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(54) Title: GALANIN AGONISTS

(57) Abstract: Galmic, a non-peptide galanin receptor agonist, affects behaviors in seizure, pain and forced swim tests.



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Galanin Agonists

Description

Technical Field:

5 The invention relates to agonists of galanin. More specifically, the invention relates to the development of molecular libraries having agonist activity with respect to the peptide hormone galanin and to the identification and synthesis of individual bioactive molecules within such libraries.

Background:

10 Galanin, a 29~30 amino acids-long neuropeptide, has been shown to affect feeding, cognitive, sexual behavior, and regulates seizure and pain thresholds when applied intraventricularly (M. E. Vrontakis, *Curr. Drug Target CNS Neurol. Disord.*, (2002) **1**, 531-541; A. Mazarati, et al., *Neuroscientist*, (2001) **7**, 506-517; and T. Bartfai, et al., *Crit. Rev. Neurobiol.*, (1993) **7**, 229-
15 274). Galanin actions are mediated through three GPCR type receptors present in the brain and the peripheral nervous system (T. A. Branchek, et al., *Trends Pharmacol. Sci.*, (2000) **21**, 109-117; S. Wang, et al., *J. Biol. Chem.*, (1997) **272**, 31949-31952; and E. Heuillet, et al., *Eur. J. Pharmacol.*, (1994) **269**, 139-147). Galanin binds to at least three different G-protein coupled
20 receptors (GalR1-3) and influences such processes as insulin secretion, gut secretion/motility, memory, sexual behavior, and pain regulation among others. Transgenic mice overexpressing galanin have much higher seizure thresholds (M. Kokaia, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2001) **98**, 14006-14011) and the GalR1 (-/-) mice have spontaneous seizures, demonstrating the role of
25 galanin and its receptor in seizure control (A. S. Jacoby, et al., *Brain Res. Mol. Brain Res.*, (2002) **107**, 195-200). Site-directed mutagenesis studies on a sixteen-amino acid fragment have shown that this peptide binds to galanin

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receptor type 1 (GalR1) through three amino acid residues (Trp², Asn⁵, Tyr⁹), thought to be in an alpha-helical conformation, as well as through the N-terminal residue.

5 Despite the wealth of biological information on galanin signaling, no progress has been made in utilizing the galanin receptor subtypes as drug targets. The lack of progress is largely due to the poor results from random screening at several major pharmaceutical companies (T. Branchek, et al., *Ann. N.Y. Acad. Sci.*, (1998) **863**, 94-107). More than 4 million compounds
10 were screened but no workable compounds for medicinal chemical optimization were identified. Both Johnson & Johnson (M. K. Scott, et al., *Bioorg. Med. Chem.*, (2000) **8**, 1383-1391) and Schering have published high micromolar compounds that had stability and other problems (T. Branchek, et al., *Ann. N.Y. Acad. Sci.*, (1998) **863**, 94-107).

15 Galnon (Figure 1) is a non-peptide galanin receptor ligand that was designed to display analogs of the three major pharmacophores of galanin: (Trp², Asn⁵, Tyr⁹) on a linear, peptide-like backbone (A. Jureus, et al., *J. Pept. Res.*, (1997) **49**, 195-200; and T. Land, et al., *Brain Res.*, (1991) **558**, 245-
20 250). Galnon is a low affinity, non-receptor subtype selective agonist that acts at both GalR1 and GalR2 type receptors (K. Saar, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2002) **99**, 7136-7141). Despite this lack of specificity, galnon has been shown to affect behavioral symptoms and neurochemical correlates in opiate withdrawal (V. Zachariou, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2003)
25 **100**, 9028-9033), pain (W. P. Wu, et al., *Eur. J. Pharmacol.*, (2003) **482**, 133-137), and seizure (K. Saar, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2002) **99**, 7136-7141) and to exert antidepressant-like effects in a forced swim test.

Chu and co-workers found that a complex natural product exhibited

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micromolar levels of inhibition (IC_{50}) against hGalR1, but this compound has not been further developed. (Chu, M.; et al., *Tetrahedron Lett.* **1978**, 387, 6111.) Bartfai and co-workers demonstrated that a twenty amino acid chimeric peptide could displace ^{125}I -labeled galanin from membrane-binding sites and
5 that this compound exhibited antagonistic activity in vivo against the neuronal actions of galanin. (Fisone, G. et al., *Proc. Natl. Acad. Sci.* **1989**, 86, 9588-9591; Bedecs, K. et al., *Neuropeptides* **1995**, 29, 137-143.) However, such peptides tend to have low in vivo stability and cannot cross the blood-brain barrier. More recently Scott et al., disclosed a library of dithiine
10 and dithiepine compounds with submicromolar IC_{50} s as antagonists of hGalR1. (U.S. Patent No. 6,407,136, **2002**.) The presence of highly reactive alpha-beta-unsaturated sulfones in these compounds make their use problematic in a clinical setting. Quite recently, a dipeptide derivative, Galnon, exhibited agonist activity at galanin receptors in addition to broad spectrum
15 anti-convulsant properties. (Saar, K., et al., U.S. Patent Application Publication US 2003/0055000; Saar, K., et al., *Proc. Natl. Acad. Sci.* **2002**, 99, 7136-7141.) Nevertheless, there remains a need in the art for galanin agonists and antagonists having the desirable properties noted above.

20 Recognition at the molecular level is a fundamental characteristic of biochemical systems. Models developed in bioorganic chemistry have revealed the importance of complementarity in size, shape, and functional groups in molecular recognition. Structures that feature a cleft are particularly effective in regard to complementarity since functional groups attached to the
25 interior of the cleft converge on substrates held inside. J. Rebek has described the use of such clefts for recognition of acids, bases, amino acids, metal ions, and neutral substrates and for providing microenvironments complementary to asymmetric molecules. (J. Rebek, Jr., *Science* (1987) **235** (4795), 1478-84).

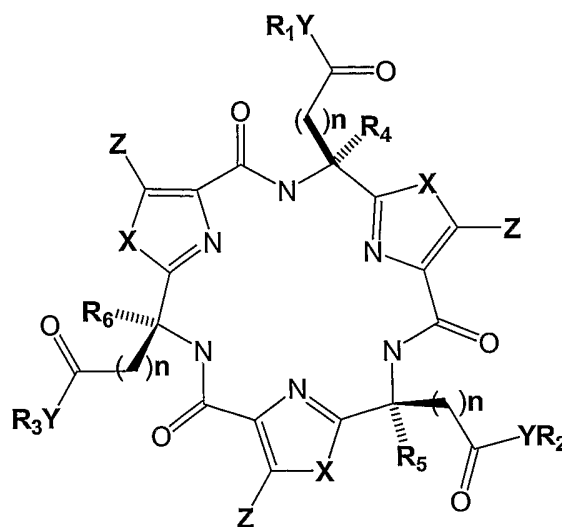
- 4 -

Summary:

The pharmacological exploitation of the galanin receptors as drug
5 targets for treatment of epilepsy, depression and pain has been hampered by
the lack of workable compounds for medicinal chemists from random screening
of large chemical libraries. The present disclosure uses the tripeptidomimetic,
galnon, and displays its presumed pharmacophores on a rigid molecular
scaffold. The scaffold is related to marine natural products and presents three
10 functional groups near one another in space, in the manner reminiscent of a
protein surface. An active compound, Galmic, was identified from a synthetic
library and tested *in vitro* and *in vivo* for its affinity and efficacy at galanin
receptors. Galmic has micromolar affinity for GalR1 ($K_i=34.2 \mu\text{M}$) and virtually
no affinity for GalR2 receptors. *In vitro*, Galmic, like galanin, suppresses
15 long-term synaptic plasticity (LTP) in the dentate gyrus; it blocks status
epilepticus when injected intrahippocampally or administered intraperitoneally.
Galmic applied intraperitoneally shows antidepressant-like effects in the forced
swim test and it is a potent inhibitor of flinching behavior in the inflammatory
pain model induced by formalin injection. These data further implicate brain
20 and spinal cord galanin receptors as drug targets and provide an example of a
systemically active compound based on a scaffold that mimics protein
surfaces.

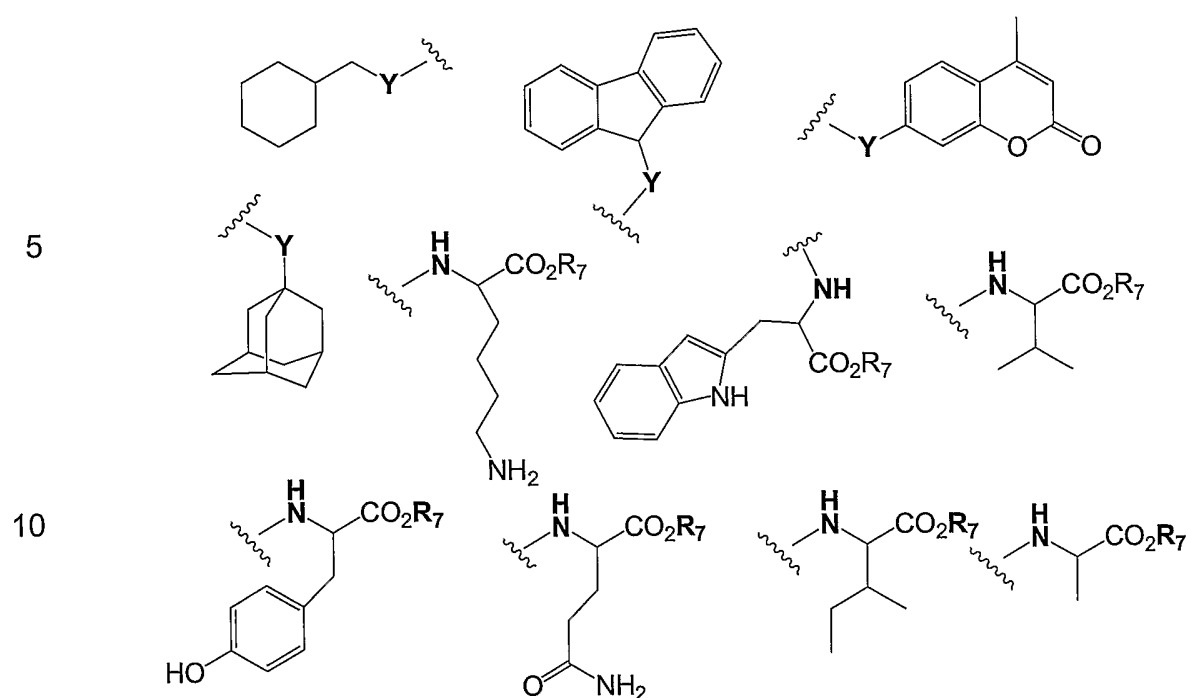
One aspect of the invention is directed to a compound represented by
25 the following structure:

- 5 -

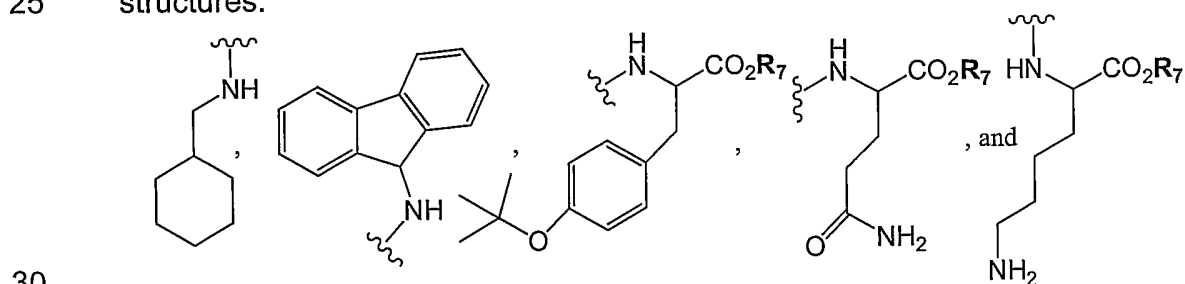


In the above structure, **X** is a diradical independently selected at each occurrence from the group consisting of -O- and -S-; **Y** is a diradical independently selected at each occurrence from the group consisting of -O- and -NH-; **Z** is a radical independently selected at each occurrence from the group consisting of -H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl; **R**₁, **R**₂, and **R**₃ are each a radical independently selected from the group consisting of -H, C₁₋₁₀ alkyl, substituted C₁₋₁₀ alkyl, -(C₀₋₄ alkyl)-(C₆₋₁₄ aryl), substituted -(C₀₋₄ alkyl)-(C₆₋₁₄ aryl), -(C₀₋₄ alkyl)-(C₁₋₁₂ heterocyclyl), and substituted -(C₀₋₄ alkyl)-(C₁₋₁₂ heterocyclyl), and if **Y** is -NH-, then **YR**₁, **YR**₂, and **YR**₃ may each optionally and independently form an amino radical of naturally occurring or non-naturally occurring amino acid; **R**₄, **R**₅ and **R**₆ are each independently C₁₋₆ alkyl, optionally substituted with 1-3 of -F, -Cl, -Br, -I, -OH, C₁₋₄ alkoxy, -NH₂, C₁₋₄ aminoalkyl, C₂₋₄ aminodialkyl, -SH, C₁₋₄ thioalkyl, or oxo; and $n = 0-5$. However, there is a proviso that at least one of **YR**₁, **YR**₂, and **YR**₃ is a presumed pharmacophore of galnon or analog thereof, selected from the group consisting of the following radicals:

- 6 -

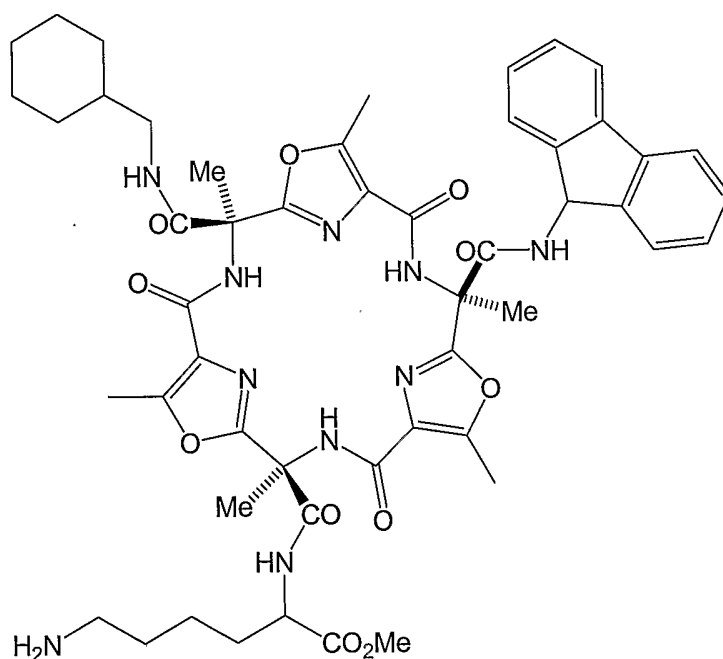


15 Within the above radicals, R_7 is a radical independently selected, at each occurrence, from the group consisting of $-H$, C_{1-6} alkyl, substituted C_{1-6} alkyl, $(C_{0-4}$ alkyl)- $(C_{6-14}$ aryl), substituted $(C_{0-4}$ alkyl)- $(C_{6-14}$ aryl), and phosphodiester linked oligonucleotide tag, wherein alkyl and aryl groups are optionally substituted with up to 3 substituents selected from the group consisting of $-F$,
 20 $-Cl$, $-Br$, $-I$, $-CN$, $-NO_2$, $-OH$, $-COOH$, $-CONH_2$, $-NH_2$, $-SH$, C_{1-4} alkyl, C_{1-4} oxyalkyl, $-C(O)(O-C_{1-4}$ alkyl), C_{1-4} aminoalkyl, C_{1-4} thioalkyl, tri(C_{1-4} alkyl)silyl. Alternatively, the presumed pharmacophore of galnon or analog thereof may be more narrowly defined so that YR_1 , YR_2 , and YR_3 are each independently selected from the group of consisting of radicals represented by the following
 25 structures:



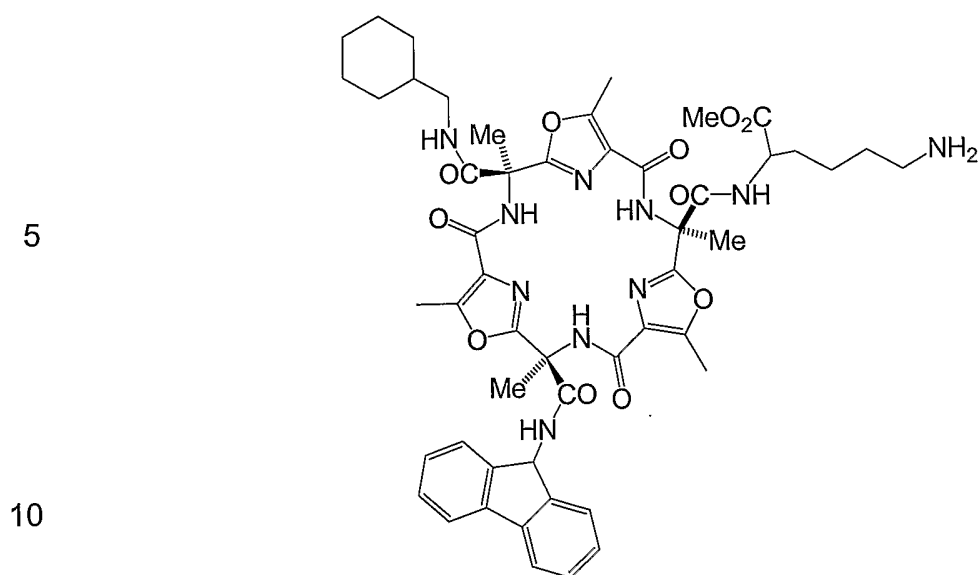
- 7 -

In a preferred embodiment of this aspect of the invention, $-YR_1$, $-YR_2$, and $-YR_3$ are each $-OR_1$, $-OR_2$, and $-OR_3$, respectively. More particularly, $-YR_1$, $-YR_2$, and $-YR_3$ are each independently C_{1-6} alkoxy, substituted C_{1-6} alkoxy, $-O-(C_{0-4}$ alkyl)-(C₆₋₁₄ aryl), or substituted $-O-(C_{0-4}$ alkyl)-(C₆₋₁₄ aryl), subject to the above proviso. In another preferred embodiment, YR_1 , YR_2 , and YR_3 are each $-NHR_1$, $-NHR_2$, and $-NHR_3$, respectively. In another preferred embodiment, R_7 is selected from the group consisting of $-H$, methyl, and benzyl. More particularly, R_7 is methyl; or R_4 , R_5 and R_6 are each methyl; or $n = 0$ or 1; or $n = 0$; or X is O; or X is S; or X is O and Z is methyl; or Z is methyl. Preferred examples include the following structures:



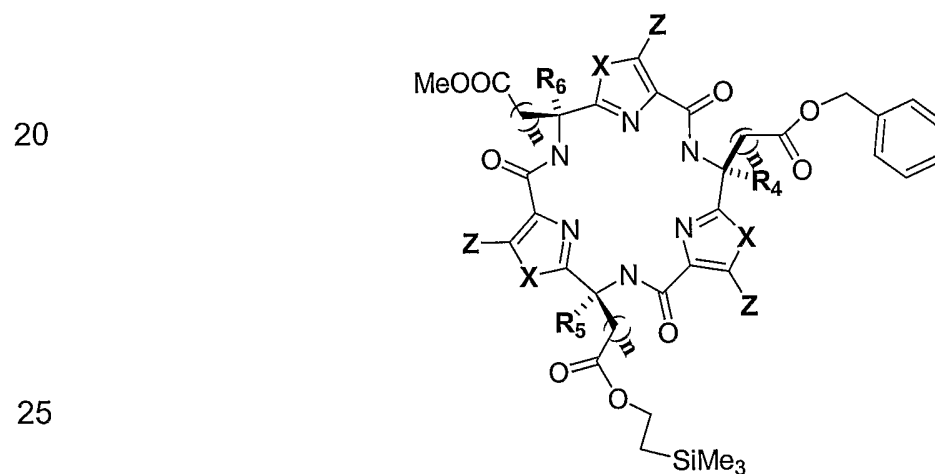
and

- 8 -



The second aspect of the invention is directed to a chemical intermediate employable for synthesizing compounds of the above first aspect of the invention. The chemical intermediate is represented by the following structure:

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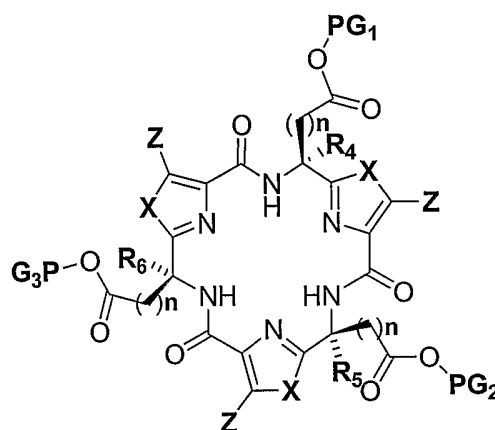
In the above structure, **X** is a diradical independently selected at each occurrence from the group consisting of -O- and -S-; **Z** is a radical independently selected at each occurrence from the group consisting of -H, **C**₁₋₆ alkyl, **C**₂₋₆ alkenyl, and **C**₂₋₆ alkynyl; **R**₄, **R**₅ and **R**₆ are each independently

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- 9 -

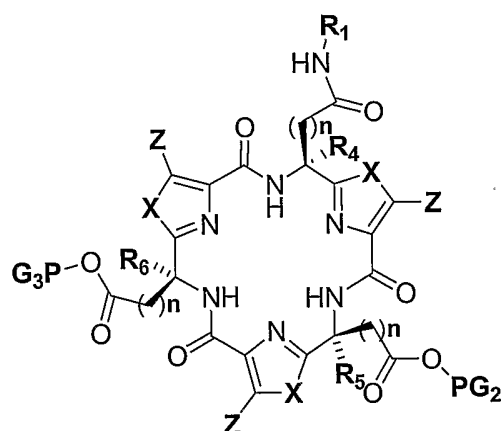
C₁₋₆ alkyl, optionally substituted with 1-3 of -F, -Cl, -Br, -I, -OH, C₁₋₄ alkoxy, -NH₂, C₁₋₄ aminoalkyl, C₂₋₄ aminodialkyl, -SH, C₁₋₄ thioalkyl, or oxo; and n = 0-5.

A third aspect of the invention is directed to a method of making a compound of claim 1. In the first step of this aspect of the invention, a compound of formula I is reacted with H₂ in the presence of a suitable catalyst, followed by reaction with R₁NH₂ in the presence of a coupling reagent to yield a compound of formula II. The compound of formula I is represented as follows:



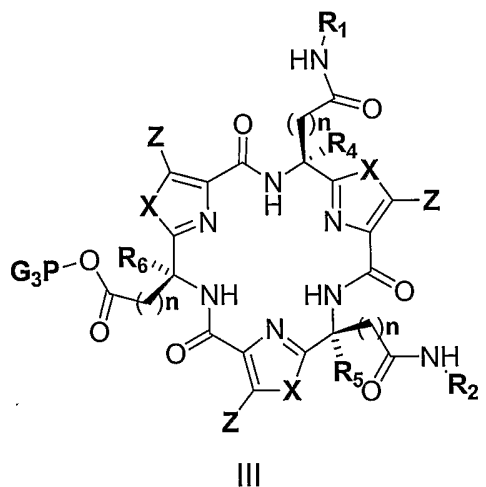
In the above structure, X, Z, n, R₄, R₅, and R₆ are each as defined in the compound that comprises the first aspect of the invention. PG₁, PG₂ and PG₃ are orthogonal protecting groups. The preferred orthogonal protecting groups are the protecting groups employed in the chemical intermediate that comprises the second aspect of the invention, viz., PG₁ is benzyl, PG₂ is trimethylsilylethyl, and PG₃ is methyl. R₁ of R₁NH₂ is the same as defined in the first aspect of the invention. The compound of formula II is represented as follows:

- 10 -



II

In the next step of this aspect of the invention, the compound of formula II is reacted with a fluoride source, followed by reaction with R_2NH_2 in the presence of a coupling agent, wherein R_2 is as defined in the first aspect of the invention, to yield a compound of formula III:



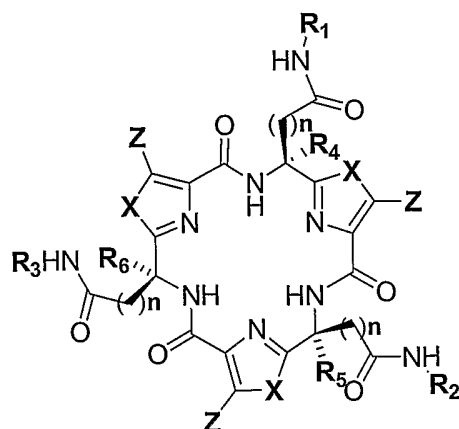
III

In the next step of this aspect of the invention, the compound of formula III is reacted with a hydrolytic agent followed by reaction with R_3NH_2 in the presence of a coupling agent, wherein R_3 is as defined in claim 1 to yield a compound of the following structure:

30

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5



Another aspect of the invention is directed to a pharmaceutical
 10 composition comprising a pharmaceutically effective amount of the compound
 of the first aspect of the invention or a pharmaceutical salt thereof, and a
 pharmaceutically acceptable carrier or diluent.

Another aspect of the invention is directed to a method for treating a
 15 mammal having a condition mediated by a galanin deficiency comprising
 administering to the mammal in need thereof the composition of the
 pharmaceutical composition described in the previous aspect of the invention.
 In a preferred mode, the mammal is suffering from at least one condition
 selected from the group consisting of Alzheimer's disease, depression,
 20 epilepsy, and an eating disorder.

Brief Description of Figures:

Figure 1 illustrates the structures of Galnon and of various platform
 25 structures that present C_3 symmetry, viz. Dendroamide C, a prior art synthetic
 scaffold (1), and Galmic.

Figure 2 illustrates a synthetic method for producing Galmic.

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Figure 3 illustrates a representation of the calculated molecular structure of Galmic.

5 Figure 4 illustrates a chart for presenting the effects of Galmic on long-term synaptic plasticity in mouse dentate gyrus (DG) slices.

Figure 5 illustrates a bar chart for presenting the anti-seizure activity of Galmic.

10 Figure 6 illustrates a graph and a bar chart representing the effects of Galmic on formalin induced flinching (A).

15 Figure 7 illustrates a bar chart for presenting the results of open field and force swim tests for Galmic.

Figure 8 illustrates a table comparing the affinity of Galmic and galnon for galanin receptors.

20 Figure 9 illustrates a procedure for synthesizing oxazole derivatives (18a and 18b) employable for making macrocycles such as compound 1 and Galmic.

Figure 10 illustrates a procedure for linking three oxazole derivatives (18a and 18b) to form a linear intermediate (21).

25 Figure 11 illustrates a procedure for cyclizing the linear intermediate (21) to form a macrocycle having three oxazoles linked by *trans* amide bonds (11).

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Figure 12 illustrates a procedure for derivatizing the cyclic intermediate (11) for producing Galmic (2) or a generic form of the invention (12).

Figure 13 illustrates an alternative method for cyclizing a linear intermediate (3a or 3b) to form a macrocycle having three oxazoles linked to one another with *trans* amide bonds, each of the linking amide bonds having an asymmetric carbon, each asymmetric carbon having a differentially protected ester (6a or 6b).

10 Detailed Description:

The present disclosure describes the synthesis and characterization of a library of triamides having galanin receptor agonist activity. The library of triamides was derived from the coupling of amines to the triacid (Figure 2). The library was screened and one agonist of particular activity was identified as being a systemically active, subtype-selective agonist of galanin receptor. The agonist is herein given the name of Galmic.

Galmic represents a second generation peptide ligand mimic that, through its interactions with galanin receptors, exhibits galanin agonist-like effects in a variety of animal models upon systemic administration.

Galmic employs a platform that was inspired by a number of biologically active and peptide-derived marine natural products such as Dendroamide C (D. Faulkner, *J. Nat. Prod. Rep.*, (1999) **16**, 155; G. R. Pettit, *Pure Appl. Chem.*, (1994) **66**, 2271; M. G. Garson, *Chem. Rev.*, (1993) **93**, 1699; B. S. Davidson, *Chem. Rev.*, (1993) **93**, 1771; and N. Fusetani, et al., *Chem. Rev.*, (1993) **93**, 1793), macrocycles that contain three oxazole or thiazole rings linked by *trans* amide bonds (G. Haberhauer, et al, *Tetrahedron Letters* (2000), **41**, 5013-5016; L. Somogyi, et al., *Tetrahedron* (2001) **57**, 1699-1708; and Haberhauer et al., *Eur. J. Org. Chem.* (2003), 3209-3218). The most favored

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conformations feature all the basic oxazole nitrogens and the hydrogens of the secondary amides directed to the center of the macrocycle. This self-complementary array of hydrogen bond donors and acceptors in the interior of the macrocycle fixes the molecule's conformation; the macrocycle is expected to be rigid and roughly planar. Little space is left within the center of the structure and only minimal hydration is expected for the inwardly-directed N-H bonds. Accordingly, the molecule is a platform that can present three functional groups on the same face. It serves as core structure for solution phase combinatorial chemistry.

Finding nonpeptide, systemically active, small ligands that act as agonists at neuropeptide receptors is a difficult task, and was it not for the example of morphine many would have given up. The approaches that lead to the development of neuropeptide antagonists have utilized random screening of large chemical libraries to find molecules that bind to the neuropeptide receptor, followed by painstaking medicinal chemistry work to improve affinity, selectivity and to afford proper pharmacokinetic and metabolic properties. This process requires that there are some compounds in the library that bind with micromolar or better affinity to the neuropeptide receptor. Such "hits" have not been easy to find for the galanin receptors (T. Branchek, et al., *Ann. N.Y. Acad. Sci.*, (1998) **863**, 94-107) and the two low affinity compounds that were found were chemically not tractable. While most neuropeptide receptor ligands that have reached clinical use are antagonists (T. Hokfelt, et al., *Lancet Neurol.*, (2003) **2**, 463-472), some neuropeptide receptor agonists have been developed from peptidomimetics, most often for cyclic peptide ligands such as somatostatin receptor agonists (A. Janecka, et al., *J. Pept. Res.*, (2001) **58**, 91-107). In the case of galanin receptors, a tripeptide mimetic library based on the pharmacophores of galanin yielded a compound galnon, which, despite its low affinity and poor receptor subtype selectivity, has rapidly become a tool in studies on the role of galanin in seizures, cognition, pain, feeding and opiate

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withdrawal (K. Saar, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2002) **99**, 7136-7141; V. Zachariou, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2003) **100**, 9028-9033; and W. P. Wu, et al., *Eur. J. Pharmacol.*, (2003) **482**, 133-137).

5 The present disclosure describes the structure of Galmic (Figure 1). Despite its high molecular weight (Mw 984), Galmic penetrates the blood brain barrier reasonably quickly as evidenced by its effects in suppressing status epilepticus after intraperitoneal injection of a 2 mg/kg i.p. dose (Figure 5C). Under conditions of intrahippocampal administration, Galmic was 6-7 fold more
10 potent than Galnon (K. Saar, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2002) **99**, 7136-7141) in inhibiting SSSE. Similarly, systemically administered Galmic has potent behavioral effects in the forced swim test where it exhibits antidepressant like activity at 15 mg/kg i.p. dose (Figure 6). Its effects are clearly observable within 40 minutes of the intraperitoneal injection. The
15 penetration of Galmic into the brain may be passive or may be mediated by some transporter. If the latter, it must have a rather high capacity and velocity, because the compound showed rapid CNS effects and, being a low affinity ligand at galanin receptors (Figure 8), a 10^{-5} M or higher concentration must be achieved in the brain to exert the observed effects in seizure and behavioral
20 models (Figure 5, 6 and 7) used here. The properties of the scaffold and of the attached side chains in relation to crossing the blood brain barrier are worth further investigation. The *in vitro* and *in vivo* effects of Galmic reported here are congruent with the action of a galanin type 1 receptor agonist and occur at similar doses as those of galnon (2-20 mg/kg i.p.), the tripeptide mimetic (K.
25 Saar, et al., *Proc. Natl. Acad. Sci. U. S. A.*, (2002) **99**, 7136-7141).

 The interpretation of the pharmacologic spectrum of the effects of Galmic is somewhat hampered by its low affinity for the GalR1 type. The K_i value of 34.2 μ M requires that high doses of the compound be used in the
30 experiments; thus, it can not be ruled out that Galmic might be able to bind to

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additional proteins such as receptors, ion channels and enzymes and thereby contribute to the observed effects. Nevertheless, the effects that are disclosed herein mimic those of galanin injected intra-hippocampally or intra-cerebroventricularly. Accordingly, the effects reported in figures 4-7 are ascribed to the galanin receptor agonist properties of Galmic.

Secondary amides such as peptides are regarded as liabilities in transport across membranes because their hydration, particularly at the N-H bonds, resists partitioning into hydrophobic environments. The activity of Galmic compound *in vivo* was, therefore, unexpected as it requires transport across membranes including the blood/brain barrier. The secondary amides that are part of the macrocyclic framework are internally solvated since, at best, a single water molecule can be accommodated in the center of the structure. The amides that attach the side chains to the periphery of the scaffold are also capable of internal solvation: the NH donors of the peripheral amides are hindered to external water but can find their complements internally in the N and O acceptors of the oxazole subunits through rotations around the bonds indicated.

There is evidence for an α -helical structure of galanin in trifluoroethanol (A. Ohman, et al., *Biochemistry (Mosc.)*, (1998) **37**, 9169-9178), but little is known about the conformation of galanin (or, as is the case at hand, of galnon) that is ultimately recognized by galanin receptor. An ingenious terphenyl scaffold devised by Hamilton (B. P. Orner, et al., *J. Am. Chem. Soc.*, (2001) **123**, 5382-5383; and J. T. Ernst, et al., *Angew. Chem. Int. Ed. Engl.*, (2002) **41**, 278-281) mimics the positions of the *i*, *i*+3 and *i*+7 of the side chains in an α -helix. At first glance, the trioxazole platform does not achieve this presentation of side chains, but rotations about the bonds indicated can shorten and lengthen the distances between the side chains. More importantly, the rigidity of the scaffold ensures that the 3 side chains all appear

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on the same face of the structure. A calculated structure is shown in Figure 3.

Materials and Methods:

It is disclosed herein that the display of side chains identified as active
5 in galanin or those of the nonpeptide galanin receptor agonist galnon on a
rigid platform creates a compound capable of recognition by the receptor
GalR1. Mixtures of 3 to 4 amines were coupled to the triacid platform (D. Mink,
et al., *Tetrahedron Lett.*, (1998) **39**, 5709-5712; G. Haberhauer, et al.,
Tetrahedron Lett., (2000) **41**, 5013-5016; and L. Somogyi, et al., *Tetrahedron*,
10 (2001) **57**, 1699-1708) and obtained small combinatorial libraries. Active
mixtures were obtained (after deblocking with trifluoroacetic acid) from the
amines shown: cyclohexylmethyl amine, **2**; fluorenyl amine **3** and
e-t-BOC-/l-lysine methyl ester **4**. This mixture (11 compounds) was fractionated
by HPLC and the most active compound (structure **5**, Galmic, or its
15 cyclodiastereomer) was identified by mass spectrometry. The individual
molecule was prepared by total synthesis which will be described elsewhere.

Physical Characterization of Galmic:

¹H NMR-300 MHz (CDCl₃): 9.01 (s, NH); 8.94 (s, NH); 8.89 (s, NH); 7.97 (s
20 broad, 3H); 7.65 (d, J=7.5 Hz, 2H); 7.55 (d, J=7.2 Hz, 1H); 7.42-7.19 (m, 5H);
6.87 (pseudo t, J=5.4 Hz, 1H); 6.15 (d, J=8.4 Hz, 1H); 4.55 (m broad, 1H); 3.61
(s, 3H); 3.38 (s broad, 1H); 3.12 (m, 1H); 2.90 (m, 3H); 2.70 (s, 3H); 2.65 (s,
6H); 2.19 (s, 3H); 2.09 (s, 3H); 2.04 (s, 3H); 2.00-1.28 (m, 14H); 1.20-1.04 (m,
3H); 0.92-0.76 (m, 2H). ¹³C NMR-75 (CDCl₃): 171.61; 168.81; 167.39; 160.44;
25 160.41; 160.11; 159.97; 159.17; 156.09; 156.03; 155.91; 143.62; 143.39;
140.50; 140.43; 128.67 (CH); 128.58 (CH); 127.85 (CH); 127.65 (CH); 124.97
(CH); 124.72 (CH); 119.87 (CH); 60.68; 60.45; 59.90; 55.34 (CH); 52.46 (CH₃);
52.16 (CH); 46.27 (CH₂); 39.55 (CH₂); 37.74 (CH); 30.63 (CH₂); 26.43 (CH₂);
26.23 (CH₂); 25.80 (CH₂); 21.69 (CH₃); 21.50 (CH₃); 21.44 (CH₃); 11.89 (3
30 CH₃). MALDI-FTMS [M+H]⁺: expected: 989.4510; observed: 989.4508.

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Receptor Binding (Cell lines and ligand binding):

The affinity of Galmic for galanin receptors was determined by competitive equilibrium binding experiments using membranes prepared from GalR1 or GalR2 expressing cells. Galmic displaces [¹²⁵I]-galanin binding in Bowes cells (hGalR1) with a K_i value of 34.2 μM, while in CHO-GalR2 cells no competition was observed with up to 100 μM Galmic. Compared with galnon, the first synthetic galanin receptor non-peptide ligand, Galmic seems to be more GalR1 selective but possesses lower affinity (Figure 8).

Stably transfected Chinese Hamster Ovary (CHO) cells expressing rat GalR2, and Bowes' melanoma cells that express human GalR1, were cultivated as described earlier (S. Wang, et al., *J. Biol. Chem.*, (1997) **272**, 31949-31952; and E. Heuillet, et al., *Eur. J. Pharmacol.*, (1994) **269**, 139-147). They were used to determine the affinity of the components of the chemical library for GalR1 and GalR2 receptors, using [¹²⁵I]porcine galanin (2200 Ci/mmol, PerkinElmer Life Science, Boston, MA, USA) (0.2 nM) as tracer. The components of the chemical library were tested as competitors at concentrations 10⁻⁸-10⁻⁴ M. The radioligand binding assay was performed in 150 μL binding buffer [50 mM Tris-Cl (pH 7.4), 5 mM MgCl₂, 0.05 % (w/v) bovine serum albumin, supplemented with peptidase and protease inhibitors: 50 μM leupeptin, 100 μM phenylmethanesulfonyl fluoride and 2 μg/mL aprotinin]. Incubations were carried out at room temperature for 45 min and terminated by rapid vacuum filtration through glass fiber filters (Packard, Meriden, CT, USA). The filters were washed three times and counted in a Gamma counter. The K_i values were determined with Prism software (GraphPad, San Diego, CA).

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Effects of Galmic on the synaptic transmission and plasticity in mouse dentate gyrus (DG) (Electrophysiology):

Administration of DMSO (0.03%, n = 6) or Galmic (1 mM, n = 7) had no significant effect on the fEPSP response of the upper one-third of the molecular layer in DG following single test stimuli at threshold, half-maximal or maximal intensity (data not shown). Similarly, application of galanin (1-29) (n=6) had no significant effect on the input-output curves (data not shown). In the paired-pulse facilitation (PPF) test, Galmic (1 mM) caused a significant reduction in the percentage ratio of the fEPSP response of the second pulse over the first one at 500 msec time interval (t-test: $p < 0.05$ – data not shown). Administration of galanin (1-29) (1 mM) (n=6) also exhibited a similar effect on PPF at the same time interval. On average, application of galanin (1-29) caused $18.3\% \pm 4$ reduction (data not shown) and Galmic reduced the PPF ratio by $15.4\% \pm 4$. The effect of Galmic was evident in six experiments out of seven. The first pulse fEPSP slope was not affected by the galanin (1-29) or Galmic compared to ACSF-treated response. The slope of the second pulse was reduced significantly by $19.6\% \pm 8$ and $14.6\% \pm 5$ following superfusion of galanin (1-29) or Galmic, respectively (t-test: $p < 0.05$). Administration of the 0.03% v/v DMSO used as vehicle had no significant effect on PPF.

The superfusion of DMSO, Galmic or galanin (1-29) was started 10 – 15 min prior to HFTs and continued until one min post LTP induction. At the concentration of 1 mM neither Galmic nor galanin (1-29) affected the baseline recording obtained prior to LTP induction compared to DMSO (0.03%v/v)-treated slices (Figure 4). The level of potentiation was attenuated following application of Galmic or galanin (1-29) in a similar pattern. The level of reduction following application of Galmic was significant at 2 – 6 min post-LTP induction (t-test: $p < 0.05$). Galmic, similar to galanin (1-29), blocked the late-phase of LTP treated slices and on average (21 – 61 min post-LTP induction) caused a $30\% \pm 1$ (Galmic) reduction in the fEPSP slope compared to DMSO treated slices (t-test: $p < 0.005$).

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The hippocampal slices were prepared from male C57Bl/6 mice (4-6 weeks old). Following 1 hour equilibrium period, a glass micropipette (1-5 M Ω) filled with ACSF or NaCl was placed in the outer one-third of the molecular layer (OML) of the inner blade of the dentate gyrus (DG), and a bipolar tungsten stimulating electrode was placed in the apex of the DG to activate the lateral perforant pathway innervating OML. The field potentials were recorded by an AxoClamp 2B (Axon Instrument), digitized and processed using a Digidata and pCLAMP acquisition software (Axon Instrument), respectively. The stability of the fEPSP was established by recording fEPSP responses stimulated at 40-50% of maximum intensity (one/min for 15 min). The input-output curve was generated by stimulating the pathway at three different intensities (threshold, half-maximal and maximally effective). A series of paired-pulse facilitation (PPF) experiments at 15, 30, 50, 100, 200 and 500 msec intervals were carried out prior to and after baseline fEPSP recordings. The baseline fEPSP recording was conducted 15 min prior to and 60 min after LTP induction at 40-50% of the maximal amplitude response. LTP was induced by two (20 sec intervals) trains of high frequency stimulus (HFTs) at 100 Hz for 1 sec at maximally effective stimulus intensity. Ten to fifteen min prior to LTP induction, DMSO, Galmic (dissolved in DMSO 30% v/v) or galanin (1-29) was applied to the bath at the final concentration of 0.03% v/v for DMSO and 1 mM for Galmic and 1 mM for galanin (1-29), and the application was continued until 1 min post-HFTs.

The amplitude of the I-O of fEPSP responses was computed using Clampfit software (Axon Instrument). The initial slope of the fEPSP (between the 0 – 50% points) was calculated and the data were normalized to baseline values. Student's t-test was used for statistical analysis.

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Effects of Galmic in experimental status epilepticus (Self-sustaining status epilepticus (SSSE) and drug administration):

When injected into DG 10 min after PPS, Galmic attenuated seizures in a dose-dependent manner (Figure 5A): anticonvulsant effects appeared at 0.1 nmoles, when SSSE duration was between 1 and 2 hours, vs. 10 - 12 hours in control, and became stronger as the dose of the drug was increased to 1 and 5 nmoles. At the dose of 5 nmoles, the compound completely and irreversibly abolished seizures within 5 to 10 min, and spikes within 2 hours after administration. At higher dose-ranges (5 and 10 nmoles), Galmic was effective in attenuating SSSE during its maintenance phase (Figure 5B). Injection of the compound of 10 nM significantly shortened SSSE duration (4 - 5 hours), attenuated its severity (time spent in seizures 20 - 40 min), and shortened the duration of spikes (under 8 hours vs. 24 hours in controls). I.p. administration of Galmic at the dose of 2 mg/kg 10 min after PPS significantly attenuated self-sustaining seizures whereas the 1 mg/kg i.p. dose was without effect (Figure 5C).

SSSE was induced in adult male Wistar rats as previously described (A. M. Mazarati, et al., *J. Neurosci.*, (1998) **18**, 10070-10077). Briefly, animals were subjected to 30-minutes perforant path stimulation (PPS) through a stimulating electrode, which had been chronically implanted into the angular bundle of perforant path, using a Grass stimulator model 8800 with the following parameters: 10 s of 20 Hz trains of 1 ms 30 V pulses delivered every min, together with the continuous 2 Hz stimulation using the same parameters. Electrographic activity was acquired through a recording electrode-guide cannula (Plastics One, Roanoke, VA), which had been chronically implanted into the dentate gyrus ipsilateral to PPS, and analyzed off-line using Harmonie Software (Stellate Systems, Montreal, Quebec) configured for automatic detection and saving of seizures and spikes. The following parameters were calculated: SSSE duration, i.e. the time between the end of PPS and the

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occurrence of the last seizure; time in seizures, i.e. cumulative time spent in software-recognized seizures during SSSE; total number of seizure episodes; spike duration, i.e. time of the occurrence of the last electrographic spike.

Galmic was dissolved in 50% v/v DMSO and was administered into the dentate gyrus using an injection cannula connected to a Hamilton microsyringe and placed into the lumen of the guide cannula. Galmic was injected during either induction phase of SSSE, 10 min after the end of PPS, or during drug-resistant maintenance phase (C. G. Wasterlain, et al., *Epilepsia*, (2000) **41 Suppl 6**, S134-143), 60 min after the end of PPS. Control animals were treated with the vehicle (DMSO). Each group included 4-5 animals. In a separate set of experiments, to test blood-brain permeability, animals received i.p. injections of Galmic, 10 min after PPS (3 animals per group). Data were analyzed using one-way ANOVA, with Bonferroni post-hoc test.

Effects of Galmic in inflammatory pain model (Formalin test):

After an injection of 20 μ l of 2.5 % formalin into the paw, mice displayed two phases of flinching behavior. Phase 1 started with initial intense flinches occurring 1 - 2 min post-injection, followed by a rapid decline at 5 - 6 min. Phase 2 began after 15 - 20 min with the maximal response typically observed around 25 - 30 min after the formalin injection (Figure 6). Injection of Galmic to mice (2.45 – 9.8 μ moles/kg) produced a dose-dependent inhibition on both Phase 1 and 2 (Figure 6). The ED₅₀ value (μ moles/kg, 95% C.I.) of inhibitory effects of Galmic on Phase 1 was 2.9 (2.1-4.1) and Phase 2 was 3.7 (2.6-5.2). Galmic up to the highest doses (9.8 μ moles/kg i.p.) did not produce abnormal sensory or motor functions in these animals. Additionally, Galmic produced antinociceptive effect in the rat formalin test with a similar potency as in mouse. In contrast, Galnon at equivalent doses had no effect in this test (data not shown).

Male C57Bl/6 mice (25-30g, Harlan Sprague Dawley, Indianapolis, IN)

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were used. To quantify formalin paw injection induced flinching/licking behavior, an automated sensing system was employed (T. L. Yaksh, et al., *J. Appl. Physiol.*, (2001) **90**, 2386-2402). Briefly, a C-shape soft metal band (4.8mm wide and 8.5mm long, 0.1g) was placed on one of the hind paws of an animal. After acclimation for 30 min, animals were gently restrained and 20µl of 2.5% formalin solution was injected subcutaneously into the dorsal surface of the banded paw with a 30-gauge needle. Data collection was initiated after the animal was placed inside of the test chamber. Nociceptive behavior was quantified by automatically counting incidences of spontaneous flinching/shaking of the injected paw (T. L. Yaksh, et al., *J. Appl. Physiol.*, (2001) **90**, 2386-2402). The flinches were counted over 1-min intervals for 60 min, and present as total numbers of flinches per min or per phase (Phase1: 1-9 min, Phase 2: 10-60 min, Phase 2A: 10-40 min and Phase 2B: 41-60 min). The animals were sacrificed with CO₂ immediately after the test. Galmic was dissolved in 30 % v/v DMSO, and given intraperitoneally (i.p.) 15 min prior to formalin paw injection.

Effects of Galmic in forced swim test and open field test:

Forced Swim Test:

In the forced swim test, vehicle treated rats displayed initially high levels of activity during the first two minutes of the procedure, followed by prolonged periods of immobility interspersed with intermittent short bouts of activity. By contrast, animals treated with Galmic (15 mg/kg i.p.) exhibited a large (55%) increase in activity in the test, similar to other antidepressant compounds (L. A. Galea, et al., *Behav. Brain Res.*, (2001) **122**, 1-9) and thus consistent with the hypothesis that Galmic displays antidepressant-like properties. The difference in time spent active between the two groups was highly significant ($t_{(15)} = 3.19$, $p = 0.006$) (Figure 7). These results are consistent with the observation that galnol produces dose-dependent increases in activity in the forced swim test (Lu et al., in preparation).

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In the open field test, vehicle treated rats displayed typical thigmotaxic behavior, in which locomotor activity was largely confined to the walled perimeter of the arena, with relatively little time spent in the center. Galmic (15 mg/kg) treated rats exhibited a similar pattern of behavior, although total levels of activity (measured as the numbers of squares crossed) was reduced by more than half – a highly significant effect ($t_{(14)} = 3.66$, $p = 0.003$) (Figure 7).

Open Field Test procedure:

One week after the forced swim test, different groups of rats were tested in the open field test. Animals were treated with Galmic (15mg/kg, i.p.) (n = 10) or vehicle (n = 6), as described above; forty minutes after injection, rats were placed in an open field apparatus. The locomotor activity over a 5 min period was recorded on videotape. Line-crossing behavior (defined as at least three paws in a square) was tallied and compared.

Animals were randomly assigned to either drug treatment group. Rats were injected i.p. either with Galmic (dissolved in 50% DMSO; 15mg/kg, i.p.) (n = 10) or vehicle (50% DMSO, n = 7), in a volume of 2 ml/kg. Forty minutes following injection, animals were subjected to the forced swim test procedure. Rats were placed singly in a cylindrical glass container (48 cm height, 21 cm diameter) that contained tap-water ($25 \pm 1^\circ\text{C}$) to a depth of 27 cm; this depth was just sufficient for the rat's tail to touch the bottom of the container. Each animal was tested for 10 min, all tests were recorded on videotape, and later scored by an experienced observer who was blind to the experimental conditions of the animals. The presence of antidepressant activity in a drug is inferred from its capacity to increase the fraction of time spent in active behavior (F. Borsini, et al., *Psychopharmacology (Berl)*, (1988) **94**, 147-160).

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Synthetic Methods:

Compounds of the invention may be synthesized as shown in Figures 9-13. Synthesis of the component rings of invention scaffolds may be carried out using standard techniques. For example, Figure 9 illustrates the synthesis of oxazole containing precursors to compounds of the invention. The amino malonate **13** is transesterified with an alkali oxide such as PG¹OLi and then reacted under the conditions shown with a hydroxy amino acid derivative such as Boc-serine or Boc-threonine methyl ester (R₄ and Z are as defined herein) to give the amide. The latter compound may be cyclized to the oxazoline by exposure to Burgess' Reagent and converted to oxazole by treatment with NiO₂ in a suitable solvent such as benzene, toluene and the like. A second transesterification may be carried out to give the orthogonally protected compound **15**. One skilled in the art will appreciate that a variety of oxazole building blocks may be prepared by slight modification of these techniques. Furthermore, the oxidation with NiO₂ may be omitted and the oxazoline transesterified to give analogous oxazoline building blocks. Oxazole and oxazoline precursors to compounds of the invention may readily be prepared by slight modification of procedures described in copending U.S. Application No. 09/053,837, hereby incorporated by reference in its entirety.

The acyclic precursor to compounds of the invention may be assembled by iterative procedures well known in the art of peptide synthesis. Thus, for example, as shown in Figure 10, **18a** may be Boc-deprotected with, e.g. TFA or another suitable acid, and coupled to carboxyl-deprotected **18b** (i.e., PG² was previously removed) in the presence of a base such as DIEA using typical peptide coupling reagents such as HBTU or PYBOP. The resulting dimer **20** may be Boc-deprotected again and coupled to a third component as shown to give compound **21**. Alternatively, the R₁, R₂, and R₃ moieties may be installed

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on the building blocks by selective deprotection of the appropriate carboxylic acid and coupling by any of the methods described above. The building blocks are then linked by coupling reactions as before. It should be understood that other suitable protecting groups may be used on the amino and carboxyl groups in the synthesis of the linear precursor so long as an orthogonal protection scheme is maintained.

Macrocyclization of the linear precursor may be effected by a variety of methods well known in the art. For example, coupling agents such as EDCI or PYBOP may be used under typical conditions (i.e., in the presence of a base such as DIEA and a suitable solvent such as DMF) to form an amide bond between the amino- and carboxyl-containing termini of the acyclic precursor in moderate yield. Preferably, the coupling agent DEPBT is used according to Li, H., et al., Organic Letters 1, 91-93 (1999). Figure 13 illustrates another preferred method of macrocyclization where the R_1 , R_2 , and R_3 moieties are either amides or orthogonally protected esters or a combination of both. First, an activated ester **4** is formed from the free terminal carboxyl using, e.g., pentafluorophenol and DCC in a suitable solvent such as EtOAC. The amino protecting group (e.g., Boc) is removed with a suitable acid such as trifluoroacetic acid to give **5**. Heating the latter compound with DMAP in a solvent such as toluene yields the macrocycle **6**.

Figure 12 exemplifies a method for installation of R_1 , R_2 , and R_3 amides from an orthogonally protected macrocycle of the invention. As shown, a first protecting group such as benzyl ester may be removed by hydrogenolysis in a suitable solvent in the presence of a suitable catalyst such as Pd on carbon and the like. The amide is formed from the free carboxyl and an amine (R^1NH_2) by use of typical peptide coupling reagents such as those described herein or others well known in the art. A second protecting group (e.g. trimethylsilylethyl ester) is removed from the resulting compound **10** by

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exposure to a fluoride source such as HF in pyridine, and the free carboxyl is coupled to an amine (R^2NH_2) as before. A third protecting group (e.g. methyl or ethyl ester) is removed from compound **11** by hydrolysis with base (e.g. LiOH, NaOH, KOH, and the like). The final amide is formed from the

5 corresponding amine (R^3NH_2) as above. While Figure 12 depicts compounds of the invention, the invention is not intended to be so limited; the same types of procedures can be used to produce scaffolds employed within compounds of the invention. For example, the techniques disclosed in copending U.S. Application No. 09/053,837 may be utilized to produce compounds of the

10 invention. Those of skill in the art will readily appreciate that a variety of orthogonally protected macrocycles may be prepared using well known techniques and subsequently functionalized in a similar fashion to produce compounds of the invention.

15

EXAMPLES

Example 1: Synthesis of Compounds of the Invention

General Considerations:

20 Reactions were performed under N_2 atmosphere. NMR spectra were recorded on Varian 300 and Bruker DRX 600 spectrometers. Chemical shifts are given in ppm relative to TMS. The spectra were referenced to deuterated solvents indicated in brackets in the analytical data. Thin Layer Chromatography (TLC) were performed on aluminium sheets of silica gel.

25 Purification by column chromatography were performed on silicagel 60 (230-400 mesh; Merck KgaA). Solvents and chemicals were used as purchased from commercial suppliers.

30 **Synthesis of oxazole macrocycles 6a and 6b:**

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To a solution of carboxylic acid **3b** (1.2 g, 1.23 mmol) in absolute ethyl acetate (10 ml) cooled to -20°C was added pentafluorophenol (237 mg, 1.29 mmol) and DCC (266 mg, 1.29 mmol). The reaction mixture was allowed to warm to room temperature overnight, filtered from urea and evaporated. The crude pentafluorophenyl ester was treated with trifluoroacetic acid (5 ml) during 1h at 0°C . Excess acid was evaporated and the residue dried under reduced pressure (ca. 10^{-3} mbar).

Crude ammonium salt was added over 30 minutes in a hot (100°C) solution of toluene containing 3 equivalents of DMAP (443 mg, 3.63 mmol). After one more hour of reaction, the mixture was cooled to room temperature, washed with (H_2O 2x100 ml), HCl 5% (2x100 ml), dried over MgSO_4 and evaporated to a white solid that was purified by flash chromatography to give 710 mg (72%) of compound **6b** as a white solid.

Macrocycle **6a** was prepared using the same procedure in 70% yield.

Characteristics of 6b: MALDI-FTMS $[\text{M}+\text{Na}]^+$: expected: 883.3385; observed: 833.3400.

Characteristics of 6a: MALDI-FTMS $[\text{M}+\text{Na}]^+$: expected: 883.3385; observed: 883.3385.

Synthesis of carboxylic acids **7a** and **7b**:

To a solution of **6b** (190 mg, 0.22 mmol) in 4.5 ml THF was added of solution of $\text{LiOH} \cdot \text{H}_2\text{O}$ (5 eq., 46 mg, 1.1 mmol) in 2 ml of water at room temperature. The reaction mixture was stirred overnight and THF was evaporated. The carboxylic acid was precipitated by addition of a solution of HCl 5% until pH 2 and extracted with ethyl acetate. Purification by flash

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chromatography (2.5% AcOH in AcOEt) gave 140 mg (75%) of compound **7b** as a white solid.

Carboxylic acid **7a** was prepared using the same procedure in 71%
5 yield (120 mg).

Characteristics of 7b: MALDI-FTMS $[M+H]^+$: expected: 847.3409; observed: 847.3405.

10 **Characteristics of 7a:** MALDI-FTMS $[M+Na]^+$: expected: 869.3229; observed: 869.3221.

Synthesis of triamides 8a(i) and 8b(i):

To a solution of **7b** (130 mg, 0.1535 mmol) in DMF (1ml) was added
15 EDCI (43 mg, 1.5 eq.) and HOBt.H₂O (35 mg, 1.5 eq.). The reaction mixture was stirred at room temperature for 30 minutes and the solution was cooled to 0°C. H-Lys(Boc)-OMe.HCl (67 mg, 1.5 eq.) and DIEA (1.5 eq., 40 µl) were added and the reaction mixture was stirred at room temperature for 20h. DMF was evaporated under high vacuum and ethyl acetate was added (6 ml). The
20 solution was washed with HCl 5% (2x 1 ml), water (2x 1 ml and NaHCO₃ (2x 1 ml), dried over MgSO₄ and evaporated to give a crude oil that was purified by flash chromatography to give 142 mg (85%) of a white solid **8b(i)**.

Triamid **8a(i)** was prepared using the same procedure in 82% yield (105
25 mg).

Characteristics of 8b(i): MALDI-FTMS $[M+Na]^+$: expected: 1111.4859; observed: 1111.4831.

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Characteristics of 8a(i): MALDI-FTMS $[M+Na]^+$: expected: 1111.4859;
observed: 1111.4843

Synthesis of triamides 8a(ii) and 8b(ii):

5 To a solution of **8b(i)** (130 mg, 0.1209 mmol) in 1 ml of dry DCM was added TFA (250 μ l). The reaction mixture was stirred for 3h at room temperature. Solvents were evaporated to give 132 mg (quantitative yield) of **8b(ii)** as a white powder.

10 Triamid **8a(ii)** was prepared using the same procedure in quantitative yield (107 mg).

Characteristics of 8b(ii): MALDI-FTMS $[M+H]^+$: expected: 989.4510;
observed: 989.4508.

15 **Characteristics of 8a(ii):** MALDI-FTMS $[M+H]^+$: expected: 989.4516;
observed: 989.4514.

20 **Synthesis of 23:**

 To a solution of 500 mg (0.48 mmol) of trichloroethyl ester **22** in mixture of acetic acid (45 ml) and water (5 ml) was added 1.5 g (50 equiv.) of zinc powder. The reaction was stirred at room temperature for 5 h, filtered and the solvents were evaporated. Ethyl acetate was added and the solution was
25 washed with 5% HCl, a saturated solution of NaCl, dried over $MgSO_4$ and evaporated to give 420 mg (96%) of a white solid. MALDI-FTMS $[M+Na]^+$: expected: 933.3308; observed: 933.3295.

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Abbreviations:

ACOH stands for acetic acid.

Bn stands for benzyl.

Boc stands for t-butyloxycarbonyl.

5 DCC stands for N,N-dicyclohexylcarbodiimide.

DCM stands for dichloromethane.

DEPBT stands for 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one.

DIEA stands for N-ethyl-N,N-diisopropylamine, also known as Hunig's base.

DMAP stands for 4-dimethylaminopyridine.

10 DMF stands for dimethylformamide.

DMPU stands for 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone.

DPPA stands for diphenylphosphine azide;

EDCI stands for 1-ethyl-3-(3-(dimethylaminopropyl) carbodiimide.

E_tOAC stands for ethyl acetate.

15 HOBt stands for 1-hydroxybenzotriazole.

PYBOP stands for benzotriazol-1-yl-oxytripyrrolidinophosphonium
hexafluorophosphate.

THF stands for tetrahydrofuran.

20 Definitions:

The phrase "protecting groups" with respect to hydroxyl groups, amine groups, carboxyl groups, and sulfhydryl groups refers to chemical moieties that are attached to and protect these functionalities from undesirable reaction during chemical syntheses. Protecting groups are well known to those skilled
25 in the art and include those set forth in *Protective Groups in Organic Synthesis*, Greene, T.W.; Wuts, P. G. M., John Wiley & Sons, New York, (3rd Edition, 1999) and *The Practice of Peptide Synthesis*, Bodanszky, M. and Bodanszky, A., Springer-Verlag, New York, (1984). Such groups can be added or removed from the functionality being protected using the procedures
30 set forth therein and others known in the art. Examples of protected hydroxyl

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groups include, but are not limited to, silyl ethers such as those obtained by reaction of a hydroxyl group with a reagent such as, but not limited to, *t*-butyldimethyl-chlorosilane, trimethylchlorosilane, triisopropylchlorosilane, triethylchlorosilane; substituted methyl and ethyl ethers such as, but not
5 limited to methoxymethyl ether, methylthiomethyl ether, benzyloxymethyl ether, *t*-butoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ethers, 1-ethoxyethyl ether, allyl ether, benzyl ether; esters such as, but not limited to, benzoylformate, formate, acetate, trichloroacetate, and trifluoroacetate. Examples of protected amine groups include, but are not limited to, amides
10 such as, formamide, acetamide, trifluoroacetamide, and benzamide; imides, such as phthalimide, and dithiosuccinimide; carbamates such as *t*-butyl carbamate (Boc), fluorenylmethyl carbamate (Fmoc), and benzyl carbamate (Cbz); and others. Examples of protected sulfhydryl groups include, but are not limited to, thioethers such as *S-t*-butyl thioether, *S*-benzyl thioether, and
15 *S*-4-picolyl thioether; substituted *S*-methyl derivatives such as hemithio, dithio and aminothio acetals; and others. Examples of protected carboxyl groups include but are not limited to esters such as methyl, ethyl, *t*-butyl, trimethylsilylethyl, benzyl, and the like.

20 A "pharmaceutically acceptable salt" includes a salt with an inorganic base, organic base, inorganic acid, organic acid, or basic or acidic amino acid. As salts of inorganic bases, the invention includes, for example, alkali metals such as sodium or potassium; alkaline earth metals such as calcium and magnesium or aluminum; and ammonia. As salts of organic bases, the
25 invention includes, for example, trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, and triethanolamine. As salts of inorganic acids, the instant invention includes, for example, hydrochloric acid, hydroboric acid, nitric acid, sulfuric acid, and phosphoric acid. As salts of organic acids, the instant invention includes, for example, formic acid, acetic
30 acid, trifluoroacetic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid,

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citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid. As salts of basic amino acids, the instant invention includes, for example, arginine, lysine and ornithine. Acidic amino acids include, for example, aspartic acid and glutamic acid.

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The term "alkyl" refers to unsubstituted alkyl groups that do not contain heteroatoms. Thus the term includes straight chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The term also includes branched chain isomers of straight chain alkyl groups, including but not limited to, the following which are provided by way of example: $-\text{CH}(\text{CH}_3)_2$, $-\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, $-\text{CH}(\text{CH}_2\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_3$, $-\text{C}(\text{CH}_2\text{CH}_3)_3$, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)_2$, $-\text{CH}_2\text{C}(\text{CH}_3)_3$, $-\text{CH}_2\text{C}(\text{CH}_2\text{CH}_3)_3$, $-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, $-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, $-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)_2$, $-\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)_3$, $-\text{CH}_2\text{CH}_2\text{C}(\text{CH}_2\text{CH}_3)_3$, $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)_2$, $-\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, and others. The term also includes cyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and such rings substituted with straight and branched chain alkyl groups as defined above. The term also includes polycyclic alkyl groups such as, but not limited to, adamantyl, norbornyl, and bicyclo[2.2.2]octyl and such rings substituted with straight and branched chain alkyl groups as defined above. Thus, the term alkyl groups includes primary alkyl groups, secondary alkyl groups, and tertiary alkyl groups. Alkyl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound. Preferred alkyl groups include straight and branched chain alkyl groups and cyclic alkyl groups having 1 to 20 carbon atoms, and more preferred such groups have from 1 to 10 carbon atoms. Even more preferred such groups, also known as lower alkyl groups, have from 1 to 5 carbon atoms. Most preferred alkyl groups

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include straight and branched chain alkyl groups having from 1 to 3 carbon atoms and include methyl, ethyl, propyl, and $-\text{CH}(\text{CH}_3)_2$.

The phrase "substituted alkyl" refers to an alkyl group as defined above
5 in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen and non-carbon atoms such as, but not limited to, a halogen atom in halides such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups,
10 sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkylarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups.
15 Substituted alkyl groups also include groups in which one or more bonds to a carbon(s) or hydrogen(s) atom is replaced by a bond to a heteroatom such as oxygen in carbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles. Preferred substituted alkyl groups include, among others, alkyl groups in which one or more bonds to a carbon or
20 hydrogen atom is/are replaced by one or more bonds to fluorine atoms. One example of a substituted alkyl group is the trifluoromethyl group and other alkyl groups that contain the trifluoromethyl group. Other alkyl groups include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a
25 hydroxyl, alkoxy, aryloxy group, or heterocycloxy group. Still other alkyl groups include alkyl groups that have an amine, alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine, diarylamine, heterocyclamine, (alkyl)(heterocycl)amine, (aryl)(heterocycl)amine, or diheterocyclamine group.

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The term "aryl" refers to unsubstituted aryl groups that do not contain heteroatoms. Thus, the term includes, but is not limited to, groups such as phenyl, biphenyl, anthracenyl, naphthenyl by way of example. Although the term "aryl" includes groups containing condensed rings such as naphthalene, it does not include aryl groups that have other groups such as alkyl or halo groups bonded to one of the ring members, as aryl groups such as tolyl are considered herein to be substituted aryl groups as described below. A preferred aryl group is phenyl. Aryl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound, however.

The phrase "substituted aryl group" has the same meaning with respect to aryl groups that substituted alkyl groups had with respect to alkyl groups. However, a substituted aryl group also includes aryl groups in which one of the aromatic carbons is bonded to one of the non-carbon or non-hydrogen atoms described above and also includes aryl groups in which one or more aromatic carbons of the aryl group is bonded to a substituted and/or unsubstituted alkyl, alkenyl, or alkynyl group as defined herein. This includes bonding arrangements in which two carbon atoms of an aryl group are bonded to two atoms of an alkyl, alkenyl, or alkynyl group to define a fused ring system (e.g. dihydronaphthyl or tetrahydronaphthyl). Thus, the phrase "substituted aryl" includes, but is not limited to tolyl, and hydroxyphenyl among others.

The term "alkenyl" refers to straight and branched chain and cyclic groups such as those described with respect to alkyl groups as defined above, except that at least one double bond exists between two carbon atoms. Examples include, but are not limited to vinyl, $-\text{CH}=\text{C}(\text{H})(\text{CH}_3)$, $-\text{CH}=\text{C}(\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)=\text{C}(\text{H})_2$, $-\text{C}(\text{CH}_3)=\text{C}(\text{H})(\text{CH}_3)$, $-\text{C}(\text{CH}_2\text{CH}_3)=\text{CH}_2$, cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl among others.

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The phrase "substituted alkenyl" has the same meaning with respect to alkenyl groups that substituted alkyl groups had with respect to alkyl groups. A substituted alkenyl group includes alkenyl groups in which a non-carbon or non-hydrogen atom is bonded to a carbon double bonded to another carbon and those in which one of the non-carbon or non-hydrogen atoms is bonded to a carbon not involved in a double bond to another carbon. Preferred substituted alkenyl groups have from 2 to 20 carbons, and more preferred such groups have from 2 to 10 carbons.

The term "alkynyl" refers to straight and branched chain groups such as those described with respect to alkyl groups as defined above, except that at least one triple bond exists between two carbon atoms. Examples include, but are not limited to $-\text{C}(\text{C}(\text{H}))$, $-\text{C}(\text{C}(\text{CH}_3))$, $-\text{C}(\text{C}(\text{CH}_2\text{CH}_3))$, $-\text{C}(\text{H}_2)\text{C}(\text{C}(\text{H}))$, $-\text{C}(\text{H})_2\text{C}(\text{C}(\text{CH}_3))$, and $-\text{C}(\text{H})_2\text{C}(\text{C}(\text{CH}_2\text{CH}_3))$ among others. Preferred alkynyl groups have from 2 to 20 carbons, and more preferred such groups have from 2 to 10 carbons.

The phrase "substituted alkynyl" has the same meaning with respect to alkynyl groups that substituted alkyl groups had with respect to alkyl groups. A substituted alkynyl group includes alkynyl groups in which a non-carbon or non-hydrogen atom is bonded to a carbon triple bonded to another carbon and those in which a non-carbon or non-hydrogen atom is bonded to a carbon not involved in a triple bond to another carbon.

The term "aralkyl" refers to alkyl groups as defined above in which a hydrogen or carbon bond of the alkyl group is replaced with a bond to an aryl group as defined above. For example, methyl ($-\text{CH}_3$) is an alkyl group. If a hydrogen atom of the methyl group is replaced by a bond to a phenyl group, such as if the carbon of the methyl were bonded to a carbon of benzene, then the compound is an aralkyl group (*i.e.*, a benzyl group). Thus the term

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includes, but is not limited to, groups such as benzyl, diphenylmethyl, and 1-phenylethyl ($-\text{CH}(\text{C}_6\text{H}_5)(\text{CH}_3)$) among others.

5 The phrase "substituted aralkyl" has the same meaning with respect to aralkyl groups that substituted aryl groups had with respect to aryl groups. However, a substituted aralkyl group also includes groups in which a carbon or hydrogen bond of the alkyl part of the group is replaced by a bond to a non-carbon or a non-hydrogen atom. Examples of substituted aralkyl groups include, but are not limited to, $-\text{CH}_2\text{C}(=\text{O})(\text{C}_6\text{H}_5)$, and $-\text{CH}_2(2\text{-methylphenyl})$ among others.

15 The term "heterocyclyl" refers to both aromatic and nonaromatic ring compounds including monocyclic, bicyclic, and polycyclic ring compounds such as, but not limited to, quinuclidyl, containing 3 or more ring members of which one or more is a heteroatom such as, but not limited to, N, O, and S. Although the term "heterocyclyl" includes condensed heterocyclic rings such as benzimidazolyl, it does not include heterocyclyl groups that have other groups such as alkyl or halo groups bonded to one of the ring members; compounds such as 2-methylbenzimidazolyl are substituted heterocyclyl groups. Examples of heterocyclyl groups include, but are not limited to: unsaturated 3 to 8 member rings containing 1 to 4 nitrogen atoms such as, but not limited to pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridinyl, dihydropyridinyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl etc.), tetrazolyl, (e.g. 1H-tetrazolyl, 2H tetrazolyl, etc.); saturated 3 to 8 member rings containing 1 to 4 nitrogen atoms such as, but not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl; condensed unsaturated heterocyclic groups containing 1 to 4 nitrogen atoms such as, but not limited to, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl; 25 30 unsaturated 3 to 8 member rings containing 1 to 2 oxygen atoms and 1 to 3

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nitrogen atoms such as, but not limited to, oxazolyl, isoxazolyl, oxadiazolyl (e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.); saturated 3 to 8 member rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, morpholinyl; unsaturated condensed heterocyclic groups containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, benzoxazolyl, benzoxadiazolyl, benzoxazinyl (e.g. 2H-1,4-benzoxazinyl etc.); unsaturated 3 to 8 member rings containing 1 to 3 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolyl, isothiazolyl, thiadiazolyl (e.g. 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.); saturated 3 to 8 member rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolodiny; saturated and unsaturated 3 to 8 member rings containing 1 to 2 sulfur atoms such as, but not limited to, thienyl, dihydrodithiiny, dihydrodithionyl, tetrahydrothiophene, tetrahydrothiopyran; unsaturated condensed heterocyclic rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, benzothiazolyl, benzothiadiazolyl, benzothiazinyl (e.g. 2H-1,4-benzothiazinyl, etc.), dihydrobenzothiazinyl (e.g. 2H-3,4-dihydrobenzothiazinyl, etc.), unsaturated 3 to 8 member rings containing oxygen atoms such as, but not limited to furyl; unsaturated condensed heterocyclic rings containing 1 to 2 oxygen atoms such as benzodioxolyl (e.g., 1,3-benzodioxolyl, etc.); unsaturated 3 to 8 member rings containing an oxygen atom and 1 to 2 sulfur atoms such as, but not limited to, dihydrooxathiiny; saturated 3 to 8 member rings containing 1 to 2 oxygen atoms and 1 to 2 sulfur atoms such as 1,4-oxathiane; unsaturated condensed rings containing 1 to 2 sulfur atoms such as benzothienyl, benzodithiiny; and unsaturated condensed heterocyclic rings containing an oxygen atom and 1 to 2 oxygen atoms such as benzoxathiiny. Heterocyclyl groups also include those described above in which one or more S atoms in the ring is double-bonded to one or two oxygen atoms (sulfoxides and sulfones). For example, heterocyclyl groups include tetrahydrothiophene oxide and tetrahydrothiophene 1,1-dioxide. Preferred

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heterocyclyl groups contain 5 or 6 ring members. More preferred heterocyclyl groups include morpholine, piperazine, piperidine, pyrrolidine, imidazole, pyrazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, thiophene, thiomorpholine, thiomorpholine in which the S atom of the thiomorpholine is bonded to one or more O atoms, pyrrole, pyridine homopiperazine, oxazolidin-2-one, pyrrolidin-2-one, oxazole, quinuclidine, thiazole, isoxazole, furan, and tetrahydrofuran.

The phrase "substituted heterocyclyl" refers to a heterocyclyl group as defined above in which one or more of the ring members is bonded to a non-hydrogen atom such as described above with respect to substituted alkyl groups and substituted aryl groups. Examples include, but are not limited to, 2-methylbenzimidazolyl, 5-methylbenzimidazolyl, 5-chlorobenzthiazolyl, 1-methyl piperazinyl, 2-phenoxy-thiophene, and 2-chloropyridinyl among others. In addition, substituted heterocyclyl groups also include heterocyclyl groups in which the bond to the non-hydrogen atom is a bond to a carbon atom that is part of a substituted and unsubstituted aryl, substituted and unsubstituted arylalkyl, or unsubstituted heterocyclyl group. Examples include but are not limited to 1-benzylpiperidinyl, 3-phenylthiomorpholinyl, 3-(pyrrolidin-1-yl)-pyrrolidinyl, and 4-(piperidin-1-yl)-piperidinyl.

The term "heterocyclylalkyl" refers to alkyl groups as defined above in which a hydrogen or carbon bond of the unsubstituted alkyl group is replaced with a bond to a heterocyclyl group as defined above. For example, methyl (-CH₃) is an alkyl group. If a hydrogen atom of the methyl group is replaced by a bond to a heterocyclyl group, such as if the carbon of the methyl were bonded to carbon 2 of pyridine (one of the carbons bonded to the N of the pyridine) or carbons 3 or 4 of the pyridine, then the compound is a heterocyclylalkyl group.

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The phrase "substituted heterocyclalkyl" has the same meaning with respect to heterocyclalkyl groups that substituted aralkyl groups had with respect to aralkyl groups. However, a substituted heterocyclalkyl group also includes groups in which a non-hydrogen atom is bonded to a heteroatom in the heterocycl group of the heterocyclalkyl group such as, but not limited to, a nitrogen atom in the piperidine ring of a piperidinylalkyl group. In addition, a substituted heterocyclalkyl group also includes groups in which a carbon bond or a hydrogen bond of the alkyl part of the group is replaced by a bond to a substituted and unsubstituted aryl or substituted and unsubstituted arylalkyl group. Examples include but are not limited to phenyl-(piperidin-1-yl)-methyl and phenyl-(morpholin-4-yl)-methyl.

The term "alkoxy" refers to a hydroxyl group (-OH) in which the bond to the hydrogen atom is replaced by a bond to a carbon atom of an alkyl group as defined above.

The phrase "substituted alkoxy" refers to a hydroxyl group (-OH) in which the bond to the hydrogen atom is replaced by a bond to a carbon atom of an otherwise substituted alkyl group as defined above.

A "pharmaceutically acceptable salt" includes a salt with an inorganic base, organic base, inorganic acid, organic acid, or basic or acidic amino acid. As salts of inorganic bases, the invention includes, for example, alkali metals such as sodium or potassium; alkaline earth metals such as calcium and magnesium or aluminum; and ammonia. As salts of organic bases, the invention includes, for example, trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, and triethanolamine. As salts of inorganic acids, the instant invention includes, for example, hydrochloric acid, hydroboric acid, nitric acid, sulfuric acid, and phosphoric acid. As salts of organic acids, the instant invention includes, for example, formic acid, acetic

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acid, trifluoroacetic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid. As salts of basic amino acids, the instant invention includes, for example, arginine, lysine and ornithine. Acidic amino acids include, for example, aspartic acid and glutamic acid.

Tautomers refers to isomeric forms of a compound that are in equilibrium with each other. The concentrations of the isomeric forms will depend on the environment the compound is found in and may be different depending upon, for example, whether the compound is a solid or is in an organic or aqueous solution. For example, in aqueous solution, ketones are typically in equilibrium with their enol forms. Thus, ketones and their enols are referred to as tautomers of each other. As readily understood by one skilled in the art, a wide variety of functional groups and other structures may exhibit tautomerism, and all tautomers of compounds having formulas I and II are within the scope of the present invention.

Compounds of the present invention include enriched or resolved optical isomers at any or all asymmetric atoms as are apparent from the depictions. Both racemic and diastereomeric mixtures, as well as the individual optical isomers can be isolated or synthesized so as to be substantially free of their enantiomeric or diastereomeric partners, and these are all within the scope of the invention.

Detailed Description of Figures:

Figure 1 illustrates the structures of Galnon and of various platform structures that present C_3 symmetry, viz. Dendroamide C, a prior art synthetic scaffold (1), and Galmic. Galnon is a prior art agonist of galanin receptor; Dendroamide C is a biologically active peptide-derived marine natural product

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having a macrocycle that contains three oxazole or thiazole rings linked by *trans* amide bonds; scaffold (1) is the prior art scaffold upon which Galmic was constructed; Galmic is a novel agonist of galanin receptor.

5 Figure 2 illustrates a synthetic method for producing a library of potential galanin agonists and for fractionating the library to obtain GalGalmic. R₁, R₂, and R₃ represent random substituents resulting from the condensation of amines **a**, **b**, and **c**. EDCI stands for 1-ethyl-3-(3- (dimethylaminopropyl) carbodiimide; HOBt stands for 1-hydroxybenzotriazole; DIEA stands for
10 N-ethyl-N,N-diisopropylamine, also known as Hunig's base; DMF stands for dimethylformamide; TFA⁻ stands for trifluoroacetate; Boc stands for t-butyloxycarbonyl.

15 Figure 3 illustrates a representation of the calculated structure of Galmic: the side chain amides have been arbitrarily rotated to show the most compact structure. In any conformation the side chains appear on the same face of the macrocycle.

20 Figure 4 illustrates a chart for presenting the effects of Galmic on long-term synaptic plasticity in mouse dentate gyrus (DG) slices. Administration of Galmic (1 μ M) for 10-15 min prior to LTP-induction attenuated the early and late phases of LTP compared to the DMSO (0.03%v/v) treated slices. The bar illustrates the duration of the vehicle (DMSO) or drug administration and the arrow indicates the time of stimulation
25 with high frequency trains (tetanus). The effect of Galmic was significantly different from DMSO- treated slices at 2-6 min and 17-60 min post-LTP induction. Galmic (1 μ M) caused a similar effect as administration of galanin (1-29) (1 μ M) in attenuating the LTP in DG. All values are mean $\pm$ SEM (t-test: ** $P < 0.005$).

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Figure 5 illustrates a bar chart for presenting effects of Galmic on self-sustaining status epilepticus (SSSE). Galmic was injected into the dentate gyrus ipsilateral to the stimulation site at 10 min (A) or 60 min (B) after PPS. Dose (nanomoles) is indicated in the legend next to the graph. (C)

5 Intraperitoneal administration of Galmic 10 min after PPS attenuated SSSE at 2mg/kg i.p., but not 1 mg/kg i.p.. Asterisk- $p < 0.05$ vs Control (One-Way ANOVA + Bonferroni t-test).

Figure 6 illustrates a graph and a bar chart representing the effects of
10 Galmic on formalin induced flinching (A). Time course over 60-min of flinch responses following formalin paw injection in mice with i.p. injection of Galmic ($9.8 \mu\text{mol/kg}$, $n=5$) or vehicle (30% DMSO, $10 \mu\text{l}$, $n=8$). Each point represents the number of flinches per min. (B) Histograms representing the dose-response effects of Galmic (doses in $\mu\text{moles/kg}$ indicated in brackets,
15 $n=5$ each dose) on formalin test phase 1 and phase 2 flinching behavior of mice. The bars represent the total number of flinches in each phase: Ph-I (1-9 min), Ph-II (10-60 min), Ph-IIA (10-40 min) and Ph-IIB (41-60 min). The data are expressed as mean $\pm$ s.e.m. * $p < 0.05$ vs. the vehicle group, one-way ANOVA followed by Dunnett's tests.

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Figure 7 illustrates a bar chart for presenting the effects of treatment of rats with Galmic in the forced swim test (left panel). Activity was measured during a 10 min test at 45 min after injection. Galmic (15 mg/kg i.p.) produced a significant increase in activity in the test, compared to vehicle treated
25 (DMSO 50 % v/v) animals, consistent an antidepressant-like profile. Right panel, Galmic (15 mg/kg i.p.) was administered in the open field test. Galmic significantly reduced the locomotor activity in this task compared to vehicle treated animals, without any obvious signs of sedation. Values represent group means ($\pm$ SEM) (independent means t -test: * $p < 0.01$ vs control).

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Figure 8 illustrates a table comparing the affinity of Galmic and galnon for galanin receptors. K_i values of Galmic and Galnon at GalR1 and GalR2 receptors were determined by displacement of [125 I] galanin from membranes prepared from human Bowes' cells (hGalR1) and stably transferred CHO cells expressing rat GalR2, respectively. [125 I] galanin concentration was 0.2 nM, and Galmic and galnon concentrations were between 10^{-8} M - 10^{-4} M. K_i values were calculated with Prism software.

Figure 9 illustrates a procedure for synthesizing oxazole derivatives (18a and 18b) employable for making macrocycles such as compound 1 and Galmic. R_4 is a substituent that corresponds to the methyl groups attached to the asymmetric carbons of Galmic. When R_4 is methyl, R_4 serves to prevent racemization of Galmic. Larger alkyl groups and substituents other than methyl may also be employed to prevent racemization. If racemization is unimportant, R_4 may be hydrogen.

Figure 10 illustrates a procedure for linking three oxazole derivatives (18a and 18b) to form a linear intermediate having three asymmetric carbons, each asymmetric carbon having a differentially protected ester (21).

Figure 11 illustrates a procedure for cyclizing the linear intermediate (21) to form a macrocycle having three oxazoles linked by *trans* amide bonds (11).

Figure 12 illustrates a procedure for derivatizing the cyclic intermediate (11) for producing Galmic (2) or a generic form of the invention (12). In the case of Galmic, R_1NH_2 , R_2NH_2 , and R_3NH_2 correspond to the amines indicated in Figure 2. In the more generic form of the invention (12), R_1NH_2 , R_2NH_2 , and R_3NH_2 correspond to the amines indicated in Figure 2 and to a broader range of amines.

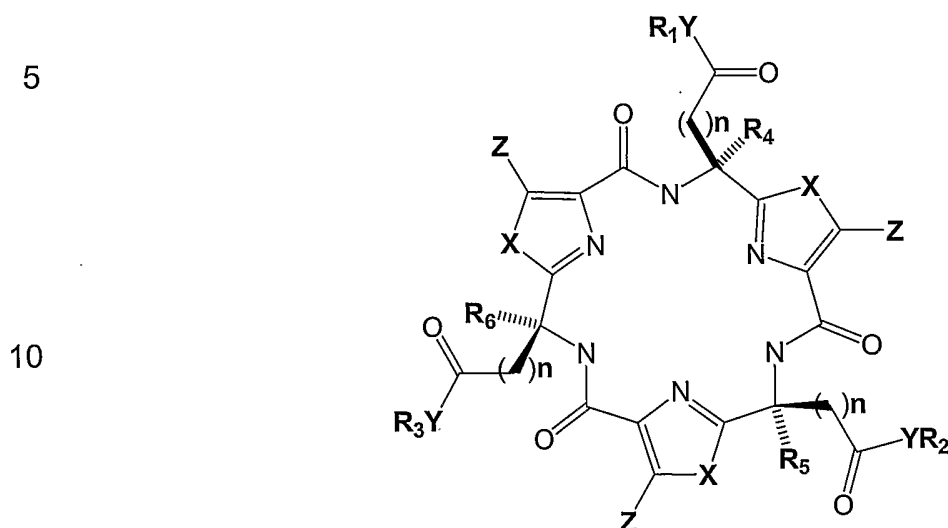
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Figure 13 illustrates an alternative method for cyclizing a linear intermediate (**3a** or **3b**) to form a macrocycle having three oxazoles linked to one another with *trans* amide bonds, each of the linking amide bonds having an asymmetric carbon, each asymmetric carbon having a differentially
5 protected ester (**6a** or **6b**). Figure 13 further illustrates the conversion of the macrocycle (**6a** or **6b**) to form a Galmic intermediate having a lysine substituent at one of the asymmetric carbons, while the two remaining asymmetric carbons remain differentially protected (**8a(ii)** or **8b(ii)**).

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What is claimed is:

1. A compound represented by the following structure:



wherein:

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X is a diradical independently selected at each occurrence from the group consisting of -O- and -S-;

Y is a diradical independently selected at each occurrence from the group consisting of -O- and -NH-;

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Z is a radical independently selected at each occurrence from the group consisting of -H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl;

R₁, **R₂**, and **R₃** are each a radical independently selected from the group consisting of -H, C₁₋₁₀ alkyl, substituted C₁₋₁₀ alkyl, -(C₀₋₄ alkyl)-(C₆₋₁₄ aryl), substituted -(C₀₋₄ alkyl)-(C₆₋₁₄ aryl), -(C₀₋₄ alkyl)-(C₁₋₁₂ heterocyclyl), and substituted -(C₀₋₄ alkyl)-(C₁₋₁₂ heterocyclyl), and if **Y** is -NH-, then **YR₁**, **YR₂**, and **YR₃** may each optionally and independently form an amino radical of naturally occurring or non-naturally occurring amino acid;

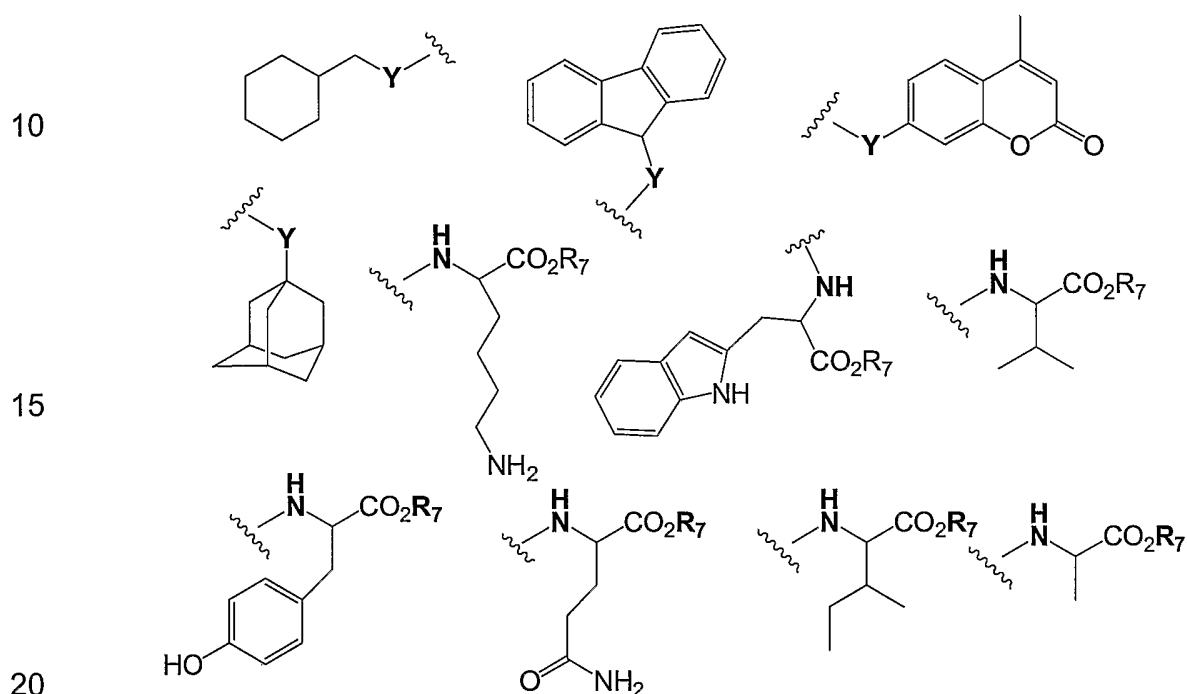
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R₄, R₅ and R₆ are each independently C₁₋₆ alkyl, optionally substituted with 1-3 of -F, -Cl, -Br, -I, -OH, C₁₋₄ alkoxy, -NH₂, C₁₋₄ aminoalkyl, C₂₋₄ aminodialkyl, -SH, C₁₋₄ thioalkyl, or oxo; and
n = 0-5;

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with a proviso that at least one of YR_1 , YR_2 , and YR_3 is selected from the group consisting of the following radicals:



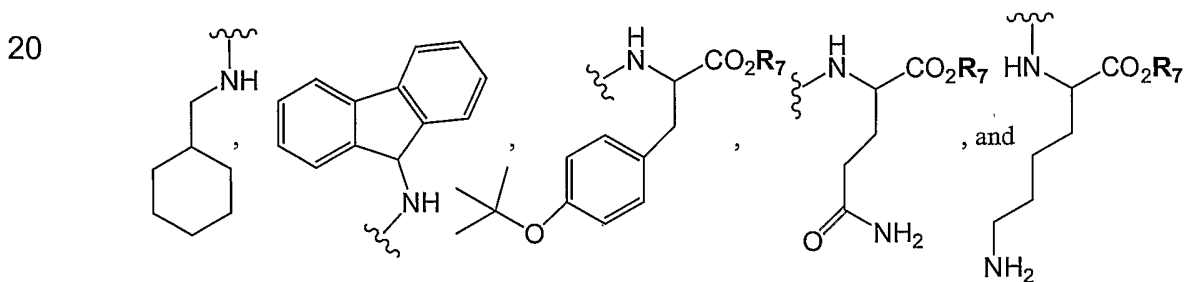
wherein

R₇ is a radical independently selected, at each occurrence, from the group consisting of -H, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, (C₀₋₄ alkyl)-(C₆₋₁₄ aryl), substituted (C₀₋₄ alkyl)-(C₆₋₁₄ aryl), and phosphodiester linked oligonucleotide tag, wherein alkyl and aryl groups are optionally substituted with up to 3 substituents selected from the group consisting of -F, -Cl, -Br, -I, -CN, -NO₂, -OH, -COOH, -CONH₂, -NH₂, -SH, C₁₋₄ alkyl, C₁₋₄ oxyalkyl, -C(O)(O-C₁₋₄ alkyl), C₁₋₄ aminoalkyl, C₁₋₄ thioalkyl, tri(C₁₋₄ alkyl)silyl.

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2. The compound of claim 1, wherein $-YR_1$, $-YR_2$, and $-YR_3$ are each $-OR_1$, $-OR_2$, and $-OR_3$, respectively.
3. The compound of claim 2, wherein $-YR_1$, $-YR_2$, and $-YR_3$ are each independently C_{1-6} alkoxy, substituted C_{1-6} alkoxy, $-O-(C_{0-4} \text{ alkyl})-(C_{6-14} \text{ aryl})$, or substituted $-O-(C_{0-4} \text{ alkyl})-(C_{6-14} \text{ aryl})$.
4. The compound of claim 1, wherein YR_1 , YR_2 , and YR_3 are each $-NHR_1$, $-NHR_2$, and $-NHR_3$, respectively.
5. The compound of claim 4, wherein R_7 is selected from the group consisting of $-H$, methyl, and benzyl.
6. The compound of claim 4, wherein R_7 is methyl.
7. The compound of claim 1, wherein YR_1 , YR_2 , and YR_3 are each independently selected from the group consisting of :



8. The compound of claim 1, wherein R_4 , R_5 and R_6 are each methyl.
9. The compound of claim 1, wherein $n = 0$ or 1.

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10. The compound of claim 1, wherein **n** = 0.

11. The compound of claim 1, wherein **X** is O.

5 12. The compound of claim 1, wherein **X** is S.

13. The compound of claim 1, wherein **X** is O and **Z** is methyl.

14. The compound of claim 1, wherein **Z** is methyl.

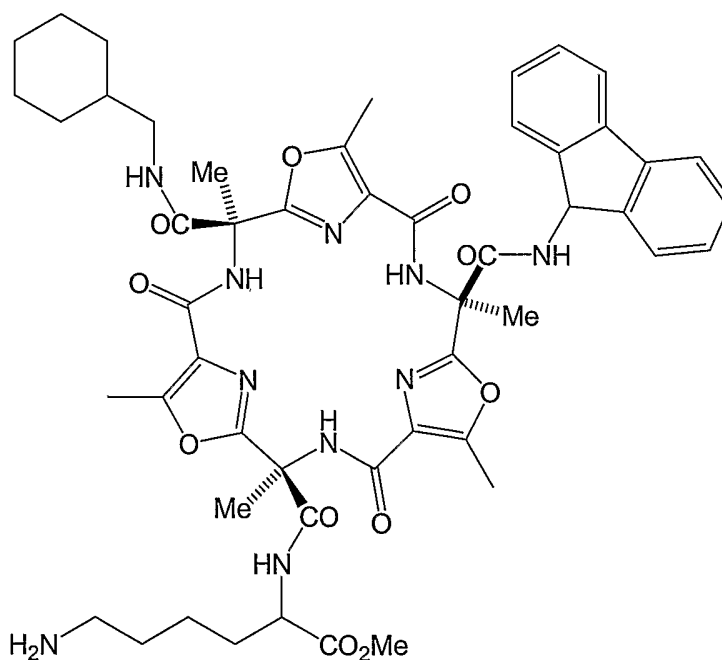
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15. The compound of claim 1 having the structure:

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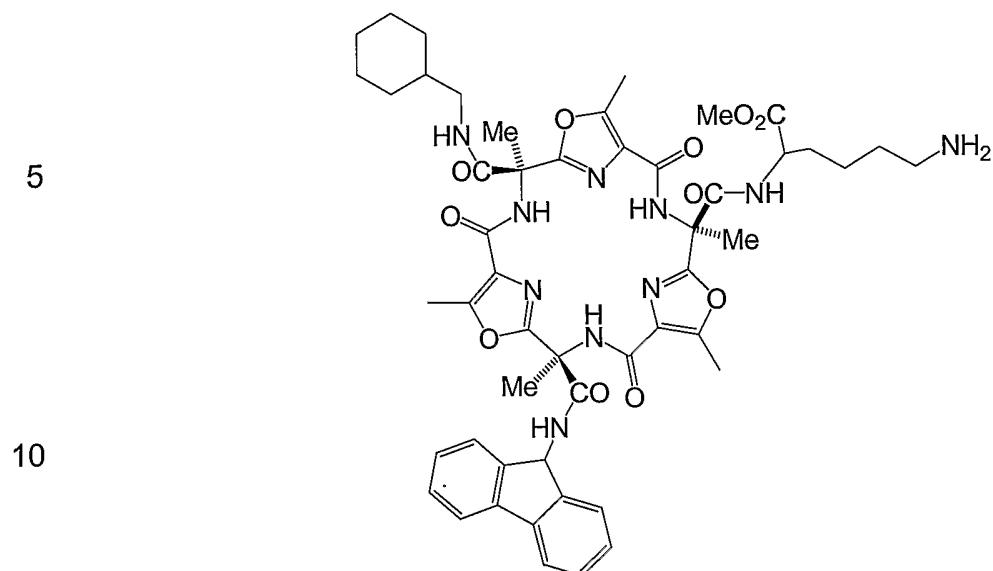
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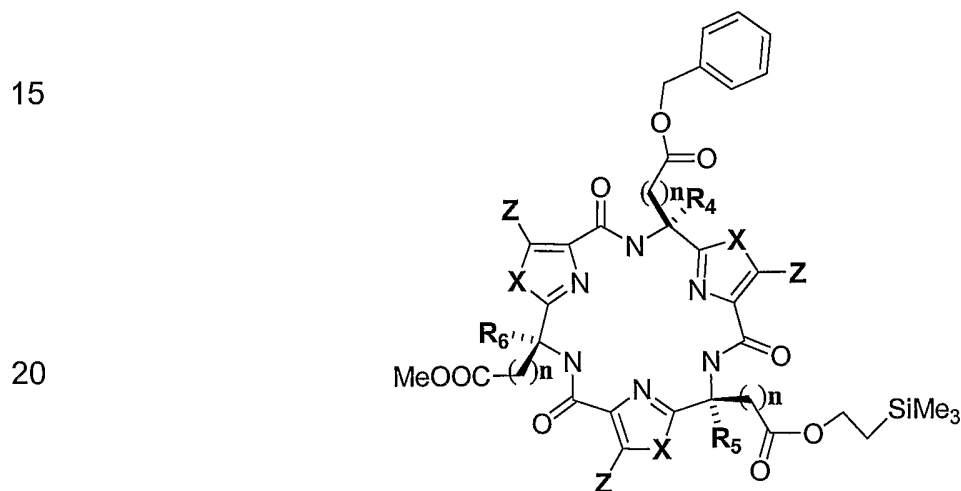


- 50 -

16. The compound of claim 1 having the structure:



17. A compound represented by the following structure:



wherein:

X is a diradical independently selected at each occurrence from the group consisting of -O- and -S-;

25

Z is a radical independently selected at each occurrence from the group consisting of -H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl;

R₄, **R₅** and **R₆** are each independently C₁₋₆ alkyl, optionally substituted with 1-3 of -F, -Cl, -Br, -I, -OH, C₁₋₄ alkoxy, -NH₂, C₁₋₄ aminoalkyl, C₂₋₄ aminodialkyl, -SH, C₁₋₄ thioalkyl, or oxo; and

30

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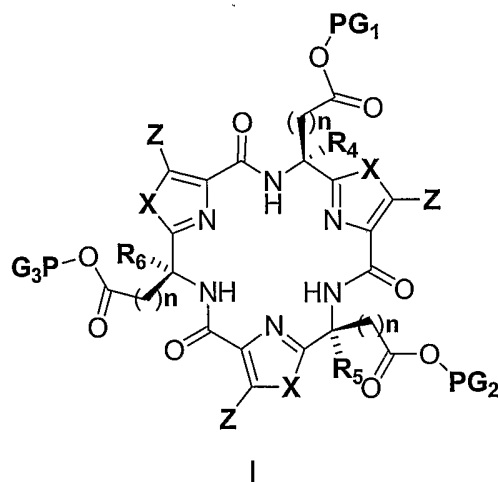
n = 0-5.

18. A method of making a compound of claim 1, comprising the following steps:

Step A: reacting a compound of formula I:

5

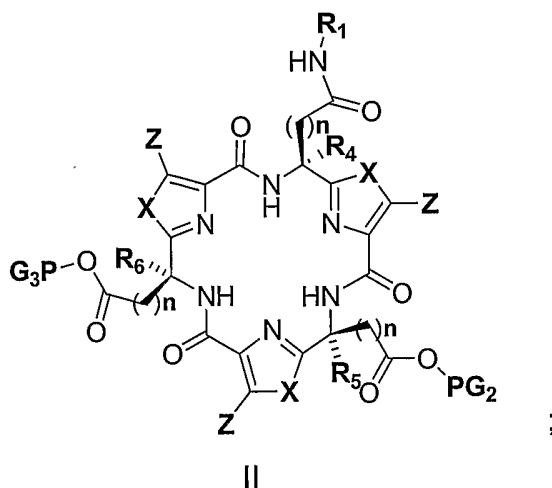
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with H_2 in the presence of a suitable catalyst, followed by reaction with R_1NH_2 in the presence of a coupling reagent, wherein X , Z , n , R_1 , R_4 , R_5 , and R_6 are each as defined in claim 1; and PG_1 , PG_2 and PG_3 are orthogonal protecting groups, to yield a compound of formula II:

20

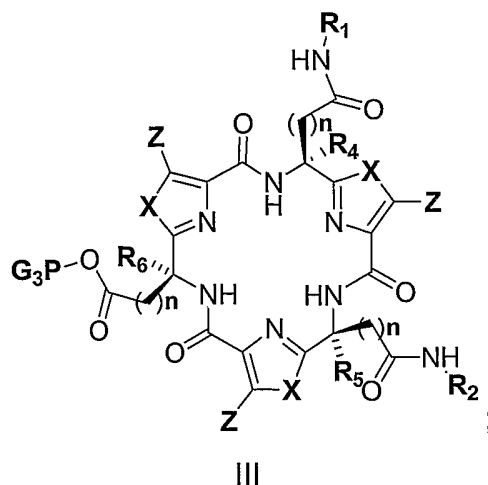
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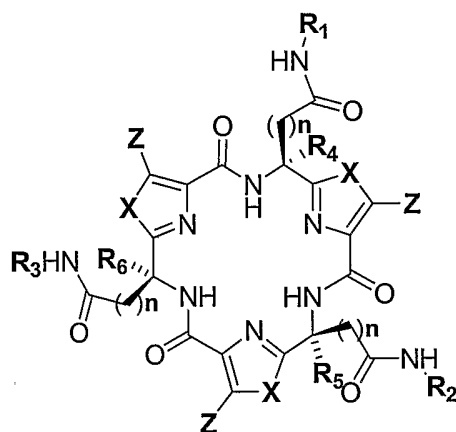
Step B: reacting the compound of formula II with a fluoride source, followed by reaction with R_2NH_2 in the presence of a coupling agent, wherein R_2 is as defined in claim 1 to yield a compound of formula III:

30

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Step C: reacting the compound yielded in said Step B with a hydrolytic agent
 followed by reaction with R_3NH_2 in the presence of a coupling agent,
 wherein R_3 is as defined in claim 1 to yield a compound of the following
 structure:



19. The method of claim 34 wherein PG_1 is benzyl, PG_2 is trimethylsilylethyl, and PG_3 is methyl.

20. A pharmaceutical composition, comprising a pharmaceutically effective amount of the compound of claim 1 or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier or diluent.

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21. A method of treating a mammal having a condition mediated by a galanin deficiency comprising administering to the mammal in need thereof the composition of claim 20.

- 5 22. The method of claim 21, wherein the mammal is suffering from at least one condition selected from the group consisting of Alzheimer's disease, depression, epilepsy, and an eating disorder.

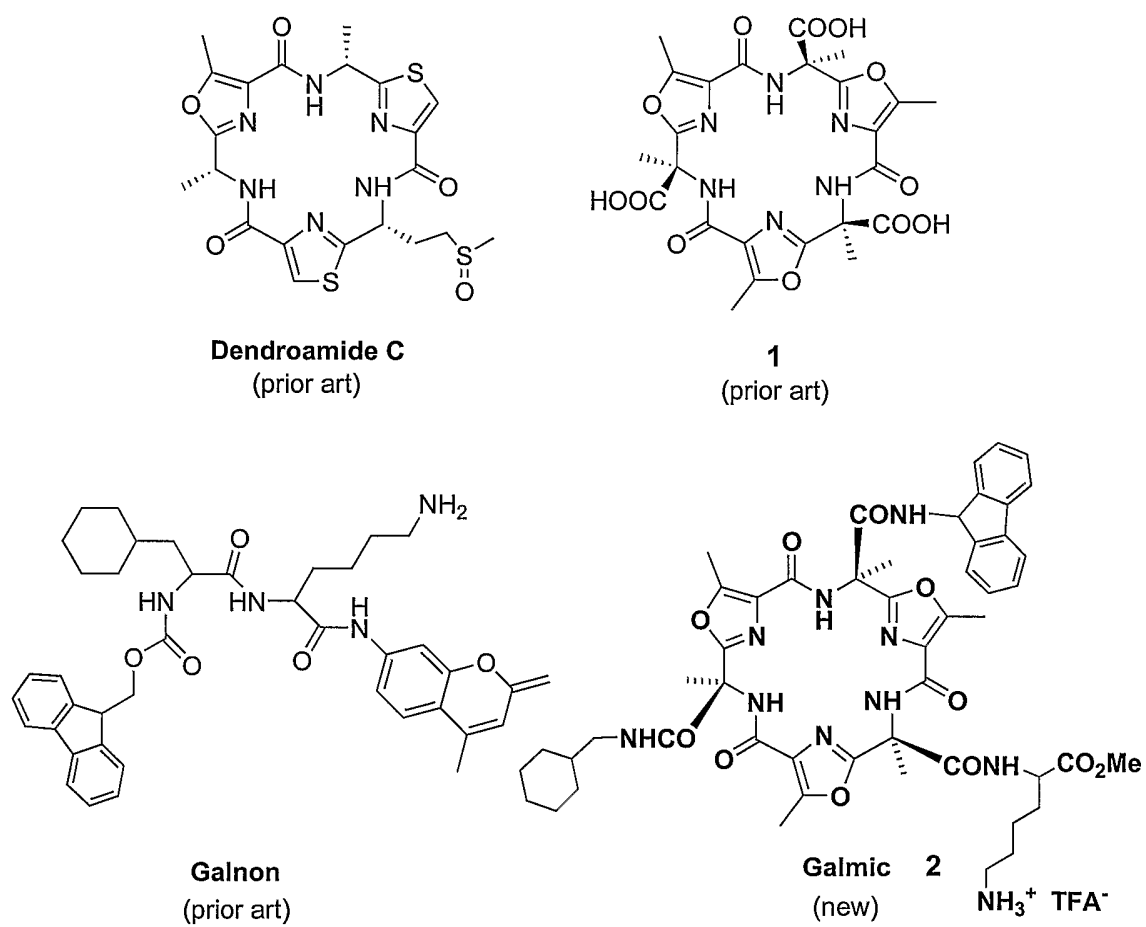


Figure 1

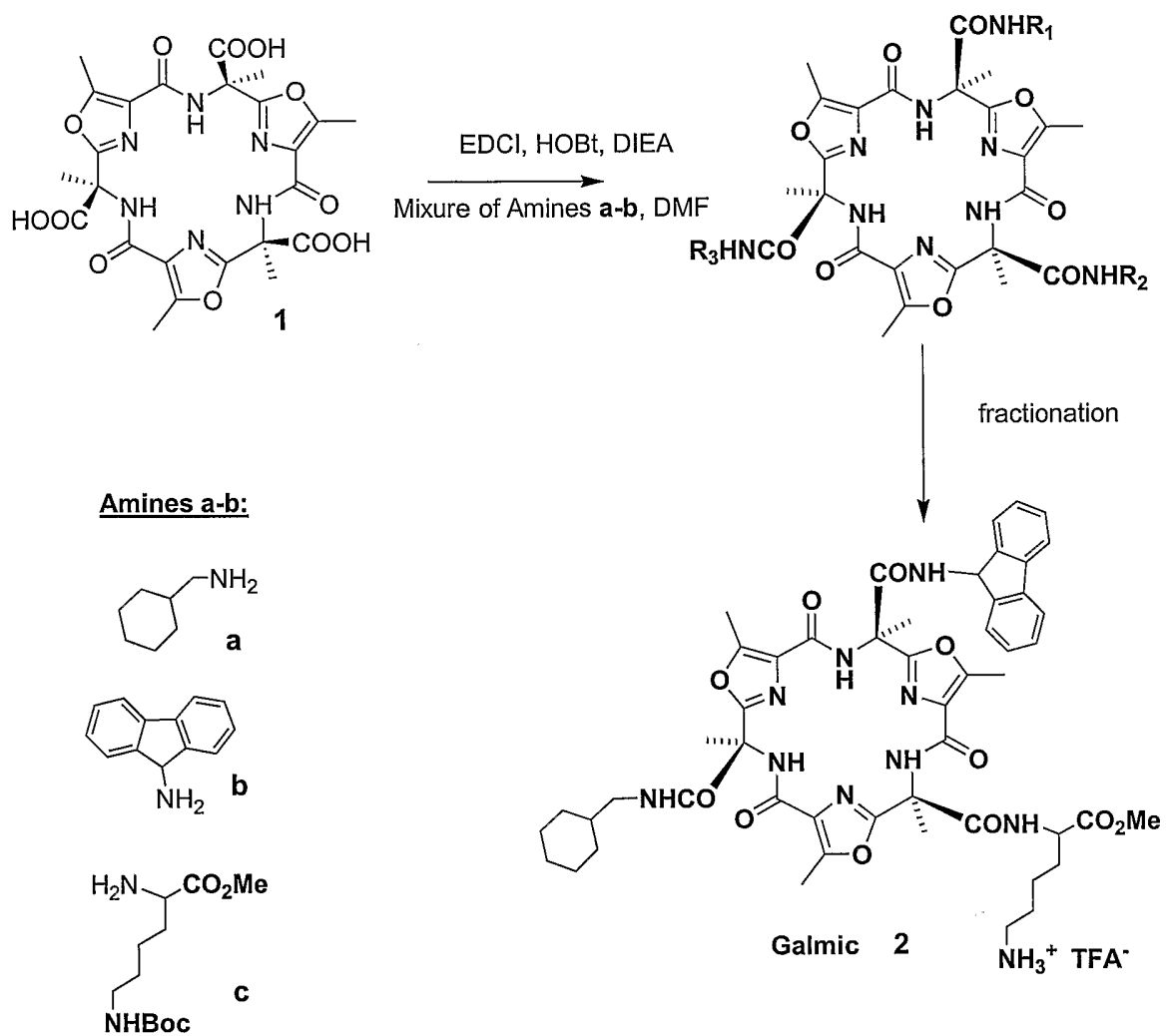


Figure 2

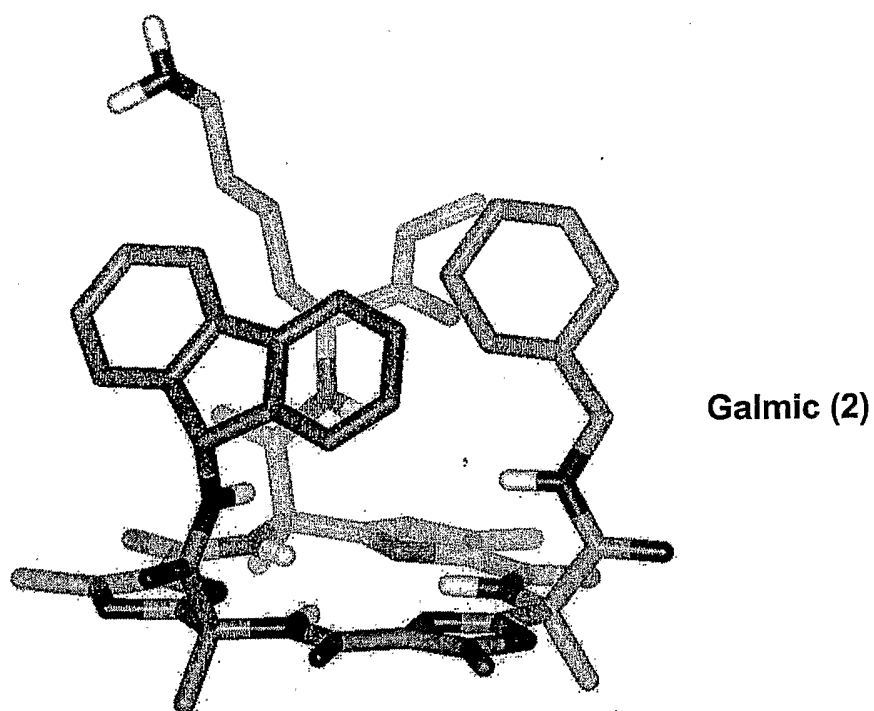


Figure 3

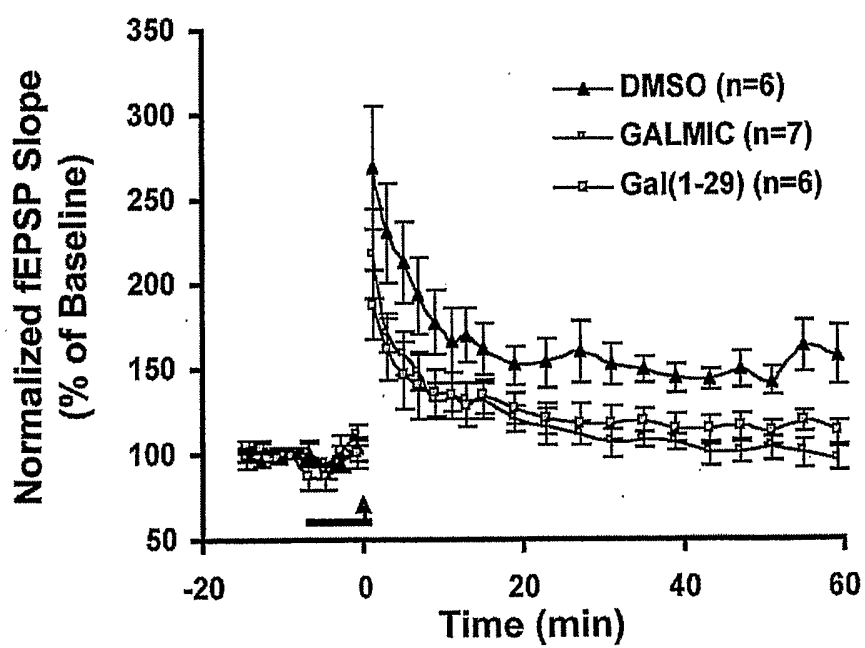


Figure 4

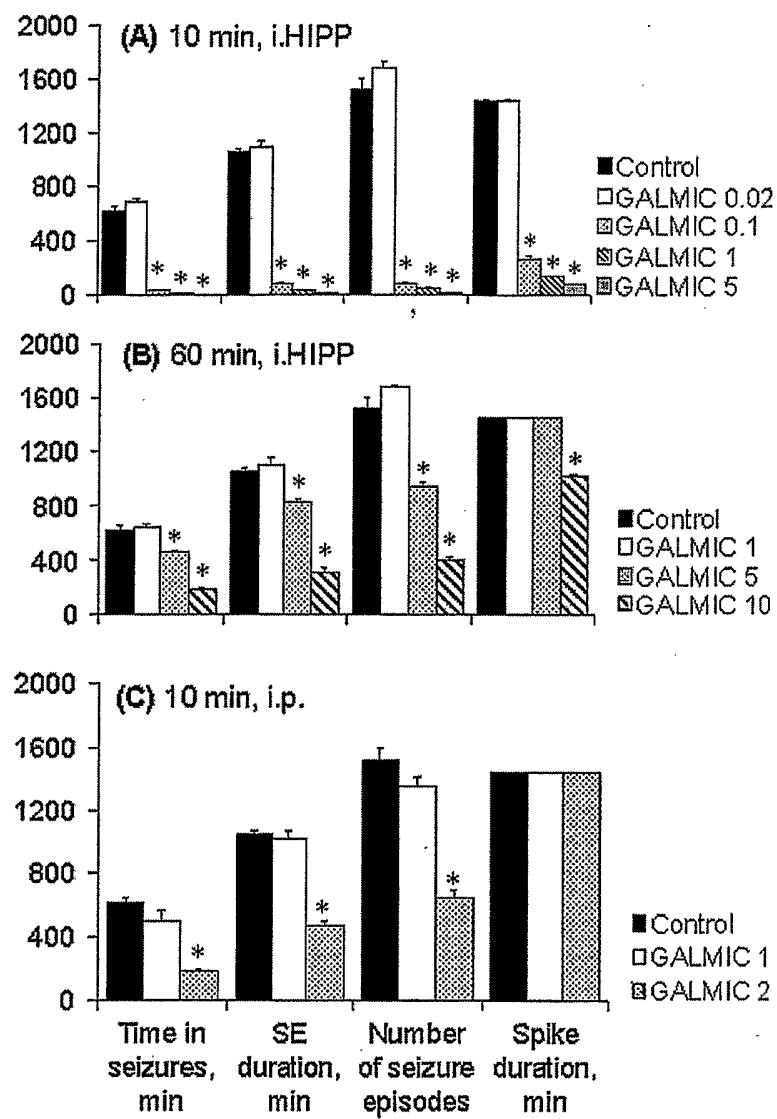


Figure 5

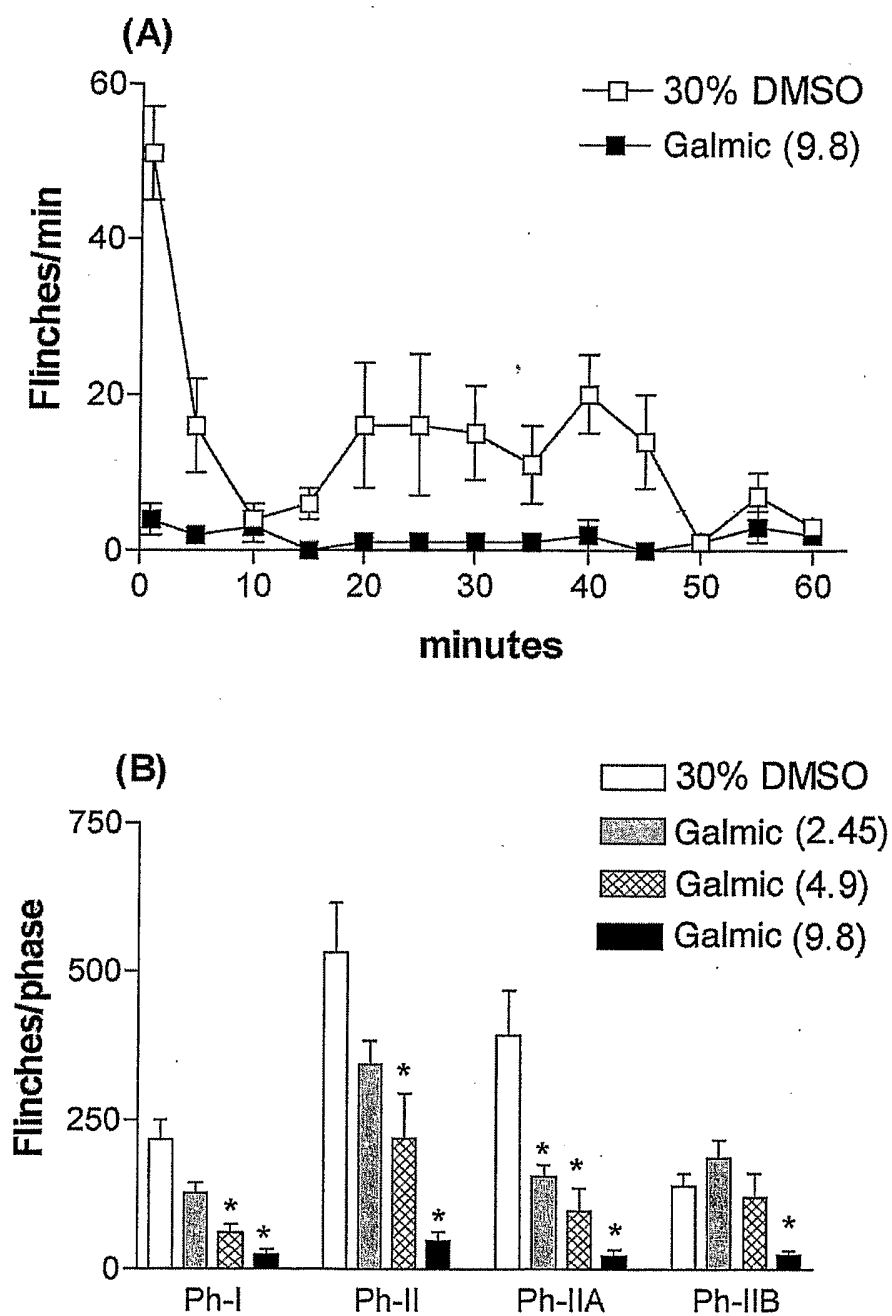
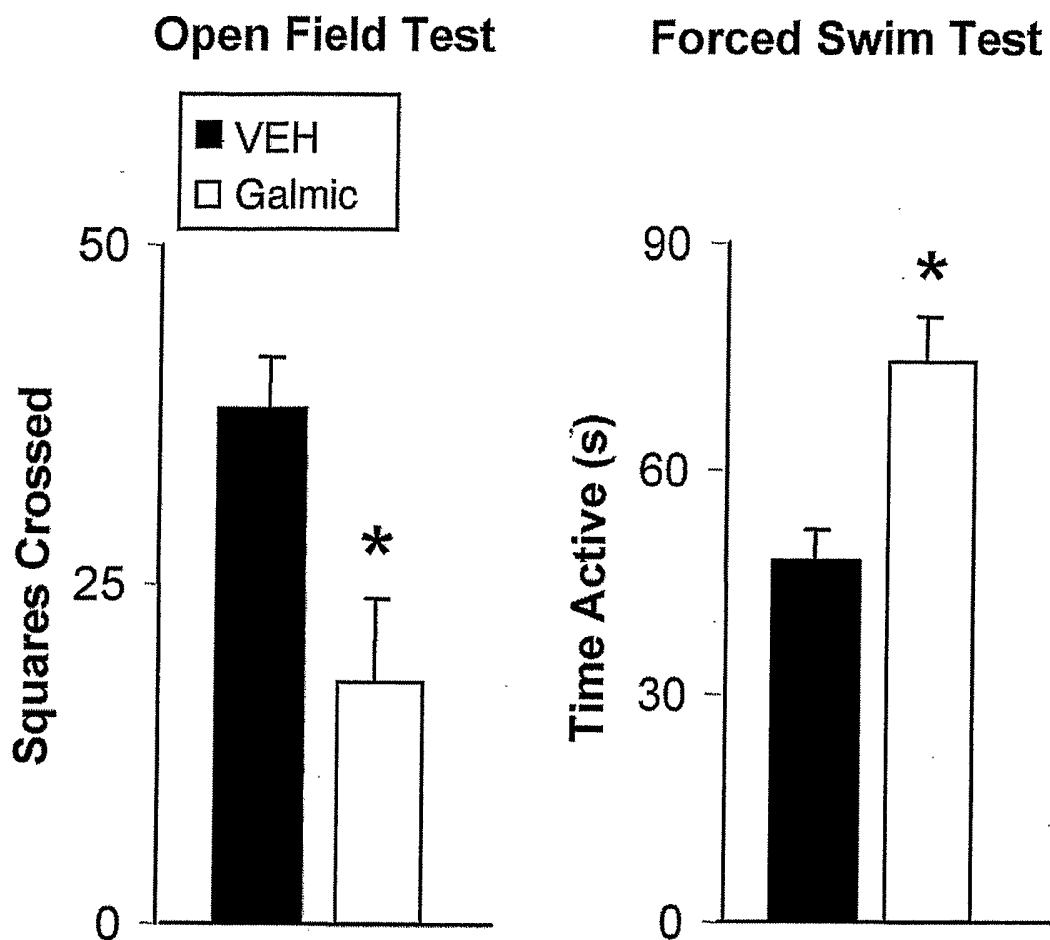


Figure 6

**Figure 7**

	Bowes Cells (hGalR1)	CHO Cells (rGalR2)
Galmic	34.2	>100
Galnon	11.7	34.1

Figure 8

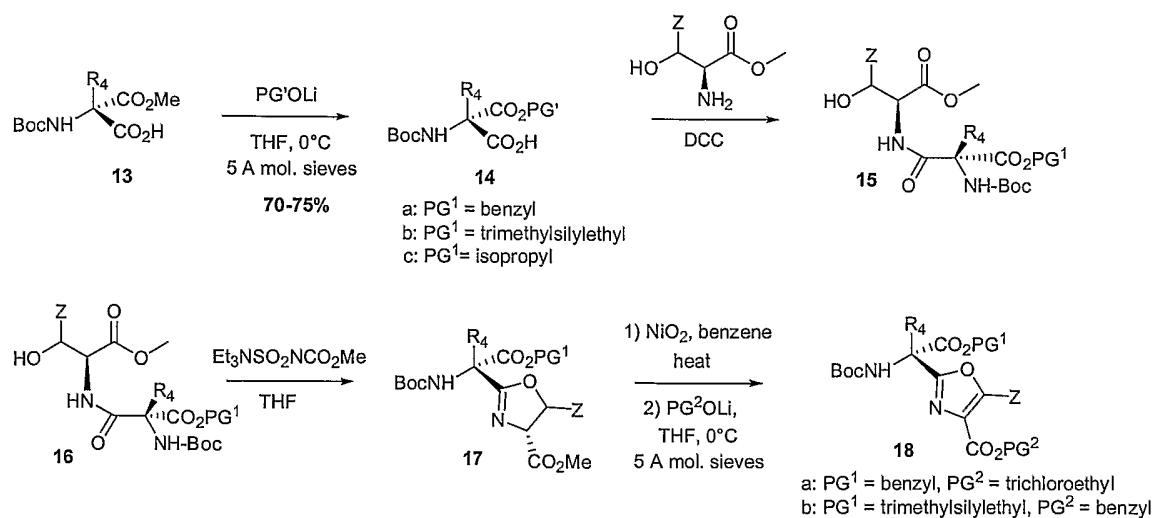


Figure 9

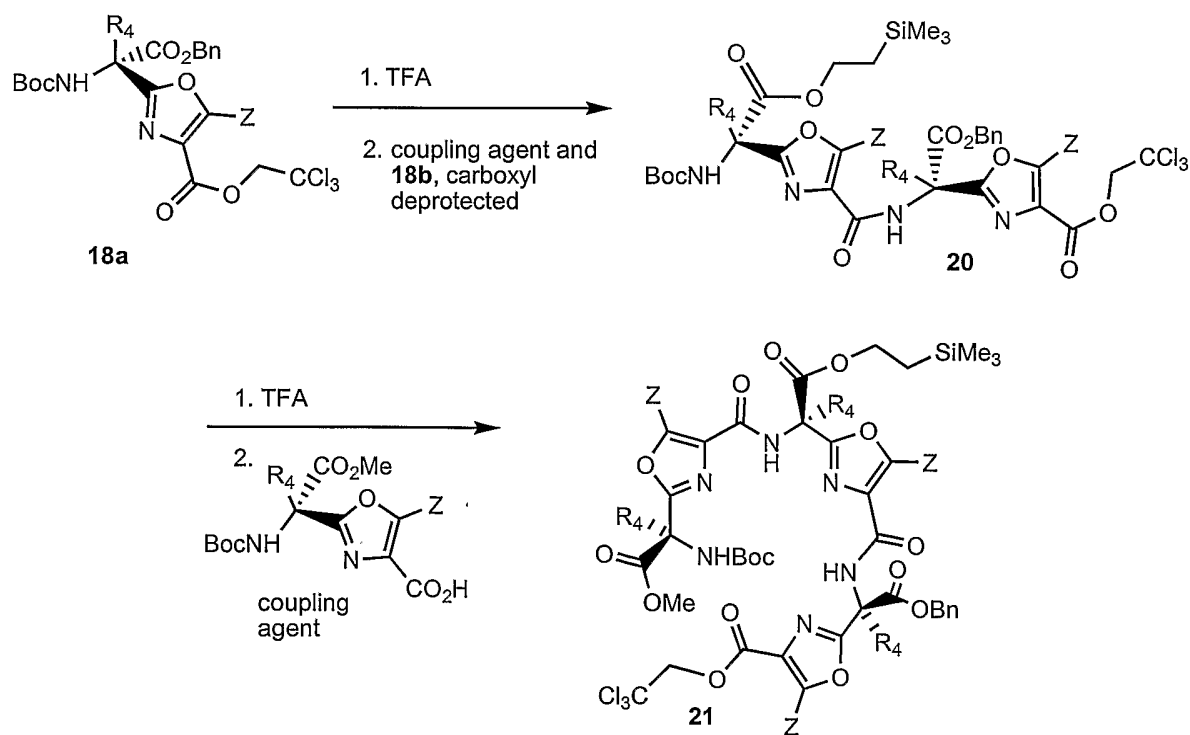


Figure 10

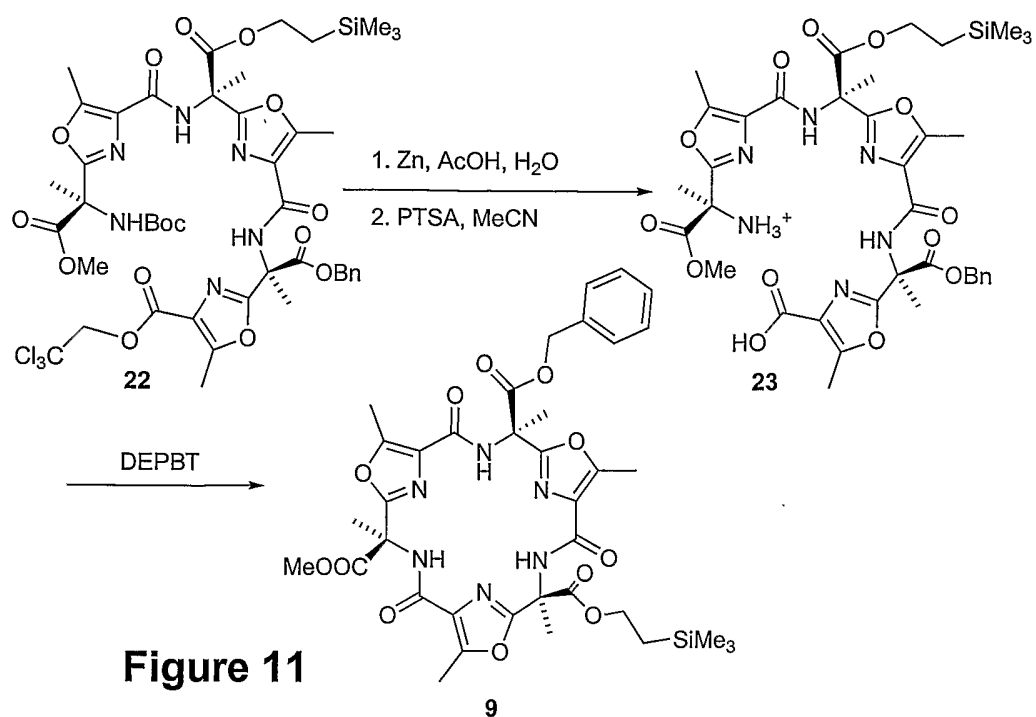


Figure 11

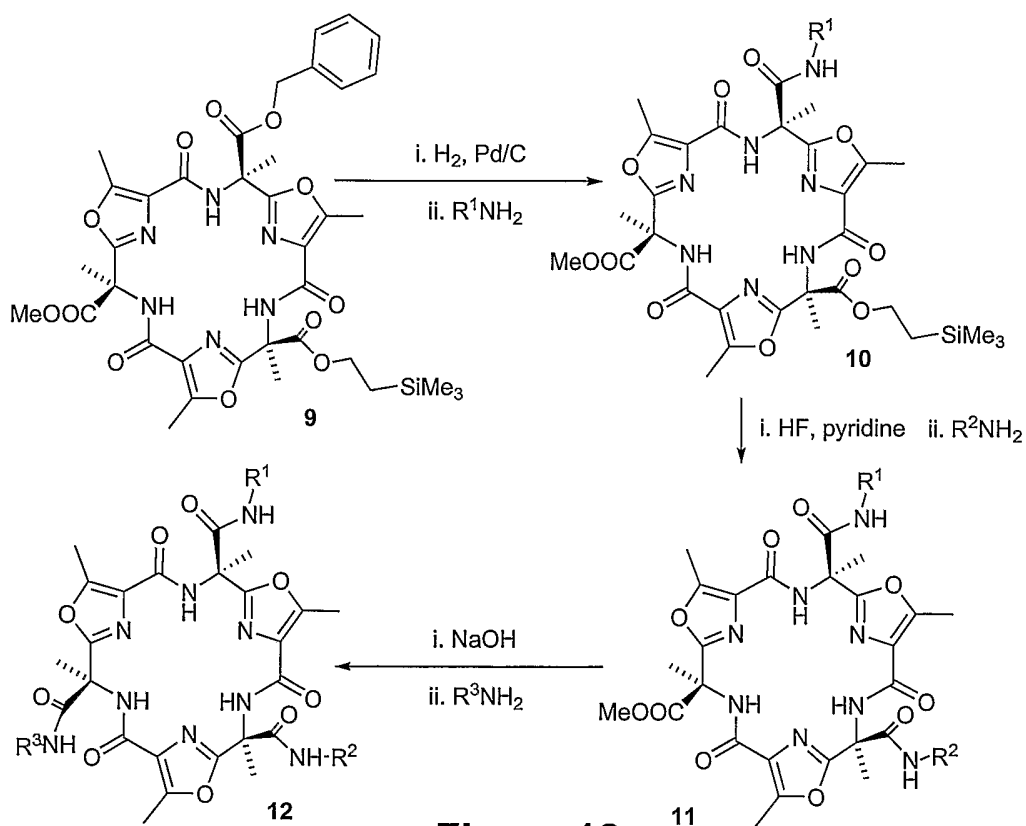


Figure 12

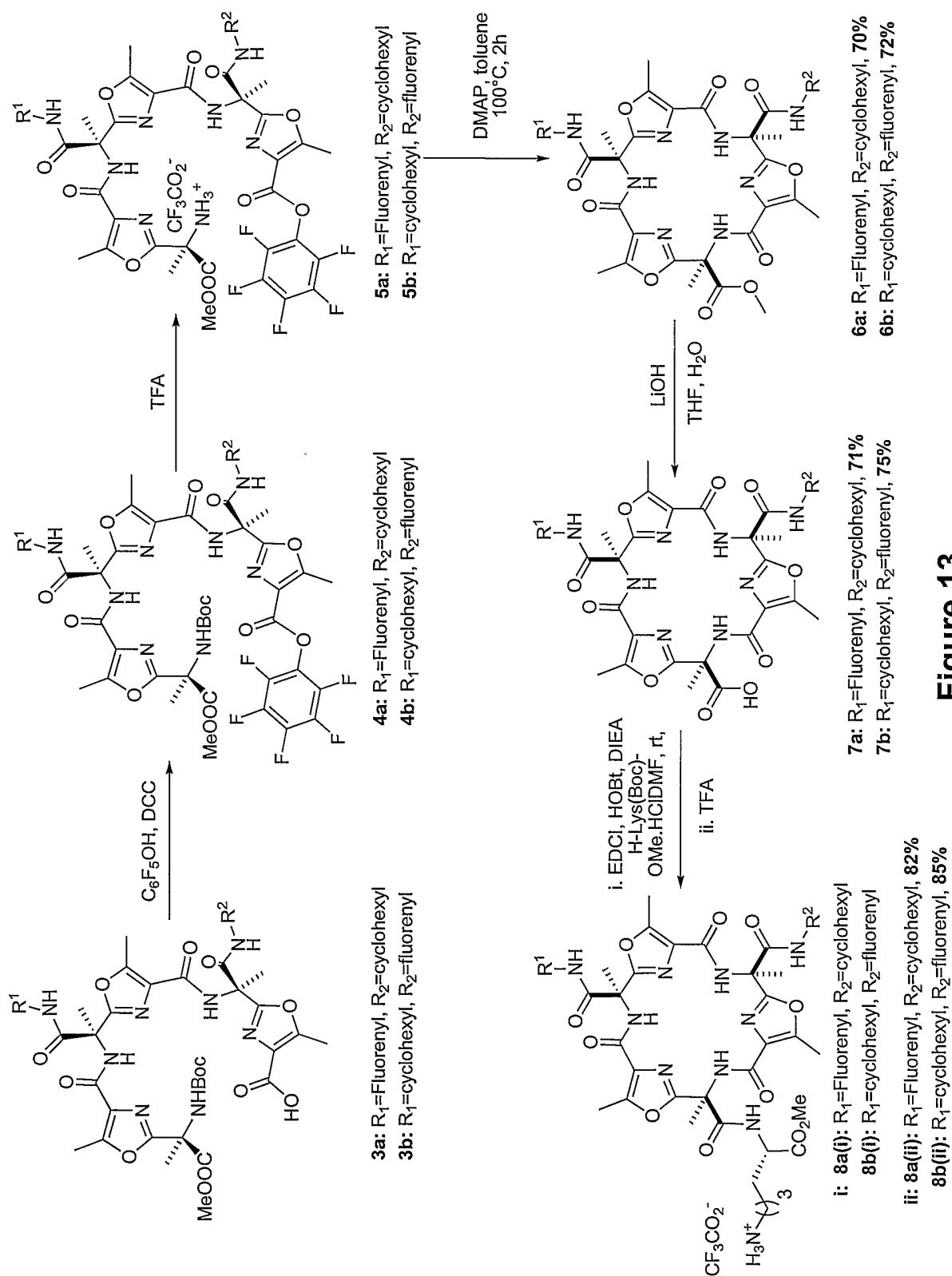


Figure 13