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SARAGOVI, H.U. et al., Current Pharmac. Design. 2002, Vol. 8, pages 2201-16 (X)
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[Continued on next page]

(54) Title: COMPOUNDS THAT MODULATE NEURONAL GROWTH AND THEIR USES

BDNF	-----APMKEANIRG---QGGLAYPGVRTHGTLSEVNGPKAGSRGL	38
NT4	-----QP---PPSTLPPFLAP-----	13
NT3	QGNMMDQRSLEFEDSLNSLIIKLIQADILKNKLSKQMVVDVKENYQSTLPEKAEAPRPERGG	60
NGF	-----EPHSESINVPAGHTI PQVHWTKLQHSLDTALRRARSAPAAIAARVAG---	47
BDNF	TSLADTFEHVIEELLDEBDQKVRPNEENKADADLYTSRVMLSSQVPLEPPLFLLEE--YK	96
NT4	-----EWDLLSPRVVLSRGAPAGPPLFLLEAGAFR	44
NT3	PAKS-----AFQPVIAMTELLRQQRRYNSPRVLLSDSTPLEPPLLYLMED--YV	108
NGF	-----QTRNITVDPRLPFKRRRLRSRVLFSTQPPREAA---DTQDLDFE	88
BDNF	NYLDA-ANMSMR-VRRHSDPA-RRGELSVCDSESEWVTAADKKTAVDMSGGTVTVLEKVP	153
NT4	ESAGAPANRSRRGVSETAPAS-RRGELAVCDVAVSGWVT--DRRTAVDLRGREVEVLGEVP	101
NT3	GSPVV-ANRTSRKRYAEHKS-HRGEYSVCDSESLWVT--DKSSAIDIRGHQVTVLGEIK	164
NGF	VGGAAPFNTRHSKRSSSHPIFHRGEFSVCDSESVVWVG--DKTTATDIKQKVMVLGEVN	146
BDNF	VSKGQ-LKQFYFETRCNPMGYTKEG-----CRGIDKRHWNSQCRTTQSYVRALTMDSK	205
NT4	AAGGSPLRQYFFETRCADNAEEGGPGAGGGGCRGVDRRHVWSECKAKQSYVRALTADAQ	161
NT3	TGNSP-VKQFYFETRCKEARPVKNG-----CRGIDDKHWNSQCKTSQTYVRALTSENN	216
NGF	INNSV-FKQYFFETRCRDPNPVDSG-----CRGIDSKHWNSYCTTHTFVKALTMDSG-	197
BDNF	KRIGWRFIRIDTSCVCTLTIKRGR--	229
NT4	GRVGRWIRIDTACVCTLLERTGRA-	186
NT3	KLVGRWIRIDTSCVCLSRKIGRT-	241
NGF	KQAARWIRIDTACVCLSRKAVRA	223

(57) Abstract: Cyclic peptides and peptidomimetics are provided that bind to and/or modulate activities associated with Trk receptors, including processes associated with the growth and repair of the central nervous system (e.g., neuronal growth and survival, axonal growth, neurite outgrowth and synaptic plasticity). Cyclic peptides and peptidomimetics are also provided that block or reduce the effect of other factors that inhibit growth and/or repair of the central nervous system. Pharmaceutical compositions and other formulations comprising these compounds are provided. In addition, the invention provides methods for using the cyclic peptides and peptidomimetics to modulate Trk mediated activities, including processes such as neuronal growth, survival and recover, axonal growth, neurite outgrowth, and synaptic plasticity. Further, the invention provides methods for promoting central nervous system (CNS) neuron growth by administering a p75 receptor binding agent.

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**COMPOUNDS THAT MODULATE NEURONAL GROWTH
AND THEIR USES**

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1. CROSS-REFERENCE TO RELATED APPLICATIONS

Priority is claimed under 35 U.S.C. 119(e) to U.S. provisional patent applications Serial No. 60/501,864, filed on September 10, 2003; Serial No. 60/559,898, filed April 5, 2004; and Serial No. 60/603,187, filed August 20, 2004. The contents of these provisional applications are hereby incorporated by reference, in their entireties.

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2. FIELD OF THE INVENTION

The present invention relates to compositions and methods for modulating the growth and repair of the central nervous system (CNS), including processes such as neuronal survival, axonal growth and synaptic plasticity. More specifically, the invention relates to compounds (including cyclic peptides and peptidomimetic compounds) that are either agonists or antagonists of a family of receptors, known as Trk receptors, that are expressed on the surface of neuronal cells and which regulate such processes of CNS growth and repair. Further, the invention relates to methods for promoting CNS growth and repair using a p75 binding agent.

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3. BACKGROUND OF THE INVENTION

Injury to the central nervous system (CNS) can have devastating consequences due to the poor regenerative capacity of neurons in that environment. This contrasts markedly with the comparatively good regenerative capacity of neurons in the peripheral nervous system. See, for example, Horner & Gage, *Nature* 2000, 407:963-970. Numerous diseases, such as Alzheimer's disease, Parkinson's disease, stroke, head and spinal cord trauma to name a few, are all associated with damage to the CNS that is often severe, even debilitating, long lasting or even permanent. No cure is presently available for these conditions, and even palliative treatments are lacking.

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3.1. Neurotrophins

It is now understood that the growth and regeneration of neurons is regulated at least in part by certain polypeptide growth factors, known as neurotrophins or "NTs," which bind to and activate cell surface receptors having an intrinsic tyrosine kinase activity. Upon neurotrophin binding, these receptors are believed become autophosphorylated on one or more amino acid residues and subsequently associate with intracellular molecules important for signal transduction. For a review, see Ulrich & Schlessinger, *Cell* 1990, 61:203-212.

The first identified neurotrophin is known in the art as nerve growth factor (NGF) and has a prominent effect on developing sensory and sympathetic neurons of the peripheral nervous system. See, Levi-Montalcini & Angeletti, *Physiol. Rev.* 1958, 48:534-569; Thoenen *et al.*, *Rev. Physiol. Biochem. Pharmacol.* 1987, 109:145-178; Thoenen & Barde, *Physiol. Rev.* 1980, 60:1284-1325; Whittemore & Seiger, *Brain Res.* 1987, 434:439-464; Angeletti & Bradshaw, *Proc. Natl. Acad. Sci. U.S.A.* 1971, 68:2417-2420; Angeletti *et al.*, *Biochemistry* 1973, 12:100-115. NGF orthologs have also been isolated and characterized in a number of other species, including mice, birds, reptiles and fishes (Scott *et al.*, *Nature* 1983, 302:538-540; Schwartz *et al.*, *J. Neurochem.* 1989, 52:1203-1209; and Hallböök *et al.*, *Neuron* 1991, 6:845-858.

A number of other NTs are also described in the art. These include brain-derived neurotrophic factor (BDNF), which is also known as neurotrophin-2 (NT-2). See, Leibrock *et al.*, *Nature* 1989, 341:149-152. Still other NTs include a factor originally called neuronal factor (NF) and now commonly referred to as neurotrophin-3 or "NT-3" (Emfors *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 1990, 87:5454-5458; Höhn *et al.*, *Nature* 1990, 344:339; Maisonnier *et al.*, *Science* 1990, 247:1446; Rosenthal *et al.*, *Neuron* 1990, 4:767; Jones & Reichardt, *Proc. Natl. Acad. Sci. U.S.A.* 1990, 87:8060-8064; and Kaisho *et al.*, *FEBS Lett.* 1990, 266:187). Neurotrophins-4 and -5 (NT-4 and NT-5) are also described in the art. See, Hallbrook *et al.*, *Neuron* 1991, 6:845-858; Berkmeier *et al.*, *Neuron* 1991, 7:857-866; IP *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 1992, 89:3060-3064. See also, U.S. Patent No. 5,364,769 issued November 15, 1994 to Rosenthal. Because it was subsequently shown to be a mammalian ortholog of the *Xenopus* NT-4 described by Hallbrook *et al.*, *supra*, the mammalian NT-5 molecule described by Berkmeier *et al.*, *supra*, is also commonly referred to as NT-4/5. An alignment of NT's BDNF, NT4, NT3, and NGF is provided in Figure 1.

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3.2. Trk Receptors

Neurotrophins mediate their effect through a family of receptor tyrosine kinases that are expressed on the surface of neuronal cells and referred to collectively as Trk-receptors. At least three different Trk-receptors are described in the art: TrkA, TrkB and TrkC. For a review, see U.S. Patent Nos. 5,844,092; 5,877,016; 6,025,166; 6,027,927; and 6,153,189 by all Presta *et al.* Although the structure and sequences of the different Trk-receptors are similar, alternate splicing increases the complexity of this family giving rise to several different isoforms of each receptor. An alignment of the different Trk-receptor amino acid sequences is provided here at Figure 2A-2C setting forth the consensus sequences and boundaries for the various domains of each receptor. See also, Figures 16A-16C in U.S. Patent No. 5,877,016.

Each of the different Trk-receptors exhibits particular binding affinity for the different neurotrophins, although there is some overlap. Hence, TrkA is believed to bind not only NGF, but also NT-3 and NT-4/5 (but not BDNF). TrkB is believed to bind BDNF, NT-3, NT-4 and NT-4/5, but not NGF. By contrast, TrkC is believed to bind only NT-3 and not any of the other neurotrophins.

A number of studies have validated the Trk-receptors as therapeutic targets for brain repair. See, for example, Liu *et al.*, *J. Neurosci.* 1999, 19:4370-4387; Menet *et al.*, *Eur. J. Neurosci.* 1998, 10:607-621; and Kobayashi *et al.*, *J. Neurosci.* 1997, 17:9583-9595. The Trk-receptors and their ligands have also been studied using X-ray crystallography to obtain three-dimensional structures of the ligand-receptor binding complexes. Wiesmann *et al.*, *Nature* 1999, 401:184-188; Banfield *et al.*, *Structure (Camb)* 2001, 9:1191-1199. These and other studies suggest that neurotrophin binding to the Trk-receptors induces dimerization of receptor monomers, resulting in an increase of the receptors' intrinsic tyrosine kinase activity. This increased activity triggers, in turn, signaling cascades that are believed to be beneficial to neurons by promoting neuronal survival, axonal growth, and synaptic plasticity. Snider, *Cell* 1994, 77:627-638; Kaplan & Miller, *Curr. Opin. Neurobiol.* 2000, 10:381-391.

There has therefore been considerable recognition that therapeutic compounds which target and activate Trk-receptors (*i.e.*, Trk-receptor "agonists") would be beneficial and desirable. See, for example, Lindsay *et al.*, *Exp. Neurol.* 1993, 124:103-118; Olson,

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Neurochem. Int. 1994, 23:1-3. Moreover, increased levels of certain neurotrophins (e.g., BDNF) are also associated with medical conditions such as epilepsy (Binder *et al.*, *Trends Neurosci.* 2001, 24:47-53). Hence, even compounds that inhibit Trk-receptor activity (*i.e.*, Trk-receptor "antagonists") would be beneficial. Despite this long felt need, such compounds have been elusive at best. As large-molecules, the therapeutic delivery of effective levels of neurotrophins themselves presents considerable, possibly insurmountable, challenges. Moreover, natural neurotrophins may interact with other receptors, such as the p75 receptor in neurons, which is associated with neuronal apoptosis and growth cone collapse. Lee *et al.*, *Curr. Opin. Neurobiol.* 2001, 11:281-286.

However, previous efforts to design peptidomimetic agonists and/or antagonists of Trk-receptors have also been unsuccessful. For example, cyclic peptides derived from loop 1 of the neurotrophin NGF have been reported to moderately mimic the survival activity of NGF. However, these peptides appear to function in a p75, rather Trk-receptor, dependent manner. Long *et al.*, *J. Neurosci. Res.* 1997, 48:1-17. Some NGF loop 4 cyclic peptides are said to show NGF-like survival activity that is blocked by a Trk antagonist. However, the maximal survival response induced by those peptides is reported to be only 10-15% of the maximal response promoted by the NGF neurotrophin itself. See, Xie *et al.*, *J. Biol. Chem.*, 2000, 275:29868-29874; and Miliarichouk *et al.*, *J. Biol. Chem.* 2000, 275:9946-9956. Bicyclic and tricyclic dimeric versions of BDNF loop 2 peptides have been shown to have BDNF-like activity. Again, however, the maximal survival response they induce is reported to be only 30% of the maximal response promoted by the natural neurotrophin. O'Leary *et al.*, *J. Biol. Chem.* 2003, 278:25738-25744 (Electronic publication May 2, 2003).

There continues to exist, therefore, a long felt need for compositions that can modulate (*i.e.*, increase or inhibit) neuronal growth and recovery. There also exists a need for processes and methods (including therapeutic methods) that effectively modulate neuronal growth and recovery.

3.21. The p75 receptor Neurotrophin Receptor

The p75 receptor plays roles in signaling complexes for neuronal apoptosis and growth inhibition. Barker, *Neuron* 2004, 42:529-533. The p75 receptor is a member of the tumor necrosis factor (TNF) superfamily and is characterized by cysteine-rich

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domains (CRDs) in its extracellular portion. These CRDs are required for neurotrophin binding, and p75 receptor serves as a low affinity receptor for neurotrophins such as NGF, BDNF, NT-3, and NT-4. Huang and Reichardt, *Annu. Rev. Biochem.* 2003, 72:609-642. NGF, BDNF, NT-3 and NT-4 can effectively compete with each other for binding to p75 receptor. In inhibitory environments, these neurotrophins can be used to compete out each other's binding to p75 receptor in order to reveal responses that depend solely on Trk signaling. Barker and Shooter, *Neuron* 1994, 13:203-215.

3.22. The p75 receptor and the NGF TDIKQKE Motif

The TDIKQKE motif that constitutes the first β hairpin loop of NGF plays a crucial role in the binding of NGF to the p75 receptor. He and Garcia, *Science* 2004, 304:870-875; Ibanez *et al.*, *Cell* 1992, 69:329-341. Furthermore, constrained TDIKQKE motifs interact with the p75 receptor and are expected to compete for neurotrophin binding to this receptor. Longo *et al.*, *J. Neurosci. Res.* 1997, 48:1-17.

The cyclic peptides and peptidomimetic compounds derived from loop 1 of NGF have been reported to moderately mimic NGF's neuron growth-promoting activity (see U.S. Patent No. 6,017,878 to Saragovi *et al.*), and these peptides appear to function in a p75 receptor-dependent manner (Longo *et al.*, *J. Neurosci. Res.* 1997, 48:1-17). Some NGF loop 4 cyclic peptides are said to show NGF-like neuron growth promotion that is blocked by a Trk antagonist. However, the maximal response induced by those peptides is reported to be only 10-15% of the maximal response promoted by the NGF neurotrophin itself. See Xie *et al.*, *J. Biol. Chem.* 2000, 275:29868-29874; and Mallatichouk *et al.*, *J. Biol. Chem.* 2000, 275:9946-9956. Bicyclic and tricyclic dimeric versions of BDNF loop 2 peptides have been shown to have BDNF-like activity. Again, however, the maximal response they induce is reported to be only 30% of the maximal response promoted by the natural neurotrophin. O'Leary *et al.*, *J. Biol. Chem.* 2003, 278:25738-25744 (Electronic publication May 2, 2003).

3.3. Inhibitory Signals

The central nervous system's limited ability to repair injuries is thought to be at least partly due to the presence of inhibitory products that prevent axonal regeneration - including inhibitors associated with damaged myelin (Berry, *Bibl. Anat.* 1982, 23:1-11). Indeed,

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biochemical studies on central myelin have identified two protein fractions that contain inhibitory activity for cell spreading (Caroni & Schwab, *J. Cell Biol.* 1988, 106:1281-1288) and monoclonal antibodies that bind to those fractions enhance the growth of cultured sensory and sympathetic neurons in what are otherwise non-permissive substrates for neurite growth (Caroni & Schwab, *Neuron* 1988, 1:85-96). Studies with these same antibodies in lesioned animals have also shown that functional recovery can be obtained by blocking the function of inhibitory molecules associated with myelin (Bregman *et al.*, *Nature* 1995, 378:498-501; Schnell & Schwab, *Nature* 1990, 343:269-272). A much more robust regeneration response has been obtained in mice immunized with whole myelin (Huang *et al.*, 1999) further demonstrating that CNS recovery and repair can be enhanced *in vivo*, by blocking inhibitory factors.

At least three myelin derived molecules are described in the art that are potent inhibitors of axonal growth: the myelin-associated glycoprotein, which is also referred to as "MAG" (described by McKerracher *et al.*, *Neuron* 1994, 13:805-811; and by Mukhopadhyay *et al.*, *Neuron* 1994, 13:757-767); Nogo-A (see Chen *et al.*, *Nature* 2000, 403:434-439; GrandPre *et al.*, *Nature* 2000, 403:439-444; and Prinjha *et al.*, *Nature* 2000, 403:383-384) and the oligodendrocyte myelin glycoprotein (Wang *et al.*, *Nature* 2002, 417:941-944). The Nogo receptor (also referred to as "NgR"), the ganglioside GT1b and the p75 neurotrophin receptor (also referred to as "p75^{NTR}" or "p75NTR") have been implicated in mediating responses to all three of these inhibitory molecules. Specifically, binding to NgR is said to be required for inhibitory activity by all three inhibitors MAG, Nogo-A and oligodendrocyte glycoprotein (Domeniconi *et al.*, 2002; Liu *et al.*, 2002; Wang *et al.*, 2002b). However, MAG can also bind directly to the GT1b receptor (Vyas & Schnaar, *Biochimie* 2001, 83:677-682). Moreover, antibody induced clustering of GT1b receptor can mimic the inhibitory response produced by MAG (see, Vinson *et al.*, *J. Biol. Chem.* 2001, 276:20280-20285; and Vyas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 2002, 99:8412-8417).

The p75 receptor is the signaling component of a multimeric receptor complex that can bind all three myelin receptors. See Domeniconi *et al.*, *Neuron* 2002, 35:283-290; Liu *et al.*, *Science* 2002, 297:1190-1193; Wang *et al.*, *Nature* 2002, 417:941-944. Interactions between the GT1b and p75^{NTR} receptors have been reported (Yamashita *et al.*, 2002), as have interactions between the NgR and p75^{NTR} receptors (see, Wang *et al.*, 2002a; and Weng *et*

al., 2002). Such interactions with p75^{NTR} are thought to be important in the transmission of inhibitory signals (*e.g.*, from MAG, Nogo-A and/or oligodendrocyte glycoprotein) across the cell membrane. For example, interactions of MAG or a Nogo-A peptide with cells that express NgR increases association of p75^{NTR} with Rho-GDI, and induces the release of RhoA from that complex (Yamashita & Tohyama, *Nat. Neurosci.* 2003, 6:461-471). This step is a pre-requisite for activation of RhoA and inhibition of growth (*Id*), and the inhibition of RhoA and/or Rho kinase (a downstream effector of RhoA) effectively circumvents inhibitory activity, *e.g.*, of myelin in cultured neurons (see, for example, Dergham *et al.*, *J. Neurosci.* 2002, 22:6570-6577; Fournier *et al.*, *J. Neurosci.* 2003, 23:1416-1423; and Lehmann *et al.*, *J. Neurosci.* 1999, 19:7537-7547).

As noted above, the various neurotrophins (*e.g.*, NGF, BDNF, NT-3 and NT-4/5) do have dramatic effects on neuronal survival and axonal growth during development. It has been recently suggested that neurotrophins and inhibitory molecules (for example, MAG, Nogo-A and oligodendrocyte glycoprotein) may have an opposing effect on the coupling of p75^{NTR} receptor to Rho-GDI (*see*, Yamashita & Tohyama, *Nat. Neurosci.* 2003, 6:461-467). Nevertheless, it has not as of yet been possible to promote robust, long range axonal regeneration using neurotrophins. This is believed to be at least partly due to the inability of neurotrophins to effectively counteract inhibitory signals such as those described above. For example, the treatment of cultured neurons with neurotrophins such as NGF, BDNF or GDNF (glial derived neurotrophic factor) does not normally counteract the inhibitory activity of myelin unless the neurons are first “primed” by exposure to the neurotrophin for several hours before exposure to the inhibitory signal (Cai *et al.*, *Neuron* 1999, 22:89-101). Such priming, however, is of limited effect, time consuming, cumbersome to apply, and impractical for clinical and other *in vivo* applications. Moreover (and as noted above), the therapeutic delivery of neurotrophins themselves, which are large molecules, presents considerable and possibly insurmountable technical challenges. Furthermore, neurotrophins may be compromised in their ability to promote regeneration because they bind to the inhibitory complex through their interaction with the p75 receptor. Neurotrophins, which are bound to p75 receptor, cannot activate Trk receptors to overcome inhibitory signaling and to promote neuronal growth.

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Hence, there additionally exists a need for compounds that can effectively modulate the effects of inhibitory signals on neuronal growth and recovery – including compounds that effectively modulate effects of inhibitory signals such as those produced by MAG, Nogo-A, oligodendrocyte glycoprotein, NgR, CNTF, p75^{NTR} and/or downstream effectors of these signaling molecules. In particular, there exists a need for compounds that can effectively counteract such inhibitory signals, and/or stimulate neuronal growth and recovery. There also exists a need for processes and methods (including therapeutic methods) that modulate effects of such inhibitory signals and, in particular, for processes and methods that counteract such inhibitory signals and/or stimulate neuronal growth and recovery.

* * * * *

The citation and/or discussion of a reference in this section and throughout the specification is provided merely to clarify the description of the present invention and is not an admission that any such reference is "prior art" to the invention described herein.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

4. SUMMARY OF THE INVENTION

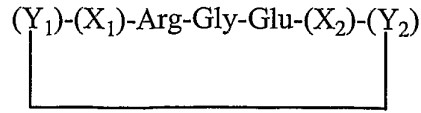
The present invention provides at least a partial solution to the above-mentioned problems in the art by providing compounds and formulations thereof which modulate (*e.g.*, enhance or inhibit) activity mediated by a Trk receptor such as TrkA, TrkB or TrkC. For example, in one embodiment the invention provides compounds that are Trk antagonists and, as such, inhibit Trk mediated activity. In other embodiments, the invention provides compounds that are Trk agonists and, as such, enhance or increase Trk mediated activity.

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As noted above, Trk receptors and their ligands (*i.e.*, neurotrophins such as NGF, BDNF, NT-3, NT-4, NT-5 and NT-4/5) are associated with the growth and repair of the central nervous system (CNS). As such, Trk modulator compounds of the present invention can be used to modulate such processes, including processes of neuronal growth and survival, axonal growth, neurite outgrowth, and synaptic plasticity. In one aspect, therefore, the present invention provides methods (including therapeutic methods) that use Trk modulator compounds of the invention to modulate (*e.g.*, enhance or inhibit) such processes.

In one particular embodiment, the invention provides cyclic peptide compounds that modulates Trk receptor mediated activity. These cyclic peptides preferably comprise, within

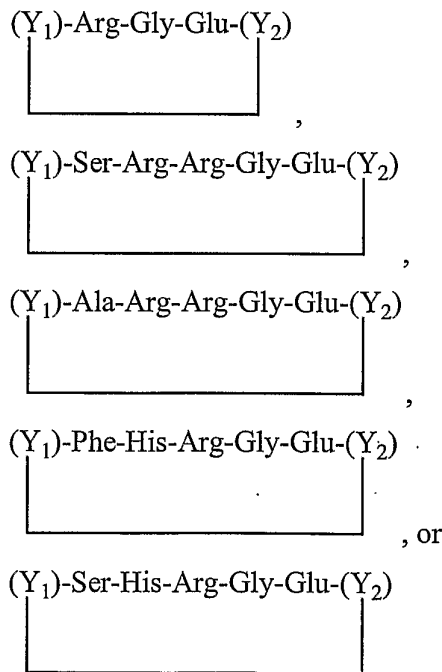
a cyclic peptide ring, the amino acid sequence: Arg-Gly-Glu. In a more particular embodiment, the cyclic peptide comprises the formula:



(Formula I)

In Formula I, the elements Y_1 and Y_2 are independently selected amino acids with a
 5 covalent bond formed between Y_1 and Y_2 . The element X_1 and X_2 are optional and, if
 present, are independently selected amino acids or sequences of amino acids joined by
 peptide bonds. Preferably X_1 and/or X_2 are each between zero and about 10 amino acids in
 length, and are more preferably about 1, 2, 3, 4 or 5 amino acids in length. Moreover, X_1 and
 X_2 are also preferably selected so that the size of the cyclic peptide ring ranges from about 5
 10 to about 15 amino acids in length, and is more preferably between about 5-10 amino acids in
 length.

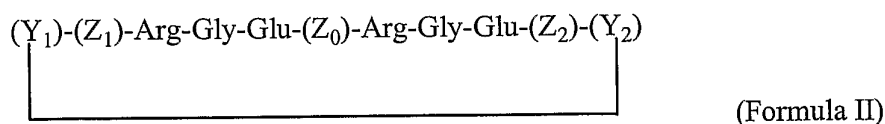
The invention further provides, in particular embodiments, cyclic peptides having the
 formula:



where Y_1 and Y_2 are as described above, for Formula I. Particularly preferred cyclic
 15 peptides of the invention are ones comprising the amino acid sequence: CSRRGEC (SEQ ID
 NO:1), N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2), CARRGEC (SEQ ID NO:3), N-Ac-

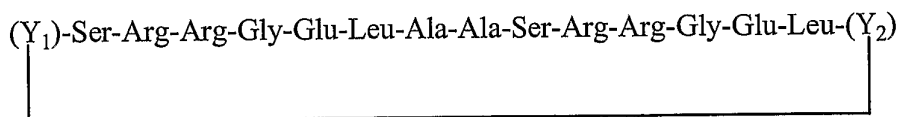
5 CARRGEC-NH₂ (SEQ ID NO:4), CFHRGEC (SEQ ID NO:5), N-Ac-CFHRGEC-NH₂ (SEQ ID NO:6), CSHRGEC (SEQ ID NO:7), N-Ac-CFHRGE-NH₂ (SEQ ID NO:8), CRGEC (SEQ ID NO:9), N-Ac-CRGEC-NH₂ (SEQ ID NO:10), N-Ac-KRGED-NH₂ (SEQ ID NO:11), H-C(O)-CRGEC-NH₂ (SEQ ID NO:12), CH₃-SO₂-NH-CRGEC-NH₂ (SEQ ID NO:13), N-Ac-CRGEC-Y-NH₂ (SEQ ID NO:14), H-C(O)-CRGEC-Y-NH₂ (SEQ ID NO:15) and CH₃-SO₂-NH-CRGEC-Y-NH₂ (SEQ ID NO:16), (where the underlined portion of each amino acid sequence indicates that portion of the peptide that is cyclized).

Preferred cyclic peptides of the above formulas and sequences are Trk antagonists. However, the invention also provides, in other embodiments, cyclic peptides that are Trk agonists. Such cyclic peptides preferably have the formula:



In Formula II, above, the elements Y₁ and Y₂ are independently selected amino acids with a covalent bond formed between Y₁ and Y₂. The elements Z₁, Z₂ and Z₀ are optional and, if present, are independently selected amino acids or sequences of amino acids joined by peptide bonds. Preferably, Z₁, Z₂ and/or Z₀ are each no more than about ten amino acids in length, and are more preferably only about 1, 2, 3, 4, 5 or 10 amino acids in length. Moreover, the lengths of Z₁, Z₂ and/or Z₀ are preferably selected so that the size of the cyclic peptide ring ranges from about 10-50 amino acids in length, and more preferably from about 10-25 or from about 15-20 amino acids in length. In particularly preferred embodiments, the elements Z₁, Z₂ and Z₀ are selected such that the tandem Arg-Gly-Glu sequences in Formula I adopt a conformation where they are adjacent and anti-parallel to each other.

In preferred embodiments, the invention provides cyclic peptides according to Formula II that have the formula



25 where the elements Y₁ and Y₂ are as set forth, *supra*, for Formula II. Particularly preferred peptides according to Formula II, which are also a part of the present invention, are cyclic peptides comprising the amino acid sequence: CSRRGELAASRRGELC (SEQ ID

NO:17) and N-Ac-CSRRGELAASRRGELC-NH₂ (SEQ ID NO:18), (where the underlined portion of each amino acid sequence indicates that portion of the peptide that is cyclized).

In accordance with the invention, cyclic peptides are provided comprising, within a cyclic ring of the cyclic peptide, the D amino acid sequence:

5 dGlu-Gly-dArg

wherein the cyclic peptide modulates Trk receptor mediated activity. Preferred cyclic peptides that modulate Trk receptor mediated activity comprising D amino acid sequences are c[dLdEGdRdRdSdLdEGdRdRdS] (SEQ ID NO:40), (where the bracketed portion of the amino acid sequence indicates that portion of the peptide that is cyclized by a peptide
10 bond) and Ac-dCdLdEGdRdRdSdAdAdLdEGdRdRdSdC-NH₂ (SEQ ID NO:41).

In a further embodiment, the invention provides the cyclic peptide having the amino acid sequence c[SRRGELSRRGEL] (SEQ ID NO:39).

In addition to the cyclic peptides, the invention also provides methods for identifying other compounds (*i.e.*, "candidate compounds") that modulate Trk receptor mediated activity
15 or are likely to modulate such activity. These methods involve comparing a three-dimensional structure of the candidate compound with the three-dimensional structure of a cyclic peptide of the invention. Similarity between the structure of the candidate compound and the structure of the cyclic peptide is indicative of the candidate compound's ability to modulate Trk receptor mediated activity. Hence, a candidate compound having a
20 substantially similar structure to the three-dimensional structure of the cyclic peptide is likely to be a compound which modulates Trk receptor mediated activity.

The above methods are ideally suited for identifying peptidomimetic compounds that modulate Trk receptor mediated activity. Accordingly, the invention provides peptidomimetic compounds that are Trk modulators, and such compounds are considered
25 another aspect of the invention. In particular, the peptidomimetic compounds of the invention are compounds having a three-dimensional structure that is substantially similar to the three-dimensional structure of a cyclic peptide of the invention (*i.e.*, a cyclic peptide that modulates Trk mediated activity and comprises, within a cyclic ring thereof, the amino acid sequence Arg-Gly-Glu).

30 The invention additionally provides methods, including therapeutic methods, that use cyclic peptides and peptidomimetic compounds to modulate Trk mediated activity. In one

such embodiment, the invention provides methods for inhibiting Trk mediated activity. Such methods involve contacting a cell (*in vitro* or *in vivo*) with an amount of a cyclic peptide or peptidomimetic compound of the invention that inhibits Trk mediated activity. The amount of the cyclic peptide or peptidomimetic compound contacted to the cell should be an amount that effectively inhibits the Trk receptor mediated activity.

In another embodiment, the invention provides methods for enhancing Trk mediated activity. Such methods involve contacting a cell (*in vitro* or *in vivo*) with an amount of a cyclic peptide or peptidomimetic compound of the invention that enhances Trk mediated activity. The amount of the cyclic peptide or peptidomimetic compound contacted to the cell should be an amount that effectively enhances the Trk receptor mediated activity.

Examples of Trk mediated activities that can be modulated (*e.g.*, enhanced or inhibited) by such methods include: neuronal growth and survival, axonal growth, neurite outgrowth and synaptic plasticity and well as other processes of central nervous system (CNS) growth and/or repair. Accordingly, the invention additionally provides methods for enhancing growth or repair of the central nervous system in an individual. These methods involve administering to the individual an amount of a cyclic peptide or a peptidomimetic compound of the invention that enhances Trk mediated activity. The amount of the cyclic peptide or peptidomimetic compound administered should be an amount that effectively enhances CNS growth or repair.

The invention additionally provides methods that use Trk agonists and antagonists to modulate responses that inhibit CNS growth and repair, including responses that normally inhibit processes such as neuronal growth, neuronal survival, axonal growth, neurite outgrowth and synaptic plasticity. In particular, Trk agonists and antagonists of the invention can be used to modulate inhibitory factors and/or inhibitory signals generated by such factors. Examples include factors associated with myelin, including the myelin associated glycoprotein (MAG), Nogo-A and the oligodendrocyte myelin glycoprotein. In general, the invention provides methods using Trk agonists and/or antagonists to modulate a CNS inhibitor response mediated by a signal cascade with one or more components that are themselves modulated by a factor or factors involved in signaling by a Trk receptor. These include, for example, components such as Rho that are modulated by protein kinase A (PKA) and/or by phosphoinositide-3 kinase (PI3K). In preferred embodiments, therefore, the

invention provides methods for reducing such "CNS inhibitor" responses by contacting a cell with a Trk agonists (*e.g.*, a cyclic peptide or peptidomimetic) of the invention in an amount that is effective for reducing the CNS-inhibitor response. The invention also provides methods for reducing a CNS inhibitor response in an individual, by administering to the individual an amount of a Trk agonists (*e.g.*, a cyclic peptide or peptidomimetic) of the invention in an amount that effectively reduces the CNS inhibitor response.

In still other embodiments, the invention provides pharmaceutical compositions that can be used in therapeutic methods, such as those described above. Such pharmaceutical compositions comprise an amount of a cyclic peptide or peptidomimetic compound of the invention, along with one or more carriers, diluents or excipients that are pharmaceutically and/or physiologically acceptable.

Further, the present invention is based on the discovery that agents which interfere with the binding of neurotrophins to the p75 receptor promote CNS neuron growth in an inhibitory environment.

Trk receptors and their ligands (*i.e.* neurotrophins such as NGF, BDNF, NT-3, NT-4 and NT-5) are associated with the growth and repair of CNS neurons. As such, when neurotrophins bind to and activate Trk receptors, Trk activity triggers signaling cascades which promote neuronal growth. However, the p75 receptor binds neurotrophins with low affinity and, when the p75 receptor is engaged in an inhibitory complex, this interaction compromises the ability of neurotrophins to promote CNS neuron growth. The invention provides methods for promoting CNS neuron growth using a p75 receptor binding agent, which interferes with the binding of a neurotrophin to the p75 receptor.

According to the present invention, a method is provided for promoting CNS neuron growth in an inhibitory environment, which comprises administering to an individual a therapeutically effective amount of a p75 receptor binding agent. In one embodiment, the p75 receptor binding agent includes a neurotrophin binding motif or a peptidomimetic thereof. In a particular embodiment, the p75 receptor binding agent comprises a cyclic peptide or peptidomimetic comprising, within a cyclic ring thereof, the amino acid sequence Thr-Asp-Ile-Lys-Gly-Lys-Glu (TDIKGKE) (SEQ ID NO:42). A preferred p75 receptor binding agent is N-Ac-CTDIKGKEC-NH₂ (SEQ ID NO:43). The individual is preferably a mammal and more preferably a human.

The present invention provides methods for promoting CNS neuron growth in an inhibitory environment, which comprise administering to an individual a therapeutically effective amount of a p75 receptor binding agent in combination with a neurotrophin. In one embodiment, the neurotrophin is selected from the group consisting of NGF, BDNF, NT-3, NT-4 and NT-5. In a further embodiment, the p75 receptor binding agent is administered in an amount about 10 to about 100 fold greater than the neurotrophin. In an aspect of the invention, the p75 receptor binding agent is a neurotrophin that interferes with another, different neurotrophin for binding to the p75 receptor, but does not interfere with binding of the another, different neurotrophin to a Trk receptor expressed on an injured neuron. In a particular aspect, the p75 receptor binding agent is NGF and the neurotrophin is BDNF wherein NGF is administered in an amount about 10 to about 100 fold greater than BDNF. The individual is preferably a mammal, and more preferably, a human.

5. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence alignment of NTs BDNF (SEQ ID NO:19), NT4 (SEQ ID NO:20), NT3 (SEQ ID NO:21), and NGF (SEQ ID NO:22). Mature chains are denoted by bold lettering and the RGE motif is underlined.

Figures 2A-2C show an alignment of the full length amino acid sequences of human TrkA (SEQ ID NO:23), TrkB (SEQ ID NO:24) and TrkC (SEQ ID NO:25) receptors. Consensus sequences for the receptors are boxed, and the boundaries of the receptors' various domains are marked by vertical lines. See also, U.S. Patent No. 5,844,092 by Presta *et al.*

Figures 3A-3B illustrate representative backbone modifications that may be present within a peptidomimetic. See also, Figures 4A and 4B in WO 01/53331.

Figure 4 illustrates representative unusual amino acids and dipeptide surrogates that may be incorporated into a peptidomimetic. See also, Figure 5 in WO 01/53331.

Figure 5 illustrates representative secondary structure mimics that may be incorporated into a peptidomimetic. See also, Figure 6 in WO 01/53331.

Figures 6A-6C illustrates the analysis of a NT/Trk crystal structure to identify linear regions of the ligand that interact with the Trk receptor. **Figure 6A** shows a ribbon image of the crystal structure (reported by Banfield *et al.*, *Structure (Camb)* 2001, 9:1191-1199) of an NT-4 dimer (denoted as chain a₁ and a₂) in a complex with two membrane proximal Ig domains from the TrkB receptor (denoted as chain b₁ and b₂). **Figure 6B** shows the results of examining this crystal structure (solid line) and the NGF/TrkA crystal structure (reported by Wiesman *et al.*, *Nature* 1999, 401:184-188) (dotted line) for linear peptide sequences (LIPs) that make contact between the a₁ and b₂ chains. **Figure 6C** shows the results of examining the NGF (dotted line) and NT-4 (solid line) crystal structures for LIPs that make contact between the a₁ and b₁ chains.

Figures 7A-7D show data from experiments where cerebellar neurons were cultured over monolayers of 3T3 cells in control media or in media supplemented with NT-4 or BDNF in the presence of various peptides for 18 hours before being fixed and stained for GAP-43. The mean length of the longest neurite was determined from between about 100-120 neurons under each culture condition. **Figure 7A** shows data from experiments testing the effects of various concentrations of NT-4 and BDNF on neurite outgrowth. **Figure 7B** shows data from experiments testing the effects of increasing concentrations of the cyclic peptide N-Ac-CRGEC-NH₂ in control media and in media containing 5 ng/ml BDNF or 5 ng/ml NT-4, as indicated. **Figure 7C** shows data from experiments testing the effects of the NT-4 derived cyclic peptide N-Ac-CSRRGEC-NH₂ (SEQ ID NO:26), the NT-3 derived cyclic peptide N-Ac-CSHRGEC-NH₂ and the NGF derived cyclic peptide N-Ac-CFHRGEC-NH₂ in cerebellar neurons cultured with 5 ng/ml BDNF. **Figure 7D** shows data from experiments identical to that shown in **Figure 7C**, but where the cerebellar neurons are cultured with 5 ng/ml NT-4.

Figure 8 illustrates results from experiments where cerebellar neurons were cultured over monolayers of 3T3 cells in control media or in media supplemented with NT-4, BDNF,

FGF2 (all at 5 ng/ml), with the CG1 receptor agonist WIN55,2122-2 (0.2 μ M) or over monolayers of 3T3 cells the express transfected N-cadherin (NCAD) on their cell surface. The experiments were performed, and data plotted, in the presence and absence of: (a) the cyclic peptide N-Ac-CRGEC-NH₂ at 440 μ M; (b) the cyclic peptide N-Ac-CSRRGEC-NH₂ at 125 μ M; and (c) the linear peptide N-Ac-SRRGELA-NH₂ (SEQ ID NO: 27) at 125 μ M.

Figures 9A-C show modeled structures of the B_{AG} peptide. **Figure 9A** shows the native structure of the SRRGELA motif from one monomer of the NT-4 dimer in the NT-4/TrkB crystal structure. The native structure of the ASRRGEL (SEQ ID NO:28) motif from the partner NT-4 monomer in that crystal structure is shown in **Figure 9C**. A modeled structure of the B_{AG} peptide N-Ac-CSRRGELAASRRGELC-NH₂ incorporating these “tandem-repeat” motifs is shown in **Figure 9B**.

Figures 10A-10B show results from neurite outgrowth experiments where cerebellar neurons were cultured in media supplemented with a range of concentrations of the B_{AG} peptide N-Ac-CSRRGELAASRRGELC-NH₂. **Figure 10A** shows the mean value of absolute neurite lengths determined from 100-120 neurons sampled in a single experiment. **Figure 10B** shows a histogram comparing effects of the B_{AG} peptide (6 μ M) with the response to established growth promoting agents, including NT-4, BDNF, and FGF2 (all at 5 ng/ml) as indicated.

Figure 11 shows results from neurite outgrowth experiments where cerebellar neurons were cultured in control media or in media supplemented with: (a) the B_{AG} peptide N-Ac-CSRRGELAASRRGELC-NH₂ at 6 μ M; (b) the TrkB antagonist peptide N-Ac-CSRRGEC-NH₂ (SEQ ID NO:29) at 125 μ M; (c) the Trk specific tyrosine kinase inhibitor K252a at 100 nM; or the linear version of the TrkB antagonist peptide N-Ac-SRRGELA-NH₂ at 125 μ M, as indicated.

Figure 12 shows a bar graph depicting results from neurite outgrowth experiments testing the effects of various agents. In particular, cerebellar neurons were cultured over monolayers of N-cadherin expressing 3T3 cells in media supplemented with a soluble MAG-

Fc fusion construct at final concentrations of 0, 5 or 25 $\mu\text{g/ml}$ (as indicated below each bar in the graph). Experiments were done in control media (*i.e.*, in media supplemented with MAG-Fc only) and in media additionally supplemented with the Rho kinase inhibitor Y27632 (10 μM final concentration) B_{AG} polypeptide (6 μM final concentration) or BDNF (5 ng/ml final concentration). Cultures were maintained for 22 hours before being fixed and stained for GAP-43. The mean length of the longest neurite was determined from measurements of between about 100-120 neurons under each culture condition. Each column of the graph depicts pooled results from a number of independent experiments (indicated above the column), and the bars indicate standard error of the mean (SEM).

10

Figure 13 illustrates a dose response curve for B_{AG} polypeptide on the MAG-Fc response in cultured neurons. In particular, each data point indicates the mean length measured from about 120-150 neurons when cultured over monolayers of N-cadherin expressing 3T3 cells in media supplemented with MAG-Fc (25 $\mu\text{g/ml}$ final concentration) and B_{AG} polypeptide at the final concentration indicated on the horizontal axis. Each point indicates data from a single, representative experiment and the bars on each point indicate the SEM.

Figure 14 shows a bar graph depicting results from experiments testing the effects of B_{AG} polypeptide and BDNF on neurite outgrowth in cerebellar neurons that were cultured over monolayers of 3T3 cells that do not express N-cadherin and in media supplemented with 0 or 25 $\mu\text{g/ml}$ final concentration MAG-Fc (as indicated in the figure). Experiments were done in control media (*i.e.*, in media supplemented with MAG-Fc only) and in media additionally supplemented with B_{AG} polypeptide (6 μM final concentration) or BDNF (5 ng/ml final concentration). Cultures were maintained for 22 hours before being fixed and stained for GAP-43. The mean length of the longest neurite was determined from measurements of between about 100-120 neurons under each culture condition. Each column of the graph depicts pooled results from three independent experiments, and the bars indicate standard error of the mean (SEM).

30

Figure 15 shows a bar graph depicting results from experiments testing the B_{AG} polypeptide's effect on neurite outgrowth in cerebellar neurons that were cultured over monolayers of N-cadherin expressing 3T3 cells in either: (C) control media without supplements; (1) media supplements with monoclonal antibody for GT1b (20 µg/ml final concentration); or (2) media supplemented with both the GT1b antibody (20 µg/ml final concentration) and B_{AG} polypeptide (6 µM final concentration). Cultures were maintained for 22 hours before fixing and staining for GAP-43. The mean length of the longest neurite was determined from between about 100 and 120 neurons under each culture condition. Each column in the figure indicates pooled results from between 7 and 10 independent experiments, and the bars on each column indicate the SEM.

Figure 16 shows a bar graph depicting results from experiments testing the effects of various agents on neurite outgrowth in cerebellar neurons that were cultured over monolayers of N-cadherin expressing 3T3 cells either in control media without supplements (column C) or in media pre-treated with antibody to p75^{NTR} (columns 1-4). These antibody treated neurons were cultured after treatment in either: (1) control media without supplements; (2) the Rho kinase inhibitor Y27632 (10 µM final concentration); (3) B_{AG} polypeptide (6 µM final concentration); or (4) BDNF neurotrophin (5 ng/ml final concentration). Cultures were maintained for 22 hours before being fixed and stained for GAP-43. The mean length of the longest neurite was determined from between about 100 and 120 neurons under each culture condition. Each column in the figure indicates pooled results from the number of independent experiments indicated above it, and the bars on each column indicate the SEM.

Figure 17 shows a bar graph depicting results from experiments testing the effects of various kinase inhibitors on neurite outgrowth in cerebellar neurons cultured under different conditions. In particular, cerebellar neurons were cultured over monolayers of 3T3 cells in media supplements with either B_{AG} polypeptide at a final concentration of 6 µM (blank bars), BDNF neurotrophin at a final concentration of 5 ng/ml (striped bars) or FGF2 at a final concentration of 5 ng/ml (black bars). To test the effects of various agents, the experiments were done as indicated in the figure using control media containing no additional supplements, or using media additionally supplement with K252a (100 nM final

concentration), a PKA inhibitor (KT5720 at 200 nM final concentration or H-89 at 400 nM final concentration), or a PI3K inhibitor (Wortmannin or Ly294002, each at a final concentration of 10 μ M). Cultures were maintained for 18 hours before being fixed and stained for GAP-43, and the mean length of the longest neurite was determined from between
5 about 100 and 120 neurons under each culture condition. Data are pooled for results obtained with each of the PKA inhibitors and each of the PI3K inhibitors (which produced the same results). Each column in the figure indicates pooled results from at least three independent experiments, and the bars on each column indicate the SEM.

10 **Figures 18A-18B** show graphs depicting the results from experiments testing the effects on neurite growth in cerebellar neurons that were cultured in an “inhibitory environment” of wells coated with polylysine at 17 μ g/ml in distilled water (dH₂O); a mixture of goat anti-human IgG (Fc-specific) and fibronectin (both at 10 μ g/ml in DMEM); and MAG-Fc at 0.25 μ g/ml in DMEM/10% FCS. Cultures were maintained for 27 hours
15 before being fixed and stained for GAP-43. **Figure 18A** shows a dose-response curve of mean neurite length of cerebellar neurons grown in the presence of hriB_{AG2}, hB_{AG2} or riB_{AG}. **Figure 18B** shows a bar graph depicting the mean neurite length of cerebellar neurons grown in the presence of BDNF, B_{AG}, hriB_{AG2}, hB_{AG2} or riB_{AG}.

20 **Figure 19** shows a bar graph depicting results from neurite outgrowth experiments testing the effects of various agents in an inhibitory environment. In particular, cerebellar neurons were cultured over monolayers of N-cadherin expressing 3T3 cells in media supplemented with a soluble MAG-Fc fusion construct at a final concentration of 25 μ g/ml. The culture was further supplemented with BDNF (1ng/ml), NGF (10 ng/ml or 100 ng/ml),
25 BDNF (1ng/ml) in combination with NGF (10 ng/ml or 100 ng/ml), a constrained monomer of the NGF loop 1 binding motif (N-Ac-CTDIKGKEC-NH₂) (SEQ ID NO:43) at 100 μ g/ml, or the NGF loop 1 peptide (at 100 μ g/ml) in combination with BDNF (at 1ng/ml). Cultures were maintained for 23 hours before being fixed and stained for GAP-43. The mean length of the longest neurite was determined from measurements of between about 100-120 neurons
30 under each culture condition. Each column of the graph depicts pooled results from a

number of independent experiments (indicated above the column), and the bars indicate standard error of the mean (SEM).

6. DETAILED DESCRIPTION

5 As noted above, the present invention provides compounds, including peptides and peptidomimetics, that modulate (*e.g.*, increase or decrease) activity mediated by Trk-receptors such as TrkA, TrkB and TrkC. Such compounds are generally referred to here as Trk-receptor modulator compounds or “Trk modulators.”

Trk modulators of the invention are useful, *e.g.*, for modulating processes such as neuronal growth and survival, axonal growth, neurite outgrowth, synaptic plasticity and other processes that are mediated, at least in part, by a Trk-receptor. These uses include therapeutic methods that may involve modulating the growth and repair of the central nervous system *in vitro* (*e.g.*, in a cell culture) or *in vivo* (such as in a patient or other individual). Trk modulators of the invention therefore have utility in the treatment of
10 diseases such as stroke, Alzheimer’s disease, Parkinson’s disease, head trauma, spinal cord injury, and epilepsy to name a few.

Applicants have discovered that a key interaction between Trk receptors and their neurotrophin ligands occurs through a conserved short linear sequence motif of three amino acid residues – Arg-Gly-Glu (*i.e.*, “RGE” in the single letter amino acid code) found at the
20 N-terminal of mature neurotrophin amino acid sequences. The RGE motif is present in all neurotrophins and, when bound to the Trk receptor, exists as half a helix in what is considered a tight loop.

Applicants have also discovered that properly constrained peptides (for example, cyclic peptides) of the small linear RGE motif have a high structural overlap with the native
25 NT structure and are able to function as Trk receptor antagonists. Similarly, peptidomimetic compounds having high structural overlap with such constrained RGE peptides are also expected to have high structural overlap with the native NT structure and, as such, can also function as Trk receptor antagonists.

As noted above, the RGE motif is conserved among all neurotrophins, and
30 interactions with this motif are important for the binding of those neurotrophins to their respective Trk receptor(s). Hence, constrained peptides and peptidomimetics comprising the

RGE motif are useful as antagonists of a wide variety of Trk receptors, including TrkA, TrkB and TrkC. However, Trk modulators of the present invention can also be targeted to specific Trk receptors, by selecting flanking amino acid sequences from an NT ligand that preferably binds to the desired Trk receptor. In preferred Trk antagonist compounds (*i.e.*, Trk
5 modulator compounds that inhibit Trk receptor mediated activity), such flanking residues preferably range in length from no more than about 0 to 10 amino acid residues in length, with sizes between about 2-5 or 2-3 amino acid residues being particularly preferred. Moreover, the size of the cyclic peptide ring (or the corresponding peptidomimetic structure) preferably ranges from only about 4 to 15 amino acid residues, with sizes from about 5 to 10
10 amino acid residues being particularly preferred.

Applicants have also determined that, in crystal structures of NT dimers in complex with their binding domain of a Trk receptor, the RGE motif runs anti-parallel to itself in the NT dimer. That is to say, the RGE helix in the first NT molecule is aligned with and in an anti-parallel orientation to the RGE helix in the second NT molecule in that dimer. See, in
15 particular, **Figures 6A-6C**. Applicants have moreover discovered that, when a tandem repeat peptide or peptidomimetic of the RGE motif is properly constrained (as in a cyclic peptide or peptidomimetic), it adopts the same anti-parallel alignment conformation and has a high structural overlap with the native NT structure. Such “tandem-repeat” RGE cyclic peptides and peptide mimetics are, surprisingly, able to function as Trk receptor agonists (*i.e.*, they are
20 able to increase activity mediated by a Trk receptor). As such, these compounds are also among the Trk modulator compounds of the invention.

As with the RGE antagonists, described, *supra*, constrained peptides and peptidomimetics comprising a tandem repeat of the RGE motif are useful as agonists for a wide variety of Trk receptors, including TrkA, TrkB and TrkC. However, the compounds
25 can also be targeted to specific Trk receptors, for example, by selecting flanking amino acid sequences from a NT ligand that preferably binds to the desired Trk receptor. In preferred Trk agonists compounds (*i.e.*, the Trk modulator compounds that increase Trk receptor mediated activity) such flanking residues preferably range in length from no more than about 0 to 10 amino acid residues in length, with sizes from 2-5 or 2-3 being more preferred.

30 Tandem repeat cyclic peptides and peptidomimetics of the invention may, optionally, contain additional amino acid residues situated between the two tandem repeats of the RGE

motif. Such additional amino acid residues therefore function as “spacer” moieties to join the two RGE motifs together in such a way that they adopt the anti-parallel alignment conformation having a high structural overlap with the RGE motif in the native NT structure(s). The exact identity of the spacer amino acid residue(s) is not important and their identities may or may not correspond to identities of amino acid residues flanking the RGE motif in a particular neurotrophin. Preferably, the spacer moiety (if present) in a tandem repeat cyclic peptide or peptidomimetic is short; *e.g.*, not longer than five amino acid residues in length, with spacer moieties between about 0-3 amino acid residues in length being more preferred. Particularly preferred spacer moieties are only about 1 or 2 amino acid residues in length.

The total size of such “tandem-repeat” cyclic peptides and peptidomimetics is moreover, typically about twice that of a Trk antagonist cyclic peptide or peptidomimetic of the invention. Hence, preferred sizes of the peptide ring (or corresponding peptidomimetic structure) are preferably from about 8 to 30 amino acid residues in length, with sizes from about 10 to 20 amino acid residues in length being particularly preferred.

Preferred cyclic peptides that comprise the RGE motif and/or tandem repeats thereof are described in Section 6.1, *infra*. Section 6.2 then describes routine experimental methods by which a person skilled in the art can determine, *e.g.*, by X-ray crystallography or NMR spectroscopy, three-dimensional “pharmacophore” structures for these and other cyclic peptides, and methods using such pharmacophore structures to design appropriate peptidomimetic compounds are provided in Section 6.3, *infra*, along with exemplary peptidomimetic modifications. Still further modifications to the Trk modulator compounds of the invention are described in Section 6.4, including pharmaceutical formulations and medicinal uses. Section 6.5 describes routine assays by which a person skilled in the art may verify the Trk modulator activity of a compound, such as a peptidomimetic compound. Section 6.6 then describes certain preferred exemplary uses of such compounds in methods for modulating Trk mediated activities such as neuronal survival, axonal growth and synaptic plasticity. Formulations of Trk modulators (including pharmaceutical formulations) that are particularly suitable for such uses are provided in Section 6.7. The specification concludes with a series of examples, at Section 7, demonstrating certain exemplary embodiments of the invention.

The present invention also provides methods for the administration of a p75 receptor binding agent to an individual, wherein the p75 receptor binding agent interferes with the binding of a neurotrophin to a p75 receptor engaged in an inhibitory complex and, thus, promotes neurotrophin binding to a Trk receptor. These methods are useful in the treatment of conditions wherein CNS neurons are damaged or injured, for example, diseases such as stroke, Alzheimer's disease, Parkinson's disease, traumatic brain injury and spinal cord injury.

The present invention is based, in part, on the discovery that agents designed to interfere with neurotrophin binding to a p75 receptor engaged in an inhibitory complex facilitate neurotrophin-mediated CNS neurite outgrowth. As described in the Examples section below, neurite outgrowth assays were performed using cultured rat cerebellar neurons in an inhibitory environment. The inhibitory culture media was supplemented with NGF, NGF-in combination with BDNF, a constrained monomer of the NGF first binding loop (N-Ac-CTDIKGKEC-NH₂) (SEQ ID NO:43), or the NGF loop 1 peptide in combination with BDNF. Cultures were maintained for 23 hours, fixed, and stained for GAP-43. The mean length of the longest neurite was then determined from 100-120 neurons under each culture condition. It was found that NGF alone and the constrained monomer alone did not promote neurite outgrowth, but the combinations of NGF with BDNF in ratios of 10:1 and 100:1 and the NGF loop 1 peptide at 100 µg/ml with BDNF at 1 ng/ml promoted neurite outgrowth. These findings show that administration of a p75 receptor binding agent, which is a neurotrophin that does not bind the expressed Trk receptor in combination with another, different neurotrophin, which binds a Trk receptor expressed on the injured neurons results in CNS neuron growth in an inhibitory environment. Here, the neurotrophin, which does not bind to an expressed Trk receptor (i.e., a p75 receptor binding agent) was administered in an amount about 10 to about 100 fold greater than the neurotrophin, which binds to an expressed Trk receptor (i.e., not a p75 receptor binding agent). These results also show that administration of a constrained monomer of the NGF first binding loop, a non-neurotrophin p75 receptor binding agent, promotes neurotrophin-mediated CNS neuron growth.

Definitions

The following defined terms are used throughout the present specification, and should be helpful in understanding the scope and practice of the present invention.

As used herein “an inhibitory environment” means an environment in which growth of a damaged or injured neuron is inhibited. An inhibitory environment is present in the milieu surrounding damaged or injured neurons. Damaged or injured neurons are present in conditions, which include, for example, diseases and disorders that are associated with damage to or impaired function of the CNS. Exemplary conditions include, but are not limited to, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophin lateral sclerosis, stroke, traumatic brain injury, and spinal cord injury. Alternatively, an inhibitory environment is an environment wherein a p75 receptor is engaged in an inhibitory complex (i.e., a p75 receptor is engaged with a molecule that binds a p75 receptor, e.g., a myelin-derived molecule such as MAG or Nogo-A), and this binding results in inhibition of neurotrophin-mediated neuron growth).

The term “therapeutically effective” means that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to an individual in need thereof. Preferably, a therapeutically effective amount can ameliorate or prevent a clinically significant deficit in the activity, function and response of the individual. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the individual. For example, “therapeutically effective” means an amount or dose of a p75 receptor inhibitor sufficient to promote the growth of a neuron in the CNS in an inhibitory environment.

As used herein, the phrase “pharmaceutically acceptable” refers to molecular entities and compositions that are “generally regarded as safe”, e.g., that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government of listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for

injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

An "individual" or "patient" as used herein is preferably a mammal and more preferably a human, but can be any animal, including a laboratory animal in the context of a
5 clinical trial or screening or activity experiment. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but not limited to domestic animals, wild animals and research animals.

10 6.1. TRK RECEPTOR MODULATORS: CYCLIC PEPTIDES

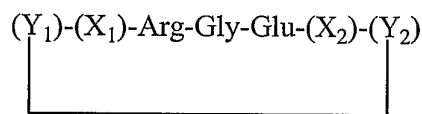
The term "cyclic peptide," as used herein, refers to a peptide or salt thereof that comprises: (1) an intramolecular covalent bond between two non-adjacent residues; and (2) at least one Trk-receptor recognition sequence RGE (*i.e.*, Arg-Gly-Glu) within a cyclic ring of the cyclic peptide. It is understood that preferred peptides of the invention which function
15 as either Trk receptor agonists or antagonists will be constrained and, hence, are preferably cyclic peptides. However, non-cyclic or "linear" peptides are also useful (*e.g.*, as intermediate compounds for making cyclic peptides of the invention). Hence, non-cyclic versions of the cyclic peptides described throughout this application are also considered part of the present invention.

20 The intramolecular bond may be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (*i.e.*, terminal functional groups of a linear peptide and/or side-chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Preferred intramolecular bonds include, but are not limited to, disulfide, amide and thioether bonds. A variety of means for cyclizing polypeptides are well known in the art, as are many other modifications that can be made to such peptides. For a general discussion,
25 see International Patent Publication Nos. WO 01/53331 and WO 98/02452. Such cyclic bonds and other modifications can also be applied to the cyclic peptides and derivative compounds of this invention. For convenience, cyclic peptides of the invention are frequently illustrated in this application showing particular cyclic bonds, which may or may
30 not be preferred. However, other embodiments of these cyclic peptides comprising

additional and/or alternative cyclic bonds will be apparent to those persons skilled in the art and are therefore considered part of this invention.

Within certain embodiments a cyclic peptide of the invention preferably comprises an N-acetyl group (*i.e.*, an amino group present on the amino terminal residue of the peptide is acetylated, preferably prior to cyclization). Alternatively, a cyclic peptide of the invention
 5 may comprise an N-formyl group (*i.e.*, the amino group present on the amino terminal residue of the peptide is formylated, preferably prior to cyclization). Alternatively, the amino group present on the amino terminal residue of the peptide may be mesylated; again, preferably prior to cyclization. The presence of such terminal groups may, for example,
 10 enhance cyclic peptide activity or stability in certain applications. In addition, within certain embodiments a cyclic peptide of the invention may comprise a C-amide group.

In certain embodiments, preferred cyclic peptides of the present invention satisfy the general formula:



(Formula I)

15 where Y_1 and Y_2 are amino acid residues whose identities are independently selected and having a covalent bond between the residues Y_1 and Y_2 . The elements X_1 and X_2 are optional and, if present, they are independently selected from the amino acid residues and combinations thereof that are linked by peptide bonds. Hence, either X_1 or X_2 or both X_1 and X_2 , if present, may be a single amino acid residue or, alternatively, may each be a sequence
 20 comprising a plurality of amino acid residues linked by peptide bonds.

In preferred embodiments, a cyclic peptide satisfying Formula I, above, will modulate one or more Trk-receptor mediated activities. For example, in certain preferred embodiments a peptide satisfying Formula I will inhibit one or more Trk-receptor mediated activities and, as such, will be a Trk antagonist. In other embodiments, a peptide satisfying Formula I will
 25 increase one or more Trk receptor mediated activities and, as such, will be a Trk agonist.

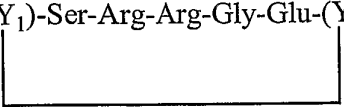

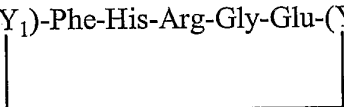
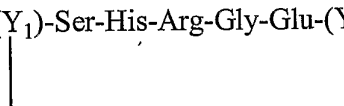
In addition to the RGE consensus sequence(s), cyclic peptides of the invention generally comprise at least one additional residue within the cyclic ring so that preferably at least one of either X_1 or X_2 in Formula I is present. Generally, the size of X_1 and/or X_2 will depend upon the desired activity of the cyclic peptide. For example, where a cyclic peptide

that is a Trk antagonist is desired, shorter peptide sequences are preferred. Accordingly, in such embodiments, X₁ and/or X₂ are each preferably between 0 and about 10 amino acids in length, with sizes of about 1, 2, 3, 4 or 5 amino acid residues being particularly preferred. Moreover, in such embodiments the lengths of X₁ and/or X₂ are also preferably selected so that the size of the cyclic peptide ring ranges from about 5 to about 15 amino acid residues, and is more preferably between about 5-10 amino acid residues in length. Peptide ring sizes of about 5-7 amino acid residues in length are particularly preferred. Such additional residues (*i.e.*, X₁ and/or X₂ in Formula I, *supra*) may be present on either the N-terminal or C-terminal side of the RGE sequence, or they may be present on both sides of the RGE sequence.

In preferred cyclic peptides of the invention, the additional residues are derived from sequences that flank the RGE sequence within one or more naturally occurring neurotrophins (*e.g.*, NGF, BDNF, NT-3, NT-4, NT-5 and NT-4/5) with or without amino acid substitutions and/or other modifications. In particular, the presence of flanking sequences from a neurotrophin may help target a cyclic peptide for a particular Trk receptor of interest. Hence, in embodiments where an antagonist for a particular Trk receptor is desired, a cyclic peptide of the invention may comprise amino acid residues flanking either the N-terminal, C-terminal or both sides of the RGE sequence that are derived from flanking sequences in a neurotrophin that preferably binds to the targeted Trk receptor.

As an example, and not by way of limitation, Table I, *infra*, list certain preferred cyclic peptides that comprise additional amino acid residues derived from particular neurotrophins whose identities are also indicated in the table. The right-hand column in Table I also indicates a Trk receptor to which the neurotrophin preferably binds (or, rather, binds with the highest binding affinity). Hence, each cyclic peptide listed in Table I may, in one embodiment, be used to inhibit the particular Trk receptor indicated along side it, in the right hand column of Table I. Those skilled in the art will appreciate, however, that there is some overlap in binding specificity of the different neurotrophin ligands for various Trk receptors. Hence, the cyclic peptides listed in Table I can also be used as antagonists of other Trk receptors. As a particular example, and not by way of limitation, it is demonstrated in the Examples, *infra*, that the cyclic peptide N-Ac-CSRRGEC-NH₂, which contains additional residues from the neurotrophin NT-4, is a more potent TrkB antagonist than are the peptides

N-Ac-CSHRGEC-NH₂ and N-Ac-CFRRGEC-NH₂, equivalent peptides designed with additional residues from the neurotrophins NT-3 and NGF, respectively.

TABLE I: TRK ANTAGONISTS		
Peptide Formula	NT	Trk-R
(Y ₁)-Ser-Arg-Arg-Gly-Glu-(Y ₂) 	NT-4	TrkB
(Y ₁)-Ala-Arg-Arg-Gly-Glu-(Y ₂) 	BDNF	TrkB
(Y ₁)-Phe-His-Arg-Gly-Glu-(Y ₂) 	NGF	TrkA
(Y ₁)-Ser-His-Arg-Gly-Glu-(Y ₂) 	NT-3	TrkC

5 Examples of particularly preferred cyclic peptide sequences of the invention, which are preferably Trk antagonists, include:

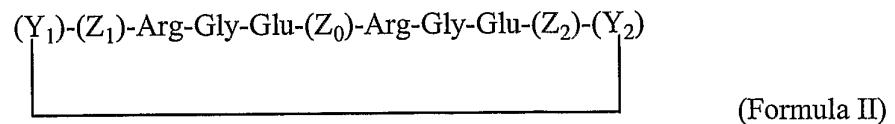
CSRRGEC (SEQ ID NO:1),
 N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2),
CARRGEC (SEQ ID NO:3),
 N-Ac-CARRGEC-NH₂ (SEQ ID NO:4),
CFHRGEC (SEQ ID NO:5),
 N-Ac-CFHRGEC-NH₂ (SEQ ID NO:6),
CSHRGEC (SEQ ID NO:7),
 N-Ac-CFHRGE-NH₂ (SEQ ID NO:8),
CRGEC (SEQ ID NO:9), and
 N-Ac-CRGEC-NH₂ (SEQ ID NO:10).

The underlined portion of each foregoing amino acid sequence indicates that portion of the peptide that is cyclized. "N-Ac" denotes an acetylated N-terminal amino group and "NH₂" denotes a C-terminal amide group.

5 Within certain embodiments, relatively small cyclic peptides of the invention that do not contain significant sequences flanking the RGE consensus sequence are particularly preferred. Such peptides may or may not contain an N-acetyl group and they may or may not contain a C-amide group. Examples of preferred, small cyclic peptides of the invention include:

N-Ac-CRGEC-NH₂ (SEQ ID NO:10),
 N-Ac-KRGED-NH₂ (SEQ ID NO:11),
 H-C(O)-CRGEC-NH₂ (SEQ ID NO:12),
 CH₃-SO₂-NH-CRGEC-NH₂ (SEQ ID NO:13),
 N-Ac-CRGEC-Y-NH₂ (SEQ ID NO:14),
 H-C(O)-CRGEC-Y-NH₂ (SEQ ID NO:15), and
 CH₃-SO₂-NH-CRGEC-Y-NH₂ (SEQ ID NO:16).

10 In other embodiments of the invention, where a Trk agonist is desired, longer peptide sequences are generally preferred. In particular, preferred cyclic peptides of the invention that are Trk agonists comprise at least one "tandem repeat" of the RGE motif. Accordingly, where such cyclic peptides satisfy Formula I, *supra*, at least one of X₁ and X₂ will be present and comprises a second RGE sequence. More specifically, such cyclic peptides of the
 15 invention preferably satisfy the following general formula:



As in Formula I, Y₁ and Y₂ are amino acid residues whose identities are independently selected and having a covalent bond between the residues Y₁ and Y₂. The elements Z₁ and Z₂ are optional and, if present, they are independently selected from the
 20 amino acid residues and combinations thereof that are linked by peptide bonds. The element Z₀ is also optional, and if present is an amino acid residue or some combination thereof, linked by peptide bonds. Hence, either Z₁, Z₂, Z₀ or any combination thereof, if present, may

each be a single amino acid residue or, alternatively, they may each be a sequence comprising a plurality of amino acid residues linked by peptide bonds.

In addition to a tandem repeat of the RGE consensus sequence, cyclic peptides of the invention generally comprise one additional residues within the cyclic ring so that, preferably, at least one of either Z_1 , Z_2 and/or Z_0 is present. In embodiments where a cyclic peptide that is a Trk agonist is desired, Z_1 , Z_2 and/or Z_0 are each preferably no more than about ten amino acid residues in length, and more preferably are each only 1, 2, 3, 4 or 5 amino acid residues in length. Moreover, the lengths of Z_1 , Z_2 and/or Z_0 are preferably selected so that the size of the cyclic peptide ring ranges from about 8-50 amino acid residues, and more preferably from about 8-25 or from about 15-20 amino acid residues.

As with the cyclic peptides of Formula I, in preferred cyclic peptides of Formula II the additional residues (*i.e.*, Z_1 , Z_2 and/or Z_0) can be derived from sequences that flank the RGE sequence within one or more naturally occurring neurotrophin (*e.g.*, NGF, BDNF, NT-3, NT-4, NT-5 or NT-4/5), with or without amino acid substitutions and/or other modifications. In particular, the presence of flanking amino acid residues from a particular neurotrophin may help target a cyclic peptide for a particular Trk receptor of interest. Hence, in embodiments where an antagonist for a particular Trk receptor is desired, a cyclic peptide of the invention may comprise amino acid residues flanking the N-terminal and/or C-terminal of one or both tandem repeat RGE sequences, and these flanking sequences may be derived from a neurotrophin that preferably binds to the targeted Trk receptor of interest.

As noted above, preferred tandem repeat cyclic peptides of the invention (including cyclic peptides according to Formula II) have the two RGE sequences aligned anti-parallel to each other. Accordingly, in preferred cyclic peptides according to Formula II the element Z_0 is present and can function as an effective "spacer moiety" to align the two RGE sequences together in an anti-parallel alignment conformation. In preferred embodiments, Z_0 is not more than 10 amino acid residues in length, and is preferably five or fewer amino acid residues in length. Preferred sizes for Z_0 are about 1, 2, 3, 4 or 5 amino acid residues in length. The exact sequence of amino acid residues in Z_0 is not critical. As such, the element Z_0 may or may not comprise a sequence of amino acid residues corresponding to a sequence from either the N-terminal or C-terminal of the RGE motif in a natural neurotrophin (*e.g.*, NGF, BDNF, NT-3, NT-4, NT-5 and NT-4/5). Where Z_0 does comprise sequences from a

neurotrophin, those sequences may or may not comprise amino acid substitutions and/or modifications.

Examples of particularly preferred cyclic peptide sequences of the invention, which are preferably Trk agonists, include:

- 5 CSRRGELAASRRGELC (SEQ ID NO:17)
 N-Ac-CSRRGELAASRRGELC-NH₂ (SEQ ID NO: 18)
 CFHRGEFSSIFHRGEFC (SEQ ID NO: 30)
 CARRGELSARRGELC (SEQ ID NO 31)
 CSHRGEYSKSHRGEYC (SEQ ID NO: 32)

- 10 The cyclic peptide sequences identified by SEQ ID NOs: 30, 31, and 32 are TrkA, TrkB, and TrkC agonists, respectively. SEQ ID NO:30 is derived from NGF. SEQ ID NO: 31 is derived from BDNF. SEQ ID NO: 32 is derived from NT-3.

- Cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. The amino acids may from natural or non-natural sources provided that at least one amino group and at least one carboxyl group are present in the molecule. A- and β-amino acids are generally preferred. The 20 L-amino acids commonly found in proteins are particularly preferred in the present invention. These amino acids are identified herein by their conventional three-letter and one-letter abbreviations, whereas the corresponding D- amino acids are designated by the prefix “d”.

- 20 In certain embodiments, cyclic peptides of the invention may comprise a sequence of D-amino acid residues that is the opposite of a sequence of L-amino acid residues provided herein. For example, the invention provides certain Trk receptor agonist polypeptides, referred to herein as riB_{AG1} and hriB_{AG2} (SEQ ID NOS:40-41) that comprise sequences of D-amino acid sequences which are the reverse sequence of another Trk receptor agonist polypeptide referred to as the B_{AG} polypeptide (SEQ ID NO:17). Hence, in addition to the polypeptides of L-amino acid residues described *supra*, the present invention also contemplates polypeptides having the reverse sequence of L-amino acid residues. Hence, in one preferred embodiment peptides and peptidomimetics of the present invention comprise sequences of L-amino acid residues including the Arg-Gly-Glu (*i.e.*, “RGE”) motif
 25
 30 described, *supra*. Accordingly, the invention also provides, in an alternative embodiment)

peptides and peptidomimetics comprising sequences of D-amino acid residues including the short linear sequence motif dGlu-Gly-dArg (*i.e.*, “dEGdR”).

Cyclic peptides may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Preferred derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal amide group (*i.e.*, the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not limited to, penicillamine, β,β -tetramethylene cysteine, β,β -pentamethylene cysteine, β -mercaptopropionic acid, β,β -pentamethylene- β -mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptothioproline, ornithine, diaminobutyric acid, α -aminoadipic acid, m-aminomethylbenzoic acid and α,β -diaminopropionic acid.

Cyclic peptides as described herein may be synthesized by methods well known in the art, including recombinant DNA methods and chemical synthesis. Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, in which a peptide linkage occurs through the direct condensation of the α -amino group of one amino acid with the α -carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (see Gross & Meienhofer, eds., “The Peptides: Analysis, Synthesis, Biology,” Vol. 1-4 (Academic Press, 1979); Bodansky & Bodansky, “The Practice of Peptide Synthesis,” 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis

requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. In particular, a high percentage of racemization may be observed when residues such as Phe-Gly are coupled. Such situations are, however, uncommon. Solubility considerations are also a factor.

5 Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield *et al.*, *J. Am. Chem. Soc.* 1963,85:2149. These methods involve assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy. The Boc strategy uses a 1% cross-linked polystyrene resin. The standard protecting group for α -amino functions is the tert-butyloxycarbonyl (Boc) group. This group can be removed with dilute solutions of strong acids such as 25% trifluoroacetic acid (TFA). The next Boc-amino acid is typically coupled to the amino acyl resin using dicyclohexylcarbodiimide (DCC). Following completion of the assembly, the peptide-resin is treated with anhydrous HF to cleave the benzyl ester link and liberate the free peptide. Side-chain functional groups are usually blocked during synthesis by benzyl-derived blocking groups, which are also cleaved by HF. The free peptide is then extracted from the resin with a suitable solvent, purified and characterized. Newly synthesized peptides can be purified, for example, by gel filtration, HPLC, partition chromatography and/or ion-exchange chromatography, and may be characterized by, for example, mass spectrometry or amino acid sequence analysis. In the Boc strategy, C-terminal amidated peptides can be obtained using benzhydrylamine or methylbenzhydrylamine resins, which yield peptide amides directly upon cleavage with HF.

In the procedures discussed above, the selectivity of the side-chain blocking groups and of the peptide-resin link depends upon the differences in the rate of acidolytic cleavage. Orthogonal systems have been introduced in which the side-chain blocking groups and the peptide-resin link are completely stable to the reagent used to remove the α -protecting group at each step of the synthesis. The most common of these methods involves the 9-fluorenylmethyloxycarbonyl (Fmoc) approach. Within this method, the side-chain protecting

groups and the peptide-resin link are completely stable to the secondary amines used for cleaving the N- α -Fmoc group. The side-chain protection and the peptide-resin link are cleaved by mild acidolysis. The repeated contact with base makes the Merrifield resin unsuitable for Fmoc chemistry, and p-alkoxybenzyl esters linked to the resin are generally
5 used. Deprotection and cleavage are generally accomplished using TFA.

Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking groups must be stable throughout the entire synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.

10 Acetylation of the N-terminal can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation is accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

Following synthesis of a linear peptide, with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the
15 art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution
20 to minimize aggregation and intermolecular side reactions. This method suffers from the disadvantage of being slow but has the advantage of only producing H₂O as a side product. Alternatively, strong oxidizing agents such as I₂ and K₃Fe(CN)₆ can be used to form disulfide linkages. Those of ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Cyclic peptides produced by this
25 method require purification using standard techniques, but this oxidation is applicable at acid pHs.

Oxidizing agents also allow concurrent deprotection/oxidation of suitable S-protected linear precursors to avoid premature, nonspecific oxidation of free cysteine.

30 DMSO, unlike I₂ and K₃Fe(CN)₆, is a mild oxidizing agent which does not cause oxidative side reactions of the nucleophilic amino acids mentioned above. DMSO is miscible with H₂O at all concentrations, and oxidations can be performed at acidic to neutral

pHs with harmless byproducts. Methyltrichlorosilane-diphenylsulfoxide may alternatively be used as an oxidizing agent, for concurrent deprotection/oxidation of S-Acm, S-Tacm or S-t-Bu of cysteine without affecting other nucleophilic amino acids. There are no polymeric products resulting from intermolecular disulfide bond formation.

5 Suitable thiol-containing residues for use in such oxidation methods include, but are not limited to, cysteine, β,β -dimethyl cysteine (penicillamine or Pen), β,β -tetramethylene cysteine (Tmc), β,β -pentamethylene cysteine (Pmc), β -mercaptopropionic acid (Mpr), β,β -pentamethylene- β -mercaptopropionic acid (Pmp), 2-mercaptobenzene, 2-mercaptoaniline and 2-mercaptoproline.

10 It will be readily apparent to those of ordinary skill in the art that, within each of these representative formulas set forth *supra*, any of the above thiol-containing residues may be employed in place of one or both of the thiol-containing residues recited.

 Within further embodiments, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (i.e., the amino and carboxy termini of a linear peptide prior to cyclization). Examples of such
15 peptides include c(SRRGE) (SEQ ID NO:33), c(ARRGE) (SEQ ID NO:34), c(FHRGE) (SEQ ID NO:35) and c(SHRGE) (SEQ ID NO:36). An example of one particularly preferred peptide having such a cyclic amide bond is the peptide c(SRRGELSRRGEL) (SEQ ID NO:39). This peptide, which is described in the Examples, *infra*, is referred to here as the
20 hB_{AG2} peptide. Within another such embodiment, the linear peptide comprises a D-amino acid. For example, the Examples, *infra*, describe another preferred peptide that is referred to as the hriB_{AG2} peptide. This peptide, which contains a cyclic amide bond as described, *supra*, comprises the following sequence of D amino acid residues:

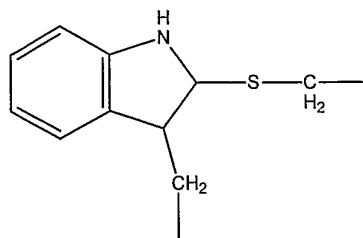
 c[dLdEdGdRdRdSdLdEdGdRdRdS] (SEQ ID NO:40). Alternatively, cyclization may be
25 accomplished by linking one terminus and a residue side chain or using two side chains, as in KRGED (SEQ ID NO:37) or KSRRGED (SEQ ID NO:38), with or without an N-terminal acetyl group and/or a C-terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α -amino adipic acid, m-aminomethylbenzoic acid, α,β -diaminopropionic acid, glutamate or aspartate.

30 Methods for forming amide bonds are well known in the art and are based on well established principles of chemical reactivity. Within one such method, carbodiimide-

mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, EDAC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. The formation of the inactive N-acylurea, resulting from an O→N migration, can be circumvented by converting the O-acylurea to an active ester by reaction with an N-hydroxy compound such as 1-hydroxybenzotriazole, 1-hydroxysuccinimide, 1-hydroxynorbornene carboxamide or ethyl 2-hydroximino-2-cyanoacetate. In addition to minimizing O→N migration, these additives also serve as catalysts during cyclization and assist in lowering racemization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Hydrazinolysis of the terminal ester necessitates the use of a t-butyl group for the protection of side chain carboxyl functions in the acylating component. This limitation can be overcome by using diphenylphosphoryl acid (DPPA), which furnishes an azide directly upon reaction with a carboxyl group. The slow reactivity of azides and the formation of isocyanates by their disproportionation restrict the usefulness of this method. The mixed anhydride method of lactam formation is widely used because of the facile removal of reaction by-products. The anhydride is formed upon reaction of the carboxylate anion with an alkyl chloroformate or pivaloyl chloride. The attack of the amino component is then guided to the carbonyl carbon of the acylating component by the electron donating effect of the alkoxy group or by the steric bulk of the pivaloyl chloride t-butyl group, which obstructs attack on the wrong carbonyl group. Mixed anhydrides with phosphoric acid derivatives have also been successfully used. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. The last few years have witnessed the development of benzotriazolylxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) and its congeners as advantageous coupling reagents. Their performance is generally superior to that of the well established carbodiimide amide bond formation reactions.

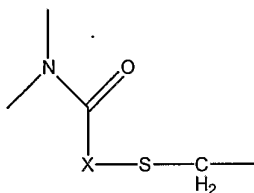
30 Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α -amino acid. By way of

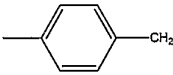
example, a lysine side chain can be coupled to bromoacetic acid through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF. Examples of thiol-containing linkages include:



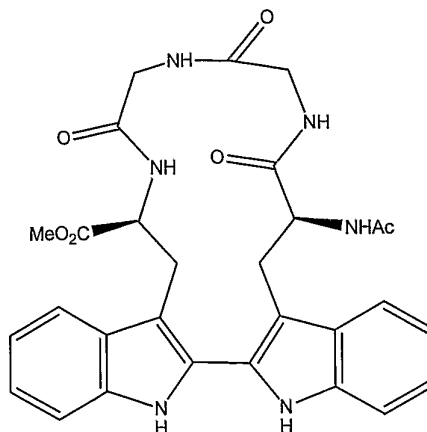
and

10



where X may be (CH₂)₄, CH₂ or 

15 Cyclization may also be achieved using $\delta_1\delta_1$ -Ditryptophan (*i.e.*, Ac-Trp-Gly-Gly-Trp-OMe), as shown below:



The structures and formulas recited herein are provided solely for the purpose of illustration, and are not intended to limit the scope of the cyclic peptides described herein.

5 6.2. TRK-RECEPTOR PHARMACOPHORES

For designing peptidomimetics, it is beneficial to obtain a three dimensional structure for the pharmacophore of one or more cyclic peptides described above. The term “pharmacophore” refers to the collection of functional groups on a compound that are arranged in three-dimensional space in a manner complementary to the target protein, and that are responsible for biological activity as a result of compound binding to the target protein. Useful three-dimensional pharmacophore models are best derived from either crystallographic or nuclear magnetic resonance structures of the target, but can also be derived from homology models based on the structures of related targets or three-dimensional quantitative structure-activity relationships derived from a previously discovered series of active compounds.

The present invention provides pharmacophores of certain representative cyclic peptides (*i.e.*, three-dimensional conformations of the neurotrophin consensus sequence RGE within such peptides). Such three-dimensional structures provide the information required to most efficiently direct the design and optimization of peptidomimetics.

In one embodiment, the three-dimensional structures of cyclic peptides are generally determined using X-ray crystallography. These techniques are well known and are within the routine skill in the art. For example, see Cantor&Schimmel, *Biophysical Chemistry* 1980 (Vols. I-III) W. H. Freeman and Company (particularly Chapters 1- 13 in Vol. I, and Chapter 13 in Vol. II). See, also, *Macromolecular Crystallography, Parts A-B* (Carter&Sweet, Eds.)

In: *Methods Enzymol.* 1997, Vols. 276-277; Jan Drenth, *Principles of Protein X-Ray Crystallography* (New York: Springer-Verlag, 1994).

5 The term "crystal" refers, generally, to any ordered (or at least partially ordered) three-dimensional array of molecules. Preferably, the ordering of molecules within a crystal is at least sufficient to produce a sharp X-ray diffraction pattern so that the molecules' three-dimensional structure may be determined.

The molecules in a crystal may be of any type, and it will be understood that a crystal may contain molecules of only one type or may comprise a plurality of different types of molecules. In preferred embodiments, crystals of the present invention comprise at least one
10 biomolecule, such as a cyclic peptide described, *supra*, in Section 6.1. Crystals of the invention may even comprise a complex or assembly of two or more proteins or other biomolecules. For example, a crystal may comprise molecules of a ligand, such as a neurotrophin, bound to molecules of a receptor, such as a Trk receptor. Typically, crystals that contain biological molecules such as proteins will contain other molecules as well, such
15 molecules of solvent (*e.g.*, water molecules) and/or salt. Other molecules such as drugs, drug candidates or compounds that bind to the protein may also be present in a crystal.

Indeed, crystal structures for the binding domain of Trk receptors complexed with a neurotrophin are already available in the art. See, for example, Wiesmann *et al.*, *Nature* 1999, 401:184; and Banfield *et al.*, *Structure (Camb.)* 2001, 9:1191. The coordinates of
20 these X-ray structures can be readily obtained, for example, from the Protein Data Bank at <www.rcsb.org> (Accession Nos. 1www and 1hcf, respectively). Hence, in particularly preferred embodiments, which are demonstrated in the Examples, *infra*, pharmacophore structures of the invention are determined using the X-ray crystal structure(s) of a neurotrophin bound to an appropriate Trk receptor (or fragment thereof). These three-
25 dimensional structures can then be used to design peptidomimetics of the invention or, alternatively, to design additional cyclic peptides that are likely to be Trk modulators.

Alternatively, the three-dimensional structures of cyclic peptides may generally be determined using nuclear magnetic resonance (NMR) techniques that are well known in the art. NMR data acquisition is preferably carried out in aqueous systems that closely mimic
30 physiological conditions to ensure that a relevant structure is obtained. Briefly, NMR techniques use the magnetic properties of certain atomic nuclei (such as ^1H , ^{13}C , ^{15}N and ^{31}P),

which have a magnetic moment or spin, to probe the chemical environment of such nuclei. The NMR data can be used to determine distances between atoms in the molecule, which can be used to derive a three-dimensional model of the molecule.

For determining three-dimensional structures of cyclic peptides (and candidate
5 peptidomimetics, as discussed below) proton NMR is preferably used. More specifically, when a molecule is placed in a strong magnetic field, the two spin states of the hydrogen atoms are no longer degenerate. The spin aligned parallel to the field will have a lower energy and the spin aligned antiparallel to the field will have a higher energy. At
10 equilibrium, the spin of the hydrogen atoms will be populated according to the Boltzmann distribution equation. This equilibrium of spin populations can be perturbed to an excited state by applying radio frequency (RF) pulses. When the nuclei revert to the equilibrium state, they emit RF radiation that can be measured. The exact frequency of the emitted radiation from each nucleus depends on the molecular environment of the nucleus and is different for each atom (except for those atoms that have the same molecular environment).
15 These different frequencies are obtained relative to a reference signal and are called chemical shifts. The nature, duration and combination of applied RF pulses can be varied greatly and different molecular properties can be probed by those of ordinary skill in the art, by selecting an appropriate combination of pulses.

For three-dimensional structure determinations, one-dimensional NMR spectra are
20 generally insufficient, as limited information pertaining to conformation may be obtained. One-dimensional NMR is generally used to verify connectivity within a molecule and yields incomplete data concerning the orientation of side chains within a peptide. Two-dimensional NMR spectra are much more useful in this respect and allow for unambiguous determination of side-chain-to-side-chain interactions and the conformation of the peptide backbone.

25 Two-dimensional NMR spectra are generally presented as a contour plot in which the diagonal corresponds to a one-dimensional NMR spectrum and the cross peaks off the diagonal result from interactions between hydrogen atoms that are directly scalar coupled. Two-dimensional experiments generally contain a preparation period, an evolution period where spins are "labeled" as they precess in the XY plane according to their chemical shift, a
30 mixing period, during which correlations are made with other spins and a detection period in which a free induction decay is recorded.

Two-dimensional NMR methods are distinguished by the nature of the correlation that is probed during the mixing period. A DQF-COSY (double quantum filtered correlation spectroscopy) analysis gives peaks between hydrogen atoms that are covalently connected through one or two other atoms. Nuclear Overhauser effect spectroscopy (NOESY) gives
5 peaks between pairs of hydrogen atoms that are close together in space, even if connected by way of a large number of intervening atoms. In total correlation spectroscopy (TOCSY), correlations are observed between all protons that share coupling partners, whether or not they are directly coupled to each other. Rotating-frame Overhauser Spectroscopy (ROESY) experiments may be thought of as the rotating frame analogue of NOESY, and yields peaks
10 between pairs of hydrogen atoms that are close together in space. One or more such methods may be used, in conjunction with the necessary water-suppression techniques such as WATERGATE and water flip-back, to determine the three-dimensional structure of a cyclic peptide or candidate peptidomimetic under aqueous conditions. Such techniques are well known and are necessary to suppress the resonance of the solvent (HDO) during acquisition
15 of NMR data.

By way of example, both TOCSY and NOESY may be applied to representative cyclic peptides for the purpose of determining the conformation and the assignment. The water solvent resonance may be suppressed by application of the WATERGATE procedure. A water flipback pulse may also be applied at the end of the mixing period for both TOCSY
20 and NOESY experiments to maintain the water signal at equilibrium and to minimize the loss of amide proton resonances due to their rapid exchange at the near neutral pH conditions (i.e., pH 6.8) used in the experiment. NMR data may be processed using spectrometer software using a squared cosine window function along both directions. Baseline corrections may be applied to the NOESY, ROESY and TOCSY spectra using the standard Bruker polynomial
25 method.

NOESY data may be acquired at several mixing times ranging from 80 ms to 250 ms. The shorter mixing time NOESY may be acquired to ensure that no diffusion effects were present in the NOESY spectrum acquired at the longer mixing times. The interproton distances may generally be determined from the 250 ms NOESY. The sequence-specific
30 assignment of the proton resonances may be determined by standard methods (see Wuthrich,

NMR of Proteins and Nucleic Acids, Wiley & Sons, New York, 1986), making use of both the results of the TOCSY and NOESY data.

For conformational calculations, the NOE cross peaks may be initially converted to a uniform distance upper and lower bounds of 1.8-5.0 angstroms regardless of the NOE intensities. The NOE distances may be refined iteratively through a comparison of computed and experimental NOEs at the various mixing times. This refinement may be much in the spirit of the PEPFLEX-II procedure (Wang *et al.*, *Techniques in Protein Chemistry IV*, 1993, Evaluation of NMR Based Structure Determination for Flexible Peptides: Application to Desmopressin p. 569), although preferably initial NOE-based distances with very loose upper bounds (*e.g.*, 5 angstroms) are used to permit the generation of a more complete set of conformations in agreement with experimental data. Dihedral-angle constraints may be derived from the values of the $^3J_{C\alpha H}$ coupling constants. A tolerance value of 40 degrees may be added to each of the dihedral angle constraints to account for the conformational flexibility of the peptide. Distance geometry calculations may be carried out utilizing fixed bond lengths and bond angles provided in the ECEPP/2 database (Ni *et al.*, *Biochemistry* 1992, 31:11551-11557). The ω -angles are generally fixed at 180 degrees, but all other dihedral angles may be varied during structure optimization.

Structures with the lowest constraint violations may be subjected to energy minimization using a distance-restrained Monte Carlo method (Ripoll & Ni, *Biopolymers* 1992, 32:359-365; Ni, *J. Magn. Reson. B* 1995, 106:147-155), and modified to include the ECEPP/3 force field (Ni *et al.*, *J. Mol. Biol.* 1995, 252:656-671). All ionizable groups may be treated as charged during constrained Monte Carlo minimization of the ECEPP/3 energy. Electrostatic interactions among all charges may be screened by use of a distance-dependent dielectric to account for the absence of solvent effects in conformational energy calculations. In addition, hydrogen-bonding interactions can be reduced to 25% of the full scale, while van der Waals and electrostatic terms are kept to full strengths. These special treatments help to ensure that the conformational search is guided primarily by the experimental NMR constraints and that the computed conformations are less biased by the empirical conformational energy parameters (Warder *et al.*, *FEBS Lett.* 1997, 411:19-26).

Low-energy conformations of the peptide from Monte Carlo calculations may be used in NOE simulations to identify proximate protons with no observable NOEs and sets of

distance upper bounds that warrant recalibration. The refined set of NOE distances including distance lower bounds derived from absent NOEs are used in the next cycles of Monte Carlo calculations, until the resulting conformations produced simulate NOE spectra close to those observed experimentally (Ning *et al.*, *Biopolymers* 1994, 34:1125-1137; Ni *et al.*, *J. Mol. Biol.* 1995, 252:656-671). Theoretical NOE spectra may be calculated using a tumbling correlation time of 1.5 ns based on the molecular weight of the peptide and the experimental temperature (Cantor & Schimmel (1980) *Biophysical Chemistry*, W. H. Freeman & Co., San Francisco). All candidate peptide conformations are included with equal weights in an ensemble-averaged relaxation matrix analysis of interconverting conformations (Ni & Zhu, *J. Magn. Reson. B* 1994, 102:180-184). NOE simulations may also incorporate parameters to account for the local motions of the methyl groups and the effects of incomplete relaxation decay of the proton demagnetizations (Ning *et al.*, *Biopolymers* 1994, 34:1125-1137). The computed NOE intensities are converted to the two-dimensional FID's (Ni, *Magn. Reson. B* 1995, 106:147-155) using the chemical shift of assignments, estimated linewidths and coupling constants for all resolved proton resonances. Calculated FIDs may be converted to simulated NOESY spectra using identical processing procedures as used for the experimental NOE data sets.

6.3. TRK RECEPTOR MODULATORS: PEPTIDOMIMETICS

As noted above, peptidomimetics are compounds in which at least a portion of the RGE sequence within a cyclic peptide is modified, such that the three dimensional structure of the peptidomimetic remains substantially the same as that of the RGE sequence. Peptidomimetics may be peptide analogues that are, themselves, cyclic peptides containing one or more substitutions or other modifications within the RGE sequence. Alternatively, at least a portion of the RGE sequence may be replaced with a nonpeptide structure, such that the three-dimensional structure of the cyclic peptide is substantially retained. In other words, one, two or three amino acid residues within the RGE sequence may be replaced by a non-peptide structure. In addition, other peptide portions of the cyclic peptide may, but need not, be replaced with a non-peptide structure. Peptidomimetics (both peptide and non-peptidyl analogues) may have improved properties (*e.g.*, decreased proteolysis, increased retention or increased bioavailability). Peptidomimetics generally have improved oral availability, which

5 makes them especially suited to treatment of conditions such as cancer. It should be noted that peptidomimetics may or may not have similar two-dimensional chemical structures, but share common three-dimensional structural features and geometry. Each peptidomimetic may further have one or more unique additional binding elements. The present invention provides methods for identifying peptidomimetics. A variety of modifications of peptide modifications (including modifications to cyclic peptides as described *supra*) are known in the art and can be used to generate peptidomimetic compounds. See, for instance, International Patent Publication No. WO 01/53331. Such modifications can also be used in the present invention to generate peptidomimetic compounds, as well as the specific
10 modifications described below.

All peptidomimetics provided herein have a three-dimensional structure that is substantially similar to a three-dimensional structure of a cyclic peptide as described above. In general, two three-dimensional structures are said to be substantially structurally similar to each other if their pharmacophore atomic coordinates have a root-mean square deviation (RMSD) less than or equal to 1 angstrom, as calculated using the Molecular Similarity
15 module within the QUANTA program (QUANTA, available from Molecular Simulations Inc., San Diego, Calif.). All peptidomimetics provided herein have at least one low-energy three-dimensional structure that is substantially similar to at least one low-energy three-dimensional structure of a cyclic peptide as described above.

20 Low energy conformations may be identified by conformational energy calculations using, for example, the CHARMM program (Brooks *et al.*, *J. Comput. Chem.* 1983, 4:187-217). The energy terms include bonded and non-bonded terms, including bond length energy, angle energy, dihedral angle energy, Van der Waals energy and electrostatic energy. It will be apparent that the conformational energy can be also calculated using any of a
25 variety of other commercially available quantum mechanic or molecular mechanic programs. A low energy structure has a conformational energy that is within 50 kcal/mol of the global minimum.

The low energy conformation(s) of candidate peptidomimetics are compared to the low energy conformations of the cyclic peptide (as determined, for example, by NMR or X-
30 ray crystallography) to determine how closely the conformation of the candidate mimics that of the cyclic peptide. In such comparisons, particular attention should be given to the

locations and orientations of the elements corresponding to the crucial side chains. If at least one of the candidate low energy conformations is substantially similar to a solution conformation of a cyclic peptide (*i.e.*, differs with a root-mean square deviation (RMSD) of 1 angstrom or less), the candidate compound is considered a peptidomimetic. Within such
5 analyses, low energy conformations of candidate peptidomimetics in solution may be studied using, for example, the CHARMM molecular mechanics and molecular dynamics program (Brooks *et al.*, *J. Comput. Chem.* 1983, 4:187-217), with the TIP3P water model (Jorgensen *et al.*, *J. Chem Phys.* 1983, 79:926-935) used to represent water molecules. The CHARMM22 force field may be used to represent the designed peptidomimetics.

10 By way of example, low energy conformations may be identified using a combination of two procedures. The first procedure involves a simulated annealing molecular dynamics simulation approach. In this procedure, the system (which includes the designed peptidomimetics and water molecules) is heated up to above room temperature, preferably around 600 K, and simulated for a period of 100 picoseconds (ps) or longer; then gradually
15 reduced to 500 K and simulated for a period of 100 ps or longer; then gradually reduced to 400 K and simulated for a period of 100 ps or longer; gradually reduced to 300 K and simulated for a period of 500 ps or longer. The trajectories are recorded for analysis. This simulated annealing procedure is known for its ability for efficient conformational search.

The second procedure involves the use of the self-guided molecular dynamics
20 (SGMD) method (Wu & Wang, *J. Physical Chemistry* 1998, 102:7238-7250). The SGMD method has been demonstrated to have an extremely enhanced conformational searching capability. Using the SGMD method, simulation may be performed at 300 K for 1000 ps or longer and the trajectories recorded for analysis.

Conformational analysis may be carried out using the QUANTA molecular modeling
25 package. First, cluster analysis may be performed using the trajectories generated from molecular dynamic simulations. From each cluster, the lowest energy conformation may be selected as the representative conformation for this cluster and may be compared to other conformational clusters. Upon cluster analysis, major conformational clusters may be identified and compared to the solution conformations of the cyclic peptide(s). The
30 conformational comparison may be carried out using the Molecular Similarity module within the QUANTA program.

Similarity in structure may also be evaluated by visual comparison of the three-dimensional structures displayed in a graphical format, or by any of a variety of computational comparisons. For example, an atom equivalency may be defined in the peptidomimetic and cyclic peptide three-dimensional structures, and a fitting operation used to establish the level of similarity. As used herein, an "atom equivalency" is a set of conserved atoms in the two structures. A "fitting operation" may be any process by which a candidate compound structure is translated and rotated to obtain an optimum fit with the cyclic peptide structure. A fitting operation may be a rigid fitting operation (*e.g.*, the cyclic peptide three-dimensional structure can be kept rigid and the three-dimensional structure of the peptidomimetic can be translated and rotated to obtain an optimum fit with the cyclic peptide). Alternatively, the fitting operation may use a least squares fitting algorithm that computes the optimum translation and rotation to be applied to the moving compound structure, such that the root mean square difference of the fit over the specified pairs of equivalent atoms is a minimum. Preferably, atom equivalencies may be established by the user and the fitting operation is performed using any of a variety of available software applications (*e.g.*, QUANTA, available from Molecular Simulations Inc., San Diego, Calif.). Three-dimensional structures of candidate compounds for use in establishing substantial similarity may be determined experimentally (*e.g.*, using NMR techniques as described herein or x-ray crystallography), or may be computer-generated using, for example, methods provided herein.

Certain peptidomimetics may be designed, based on the cyclic peptide structure. For example, such peptidomimetics may mimic the local topography about the cleavable amide bonds (amide bond isosteres). Examples of backbone modifications are given in **Figures 3A** and **3B** (see also, Figures 4A-4B in WO 01/53331). These mimetics often match the peptide backbone atom-for-atom, while retaining functionality that makes important contacts with the binding sites. Amide bond mimetics may also include the incorporation of unusual amino acids or dipeptide surrogates. Examples of such unusual amino acids and dipeptide surrogates are illustrated here, in **Figure 4** (see also Figure 5 in WO 01/53331). Still other examples are well known in the art (see, for example, in Gillespie *et al.*, *Biopolymers* 1997, 43:191-217). The conformationally rigid substructural elements found in these types of mimetics are believed to result in binding with highly favorable entropic driving forces, as

compared to the more conformationally flexible peptide linkages. Backbone modifications can also impart metabolic stability towards peptidase cleavage relative to the parent peptide. Other peptidomimetics may be secondary structure mimics. Such peptidomimetics generally employ non-peptide structures to replace specific secondary structures, such as β -turns, β -sheets and α -turns (see **Figure 5**).

To design a peptidomimetic, heuristic rules that have been developed through experience may be used to systematically modify a cyclic peptide. Within such modification, empirical data of various kinds are generally collected throughout an iterative refinement process. As noted above, optimal efficiency in peptidomimetic design requires a three-dimensional structure of the pharmacophore.

Pharmacophores as provided herein permit structure-based peptidomimetic design through, for example, peptide scaffold modification as described above. Certain peptidomimetics may be identified through visual inspection of one or more pharmacophores, as compared to the neurotrophin RGE conformation. Peptidomimetics can also be designed based on a visual comparison of a cyclic peptide pharmacophore with a three-dimensional structure of a candidate compound, using knowledge of the structure-activity relationships of the cyclic peptide. Structure-activity studies have established important binding elements in the cyclic peptides, and have permitted the development of pharmacophore models. Peptidomimetics designed in this manner should retain these binding elements.

Peptidomimetics may also be designed around replacing the disulfide bond ($--S--S--$) with a thioether ($--S--CH_2--C(O)--$). The disulfide bond in general is not very stable as it can readily be reduced under acidic conditions. Replacing the disulfide bond with a thioether moiety ($--S--CH_2--C(O)--$) can significantly improve the stability of the peptide and therefore the oral availability.

As an alternative to design by visual inspection, libraries (*e.g.*, containing hydantoin and/or oxopiperazine compounds) may be made using combinatorial chemical techniques. Combinatorial chemical technology enables the parallel synthesis of organic compounds through the systematic addition of defined chemical components using highly reliable chemical reactions and robotic instrumentation. Large libraries of compounds result from the combination of all possible reactions that can be done at one site with all the possible

reactions that can be done at a second, third or greater number of sites. Combinatorial chemical methods can potentially generate tens to hundreds of millions of new chemical compounds as mixtures, attached to a solid support, or as individual compounds.

Pharmacophores can be used to facilitate the screening of such chemical libraries. For example, instead of producing all possible members of every library (resulting in an unwieldy number of compounds), library synthesis can focus on the library members with the greatest probability of interacting with the target. The integrated application of structure-based design and combinatorial chemical technologies can produce synergistic improvements in the efficiency of drug discovery.

Further peptidomimetics are compounds that appear to be unrelated to the original peptide, but contain functional groups positioned on a nonpeptide scaffold that serve as topographical mimics. This type of peptidomimetic is referred to herein as a "non-peptidyl analogue." Such peptidomimetics may be identified using library screens of large chemical databases. Such screens use the three-dimensional conformation of a pharmacophore to search such databases in three-dimensional space. A single three-dimensional structure may be used as a pharmacophore model in such a search. Alternatively, a pharmacophore model may be generated by considering the crucial chemical structural features present within multiple three-dimensional structures.

Any of a variety of databases of three-dimensional structures may be used for such searches. A database of three-dimensional structures may be prepared by generating three-dimensional structures of a database of compounds, and storing the three-dimensional structures in the form of data storage material encoded with machine-readable data. The three-dimensional structures can be displayed on a machine capable of displaying a graphical three-dimensional representation and programmed with instructions for using the data. Within preferred embodiments, three-dimensional structures are supplied as a set of coordinates that define the three-dimensional structure.

Preferably, the 3D-database contains at least 100,000 compounds, with small, non-peptidyl molecules having relatively simple chemical structures particularly preferred. It is also important that the 3D co-ordinates of the compounds in the database be accurately and correctly represented. The National Cancer Institute (NCI) 3D-database (Milne *et al.*, *J. Chem. Inf. Comput. Sci.* 1994, 34:1219-1224) and the Available Chemicals Directory (ACD;

available from MDL Information Systems, San Leandro, Calif.) are two excellent databases that can be used to generate a database of three-dimensional structures, using molecular modeling, as discussed above. For flexible molecules, which can have several low-energy conformations, it is desirable to store and search multiple conformations. The Chem-X
5 program (Oxford Molecular Group PLC; Oxford UK) is capable of searching thousands or even millions of conformations for a flexible compound. This capability of Chem-X provides a real advantage in dealing with compounds that can adopt multiple conformations. Using this approach, although the NCI-3D database presently contains a total of 465,000 compounds, hundreds of millions of conformations can be searched in a 3D-pharmacophore
10 searching process.

A pharmacophore search typically involves three steps. The first step is the generation of a pharmacophore query. Such queries may be developed from an evaluation of critical distances in the three dimensional structure of a cyclic peptide. Using the pharmacophore query of interest, a distance bit screening is performed on the database to
15 identify compounds that fulfill the required geometrical constraints. In other words, compounds that satisfy the specified critical pair-wise distances are identified. After a compound passed the distance bit screening step, the program next checks whether the compound meets the substructural requirements as specified in the pharmacophore query. After a compound passes this sub-structural check, it is finally subjected to a conformational
20 analysis. In this step, conformations are generated and evaluated with regard to geometric requirements specified in the pharmacophore query. Compounds that have at least one conformation satisfying the geometric requirements, are considered as 'hits' and are recorded in a result database.

Other criteria, which will be apparent to those of ordinary skill in the art, may also be
25 considered when selecting specific compounds for particular applications, such as the simplicity of the chemical structure, low molecular weight, chemical structure diversity and water solubility. The application of such criteria is well understood by medicinal, computational and structural chemists.

It will be apparent that a compound structure may be optimized using screens as
30 provided herein. Within such screens, the effect of specific alterations of a candidate compound on three-dimensional structure may be evaluated, in order to optimize three-

dimensional similarity to a cyclic peptide. Such alterations include, for example, changes in hydrophobicity, steric bulk, electrostatic properties, size and bond angle.

Biological testing of candidate compounds may be used to confirm peptidomimetic activity. In general, peptidomimetics should function in a substantially similar manner as a structurally similar cyclic peptide. In other words, a peptidomimetic of the cyclic peptide N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2) should bind to a TRK with an affinity that is at least half the affinity of the cyclic peptide N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2), as measured using standard binding assays. Further, a peptidomimetic of the cyclic peptide N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2) should modulate a TRK-mediated function using a representative assay provided herein at a level that is at least half the level of modulation achieved using N-Ac-CSRRGEC-NH₂ (SEQ ID NO:2).

Once an active peptidomimetic has been identified, related analogues may be identified using two-dimensional similarity searching. Such searching may be performed, for example, using the program ISIS Base (Molecular Design Limited). Two-dimensional similarity searching permits the identification of other available, closely related compounds, which may be readily screened to optimize biological activity.

6.4. TRK MODULATING AGENTS

As noted above, the term "Trk modulator" is used here to describe any molecule comprising at least one cyclic peptide or peptidomimetic compound of the invention containing the neurotrophin motif RGE (*i.e.*, Arg-Gly-Glu). Multiple cyclic peptides and/or peptidomimetics can be present in a modulating agent of the invention. Moreover, additional RGE sequences (for example, tandem repeats of RGE sequences) may be included in a modulating agent.

Linkers may or may not be used to separate RGE sequences in a Trk modulator, including tandem repeats of RGE sequences (such as in preferred Trk agonists of the invention). Linkers can also be used to attach a modulating agent of the invention to a solid support or material, as described below.

A linker may be any molecule (including peptide and/or non-peptide sequences as well as single amino acids or other molecules), that does not contain a RGE sequence and that can be covalently linked to at least two peptide sequences and/or peptidomimetics.

Using a linker, peptidomimetics and other peptide or protein sequences may be joined in a variety of orientations.

Linkers preferably produce a distance between CAR sequences and/or peptidomimetics between 0.1 to 10,000 nm, more preferably about 0.1-400 nm. A separation
5 distance between recognition sites may generally be determined according to the desired function of the modulating agent. For Trk antagonists, the linker distance should be small (0.1-400 nm). For Trk agonists, the linker distance should be 400-10,000 nm. One linker that can be used for such purposes is $(\text{H}_2\text{N}(\text{CH}_2)_n\text{CO}_2\text{H})_m$, or derivatives thereof, where n ranges from 1 to about 10 and m ranges from 1 to about 4000. For example, if glycine
10 $(\text{H}_2\text{NCH}_2\text{CO}_2\text{H})$ or a multimer thereof is used as a linker, each glycine unit corresponds to a linking distance of about 2.45 angstroms, or 0.245 nm, as determined by calculation of its lowest energy conformation when linked to other amino acids using molecular modeling techniques. Similarly, aminopropanoic acid corresponds to a linking distance of about 3.73 angstroms, aminobutanoic acid to about 4.96 angstroms, aminopentanoic acid to about 6.30
15 angstroms and amino hexanoic acid to about 6.12 angstroms. Other linkers that may be used will be apparent to those of ordinary skill in the art and include, for example, linkers based on repeat units of 2,3-diaminopropanoic acid, lysine and/or ornithine. 2,3-Diaminopropanoic acid can provide a linking distance of either 2.51 or 3.11 angstroms depending on whether the side-chain amino or terminal amino is used in the linkage. Similarly, lysine can provide
20 linking distances of either 2.44 or 6.95 angstroms and ornithine 2.44 or 5.61 angstroms. Peptide and non-peptide linkers may generally be incorporated into a modulating agent using any appropriate method known in the art.

Modulating agents that are Trk antagonists may contain one or more peptidomimetics. Preferably such peptidomimetics are adjacent to one another (*i.e.*, without
25 intervening sequences) or are in close proximity (*i.e.*, separated by peptide and/or non-peptide linkers to give a distance between the peptidomimetics that ranges from about 0.1 to 400 nm). It will be apparent that other neurotrophin sequences, as discussed above, may also be included.

As noted above, a modulating agent may consist entirely of one or more
30 peptidomimetics, or may contain additional peptide and/or non-peptide components. Peptide portions may be synthesized as described above or may be prepared using recombinant

methods. Within such methods, all or part of a modulating agent can be synthesized in living cells, using any of a variety of expression vectors known to those of ordinary skill in the art to be appropriate for the particular host cell. Suitable host cells may include bacteria, yeast cells, mammalian cells, insect cells, plant cells, algae and other animal cells (*e.g.*, hybridoma, CHO, myeloma). The DNA sequences expressed in this manner may encode portions of an endogenous neurotrophin. Such sequences may be prepared based on known Cdna or genomic sequences, or from sequences isolated by screening an appropriate library with probes designed based on the sequences of known cadherins. Such screens may generally be performed as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using oligonucleotide primers in methods well known in the art, to isolate nucleic acid molecules encoding all or a portion of an endogenous neurotrophin. To generate a nucleic acid molecule encoding a peptide portion of a modulating agent, an endogenous sequence may be modified using well known techniques. Alternatively, portions of the desired nucleic acid sequences may be synthesized using well known techniques, and then ligated together to form a sequence encoding a portion of the modulating agent.

Trk modulating agents of the present invention may additionally comprise an antibody, or antigen-binding fragment thereof, that specifically binds to a NT sequence or, alternatively an antibody or antigen-binding fragment thereof that specifically binds to a Trk receptor sequence. As used herein, an antibody, or antigen-binding fragment thereof, is said to “specifically bind” to a NT or Trk sequence (with or without flanking amino acids) if it reacts at a detectable level (within, for example, an ELISA, as described by Newton *et al.*, *Develop. Dynamics* 1993, 197:1-13) with a peptide containing that sequence, and does not react detectably with peptides containing a different NT or Trk sequence, nor with a sequence in which the order of amino acid residues in the NT (or Trk) and/or flanking sequence is altered.

Antibodies and fragments thereof may be prepared using standard techniques. See, *e.g.*, Harlow & Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising a NT or Trk sequence is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats).

Small immunogens (*i.e.*, less than about 20 amino acids) are preferably joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. Following one or more injections, the animals are bled periodically. Polyclonal antibodies specific for the NT or Trk sequence may then be purified from such antisera by, for example, affinity chromatography using the modulating agent or antigenic portion thereof coupled to a suitable solid support.

Monoclonal antibodies specific for a NT (or Trk) sequence may be prepared, for example, using the technique of Kohler & Milstein, (*Eur. J. Immunol.* 1976, 6:511-519) and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single colonies are selected and their culture supernatants tested for binding activity against the modulating agent or antigenic portion thereof. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to enhance the yield. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified using immunofluorescence analyses of tissue sections, cell or other samples where the target cadherin is localized.

Within certain embodiments, monoclonal antibodies may be specific for particular NTs or, alternatively, for particular Trk receptors. For example, the antibody may bind to NGF, but do not bind to BDNF, or *vice versa*. As another example, a monoclonal antibody may bind specifically to TrkB and not bind specifically to TrkA, or *vice versa*. Such antibodies may be prepared as described above, using (to generate antibodies for a particular NT) an immunogen that comprises the RGE sequence and also sufficient flanking sequence to generate the desired specificity (*e.g.*, 5 amino acids on each side is generally sufficient). To evaluate the specificity of a particular antibody, representative assays as described herein and/or conventional antigen-binding assays may be employed. Such antibodies may generally be used for therapeutic, diagnostic and assay purposes, as described herein. For

example, such antibodies may be linked to a drug and administered to a mammal to target the drug to a particular Trk -expressing cell, such as a particular neuronal cell.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow & Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; see especially page 309) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns (Harlow & Lane, 1988, pages 628-29).

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6.5. EVALUATION OF TRK MODULATOR ACTIVITY

As noted above, peptidomimetics, cyclic peptides and other Trk modulators of the invention are capable of modulating (*i.e.*, enhancing or inhibiting) Trk mediated activities including, for example, neuronal survival, axonal growth and synaptic plasticity. Hence, the ability of a modulating agent (or a suspected modulating agent) to modulate Trk mediated activity can generally be evaluated either *in vitro* or *in vivo* by assaying one or more of these effects. Generally speaking, a test compound is a Trk antagonist if, within such a representative assay, contact of test cells with the candidate results in a discernible disruption of the Trk mediated activity being measured. A candidate compound is generally considered a Trk agonist if, within such a representative assay, contact of test cells with the candidate compound results in a discernible increase of the Trk mediated activity measured.

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In particular, preferred embodiments of the invention, the activity of a Trk modulator or candidate compound is evaluated *in vivo* in a neurite outgrowth assay. Within a representative neurite outgrowth assay, which is demonstrated in the Examples, *infra*, neurons may be cultured on a monolayer of cells (preferably 3T3 cells or cell lines derived therefrom). As an example, monolayers of 3T3 fibroblasts can be established by overnight culture of cells (preferably about 80,000) in individual wells of an 8-chamber well tissue culture slide. Approximately 3,000 cerebellar neurons isolated from post natal day 3 (PND3) mouse brains may be cultured for 18 hours on the various monolayers in control media (SATO/2% FCS) or in media supplemented with various concentrations of the candidate modulating agent. Alternatively, the cells may be cultured in media supplemented with a

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control peptide (for example, a non-cyclic, linear peptide having the same amino acid sequence as a Trk modulator cyclic peptide) or with a neurotrophin (*e.g.*, NGF, BDNF, NT-3, NT-4, NT-5 or NT-4/5).

5 The cell cultures may then be fixed and stained for GAP43 or with some other agent that specifically binds neurons and their neurites. The length of the longest neurite on each GAP43 positive neuron may then be measured, preferably using computer assisted morphometry. A compound that is a Trk modulator will generally modulate (*e.g.*, inhibit or enhance) neurite outgrowth in such a cell culture assay.

10 6.6. USES OF TRK RECEPTOR MODULATORS

In general, modulating agents and compositions of the present invention can be used for modulating (*e.g.*, inhibiting or enhancing) activities that are mediated by a Trk receptor – including activities mediated by TrkA, TrkB and/or TrkC. Trk receptors are implicated in the growth and repair of the central nervous system (CNS) and mediate, at least in part, such
15 processes as neuronal survival, axonal growth, neurite outgrowth, synaptic plasticity and, more generally, neurological growth. Hence, modulating agents and compositions of the invention can be used to modulate any of these processes. Such uses include, *inter alia*, therapeutic methods and pharmaceutical compositions for treating conditions, diseases and disorders that are associated with such processes. Exemplary conditions, diseases and
20 disorders include Alzheimer's disease, Parkinson's disease, stroke, and head and spinal cord injury to name a few.

In one embodiment of the invention, Trk agonist of the invention can be used to increase or enhance activities that are mediated by a Trk receptor. Hence, Trk agonist of the invention may be used, *e.g.*, to increase or enhance the growth and/or repair of the CNS, for
25 example by increasing or enhancing such processes a neuronal growth, neuronal survival, axonal growth, neurite outgrowth and synaptic plasticity. Trk agonists of the invention are therefore useful, *e.g.*, in therapeutic methods for treating diseases and disorders that involve or are otherwise associated with damage to or impaired function of the central nervous system. These include, *inter alia*, the disease and disorders listed above.

30 In other embodiments, Trk antagonists of the invention can be used to decrease or inhibit activity mediated by a Trk receptor. Thus, Trk antagonists may inhibit processes such

as neuronal growth, neuronal survival, axonal growth, neurite outgrowth and synaptic plasticity. Trk antagonists are also useful in therapeutic methods, for example to treat or ameliorate diseases and disorders (for example, epilepsy) that are associated either with increased Trk receptor activity, or with increased activity of a neurotrophin (for example, 5 BDNF) that binds to and activates a Trk receptor.

In still other embodiments, Trk agonists and antagonists of the invention can also be used to modulate responses that inhibit CNS growth and repair (*i.e.*, “CNS inhibitors”), including responses that inhibit processes such as neuronal growth, neuronal survival, axonal growth, neurite outgrowth and synaptic plasticity. In particularly preferred embodiments, 10 Trk agonists of the invention (for example a B_{AG} or other agonist polypeptide or peptide mimetic) can be used to block or reduce a CNS inhibitor response. In other embodiments of the invention, Trk agonists (for example, a B_{AG} or other agonist polypeptide or peptide mimetic) can be used to enhance and/or promote neuronal growth and recovery, even administered in an inhibitory environment such as in the presence of one or more CNS 15 inhibitors.

As a particular example, it is understood that inhibitory factors, such as those associated with myelin, exists which can inhibit or even prevent processes of CNS growth and repair, including those recited above. Examples of such inhibitors include, but are not limited to, the myelin associated glycoprotein (also referred to as “MAG”), Nogo-A and the 20 oligodendrocyte myelin glycoprotein. For a more complete description of such inhibitors, see also Section 3.3 above. Trk agonists and antagonists of the invention can be used to modulate responses that are produced by these and other CNS inhibitors.

Without being limited to any particular theory or mechanism of action, it is understood that Trk receptors modulate CNS growth and repair at least in part by a 25 mechanism or mechanisms that involve protein kinase A (PKA) and phosphoinositide 3-kinase (PI3K). Accordingly, Trk agonists and antagonists of the invention can, in preferred embodiments, modulate effects of inhibitory signals that are mediated by one or more components which are themselves modulated by either PKA or PI3K. As an example, and not by way of limitation, PKA is understood to activate Rho by direct phosphorylation on 30 Ser188 of that molecule (Ellerbroek *et al.*, *J. Biol. Chem.* 2003, 278:19023-19031). Hence, Trk agonists and antagonists of the present invention can be used to modulate signals

mediated by inhibitory cascades involving Rho. These include, *inter alia*, inhibitory signals mediated by myelin inhibitors such as MAG (and MAG fusion constructs such as MAG-Fc), Nogo-A, the oligodendrocyte myelin glycoprotein, NgR, GT1b and p75^{NTR}. Other CNS inhibitors involving Rho include signals mediated by chondroitin sulfate proteoglycans from CNS glial scar (Monnier *et al.*, *Neurosci.* 2003, 22:319-330) and, as such, these CNS inhibitors can also be modulated by Trk agonists and antagonists of the invention. As another non-limiting example, activation of PI3K is expected to overcome inhibitory activity of semaphorins (Eickholt *et al.*, *J. Cell Biol.* 2002, 157:211-217). Hence, Trk agonists and antagonists of the present invention can additionally be used to modulate these CNS inhibitors.

In general, methods of the invention involve contacting a cell expressing a Trk receptor (typically a neuronal cell) with a Trk modulating agent either *in vivo* or *in vitro*. The amount of Trk modulating agent administered should be an “effective amount” – that is to say, it should be an amount that effectively modulates a Trk mediated activity of interest or, alternatively, an amount that effectively modulates a CNS inhibitor of interest.. In embodiments where the Trk modulator is administered as part of a therapeutic method, amount administered should be an amount that effectively ameliorates (but does not necessarily eliminate or cure) the condition, disease or disorder being treated. Alternatively, the amount administered may be an amount effective to ameliorate (but not necessarily eliminate) one or more symptoms associated with the condition, disease or disorder being treated.

As a particular, non-limiting example, a Trk modulating agent of the invention can be used to modulate (*e.g.*, inhibit or enhance) neurological growth, such as neurite outgrowth. In such methods, neurite outgrowth may be enhanced and/or directed by contacting a neuron with one or more Trk agonists of the invention (*e.g.*, the cyclic peptide N-Ac-CSRRGELLAASRRGELC-NH₂). Alternatively, neurite outgrowth may be inhibited and/or decreased by contacting a neuron with one or more Trk antagonists of the invention (*e.g.*, the cyclic peptide N-Ac-CSRRGEC-NH₂). Preferred modulating agents for use within such methods are preferably linked to a polymeric matrix or other support, and comprise a cyclic peptide as described in Section 6.1, *supra*, or a peptidomimetic thereof (as described in

Section 6.3). Modulating agents comprising antibodies, or fragments thereof, may also be used in such methods, with or without the use of linkers or support materials.

The method of achieving contact to the neuronal cell and the amount of Trk modulating agent administered will depend upon the location of the neuron as well as the
5 extent and nature of desired outgrowth (or, where Trk antagonists are administered, the extent and nature of desired inhibition). For example, a neuron may be contacted (*e.g.*, via implantation) with one or more Trk modulating agents linked to a support material such as a suture, fiber nerve guide or other prosthetic device so that the neurite outgrowth is directed along the support material. Alternatively, a tubular nerve guide may be employed in which
10 the lumen of the nerve guide contains a composition comprising the modulating agent or agents. *In vivo*, such nerve guides or other supported modulating agents may be implanted using well known techniques to, for example, facilitate the growth of severed neuronal connections and/or to treat spinal cord injuries. It will be apparent to those of ordinary skill in the art that the structure and composition of the support should be appropriate for the
15 particular injury being treated. *In vitro*, a polymeric matrix may similarly be used to direct the growth of neurons onto patterned surfaces as described, for example, in U.S. Patent No. 5,510,628.

6.7. TRK RECEPTOR MODULATORS: FORMULATIONS

20 In certain embodiments, a modulating agent as described herein may, but need not, be linked to one or more additional molecules. For example, it may be beneficial for certain applications to link multiple modulating agents (which may, but need not, be identical) to a support molecule (*e.g.*, keyhole limpet hemocyanin) or a solid support, such as a polymeric matrix (which may be formulated as a membrane or microstructure, such as an ultra thin
25 film), a container surface (*e.g.*, the surface of a tissue culture plate or the interior surface of a bioreactor), or a bead or other particle, which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred, such as cellulose and derivatives thereof, collagen, spider silk or any of a variety of polyesters (*e.g.*, those derived from hydroxy acids and/or lactones) or sutures
30 (see U.S. Pat. No. 5,245,012).

Suitable methods for linking a modulating agent to a support material will depend upon the composition of the support and the intended use, and will be readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may
5 be a direct linkage between a modulating agent and functional groups on the support, or may be a linkage by way of a cross-linking agent or linker). Attachment of a modulating agent by adsorption may be achieved by contact, in a suitable buffer, with a solid support for a suitable amount of time. The contact time varies with temperature, but is generally between about 5 seconds and 1 day, and typically between about 10 seconds and 1 hour.

10 Covalent attachment of a modulating agent to a molecule or solid support may generally be achieved by first reacting the support material with a bifunctional reagent that will also react with a functional group, such as a hydroxyl, thiol, carboxyl, ketone or amino group, on the modulating agent. For example, a modulating agent may be bound to an appropriate polymeric support or coating using benzoquinone, by condensation of an
15 aldehyde group on the support with an amine and an active hydrogen on the modulating agent or by condensation of an amino group on the support with a carboxylic acid on the modulating agent. A preferred method of generating a linkage is via amino groups using glutaraldehyde. A modulating agent may be linked to cellulose via ester linkages. Similarly, amide linkages may be suitable for linkage to other molecules such as keyhole limpet
20 hemocyanin or other support materials. Multiple modulating agents and/or molecules comprising other NT and/or Trk receptor sequences may be attached, for example, by random coupling, in which equimolar amounts of such molecules are mixed with a matrix support and allowed to couple at random.

Although modulating agents as described herein may preferentially bind to specific
25 tissues or cells (*i.e.*, neuronal cells and tissues), and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent may also, or alternatively, be linked to a modulating agent to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a modulating
30 agent enhances the transport of the modulating agent to a target tissue, thereby increasing the local concentration of the modulating agent. Targeting agents include antibodies or

fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that

5 bind to a desired target site. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')₂, Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers.

10 Within other embodiments, it may also be possible to target a polynucleotide encoding a modulating agent to a target tissue, thereby increasing the local concentration of modulating agent. Such targeting may be achieved using well known techniques, including retroviral and adenoviral infection.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a

15 modulating agent. As used herein, the term "drug" refers to any bioactive agent intended for administration to a mammal to prevent or treat a disease or other undesirable condition. Drugs include hormones, growth factors, proteins, peptides and other compounds. The use of certain specific drugs within the context of the present invention is discussed below.

Within certain aspects of the present invention, one or more modulating agents as

20 described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more modulating agents in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins,

25 polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. A modulating agent (alone or in combination with a targeting agent and/or drug) may, but need not, be encapsulated within liposomes using well known technology. Compositions of the

30 present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or

intramuscular administration. For certain topical applications, formulation as a cream or lotion, using well known components, is preferred.

Optionally, a pharmaceutical composition may also contain one or more drugs, which may be linked to a modulating agent or may be free within the composition. Virtually any
5 drug may be administered in combination with a modulating agent as described herein, for a variety of purposes as described below. Examples of types of drugs that may be administered with a modulating agent include but are not limited to analgesics, anesthetics, antianginals, antifungals, antibiotics, anticancer drugs (e.g., taxol or mitomycin C), antiinflammatories (e.g., ibuprofen and indomethacin), anthelmintics, antidepressants,
10 antidotes, antiemetics, antihistamines, antihypertensives, antimalarials, antimicrotubule agents (e.g., colchicine or vinca alkaloids), antimigraine agents, antimicrobials, antipsychotics, antipyretics, antiseptics, anti-signaling agents (e.g., protein kinase C inhibitors or inhibitors of intracellular calcium mobilization), antiarthritics, antithrombin agents, antituberculotics, antitussives, antivirals, appetite suppressants, cardioactive drugs,
15 chemical dependency drugs, cathartics, chemotherapeutic agents, coronary, cerebral or peripheral vasodilators, contraceptive agents, depressants, diuretics, expectorants, growth factors, hormonal agents, hypnotics, immunosuppression agents, narcotic antagonists, parasympathomimetics, sedatives, stimulants, sympathomimetics, toxins (e.g., cholera toxin), tranquilizers and urinary antiinfectives.

20 For imaging purposes, any of a variety of diagnostic agents may be incorporated into a pharmaceutical composition, either linked to a modulating agent or free within the composition. Diagnostic agents include any substance administered to illuminate a physiological function within a patient, while leaving other physiological functions generally unaffected. Diagnostic agents include metals, radioactive isotopes and radioopaque agents
25 (e.g., gallium, technetium, indium, strontium, iodine, barium, bromine and phosphorus-containing compounds), radiolucent agents, contrast agents, dyes (e.g., fluorescent dyes and chromophores) and enzymes that catalyze a calorimetric or fluorometric reaction. In general, such agents may be attached using a variety of techniques as described above, and may be present in any orientation.

30 The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of

modulating agent following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane (see, *e.g.*, European Patent Application 710,491A). Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of modulating agent release. The amount of modulating agent contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). Appropriate dosages and the duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the modulating agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Within particularly preferred embodiments of the invention, a modulating agent or pharmaceutical composition as described herein may be administered at a dosage ranging from 0.001 to 50 mg/kg body weight, preferably from 0.1 to 20 mg/kg, on a regimen of single or multiple daily doses. For topical administration, a cream typically comprises an amount of modulating agent ranging from 0.00001% to 1%, preferably 0.0001% to 0.2%, and more preferably from 0.0001% to 0.002%. Fluid compositions typically contain about 10 ng/ml to 5 mg/ml, preferably from about 10 .mu.g to 2 mg/ml peptidomimetic. Appropriate dosages may generally be determined using experimental models and/or clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

Trk receptor modulators can also be formulated according to the description provided in section 6.9, *infra*.

6.8. p75 BINDING AGENTS

The term "p75 receptor binding agent" is used herein to describe a naturally-occurring or synthetic (e.g., recombinant) molecule, which binds to a p75 receptor engaged in an inhibitory complex, and interferes with p75 receptor:neurotrophin interaction but not neurotrophin:Trk receptor interaction. Thus, a p75 receptor binding agent facilitates
5 neurotrophin-mediated neuron growth in an inhibitory environment. A p75 receptor is engaged in an inhibitory complex when it interacts with a nogo receptor and any of the myelin-associated proteins (e.g., MAG, Nogo-A, oligodendocyte myelin glycoprotein). Examples of p75 receptor binding agents include, but are not limited to, neurotrophins, such as NGF, and agents derived from neurotrophins, such as the NGF binding loop-derived N-
10 Ac-CTDIKGKEC-NH₂ (SEQ ID NO:43). A neurotrophin is a p75 receptor binding agent according to the invention if it interferes with binding of another, different neurotrophin to the p75 receptor and does not interact with the Trk receptor expressed on the injured neuron. For example, in the case of neurons that express TrkB but not TrkA, the neurotrophin NGF is a p75 receptor binding agent because NGF will compete (i.e., interfere) with a neurotrophin
15 that binds TrkB (e.g., BDNF) for p75 receptor binding but will not interfere with neurotrophin binding (e.g., BDNF) to the TrkB receptor.

In a preferred embodiment, a p75 receptor binding agent comprises at least one cyclic peptide or peptidomimetic compound containing the NGF motif TDIKGKE (*i.e.*, Thr-Asp-Ile-Lys-Gly-Lys-Glu) (SEQ ID NO:42) within a cyclic ring of the cyclic peptide or
20 peptidomimetic compound. An especially preferred p75 receptor binding agent is N-Ac-CTDIKGKEC-NH₂ (SEQ ID NO:43). As noted previously, underlined peptide sequences denote a peptide that has been cyclized by a covalent bond between the two last underlined residues. In these examples, the p75 binding agents were cyclized by a disulfide bond between two cysteine residues, acetylated and amide blocked. It is understood that preferred
25 peptides which bind to a p75 receptor will be constrained and, hence, are preferably cyclic peptides. Methods for cyclization of peptides are described in section 6.1, *supra*.

Multiple cyclic peptides and/or peptidomimetics can be present in a p75 receptor binding agent. Moreover, additional TDIKGKE sequences (for example, tandem repeats of TDIKGKE sequences) may be included in a p75 receptor binding agent.

30 Linkers may or may not be used to separate p75 receptor binding sequences in a p75 receptor binding agent, including tandem repeats of p75 receptor binding sequences. A

linker may be any molecule (including peptide and/or non-peptide sequences as well as single amino acids or other molecules), that can be covalently linked to at least two peptide sequences and/or peptidomimetics, and does not contain a p75 receptor binding sequence.

Using a linker, peptidomimetics and other peptide or protein sequences may be joined in a
5 variety of orientations.

p75 receptor binding agents may contain one or more peptidomimetics. Preferably such peptidomimetics are adjacent to one another (*i.e.*, without intervening sequences) or are in close proximity (*i.e.*, separated by peptide and/or non-peptide linkers to give a distance between the peptidomimetics that ranges from about 0.1 to 400 nm). A p75 receptor binding
10 agent may consist entirely of one or more peptidomimetics, or may contain additional peptide and/or non-peptide components. Methods for making a peptidomimetic are described in sections 6.2 and 6.3, *supra*.

All or part of a p75 receptor binding agent can be synthesized in living cells, using any of a variety of expression vectors known to those of ordinary skill in the art to be
15 appropriate for the particular host cell. Suitable host cells may include bacteria, yeast cells, mammalian cells, insect cells, plant cells, algae and other animal cells (*e.g.*, hybridoma, CHO, myeloma). The DNA sequences expressed in this manner may encode portions of an endogenous neurotrophin. Such sequences may be prepared based on known cDNA or genomic sequences, or from sequences isolated by screening an appropriate library. Such
20 screens may generally be performed as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using oligonucleotide primers in methods well known in the art, to isolate nucleic acid molecules encoding all or a portion of an endogenous neurotrophin. To generate a nucleic acid
25 molecule encoding a peptide portion of a modulating agent, an endogenous sequence may be modified using well known techniques. Alternatively, portions of the desired nucleic acid sequences may be synthesized using well known techniques, and then ligated together to form a sequence encoding a portion of the p75 receptor binding agent.

30 6.9. p75 BINDING AGENTS: METHODS FOR PROMOTING CNS GROWTH

The present invention provides methods for promoting CNS growth, which comprise administering a p75 receptor binding agent. Trk receptors are implicated in the growth and repair of the CNS and mediate such processes as neuronal survival, axonal growth, neurite outgrowth, synaptic plasticity, and more generally, neurological growth. p75 receptors bind neurotrophins with low affinity and this binding compromises the ability of neurotrophins to activate Trk receptors in the situation where the p75 receptor is engaged in an inhibitory complex. Hence, methods which interfere with the binding of neurotrophins to p75 receptor allows neurotrophins to bind to and activate Trk receptors, and thus promote CNS neuron growth in an inhibitory environment.

In an aspect of the present invention, a method is provided which comprises administering to an individual a therapeutically effective amount of a p75 receptor binding agent in combination with at least one neurotrophin. A preferred neurotrophin is NGF, BDNF, NT-3, NT-4 or NT-5. In one embodiment, the p75 receptor binding agent is administered in an amount about 10 to about 100 fold greater than that of the neurotrophin. In another embodiment, the p75 receptor binding agent is NGF and the neurotrophin is BDNF. The methods of the present invention can be used to treat conditions, diseases and disorders that are associated with damage to or impaired function of the CNS. Exemplary conditions include, but are not limited to, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophin lateral sclerosis, stroke, traumatic brain injury, and spinal cord injury.

According to the methods of the present invention, a neurotrophin is a p75 receptor binding agent when the neurotrophin interferes with the binding of another, different neurotrophin to a p75 receptor engaged in an inhibitory complex, but does not interfere with the binding of the another, different neurotrophin to a Trk receptor expressed on an injured CNS neuron.. For example, NGF is a p75 receptor binding agent according to the present invention if it is co-administered with BDNF to an individual with neurons that express the TrkB receptor because NGF competes with BDNF for binding to the p75 receptor but does not compete with BDNF for binding to the TrkB receptor.

A p75 receptor agent as described herein can be present within a pharmaceutical composition. A pharmaceutical composition comprises a p75 receptor binding agent in combination with one or more pharmaceutically or physiologically acceptable carriers,

diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or
5 preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. A p75 receptor binding agent (alone or in combination with a targeting agent and/or drug) can be encapsulated within liposomes using well known technology.

10 Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. Pharmaceutical compositions comprising a p75 receptor binding agent can be administered by any means that allows the p75 receptor binding agent to reach and bind with p75 receptors in the body of an individual.

15 Sterile injectable forms of pharmaceutical compositions comprising a p75 receptor binding agent can be aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. A sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be employed are sterile water, lactated Ringer's
20 solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives, are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their
25 polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant. A p75 receptor binding agent can also be formulated according to the description provided in section 6.7, *supra*.

30 p75 receptor binding agents can be administered topically. For example, a p75 receptor binding agent may be applied topically to the exposed spinal cord of an individual following spinal cord injury or during surgery. For topical application, a pharmaceutical composition can be formulated in a suitable ointment containing the p75 receptor binding

agent suspended or dissolved in one or more carriers. Carriers for topical administration of p75 receptor binding agents include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, emulsifying wax, water, or absorbable materials, such as, for example, Type I collagen gel or gelatin hemostasis sponge (Gelfoam®, Pharmacia & Upjohn, Kalamazoo, MI).

Appropriate dosages and the duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the p75 binding agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Various considerations for determining appropriate dosages are described, *e.g.*, in Gilman *et al.* (eds), *The Pharmacological Bases of Therapeutics*, 8th Ed. (1990), Pergamon Press. Appropriate dosages may generally be determined using experimental models and/or clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients can be monitored for therapeutic effectiveness using physical examination, imaging studies, or assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art. Dose adjustments can be made based on the monitoring findings. For example, an individual with a spinal cord injury associated with loss of sensation in an arm can be monitored, following administration of a p75 receptor binding agent according to the invention, for return of sensation to the arm by physical examination.

Compositions comprising a p75 receptor binding agent may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of p75 receptor binding agent following administration). Such formulations may generally be prepared using well known technology and administered by, for example, subcutaneous implantation or implantation at the desired target site. Sustained-release formulations may contain a p75 receptor binding agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate-controlling membrane (*see, e.g.*, European Patent Application 710,491A). Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of binding agent release. The amount of binding agent contained within a sustained

release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated.

Although a p75 receptor binding agent as described herein may preferentially bind to specific tissues or cells (*i.e.*, neuronal cells and tissues), and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent can be linked to a p75 receptor binding agent to facilitate targeting to one or more specific tissues. As used herein, a p75 receptor “targeting agent” may be any substance (such as a compound or cell) that, when linked to a p75 receptor binding agent, enhances the transport of the p75 receptor binding agent to a target tissue (*i.e.*, a damaged neuron), thereby increasing the local concentration of the p75 receptor binding agent.

Targeting agents can include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. For example, a MAG, Nogo-A or myelin glycoprotein antibody can be a targeting agent. Examples of antibody fragments are F(ab')₂, Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Within other embodiments, it may also be possible to target a polynucleotide encoding a binding agent to a target tissue, thereby increasing the local concentration of binding agent. Such targeting may be achieved using well known techniques, including retroviral and adenoviral infection.

For certain embodiments, it may be beneficial to link a drug to a p75 receptor binding agent. For a description of drugs suitable for linking to a p75 receptor binding agent, see section 6.7, *supra*.

7. EXAMPLES

The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any
5 exemplified term. Likewise, the invention is not limited to any particular preferred
embodiments described here. Indeed, many modifications and variations of the invention
may be apparent to those skilled in the art upon reading this specification, and such variations
can be made without departing the invention in spirit or in scope. The invention is therefore
to be limited only by the terms of the appended claims along with the full scope of
10 equivalents to which those claims are entitled.

7.1. EXPERIMENTAL PROCEDURES

7.1.1 Neurite Outgrowth Assays

Co-cultures of cerebellar neurons on monolayers of either parental 3T3 cells or LK8
15 cells (an established transfected 3T3 cell line that expresses physiological levels of chick N-
cadherin; see Doherty *et al.*, *Neuron* 1991, 6:247-258) were established as previously
described by Williams *et al.* (*Neuron* 1994, 13:583-594). The cells were maintained in
Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (FCS).

For establishment of the co-cultures, about 80,000 3T3 cells (or LK8 cells) were
20 plated into individual chambers of an eight-chamber tissue culture slide coated with poly-L-
lysine and fibronectin. The plated cells were maintained overnight in Dulbecco's modified
Eagle's medium ("DMEM") supplemented with 10% FCS to allow for the formation of
confluent monolayers. The medium was removed and about 6,000 dissociated cerebellar
neurons (taken from post-natal day 9 rats) were plated into each well in SATO medium
25 supplemented with 2% FCS. Test reagents were added as indicated in the text and the co-
cultures maintained for 18 hours. The co-cultures were then fixed and stained for GAP-43
immunoreactivity. The mean length of the longest neurite per cell was measured for between
about 120 and 150 neurons, again as previously described by Williams *et al.* (*Neuron* 1994,
13:583-594).

30

7.1.2 Molecular Modeling of Trk Receptor-Ligand Structures

X-ray crystallography structures of the NGF/TrkA and NT-4/TrkB complexes were used for molecular modeling. These structures have been previously described (See, respectively, Wiesmann *et al. Nature* 1999, 401:184-188; and Banfield *et al. Structure (Camb)* 2001, 9:1191-1199) and can be readily accessed, *e.g.*, over the internet from the Protein Data Bank (PDB) under the accession numbers 1www (for NGF/TrkA) and 1hcf (for NT-4/TrkB). Swiss PDB software packages were used to isolate the structures of various motifs from the binding interfaces of the crystals, and Accelrys software was used to generate images.

Contact profiles were generated from various ligand/receptor interfaces at the NT-4/TrkB crystal structure by measuring the average number of receptor contacts per ligand residue against the ligand residue sequence number. The average was taken over a three residue window and the contact number is the number of receptor residues that are within five Angstroms of the given ligand amino acid.

7.1.3 Reagents

Recombinant human FGF2, BDNF and NT-4/5 were all obtained from R&D systems (Minneapolis, MN). The CB1 cannabinoid receptor agonist WIN55,2122-2 mesylate was obtained and used as previously described (see, Williams *et al., J. Cell. Biol.* 2003, 160:481-486). The Trk receptor antagonist K252a was obtained from Calbiochem (San Diego, CA). The PD173074 FGFR antagonist (Mohammadi *et al., Embo J.* 1998, 17:5986-5904) was synthesized and used as previously described by Skaper *et al. (J. Neurochem.* 2000, 75:1520-1527) and by Hamby *et al., J. Med. Chem.* 1997, 40:2296-2303).

Synthetic peptides were all obtained from a commercial supplier (Multiple Peptide Systems, San Diego, CA). All peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) according to routine methods, and obtained at the highest level of purity (*i.e.*, greater than 95% pure). Where peptide sequences are underlined (throughout this specification) denotes a peptide that has been cyclised by a covalent bond between the two last underlined residues. In these examples, peptides were cyclized by a disulfide bond between two cysteine residues, acetylated and amide blocked.

7.2. IDENTIFICATION OF A NT-4 LINEAR PEPTIDE SEQUENCE THAT INTERACTS WITH TRKB RECEPTOR

This section describes molecular modeling experiments that identify a suitable linear peptide (LIP) sequence from natural neurotrophin ligands that binds to a key site in a Trk
5 receptor.

Considerable evidence suggests that the membrane proximal immunoglobulin (Ig) domain (D5) of the Trk receptor is directly involved in the binding of NTs. See, for example, Perez *et al.*, *Mol. Cell Neurosci.* 1995, 6:97-105; and Urfer *et al.*, *Embo J.* 1995, 14:2795-2805. Crystal structures of the NGF/TrkA complex (Wiesmann *et al.*, *Nature* 1999, 401:184-188) and of the NT-4/TrkB complex (Banfield *et al.*, *Structure (Camb)* 2001, 1191-1199)
10 have been solved which support this view. In both structures, a single NT dimer engages to Trk receptor molecules with each NT molecule in the dimer interacting, in turn, with each Trk receptor molecule.

Both crystal structures were analyzed with an algorithm designed to highlight linear
15 regions on the ligand that interact with the receptor (Doherty *et al.*, *Mol. Cell Neurosci* 2000, 16:283-295). To illustrate this analysis, the NT-4/TrkB(D5) crystal structure is shown here in **Figure 6A**. Within this complex, an individual NT monomer (labeled a₁) makes linear contacts with both TrkB receptor monomers (labeled b₁ and b₂). Both of these two interfaces were analyzed, and this analysis is illustrated in **Figures 6B-6C**. In these figures, interfaces
20 between NGF and the TrkA receptor are shown by dotted lines, whereas the interfaces between NT-4 and TrkB are shown by a solid line. As can be seen from inspection of these two figures, the interfaces overlap considerably for these two ligand-receptor complexes.

The contact profile analysis indicates that the N-terminal of the NT ligand makes the most intimate contact with the Trk receptor (**Figure 6C**). Moreover, a small linear motif
25 (SRRGE) situated at the dominant peak in the contact profile exists as half a helix and can be considered a tight loop. The sequence is closely conserved in BDNF (ARRGE), which also binds to the TrkB receptor, and is partially conserved in NGF (FHRGE) and NT-3 (SHRGE), ligands for the TrkA and TrkC receptors, respectively. Interesting, this region of neurotrophins is disordered in crystal structures of unbound NT (see, McDonald *et al.*,
30 *Nature* 1991, 354:411-414) and therefore has not previously been the subject of peptide studies.

The a_1/b_2 interface contact profile is illustrated in **Figure 6B**. NT loops 1-4, which others have implicated in the NT/Trk interaction (LeSauter *et al.*, *J. Biol. Chem.* 1995, 270:6564-6569) are highlighted. However, none of these loops figure in the a_1/b_1 interface and only loop 1 is involved in the a_1/b_2 interface.

5

7.3. DEVELOPMENT OF A TRKB ANTAGONIST PEPTIDE

Cerebellar neurons, isolated from rat pups at PND2, were cultured over monolayers of 3T3 fibroblasts in either control media or in media supplemented with a range of BDNF and/or NT-4 concentrations. After 16 hours, the co-cultures were fixed and mean neurite
10 length determined as has been previously described (Williams *et al.*, *J. Biol. Chem.* 2000, 275:4007-4012). The results, which are illustrated in **Figure 7A**, show that both ligands stimulate neurite outgrowth in a dose-dependent manner with a maximal response seen at between about 1 and 10 ng/ml.

The results in Section 7.2, *supra*, suggest that a properly constrained peptide of the
15 small linear RGE motif that is present in all NTs might have a high structural overlap with the native NT structure and thereby function as a Trk receptor antagonist. To test this hypothesis, a cyclic version of the LIP was designed that was constrained by a disulfide bond and has the amino acid sequence: N-Ac-CRGE C -NH $_2$. The effect of this peptide on the BDNF and NT-4 response was tested in the above-described neurite outgrowth assay, with
20 both NT ligands used at concentrations of 5 ng/ml. These results, which are illustrated in **Figure 7B** show that the peptide antagonizes both the BDNF and the NT-4 response, with a 50% inhibition seen at $144 \pm 23 \mu\text{M}$ for the BDNF response and $112 \pm 22 \mu\text{M}$ for the NT-4 response. In contrast, the peptide has no effect on basal neurite outgrowth when tested at concentrations greater than about 400 μM and in the absence of any natural NT ligand.
25 These results suggest that the cyclic peptide itself has no specific effects on neuronal survival and neurite outgrowth.

7.4. AN NT-4 LIP IS A MORE POTENT TRKB ANTAGONIST THAN EQUIVALENT NT3 AND NGF LIPS

30 The RGE sequence motif is shared by all the NTs. However, the amino sequences flanking this motif differ between the TrkA, TrkB and TrkC ligands. A series of

“equivalent” peptides, designed from sequences of different NT ligands, was therefore tested to determine if an extended peptide from the TrkB ligand might be a more active TrkB receptor antagonist.

Molecular modeling studies suggest that the peptide N-Ac-CSRRGEC-NH₂ shares structural overlap with the natural SRRGE motif of NT-4. Accordingly, this peptide
5 sequence was tested alongside equivalent peptides derived from NGF (N-Ac-CFHRGEC-NH₂) and NT-3 (N-Ac-CSHRGEC-NH₂) for their ability to inhibit the BDNF and NT-4 responses in the neurite outgrowth assay described in Section 7.3, *supra*. Surprisingly, the N-Ac-CSRRGEC-NH₂ derived from NT-4 was approximately 5-fold better than the NGF
10 and NT-3 derived peptides at inhibiting the BDNF response, with a 50% inhibition seen at $27 \pm 6 \mu\text{M}$. These results are illustrated in **Figure 7C**.

As noted, above, the addition of as little as two flanking amino acid residues from NT-4 increased the efficacy of the peptide up to five-fold against a TrkB response. Addition of the equivalent amino acids from either NGF or NT-3 had no discernable effect on the
15 efficacy of the original cyclic RGE peptide, suggesting that the selectivity of NT binding may be determined, at least in part, by the nature of amino acid residues that immediately flank the RGE motif. Indeed, a considerable body of evidence suggests that specificity of Trk receptor binding is encoded by the amino terminal sequences of the NTs. See, for example, Urfer *et al.*, *Embo J.* 1994, 13:5896-5909; and McInnes & Sykes, *Biopolymers*
20 1997, 43:339-366. These findings suggests that cyclic peptides and peptidomimetics of the invention can be targeted to particular Trk receptors (*i.e.*, to a TrkA, TrkB or TrkC receptor) by selecting the RGE flanking amino acid sequences from an NT ligand that preferably binds to the desired Trk receptor.

The same qualitative response described *supra*, was seen when the peptides were tested against NT-4 (see, **Figure 7D**). However, whereas 50% inhibition of the BDNF
25 response could be obtained with only about $25 \mu\text{M}$ of the peptide N-Ac-CSRRGEC-NH₂, the same level of inhibition of the NT-4 response required the peptide to be used at about $55 \pm 4 \mu\text{M}$.

As with the cyclic peptide N-Ac-CRGEC-NH₂, neither the N-Ac-CSRRGEC-NH₂ peptide nor its NGF or NT-3 equivalents had any effect on basal neurite outgrowth in control
30 cultures not supplemented with a NT ligand. The peptides were also tested for their ability to

inhibit the neurite outgrowth response stimulated by other agents, including N-cadherin, FGF2 or a CB1 receptor agonist. Cell growth responses produced by these agents have been described elsewhere (Williams *et al.*, *J. Cell Biol.* 2003, 160:481-486) and, in particular, are not believed to involve Trk receptors. The results from those experiments are shown in

5 **Figure 8.** In particular, the cyclic peptides did not inhibit any of these responses, even when administered at concentrations that fully inhibited the NT-4 and BDNF responses. These data confirm that the cyclic peptides and peptide mimetics of this invention can fully inhibit

Trk receptor function without any non-specific effects on neurite outgrowth.

The effects of the linear peptide N-Ac-SRRGELA-NH₂ were also evaluated in the

10 neurite outgrowth assay against NT-4, BDNF and the other agents mentioned, *supra*. These results are also shown in **Figure 8.** As expected, whereas the cyclic version of this peptide was a potent inhibitor of both the NT-4 and the BDNF responses, the linear peptide did not inhibit any of the response even when tested at concentrations up to 125 μM. Hence, peptides and peptidomimetics containing the RGE motif need to be constrained, *e.g.* by

15 disulfide bonds, to be functional Trk receptor antagonists.

7.5. DEVELOPMENT OF A TRKB AGONIST PEPTIDE

In crystal structures of the NT-4/TrkB receptor complex, the SRRGE motif in NT-4 runs anti-parallel to itself in the NT-4 dimer. The corresponding motif exhibits a similar anti-

20 parallel alignment in crystal structures of the NGF/TrkA receptor complex. Previously, a “tandem-repeat” mimetic approach has been used to develop peptide agonist of N-cadherin. See, Williams *et al.*, *J. Biol. Chem.* 2002, 277:4361-4367. The anti-parallel arrangement of the RGE motif in neurotrophins suggests that the “tandem-repeat” approach might also be used to develop Trk receptor agonist peptides.

25 Molecular modeling supports the hypothesis that a tandem repeat of the NT-4 SRRGEL sequence might be constrained in the cyclic peptide N-Ac-CSRRGELAASRRGELC-NH₂ (this peptide is also referred herein to as the “B_{AG}” peptide) in a manner that would allow for simultaneous engagement of two TrkB receptor monomers. A modeled structure of the B_{AG} peptide, which emphasizes this point, is shown here at

30 **Figure 9.** The effect of the B_{AG} peptide on neurite outgrowth was therefore tested in the assay described in Section 7.3 *supra*.

The results from such an experiment are illustrated in **Figure 10A**. The peptide can be seen to stimulate neurite outgrowth in a dose-dependent manner, with an EC₅₀ of about 300 nM and a near maximal response at about 600 nM. As with the result to the natural ligands BDNF and NT-4, the response of neurite outgrowth to the B_{AG} peptide is biphasic (compare **Figures 7A** and **10A**). Next, the B_{AG} peptide's ability to stimulate axonal growth was compared to that of established growth promoting peptides. The data from these experiments, which are illustrated in **Figure 10B**, demonstrate that at 6 μM the B_{AG} peptide promotes axonal growth by the same extent as maximally active concentrations of NT-R, BDNF and FGF2.

10

7.6. TRKB ANTAGONISTS INHIBIT THE AGONIST PEPTIDE RESPONSE

To verify that the B_{AG} peptide activates Trk receptor by binding to the same site as the monomeric peptide antagonists (described in Sections 7.3-7.4, *supra*), experiments were conducted to determine whether the peptide antagonist N-Ac-CSRRGEC-NH₂ could inhibit the B_{AG} peptide's effects on neurite outgrowth. The results are shown in **Figure 11**.

15

At 125 μM, the TrkB antagonist peptide can fully inhibit the activity of a maximally active concentration of the B_{AG} peptide. In contrast, the linear version of this peptide (*i.e.*, the peptide N-Ac-SRRGELA-NH₂) had very little to no effect on B_{AG} peptide activity in the neurite outgrowth assay. K252a, a compound which is reported to be a relatively specific Trk receptor antagonist (Tapley *et al.*, *Oncogene* 1992, 7:371-381), also fully inhibited the response of neurite outgrowth to the B_{AG} peptide. However, PD17304, a specific FGF receptor antagonist, did not inhibit the response.

20

These data establish that "tandem-repeat" cyclic peptides and peptidomimetics, based on the RGE motif, are specific and effective agonist of Trk receptors.

25

7.7. TRK AGONISTS OVERCOME INHIBITORS OF NEURONAL GROWTH

This example describes additional experiments investigating the effect of Trk receptor agonists under conditions that normally inhibit neuronal growth. In particular, the experiments demonstrate that, unlike the natural Trk receptor ligand, Trk receptor agonists of the invention can counteract the activity of inhibitory molecules and/or their receptors.

30

7.7.1 Materials and Methods

Reagents and culture treatment. Unless otherwise noted here, reagents in the experiments set forth in this Section were obtained and as set forth *supra*, in Section 7.1.3 *et seq.* In particular, recombinant human FGF2 and BDNF were obtained from R&D systems (Minneapolis, MN) and used at final concentrations of 5 ng/ml. The Trk receptor agonist K252a was obtained from Calbiochem (San Diego, CA) and used at a final concentration of 100 nM. The Trk agonist peptide B_{AG} (SEQ ID NO:18) was obtained from a commercial supplier (Multiple Peptide Systems, San Diego CA). Recombinant MAG-Fc chimera was obtained from R&D Systems (Minneapolis, MN) and used at a final concentration of 5-
5 25 µg/ml. Monoclonal antibody to GT1b (clone GMR5) was obtained from Seikagaku America (Falmouth, MA) and used at a final concentration of 20 µg/ml. A p75^{NTR} rabbit polyclonal antibody was raised against the extracellular domain of that receptor as previously described (*see*, Huber & Chao, *Dev. Biol.* 1995, 167:227-238) and used at a 1:200 dilution of serum. The known PKA inhibitors KT5720 and H-89 were obtained from Calbiochem (San
15 Diego, CA) and used at final concentrations of 200 and 400 nM, respectively. The known PI3K inhibitors Wortmannin and LY294002 were also obtained from Calbiochem (San Diego, CA) and were both used at final concentrations of 10 µM. The Rho kinase inhibitor Y27632 was obtained from Tocris (Bristol, UK) and used at 10 µM final concentration.

All reagents were diluted into the co-culture media and in general added to the
20 cultures just prior to plating of the neurons. The exception was antiserum raised against the p75^{NTR} receptor. Instead, a high density neuronal suspension was treated with a 1:200 dilution of the serum for 60 minutes. The neurons were then diluted by a factor of about 20, and seeded out for culture. The residual amount of p75^{NTR} antibody in the cultures is estimated to have been approximately a 1:5000 dilution of the serum. Separate control
25 experiments demonstrated that this antibody had no effect on neurite outgrowth at a dilution of 1:1000, establishing that the 1:5000 dilution used in these experiments has, at most, a negligible effect.

Neurite outgrowth assays. Neurite outgrowth assays were performed as described in
30 Section 7.1.1, *supra*.

7.7.2 Results

The Trk receptor agonist B_{AG} block MAG inhibitory activity. Myelin associated glycoprotein (MAG) has been previously shown to inhibit neurite outgrowth response from post-natal day 2-3 rat cerebellar neurons when presented to those cells as either a transfected molecule in the cellular substrate (Mukhopadhyay *et al.*, *Neuron* 1994, 13:757-767) or when
5 added as a soluble Fc chimeric protein (Tang *et al.*, *Mol. Cell. Neurosci.* 1997, 9:333-346). In furtherance of those studies, post-natal day 3 cerebellar neurons were cultured over monolayers of LK8 cells, a 3T3 fibroblast cell line that express transfected N-cadherin and have been previously shown to promote a robust neurite outgrowth response (Williams *et al.*,
10 *Neuron* 1994, 13:583-594). Cells were cultured with soluble MAG-Fc fusion protein present in the culture medium at concentrations of 0, 5 or 25 µg/ml. As expected, MAG-Fc inhibited neurite outgrowth in a dose-dependent manner, with an approximately 40% inhibition response when present in the culture medium at a concentration of 25 µg/ml (see, **Figure 12**).

Previous reports have suggested that inhibitors such as MAG mediated their effect(s)
15 by activation of RhoA and/or its downstream effector Rho kinase. See, for example, Dergham *et al.*, *J. Neurosci.* 2002, 22:6570-6570; Fournier *et al.*, *J. Neurosci.* 2003, 23:1416-1423; and Lehmann *et al.*, *J. Neurosci.* 1999, 19:7537-7547. To confirm these reports, cells were also cultured with the known Rho kinase inhibitor Y27632 (Narumiya *et al.*, *Methods Enzymol.* 2000, 325:273-284; Davies *et al.*, *Biochem. J.* 2000, 351:95-105)
20 included in the culture medium at a concentration of 10 µM. As expected, MAG-Fc does not inhibit neurite outgrowth under these conditions, even when present in the culture medium at concentrations as high as 25 µg/ml (**Figure 12**).

Additional neurite outgrowth experiments were performed to investigate what effect, if any, a Trk agonist might have on inhibitors such as MAG. In these experiments, neurons
25 were cultured with the Trk agonist polypeptide B_{AG} (SEQ ID NO:18, described in Section 7.5 above) present in the culture medium at a concentration of 6 µM. Surprisingly, MAG-Fc failed to inhibit neurite outgrowth under these conditions, even when present in the culture medium at concentrations as high as 25 µg/ml (**Figure 12**). By contrast, when the neurotrophin BDNF was present in the culture medium at a concentration of 5 ng/ml there
30 was no measurable effect on the MAG response – *i.e.*, MAG-Fc continued to inhibit neurite outgrowth (**Figure 12**). This result is consistent with previous reports that neuron cells must

be “primed” with neurotrophins to circumvent the inhibitor activity of MAG and myelin (*see, Cai et al., Neuron* 1999, 22:89-101).

To further investigate the B_{AG} polypeptide’s ability to block MAG inhibitor activity, the polypeptide was tested in neurite outgrowth assays at a variety of different concentrations
5 in the culture medium. Results from these experiments are depicted graphically in **Figure 13**. These data show that the B_{AG} polypeptide effectively blocks MAG inhibitor activity when present in the culture medium at concentrations as low as 30 nM, with a half maximal response when present at concentrations of between about 100 and 200 nM.

To confirm that the results from these experiments were not caused by any specific
10 MAG inhibition of the N-cadherin component in neurite outgrowth, experiments were also performed with neurons cultured over monolayers of 3T3 fibroblasts cells that do not express transfected N-cadherin. Bar graphs showing data from these experiments are shown in **Figure 14**. Although the basal neurite outgrowth response is lower when cells are cultured under these conditions, MAG-Fc nevertheless produces a measurable and substantial
15 inhibition of neurite outgrowth when present at 25 µg/ml. In the absence of MAG-Fc, basal levels of neurite outgrowth are already robust, and the B_{AG} polypeptide does not have a substantial effect when present in the culture medium at a concentration of 6 µM. Inspection of **Figure 14**, however, reveals that the Trk-receptor agonist at this concentration does effectively block the MAG response, so that MAG-Fc fails to inhibit neurite outgrowth when
20 present at a final concentration of 25 µg/ml. As before, and again in contrast to the effect of B_{AG}, the neurotrophin BDNF has no apparent effect on the inhibitory response stimulated by MAG-Fc when present at a concentration of 5 ng/ml.

These experiments demonstrate that Trk receptor agonists such as the B_{AG} polypeptide can be used to effectively prevent or reduce inhibitory responses produced by
25 signaling molecules such as MAG. The results from these experiments additionally show that Trk receptor agonists (*e.g.*, B_{AG}) promote neuronal growth and recovery, even when administered in an inhibitory environment, such as in the presence of the inhibitory signaling molecule MAG.

30 ***B_{AG} blocks inhibition by GT1b.*** The B_{AG} polypeptide’s ability to circumvent inhibitory activity of GT1b was also treated in neurite outgrowth assays. Previous reports

have described multivalent IgM antibodies to GT1b that can inhibit neurite outgrowth from cerebellar granule cells (Vinson *et al.*, *J. Biol. Chem.* 2001, 276:20280-20285). To confirm these reports, cerebellar neurons were cultured over monolayers of N-cadherin expressing 3T3 cells in both control media and in media containing 20 µg/ml of monoclonal antibody for GT1b. Data from these experiments are shown in the bar graph at **Figure 15**. Consistent with previous reports, co-culturing the cells with 20 µg/ml of antibody robustly inhibits neurite outgrowth under these conditions. Compare the column labeled (1) in **Figure 15** to column C in that same figure. Co-culturing cells with 10 µM of the Rho kinase inhibitor Y27632 effectively abolishes this effect, confirming previous reports that the GT1b receptor involves Rho kinase as a downstream effector in its signal cascade (*see*, Vinson *et al.*, *J. Biol. Chem.* 2001, 276:20280-20285). Surprisingly, when the Trk-receptor agonist B_{AG} is present in the culture medium (6 µM) with antibody to GT1b, the antibody's inhibitor effect is effectively eliminated; *i.e.*, a level of neurite outgrowth is observed which is comparable to that seen when antibody is not present in the culture medium. Compare the column labeled (2) in **Figure 15** to column C in that same figure.

These results show that Trk receptor agonists such as the B_{AG} polypeptide can be used to effectively reduce or prevent inhibitory activity produced by such receptors such as GT1b. The results from these experiments additionally show that Trk receptor agonists (*e.g.*, B_{AG}) promote neuronal growth and recovery, even when administered in an inhibitory environment, such as in the presence of the inhibitory signaling by GT1b.

B_{AG} blocks inhibition by p75^{NTR}. Because inhibitory molecules in myelin are believed to signal either directly or indirectly via the p75^{NTR} receptor, the B_{AG} peptide's ability to circumvent that receptor's inhibitory activity was also investigated. To verify, first, that signaling from this receptor does inhibit neurite outgrowth, cerebellar neurons were cultured over monolayers of N-cadherin expressing 3T3 cells in both control media and in media containing polyclonal antibody to p75^{NTR} (1:200 serum dilution). Data from these experiments are presented in the bar graph at **Figure 16**. Pretreatment of the cells with antibody for 60 minutes effectively inhibits the subsequent outgrowth of neurons, as can be seen by visually comparing the columns labeled (1) and C in the bar graph at **Figure 16**. As with MAG and GT1b, antibody to p75^{NTR} does not elicit an inhibitory

response when the Rho kinase inhibitor Y27632 is added to neurons at a final concentration of 10 μM immediately after antibody treatment (see column (2) in **Figure 16**). Likewise, culturing the neurons with a final B_{AG} polypeptide concentration of 6 μM also effectively blocks the $p75^{\text{NTR}}$ antibody's inhibitory effect. However, culture the cells with the neurotrophin BDNF (5 ng/ml final concentration) has no significant effect on the inhibitory response elicited by $p75^{\text{NTR}}$ antibody.

Because the cell cultures may contain some residual amount of antibody (estimated to be no more than approximately 1:5000 serum dilution) after treatment, control experiments were performed in which cells were cultured with polyclonal antibody in the media at a 1:1000 serum dilution. The presence of antibody at this level had no measurable effect on neurite outgrowth, demonstrating that the effects observed in these experiments are not caused by the very low levels of residual antibody that may remain after treatment.

The results from these experiments demonstrate that Trk receptor agonists such as the B_{AG} polypeptide can be used to effectively reduce or prevent inhibitory responses produced by the $p75^{\text{NTR}}$ pathway. The results additionally show that Trk receptor agonists (e.g., B_{AG}) promote neuronal growth and recovery, even when administered in an inhibitory environment, such as in the presence of the inhibitory signaling by $p75^{\text{NTR}}$.

B_{AG} signaling is mediated by PKA and PI3K. To further investigate mechanisms by which a Trk receptor agonists may block inhibitory signals, cerebellar neurons were cultured for 18 hours over 3T3 monolayers in control media or in media supplemented with what had been determined to be maximally active concentrations of either the B_{AG} polypeptide (6 μM final concentration), the neurotrophin BDNF (5 ng/ml final concentration) or FGF2 (5 ng/ml final concentration). Findings from these experiments are depicted in the bar graph at **Figure 17**. Under these conditions, each of the three factors (B_{AG} , BDNF and FGF2) enhances neurite length by about 60-70% compared to the control culture. When K252a, a compound which is reported to be a relatively specific Trk receptor antagonist (Tapley *et al.*, *Oncogene* 1992, 7:371-381), was included in the culture media at a final concentration of 100 nM, the outgrowth response produced by both B_{AG} and BDNF were essentially abolished. However, the outgrowth response produced by FGF2 was unaffected, confirming reports suggesting that FGF2 promotes neurite outgrowth by a signaling cascade that is

distinct from that of Trk receptors and, in particular, does not involve either PKA or PI3K (see, Williams *et al.*, *Cell Biol.* 2003, 160:481-486).

In similar experiments, neuronal cells were cultured either with the protein kinase A (PKA) inhibitor KT5720 (200 nM final concentration) or H-89 (400 nM final concentration),
5 or with the phosphoinositide 3-kinase (PI3K) inhibitor Wortmannin (10 μ M final concentration) of LY294002 (10 μ M final concentration) in the culture media. As with the Trk receptor antagonist, the neurite outgrowth response to both B_{AG} and BDNF was essentially abolished by these kinase inhibitors. As expected, the neurite outgrowth response to FGF2 was unaffected.

10 These results demonstrate that the activated Trk receptor stimulates neurite growth by a mechanism or mechanisms that involve activation of both PKA and PI3K. Hence, Trk agonists of this invention (*e.g.*, the B_{AG} polypeptide) can be effective at blocking or reducing a wide variety of inhibitory signals. In particular, Trk agonists of the invention can be effective at blocking inhibitory signals mediated by signal cascades with one or more
15 components that are inhibited or inactivated by either PKA or PI3K.

As an example, and not be way of limitation, PKA is reported to inactivate Rho by direct phosphorylation on Ser188 of that molecule (Ellerbroek *et al.*, *J. Biol. Chem.* 2003, 278:19023-19031). Hence, Trk agonists of the present invention can be used to block or reduce signals mediated by inhibitory cascades involving Rho. These include, *inter alia*,
20 inhibitory signals mediated by myelin inhibitors such as MAG (or by MAG fusion constructs such as an MAG-Fc), Nogo-A, the oligodendrocyte myelin glycoprotein, NgR, GT1b and p75^{NTR} as well as signals mediated by chondroitin sulfate proteoglycans from the CNS glial scar (Monnier *et al. Neurosci.* 2003, 22:319-330). As another non-limiting example, activation of PI3K is expected to overcome inhibitory activity of semaphorins (Eickholt *et al.*, *J. Cell Biol.* 2002, 157:211-217). Indeed, neurotrophins are reported to overcome such
25 inhibitor signaling by activating a Trk-PI3K cascade in neurons (Atwal *et al.*, *J. Neurosci.* 2003, 23:7602-7609). Hence, Trk agonists of the present invention can be used to block or reduce these inhibitory signals as well.

7.8. ADDITIONAL TRK AGONIST COMPOUNDS

This example describes additional peptides and peptidomimetic compounds that are either based on or derived from the B_{AG} polypeptide described in the preceding examples. Data from biological assays are also presented, demonstrating that these novel compounds
5 also exhibit activity as Trk receptor agonists.

7.8.1 Novel Trk receptor agonists

The following peptides and peptidomimetics were designed based on the amino acid sequence of the B_{AG} polypeptide described *supra* – i.e., CSRRGELAASRRGELC (SEQ ID
10 NO:17). These novel compounds, which are referred to here as hB_{AG2}, riB_{AG1} and hriB_{AG2}, are set forth in Table 1, below.

TABLE I: TRK RECEPTOR AGONIST COMPOUNDS

Identifier	Sequence	Key
hB _{AG2}	c(SRRGELSRRGEL) (SEQ ID NO:39)	<ul style="list-style-type: none"> • cyclized peptide bond.
riB _{AG1}	Ac-dCdLdEGdRdRdSdAdAdLdEGdRdRdSdC-NH ₂ (SEQ ID NO:40)	<ul style="list-style-type: none"> • D-amino acid residues. • Cyclized by cysteine disulfide bonds.
hriB _{AG2}	c(dLdEGdRdRdSdLdEGdRdRdS) (SEQ ID NO:41)	<ul style="list-style-type: none"> • D-amino acid residues. • Cyclized peptide bond.

In Table I, above, the lowercase “c” is used to denote a cyclization by a peptide or
15 amide bond joining the amino-terminal amino acid residue to the carboxy-terminal amino acid residue. Hence, the hB_{AG1} polypeptide (SEQ ID NO:39) preferably comprises an amide bond joining the N-terminal serine residue to the C-terminal leucine residue. Similarly, the peptide hriB_{AG2} (SEQ ID NO:41) preferably comprises an amid bond joining the N-terminal leucine residue to the C-terminal serine residue.

20 The lowercase “d” in front of an amino acid residue in Table I denotes that the residue is a D-amino acid residue (as opposed to an L-amino acid residue). Hence, the polypeptides riB_{AG1} and hriB_{AG2} preferably comprise D-amino acid residues. Indeed, all of

the amino acid residues in these polypeptides (with the exception of the glycine residues, which are neither L nor D amino acid residues) are preferably D-amino acid residues.

It is readily apparent, upon visual inspection of the riB_{AG1} and hriB_{AG2} amino acid sequences (SEQ ID NOS:40 and 41), that these sequences are reverse sequences of the B_{AG} polypeptide sequence (SEQ ID NO:17). In particular, and as will be appreciated by those of skill in the art, polypeptides of the invention which comprise a sequence of D-amino acid residues are expected to adopt three dimensional structures (*i.e.*, “conformations”) that are substantially similar or identical to the three dimensional conformation of a polypeptide comprising the reverse sequence of L-amino acid. Hence, in addition to the polypeptides of L-amino acid residues described *supra*, the present invention also contemplates polypeptides having the reverse sequence of D-amino acid residues. Hence, in one preferred embodiment peptides and peptidomimetics of the present invention comprise sequences of L-amino acid residues including the Arg-Gly-Glu (*i.e.*, “RGE”) motif described, *supra*. Accordingly, the invention also provides, in an alternative embodiment) peptides and peptidomimetics comprising sequences of D-amino acid residues including the short linear sequence motif dGlu-Gly-dArg (*i.e.*, “dEGdR”).

Peptides and peptidomimetics of the invention that comprise such D-amino acid residues are expected to be more stable and less readily degraded *in vivo*, *e.g.*, by proteolytic enzymes. Similarly, cyclic amide bonds, such as those used in the hB_{AG2} and hriB_{AG2} polypeptides, are also expected to be less readily degraded *in vivo*. Shortened peptides (*e.g.*, hB_{AG}, which lacks two terminal cysteines and two central alanines compared to B_{AG}) are more likely to cross the blood-brain barrier. Accordingly, such peptides may be preferred, *e.g.*, for use in pharmaceutical compositions and administration to an individual.

25 7.8.2 Biological Activity

The hB_{AG2}, riB_{AG1} and hriB_{AG2} polypeptides were tested in a substrate based assay, to evaluate their ability to promote neurite outgrowth in an inhibitory environment. In particular, and as discussed above, the myelin associated glycoprotein (MAG) has been previously shown to inhibit neurite outgrowth response. See, for example, Mukhopadhyay *et al.*, *Neuron* 1994, 13:757-767; and Tang *et al.*, *Mol. Cell. Neurosci.* 1997, 9:333-346. As demonstrated in the examples, *supra*, Trk receptor agonists such as the B_{AG} polypeptide are

able to block MAG inhibitory activity, and promote neurite outgrowth in that inhibitory environment (*i.e.*, in the presence of MAG). The data presented in the experiments described here, demonstrate that the hB_{AG2}, riB_{AG1} and hriB_{AG2} polypeptides also block MAG inhibitory activity and promote neurite outgrowth.

5

Materials and Methods.

Briefly, standard plastic 8 chamber tissue-culture slides were coated as follows with either: (a) polylysine; (b) polylysine and a mixture of goat anti-human IgG and fibronectin; or (c) polylysine, a mixture of goat anti-human IgG (Fc-specific) and fibronectin and MAG-Fc. First, slides are coated with polylysine at 17 µg/ml in distilled water ("dH₂O") for thirty (30) minutes at room temperature. After aspirating the wells, a mixture of anti-human IgG and/or fibronectin (both at 10 µg/ml in DMEM) is added to wells to be coated with those compounds, and incubated for 120 minutes. The wells are again aspirated and (for wells coated with MAG-Fc) incubated for sixty (60) minutes with MAG-Fc (0.25 µg/ml in DMEM and 10% FCS). PND2/3 rat cerebellar neurons are then added at 15K to each well in DMEM, 10% FCS, 25 mM KCl and 5 ng/ml FGF2, bringing the final media volume to 300 µl in each well. The cerebellar neurons are cultured for 27 hours before fixing and staining for GAP-43. Polylysine, goat anti-human IgG (Fc-specific) and fibronectin are available from SIGMA (St. Louis, Missouri).

20

Results. The mean length of the longest neurite per neuron was determined. Basal neurite growth of about 9 µm was observed on the polylysine substrate. Neurite growth increased to about 24 µm on the polylysine/fibronectin substrate. Neurite growth decreased to about 15 µm in the wells that had the additional MAG-Fc coating. **Figure 18A** shows a dose response curve for the three peptidomimetics. Peptidomimetic hriB_{AG2} (SEQ ID NO:40) promoted substantial dose-dependent neurite growth in the inhibitory environment. A neurite growth response can be observed at a dose of about 10 µg/ml and is almost double the value seen in the inhibitory environment without the peptidomimetic at a dose of 33 µg/ml (the highest concentration tested). hB_{AG2} (SEQ ID NO:39) promotes neurite growth at a dose of 33 µg/ml. riB_{AG1} (SEQ ID NO: 41) does not promote growth at the same concentration.

30

Figure 18B shows a bar graph depicting neurite growth in the inhibitory environment in the presence of BDNF, B_{AG}, hriB_{AG2}, hB_{AG2} or riB_{AG}. BDNF has no effect on neurite growth at concentrations of 10 µg/ml and 100 µg/ml. B_{AG} peptide promotes neurite growth at concentrations of both 10 µg/ml and 100 µg/ml. hriB_{AG2} at a concentration of 33 µg/ml promoted neurite growth to a substantially greater extent than BDNF, B_{AG} peptide, and the hB_{AG2} and riB_{AG1} at any concentration. hB_{AG2} at a concentration of 33 µg/ml promoted neurite growth to an extent comparable to B_{AG} polypeptide at a concentration of 10 µg/ml.

These results show that other peptides and peptidomimetics, such as the B_{AG} peptide derivatives of this application, can promote neurite growth in an inhibitory environment and to an extent that is comparable or even superior to that of the B_{AG} polypeptide.

7.9. p75 RECEPTOR BINDING AGENTS OVERCOME INHIBITION OF NEURONAL GROWTH

This example describes experiments investigating the effect of p75 receptor binding agents under conditions that normally inhibit neuronal growth. In particular, the experiments demonstrate that p75 receptor binding agents counteract the activity of inhibitory molecules and/or their receptors.

Neurite Outgrowth Assays

Co-cultures of cerebellar neurons on monolayers of either parental 3T3 cells or LK8 cells (an established transfected 3T3 cell line that expresses physiological levels of chick N-cadherin; *see Doherty et al., Neuron* 1991, 6:247-258) were established as previously described by Williams *et al. (Neuron* 1994, 13:583-594). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). These cerebellar neurons express the TrkB receptor and do not express functional levels of the TrkA receptor.

For establishment of the co-cultures, about 80,000 3T3 cells (or LK8 cells) were plated into individual chambers of an eight-chamber tissue culture slide coated with poly-L-lysine and fibronectin. The plated cells were maintained overnight in Dulbecco's modified Eagle's medium ("DMEM") supplemented with 10% FCS to allow for the formation of confluent monolayers. The medium was removed and about 6,000 dissociated cerebellar neurons (taken from post-natal day 2/3 rats) were plated into each well in SATO medium

supplemented with 2% FCS. Test reagents were added as indicated in the text and the co-cultures maintained for 23 hours. The co-cultures were then fixed and stained for GAP-43 immunoreactivity. The mean length of the longest neurite per cell was measured for between about 120 and 150 neurons, again as previously described by Williams *et al.* (*Neuron* 1994, 13:583-594).

Reagents

Recombinant human NGF and BDNF were obtained from R&D systems (Minneapolis, MN). Synthetic peptides were all obtained from a commercial supplier (Multiple Peptide Systems, San Diego, CA). All peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) according to routine methods, and obtained at the highest level of purity (*i.e.*, greater than 95% pure).

The p75 receptor binding agents promote neurotrophin-mediated neuron growth in an inhibitory environment. Myelin associated glycoprotein (MAG) has been previously shown to inhibit neurite outgrowth response from post-natal day 2-3 rat cerebellar neurons when presented to those cells as either a transfected molecule in the cellular substrate (Mukhopadhyay *et al.*, *Neuron* 1994, 13:757-767) or when added as a soluble Fc chimeric protein (Tang *et al.*, *Mol. Cell. Neurosci.* 1997, 9:333-346). In furtherance of those studies, post-natal day 3 cerebellar neurons were cultured over monolayers of LK8 cells, a 3T3 fibroblast cell line that express transfected N-cadherin and have been previously shown to promote a robust neurite outgrowth response (Williams *et al.*, *Neuron* 1994, 13:583-594). Cells were cultured with soluble MAG-Fc fusion protein present in the culture medium at concentrations of 0, 5 or 25 $\mu\text{g/ml}$. As expected, MAG-Fc inhibited neurite outgrowth in a dose-dependent manner, with an approximately 40% inhibition response when present in the culture medium at a concentration of 25 $\mu\text{g/ml}$. Thus, this culture medium, containing soluble MAG-Fc fusion protein, is an inhibitory culture medium.

The inhibitory culture medium was further supplemented with BDNF at 1 ng/ml, NGF at 10 ng/ml or 100 ng/ml, BDNF (at 1 ng/ml) in combination with NGF (at 10 ng/ml or 100 ng/ml), a constrained monomer of the loop 1 motif in NGF which binds to the p75 receptor (N-Ac-CTDIKGKEC-NH₂) at 100 $\mu\text{g/ml}$, or the NGF loop 1 peptide (at 100 $\mu\text{g/ml}$)

in combination with BDNF (at 1 ng/ml). The growth media containing MAG-Fc alone was the control. When the neurotrophin BDNF was present in the inhibitory culture medium at a concentration of 1 ng/ml there was no measurable effect on the MAG response – *i.e.*, MAG-Fc continued to inhibit neurite outgrowth. Like BDNF, NGF at concentrations of either 10
5 ng/ml or 100 ng/ml did not stimulate neurite outgrowth in the presence of MAG-Fc (**Figure 19**). In data from individual experiments, the results obtained with NGF at 10 ng/ml and 100 ng/ml were not obviously different, and these data were therefore pooled. These results are consistent with previous reports that neuron cells must be “primed” with neurotrophins to circumvent the inhibitor activity of MAG and myelin (*see, Cai et al., Neuron* 1999, 22:89-
10 101).

The constrained monomer of the NGF loop 1 binding motif alone also had no effect on neurite outgrowth in the presence of MAG-Fc (**Figure 19**). However, the NGF loop 1 peptide in combination with BDNF produced a significant neurite outgrowth response. Additionally, a significant neurite outgrowth response was also observed when BDNF and
15 NGF were added together (**Figure 19**) in a ratio of 1:10 or 1:100 (BDNF to NGF).

The results from these experiments suggest that, when NGF and BDNF are administered to an inhibitory environment, NGF allows BDNF to promote neurite outgrowth. The results additionally show that administration of a constrained monomer of the first β hairpin loop in NGF allows BDNF to promote CNS neuron growth in an inhibitory
20 environment.

7.10. TREATMENT OF A PATIENT WITH SPINAL CORD INJURY

A patient is diagnosed with a thoracic spinal cord injury and has loss of sensation and motor activity in his legs. The patient undergoes surgery to stabilize the thoracic spine.
25 Following debridement of soft tissue and bone, the damaged spinal cord is exposed. A sterile pharmaceutical powder comprising a p75 receptor binding agent is mixed with sterile normal saline to form a gel. The surgeon topically applies the p75 receptor binding agent gel to the exposed surface of the cord. The stabilization procedure is completed in the usual fashion. Post-operatively, the patient is monitored for improvement in sensation and/or motor activity
30 in the lower extremities.

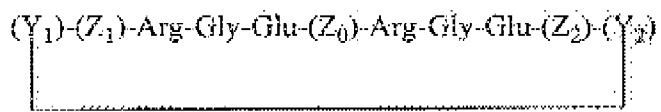
8. REFERENCES CITED

Numerous references, including patents, patent applications and various publications, are cited and discussed in the description of this invention. The citation and/or discussion of such references is provided merely to clarify the description of the present invention and is
5 not an admission that any such reference is “prior art” to the invention described here. All references cited and/or discussed in this specification (including references, *e.g.*, to biological sequences or structures in the GenBank, PDB or other public databases) are incorporated herein by reference in their entirety and to the same extent as if each reference was individually incorporated by reference.

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The claims defining the invention are as follows:

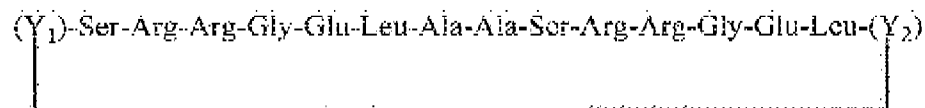
1. A cyclic peptide having the formula:



wherein:

- (a) Y_1 and Y_2 are independently selected amino acids with a covalent bond formed between Y_1 and Y_2 ; and
- (b) Z_1 , Z_2 , and Z_0 are optional and, if present, are independently selected amino acids or sequences of amino acids joined by peptide bonds.
2. The cyclic peptide according to claim 1, wherein the size of the cyclic peptide ring ranges from about 8-50 amino acid residues.

3. The cyclic peptide according to claim 1, said cyclic peptide having the formula:



4. The cyclic peptide according to claim 1, said cyclic peptide having the amino acid sequence:

Cys-Ser-Arg-Arg-Gly-Glu-Leu-Ala-Ala-Ser-Arg-Arg-Gly-Glu-Leu-Cys (SEQ ID NO:17),

wherein a covalent bond joins the N-terminal and C-terminal cysteines in said amino acid sequence.

5. The cyclic peptide according to claim 1, said cyclic peptide having the amino acid sequence:

Ser-Arg-Arg-Gly-Glu-Leu-Ser-Arg-Arg-Gly-Glu-Leu (SEQ ID NO:39);

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wherein the terminal serine and the terminal leucine are covalently linked by a peptide bond.

6. The cyclic peptide according to any one of claims 1-3, wherein the cyclic peptide enhances Trk mediated activity.

7. The cyclic peptide according to any one of claims 1-4, which cyclic peptide further comprises, on the amino terminal residue, an N-acetyl group, an N-formyl group, or an N-mesyl group.

8. The cyclic peptide according to any one of claims 1-4, and 6, which cyclic peptide further comprises, on the C-terminal residue, a C-amide group.

9. The cyclic peptide according to any one of claims 1-3, wherein Y_1 and Y_2 are covalently linked by disulfide bonds.

10. The cyclic peptide according to claim 9, wherein Y_1 and Y_2 are independently selected from the group consisting of: penicillamine, β,β -tetramethylene cysteine, β,β -pentamethylene cysteine, β -mercaptopropionic acid, β,β -pentamethylene- β -mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercapto proline, and derivatives thereof.

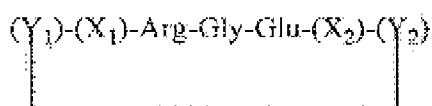
11. The cyclic peptide according to claim 9, wherein Y_1 and Y_2 are each cysteine or a derivative thereof.

12. The cyclic peptide according to claim 1 or 3, wherein Y_1 and Y_2 are covalently linked by an amide bond.

13. The cyclic peptide according to claim 12, wherein the amide bond is formed between terminal functional groups.

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14. The cyclic peptide according to claim 12, wherein the amide bond is formed between one terminal functional group and one residue side chain.
15. The cyclic peptide according to any one of claims 12-14 wherein:
 (a) Y_1 is selected from the group consisting of lysine, ornithine and derivatives thereof;
 and
 (b) Y_2 is selected from the group consisting of aspartate, glutamine and derivatives thereof.
16. The cyclic peptide according to any one of claims 12-14 wherein:
 (a) Y_1 is selected from the group consisting of aspartate, glutamine and derivatives thereof; and
 (b) Y_2 is selected from the group consisting of lysine, ornithine and derivatives thereof.
17. The cyclic peptide according to any one of claims 1-3, wherein Y_1 and Y_2 are covalently linked by a thioether bond.
18. The cyclic peptide according to any one of claims 1-3, wherein:
 (a) Y_1 and Y_2 are each tryptophan or derivatives thereof; and
 (b) the covalent bond between Y_1 and Y_2 forms a $\delta_1\delta_1$ -dityryptophan or a derivative thereof.
19. A peptidomimetic that modulates Trk receptor mediated activity, wherein the peptidomimetic has a three-dimensional structure that is substantially similar to a three-dimensional structure of a cyclic peptide that modulates Trk mediated activity, wherein the cyclic peptide comprises the formula:



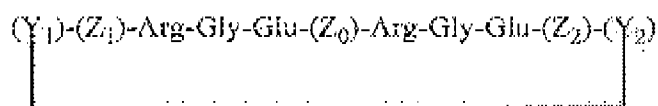
wherein:

- (a) Y_1 and Y_2 are independently selected amino acids with a covalent bond formed between Y_1 and Y_2 ; and

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(b) X_1 and X_2 are optional and, if present, are independently selected amino acids or sequences of amino acids joined by peptide bonds.

20. A peptidomimetic that modulates Trk receptor mediated activity, wherein the peptidomimetic has a three-dimensional structure that is substantially similar to a three-dimensional structure of a cyclic peptide that modulates Trk mediated activity, wherein the cyclic peptide comprises the formula:



wherein:

(a) Y_1 and Y_2 are independently selected amino acids with a covalent bond formed between Y_1 and Y_2 ; and

(b) Z_1 , Z_2 , and Z_0 are optional and, if present, are independently selected amino acids or sequences of amino acids joined by peptide bonds.

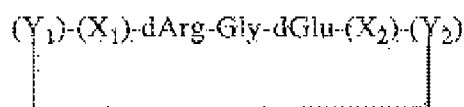
21. A cyclic peptide comprising, within a cyclic ring of the cyclic peptide, the D-amino acid sequence:



wherein the cyclic peptide modulates Trk receptor mediated activity.

22. A cyclic peptide according to claim 21 that promotes neurite growth.

23. A cyclic peptide according to claim 21, the cyclic peptide comprising the formula:



wherein:

(a) Y_1 and Y_2 are independently selected amino acids with a covalent bond formed between Y_1 and Y_2 ; and

(b) X_1 and X_2 are optional and, if present, are independently selected amino acids or sequences of amino acids joined by peptide bonds.

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24. The cyclic peptide according to claim 23, wherein the covalent bond formed between Y_1 and Y_2 is a peptide bond.

25. The cyclic peptide according to claim 24, said cyclic peptide having the amino acid sequence:

dLeu-dGlu-Gly-dArg-dArg-dSer-dLeu-dGlu-Gly-dArg-dArg-dSer (SEQ ID NO:40).

26. The cyclic peptide according to claim 23 comprising the formula having the amino acid sequence:

dCys-dLeu-dGlu-Gly-dArg-dArg-dSer-dAla-dAla-dLeu-dGlu-Gly-dArg-dArg-dSer-dCys (SEQ ID NO:41);

wherein the terminal cysteines are covalently linked by disulfide bonds.

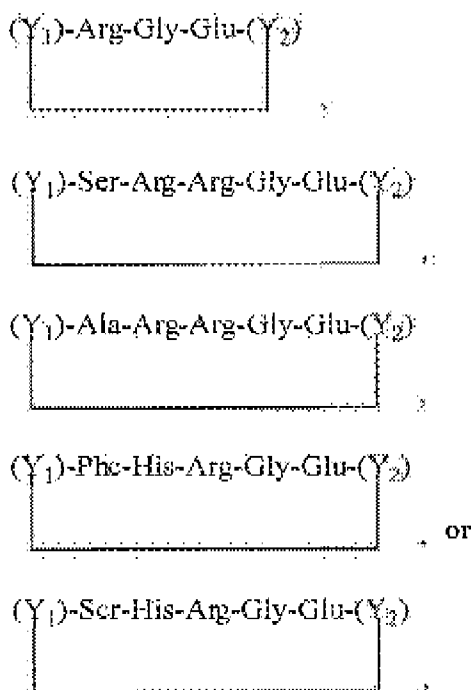
27. A pharmaceutical composition comprising:

(a) an amount of a cyclic peptide or peptidomimetic according to any one of claims 1-26, which amount is effective for modulating a Trk receptor mediated activity; and

(b) one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

28. A method for inhibiting a Trk receptor mediated activity, which method comprises contacting a cell with an amount of a cyclic peptide having the formula:

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or a peptidomimetic according to claim 19,
 wherein the amount of cyclic peptide or peptidomimetic contacted with the cell
 effectively inhibits the Trk receptor mediated activity.

29. The method according to claim 28, wherein the cell is contacted with a cyclic peptide
 having the amino acid sequence:

- Cys-Arg-Gly-Glu-Cys; (SEQ ID NO:9)
- Cys-Ser-Arg-Arg-Gly-Glu-Cys; (SEQ ID NO:1)
- Cys-Ala-Arg-Arg-Gly-Glu-Cys; (SEQ ID NO:3)
- Cys-Phe-His-Arg-Gly-Glu-Cys; (SEQ ID NO:5) or
- Cys-Ser-His-Arg-Gly-Glu-Cys; (SEQ ID NO:7),

wherein a covalent bond joins the N-terminal and C-terminal cysteines in said amino acid
 sequence.

30. A method for enhancing a Trk receptor mediated activity, which method comprises
 contacting a cell with an amount of a cyclic peptide according to any one of claims 1-18 or 21-

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26, or a peptidomimetic according to claim 20, wherein the amount of the cyclic peptide or peptidomimetic contacting with the cell effectively enhances the Trk receptor mediated activity.

31. The method according to any one of claims 28-30, wherein the Trk receptor mediated activity is selected from the group consisting of: neuronal growth, neuronal survival, axonal growth, synaptic plasticity, and neurite outgrowth.

32. A method for enhancing central nervous system (CNS) growth or repair in an individual, which method comprises administering to the individual an amount of a cyclic peptide according to any one of claims 1-18 or 21-26, or a peptidomimetic according to claim 20, wherein the amount of the cyclic peptide or peptidomimetic administered effectively enhances CNS growth or repair.

33. A method for reducing a CNS inhibitor response, which method comprises contacting a cell with an amount of a cyclic peptide according to any one of claims 1-18 or 21-26, or a peptidomimetic according to claim 20, wherein the amount of the cyclic peptide or peptidomimetic contacted with the cell effectively reduces the CNS inhibitor response.

34. A method for reducing a CNS inhibitor response in an individual, which method comprises administering to the individual an amount of a cyclic peptide according to any one of claims 1-18 or 21-26, or a peptidomimetic according to claim 20, wherein the amount of the cyclic peptide or peptidomimetic administered effectively reduces the CNS inhibitor response.

35. The method according to claim 33 or 34, wherein the CNS inhibitor response is mediated by a signal cascade with one or more components that are modulated by protein kinase A (PKA) or phosphoinositide-3 kinase (PI3K).

36. The method according to claim 33 or 34, wherein the CNS inhibitor response is mediated by a signal cascade involving Rho.

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37. The method according to claim 33 or 34, wherein the CNS inhibitor response is mediated by MAG, Nog₆-A, an oligodendrocyte myelin glycoprotein, NgR, GT1b, p75^{NTR}, a chondroitin sulfate proteoglycan or a semaphorin.
38. A method for screening a candidate compound for the ability to modulate Trk receptor mediated activity, which method comprises comparing a three-dimensional structure of the candidate compound to a three-dimensional structure of a cyclic peptide that modulates Trk receptor mediated activity, wherein:
- (a) said cyclic peptide comprises, within a cyclic ring thereof, the amino acid sequence Arg-Gly-Glu, and
 - (b) similarity between the structure of the candidate compound and the structure of the cyclic peptide is indicative of the candidate compound's ability to modulate Trk receptor mediated activity.
39. The cyclic peptide according to claim 1 or claim 21 substantially as hereinbefore described with reference to the Examples and Figures.
40. The peptidomimetic according to claim 19 or claim 20 substantially as hereinbefore described with reference to the Examples and Figures.
41. The method according to claim 38 substantially as hereinbefore described with reference to the Examples and Figures.

BDNF	-----APMKEANIRG---QGGLAYPGVTRHTGTLSEVNGPKAGSRGL	38
NT4	-----QP---PPSTLPPFLAP-----	13
NT3	QGNNDQRLPQSLPQSLNLSLIIKLIQADILKNKLSKQMVQVQSTLPKAEAPREPERGG	60
NGF	-----EPHSESNVPAGHTIIPQVHWTKLQHSALDTRRRARSAPAAAIAARVAG---	47
BDNF	TSLADTFEHVIEELLDEQKVRPNEENNKDADLYTSRVMLSSQVPLEPPLLFLEE--YK	96
NT4	-----EWDLLSPRVLSRGAPAGPPLLFLEAGAFR	44
NT3	PAKS-----AFQPVIAMDTELLRQRRYNSPRVLLSDSTPLEPPPLYLMED--YV	108
NGF	-----QTRNITVDPRLFKKRRLRSRVLFSTQPPREAA---DTQDLDFE	88
BDNF	NYLDA-ANMSMR-VRRHSDPA-RRGELSVCDSEWVTAADKKTAVDMSGGTVTVLEKVP	153
NT4	ESAGAPANRSRRGVSETAPAS-RRGELAVCDVSGWVT--DRRTAVDLRGREVEVLGEVP	101
NT3	GSPVV-ANRTSRKRYAEHKS-HRGEYSVCDSESLWVT--DKSSAIDIRGHQVTVLGEIK	164
NGF	VGGAAPFNRTHRSKRSSHPHFHRGEFSVCDSESVVWVG--DKTTATDIKGEKVMVLGEVN	146
BDNF	VSKGQ-LKQYFFETKCNPMGYTKEG-----CRGIDKRHWNSQCRTTQSYVRALTMDSK	205
NT4	AAGGSPLRQYFFETRCKADNAEBEGGPGAGGGGCRGVDRRHVSECKAKQSYVRALTADAQ	161
NT3	TGNSP-VKQYFFETRCKEARPVKNG-----CRGIDDKHWNSQCKTSQTYVRALTSENN	216
NGF	INNSV-FKQYFFETKCRDPNPVDSG-----CRGIDSKHWNSYCTTHTTFVKALTMDSG-	197
BDNF	KRIGWRFRIDTSCVCTLTIKRGR--	229
NT4	GRVGWRWIRIDTACVCTLLSRTGRA-	186
NT3	KLVGWRWIRIDTSCVCLSRKIGRT-	241
NGF	KQAARWFRIDTACVCLSRKAVRRA	223

Figure 1

		Signal	
<i>trkA</i>	1	MLRGGRRGOLGWHSWAAGPSSLAWLILAS	-----AGAACPDA CCP
<i>trkB</i>	1	-----HSSWIRWHGPAMARLWGFCLVVGFW	-----RAAFACPTIS-CK
<i>trkC</i>	1	-----HDYSL	-----FLLGSVWLDYVGSVLACPAN-CV
		Cysteine Rich I	
		HGSSGLRCTR	-DGALDSLHLHPG-----AENLTELYIEMOO
		CSASRIWCSDPSPGIVAFPRLEPNSVD	-----PENITEIFIAHOK
		CSKTEINCRRPDDGNL	-FPLLEGODSGMSNGNAINITDISRNITSIHIEHWR
			Leucine Rich
<i>trkA</i>	78	HLQHLELRDLRGLGELRNLTIVKSGLRFVAPDA	FHFPTPRLSRLMLSF
<i>trkB</i>	78	RLEIINEDDVEAYVGLRNLTIVDSGLKIFYAHKAF	LKNSNLOHINFTR
<i>trkC</i>	90	SLHTLHAYDMELYTGLOKLTIKNSGLRSIOPRA	FAKNPHLRYINLSS
			Cysteine Rich II
		NALLESLSWKIVQGLSLOELVLSGNPLHCS	CALRHLORWEEGLGGVPEQKLQC
		NKLTLSLRKHFRRHLDLSELILVGNPFT	CSCDIMHIKTILOE-AKSSPDIQDLYC
		NRLTTLSSWOLFQTLSLRELOLEQNF	FFNCSCDIRWMOLWEOEGEAKLNSONLYC
<i>trkA</i>	178	HGQG	-----PLAHKPNASC
<i>trkB</i>	177	LNES	KNIPLANLOIPHCGLPSANLAA
<i>trkC</i>	190	INAD	GSQPLFRHNSQCDLPEISVSHVNLTYREGDNAYITCNGSGS
			Immunoglobulin I
		GLEQAGWILTELEOSA	TYMKS---GGLP
		PVPMHYWDVGNLVSKH	MNET---SHTQG-SLRITN
		PLPDVDWIVTGLOS	INTHOTNLNWTNVHAINLTLVNVITSE
			DNNGFTLTICIAENY

Figure 2A

```

trkA 271 V G R A E V S V O V N V S F P A S V O - L H T A V E M H H H C I P F S Y D G O P A P S L R W L
trkB 272 V G E D O D S Y N L T V H F A P T I T F L E S P T S D H H W C I P F T V Y K G N P K P A L O W F
trkC 290 V G H S N A S V A L T Y Y P P R V S L E E P E L R L E H C I E F V Y R G N P P P T L H W L
      Immunoglobulin II
      F N G S V L N E T S F I F T E F L E P A A N E T V R H G C L R L N O P T H Y N H G N Y T L L A A N P F G Q
      Y N G A I L N E S K Y I C T K I H - - Y T N H T E Y H G C L O L D N P I T H M N G D Y T L I A K H E Y G K
      H N G Q P L R E S K I I H V E Y Y - - Q E G E I S - E G C L L F N K P T H Y N H G N Y T L I A K N P L G T
trkA 370 A S A S I M A A F M - - - - - D N P F - - - - - E F - N P E D P I P D I N S - - - - -
trkB 370 D E K O I S A H F M G W P G I D D G A N P N Y P D V I Y E D Y G T A A N D I G D T I N R S M E
trkC 387 A N Q T I N G H F L - - - - - K E P F P E S T - D N F - I L F D E V S P T - - - - -
      Transmembrane
      - T S G D P V E K K D E T - - - P F G V S V A V G L A V F A C L F L S T L L V L N K C G R R R K F G I N
      I P S I D V I D K T G R E H L S V Y A V Y I A S V V G F - C - L L V M L F L L - - K L A R H S K F G M K
      - P P I T V T H K P E E D - - - T F G V S I A V G L A A F A C Y L L V Y L F V M I N K Y G R R R S K F G M K
      Juxtamembrane
trkA 446 R P - A V L A P E D G L A M S L H F M T L G G S S L S P T E - G K G S G L Q G - - - - H I I E
trkB 466 G P A S V I S N D D D S A S P L H H I S N G S H I P S S S E G G P D A V I I G M T K I P V I E
trkC 466 G P V A V I S G E E D S A S P L H H I N H G I T I P S S L D A G P D T V V I G M T R I P V I E
      N P Q Y F - - - - - S D A C V H H I K R R D I V L K W E L G E G A F G K V F L A E C H N L L P E O D
      N P Q Y F G I T N S O L K P D T F V Q H I K R R H N I V L K R E L G E G A F G K V F L A E C Y N L C P E O D
      N P O Y F R O G H N C H K P D I Y V Q H I K R R D I V L K R E L G E G A F G K V F L A E C Y N L S P T K D

```

Figure 2B

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532 KHLVAVKALKEASESARQDFOREAELLIMLOHOHIVRFFFGVCTEGRP
566 KILVAVKTLKSDASDNARKDFHREAELLIMLOHEHIVKIFYGVCVEGDP
588 KMLVAVKALKDPTLAARKDFOREAELLINLOHEHIVKIFYGVC6D6DP
Tyrosine Kinase
LLHVFEYMRHGGDLNRF LRS HGPDAKLLAGGEDV-APGPLGLGOLLAVASQVAA
LIMVFEYMKKHGDLNKF LRA HGPDAVLM AEGMPP--TELTSOMLHIAQQOIAA
LIMVFEYMKKHGDLNKF LRA HGPDAHILVDGQPRQAKGELGLSOMLHIASOIAS
631 GMVYLAGLHFVHRDLATRNC LYGOGLVVKIGDFGMSRDIYSTDYR-
663 GMVYLASQHFVHRDLATRNC LYGENLVKIGDFGMSRDVYSTDYR-
688 GMVYLASQHFVHRDLATRNC LYGANLVKIGDFGMSRDVYSTDYR L
- - - - - VGGRIHMLPIRWHMPPE SILYRKFTTESDYWSFGVYLWEIFT
- - - - - VGGHTMLPIRWHMPPE SIMYRKFTTESDYWSLGVYLWEIFT
FNPSGNDFCIMCEYGGHTMLPIRWHMPPE SIMYRKFTTESDYWSFGVYLWEIFT
717 YGKOPWYQLSNTAEIDCITGRELERPRACPEVYAIHRGCWOREPO
749 YGKOPWYQLSNEVIECITGRYLORPRTCPOEVYELMLGCHOREPH
788 YGKOPWFQLSNTVEIECITGRYLERPRVCPKEYDYDMLGCHOREPO
QRHSIKDVHARLQALAOAPPVYLDVVLG
MRKNIKGIHTLLQNLAKASPVYLDILG
ORLNIKEIYKILHALGKATPIYLDILG

```

Figure 2C

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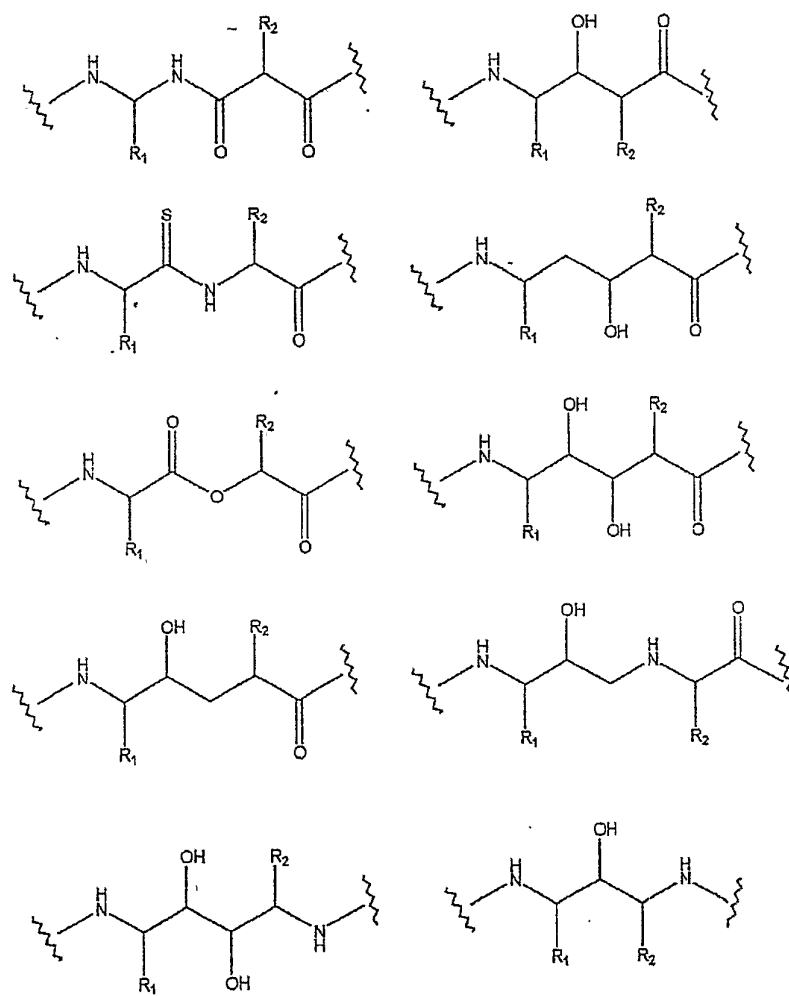


Figure 3A

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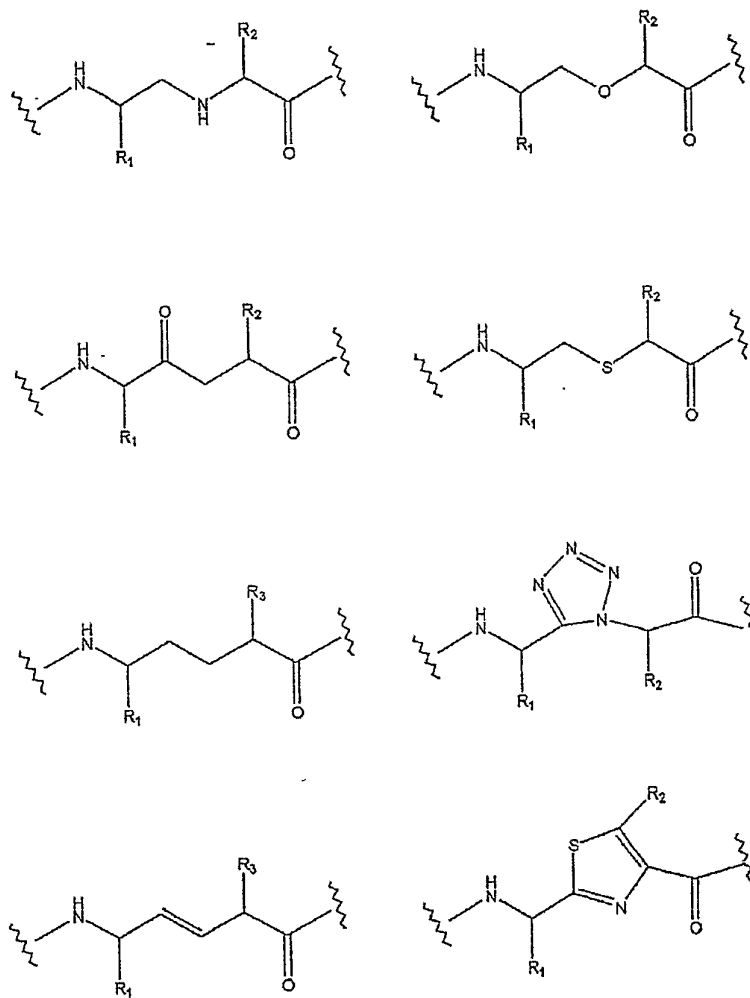


Figure 3B

7/22

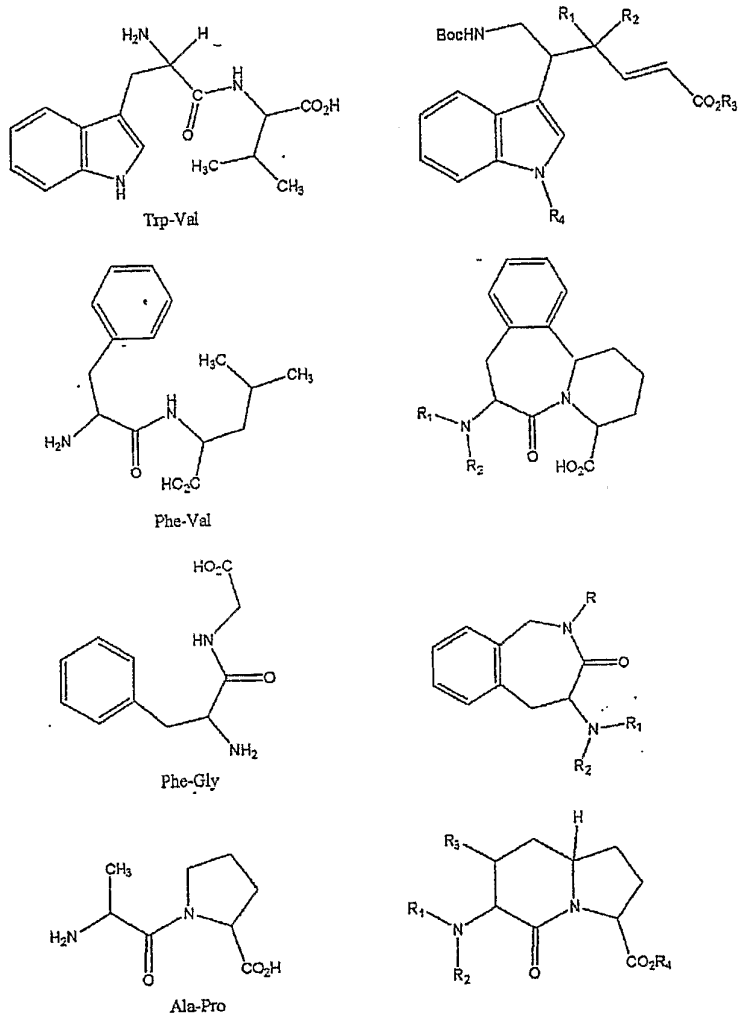


Figure 4

8/22

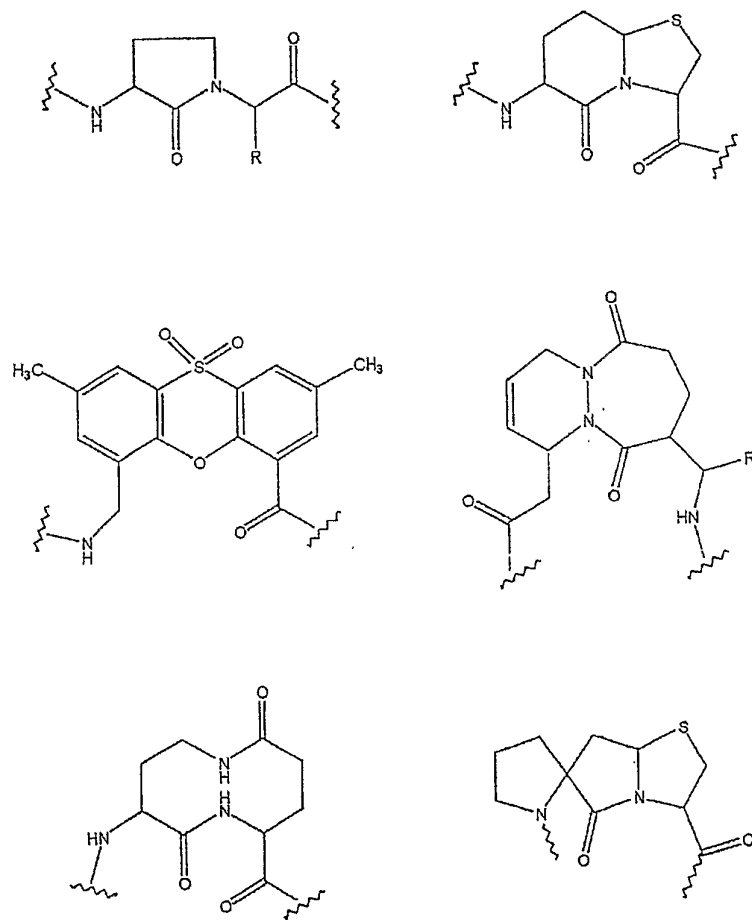


Figure 5

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Figure 6 a

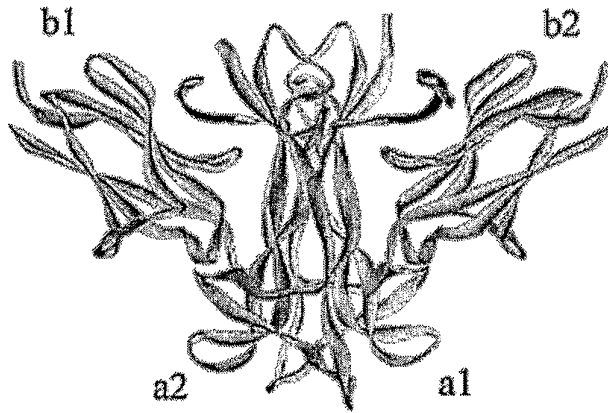


Figure 6 b

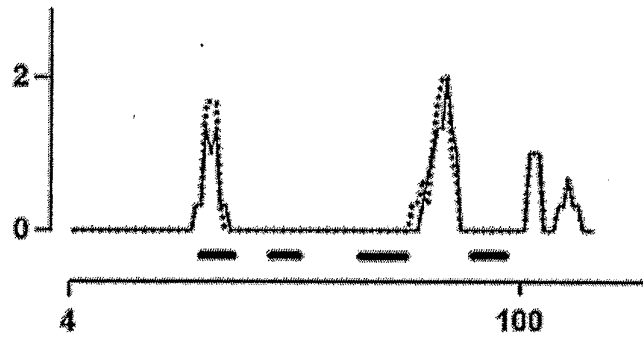
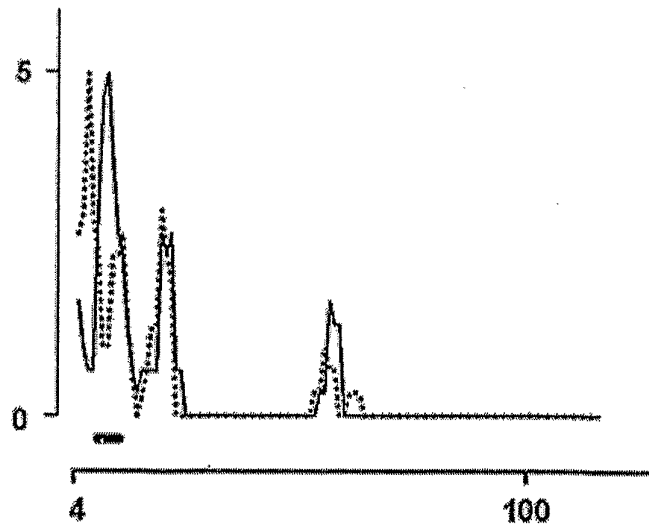


Figure 6 c



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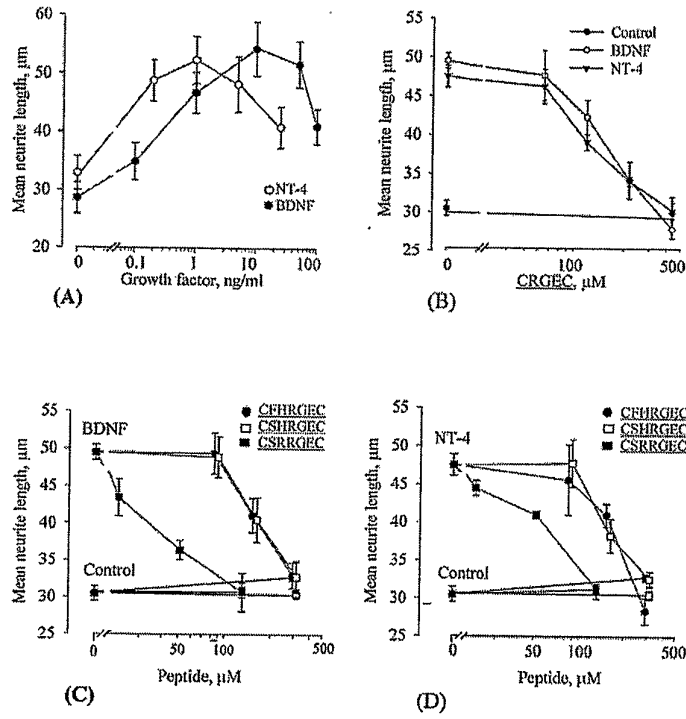


Figure 7

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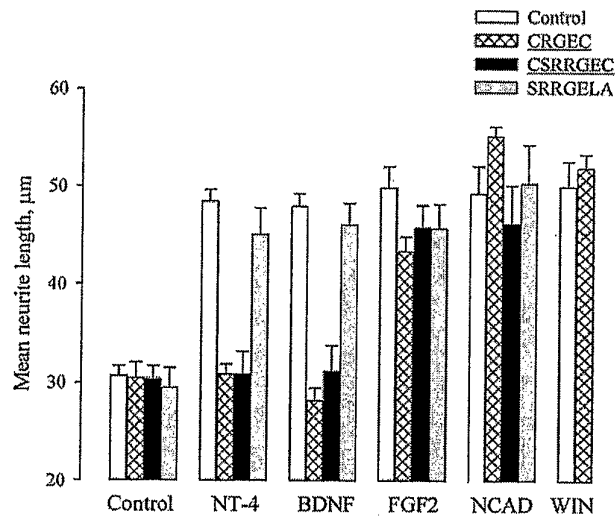


Figure 8

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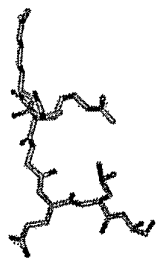


Figure 9A

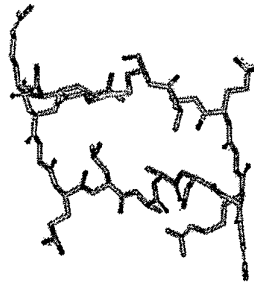


Figure 9B

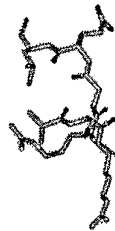


Figure 9C

Figure 10A

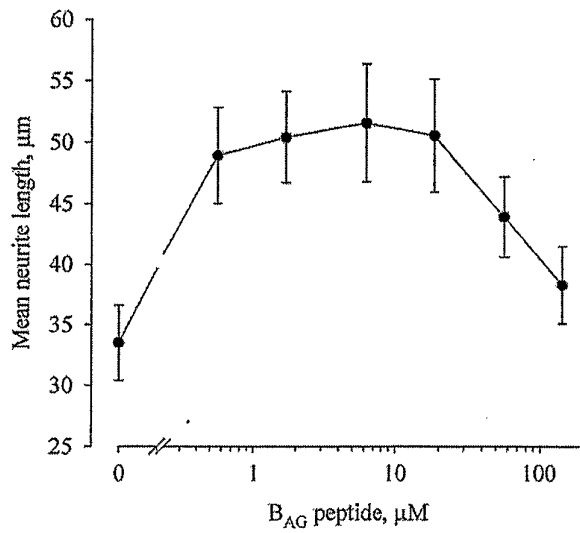
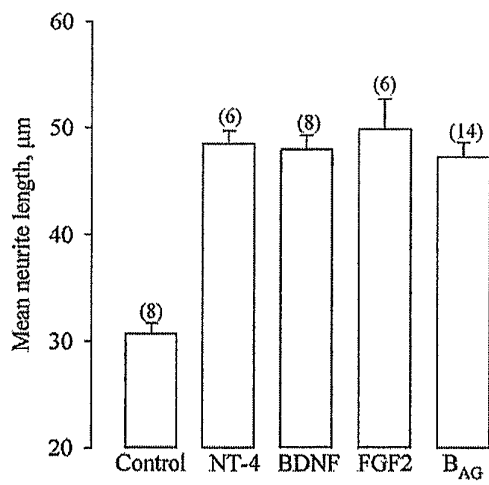


Figure 10B



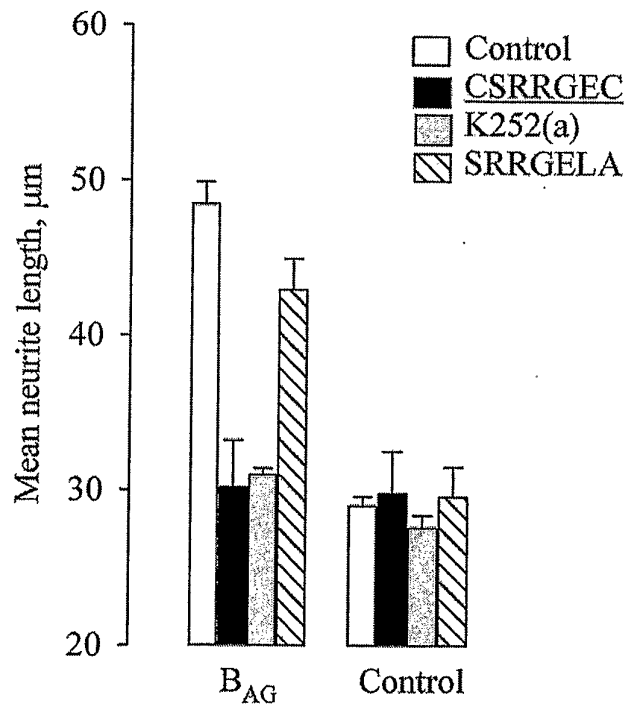


Figure 11

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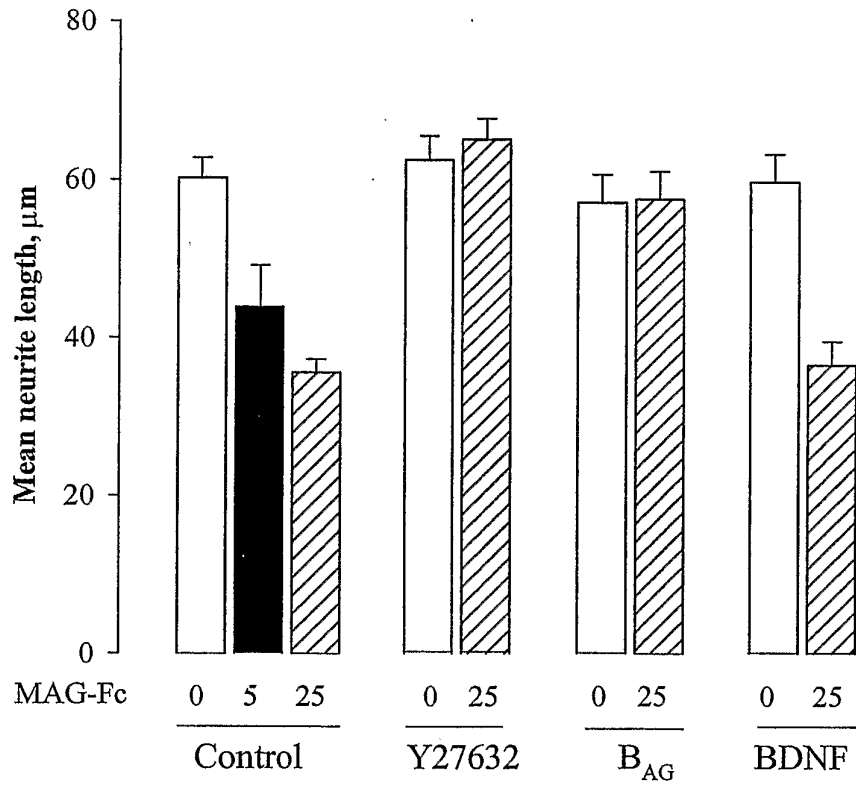


Figure 12

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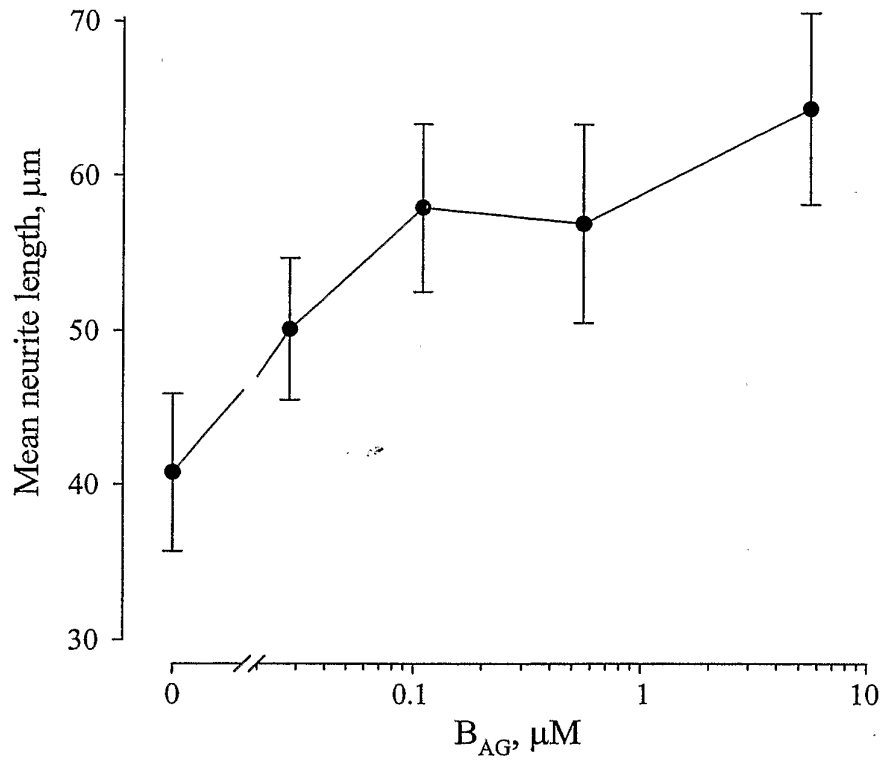


Figure 13

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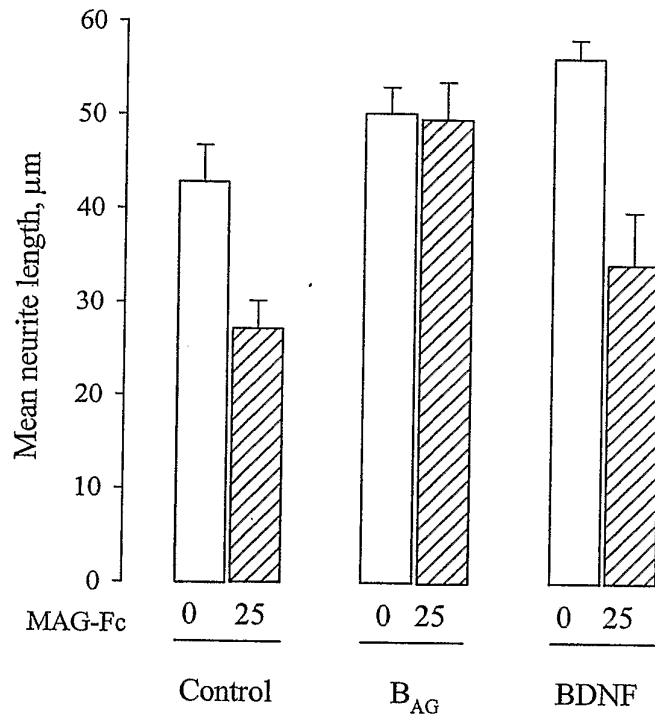


Figure 14

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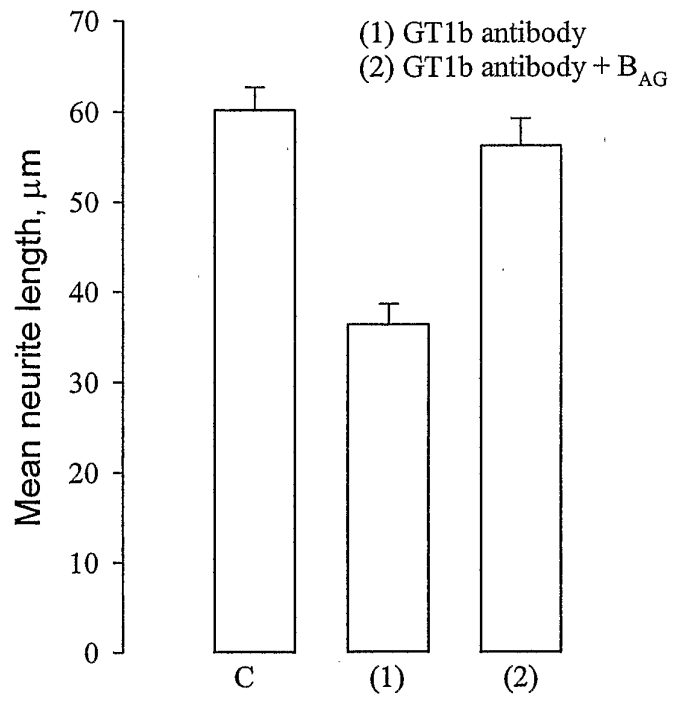


Figure 15

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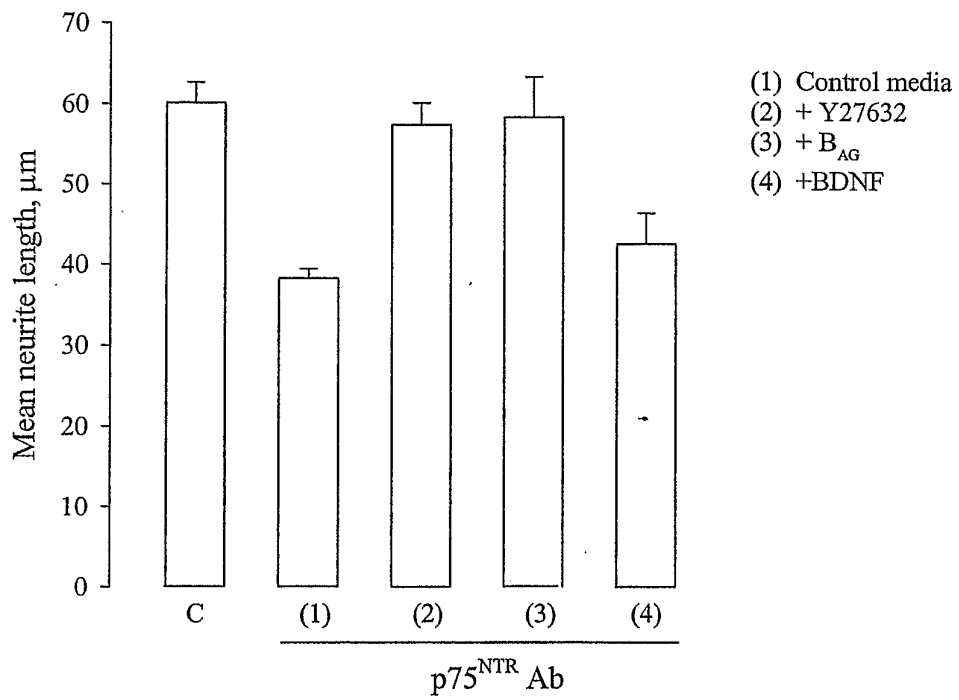


Figure 16

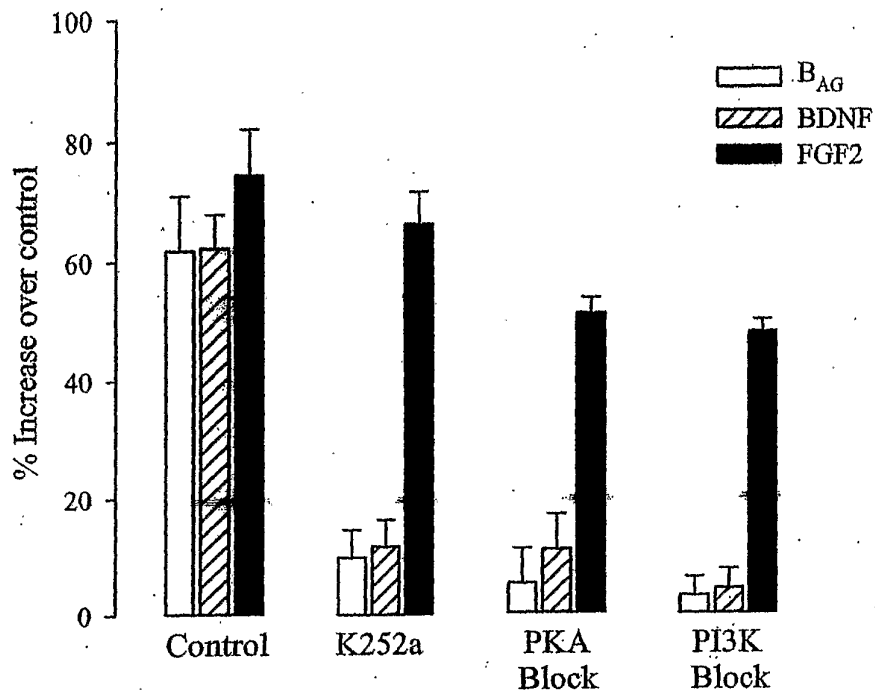


Figure 17

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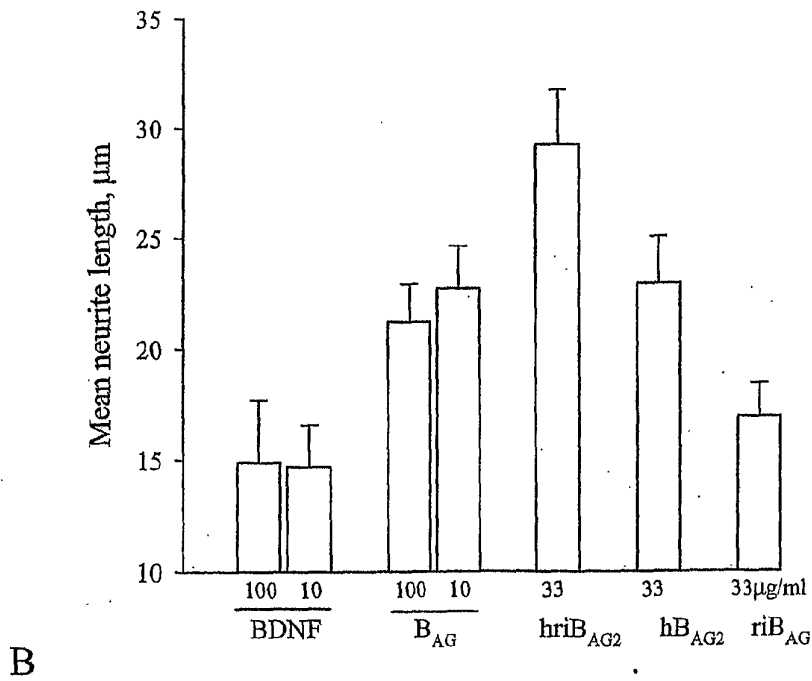
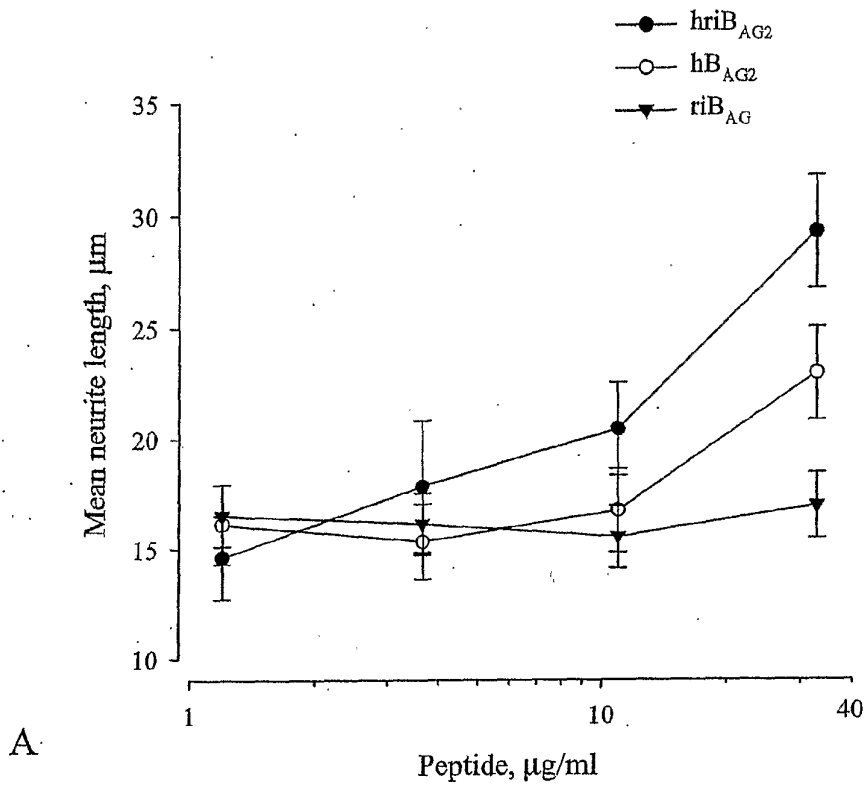


Figure 18 A & B

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BDNF	-----APMKEANIRG---QGGLAYPGVIRTHGTLESVNGPKAGSRGL	38
NT4	-----QP---PPSTLPPFLAP-----	13
NT3	QGNNMDQRSLPEDSLNSLI IKLIQADILKNKLSKQMV DVKENYQSTLPKAEAPREPERGG	60
NGF	-----EPHSESNVPAHGHTIPQVHWTKLQHS LDTALRRARSAPAAAAIARVAG---	47
BDNF	TSLADTFEHVIEELLEDEDQKVRPNEENNKDADLYTSRVMLSSQVPLEPPLFLLEE--YK	96
NT4	-----EWDLLSPRVLSRGAPAGPPLFLLEAGAFR	44
NT3	PAKS-----AFQPVIAMDTLLRQRRYNSPRVLLSDSTPLEPPPLYLMED--YV	108
NGF	-----QTRNITVDPRLPFKRRLRSRVLFSTQPPREAA---DTQDLDFE	88
BDNF	NYLDA-ANMSMR-VRRHSDPA-RRGELSVCDSEWVTAADKKTAVDMSGGTVTVLEKVP	153
NT4	ESAGAPANRSRRGVSETAPAS-RRGELAVCDVAVSGWVT--DRR.TAVDLRGREVEVLGEVP	101
NT3	GSPVV-ANRTSRKRKYAEHKS-HRGEYSVCDSESLWVT--DKSSAIDIRGHQVTVLGEIK	164
NGF	VGGAAPFNRTHRSKRSSHPIFHRGEFSVCDSESVVWVG--DKTTATDIKKEVMVLGEVN	146
BDNF	VSKGQ-LKQYFYETKCNPMGYTKEG-----CRGIDKRHWNSQCRTTQSYVRALTMDSK	205
NT4	AAGGSPLRQYFFETRCKADNAEEGGPGAGGGGCRGVDRRHVSECKAKQSYVRALTADAQ	161
NT3	TGNSP-VKQYFYETRCKEARPVKNG-----CRGIDDKHWNSQCKTSQTYVRALTSENN	216
NGF	INNSV-FKQYFFETKCRDPNPVDSG-----CRGIDSKHWNSYCTTTHTFVKALTMDSG-	197
BDNF	KRIGRWFIRIDTSCVCTLTIKRGR--	229
NT4	GRVGRRWIRIDTACVCTLLSRTGRA-	186
NT3	KLVGRRWIRIDTSCVCALSRKIGRT-	241
NGF	KQAARWFIRIDTACVCLSRKAVRRA	223

Figure 19