-azidohomoalanine and X_{AA}^{12} is (S)-homopropargyl glycine. In a specific embodiment, X_{AA}^3 is (S)-azidohomoalanine and X_{AA}^{12} is (S)-homopropargyl glycine.

[00317] In a specific embodiment, X is X_{AA}^1 . In a specific embodiment, X is $X_{AA}^1X_{AA}^2$.

[00318] In a specific embodiment, X_{AA}^1 is Tyr, Phe, Trp, or a D- isomer of Tyr, Phe, or Trp. In a specific embodiment, X_{AA}^1 is Tyr, Phe, or Trp. In a specific embodiment, X_{AA}^1 is Tyr, D-Tyr, or Phe. In a specific embodiment, X_{AA}^1 is Tyr or D-Tyr. In a specific embodiment, X_{AA}^1 is Tyr or Phe. In a specific embodiment, X_{AA}^1 is D-Tyr or Phe. In a specific embodiment, X_{AA}^1 is Phe. In a specific embodiment, X_{AA}^1 is D-Phe. In a specific embodiment, X_{AA}^1 is Trp. In a specific embodiment, X_{AA}^1 is D-Trp. In a specific embodiment, X_{AA}^1 is D-Tyr. In a preferred embodiment, X_{AA}^1 is Tyr.

[00319] In a specific embodiment, X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr. In a specific embodiment, X_{AA}^2 is N-Me-Gly or D-Tyr. In a specific embodiment, X_{AA}^2 is D-Tyr or N-Me-Tyr. In a specific embodiment, X_{AA}^2 is N-Me-Gly or N-Me-Tyr. In a specific embodiment, X_{AA}^2 is N-Me-Gly. In a specific embodiment, X_{AA}^2 is N-Me-Tyr. In a specific embodiment, X_{AA}^2 is D-Tyr. In a specific embodiment, X_{AA}^2 is optionally present. In a specific embodiment, X_{AA}^2 is present. In a specific embodiment, X_{AA}^2 is absent.

[00320] In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), (Tyr)-(N-Me-Tyr), N-Me-Tyr, D-Arg, N-Me-D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is selected from the group consisting of Tyr, Phe, D-Tyr, (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of Tyr, Phe, and D-Tyr. In a specific embodiment, X is selected from the group consisting of (Tyr)-(D-Tyr), (Tyr)-(N-Me-Gly), and (Tyr)-(N-Me-Tyr). In a specific embodiment, X is selected from the group consisting of N-Me-Tyr, D-Arg, N-Me-

D-Tyr, beta-Tyr, and N-Me-Arg. In a specific embodiment, X is Tyr. In a specific embodiment, X is Phe. In a specific embodiment, X is D-Tyr. In a specific embodiment, X is (Tyr)-(D-Tyr). In a specific embodiment, X is (Tyr)-(N-Me-Gly). In a specific embodiment, X is (Tyr)-(N-Me-Tyr). In a specific embodiment, X is N-Me-Tyr. In a specific embodiment, X is D-Arg. In a specific embodiment, X is N-Me-D-Tyr. In a specific embodiment, X is beta-Tyr. In a specific embodiment, X is N-Me-Arg.

[00321] In a specific embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group. In one preferred embodiment, the C-terminus of the conotoxin peptide analog is OH. In a specific embodiment, the C-terminus of the conotoxin peptide analog is NH₂.

[00322] In specific embodiments, X_{AA}³ is (S)-propargyl glycine, X_{AA}¹² is (S)-azidonorvaline, X is Tyr. In one embodiment, the C-terminus of the conotoxin peptide analog is a carboxylic acid group. In another embodiment, the C-terminus of the conotoxin peptide analog is an amide group.

[00323] In specific embodiments, X_{AA}³ is (S)-homopropargyl glycine, X_{AA}¹² is (S)-azidonorvaline, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00324] In specific embodiments, X_{AA}³ is (S)-homopropargyl glycine, X_{AA}¹² is (S)-azidohomoalanine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00325] In specific embodiments, X_{AA}³ is (S)-azidohomoalanine, X_{AA}¹² is (S)-homopropargyl glycine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00326] In specific embodiments, X_{AA}³ is (S)-azidoalanine, X_{AA}¹² is (S)-bishomopropargyl glycine, X is Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00327] In specific embodiments, X_{AA}³ is (S)-propargyl glycine, X_{AA}¹² is (S)-azidonorvaline, X is Phe; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00328] In specific embodiments, X_{AA}³ is (S)-propargyl glycine, X_{AA}¹² is (S)-azidonorvaline,

X is D-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00329] In specific embodiments, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-N-Me-Gly; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00330] In specific embodiments, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-D-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00331] In specific embodiments, X_{AA}^3 is (S)-propargyl glycine, X_{AA}^{12} is (S)-azidonorvaline, X is Tyr-N-Me-Tyr; and wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid group.

[00332] In another aspect, provided herein is an intermediate conotoxin peptide analog or a salt thereof, wherein the amino acid sequence of the conotoxin peptide analog is

Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 - X_{AA}^{10} -Gln- X_{AA}^{12} -Tyr (SEQ ID NO:107),

wherein

X_{AA}^3 is (S)-propargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{10} is 3-iodo-Tyr;

X_{AA}^{12} is (S)-azidonorvaline;

wherein the C-terminus of the intermediate conotoxin peptide analog is a carboxylic acid or an amide group.

[00333] In a specific embodiment, the C-terminus of the intermediate conotoxin peptide analog is a carboxylic acid group.

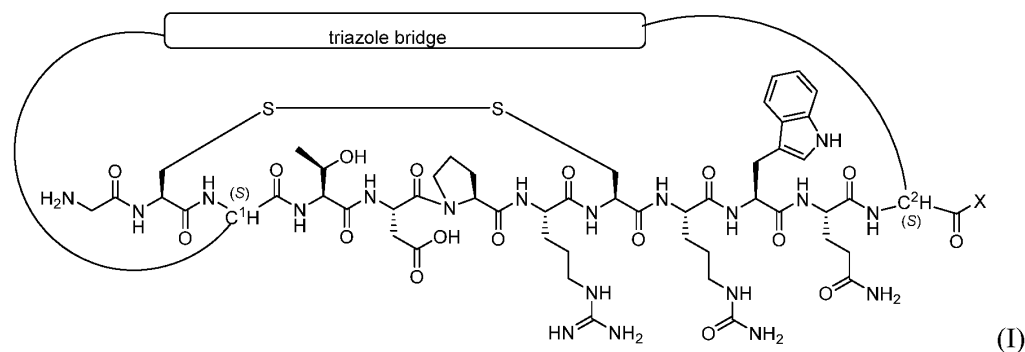
[00334] In a specific embodiment, the C-terminus of the intermediate conotoxin peptide analog is an amide group.

5.8.2. Triazole Bridge Formation

[00335] Following the synthesis of an intermediate conotoxin peptide with the necessary groups for forming a triazole bridge at the 3- and 12- positions (bearing one acetylene group and

one azide group, respectively), the acetylene group and the azide group react under triazole formation conditions to afford a triazole thereby forming a triazole bridge in the resulting conotoxin peptide analog.

[00336] In a specific embodiment, provided herein is a method of making a conotoxin peptide analog of Formula (I) (SEQ ID NO:93) or a pharmaceutically acceptable salt thereof,



wherein

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the conotoxin peptide analog of Formula (I) is a carboxylic acid or an amide group;

comprising subjecting an intermediate conotoxin peptide analog or a salt thereof to triazole formation conditions, wherein the amino acid sequence of the intermediate conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 -Trp-Gln- X_{AA}^{12} -X (SEQ ID NO:106),

wherein

X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-azidoalanine, (S)-homopropargyl glycine, (S)-azidohomoalanine, (S)-azidonorvaline and (S)-bishomopropargyl glycine;

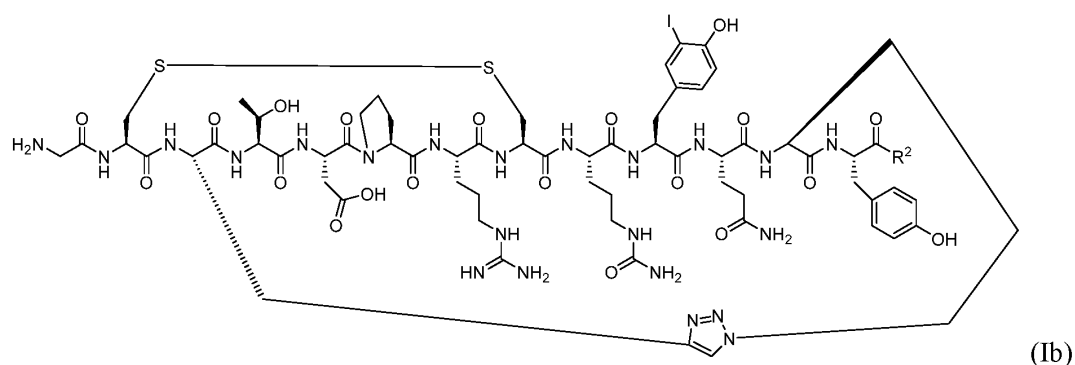
X_{AA}^9 is Citrulline;

X_{AA}^{12} is selected from the group consisting of (S)-azidohomoalanine, (S)-homopropargyl glycine, (S)-azidonorvaline, and (S)-bishomopropargyl glycine;

wherein when X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}^{12} is (S)-azidohomoalanine or

(S)-azidonorvaline; when X_{AA}^3 is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}^{12} is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;
X is as defined above for the conotoxin peptide analog of Formula (I); and
wherein the C-terminus of the intermediate conotoxin peptide analog is as defined above for the conotoxin peptide analog of Formula (I); and
wherein under said triazole formation conditions X_{AA}^3 reacts with X_{AA}^{12} to form the triazole bridge in the conotoxin peptide analog of Formula (I).

[00337] In yet another aspect, provided herein is a method of making a conotoxin peptide analog of Formula (Ib) (SEQ ID NO:104) or a pharmaceutically acceptable salt thereof,



wherein the C-terminus of the conotoxin peptide analog of Formula (Ib) is a carboxylic acid or an amide group,

comprising subjecting an intermediate conotoxin peptide analog or a salt thereof to triazole formation conditions, wherein the amino acid sequence of the intermediate conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 - X_{AA}^{10} -Gln- X_{AA}^{12} -Tyr (SEQ ID NO:107), wherein

X_{AA}^3 is (S)-propargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{10} is 3-iodo-Tyr;

X_{AA}^{12} is (S)-azidonorvaline; and

wherein the C-terminus of the intermediate conotoxin peptide analog is as defined above for the conotoxin peptide analog of Formula (Ib); and
wherein under said triazole formation conditions X_{AA}^3 reacts with X_{AA}^{12} to form a triazole bridge as depicted in the conotoxin peptide analog of Formula (Ib).

[00338] In a specific embodiment, the triazole formation conditions are conditions for “Click Chemistry” (i.e., 1,3-dipolar cycloaddition of azides and terminal alkynes) described in Hein et al. Pharm. Res. 2008, 25(10): 2216-2230. In a specific embodiment, the triazole formation conditions include presence of a copper catalyst. In a specific embodiment, the triazole formation conditions include presence of a copper catalyst and a reducing reagent. In one embodiment, the copper catalyst is a Cu(II) salt. In one embodiment, the copper catalyst is $CuSO_4$. In one embodiment, the reducing reagent is L-ascorbic acid. In another embodiment, the reducing reagent is sodium ascorbate. In one embodiment, the triazole formation conditions include presence of a ruthenium catalyst. In one embodiment, the ruthenium catalyst is $Cp^*RuCl(PPh_3)$. In certain embodiments, the triazole formation conditions are catalyst-free conditions.

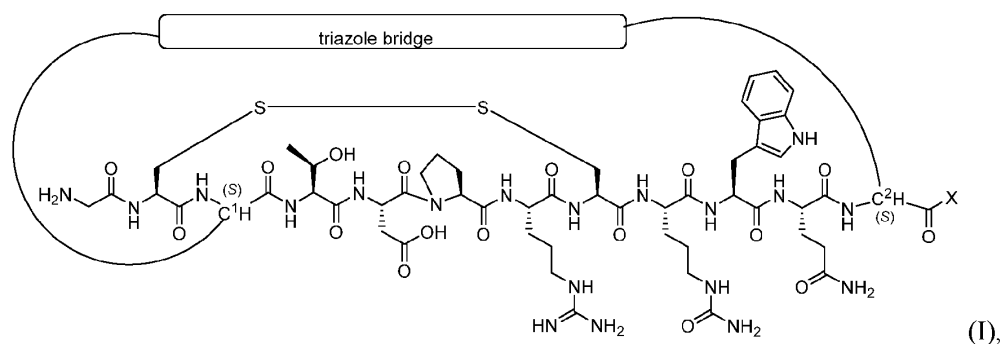
[00339] In a specific embodiment, a salt of the conotoxin peptide analog of Formula (I) or (Ib) can be subjected to salt exchange steps to afford a pharmaceutically acceptable salt. In one embodiment, for example, a TFA salt of a conotoxin peptide analog of Formula (I) reacts with a base in an aqueous solution (pH 7.0-8.0) and then reacts with an appropriate acid to afford a pharmaceutically acceptable salt of the conotoxin peptide analog of Formula (I). In one embodiment, the base is NH_4HCO_3 (aq.). In one embodiment, after reaction with a base in an aqueous solution, the conotoxin peptide analog reacts with acetic acid to afford an acetate salt of the conotoxin peptide analog.

5.8.3. PEGylation

[00340] The synthesized conotoxin peptide analogs can further be covalently conjugated to one or more polyethylene glycol (PEG) polymers. The conotoxin peptide analogs can, for example, be attached directly or via a linking group to one or more PEG polymers.

[00341] In a specific embodiment, provided herein is a method of making a PEGylated

conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent bond with the conotoxin peptide analog whereby the conotoxin peptide analog is attached directly or via a linking group to the one or more PEG polymers. In one embodiment, the conotoxin peptide analog is of Formula (I) (SEQ ID NO:93):



wherein

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group; and

wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one or more polyethylene glycol (PEG) polymers.

[00342] In a specific embodiment, provided herein is a method of making a PEGylated conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent bond with the conotoxin peptide analog whereby the conotoxin peptide analog

(Ib).

[00343] In a specific embodiment, provided herein is a method of making a PEGylated conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent bond with the conotoxin peptide analog whereby the conotoxin peptide analog is covalently attached directly or via a linking group to the one or more PEG polymers, wherein the amino acid sequence of the conotoxin peptide analog is Gly-Cys-X_{AA}³-Thr-Asp-Pro-Arg-Cys-X_{AA}⁹-Trp-Gln-X_{AA}¹²-X (SEQ ID NO:106), wherein

X_{AA}⁹ is Citrulline;

74

wherein when X_{AA}^3 is selected from the group consisting of (S)-propargyl glycine, (S)-homopropargyl glycine, and (S)-bishomopropargyl glycine, X_{AA}^{12} is (S)-azidohomoalanine or (S)-azidonorvaline; when X_{AA}^3 is selected from the group consisting of (S)-azidoalanine, (S)-azidohomoalanine, and (S)-azidonorvaline, X_{AA}^{12} is (S)-homopropargyl glycine or (S)-bishomopropargyl glycine;

X is X_{AA}^1 or $X_{AA}^1X_{AA}^2$; wherein X_{AA}^1 is Tyr, Phe, Trp, or a D-isomer of Tyr, Phe, or Trp, and X_{AA}^2 is N-Me-Gly, D-Tyr, or N-Me-Tyr; and

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00344] In yet another aspect, provided herein is a method of making a PEGylated conotoxin peptide analog or a pharmaceutically acceptable salt thereof, comprising contacting under reaction conditions a conotoxin peptide analog or a salt thereof, with one or more reactive polyethylene glycol (PEG) polymers to form a PEGylated conotoxin peptide analog, wherein the reactive PEG polymers each comprise a reactive group covalently linked, optionally via a linking group, to a PEG polymer, and wherein each reactive group reacts under the reaction conditions to form a covalent bond with the conotoxin peptide analog whereby the conotoxin peptide analog is covalently attached directly or via a linking group to the one or more PEG polymers, wherein the amino acid sequence of the conotoxin peptide analog is Gly-Cys- X_{AA}^3 -Thr-Asp-Pro-Arg-Cys- X_{AA}^9 - X_{AA}^{10} -Gln- X_{AA}^{12} -Tyr (SEQ ID NO:107),

wherein

X_{AA}^3 is (S)-propargyl glycine;

X_{AA}^9 is Citrulline;

X_{AA}^{10} is 3-iodo-Tyr;

X_{AA}^{12} is (S)-azidonorvaline;

wherein the C-terminus of the conotoxin peptide analog is a carboxylic acid or an amide group.

[00345] In a specific embodiment, the reaction conditions are standard PEGylation conditions. In one embodiment, the reaction conditions are conditions are standard amide formation conditions. Certain standard amide formation conditions are described in Valeur and Bradley, Chemical Society Reviews, 2009, 38:606-631. In one embodiment, the amide formation conditions include presence of a coupling reagent such as dicyclohexylcarbodiimide (DCC), 1-

hydroxy-1*H*-benzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), or 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU). In another embodiment, the reaction conditions are conditions are standard amine formation conditions. In one embodiment, the amine formation conditions are reductive amination conditions.

6. EXAMPLES

[00346] The following non-limiting examples disclose preparations and tests of conotoxin peptide analogs of conotoxin RgIA and PEGylated conotoxin peptide analogs of conotoxin RgIA.

[00347] It should be understood that the following examples are illustrative and not limiting.

6.1. Example 1: Disulfide Shuffling of RgIA Conotoxin Peptide Analogs **CSP-4-OH** and **CSP-4-NH2**

[00348] RgIA conotoxin peptide analogs **CSP-4-OH** and **CSP-4-NH2** have an amino acid sequence of Gly-Cys-Cys-Thr-Asp-Pro-Arg-Cys-(Cit)-(3-iodo-Tyr)-Gln-Cys-Tyr; where **CSP-4-OH** (SEQ ID NO:4) has a carboxylic acid group at the C-terminus and **CSP-4-NH2** has an amide group at the C-terminus. **CSP-4-desTyr-OH** has an amino acid sequence of Gly-Cys-Cys-Thr-Asp-Pro-Arg-Cys-(Cit)-(3-iodo-Tyr)-Gln-Cys (SEQ ID NO:5), with a carboxylic acid at the C-terminus.

[00349] When incubated in human and rat plasma and serum, conotoxin peptide analogs **CSP-4-OH** and **CSP-4-NH2** were found to be susceptible to disulfide shuffling. Such disulfide rearrangement resulted in the formation of isomers of the RgIA derivatives that lose activity on the $\alpha 9\alpha 10$ nAChR.

[00350] The active conformation of **CSP-4-NH2** consist of two disulfide bonds, one between Cys2 and Cys8, and a second between Cys3 and Cys12. The Cys2-8, Cys3-12 “native” form of **CSP-4-NH2** is active on both the human and rat $\alpha 9\alpha 10$ nAChR. Upon exposure to human and rat serum or plasma, a significant portion of the native form of **CSP-4-NH2** underwent isomerization into a “ribbon” form, containing alternative disulfide bonds Cys2-Cys12 and Cys3-Cys8 and a “bead” form containing alternative Cys2-Cys3 and Cys8-Cys12 disulfide bonds.

[00351] In plasma and serum samples, **CSP-4-NH2** principally isomerized between the native and ribbon forms, with very little bead formation (**Fig 1A, B**). Neither the ribbon nor bead form were active on the human or rat $\alpha 9\alpha 10$ nAChR, suggesting that disulfide shuffling contributes to a reduction in potency of the molecule. Furthermore, the presence of a C-terminal amide in **CSP-4-NH2** did not reduce isomerization relative to a C-terminal carboxylic acid in **CSP-4-OH**. Finally, disulfide isomerization also occurred *in vivo* following intravenous and subcutaneous injection of a 1 mg/kg dose of compound **6** to Sprague Dawley rats. At 1 h following injection, only 40 – 50 % of the recovered peptide from blood remained in the native form by HPLC-MS/MS analysis, indicating that disulfide isomerization occurs *in vivo* similar to observations *in vitro*.

[00352] Disulfide shuffling of conotoxin peptide analogs **CSP-4-OH** and **CSP-4-NH2** was demonstrated by incubating the peptides at a final concentration of 0.1 – 0.5 mg/mL in serum or plasma from Sprague Dawley rat and human *in vitro* at 37 °C up to 24 h. Plasma samples were treated with anticoagulants including citrate, K₂EDTA and heparin. Samples of the incubations were removed at several timepoints and for each timepoint, the resulting peptide isomers were extracted from the matrix by protein precipitation (3 volumes of methanol) followed by centrifugation of the precipitated sample at 10,000 x g through a Millipore Ultrafree-MC GV centrifugal filter. The resulting clarified sample was recovered and 5 μ L was injected on an Agilent 1260 Infinity HPLC fitted with a Phenomenex Aeris C-18 column (2.1 x 150 mm, 3.6 μ m). Peptides were separated by gradient elution at 0.3 mL/min with 0 to 50% B over 45 min; mobile phase A (19:1:0.01 H₂O/CH₃CN/TFA) and mobile phase B (CH₃CN with 0.05% TFA). Under these conditions, the relative retention time of the various isomers of **CSP-4-NH2** was as follows: ribbon < bead < native (**FIG. 1B**).

[00353] Treatment of **CSP-4-NH2** (40 μ g/mL) with a 1:1 mixture of oxidized glutathione and reduced glutathione (10 mM each) for 1 h, followed by treatment (quenching) with 8% formic acid, resulted in formation of the ribbon isomer with the same mass and chromatography retention time as the product observed in serum or plasma, indicating that disulfide isomerization mediated peptide instability in biological matrices (**FIG. 1D**). Glutathione reduction resulted in a mixture of native and ribbon form.

[00354] Following the resolution, collection and lyophilization of each HPLC peak as above, the identity of each **CSP-4-OH** and **CSP-4-NH₂** isomer was determined using LC-MS analysis on an Orbitrap Elite (**FIG. 1C**). Aliquots of non-reduced sample were diluted 1:100 in water:acetonitrile:formic acid (98:2:0.1%) to an estimated concentration of 1.2-1.5 pmol/ μ L. A 10 μ L injection was made by an Easy-nLC II HPLC system (Thermo Scientific) onto a 75 μ m i.d. PicoTip™ 25 cm long fused silica nano-column (New Objective) coupled to a 2 cm long, 100 μ m i.d. IntegraFrit™trap (New Objective). The column and the trap were configured in a vented configuration (Licklider, *et al.*, Analytical Chemistry, 2002, 74, 3076-3082), with the column packed with 5 μ m size Magic C18 AQ reverse-phased media (100Å pore size, Michrom Bioresources, Auburn, CA) and the trap packed with the same material (200Å pore size, Michrom). Samples were resolved on the column using a gradient consisting Mobile Phase A [ultrapure grade water/formic acid (0.1% by volume)] and Mobile Phase B [acetonitrile/formic acid (0.1% by volume)] at a flow rate of 400 nL/min according to the following: beginning with 95% “A” and 5% “B”, 0 to 2 minutes where Mobile Phase “B” is ramped from 5% to 7%, followed by a ramp of 7% “B” to 35% “B” over 60 minutes, followed by a ramp of 35% “B” to 50% “B” over 2 minutes where “B” is held for 1 minute, followed by a ramp of 50% “B” to 90% “B” over 5 minutes and held for 8 minutes, and finally followed by a period where “B” is ramped back down to 5% over 1 minute for re-equilibration to starting conditions. The mass spectrometer was operated in the data dependent mode over the range of 400-1800 m/z. For each cycle of the instrument, the top 3 most abundant ions were selected from a precursor scan (with the orbitrap resolution set at 120k in the profile mode). MS/MS data were collected in the centroid mode using the same settings as listed above, with the exception that the isolation width was set to 2.0 m/z. The dynamic exclusion settings used were as follows: repeat count of 1, a 15 second count duration, an exclusion list size of 500 and an exclusion duration of 30 seconds. The native and ribbon isomers had the same mass-to-charge ratio as determined using mass spectrometry, indicating that the two HPLC peaks have the same identity (**FIG. 1C**).

[00355] Isolated ribbon isomer of **CSP-4-NH₂** was found to have no blocking activity on the human $\alpha 9\alpha 10$ nAChR. **CSP-4-NH₂** was incubated in human serum for one hour as described above, followed by preparative HPLC and collection of the peak corresponding to the ribbon

isomer. The ribbon isomer was also chemically synthesized with the Cys2-Cys12 and Cys3-Cys8 disulfide connectivity. Chemically synthesized ribbon isomer and isomer isolated following incubation in human serum were tested for activity on the human $\alpha 9\alpha 10$ nAChR using two electrode voltage clamp electrophysiology.

[00356] The ability of each analog to block acetylcholine-induced $\alpha 9\alpha 10$ nAChR currents in *Xenopus* oocytes was measured. As shown in the **Table 1**, native **CSP-4-NH2** at a concentration of 100 nM blocked >99% of ACh-evoked currents, whereas both the chemically synthesized and serum-derived ribbon isomers of **CSP-4-NH2** were inactive at concentrations of 0.1 – 10 μ M. As shown in **FIG. 1A** and **1B**, in plasma and serum samples, conotoxin peptide analog **CSP-4-NH2** isomerized between the native and ribbon forms, with very little bead formation.

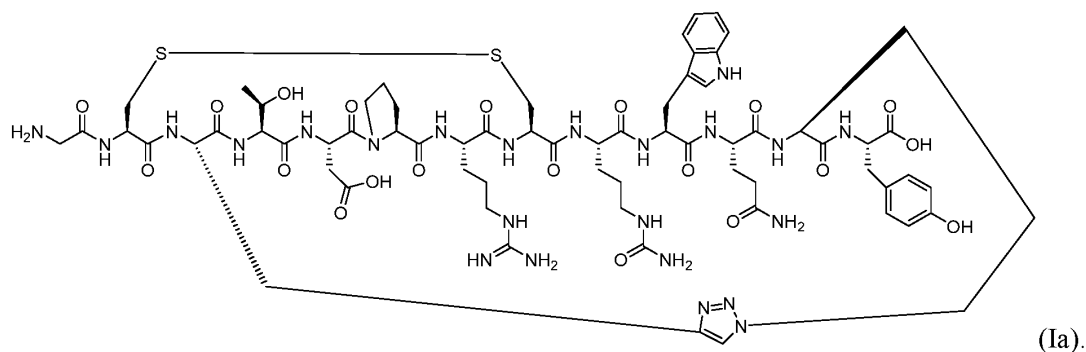
[00357] The reduced activity of the ribbon form on the human or rat $\alpha 9\alpha 10$ nAChR suggested that disulfide shuffling contributes to a reduction in potency of the molecule. Furthermore, the presence of a C-terminal amide in **CSP-4-NH2** did not reduce isomerization relative to a C-terminal carboxylic acid in **CSP-4-OH**. Finally, disulfide isomerization also occurred *in vivo* following intravenous and subcutaneous injection of a 1 mg/kg dose of **CSP-4-OH** to Sprague Dawley rats. At 1 h following injection, only 40 – 50 % of the recovered peptide from blood remains in the native form by HPLC-MS/MS analysis, indicating that disulfide isomerization occurs *in vivo* similar to observations *in vitro*.

Table 1: Activity of native and ribbon isomers of CSP-4-NH2 on human $\alpha 9\alpha 10$ nAChR expressed in *Xenopus* oocytes (Percent of 100 μ M acetylcholine-induced current remaining in the presence of compound compared to control response in the absence of compound)

human $\alpha 9\alpha 10$ nAChRs					
	Oocyte Number				
Compound (μ M)	1	2	3	4	Mean $\pm$ SEM
0.1 μ M native CSP-4-NH2	0.4	0.21	0.59		0.4 $\pm$ 0.11
0.1 μ M synthetic ribbon CSP-4-NH2	112.25	102.48			107.4 $\pm$ 4.9
10 μ M synthetic ribbon CSP-4-NH2	95	102.48	84.56	105.07	96.8 $\pm$ 4.6
1 μ M serum-isolated ribbon CSP-4-NH2	130.35	98.77	101.21		110.1 $\pm$ 10.1

6.2. Example 2: Preparation of Conotoxin Peptide Analogs

[00358] Example 1.1 Conotoxin Peptide Analog Ia (L-tyrosine, glycyl-L-cysteiny-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteiny-L-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminy-L-norvalyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:6):



[00359] 2-Chlorotrityl resin loaded with Fmoc-Tyr(OtBu)-OH (1): 2-Chlorotrityl chloride resin (MFCD00040399, 15.0 g, 18 mmol) was added to a reaction vessel and was washed with CH₂Cl₂ (100 mL) and then swelled in CH₂Cl₂ (100 mL) for 10 min. After draining the reaction vessel, a solution of Fmoc-Tyr(OtBu)-OH (2.5 g, 5.4 mmol) in CH₂Cl₂ (150 mL) was added, followed by dropwise addition of DIPEA (1.8 mL, 10.8 mmol). The reaction vessel was then shaken at room temperature for 1.5 h. The solvent was drained from the resin, which was subsequently washed with DMF (6x100 mL), followed by MeOH (2x100 mL) and finally Et₂O (2x100 mL). The resin was dried under vacuum for 2 h and the Fmoc loading test indicated that peptide loading was 0.29 mmol/g.

[00360] Solid phase peptide synthesis (SPPS) of peptide sequence Fmoc-Gly-Cys(Trt)-Pra-Thr(tBu)-Asp(OtBu)-Pro-Arg(Pbf)-Cys(Trt)-Cit-Trp(Boc)-Gln(Trt)-5-azido-NVa-Tyr(tBu)-O-(2-Chlorotrityl) on polystyrene support (2): The Fmoc-Tyr(OtBu)-loaded 2-chlorotrityl resin (1, 17.3 g, 5 mmol) was washed with DMF (100 mL) and then drained completely. The Fmoc group was removed from the tethered Tyr group by treatment with 1:4

piperidine/DMF (2x100 mL, 15 min each), and then the resin was washed with DMF (6x100 mL). Then, HATU (3.80 g, 10 mmol), HOAt (1.35 g, 10 mmol), and DIPEA (2.5 mL, 15 mmol) were added with Fmoc-5-azido-Nva-OH (CAS# 1097192-04-5, 3.80 g, 10 mmol) in DMF (100 mL). The reaction vessel was shaken for a minimum of 2.5 h, followed by washing with DMF (6x100 mL). Reaction completeness was determined by the Ninhydrin test. If negative (colorless), the coupling was considered complete and the synthesis was continued. If positive (blue), a second equivalent of the same Fmoc-amino acid (10 mmol) in DMF (100 mL) was coupled using the resin and HATU/HOAt (10 mmol/10 mmol) as described above. Once the Ninhydrin test indicated the reaction was complete, the Fmoc was removed from each of the sequentially added amino acid, followed by coupling of the subsequent Fmoc-amino acid-OH in the peptide sequence as described above. This was successively carried out in the following order with Fmoc-Gln(Trt)-OH (CAS#132327-80-1, 6.1 g, 10 mmol), Fmoc-Trp(Boc)-OH (CAS#143824-78-6, 5.2 g, 10 mmol), Fmoc-Cit-OH (CAS# 133174-15-9, 3.9 g, 10 mmol), Fmoc-Cys(Trt)-OH (CAS# 103213-32-7, 5.8 g, 10 mmol), Fmoc-Arg(Pbf)-OH (CAS# 154445-77-9, 6.4 g, 10 mmol), Fmoc-Pro-OH (CAS# 71989-31-6, 3.3 g, 10 mmol), Fmoc-Asp(OtBu)-OH (CAS# 71989-14-5, 4.1 g, 10 mmol), Fmoc-Thr(tBu)-OH (CAS# 71989-35-0, 3.9 g, 10 mmol), Fmoc-Pra-OH (CAS# 198561-07-8, 3.3 g, 10 mmol), Fmoc-Cys(Trt)-OH (5.8 g, 10 mmol), and Fmoc-Gly-OH (CAS# 29022-11-5, 2.9 g, 10 mmol). After the linear peptide couplings were complete, the resin was washed with DMF (100 mL × 6), followed by MeOH (100 mL × 2) and finally Et₂O (100 mL × 2), and then dried under vacuum for 2 h.

[00361] Resin cleavage of amino acid sequence H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-OH (3) (SEQ ID NO:7): To the dry resin (**2**, 28.0 g, 5.0 mmol) in a flask was added 230 mL of cleavage solution (TFA/EDT/Thioanisole/Anisole: 90:3:5:2) and the mixture was shaken for 2 h at room temperature. After this time, the resin was filtered and washed with TFA (2 x 50 mL). The filtrates were combined, and 10-fold volume of cold (0 °C) Et₂O was added, which resulted in precipitation of the peptide. The precipitated peptide was centrifuged at 5,000 rpm for 10 min and washed with cold (0 °C) Et₂O (3 x 50 mL). The crude product was dried under vacuum for 2 h to obtain **3** (6.8 g, 4.2 mmol, 84%), which was used

without further purification for the next step.

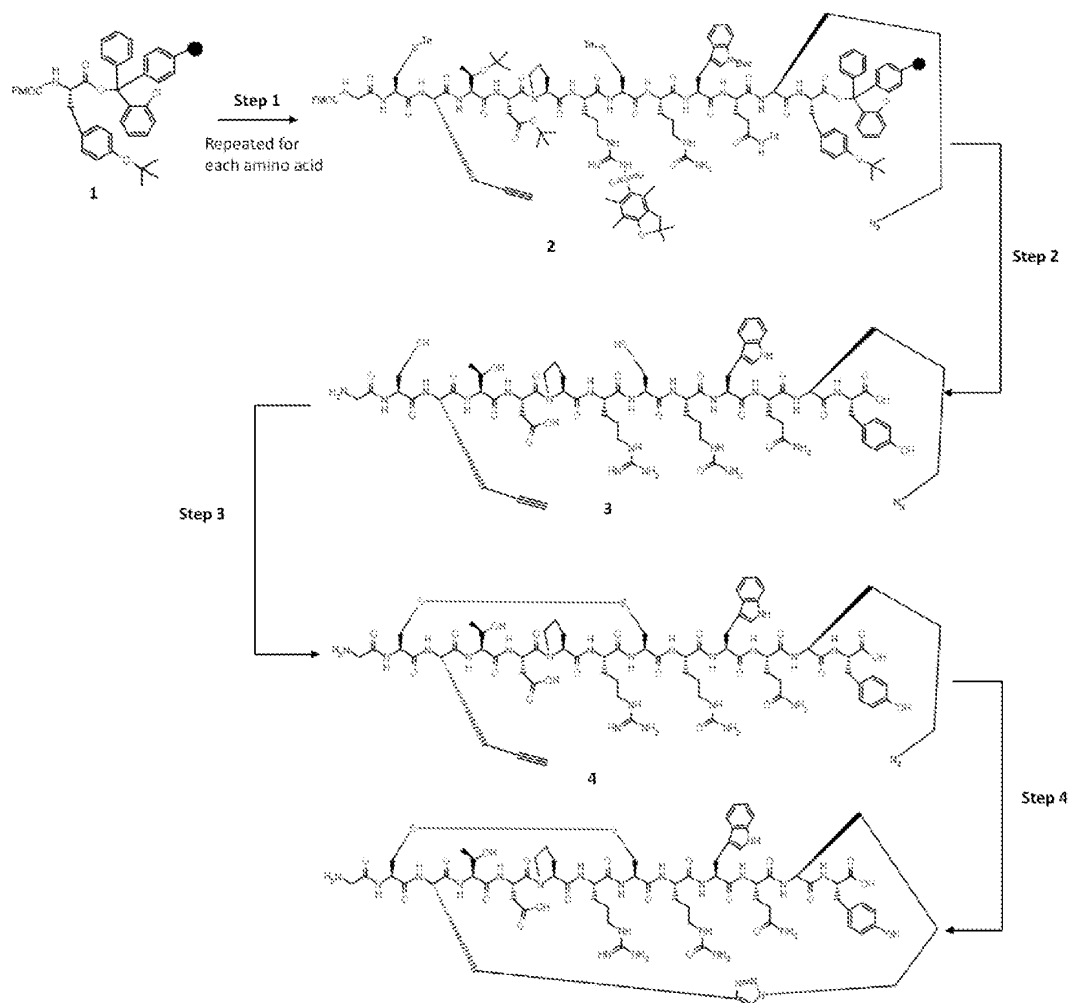
[00362] Cysteine disulfide bridge formation to produce H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-OH, (Cys2→Cys8) disulfide bridge (4) (SEQ ID NO:8): Peptide **3** (2.0 g, 1.2 mmol) was dissolved in CH₃CN (100 mL), DMSO (100 mL) and H₂O (1800 mL). Then H₂O₂ (30% in H₂O, 0.25 mL) was added dropwise and the mixture was stirred at room temperature for 1.5 h. After this time, the mixture was filtered, and the filtrate was purified by HPLC Method J to afford 800 mg of the title compound **4** as a white solid (yield: 38%, TFA salt). LC-MS (ESI) m/z: 540.7 [M+3H]/3⁺, 810.3 [M+2H]/2⁺, Purity 95% (214 nm). The remaining 4.8 g of **3** was distributed into two batches and oxidized and purified as described to obtain a total of 2.5 g of **4** (overall yield: 35%).

[00363] 1,3-Dipolar cyclization / Click reaction (triazole bridge formation) to produce conotoxin peptide analog Ia: Peptide **4** (400 mg, 0.25 mmol) was dissolved in iPrOH (100 mL) and H₂O (300 mL). Then a solution of CuSO₄ (157 mg, 1 mmol) in H₂O (2 mL) was added, followed by the dropwise addition of L-ascorbic acid (176 mg, 1 mmol) in H₂O (2 mL). The mixture was stirred at room temperature for 2 h. Then, the solution was loaded directly onto a flash column (e.g. Biotage® SNAP Ultra C18, 120 g) and eluted with water (0.1% TFA) and CH₃CN. The eluate fractions containing conotoxin peptide analog **Ia** were combined and lyophilized to obtain 305 mg of the TFA salt of conotoxin peptide analog **Ia** as a white solid (305 mg, TFA salt, yield: 69%). LC-MS (ESI) m/z: 540.7 [M+3H]/3⁺, 810.3 [M+2H]/2⁺, Purity 90% (214 nm). The remaining 2.1 g of **4** was distributed into three batches and each was treated with the conditions described above, affording 1.7 g of the title conotoxin peptide analog **Ia** (overall combined yield: 61%, TFA salt).

[00364] The obtained TFA salt of conotoxin peptide analog **Ia** (1.0 g) was dissolved in H₂O (20 mL) and CH₃CN (2 mL). Then a 20 mM solution of NH₄HCO₃ (aq.) was added drop wise to adjust the pH 7.0-8.0. The mixture was then purified using HPLC method H. Product containing fractions were lyophilized to give the acetate salt of conotoxin peptide analog **Ia** a white solid (650 mg, acetate salt, overall yield: 62%). The remaining 700 mg of conotoxin peptide analog **Ia**-TFA salt was purified and converted to the acetate salt in same manner to afford 350 mg (48%, acetate salt). The overall combined amount obtained was 1.0 g acetate salt

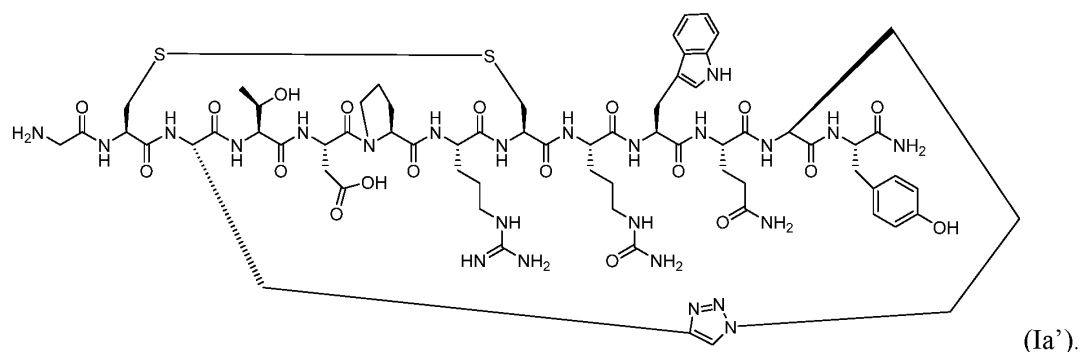
of conotoxin peptide analog **1a** (56%). LC-MS (ESI-TOF) m/z : 540.2 $[M+3H]/3^+$, 810.0 $[M+2H]/2^+$, 1617.66 $[M+H]^+$; HPLC method A; retention time: 10.1 min; purity 95.7%.

[00365] The synthesis of Conotoxin Peptide Analog **1a** is illustration in Scheme 1.



[00366] **Example 1.2 Conotoxin Peptide Analog 1a'** (L-tyrosinamide, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-or-nithyl-L-tryptophyl-L-glutaminyl-L-norvalyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12⁵-(1H-

1,2,3-triazole-4,1-diyl)) (SEQ ID NO:9):



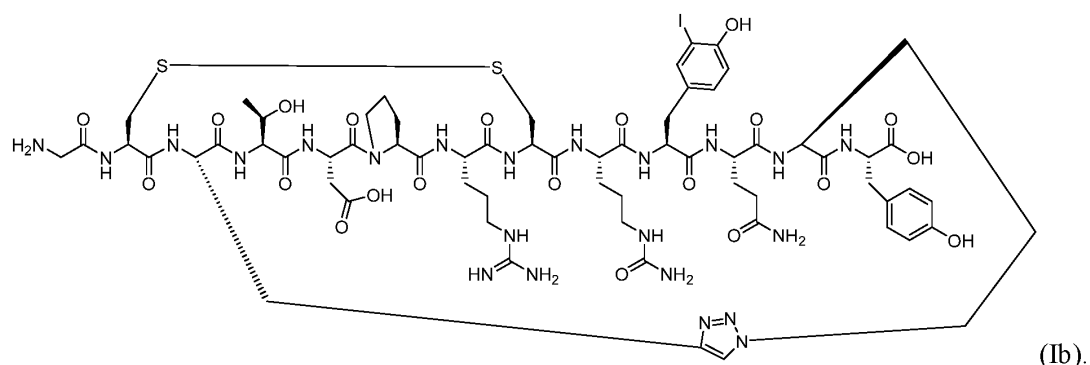
[00367] Intermediate conotoxin peptide analog **Ya'** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-Tyr-NH₂**) (SEQ ID NO:10): Intermediate conotoxin peptide analog **Ya'** was synthesized by employing the same procedure described for compound **3** using a Rink Amide resin MFCD00677976 in lieu of 2-Chlorotrityl chloride resin MFCD00040399.

[00368] Intermediate conotoxin peptide analog **Za'** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-Tyr-NH₂, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:11):

Intermediate conotoxin peptide analog **Za'** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Ya'** in lieu of compound **3**.

[00369] Conotoxin peptide analog **Ia'** was synthesized by employing the same procedure described for Conotoxin peptide analog **Ia'** using intermediate conotoxin peptide analog **Za'** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 1617.5 (M+1H)⁺ (calculated MW: 1616.5); HPLC method E; retention time: 14.0 min; purity 96.9%.

[00370] **Example 1.3 Conotoxin Peptide Analog Ib** (L-tyrosine, glycyl-L-cysteiny-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteiny-L-N⁵-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminy-L-norvalyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:12):

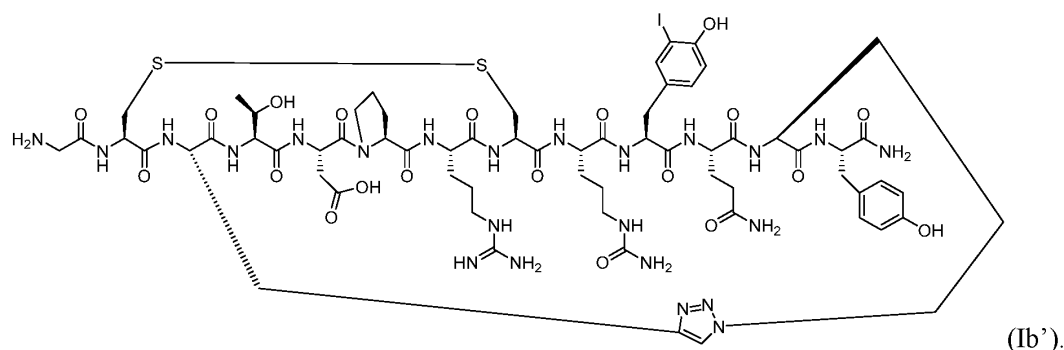


[00371] Intermediate conotoxin peptide analog **Yb** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-5-azidoNVa-Tyr-OH**) (SEQ ID NO:13): Intermediate conotoxin peptide analog **Yb** was synthesized by employing the same procedure described for compound **3** using Fmoc-3-I-Tyr-OH in lieu of Fmoc-Trp(Boc)-OH in the peptide synthesis of the 10-position residue.

[00372] Intermediate conotoxin peptide analog **Zb** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-5-azidoNVa-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:14): Intermediate conotoxin peptide analog **Zb** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yb** in lieu of compound **3**.

[00373] Conotoxin peptide analog **Ib** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zb** in lieu of compound **4**. LC-MS (ESI-TOF) m/z : 574.7 $[M+3H]/3^+$, 861.5 $[M+2H]/2^+$ (calculated MW: 1720.54); HPLC method C; retention time: 11.7 min; purity 94.8%.

[00374] **Example 1.4 Conotoxin Peptide Analog Ib'** (L-tyrosinamide, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N⁵-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutamyl-L-norvalyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:15):

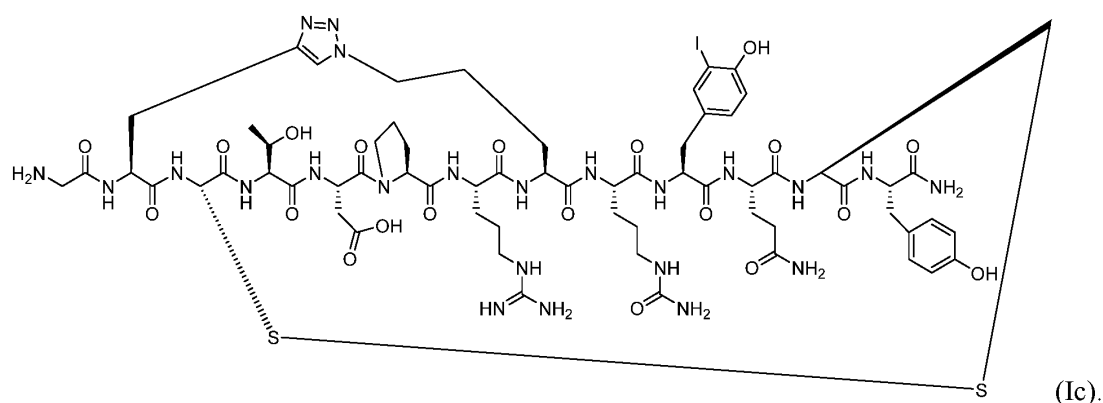


[00375] Intermediate conotoxin peptide analog **Yb'** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-5-azidoNVa-Tyr-NH₂**) (SEQ ID NO:16): Intermediate conotoxin peptide analog **Yb'** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Yb** using a Rink Amide resin MFCD00677976 in lieu of 2-Chlorotriyl chloride resin MFCD00040399 in the solid phase peptide synthesis.

[00376] Intermediate conotoxin peptide analog **Zb'** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-5-azidoNVa-Tyr-NH₂**, (Cys2→Cys8) disulfide bridge) (SEQ ID NO:17): Intermediate conotoxin peptide analog **Zb'** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yb'** in lieu of compound **3**.

[00377] Conotoxin peptide analog **Ib'** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zb'** in lieu of compound **4**. LC-MS (ESI-TOF) m/z: 574.2 [M+3H]/3⁺, 860.8 [M+2H]/2⁺ (calculated MW: 1719.55); HPLC method B; retention time: 10.5 min; purity 93.8%.

[00378] **Example 1.4 Conotoxin Peptide Analog Ic** (L-tyrosinamide, glycyl-L-alanyl-L-cysteinyl-L-threonyl-L-α-aspartyl-L-prolyl-L-arginyll-L-norvalyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminyll-L-cysteinyll-cyclic 2³,8⁵-(1H-1,2,3-triazole-4,1-diyl)-cyclic -(3→12)-disulfide) (SEQ ID NO:18):

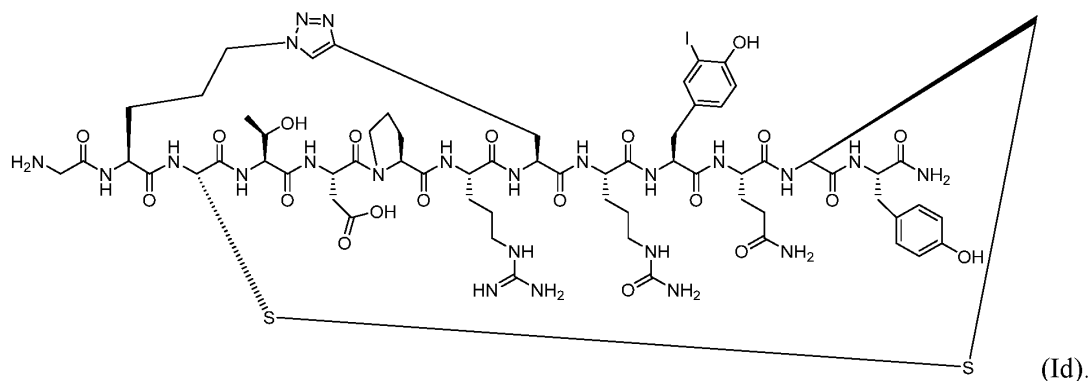


[00379] Intermediate conotoxin peptide analog **Yc** (**H-Gly-Pra-Cys-Thr-Asp-Pro-Arg-5-azidoNva-Cit-3-I-Tyr-Gln-Cys-Tyr-NH₂**) (SEQ ID NO:19): Intermediate conotoxin peptide analog **Yc** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Yb'** using Fmoc-Cys(Trt)-OH in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue), Fmoc-5-azido-Nva-OH in lieu of Fmoc-Cys(Trt)-OH (the 8-position residue), Fmoc-Cys(Trt)-OH in lieu of Fmoc-Pra-OH (the 3-position residue), and Fmoc-Pra-OH in lieu of Fmoc-Cys(Trt)-OH (the 2-position residue) in the solid phase peptide synthesis.

[00380] Intermediate conotoxin peptide analog **Zc** (**H-Gly-Pra-Cys-Thr-Asp-Pro-Arg-5-azidoNva-Cit-3-I-Tyr-Gln-Cys-Tyr-NH₂, (Cys3→Cys12) disulfide bridge**) (SEQ ID NO:20): Intermediate conotoxin peptide analog **Zc** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yc** in lieu of compound **3**.

[00381] Conotoxin peptide analog **1c** was synthesized by employing the same procedure described for conotoxin peptide analog **1b'** using intermediate conotoxin peptide analog **Zc** in lieu of Intermediate conotoxin peptide analog **Zb'**. LC-MS (ESI-TOF) *m/z*: 574.2 [M+3H]³⁺, 860.7 [M+2H]²⁺ (calculated MW: 1719.55); HPLC method B; retention time: 10.2 min; Purity 98.3%.

[00382] Example 1.5 Conotoxin Peptide Analog Id (L-tyrosinamide, glycyl-L-norvalyl-L-cysteinyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-alanyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminyl-L-cysteinyl-cyclic 2⁵,8³-(1H-1,2,3-triazole-1,4-diyl)-cyclic -(3 $\rightarrow$ 12)-disulfide) (SEQ ID NO:21):



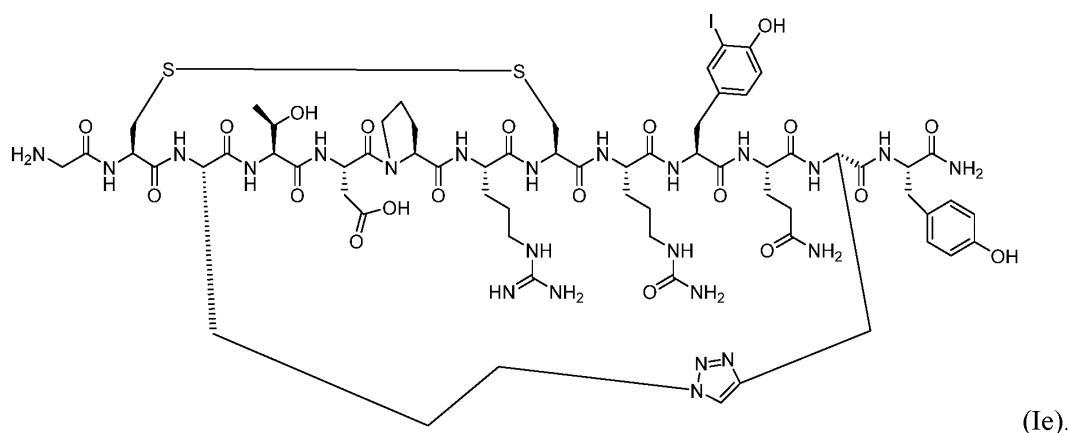
[00383] Intermediate conotoxin peptide analog **Yd** (**H-Gly-5-azidoNva-Cys-Thr-Asp-Pro-Arg-Pra-Cit-3-I-Tyr-Gln-Cys-Tyr-NH₂**) (SEQ ID NO:22): Intermediate conotoxin peptide analog **Yd** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Yb'** using Fmoc-Cys(Trt)-OH in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue), Fmoc-Pra-OH in lieu of Fmoc-Cys(Trt)-OH (the 8-position residue), Fmoc-Cys(Trt)-OH in lieu of Fmoc-Pra-OH (the 3-position residue), and Fmoc-5-azido-Nva-OH in lieu of Fmoc-Cys(Trt)-OH (the 2-position residue) in the solid phase peptide synthesis.

[00384] Intermediate conotoxin peptide analog **Zd** (**H-Gly-5-azidoNva-Cys-Thr-Asp-Pro-Arg-Pra-Cit-3-I-Tyr-Gln-Cys-Tyr-NH₂**, (Cys3 $\rightarrow$ Cys12) disulfide bridge) (SEQ ID NO:23): Intermediate conotoxin peptide analog **Zd** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Zb'** using intermediate conotoxin peptide analog **Yd** in lieu of intermediate conotoxin peptide analog **Yb'**.

[00385] Conotoxin peptide analog **Id** was synthesized by employing the same procedure described for conotoxin peptide analog **Ib'** using intermediate conotoxin peptide analog **Zd** in

lieu of intermediate conotoxin peptide analog **Zb'**. LC-MS (ESI-TOF) m/z: 860.7 [M+2H]/2⁺ (calculated MW: 1719.55); HPLC method B; retention time: 10.2 min; purity 99.2%.

[00386] Example 1.6 Conotoxin Peptide Analog Ie (L-tyrosinamide, glycyl-L-cysteinyl-L-norvalyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminyl-L-alanyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3⁵,12³-(1H-1,2,3-triazole-1,4-diyl)) (SEQ ID NO:24):

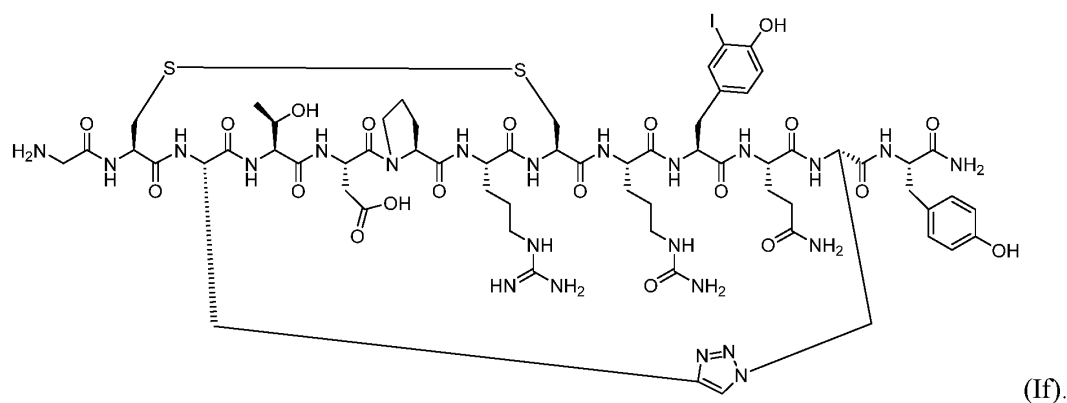


[00387] Intermediate conotoxin peptide analog **Ye** (H-Gly-Cys-5-azidoNva-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-Pra-Tyr-NH₂) (SEQ ID NO:25): Intermediate conotoxin peptide analog **Ye** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Yb'** using FMOC-Pra-OH in lieu of FMOC-5-azido-Nva-OH (the 12-position residue) and FMOC-5-azido-Nva-OH in lieu of FMOC-Pra-OH (the 3-position residue) in the solid phase peptide synthesis.

[00388] Intermediate conotoxin peptide analog **Ze** (H-Gly-Cys-5-azidoNva-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-Pra-Tyr-NH₂, (Cys2 $\rightarrow$ Cys8) disulfide bridge) (SEQ ID NO:26): Intermediate conotoxin peptide analog **Ze** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Zb'** using intermediate conotoxin peptide analog **Ye** in lieu of intermediate conotoxin peptide analog **Yb'**.

[00389] Conotoxin peptide analog **Ie** was synthesized by employing the same procedure described for conotoxin peptide analog **Ib'** using intermediate conotoxin peptide analog **Ze** in lieu of intermediate conotoxin peptide analog **Zb'**. LC-MS (ESI-TOF) m/z : 574.4 $[M+3H]/3^+$, 861.1 $[M+2H]/2^+$ (calculated MW: 1719.55); HPLC method C; retention time: 12.6 min; purity 92.1%.

[00390] Example 1.7 Conotoxin Peptide Analog If (L-tyrosinamide, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminyl-L-alanyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12³-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:27):



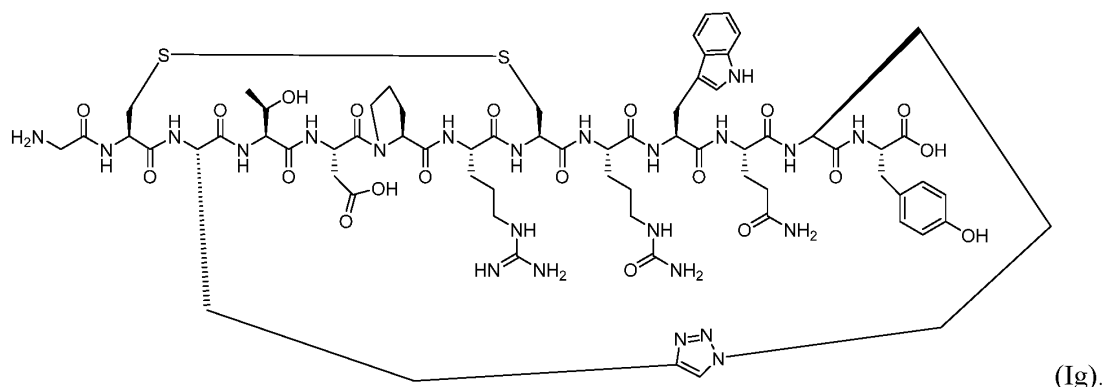
[00391] Intermediate conotoxin peptide analog **Yf** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-3-azidoAla-Tyr-NH₂) (SEQ ID NO:28): Intermediate conotoxin peptide analog **Yf** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Yb'** using Fmoc-3-azido-Ala-OH in lieu of Fmoc-Pra-OH (the 12-position residue) and Fmoc-Pra-OH in lieu of Fmoc-5-azido-Nva-OH (the 3-position residue) in the solid phase peptide synthesis.

[00392] Intermediate conotoxin peptide analog **Zf** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-3-I-Tyr-Gln-3-azidoAla-Tyr-NH₂, (Cys2 $\rightarrow$ Cys8) disulfide bridge) (SEQ ID NO:29): Intermediate conotoxin peptide analog **Zf** was synthesized by employing the same procedure described for intermediate conotoxin peptide analog **Zb'** using intermediate conotoxin peptide

analog **Yf** in lieu of intermediate conotoxin peptide analog **Yb'**.

[00393] Conotoxin peptide analog **If** was synthesized by employing the same procedure described for conotoxin peptide analog **Ib'** using intermediate conotoxin peptide analog **Zf** in lieu of intermediate conotoxin peptide analog **Zb'**. LC-MS (ESI-TOF) m/z: 847.1 [M+2H]/2⁺; 1693.1 [M+1H]⁺ (calculated MW: 1691.52); HPLC method E; retention time: 12.3 min; purity 94.6%.

[00394] Example 1.8 Conotoxin Peptide Analog Ig (L-tyrosine, glycyl-L-cysteinyl-(2S)-2-aminobutanoyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3⁴,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:30):



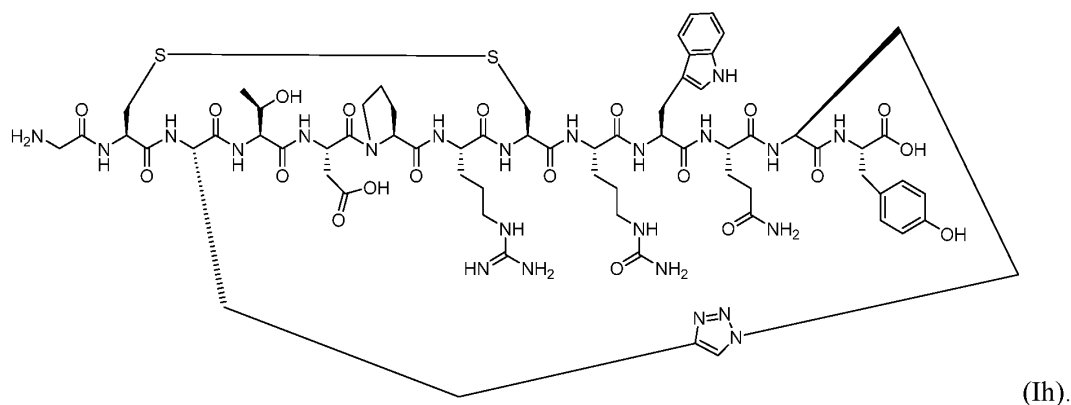
[00395] Intermediate conotoxin peptide analog **Yg** (**H-Gly-Cys-homoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-OH**) (SEQ ID NO:31): Intermediate conotoxin peptide analog **Yg** was synthesized by employing the same procedure described for compound **3** using Fmoc-homopropargyl glycine in lieu of Fmoc-Pra-OH (the 3-position residue) in the solid phase peptide synthesis.

[00396] Intermediate conotoxin peptide analog **Zg** (**H-Gly-Cys-homoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-OH, (Cys2 $\rightarrow$ Cys8) disulfide bridge**) (SEQ ID NO:32): Intermediate conotoxin peptide analog **Zg** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yg** in lieu of

compound **3**.

[00397] Conotoxin peptide analog **Ig** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zg** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 545.0 $[M+3H]/3^+$, 817.3 $[M+2H]/2^+$ (calculated MW: 1631.67); HPLC method A; retention time: 12.5 min; purity 98.2%.

[00398] **Example 1.9 Conotoxin Peptide Analog Ih** (L-tyrosine, glycyl-L-cysteinyl-(2S)-2-aminobutanoyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminyll-L-(2S)-2-aminobutanoyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3⁴,12⁴-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:33):



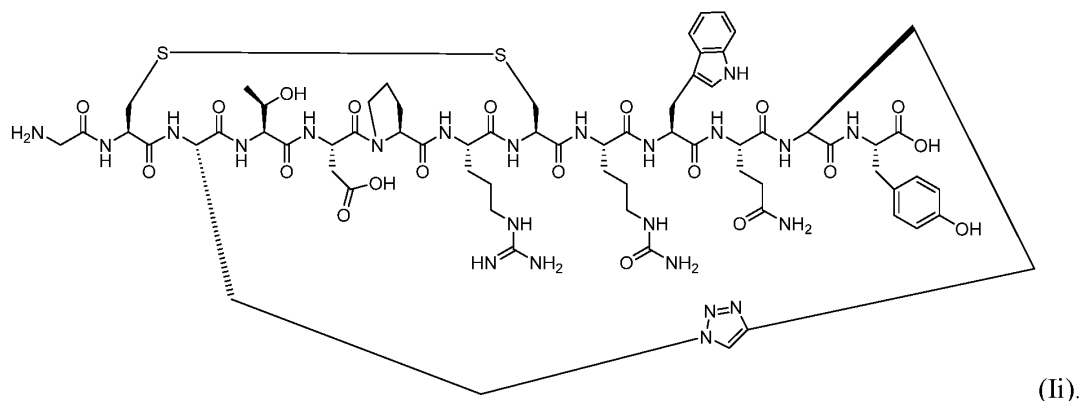
[00399] Intermediate conotoxin peptide analog **Yh** (H-Gly-Cys-homoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-azidohomoAla-Tyr-OH) (SEQ ID NO:34): Intermediate conotoxin peptide analog **Yh** was synthesized by employing the same procedure described for compound **3** using Fmoc-homopropargyl glycine in lieu of Fmoc-Pra-OH (the 3-position residue) and Fmoc- γ -azido-homoalanine in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue) in the solid phase peptide synthesis.

[00400] Intermediate conotoxin peptide analog **Zh** (H-Gly-Cys-homoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-azidohomoAla-Tyr-OH, (Cys2 $\rightarrow$ Cys8) disulfide bridge) (SEQ ID NO:35): Intermediate conotoxin peptide analog **Zh** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yh** in lieu of

compound **3**.

[00401] Conotoxin peptide analog **Ih** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zh** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 540.6 [M+3H]/3⁺, 809.9 [M+2H]/2⁺ (calculated MW: 1617.66); HPLC method A; retention time: 12.4 min; purity 83.9%.

[00402] **Example 1.10 Conotoxin Peptide Analog Ii** (L-tyrosine, glycyl-L-cysteinyl-(2S)-2-aminobutanoyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-(2S)-2-aminobutanoyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3⁴,12⁴-(1H-1,2,3-triazole-1,4-diyl)) (SEQ ID NO:36):



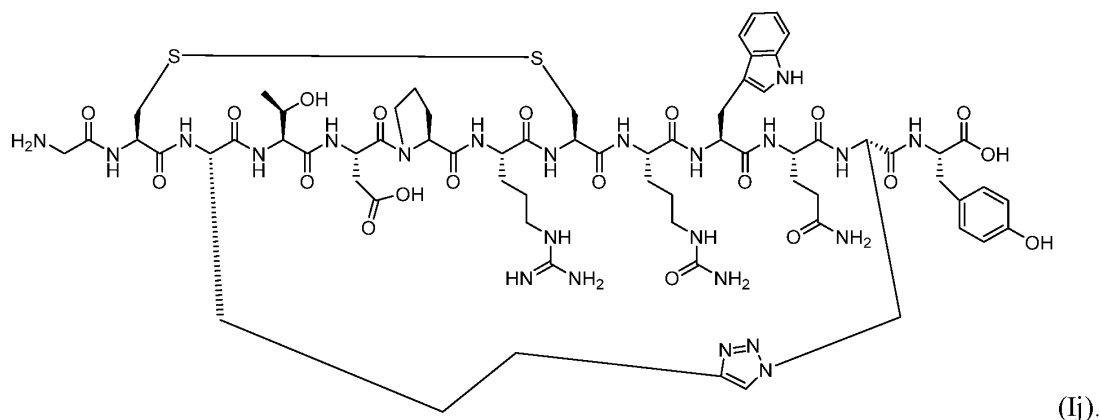
[00403] Intermediate conotoxin peptide analog **Yi** (**H-Gly-Cys-azidohomoAla-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-homoPra-Tyr-OH**) (SEQ ID NO:37): Intermediate conotoxin peptide analog **Yi** was synthesized by employing the same procedure described for compound **3** using Fmoc- γ -azido-homoalanine in lieu of Fmoc-Pra-OH (the 3-position residue) and Fmoc-homopropargyl glycine in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue) in the solid phase peptide synthesis.

[00404] Intermediate conotoxin peptide analog **Zi** (**H-Gly-Cys-azidohomoAla-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-homoPra-Tyr-OH, (Cys2 $\rightarrow$ Cys8) disulfide bridge**) (SEQ ID

NO:38): Intermediate conotoxin peptide analog **Zi** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yi** in lieu of compound **3**.

[00405] Conotoxin peptide analog **Ii** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zi** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 540.7 $[M+3H]/3^+$, 810.5 $[M+2H]/2^+$ (calculated MW: 1617.66); HPLC method A; retention time: 12.3 min; purity 85.8%.

[00406] Example 1.11 Conotoxin Peptide Analog Ij (L-tyrosine, glycyl-L-cysteinyl-(2S)-2-aminopentanoyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutaminyl-L-alanyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3⁵,12³-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:39):

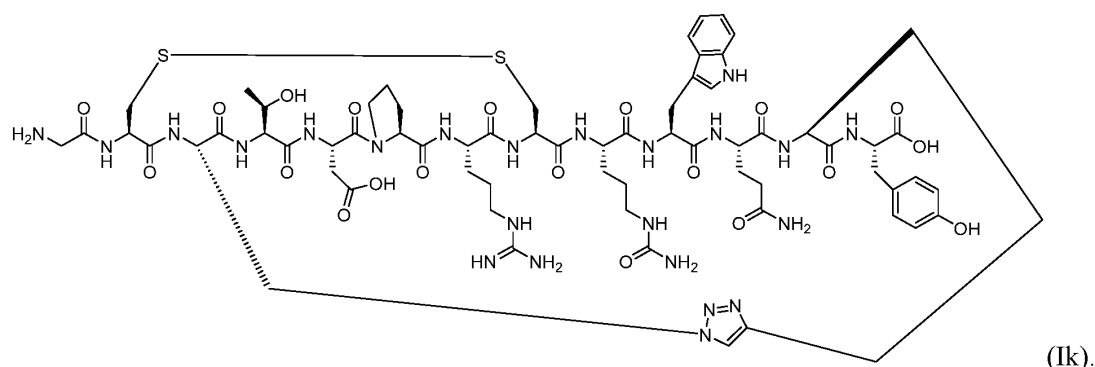


[00407] Intermediate conotoxin peptide analog **Yj** (**H-Gly-Cys-bishomoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-3-azidoAla-Tyr-OH**) (SEQ ID NO:40): Intermediate conotoxin peptide analog **Yj** was synthesized by employing the same procedure described for compound **3** using Fmoc-bis-homopropargyl glycine in lieu of Fmoc-Pra-OH (the 3-position residue) and Fmoc-3-azido-Ala-OH in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue) in the solid phase peptide synthesis.

[00408] Intermediate conotoxin peptide analog **Zj** (**H-Gly-Cys-bishomoPra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-3-azidoAla-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:41): Intermediate conotoxin peptide analog **Zj** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yj** in lieu of compound **3**.

[00409] Conotoxin peptide analog **Ij** was synthesized by employing the same procedure described for conotoxin peptide analog **1a** using intermediate conotoxin peptide analog **Zj** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 540.5 $[M+3H]/3^+$, 810.1 $[M+2H]/2^+$ (calculated MW: 1617.66); HPLC method A; retention time: 12.04 min; purity 97.6%.

[00410] Example 1.12 Conotoxin Peptide Analog Ik (L-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-(2S)-2-aminopentanoyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-1,4-diyl)) (SEQ ID NO:42):

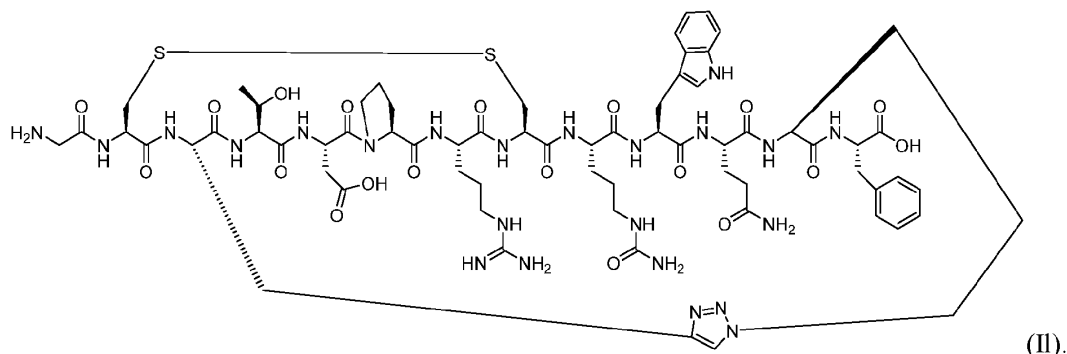


[00411] Intermediate conotoxin peptide analog **Yk** (**H-Gly-Cys-3-azidoAla-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-bishomoPra-Tyr-OH**) (SEQ ID NO:43): Intermediate conotoxin peptide analog **Yk** was synthesized by employing the same procedure described for compound **3** using Fmoc-3-azido-Ala-OH in lieu of Fmoc-Pra-OH (the 3-position residue) and Fmoc-bis-homopropargyl glycine in lieu of Fmoc-5-azido-Nva-OH (the 12-position residue) in the solid phase peptide synthesis.

[00412] Intermediate conotoxin peptide analog **Zk** (**H-Gly-Cys-3-azidoAla-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-bishomoPra-Tyr-OH**, (Cys2→Cys8) disulfide bridge) (SEQ ID NO:44): Intermediate conotoxin peptide analog **Zk** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yk** in lieu of compound **3**.

[00413] Conotoxin peptide analog **Ik** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zk** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 540.6 [M+3H]/3⁺, 810.0 [M+2H]/2⁺ (calculated MW: 1617.66); HPLC method A; retention time: 12.24 min; purity 97.6%.

[00414] **Example 1.13 Conotoxin Peptide Analog II** (L-phenylalanine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N⁵-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:45):



[00415] 2-Chlorotrityl resin loaded Fmoc-Phe-OH (**5**): 2-Chlorotrityl resin loaded Fmoc-Phe-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin **1** using Fmoc-Phe-OH in lieu of Fmoc-Tyr(OtBu)-OH.

[00416] Intermediate conotoxin peptide analog **Yl** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-Phe-OH**) (SEQ ID NO:46): Intermediate conotoxin peptide analog **Yl** was synthesized by employing the same procedure described for compound **3** using Fmoc-

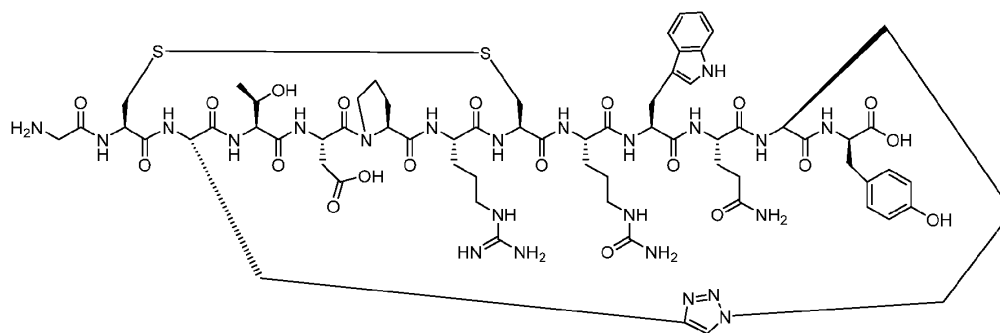
Phe-OH-loaded 2-chlorotrityl resin (**5**) in lieu of the FMOC-Tyr(OtBu)-loaded 2-chlorotrityl resin (**1**) as the C-terminus start in the solid phase peptide synthesis.

[00417] Intermediate conotoxin peptide analog **ZI** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-Phe-OH**, (**Cys2→Cys8**) **disulfide bridge**) (SEQ ID NO:47):

Intermediate conotoxin peptide analog **ZI** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **YI** in lieu of compound **3**.

[00418] Conotoxin peptide analog **II** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **ZI** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 535.2 $[M+3H]/3^+$, 802.2 $[M+2H]/2^+$ (calculated MW: 1601.66); HPLC method A; retention time: 13.47 min; purity 95.6%.

[00419] **Example 1.14 Conotoxin Peptide Analog Im** (D-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N⁵-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:48):



(**Im**).

[00420] 2-Chlorotrityl resin loaded FMOC-D-Tyr(OtBu)-OH (**6**): 2-Chlorotrityl resin loaded FMOC-D-Tyr(OtBu)-OH was synthesized by employing the same procedure described for 2-

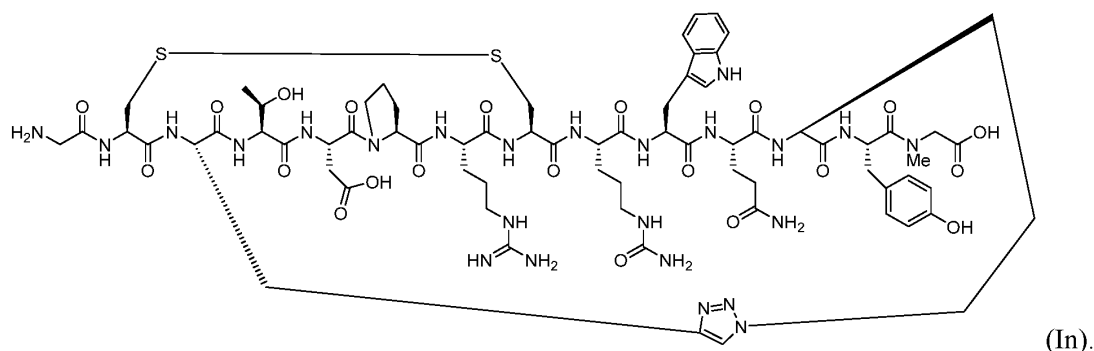
Chlorotrityl resin 1 using Fmoc-D-Tyr(OtBu)-OH in lieu of Fmoc-Tyr(OtBu)-OH.

[00421] Intermediate conotoxin peptide analog **Ym** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-D-Tyr-OH**) (SEQ ID NO:49): Intermediate conotoxin peptide analog **Ym** was synthesized by employing the same procedure described for compound **3** using Fmoc-D-Tyr(OtBu)-OH-loaded 2-chlorotrityl resin (**6**) in lieu of the Fmoc-Tyr(OtBu)-loaded 2-chlorotrityl resin (**1**) as the C-terminus start in the solid phase peptide synthesis.

[00422] Intermediate conotoxin peptide analog **Zm** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-D-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:50): Intermediate conotoxin peptide analog **Zm** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Ym** in lieu of compound **3**.

[00423] Conotoxin peptide analog **Im** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zm** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 540.6 [M+3H]/3⁺, 810.0 [M+2H]/2⁺ (calculated MW: 1617.66); HPLC method A; retention time: 12.07 min; purity 86.6%.

[00424] **Example 1.15 Conotoxin Peptide Analog In** (glycine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-L-tyrosyl-N-methyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:51):



[00425] 2-Chlorotrityl resin loaded Fmoc-N-Me-Gly-OH (**7**): 2-Chlorotrityl resin loaded

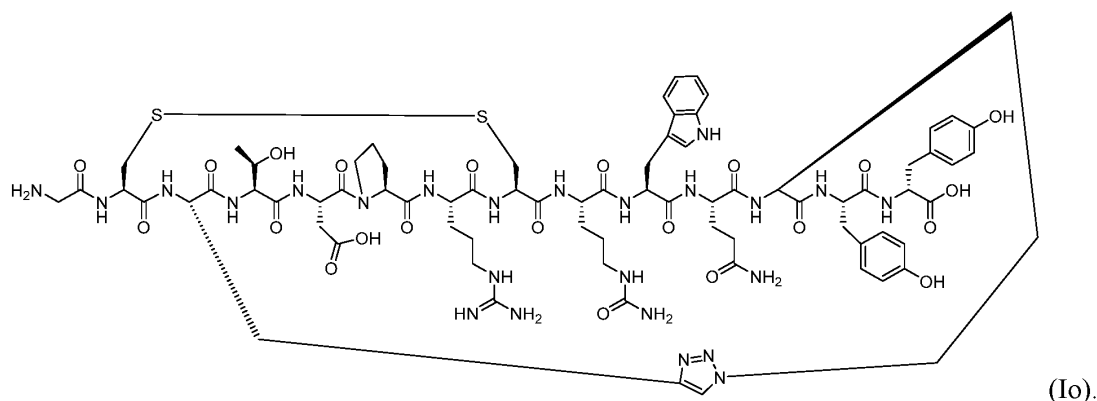
FMOC-N-Me-Gly-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin **1** using FMOC-N-Me-Gly-OH in lieu of FMOC-Tyr(OtBu)-OH.

[00426] Intermediate conotoxin peptide analog **Yn** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-N-Me-Gly-OH**) (SEQ ID NO:52): Intermediate conotoxin peptide analog **Yn** was synthesized by employing the same procedure described for compound **3** using FMOC-N-Me-Gly-OH-loaded 2-chlorotrityl resin (**7**) to sequentially add amino acid, carried out in the following order with FMOC-Tyr(OtBu)-OH, FMOC-5-azido-Nva-OH, FMOC-Gln(Trt)-OH, FMOC-Trp(Boc)-OH, FMOC-Cit-OH, FMOC-Cys(Trt)-OH, FMOC-Arg(Pbf)-OH, FMOC-Pro-OH, FMOC-Asp(OtBu)-OH, FMOC-Thr(tBu)-OH, FMOC-Pra-OH, FMOC-Cys(Trt)-OH, and FMOC-Gly-OH.

[00427] Intermediate conotoxin peptide analog **Zn** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-N-Me-Gly-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:53): Intermediate conotoxin peptide analog **Zn** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yn** in lieu of compound **3**.

[00428] Conotoxin peptide analog **In** was synthesized by employing the same procedure described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zn** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 564.2 [M+3H]/3⁺, 846.0 [M+2H]/2⁺ (calculated MW: 1688.69); HPLC method A; retention time: 11.63 min; purity 95.8%.

[00429] **Example 1.16 Conotoxin Peptide Analog Io** (D-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-L-tyrosyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:54):



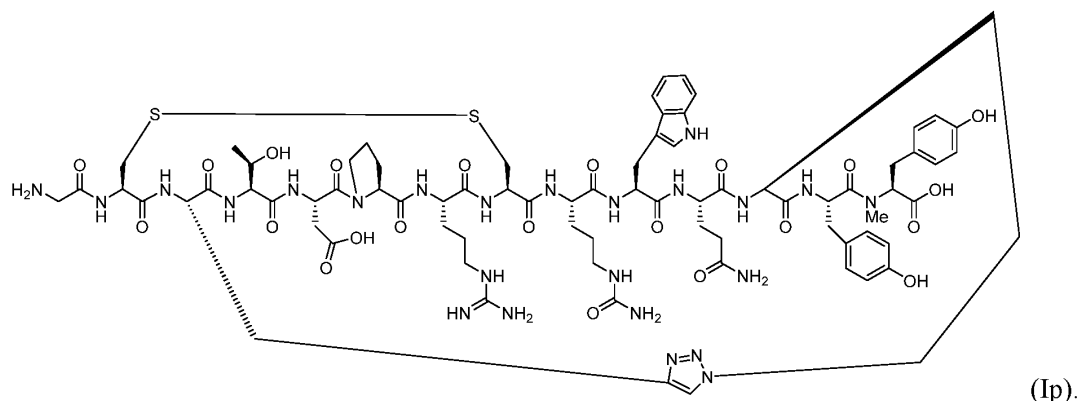
[00430] Intermediate conotoxin peptide analog **Yo** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-D-Tyr-OH**) (SEQ ID NO:55): Intermediate conotoxin peptide analog **Yo** was synthesized by employing the same procedure described for compound **3** using Fmoc-D-Tyr(OtBu)-OH-loaded 2-chlorotrityl resin to sequentially add amino acid, carried out in the following order with Fmoc-Tyr(OtBu)-OH, Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00431] Intermediate conotoxin peptide analog **Zo** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-D-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:56): Intermediate conotoxin peptide analog **Zo** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yo** in lieu of compound **3**.

[00432] Conotoxin peptide analog **Io** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zo** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 595.0 $[M+3H]/3^+$, 891.7 $[M+2H]/2^+$ (calculated MW: 1780.72); HPLC method A; retention time: 12.43 min; purity 97.4%.

[00433] **Example 1.17 Conotoxin Peptide Analog Ip** (L-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-

L-tryptophyl-L-glutaminyll-L-norvalyl-L-tyrosyl-N-methyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:57):



[00434] 2-Chlorotrityl resin loaded Fmoc-N-Me-Tyr-OH (**8**): 2-Chlorotrityl resin loaded Fmoc-N-Me-Tyr-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin 1 using Fmoc-N-Me-Tyr-OH in lieu of Fmoc-Tyr(OtBu)-OH.

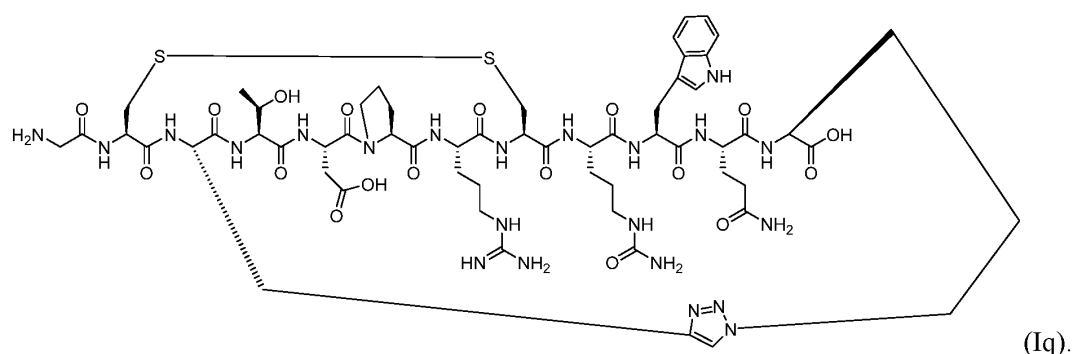
[00435] Intermediate conotoxin peptide analog **Yp** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-N-Me-Tyr-OH) (SEQ ID NO:58): Intermediate conotoxin peptide analog **Yp** was synthesized by employing the same procedure described for compound **3** using Fmoc-N-Me-Tyr-OH-loaded 2-chlorotrityl resin (**8**) to sequentially add amino acid, carried out in the following order with Fmoc-Tyr(OtBu)-OH, Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00436] Intermediate conotoxin peptide analog **Zp** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-Tyr-N-Me-Tyr-OH, (Cys2→Cys8) disulfide bridge) (SEQ ID NO:59): Intermediate conotoxin peptide analog **Zp** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yp** in lieu of compound **3**.

[00437] Conotoxin peptide analog **Ip** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zp** in

lieu of compound **4**. LC-MS (single quad ESI) m/z : 599.7 $[M+3H]/3^+$, 898.7 $[M+2H]/2^+$ (calculated MW: 1794.74); HPLC method A; retention time: 12.56 min; purity 95.5%.

[00438] Example 1.18 Conotoxin Peptide Analog Iq (L-norvaline, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminyL-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:60):



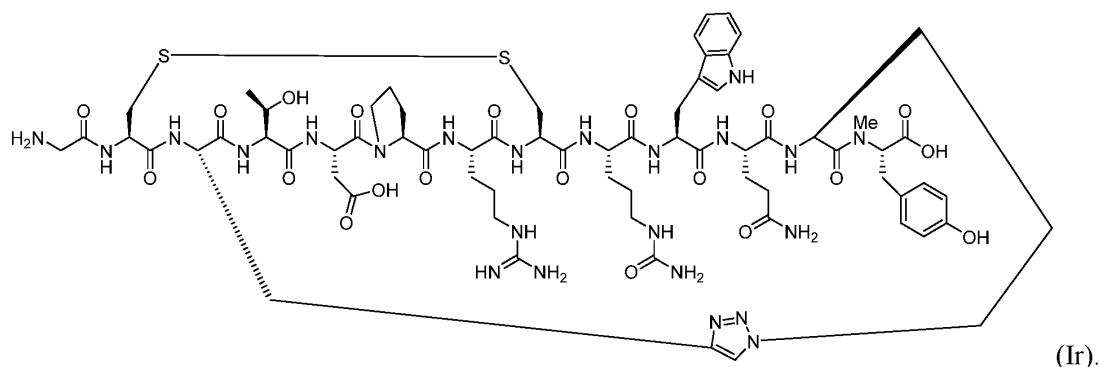
[00439] 2-Chlorotrityl resin loaded FMOC-5-azido-Nva-OH (**9**): 2-Chlorotrityl resin loaded FMOC-5-azido-Nva-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin **1** using FMOC-5-azido-Nva-OH in lieu of FMOC-Tyr(OtBu)-OH.

[00440] Intermediate conotoxin peptide analog **Yq** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-OH) (SEQ ID NO:61): Intermediate conotoxin peptide analog **Yq** was synthesized by employing the same procedure described for compound **3** using FMOC-5-azido-Nva-OH-loaded 2-chlorotrityl resin (**9**) to sequentially add amino acid, carried out in the following order with FMOC-Gln(Trt)-OH, FMOC-Trp(Boc)-OH, FMOC-Cit-OH, FMOC-Cys(Trt)-OH, FMOC-Arg(Pbf)-OH, FMOC-Pro-OH, FMOC-Asp(OtBu)-OH, FMOC-Thr(tBu)-OH, FMOC-Pra-OH, FMOC-Cys(Trt)-OH, and FMOC-Gly-OH.

[00441] Intermediate conotoxin peptide analog **Zq** (H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-OH, (Cys2 $\rightarrow$ Cys8) disulfide bridge) (SEQ ID NO:62): Intermediate conotoxin peptide analog **Zq** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yq** in lieu of compound **3**.

[00442] Conotoxin peptide analog **Iq** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zq** in lieu of compound **4**. LC-MS (ESI-TOF) m/z: 1455.5 [M+1H]⁺ (calculated MW: 1454.59); HPLC method A; retention time: 11.4 min; purity 95.0%.

[00443] Example 1.19 Conotoxin Peptide Analog Ir (L-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminy-L-norvalyl-N-methyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:63):



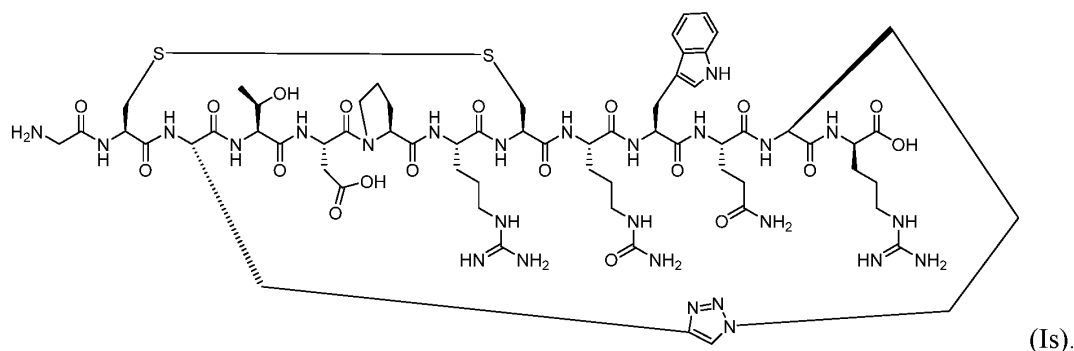
[00444] Intermediate conotoxin peptide analog **Yr** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-N-Me-Tyr-OH**) (SEQ ID NO:64): Intermediate conotoxin peptide analog **Yr** was synthesized by employing the same procedure described for compound **3** using Fmoc-N-Me-Tyr-OH-loaded 2-chlorotrityl resin to sequentially add amino acid, carried out in the following order with Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00445] Intermediate conotoxin peptide analog **Zr** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-N-Me-Tyr-OH, (Cys2 $\rightarrow$ Cys8) disulfide bridge**) (SEQ ID NO:65): Intermediate conotoxin peptide analog **Zr** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yr** in lieu of

compound **3**.

[00446] Conotoxin peptide analog **Ir** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zr** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 545.3 $[M+3H]/3^+$, 817.0 $[M+2H]/2^+$ (calculated MW: 1631.67); HPLC method A; retention time: 12.01 min; purity 96.4%.

[00447] **Example 1.20 Conotoxin Peptide Analog Is** (D-arginine, glycyl-L-cysteiny-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteiny-L-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminy-L-norvalyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:66):



[00448] 2-Chlorotrityl resin loaded Fmoc-D-Arg-OH (**10**): 2-Chlorotrityl resin loaded Fmoc-D-Arg-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin 1 using Fmoc-D-Arg-OH in lieu of Fmoc-Tyr(OtBu)-OH.

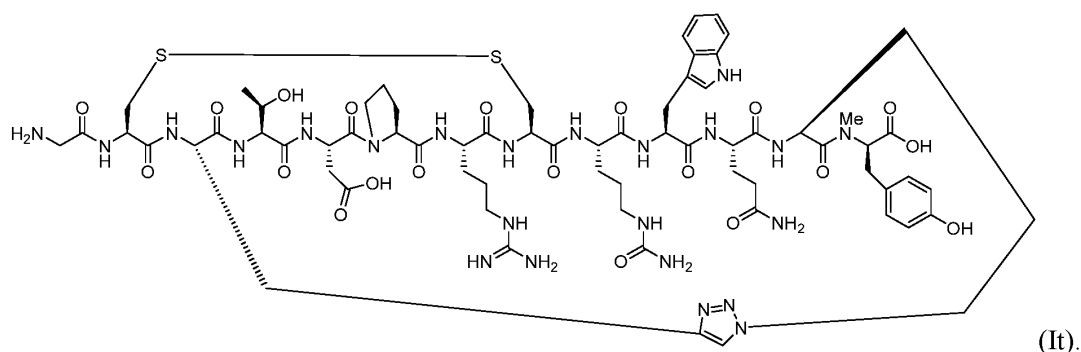
[00449] Intermediate conotoxin peptide analog **Ys** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-D-Arg-OH**) (SEQ ID NO:67): Intermediate conotoxin peptide analog **Ys** was synthesized by employing the same procedure described for compound **3** using Fmoc-D-Arg-OH-loaded 2-chlorotrityl resin (**10**) to sequentially add amino acid, carried out in the following order with Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00450] Intermediate conotoxin peptide analog **Zs** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-D-Arg-OH**, (**Cys2→Cys8**) **disulfide bridge**) (SEQ ID NO:68):

Intermediate conotoxin peptide analog **Zs** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Ys** in lieu of compound **3**.

[00451] Conotoxin peptide analog **Is** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zs** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 539.2 $[M+3H]/3^+$, 807.0 $[M+2H]/2^+$ (calculated MW: 1610.69); HPLC method A; retention time: 10.52 min; purity 97.1%.

[00452] **Example 1.21 Conotoxin Peptide Analog It** (D-tyrosine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-N-methyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:69):



[00453] 2-Chlorotrityl resin loaded Fmoc-N-Me-D-Tyr-OH (**11**): 2-Chlorotrityl resin loaded Fmoc-N-Me-D-Tyr-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin **1** using Fmoc-N-Me-D-Tyr-OH in lieu of Fmoc-Tyr(OtBu)-OH.

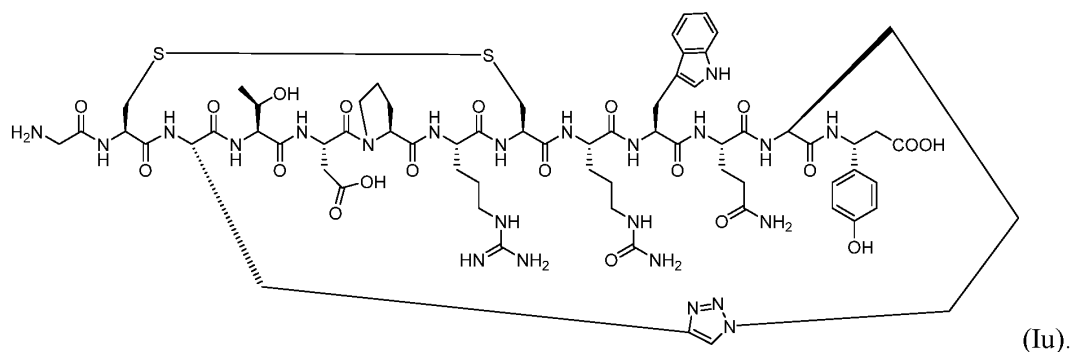
[00454] Intermediate conotoxin peptide analog **Yt** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNVa-N-Me-D-Tyr-OH**) (SEQ ID NO:70): Intermediate conotoxin peptide analog **Yt** was synthesized by employing the same procedure described for compound **3** using Fmoc-N-Me-D-Tyr-OH-loaded 2-chlorotrityl resin (**11**) to sequentially add amino acid, carried

out in the following order with FMOC-5-azido-Nva-OH, FMOC-Gln(Trt)-OH, FMOC-Trp(Boc)-OH, FMOC-Cit-OH, FMOC-Cys(Trt)-OH, FMOC-Arg(Pbf)-OH, FMOC-Pro-OH, FMOC-Asp(OtBu)-OH, FMOC-Thr(tBu)-OH, FMOC-Pra-OH, FMOC-Cys(Trt)-OH, and FMOC-Gly-OH.

[00455] Intermediate conotoxin peptide analog **Zt** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-N-Me-D-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:71): Intermediate conotoxin peptide analog **Zt** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yt** in lieu of compound **3**.

[00456] Conotoxin peptide analog **It** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zt** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 545.3 $[M+3H]/3^+$, 817.0 $[M+2H]/2^+$ (calculated MW: 1631.67); HPLC method A; retention time: 10.72 min; purity 96.6%.

[00457] Example 1.22 Conotoxin Peptide Analog Iu ((R)-3-(amino)-3-(4-hydroxyphenyl)propionic acid, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyll-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutaminyll-L-norvalyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:72):



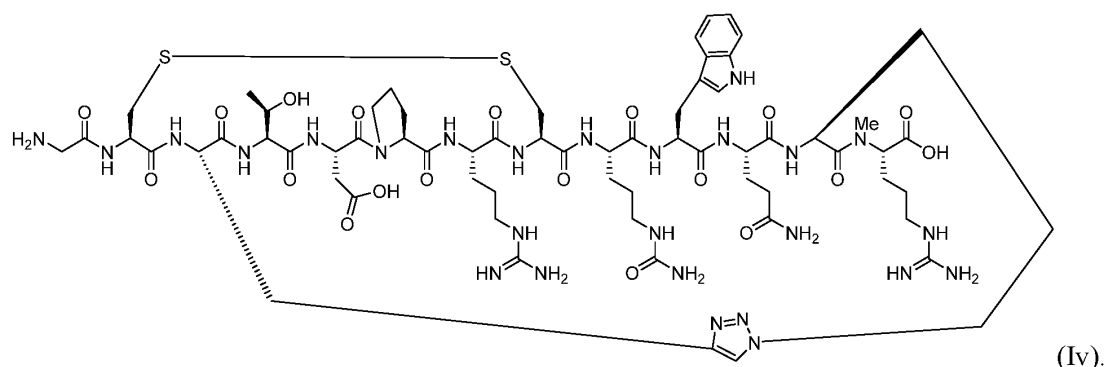
[00458] 2-Chlorotrityl resin loaded FMOC-beta-Tyr-OH (**12**): 2-Chlorotrityl resin loaded FMOC-beta-Tyr-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin 1 using FMOC-beta-Tyr-OH in lieu of FMOC-Tyr(OtBu)-OH.

[00459] Intermediate conotoxin peptide analog **Yu** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-beta-Tyr-OH**) (SEQ ID NO:73): Intermediate conotoxin peptide analog **Yu** was synthesized by employing the same procedure described for compound **3** using Fmoc-beta-Tyr-OH-loaded 2-chlorotrityl resin (**12**) to sequentially add amino acid, carried out in the following order with Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00460] Intermediate conotoxin peptide analog **Zu** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-beta-Tyr-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:74): Intermediate conotoxin peptide analog **Zu** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yu** in lieu of compound **3**.

[00461] Conotoxin peptide analog **Iu** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zu** in lieu of compound **4**. LC-MS (single quad ESI) m/z: 544.5 [M+3H]/3⁺, 816.3 [M+2H]/2⁺ (calculated MW: 1631.67); HPLC method A; retention time: 10.28 min; purity 98.8%.

[00462] **Example 1.23 Conotoxin Peptide Analog Iv** (L-arginine, glycyl-L-cysteinyl-L-alanyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-tryptophyl-L-glutamyl-L-norvalyl-N-methyl-cyclic (2→8)-disulfide-cyclic 3³,12⁵-(1H-1,2,3-triazole-4,1-diyl)) (SEQ ID NO:75):



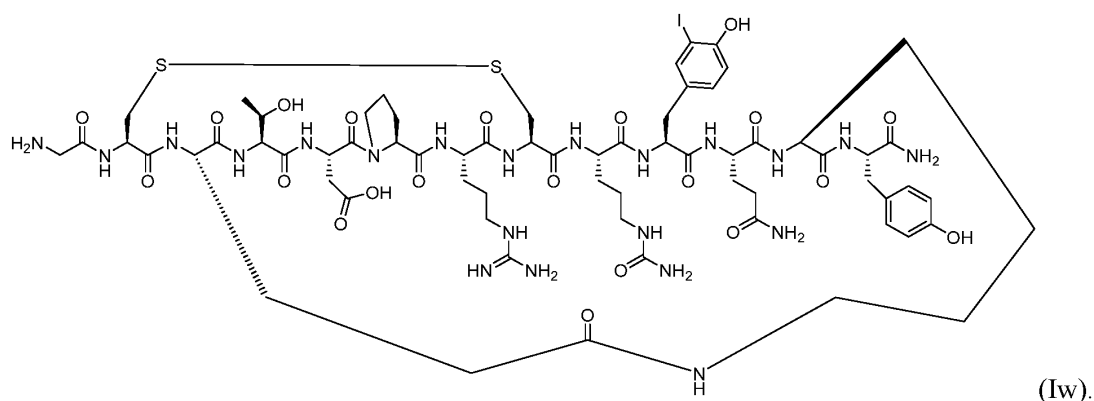
[00463] 2-Chlorotrityl resin loaded Fmoc-N-Me-Arg-OH (**13**): 2-Chlorotrityl resin loaded Fmoc-N-Me-Arg-OH was synthesized by employing the same procedure described for 2-Chlorotrityl resin **1** using Fmoc-N-Me-Arg-OH in lieu of Fmoc-Tyr(OtBu)-OH.

[00464] Intermediate conotoxin peptide analog **Yv** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-N-Me-Arg-OH**) (SEQ ID NO:76): Intermediate conotoxin peptide analog **Yv** was synthesized by employing the same procedure described for compound **3** using Fmoc-N-Me-Arg-OH-loaded 2-chlorotrityl resin (**13**) to sequentially add amino acid, carried out in the following order with Fmoc-5-azido-Nva-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Cit-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Pra-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Gly-OH.

[00465] Intermediate conotoxin peptide analog **Zv** (**H-Gly-Cys-Pra-Thr-Asp-Pro-Arg-Cys-Cit-Trp-Gln-5-azidoNva-N-Me-Arg-OH, (Cys2→Cys8) disulfide bridge**) (SEQ ID NO:77): Intermediate conotoxin peptide analog **Zv** was synthesized by employing the same procedure described for compound **4** using intermediate conotoxin peptide analog **Yv** in lieu of compound **3**.

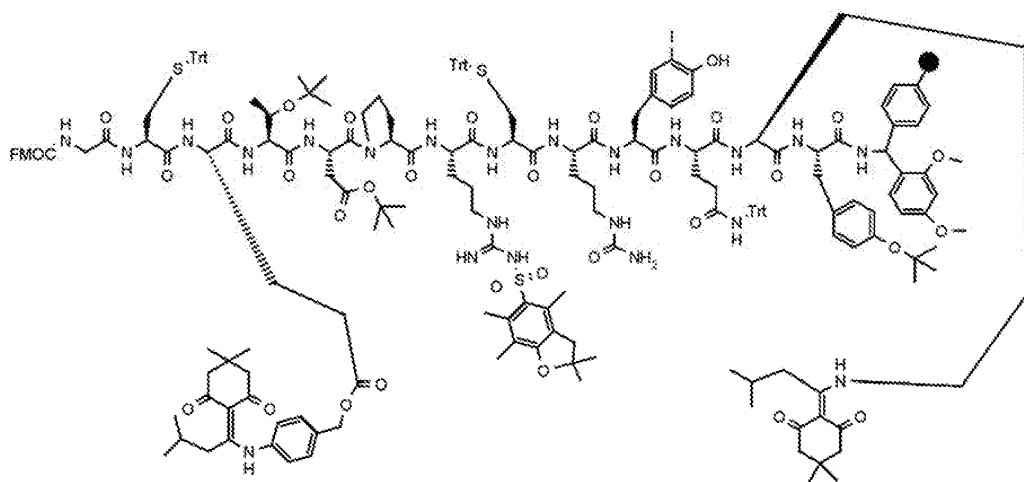
[00466] Conotoxin peptide analog **Iv** was synthesized by employing the same procedures described for conotoxin peptide analog **Ia** using intermediate conotoxin peptide analog **Zv** in lieu of compound **4**. LC-MS (single quad ESI) m/z : 542.9 $[M+3H]/3^+$, 813.5 $[M+2H]/2^+$ (calculated MW: 1624.71); HPLC method A; retention time: 9.29 min; purity 95.9%.

[00467] Example 1.24 Conotoxin Peptide Analogs Iw (L-tyrosinamide, glycyl-L-cysteinyl-L-glutamyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutamyl-L-lysyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic-(3 $\rightarrow$ 12)-lactam) (SEQ ID NO:78):



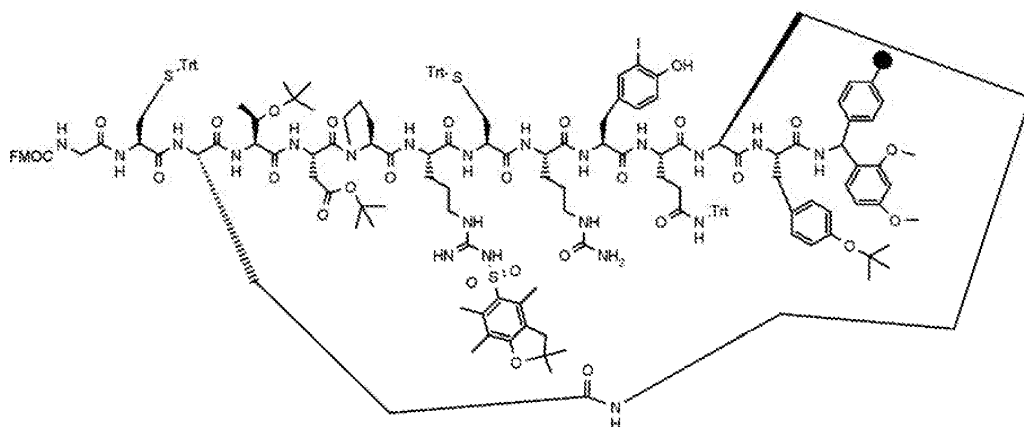
[00468] Synthesis of Linear Peptide Intermediate (X1) (SEQ ID NO:79): Fmoc-Tyr(OtBu)-loaded Rink amide MBHA resin (1.7 g, 0.5 mmol) was washed with DMF (10 ml) and then drained completely. The Fmoc group was removed from the tethered Tyr group by treatment with 1:4 piperidine/DMF (2x10 mL, 15 min each), and then the resin was washed with DMF (6x10 mL). Then, HATU (CAS#148893-10-1, 0.38 g, 1 mmol), HOAt (CAS# 39968-33-7, 0.14 g, 1 mmol), and DIPEA (0.25 mL, 1.5 mmol) were added with Fmoc-Lys(ivDde)-OH (CAS# 204777-78-6, 0.57 g, 1 mmol) in DMF (10 mL). The reaction vessel was shaken for a minimum of 2.5 h, followed by washing with DMF (6x10 mL). Reaction completeness was determined by the Ninhydrin test. If negative (colorless), the coupling was considered complete and the synthesis was continued. If positive (blue), a second equivalent of the same Fmoc-amino acid (1 mmol) in DMF (10 mL) was coupled using the resin and HATU/HOAt (1 mmol/1 mmol) as described above. Once the Ninhydrin test indicated the reaction was complete, the Fmoc was removed from each of the sequentially added amino acid, followed by coupling of the subsequent Fmoc-amino acid-OH in the peptide sequence as described above. This was successively carried out in the following order with Fmoc-Gln(Trt)-OH (CAS# 132327-80-1, 0.61 g, 1 mmol), Fmoc-(3-I-Tyr)-OH (CAS# 134486-00-3, 0.53 g, 1 mmol), Fmoc-Cit-OH

(CAS# 133174-15-9, 0.39 g, 1 mmol), Fmoc-Cys(Trt)-OH (CAS# 103213-32-7, 0.58 g, 1 mmol), Fmoc-Arg(Pbf)-OH (CAS# 154445-77-9, 0.64 g, 1 mmol), Fmoc-Pro-OH (CAS# 71989-31-6, 0.33 g, 1 mmol), Fmoc-Asp(OtBu)-OH (CAS# 71989-14-5, 0.41 g, 1 mmol), Fmoc-Thr(tBu)-OH (CAS# 71989-35-0, 0.39 g, 1 mmol), Fmoc-Glu(ODmab) (CAS# 268730-86-5, 0.68 g, 1 mmol), Fmoc-Cys(Trt)-OH (0.58 g, 1 mmol), and Fmoc-Gly-OH (CAS# 29022-11-5, 0.29 g, 1 mmol). After the linear peptide couplings were complete, the resin was washed with DMF (10 mL $\times$ 6), followed by MeOH (10 mL $\times$ 2) and finally Et₂O (10 mL $\times$ 2), and then dried under vacuum for 2 h.



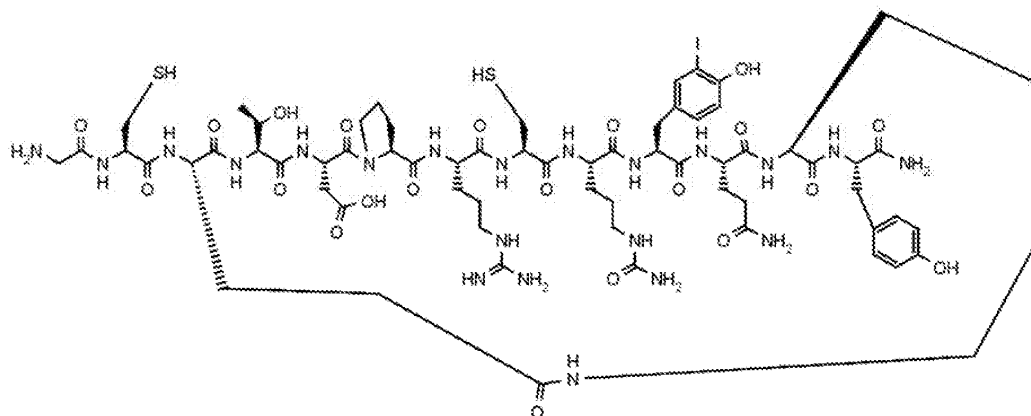
X1

[00469] Lactam formation to produce Intermediate X2 on resin (SEQ ID NO:80): To remove the ivDde and ODmab protecting groups from the Lys12 and Glu3 residues, respectively, the peptide resin was washed with DMF (10 mL) and then drained completely, then the resin was incubated with 2% hydrazine hydrate in DMF (10 mL) for 1 h at rt. The resin was subsequently washed with DMF (6 $\times$ 10 mL) and then resuspended in DMF (10 mL) followed by treatment with HATU (0.38g, 1 mmol), HOAt (0.14 g, 1 mmol), and DIPEA (0.25 mL, 1.5 mmol). The reaction vessel was shaken for a minimum of 2.5 h, followed by washing with DMF (6 $\times$ 10 mL), then MeOH (10 mL $\times$ 2) and finally Et₂O (10 mL $\times$ 2), and then dried under vacuum for 2 h.



X2

[00470] Cleavage from resin to afford Intermediate X3 (SEQ ID NO:81): The lactam was cleaved from the resin by incubating Intermediate **X2** in 23 mL of cleavage solution (TFA/EDT/Thioanisole/Anisole: 30:3:5:2) and the mixture was shaken for 2h at room temperature. After this time the resin was filtered and washed with TFA (2 x 5 mL). The filtrates were combined, and 10-fold volume of cold (0 °C) Et₂O was added, which resulted in precipitation of the peptide. The precipitated peptide was centrifuged at 5,000 rpm for 10 min and washed with cold (0 °C) Et₂O (3 x 5 mL). The crude **X3** was dried under vacuum for 2 h to obtain the crude monocyclic 3→12 lactam peptide intermediate (**X3**, MW = 1727.7 g/mol, 0.4 g, 0.23 mmol, 46%), which was used without further purification.



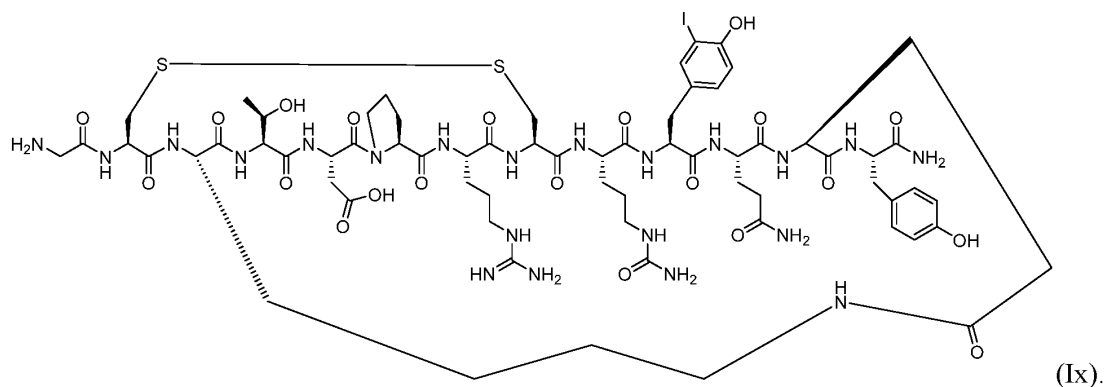
X3

[00471] Disulfide bridge formation to afford conotoxin peptide analog Iw: Intermediate

X3 (0.4 g, 0.23 mmol) was dissolved in CH₃CN (10 mL), DMSO (10 mL) and H₂O (180 mL). Then H₂O₂ (30% in H₂O, 25 μ L) was added dropwise and the mixture was stirred at room temperature for 1.5 h. After this time, the mixture was filtered, and the filtrate was purified by HPLC Method J to afford 50 mg of the conotoxin peptide analog **Iw** as a white solid (MW = 1725.6 g/mol, 29 μ mol, yield this step: 12.6%, TFA salt). Purity 82% (230 nm).

[00472] The peptide was purified further while exchanging the TFA anion for acetate by preparative HPLC according to the method described for conotoxin peptide analogs **Ia**. The isolated yield of conotoxin peptide analog **Iw** (acetate salt) was 20 mg (12 μ mol, 2.4 % overall yield). LC-MS (single quad ESI) m/z: 576.1 [M+3H]/3⁺, 863.5 [M+2H]/2⁺, (calculated MW: 1723.56); HPLC method K; retention time: 7.0 min; purity 97%.

[00473] Example 1.25 Conotoxin Peptide Analogs Ix (L-tyrosinamide, glycyl-L-cysteinyl-L-lysyl-L-threonyl-L- α -aspartyl-L-prolyl-L-arginyl-L-cysteinyl-N5-(aminocarbonyl)-L-ornithyl-L-3-iodotyrosyl-L-glutamyl-L-glutamyl-cyclic (2 $\rightarrow$ 8)-disulfide-cyclic-(3 $\rightarrow$ 12)-lactam) (SEQ ID NO:82):



[00474] Conotoxin peptide analog **Ix** was synthesized by employing the same procedures described for conotoxin peptide analog **Iw** using FMOC-Glu(ODmab) in lieu of FMOC-Lys(ivDde)-OH and FMOC-Lys(ivDde)-OH in lieu of FMOC-Glu(ODmab). LC-MS (ESI-ion trap) m/z: 862.9 [M+2H]/2⁺, 1724.3 [M+H]/⁺, (calculated MW: 1723.56); HPLC method K;

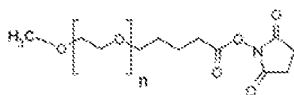
retention time: 7.1 min; purity 95%.

6.3. Example 2: Preparation of PEGylated Conotoxin Peptide Analogs

[00475] **Example 2.1 PEGylated Conotoxin Peptide Analog IIa (SEQ ID NO:83) (30 kDa-mPEG-valerate (VA)-conotoxin peptide analog Ia):**

(IIa)

[00476] The acetate salt of conotoxin peptide analog **Ia** (25 mg, 0.015 mmol) was dissolved in PBS buffer (pH=8.0, 12.5 mL) followed by the addition of mPEG-30 kDa-VA-NHS ester (560 mg, 0.018 mmol; NOF America, SUNBRIGHT ME-300HS; Final linker = valeric acid amide



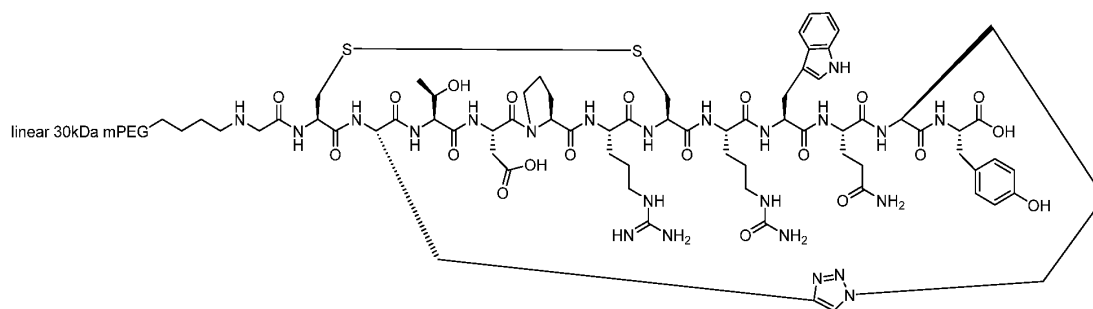
(“VA”); ; average $n = 675$) in H₂O (20 mL). After shaking the reaction vessel for 2 h at room temperature, the reaction progress was monitored using analytical HPLC. When >90% of conotoxin peptide analog **Ia** had been consumed, as determined by HPLC analysis, the resulting PEGylated peptide, PEGylated conotoxin peptide analog **IIa** was purified by HPLC Method I to remove unreacted conotoxin peptide analog **Ia** (RT = 5.0 min). The HPLC peak for PEGylated conotoxin peptide analog **IIa** was fractionated into thirds and each fraction was analyzed by the HPLC Method I with ELSD detection (in-line, post UV detector) to determine the peptide purity (UV RT = 16.65 min, ELSD retention time = 16.75 min) amount of residual free PEG (ELSD RT=17.00 min). Fractions with >95% peptide purity that contained <5% free PEG, based on the ELSD signal (free PEG relative to the ELSD signal of peptide), were combined and lyophilized. Overall isolated yield 0.21g (44%); HPLC Method I:

purity: 95% (UV 214nm), 97% (ELSD). The characterization data for PEGylated conotoxin peptide analog **IIa** are listed in Table 2 below.

Table 2. Characterization Data for PEGylated conotoxin peptide analog **IIa**

Analysis	Result	Method
Experimentally Determined Mass	32254.6	MALDI-TOF
Copper content	<11 ppm	ICP-AES
Karl Fischer Water content	0.40 %	Coulometric KF analysis
Nitrogen content	0.93 %	Elemental Analysis
Peptide content	91.0 % (based on calculated MW: 31512)	Calculation using nitrogen content in elemental analysis
AcOH content	0.549%	Ion Chromatography
TFA content	0.361%	Ion Chromatography

[00477] **Example 2.2 PEGylated Conotoxin Peptide Analog IVa (SEQ ID NO:84) (30 kDa-mPEG-bAmine-conotoxin peptide analog Ia):**



(IVa)

[00478] The acetate salt of conotoxin peptide analog **Ia** (25 mg, 0.013 mmol) was dissolved in

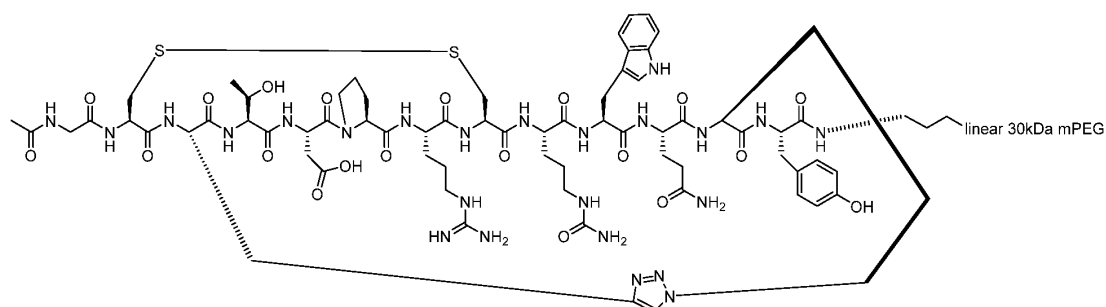
MeOH (10 mL) followed by the addition of a solution of mPEG-30 kDa-butyraldehyde (CAS No. 9004-74-4, 414 mg, 0.013 mmol) in MeOH (10 mL). The mixture was shaken at room temperature for 3 h. After this time, a solution of PPTS (3.3 mg, 0.013 mmol) in MeOH (0.1 mL) was added, followed by addition of a solution of NaCNBH₃ (1.6 mg, 0.026 mmol) in MeOH (0.1 mL). Shaking was continued for 12 h, after this time the reaction mixture was purified directly by prep-HPLC method G to provide TFA salt of PEGylated conotoxin peptide analog **IVa** (92 mg, overall yield, 21%, TFA salt). HPLC Purity: 95.7% (UV 214 nm).

[00479] A subsequent salt exchange was achieved by dissolving TFA salt of PEGylated conotoxin peptide analog **IVa** (92 mg, 0.0027 mmol) from above in 3 mL of water. To this solution, the dropwise addition of NH₄HCO₃ (aq) was added to adjust the pH 7.0-8.0. The mixture was then purified directly by preparative RP-HPLC (Mobile Phase: A: 0.1% AcOH in Water, B: 0.1% AcOH in ACN), and lyophilized to provide acetate salt of PEGylated conotoxin peptide analog **IVa** as white solid (31 mg, overall yield, 7%, acetate salt). LC/MS Purity: 95.5% (UV 214 nm), 98.7% (ELSD). The characterization data for PEGylated conotoxin peptide analog **IVa** are listed in Table 3 below.

Table 3. Characterization Data for PEGylated conotoxin peptide analog **IVa**

Analysis	Result	Method
Experimentally Determined Mass	33465.4	MALDI-TOF
Nitrogen content	1.00 %	C,H,N Combustion Analysis
Peptide content	98.05 % (based on calculated MW: 31571.8)	Calculation using nitrogen content in elemental analysis

[00480] **Example 2.3** C-Terminus PEGylated Conotoxin Peptide Analog Va (SEQ ID NO:85) (conotoxin peptide analog Ia-30 kDa-PEG)



(Va)

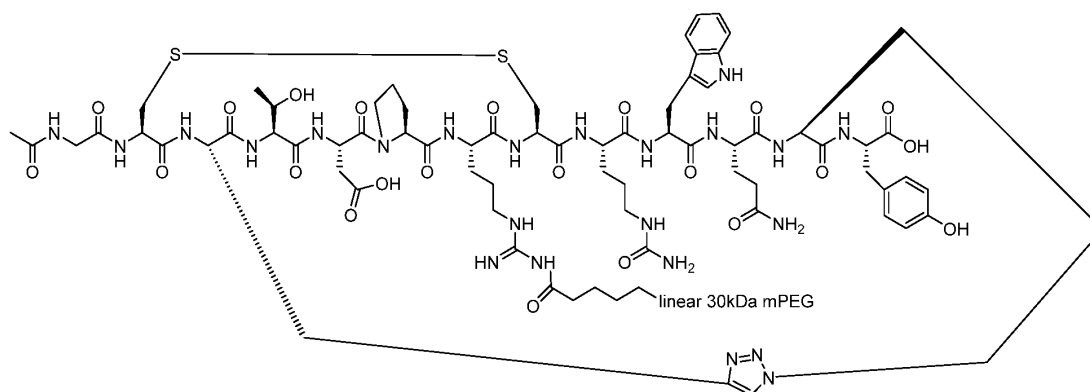
[00481] N-terminus N-acetyl conotoxin peptide analog **1a** intermediate (**14**): conotoxin peptide analog **1a** (75 mg, 46 μ mol) was dissolved in anhydrous DMF (35 mL) and then Ac₂O (7.5 μ L, 0.3 mmol) and anhydrous pyridine (32 μ L, 0.4 mmol) were added. The solution stirred for 2 h at room temperature. Then, the product was purified by preparative HPLC method G (Table 7). After lyophilization of the product containing fractions, compound **14** was obtained as an off-white solid (44.5 mg, 27 μ mol, 98% purity, 59% yield). LC-MS (ESI) m/z: 554.6 [M+3H]/3⁺, 831.0 [M+2H]/2⁺, Purity 98% (UV 214 nm).

[00482] C-Terminus PEGylated Conotoxin Peptide Analog **Va**: Compound **14** (15 mg, 9.0 μ mol) was dissolved in anhydrous DMF (2.5 mL) and a solution of 30 kDa-mPEG-propylamine (300 mg, 10 μ mol, 1.1 equiv.) in anhydrous DMF (2.5 mL) was added. Then, a solution of HBTU (3.7 mg, 9.7 μ mol) in DMF (0.3 mL) and a solution of DIPEA (458 μ L, 26 μ mol) in anhydrous DMF (45 mL) were each added. The mixture was stirred at room temperature for 2 h then purified by HPLC method G (Table 7). Lyophilization of the purified product afforded TFA salt of C-terminus PEGylated conotoxin peptide analog **Va** (100 mg, 3.1 μ mol, 35% yield), as a TFA salt (Purity 91% at 214 nm). This TFA salt was then dissolved in H₂O:CH₃CN (3 mL: 1 mL), then a dilute solution of NH₄HCO₃ (aq.) was added dropwise to obtain a pH = 7.0-8.0. The mixture was then purified by HPLC method H (Table 7) to afford acetate salt of C-terminus PEGylated conotoxin peptide analog **Va**. After lyophilization, a white solid was obtained, (50 mg, yield: 15.8 μ mol, 17.5%, acetate salt). LC- Purity: 90.4% (UV 214 nm), 99.3% (ELSD). The characterization data for PEGylated conotoxin peptide analog **Va** are listed in Table 4 below.

Table 4. Characterization Data for PEGylated conotoxin peptide analog Va

Analysis	Result	Method
Experimentally Determined Mass	31307.7	MALDI-TOF
Nitrogen content	0.71 %	C,H,N Combustion Analysis
Peptide content	66.77 % (based on calculated MW: 31598.8)	Calculation using nitrogen content in elemental analysis

[00483] Example 2.4 7-Arg-PEGylated Conotoxin Peptide Analog VIa (SEQ ID NO:86) (Ac-conotoxin peptide analog Ia (Arg7-VA-30 kDa-PEG)



(VIa)

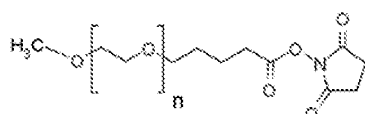
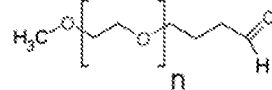
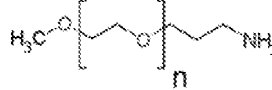
[00484] N-terminus N-acetyl conotoxin peptide analog **Ia** intermediate **14** (15 mg, 9.0 μmol) was dissolved in anhydrous DMF (2.5 mL) followed by addition of a solution of the mPEG-30 kDa-valeric Acid-NHS ester reagent (0.54 g, 18 μmol) in anhydrous DMF (2.5 mL). Then, a solution of DBU (4 μL , 27 μmol) in DMF (0.3 mL) was added. The mixture was heated to 40 $^{\circ}\text{C}$ for 4 h and then the product was purified by HPLC method G (Table 7). Subsequent lyophilization afforded 35 mg of acetate salt of 7-Arg-PEGylated conotoxin peptide analog **VIa** (Table 6; 35 mg, 1.1 μmol , 12.2%, TFA salt). Purity 80% (UV 214 nm). The salt exchange was

carried out as described for to afford acetate salt of 7-Arg-PEGylated conotoxin peptide analog **VIa** as a white solid (28 mg, 9.8% overall yield, acetate salt). Purity: 99.1% (UV 214 nm), 81.4% (ELSD). The characterization data for PEGylated conotoxin peptide analog **VIa** are listed in Table 5 below.

Table 5. Characterization Data for PEGylated conotoxin peptide analog VIa

Analysis	Result	Method
Experimentally Determined Mass	32102.8	MALDI-TOF
Nitrogen content	0.55 %	C,H,N Combustion Analysis
Peptide content	54.0 % (based on calculated MW: 31641.9)	Calculation using nitrogen content in elemental analysis

[00485] Table 6. PEGylation reagents

PEGylation reagent	Structure
mPEG-30 kDa-valeric acid-NHS ester average n = 675. Final linker = valeric acid amide ("VA")	
mPEG-30 kDa-butyraldehyde Final linker = butylamine linker ("bAmine") average n = 678	
mPEG-30 kDa-propylamine Final linker = propylamide ("pAmide") average n = 678	

[00486] The HPLC methods described herein are summarized in Table 7 below.

Table 7: Summary of HPLC Methods

<u>HPLC Method</u>	<u>Description</u>
A	UV 214 nm; Column: XBridge Peptide BEH C18, 4.6x150 mm, 3.5 μ m; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (5), 20 min (65); Flow rate: 1.0 mL/min; 40 °C column temperature.
B	UV 220 nm; Column: Phenomenex Jupiter, 4.6x250 mm, 5 μ m, C18, 300 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (10), 30 min (70); Flow rate 1.0 mL/min; room temperature.
C	UV 220 nm; Column: YMC-Pack-ODS-A, 4.6x250 mm, 5 μ m, C18, 200 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (5), 30 min (65); 1.0 mL/min; room temperature.
D	UV 220 nm; Column: Phenomenex Jupiter, 4.6x250 mm, 5 μ m, C18, 300 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (10), 30 min (70); Flow rate 1.0 mL/min; room temperature.
E	UV 220 nm; Column: YMC-Pack-ODS-A, 4.6x250 mm, 5 μ m, C18, 200 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (5), 30 min (65); 1.0 mL/min; room temperature.
F	UV 215 nm; Column: YMC-Pack-ODS-A, 4.6x250 mm, 5 μ m, C18, 200 Å; Eluent A: 0.1% TFA in water; Eluent B: 0.1% TFA in CH ₃ CN; Time (%B): 0 min (10), 30 min (40); 1.0 mL/min; room temperature.
G	UV 215 nm; Column: XBridge Peptide BEH C18, 19x250 mm, 10 μ m, 300 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (17), 3 min (32), 23 min (42), 28 (95); Flow rate: 25 mL/min; room temperature.
H	UV 215 nm; Column: XBridge Peptide BEH C18, 19x250 mm, 10 μ m, 300 Å; Eluent A: 0.1% AcOH in water; Eluent B: 0.1% AcOH in CH ₃ CN; Time (%B):

	0 min (5), 5 min (25), 15 min (80), 20 (95); Flow rate: 20 mL/min; room temperature.
I	UV 214 nm and ELSD detection; Column: XBridge Peptide BEH C18, 4.6x150 mm, 3.5 μ m, 300 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (15), 3 min (15), 20 min (75); Flow rate: 1.0 mL/min; 40°C column temperature.
J	UV 215 nm; Column: XBridge Peptide BEH C18, 19x250 mm, 10 μ m, 130 Å; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Flow rate: 20.0 mL/min; gradient elution 5 to 100% B; room temperature.
K	UV 230 nm; Column: XBridge Peptide BEH C18, 2.1x150 mm, 3.5 μ m; Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in CH ₃ CN; Time (%B): 0 min (5), 8 min (100); Flow rate: 0.3 mL/min; 25 °C column temperature.

6.4. Stability Analysis

[00487] To address disulfide instability in the RgIA derivatives, conotoxin peptide analogs with a disulfide bridge replaced by a triazole bridge were synthesized and were evaluated in human and Sprague Dawley rat plasma and serum.

[00488] Reverse phase HPLC analysis of samples from each of the triazole-stabilized conotoxin peptide analogs revealed a single isolated peak consistent with the native conformation of the peptide. For example, **FIG. 2A** shows that conotoxin peptide analog **Ia** shows a single isolated peak on reverse phase HPLC in rat plasma at 0 h, 8 h and 24 h. There was no evidence for disulfide rearrangement, suggesting that substitution of one native disulfide bond with a triazole mimetic is adequate to prevent disulfide shuffling of RgIA derivatives in plasma.

[00489] In contrast, as shown in the reverse phase HPLC traces of conotoxin peptide analog **CSP-4-NH₂** in **FIG. 2B**, a significant portion of conotoxin peptide analog **CSP-4-NH₂** (“native” form, consisting of two disulfide bonds, one between Cys2 and Cys8, and a second between Cys3 and Cys12) underwent isomerization into a “ribbon” form, containing alternative disulfide bonds Cys2-Cys12 and Cys3-Cys8 and a “bead” form containing alternative Cys2-Cys3 and

Cys8-Cys12 disulfide bonds. In plasma and serum samples, conotoxin peptide analog **CSP-4-NH₂** principally isomerized between the native and ribbon forms, with very little bead formation (**FIG. 2B**).

6.5. Biological Assays

6.5.1. Two-Electrode Voltage Clamp Method for Rat and Human nAChRs

Example 4.1

Assay:

[00490] *Xenopus laevis* oocytes (Xenopus 1, MI) were used to heterologously express cloned rat or human nAChR subtypes. Recordings were made 1-5 days post-injection. Briefly, an oocyte was placed in a 100 μ L chamber (4 mm diameter 62 mm deep) fabricated from Sylgard and gravity-perfused with ND96 (96 mM NaCl, 1.8 mM CaCl₂, 2.0 mM KCl, 1.0 mM MgCl₂, 5 mM HEPES, pH 7.1–7.5) at a constant flow rate (2 mL/min). The oocyte's membrane potential was held at -70 mV using a GeneClamp 500 two-electrode voltage clamp amplifier. nAChRs were stimulated with 1-sec pulses of 100 μ M acetylcholine (ACh) once every minute and ACh-gated currents were acquired. After a steady baseline of ACh pulses was achieved using ND96, the solution was switched to ND96 containing various compound concentrations and ACh pulses were observed for a blocking response. Responses to ACh of nAChRs after peptide application were calculated as the “% response” of that observed at baseline. Concentration-response curves for inhibition of ACh-gated currents were generated by fitting each oocyte data to the Hill equation: % response = $100 / \{1 + ([\text{toxin}] / \text{IC}_{50})^{n_H}\}$ by non-linear regression analysis and using constraining at bottom equal to 0 and top equal to 100 (GraphPad Prism). Independent concentration response curves were fitted per oocyte and the IC₅₀ values were averaged. IC₅₀ average values were reported as means $\pm$ SEM. Two-tailed unpaired t-test was used for significance differences (GraphPad Prism).

Results:

6.5.1.1. Replacement of disulfide bridge with a triazole bridge improves stability and maintains adequate activity on both rat and human nAChRs

Table 8. Inhibition of the rat and human $\alpha 9\alpha 10$ nicotinic acetylcholine receptors (nAChRs) by conotoxin peptide RgIA and its analogs

Conotoxin Peptide / Analog	$\alpha 9\alpha 10$ nAChR IC ₅₀ (nM)	
	Rat	Human
RgIA	2.4 ± 0.7	>10,000
CSP-4-OH	0.9 ± 0.6	1.5 ± 0.5
CSP-4-NH ₂	0.4 ± 0.2	3.5 ± 1.5
CSP-4-desTyr	NT	42 ± 0.1
Ia	14.3 ± 1.8	0.7 ± 0.03
Ia'	12.61 ± 0.08	26 ± 4
Ib	7 ± 0.7	37 ± 1.3
Ib'	2.3 ± 0.5	48 ± 9
Ic	69 ± 8.5	152 ± 71
Id	≥ 1000	≥ 1000
Ie	6.6 ± 1.7	115 ± 56
If	12.5 ± 8.7	1521 ± 116
Ig	22 ± 1.1	19 ± 1.2
Ih	8.5 ± 1.1	8 ± 1.5
Ii	1.6 ± 0.6	3.4 ± 1.4
Ij	69 ± 8.5	152 ± 71
Ik	9.5 ± 1.3	16.6 ± 1.1
Il	NT	11 ± 1.4
Im	NT	50 ± 1.2
In	≤ 10 nM	37 ± 15
Io	≤ 10 nM	59
Ip	≤ 10 nM	26 ± 1.4
Iq	69 ± 2	>300 nM
Ir	31 ± 0.7	~100
Is	NT	59 ± 1.7
It	NT	~100 nM
Iu	<30	~100 nM

Iv	<30	~100 nM
Iw	36 ± 9	>300
Ix	21 ± 1.4	123 ± 22

Note: NT = not tested.

[00491] The activities of the conotoxin peptide analogs and the PEGylated conotoxin peptide analogs prepared in **Sections 6.2** and **6.3** on both rat and human nAChRs were determined and the results are summarized in **Tables 8** and **9** respectively.

[00492] As shown in **Table 8**, **CSP-4–NH2** showed nanomolar activities on both rat and human nAChRs (IC_{50} : 0.4 ± 0.2 nM on rat $\alpha 9\alpha 10$ nAChR and 3.5 ± 1.5 nM on human $\alpha 9\alpha 10$ nAChR). Nevertheless, because **CSP-4–NH2** has two disulfide bridges (one disulfide bridge between cysteine residues at 2- and 8-positions; another one disulfide bridge between cysteine residues at 3- and 8-positions), **CSP-4–NH2** suffered from a disulfide shuffling issue and has poor stability (see **FIG. 1A–FIG. 1D** and **FIG. 2B**).

[00493] As shown in **Table 8**, conotoxin peptide analogs **Iw** and **Ix** with the 3- and 12-position disulfide bridge replaced by a lactam bridge, did not maintain adequate activities on the human $\alpha 9\alpha 10$ nAChR. Conotoxin peptide analogs **Iw** and **Ix** showed IC_{50} 's of >300 nM and 123 ± 22 nM, respectively. Certain conotoxin peptide analogs that had a triazole bridge formed between a Pra at 3- position and an AzidoNva at 12- position maintained adequate biological activities on the human $\alpha 9\alpha 10$ nAChR (e. g., $IC_{50} < 100$ nM). For example, the IC_{50} 's of conotoxin peptide analogs **Ia** and **Ia'** on the human $\alpha 9\alpha 10$ nAChR were measured to be 0.7 ± 0.03 nM and 26 ± 4 nM, respectively. The IC_{50} 's of conotoxin peptide analogs **Ib** and **Ib'** on the human $\alpha 9\alpha 10$ nAChR were measured to be 37 ± 1.3 nM and 48 ± 9 nM, respectively. Conotoxin peptide analogs **Ia** and **Ib**, with a carboxylic acid at the C-terminus, and conotoxin peptide analogs **Ia'** and **Ib'**, with an amide group at the C-terminus, maintained adequate activities. Conotoxin peptide analogs **Ia** and **Ib**, both of which have an amide group at the C-terminus, each showed lower IC_{50} 's than the corresponding conotoxin peptide analog with a carboxylic acid at the C-terminus (**Ia** vs. **Ia'** and **Ib** vs. **Ib'**).

[00494] In contrast, conotoxin peptide analogs **Ic** and **Id** having a triazole bridge formed between the 2- and 8-positions, did not maintain adequate activity on the human $\alpha 9\alpha 10$ nAChR. The IC_{50} 's of conotoxin peptide analogs **Ic** and **Id** were measured to be 152 ± 71 nM and $\geq$

1000 nM, respectively. Also in contrast, conotoxin peptide analogs **If** and **Ij** having a triazole bridge formed between the 3- and 12-positions, but having a triazole bridge formed from a Pra or an AzidoAla, respectively, at the 12-position did not maintain adequate activity on the human $\alpha 9\alpha 10$ nAChR. The IC_{50} 's of conotoxin peptide analogs **If** and **Ij** were measured to be 1521 ± 116 nM and 152 ± 71 nM, respectively.

[00495] Deletion of the terminal amino acid of RgIA and replacement with an amide (Cys12-amide) has previously been demonstrated to have no substantial impact on binding of the peptide to the rat $\alpha 9\alpha 10$ nAChR (Ellison et al, 2008, J. Mol. Biol. 377:1216-1227; Armishaw, 2010, Toxins 2:1471-1499; US 20120220539 A1). Further, other alpha-conotoxins such as Vc1.1 and ImI do not have an amino acid at position 13 (see **FIG. 4**), suggesting that this residue would not be essential for channel binding.

[00496] However, C-terminal amino acid deletion or replacement was found unexpectedly to affect human $\alpha 9\alpha 10$ nAChR potency in the context of conotoxin peptide analog **1a**. Specifically, as shown in **Table 8**, when the Tyr at the 13-position was replaced with a Phe (conotoxin peptide analog **II**) or D-Tyr (conotoxin peptide analog **Im**), the potency was reduced. The IC_{50} 's of conotoxin peptide analogs **II** and **Im** were measured to be 11 ± 1.4 nM and 50 ± 1.2 nM, respectively. In contrast, when the Tyr at the 13-position was deleted (conotoxin peptide analog **Iq**), replaced by an N-Me-Tyr (conotoxin peptide analog **Ir**), replaced by a D-Arg (conotoxin peptide analog **Is**), replaced by an N-Me-D-Tyr (conotoxin peptide analog **It**), replaced by a beta-Tyr (conotoxin peptide analog **Iu**) or replaced by N-Me-Arg (conotoxin peptide analog **Iv**), it afforded conotoxin peptide analogs with lower potencies on the human $\alpha 9\alpha 10$ nAChR.

[00497] The impact of deletion of the amino acid residue at the 13-position of conotoxin peptide analogs with a triazole bridge formed between the 3- and 12-positions on their potency on the human $\alpha 9\alpha 10$ nAChR was evaluated relative to the impact of deletion of the 13-position residue of **CSP-4-OH** (the corresponding conotoxin peptide analog with a 3- and 12-positions disulfide bridge). Unexpectedly, with respect to **CSP-4-OH** (Cys3,12 disulfide bridge), deletion of Tyr at 13-position was found to result in a greater than 20-fold reduction in binding affinity for the human $\alpha 9\alpha 10$ nAChR (**FIG. 5A**). However, with respect to conotoxin peptide analog **1a** (replacement with a triazole bridge between 3- and 12- position), deletion of amino acid 13

resulted in an approximately 10-fold loss in activity on the rat channel and a greater than 100-fold loss of activity on the human channel (conotoxin peptide analogs **1a** vs **1q**) (**Table 8, FIG. 5B**). These data showed that deletion of an amino acid at C-terminus (position 13) decreased the activities of conotoxin peptide analogs with a triazole bridge between the 3- and 12- positions, but not for those with a disulfide bridge between the 3- and 12- positions. These data suggested that interactions throughout loop 2 and the carboxy-terminus of the peptide are important for determining human channel potency in the context of a stabilized disulfide mimetic and that changes to the secondary structure of loop 2 and the carboxy terminus create unpredictable interactions between conotoxin peptide RgIA analogs and the $\alpha 9\alpha 10$ nAChR.

[00498] Moreover, addition of one additional amino acid to C-terminus of conotoxin peptide analog **1a** (e.g., an N-Me-Gly, D-Tyr, or N-Me-Tyr) to afford conotoxin peptide analogs **1n**, **1o**, and **1p** of 14 residues maintained potency on the human $\alpha 9\alpha 10$ nAChR. As illustrated in **Table 8**, for example, the IC_{50} 's of conotoxin peptide analogs **1n** (additional an N-Me-Gly at the 14- position compared to **1a**), **1o** (additional a D-Tyr at the 14- position compared to **1a**) and **1p** (additional an N-Me-Tyr at the 14- position compared to **1a**) were measured to be 37 ± 15 nM, 59 nM and 26 ± 1.4 nM, respectively.

[00499] **Channel binding selectivity:** in an evaluation of potential off-target effects involving 88 receptors, transporters, enzymes and kinases, triazole-containing conotoxin peptide analogs **1a'** and **1b'** demonstrated no off-target effects at 10 μ M, except for partial inhibition (52% and 56%, respectively) of the $\alpha 7$ nAChR. In this respect, triazole bridge replacement of disulfide bridge in conotoxin peptide RgIA did not significantly alter the selectivity of the conotoxin peptide analogs.

6.5.1.2 PEGylation of conotoxin peptide analog **1a** at the N-terminus

unexpectedly retains adequate activity on both rat and human nAChRs

[00500] The addition of a 30 kDa valerate-linear PEG (N-terminal acylation) to the N-terminus of conotoxin peptide analog **1a** resulted in PEGylated conotoxin peptide analog **11a**. When evaluated in its activity against the human and rat nAChRs, PEGylated conotoxin peptide analog **11a** produced similar blockade on the rat and human $\alpha 9\alpha 10$ nAChRs (**FIG. 6A** and **FIG. 6B**) with low nanomolar potencies. On the human $\alpha 9\alpha 10$ nAChR, PEGylated conotoxin peptide

analog **IIa** resulted in nearly identical IC_{50} (**Table 9**; 0.7 ± 0.1 nM) to the unconjugated conotoxin peptide analog **Ia** (0.7 ± 0.03 nM). In contrast to the literature teaching that PEGylation would potentially create a structural constraint resulting in a reduction of peptide binding potency to rodent and human channels (Fishburn, 2008, J. Pharm. Sci. 97:4167-4183; Parrott and DeSimone, 2011, Nat. Chem. 4:13-14), the PEGylated conotoxin peptide analog **IIa** with an about 18.5-fold molecular weight increase by the attachment to the PEG polymer unexpectedly retained the potency of conotoxin peptide **Ia** (0.7 ± 0.1 nM on human $\alpha 9\alpha 10$ nAChR)

Table 9. Inhibition of the rat and human $\alpha 9\alpha 10$ nicotinic acetylcholine receptors (nAChRs) by conotoxin peptide analog **Ia** and its PEGylated conotoxin peptide analogs **IIa**, **IIIa**, **IVa**, **Va** and **VIa**

Conotoxin Peptide / Analog	$\alpha 9\alpha 10$ nAChR IC_{50} (nM)	
	Rat	Human
RgIA	2.4 ± 0.7	>10,000
CSP-4-OH	1.5 ± 0.5	0.9 ± 0.6
CSP-4-NH ₂	0.4 ± 0.2	3.5 ± 1.5
Ia	14.3 ± 1.8	0.7 ± 0.03
Ia'	7.7 ± 0.7	26 ± 4
IIa	NT	0.7 ± 0.1
IVa	NT	100
Va	NT	~300
VIa	NT	300

Note: NT = not tested.

[00501] The impact of PEGylation at different attachment positions was evaluated by testing the activities of PEGylated conotoxin peptide analogs **Va** (in which a linear mPEG polymer is covalently attached to the C-terminus of conotoxin peptide analog **Ia**) and **VIa** (in which a linear mPEG polymer is covalently attached to the Arginine at the 7-position of conotoxin peptide analog **Ia**) against the human $\alpha 9\alpha 10$ nAChR. Substantially higher IC_{50} against the human nAChR was observed for both PEGylated conotoxin peptide analogs **Va** ($IC_{50} = \sim 300$ nM) and **VIa** ($IC_{50} = 300$ nM), compared to that of PEGylated conotoxin peptide analogs **IIa** ($IC_{50} = 0.7 \pm$

0.1 nM), which has a linear mPEG polymer covalently attached to the N-terminus of conotoxin peptide analog **Ia**. Conjugating to the PEG at the C-terminus (**Va**) or at position 7 (**VIa**) of conotoxin peptide analog **Ia** did not afford PEGylated conotoxin peptide analogs with adequate activities.

[00502] The potency of a PEGylated conotoxin peptide analog with a different linker was also studied. The activity of the PEGylated conotoxin peptide analog **IVa**, with a linear mPEG polymer attached to the N-terminus of conotoxin peptide analog **Ia** via a butylene linker) against the human $\alpha 9\alpha 10$ nAChR was tested. As shown in **Table 9**, the IC_{50} of PEGylated conotoxin peptide analog **IVa** was more than 100-fold higher ($IC_{50} = \sim 100$ nM) than that of PEGylated conotoxin peptide analogs **IIa** ($IC_{50} = 0.7 \pm 0.1$ nM), in which the PEG polymer is attached to the N-terminus of the peptide (conotoxin peptide analog **Ia**) via a valerate linker. These results illustrated that the choice of linker type and of the position on the peptide to which the PEG polymer is attached can be crucial to the PEGylation derivatization of conotoxin peptide analog **Ia** for retaining its potency against the human $\alpha 9\alpha 10$ nAChR. Unexpectedly, the PEGylated conotoxin peptide analog **IIa**, with a 30 kDa linear mPEG covalently attached to the N-terminus of conotoxin peptide analog **Ia** via a valerate linker was found to be the optimal PEGylated conotoxin peptide analog.

[00503] A similar unexpected result was also demonstrated with PEGylated **CSP-4-OH**. With the addition of a 30 KDa PEG to the N-terminus of **CSP-4-OH** via a valerate linker, the resulting PEGylated conotoxin peptide analog **XI** (linear 30 kDa mPEG-VA-**CSP-4-NH2**) maintained low nanomolar potency on the rat $\alpha 9\alpha 10$ nAChR ($IC_{50} = 6.0 \pm 0.1$ nM), suggesting that the N-termini of these peptides are permissive to the addition of bulky molecules without significantly altering their *in vitro* biological potency.

6.5.2. Pharmacokinetics Studies of Conotoxin Peptide Analogs and PEGylated Conotoxin Peptide Analogs in Rats

[00504] **Pharmacokinetics Studies of Conotoxin Peptide Analog Ia in rats:** conotoxin peptide analog **Ia** was injected intravenously or subcutaneously in 10-week-old male Sprague Dawley rats (n=3 per group) at 1 mg/kg in vehicle (10 mM sodium phosphate, 0.8% sodium

chloride, 0.05% Tween 20, pH 6.0). Blood was collected at various time points (pre-dose and from 0.083 to 24 hours post dose) via tail vein into K₂EDTA collection tubes, centrifuged and plasma was stored at -60 to -90°C until analysis. The concentrations of conotoxin peptide analog **1a** were determined using a peptide specific LC-MS/MS assay as described below. Raw data were generated and plotted using a nonlinear regression with a sigmoidal dose-response (variable slope) with GraphPad Prism. Pharmacokinetic parameters were calculated using PKSolver 2.0 software.

[00505] LC-MS/MS analysis for conotoxin peptide analog 1a plasma PK samples:

conotoxin peptide analog **1a** sample analyses were carried out with a Shimadzu liquid chromatography system (Shimadzu UFLC-XR) and an AB Sciex API 5000 triple quadrupole tandem mass spectrometer. The HPLC system consisted of Shimadzu liquid chromatography system equipped with two LC-20AC XR pumps, a CBM-20A Communication Module, a SIL-20AC XR auto-sampler, a CTO-20A Column Thermostat and in-line SPD-20A UV detector. Chromatographic separation was carried out on a Thermo Scientific Fluophase PFP column (2.1 x 50 mm, 5 µm, 100 Å) at 40°C. The mobile phase A was water with 0.05% acetic acid and mobile phase B was acetonitrile with 0.05% acetic acid. At a flow rate of 0.3 mL/min, a linear gradient of B (5%-90%) was applied over 0-6 min, then 90% over 6.0-7.8 min, and then returned to 5% B for 2 min for column equilibration. The samples were kept at 4°C in the auto-sampler and a volume of 5 µL was injected onto the HPLC system for each analysis.

[00506] Mass spectrometric detection was performed on a triple quadrupole tandem mass spectrometer API 5000) equipped with a turbo ion spray source operated in the negative ionization mode. The MS operating conditions were optimized as follows: The ion spray voltage was set at -4500 KV and the source temperature was maintained at 500°C; The collision energy was set at -35V. Nitrogen was used as the collision gas. The flow rates of the curtain gas, ion source gas1 and gas2 were set at 10, 40 and 40 L/min, respectively. The operation of the LC-MS/MS and data analysis were performed using the Analyst 2.1 software (AB Sciex). Quantification data was obtained by using multiple reaction monitoring (MRM) mode of conotoxin peptide analog **1a** transition at m/z 809.0 / 792.5.

[00507] A standard curve of conotoxin peptide analog **1a** (10-5000 ng/mL) was made in

K₂EDTA rat plasma (Bioreclamation) from a 1.0 mg/mL stock solution of accurately weighed, purity corrected peptide in DMSO using a HP D300 Digital Dispenser (Tecan). 50 µL of PK sample plasma, conotoxin peptide analog **Ia** standards, and conotoxin peptide analog **Ia** QC samples were aliquoted onto a Phree Phospholipid Removal Plate (Phenomenex), treated with 450 µL of methanol (1:10 dilution) and thoroughly mixed. Samples were then filtered through the Phree Plate into 96-deep-well sample plate with a vacuum manifold and analyzed using the LC-MS/MS methods described. The concentration of conotoxin peptide analog **Ia** was derived from the standard curve using one parameter nonlinear regression with AB-Sciex MultiQuant software, and PK parameters were calculated using PKSolver 2.0 software.

[00508] Pharmacokinetics (PK) studies of PEGylated conotoxin peptide analog **Ia in rats:** PEGylated conotoxin peptide analog **Ia** was injected intravenously (“IV”) or subcutaneously (“SC”) in 10-week-old male Sprague Dawley rats (n=3 per group) at 1 mg/kg (core peptide content, ~19 mg/kg PEGylated peptide content) in vehicle (10 mM sodium phosphate, 0.8% sodium chloride, 0.05% Tween 20, pH 6.0). Blood was collected at various time points (pre-dose and from 0.083 – 120 h post dose) via tail vein into K₂EDTA collection tubes, centrifuged and plasma was stored at -60 to -90 °C until analysis. Amount of conotoxin peptide analog **Ia** was quantified by a PEG-conjugate specific ELISA assay as described below. Derived plasma concentrations (net peptide) were generated using a nonlinear regression with a sigmoidal dose-response (variable slope) with GraphPad Prism. PK parameters were calculated using PKSolver 2.0 software.

[00509] ELISA antibodies, reagents and methods for PEGylated conotoxin peptide analog **Ia PK sample analysis:** Rabbit polyclonal anti-conopeptide capture antibodies were generated by immunizing naïve NZW rabbits with conotoxin peptide analog **Ia** conjugated to a KLH carrier protein and boosted with conotoxin peptide analog **Ia**. Polyclonal IgG antibodies were then purified from immune serum using an affinity Protein G Sepharose (GE Healthcare Life Sciences) gravity column followed by polishing and buffer exchange on a 16/600 Superdex 75pG Size-Exclusion Chromatography (SEC) column (GE Healthcare Life Sciences) using PBS as the mobile phase. SEC fractions containing purified antibody were pooled and concentrations were calculated by bicinchoninic acid assay (BCA assay). The purified polyclonal antibodies

have demonstrated cross reactivity to recognize conotoxin peptide analog **Ia** and conotoxin peptide analog **IIa**. The microtiter plate wells were coated with 50 μ L per well of capture antibody (Purified anti-conotoxin peptide analog **Ia** 7626R pAb, Lot# 26R-1-D54-0618, 5 μ g/mL) in coating buffer (0.1M NaHCO₃ pH 9.6) for 12 h at 4°C or at 37°C with shaking for 1 h and then blocked with 200 μ L per well of 5% BSA in PBS for 2 h at room temperature. Plasma samples, QC samples and standards (50 μ L) were loaded in duplicate and incubated for 1.5-2 h at room temperature with shaking, followed by the addition of 50 μ L per well of anti-PEG antibody (1 μ g/mL) in diluent buffer (0.5% BSA in PBS) for an additional 1 h at room temperature with shaking. 50 μ L per well of streptavidin HRP diluted 1:200 in diluent buffer was added to the plate and incubated for 1 h at room temperature with shaking, followed by the addition of 50 μ L of the chromogenic substrate (TMB) which was incubated at room temperature for 5-6 min. The reaction was stopped with 100 μ L H₂SO₄ and absorbance at 450 nm was measured using ELISA plate reader. Plates were washed 3 times with washing buffer (PBST, pH 7.4, containing 0.1% (v/v) Tween 20) then 3-6 times with PBS after each step. As a reference for quantification, a standard curve was established by a serial dilution of PEGylated conotoxin peptide analog **IIa** in 10% K₂EDTA plasma (rat or monkey) (400 ng/mL-0.06 ng/mL, weight based on core peptide content). The amount of PEGylated conotoxin peptide analog **IIa** in each sample was quantified by extrapolating the signal of the sample into the linear range (signal vs concentration) of the standard curve.

[00510] Results: As discussed below, PEGylation of conotoxin peptide analog **Ia** was shown to prolong its circulation time. The pharmacokinetic profile of PEGylated conotoxin peptide **IIa** was evaluated in rats and compared to the PK profile and clearance of conotoxin peptide analog **Ia**. Plots of mean concentrations of conotoxin peptide analog **Ia** and PEGylated conotoxin peptide **IIa** vs. time in plasma following a single 1 mg/kg IV or SC dose are shown in **FIG. 7A** and **FIG. 7B**. Two enhancements due to PEGylation were clear when comparing the PEG-conjugate PK profile to the profile of the unconjugated conotoxin peptide analog **Ia** alone: (i) the half-life of conotoxin peptide analog **Ia** was substantially increased by the addition of a PEG group, and (ii) the total exposure of PEGylated conotoxin peptide **IIa** was much greater than that for unconjugated conotoxin peptide analog **Ia**. For SC administered compounds, the elimination

half-life of PEGylated conotoxin peptide **IIa** was approximately 42-times greater than the unconjugated conotoxin peptide analog **Ia** (21 h vs 0.5 h respectively). The C_{max} and AUC for PEGylated conotoxin peptide **IIa** were also improved, with the C_{max} of PEGylated conotoxin peptide **IIa** being nearly four-fold that of the unconjugated conotoxin peptide analog **Ia** and the AUC for PEGylated conotoxin peptide **IIa** being nearly 140-times greater than the AUC for conotoxin peptide analog **Ia** (C_{max}: PEGylated conotoxin peptide **IIa**: 2518 ng/mL; conotoxin peptide analog **Ia**: 648 ng/mL) (AUC₀₋₄: PEGylated conotoxin peptide **IIa**: 137729 ng/mL*hr; conotoxin peptide analog **Ia**: 981 ng/mL*hr). Pharmacokinetics (PK) and pharmacodynamics (PD) parameters for conotoxin peptide analog **Ia**: and PEGylated conotoxin peptide **IIa** in rats are shown in **Table 10** below.

Table 10. Pharmacokinetics (PK) and pharmacodynamics (PD) parameters for conotoxin peptide analog **Ia**: and PEGylated conotoxin peptide **IIa** in rats

Parameter	Conotoxin peptide analog Ia	PEGylated Conotoxin peptide analog IIa
In vitro potency (TEVC Human $\alpha 9\alpha 10$)	0.7 nM	0.7 nM
PK (Rat, SC - 1 mg/kg) C _{max}	648 ng/ml	2518 ng/ml
PK (Rat, SC - 1 mg/kg) C _{last}	20 ng/ml	166 ng/ml
PK (Rat, SC - 1 mg/kg) T _{max}	0.25 h	24 h
PK (Rat, SC - 1 mg/kg) T _{last}	3 h	120 h
PK (Rat, SC - 1 mg/kg) AUC	981 ng/mL*h	137729 ng/mL*h
PK (Rat, SC - 1 mg/kg) T _{1/2}	0.5 h	21 h
PD (Rat CIPN) Required dose to reach max efficacy	0.5 mg/kg (net peptide)	0.5 mg/kg (net peptide)
PD (Rat CIPN) Duration of PD effect	4-24 h	48-72 h

Metabolic stability (Rat SC dose, % intact peptide remaining at Tmax relative to loss of Tyr13)	66% (percent intact peptide at 0.25 h)	82% (percent intact peptide at 104 h)
---	--	---------------------------------------

[00511] The enhancement of PK profile by PEGylation of conotoxin peptide analog **Ia** was also observed in monkeys. Male cynomolgus macaque monkeys were administered PEGylated conotoxin peptide **Ila** at 1 mg/kg (net peptide concentration) IV and SC for a PK study. After IV administration, the PEGylated conotoxin peptide **Ila** highest exposure was at 0.083 h post-dose with a C_{max} of 42,200 ng/mL and decreased to 109 ng/mL at 168 h post-dose. The AUC was 603,594 ng/ml*h. After SC administration, the PEGylated conotoxin peptide **Ila** highest exposure was at 24 h post-dose with a C_{max} of 7,867 ng/mL and decreased to 216 ng/mL at 168 h post-dose. The AUC was 453,077 ng/ml*h and the bioavailability of PEGylated conotoxin peptide **Ila** after SC dosing was 75%. PK profiles and parameters for PEGylated conotoxin peptide **Ila** in monkeys are shown in **FIG. 8** and **Table 11**.

Table 11. Pharmacokinetic parameters of PEGylated conotoxin peptide **Ila** after a single 1 mg/kg dose administration (IV or SC) in monkeys

Parameter		Unit	PEGylated conotoxin peptide Ila 1 mg/kg IV	PEGylated conotoxin peptide Ila 1 mg/kg SC
Peak plasma concentration	C _{max}	ng/mL	41764	7867
Last plasma concentration	C _{last}	ng/mL	109	101
Time of peak plasma concentration	T _{max}	h	0.083	24
Time of last plasma concentration	T _{last}	h	168	216
Elimination half life	T _{1/2}	h	28.3	29.8
Area under the curve	AUC _{0-t}	ng/mL*h	599144	448716
AUC extrapolated to infinity	AUC _{0-inf obs}	ng/mL*h	448716	453077
Bioavailability	F	%	NT	87

Note: NT = not tested.

6.5.3. Analgesic Activity in Animal Pain Models

[00512] The chronic constriction injury (CCI) model is a model of mononeuropathy induced by ligation to the sciatic nerve (Bennett and Xie, 1988, *Pain*, 33(1):87-107). Damage to the peripheral nerves is involved, with infiltration by mast cells, granulocytes, macrophages and T lymphocytes. These cells, via secretion of inflammatory mediators (e.g. proinflammatory cytokines and chemokines), can contribute to the generation and maintenance of neuropathic pain. The CCI is one of the common models for peripheral nerve injury.

[00513] **Assay:** male Sprague Dawley rats were anesthetized in an induction chamber using 5% Isoflurane (O₂ at 1 L/ min) on Day 0. Once the appropriate anesthetic plane had been reached, a constant flow of isoflurane at a maintenance level of 3% was administered by nose cone. A 2 cm skin incision was made on the left hind leg parallel to the femur. The sciatic nerve was exposed, and proximal to the trifurcation of the sciatic nerve, four loose ligatures of 4-0 chromic gut were tied approximately 1 mm apart. The muscle and skin were closed, and animals were allowed to recover. Beginning on the day of surgery and daily for 14 days thereafter, 0.1 mg/kg of conotoxin peptide analog **1b'** was administered in a vehicle composed of 10 mM sodium phosphate, 0.8% NaCl, 0.05% tween 20 by subcutaneous injection.

[00514] **Results:** Data from animals treated with conotoxin peptide analog **1b'** were compared to animals treated with vehicle control. Mechanical hyperalgesia was measured in the left hind paw (n=3 times) using an Ugo Basile Analgesimeter 7 and 14 days post-surgery. Statistical analysis was performed by two-way ANOVA. As shown in **FIG. 9**, daily treatment with 0.1 mg/kg conotoxin peptide analog **1b'** resulted in a statistically significant improvement in paw withdrawal thresholds relative to vehicle-treated control animals on both measurement days. These data indicated that a triazole-substituted RgIA analog effectively treated neuropathic pain in the rat CCI model.

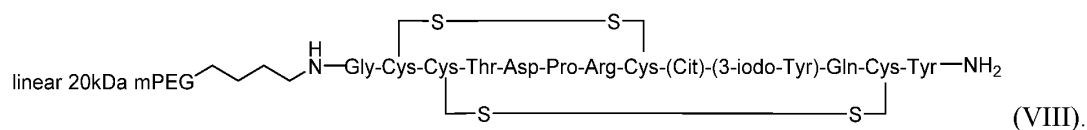
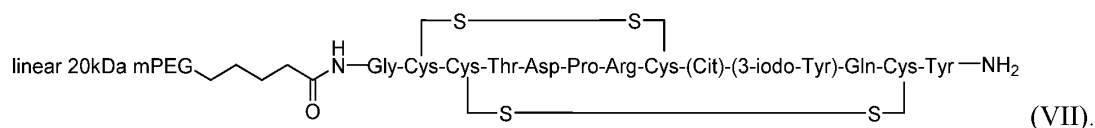
6.5.4. Chemotherapy Induced Peripheral Neuropathy Model

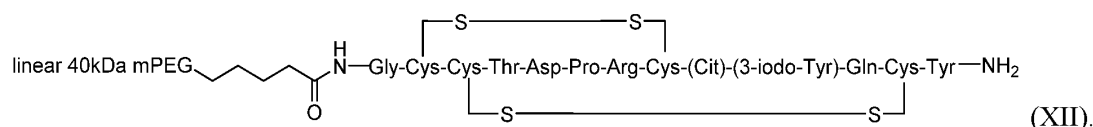
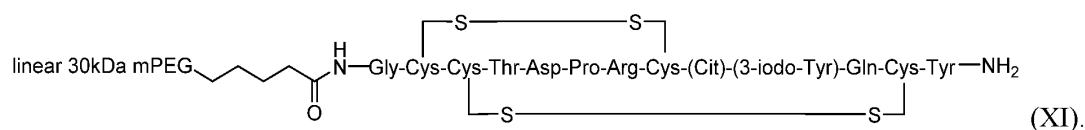
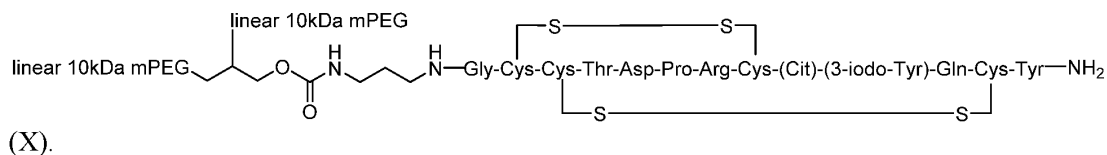
[00515] **Assay:** Chemotherapy induced peripheral neuropathy (CIPN) is common in patients undergoing chemotherapy with platinum salts. Chemotherapy induced neuropathy in male Sprague Dawley rats was induced by treatment with 2.4 mg/kg intravenous (i.v.) oxaliplatin

twice per week for 3 weeks (6 i.v. injections). The nociceptive thresholds in rats were determined with an analgesimeter (Ugo Basile, Varese, Italy). Briefly, a constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical probe. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred. Vocalization or withdrawal reflex thresholds were expressed in grams. For analgesia measures, mechanical pressure application was stopped at 120 g. Throughout the study period, experimenters were blinded as to the identity of the injected compounds. Data were analyzed with one-way ANOVA using Dunnett's multiple comparison test (GraphPad Prism). Neuropathic pain was measured on day 14 following administration of a single subcutaneous dose (e.g., 0.5 mg/kg) of conotoxin peptide analog.

6.5.4.1. Evaluation of PEGylated conotoxin peptide analog derivatives with different PEG polymers

[00516] Different PEGylated derivatives of **CSP-4-NH₂** were prepared, including linear 20 kDa mPEG-VA-**CSP-4-NH₂** (PEGylated conotoxin peptide analog **VII**, Formula (VII), SEQ ID NO:87), linear 20 kDa mPEG-bAmine-CSP-4-NH₂ (PEGylated conotoxin peptide analog **VIII**, Formula (VIII), SEQ ID NO:88), branched 2x10 kDa mPEG-amine-**CSP-4-NH₂** (PEGylated conotoxin peptide analog **IX**, Formula (IX), SEQ ID NO:89), branched 2x10 kDa mPEG-carbonate-**CSP-4-NH₂** (PEGylated conotoxin peptide analog **X**, Formula (X), SEQ ID NO:90), linear 30 kDa mPEG-VA-**CSP-4-NH₂** (PEGylated conotoxin peptide analog **XI**, Formula (XI), SEQ ID NO:91), and linear 40 kDa mPEG-VA-**CSP-4-NH₂** (PEGylated conotoxin peptide analog **XII**, Formula (XII), SEQ ID NO:92).





135

was no additional benefit obtained by conjugation with a PEG molecule larger than a 30 kDa linear PEG (XII) or with a branched chain PEG (IX and X) (FIG. 10C).

6.5.4.2. Evaluation of efficacy of conotoxin peptide analogs Ia and Ia' and PEGylated conotoxin peptide analog IIa

[00518] As shown in FIG. 11A, a single subcutaneous dose (0.5 mg/kg) of conotoxin peptide analog Ia' resulted in statistically significant improvements in mechanical hyperalgesia at 0.5 h, 4 h and 24 h post-dose. As shown in FIG. 11B, single subcutaneous injection of conotoxin peptide analog Ia' resulted in a statistically significant improvement in mechanical hyperalgesia at 0.5 h, 4 h and 24 h post-dose relative to vehicle treated animals. FIG. 11C shows administration of a single 0.5 mg/kg dose of conotoxin peptide analog Ia or PEGylated conotoxin peptide analog IIa to rats 14 days following induction of oxaliplatin-induced peripheral neuropathy resulted in a significant reduction in mechanical hyperalgesia (up to 4 h for Ia or up to 72 h for IIa). These data further indicated the therapeutic potential for treating or preventing neuropathic pain with these triazole stabilized RgIA derivatives.

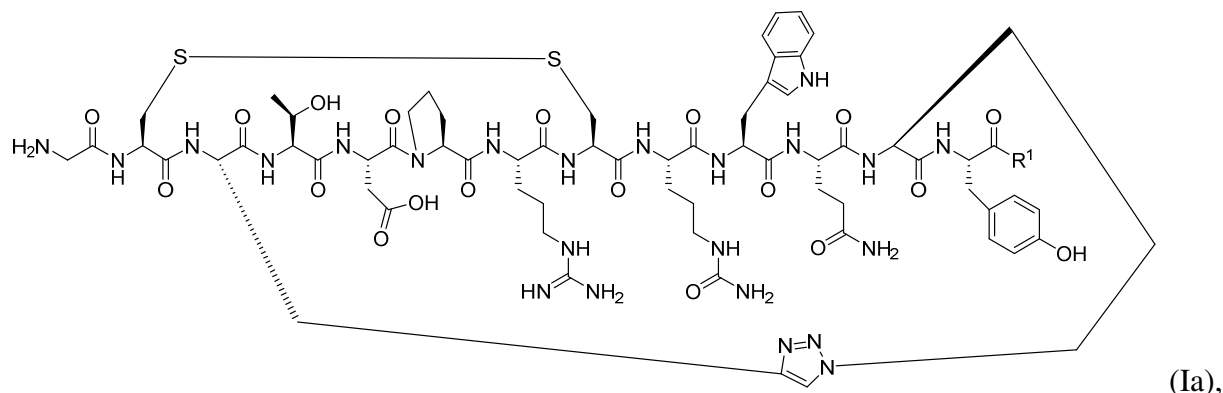
7. Incorporation by reference

[00519] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00520] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

CLAIMS

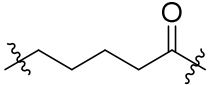
1. A PEGylated conotoxin peptide analog or pharmaceutically acceptable salt thereof, wherein the conotoxin peptide analog is of Formula (Ia) (SEQ ID NO:94)



wherein R¹ is OH,
 wherein the conotoxin peptide analog is covalently attached directly or via a linking group to one polyethylene glycol (PEG) polymer;
 wherein the PEG polymer is covalently attached to the N-terminus of the conotoxin peptide analog; and
 wherein the PEG polymer has a molecular weight in the range of 10 kDa to 40 kDa.

2. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1, wherein the PEG polymer is covalently attached to the conotoxin peptide analog via a linking group.

3. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 2,

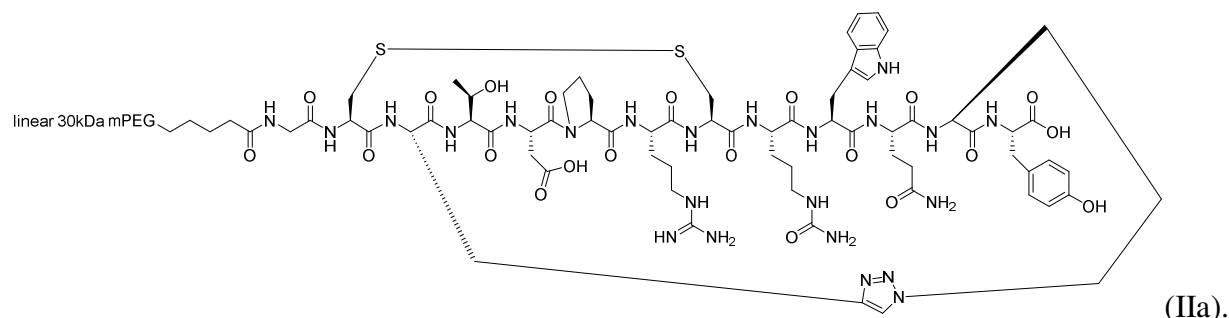
wherein the linking group is a valerate linker having a formula of .

4. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1, wherein the PEG polymer is a linear PEG polymer.

5. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1, wherein the PEG polymer has a molecular weight of 30 kDa.

6. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 5, wherein the PEG polymer is a linear PEG polymer.

7. The PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1, wherein the PEGylated conotoxin peptide analog is of Formula (IIa) (SEQ ID NO:83):



8. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 7, and a pharmaceutically acceptable carrier.

9. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 7 in the manufacture of a medicament for treating pain in a subject.

10. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1, and a pharmaceutically acceptable carrier.

11. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1 in the manufacture of a medicament for treating pain in a subject.

12. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 2, and a pharmaceutically acceptable carrier.

13. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 2 in the manufacture of a medicament for treating pain in a subject.

14. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 3, and a pharmaceutically acceptable carrier.
15. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 4, and a pharmaceutically acceptable carrier.
16. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 5, and a pharmaceutically acceptable carrier.
17. A pharmaceutical composition comprising the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 6, and a pharmaceutically acceptable carrier.
18. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 3 in the manufacture of a medicament for treating pain in a subject.
19. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 4 in the manufacture of a medicament for treating pain in a subject.
20. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 5 in the manufacture of a medicament for treating pain in a subject.
21. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 6 in the manufacture of a medicament for treating pain in a subject.
22. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 1 in the manufacture of a medicament for treating inflammation in a subject.

23. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 7 in the manufacture of a medicament for treating inflammation in a subject.
24. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 2 in the manufacture of a medicament for treating inflammation in a subject.
25. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 3 in the manufacture of a medicament for treating inflammation in a subject.
26. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 4 in the manufacture of a medicament for treating inflammation in a subject.
27. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 5 in the manufacture of a medicament for treating inflammation in a subject.
28. Use of a therapeutically effective amount of the PEGylated conotoxin peptide analog or pharmaceutically acceptable salt of claim 6 in the manufacture of a medicament for treating inflammation in a subject.