Synthesis and Biological Evaluation of Analogues of Pro-Leu-Gly-NH_2 Modified at the Leucyl Residue

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Received December 12, 1989

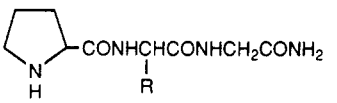
A series of analogues of Pro-Leu-Gly-NH_2 (PLG) in which the leucine residue has been replaced with the aliphatic amino acids L-isoleucine, L-2-aminohexanoic acid (Ahx), L-2-aminopentanoic acid, and L-2-aminobutanoic acid and the aromatic amino acids L-phenylalanine, L-phenylglycine, L- and D-2-amino-4-phenylbutanoic acid, L-*O*-methyltyrosine, and L-4-nitrophenylalanine have been synthesized. These analogues were tested for their ability to enhance the binding of the dopamine receptor agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to striatal dopamine receptors. Two of the above analogues, Pro-Ahx-Gly-NH_2 (**3**) and Pro-Phe-Gly-NH_2 (**6**), showed significant activity in this assay system. Pro-Ahx-Gly-NH_2 produced a 16% enhancement of ADTN binding at 0.1 μM, while Pro-Phe-Gly-NH_2 enhanced the binding of ADTN by 31% at a concentration of 1 μM.

Numerous pharmacological studies have demonstrated that the tripeptide Pro-Leu-Gly-NH_2 (PLG, **1**) is capable of modulating dopamine receptors within the central nervous system.[1] For example, we and others have demonstrated with radioligand binding studies that PLG selectively enhances the affinity of dopamine agonists such as 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN),[2,3] apomorphine,[4-6] and *n*-propylnorapomorphine[7,8] to dopamine receptors, but does not affect the binding of dopamine receptor antagonists such as spiroperidol. PLGs modulation of dopamine receptors is also illustrated by this tripeptide's ability to either prevent or reverse the development of D_2 dopamine receptor supersensitivity that is produced by the long-term administration of neuroleptic drugs such as haloperidol. This has been shown through both biochemical[9,10] and behavioral[11-13] studies. In fact, the ability of PLG to "down-regulate" supersensitized dopamine receptors appears to be a general phenomenon since it is observed in several other pharmacological paradigms.[7,14-16]

A number of structure–activity relationship studies have been carried out on PLG. Several groups including our own have shown through the use of a variety of pharmacological assay systems that the Pro residue of PLG can be replaced with other residues to afford pharmacologically active analogues.[3,17] We have also shown that the Gly-NH_2 residue can be replaced with a number of cyclic and heterocyclic amino acid residues to give active PLG analogues.[2] However, very few analogues of PLG in which the Leu residue has been modified have been evaluated for their ability to modulate dopamine receptors.[18,19] In an effort to further elucidate the structural requirements of PLG with respect to its ability to modulate dopamine receptors we have synthesized, in the present study, the PLG analogues **2–11** in which the Leu residue has been replaced with the aliphatic amino acids L-Ile, L-2-aminohexanoic acid (Ahx), L-2-aminopentanoic acid (Ape), and L-2-aminobutanoic acid (Abu) and the aromatic amino acids L-Phe, L-phenylglycine (Phg), L- and D-2-amino-4-phenylbutanoic acid (Hph), L-*O*-methyltyrosine, and L-4-nitrophenylalanine. These analogues have been evaluated for their ability to increase the binding of the dopamine agonist ADTN to striatal dopamine receptors.

Results and Discussion

Chemical Syntheses. The synthetic route that was used to make compounds **2–10** is depicted in Scheme I. The *N*-*tert*-butoxycarbonyl-protected amino acid derivatives were coupled by the mixed anhydride method[20,21] to either Gly-OEt, Gly-OMe, or Gly-NH_2 to give the dipeptide derivatives **12a–d**, **12e**, and **12f–i**, respectively (Table I). The *N*-*tert*-butoxycarbonyl group was removed from each



R =

1: $CH_2CH(CH_3)_2$

2: $CH(CH_3)CH_2CH_3$

3: $CH_2CH_2CH_2CH_3$

4: $CH_2CH_2CH_3$

5: CH_2CH_3

6: CH_2Ph

R =

7: Ph

8: CH_2CH_2Ph (L-isomer)

9: CH_2CH_2Ph (D-isomer)

10: CH_2–C$_6$H$_4$–OCH_3

11: CH_2–C$_6$H$_4$–NO_2

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0022-2623/90/1833-1828$02.50/0

Table I. Physical Properties of Protected Dipeptides **12a–j**

no.	compd	yield, %	mp, °C	$[\alpha]^{23}_D$, deg(*c*, solvent)	formula[a]
12a	Boc-Ile-Gly-OEt	78	101–102[b]	−9.4 (0.9, $CHCl_3$)	$C_{15}H_{28}N_2O_5$
12b	Boc-Ahx-Gly-OEt	54	wax[c]	−14.0 (0.6, MeOH)	$C_{15}H_{28}N_2O_5$
12c	Boc-Ape-Gly-OEt	97	74–75[b]	−25.1 (0.8, MeOH)	$C_{14}H_{26}N_2O_5$
12d	Boc-Abu-Gly-OEt	82	gum[c]	−16.9 (0.9, $CHCl_3$)	$C_{13}H_{24}N_2O_5$
12e	Boc-Phe-Gly-OMe	80	104–105[b]	−6.8 (1.46, AcOH)	$C_{17}H_{24}N_2O_5$
12f	Boc-Phg-Gly-NH_2	69	60–62[d]	+94.5 (0.5, MeOH)	$C_{15}H_{21}N_3O_4$
12g	Boc-L-Hph-Gly-NH_2	84	glass[d]	−4.8 (1.2, MeOH)	$C_{17}H_{25}N_3O_4$
12h	Boc-D-Hph-Gly-NH_2	87	glass[d]	+5.1 (1.7, MeOH)	$C_{17}H_{25}N_3O_4$
12i	Boc-Tyr(OMe)-Gly-NH_2	76	glass[e]	−13.2 (0.9, MeOH)	$C_{17}H_{25}N_3O_5$
12j	Boc-Phe(4-NO_2)-Gly-NH_2	68	173–175[d]	+53.5 (1.5, MeOH)	$C_{16}H_{22}N_4O_6$

[a] C, H, N analyses were within ±0.4% of the theoretical values unless otherwise indicated. [b] Recrystallized from EtOAc/hexanes. [c] Purified by flash chromatography (hexane/EtOAc, 3:1). [d] Purified by column chromatography (EtOAc/MeOH, 10:1). [e] Purified by column chromatography (EtOAc/MeOH, 1:1).

Table II. Physical Properties of Protected Tripeptides **13a–e** and **14a–j**

no.	compd	yield, %	mp, °C	$[\alpha]^{23}_D$, deg(*c*, solvent)	formula[a]
13a	Z-Pro-Ile-Gly-OEt	75	157–158[b]	−70.0 (1.3, $CHCl_3$)	$C_{23}H_{33}N_3O_6$
13b	Z-Pro-Ahx-Gly-OEt	66	134–135[b]	−70.1 (1.3, MeOH)	$C_{23}H_{33}N_3O_6$
13c	Z-Pro-Ape-Gly-OEt	69	152–153[b]	−87.0 (1.1, MeOH)	$C_{22}H_{31}N_3O_6$
13d	Z-Pro-Abu-Gly-OEt	63	109–110[c]	−88.4 (1.4, MeOH)	$C_{21}H_{29}N_3O_6$
13e	Z-Pro-Phe-Gly-OMe	77	144–147[d]	−69.9 (1.07, MeOH)[d]	$C_{25}H_{29}N_3O_6$[e]
14a	Z-Pro-Ile-Gly-NH_2	87	180–181[f]	−66.3 (1.6, MeOH)	$C_{21}H_{30}N_4O_5$
14b	Z-Pro-Ahx-Gly-NH_2	84	167–168[f]	−71.6 (1.0, MeOH)	$C_{21}H_{30}N_4O_5$
14c	Z-Pro-Ape-Gly-NH_2	84	189–191[g]	−75.5 (1.0, MeOH)	$C_{20}H_{28}N_4O_5$
14d	Z-Pro-Abu-Gly-NH_2	90	170–172[h]	−75.2 (0.8, $CHCl_3$)	$C_{19}H_{26}N_4O_5$
14e	Z-Pro-Phe-Gly-NH_2	84	174–176[f]	−73.7 (1.0, MeOH)	$C_{24}H_{28}N_4O_5$[i]
14f	Z-Pro-Phg-Gly-NH_2	52	213–217[g]	+40.0 (0.3, MeOH)	$C_{23}H_{26}N_4O_5$
14g	Z-Pro-L-Hph-Gly-NH_2	75	113–116[g]	−55.0 (1.0, MeOH)	$C_{25}H_{30}N_4O_5$
14h	Z-Pro-D-Hph-Gly-NH_2	60	165–167[f]	−21.9 (1.0, MeOH)	$C_{25}H_{30}N_4O_5$
14i	Z-Pro-Tyr(OMe)-Gly-NH_2	66	172–175[j]	−58.8 (1.0, MeOH)	$C_{25}H_{30}N_4O_6$
14j	Boc-Pro-Phe(4-NO_2)-Gly-NH_2	73	190–192[g]	−65.0 (0.8, MeOH)	$C_{21}H_{29}N_5O_7$

[a] C, H, N analyses were within ±0.4% of the theoretical values unless otherwise indicated. [b] Recrystallized from EtOAc. [c] Purified by flash chromatography (EtOAc/hexanes, 3:2) and recrystallization from $CHCl_3$/hexanes. [d] Lit.[23] reports mp 135 °C, $[\alpha]_D$ −67° (*c* 1.0, MeOH). [e] Not determined. [f] Recrystallized from MeOH/Et_2O. [g] Recrystallized from MeOH. [h] Recrystallized from acetone/hexanes. [i] C: calcd, 63.70; found, 62.78. [j] Recrystallized from *i*-PrOH.

Table III. Physical Properties of PLG Analogues **2–11**

no.	compd	yield, %	mp, °C	$[\alpha]^{23}_D$, deg(*c*, solvent)	formula[a]
2	Pro-Ile-Gly-NH_2	82	167–169[b]	−48.7 (1.5, MeOH)	$C_{13}H_{24}N_4O_3$
3	Pro-Ahx-Gly-NH_2	92	153–154[b]	−40.3 (0.9, MeOH)	$C_{13}H_{24}N_4O_3$
4	Pro-Ape-Gly-NH_2	91	foam[c]	−47.7 (1.7, MeOH)	$C_{12}H_{22}N_4O_3 \cdot {}^1/_4H_2O$
5	Pro-Abu-Gly-NH_2	88	foam[c]	−56.1 (0.5, MeOH)	$C_{11}H_{20}N_4O_3 \cdot {}^1/_4H_2O$
6	Pro-Phe-Gly-NH_2·HCl	70	163–165	−9.1 (1.1, MeOH)	$C_{16}H_{23}N_4O_3Cl \cdot {}^1/_4H_2O$
7	Pro-Phg-Gly-NH_2	55	glass[c]	+71.3 (0.47, MeOH)	$C_{15}H_{20}N_4O_3$
8	Pro-L-Hph-Gly-NH_2	75	78–81[b]	−30.8 (1.0, MeOH)	$C_{17}H_{24}N_4O_3 \cdot {}^1/_2H_2O$
9	Pro-D-Hph-Gly-NH_2	79	79–81[b]	−30.1 (1.0, MeOH)	$C_{17}H_{24}N_4O_3 \cdot {}^1/_2H_2O$
10	Pro-Tyr(OMe)-Gly-NH_2	27	glass[c]	−4.1 (0.22, MeOH)	$C_{17}H_{24}N_4O_4$[d]
11	Pro-Phe(4-NO_2)-Gly-NH_2·HCl	87	280 dec	−7.6 (0.3, MeOH)	$C_{16}H_{22}N_5O_5Cl$

[a] C, H, N analyses were within ±0.4% of the theoretical values unless otherwise indicated. [b] Recrystallized from MeOH/Et_2O. [c] Purified by medium-pressure reverse-phase chromatography (C-8; MeOH/H_2O, 1:1). [d] Elemental analyses not carried out due to the small amount of material obtained. TLC analysis showed the material to be homogeneous: R_f = 0.07 (EtOAc/MeOH/H_2O, 16:4:1, silica gel), R_f = 0.15 (MeOH/H_2O, 2:1, C-18).

of the dipeptides **12a–e** with 4 N HCl in dioxane and the resulting deprotected species then was coupled to Z-Pro-OH by the mixed-anhydride method to provide tripeptides **13a–e** (Table II). These esters were converted to the corresponding primary amides **14a–e** (Table II) with methanolic ammonia. The tripeptides **14f–i** (Table II) were obtained directly from the dipeptides **12f–i** by coupling the corresponding deprotected dipeptide derivatives to Z-Pro-OH by the mixed anhydride method. Hydrogenolysis (H_2, Pd/C) of the tripeptide **14a–i** yielded the Leu modified PLG analogues **2–10** (Table III).

The PLG analogue Pro-Phe(4-NO_2)-Gly-NH_2 (**11**) was obtained in an analogous manner except that after the dipeptide Boc-Phe(4-NO_2)-Gly-NH_2 (**12j**) was deprotected it was coupled to Boc-Pro-OH to give Boc-Pro-Phe(4-NO_2)-Gly-NH_2 (**14j**). Removal of the *N-tert*-butoxycarbonyl protecting group from **14j** with 4 N HCl in dioxane yielded the desired PLG analogue **11**.

Pharmacological Studies. The Leu-modified PLG analogues, compounds **2–11**, were evaluated for their ability to enhance the binding of [^{3}H]ADTN to D_2 dopamine receptors isolated from bovine caudate membranes. This assay, which has been described previously by us,[2,3,22] has been used to evaluate the ability of PLG and its analogues to modulate dopamine receptors. In this assay, PLG possesses a dose–response curve that is bell-shaped with the maximum effect occurring at a PLG concentration of 1 μM (Figure 1). At this dose level, PLG enhances the binding of ADTN to dopamine receptors by 27%. This enhancement in binding has been shown previously to be due to a decrease in the dissociation constant of the agonist

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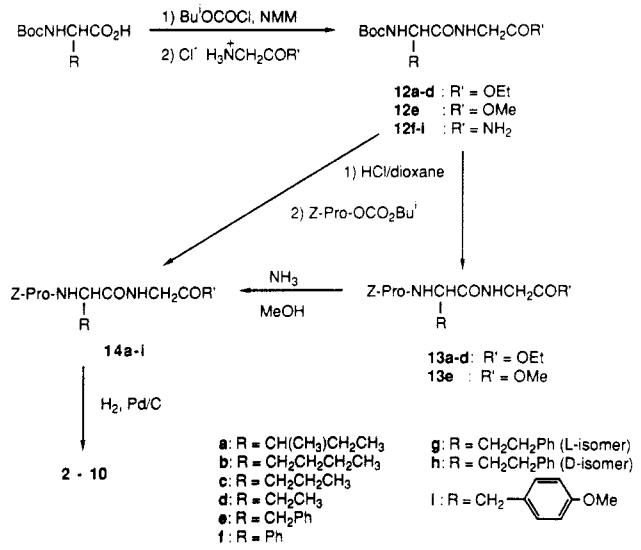
Scheme I



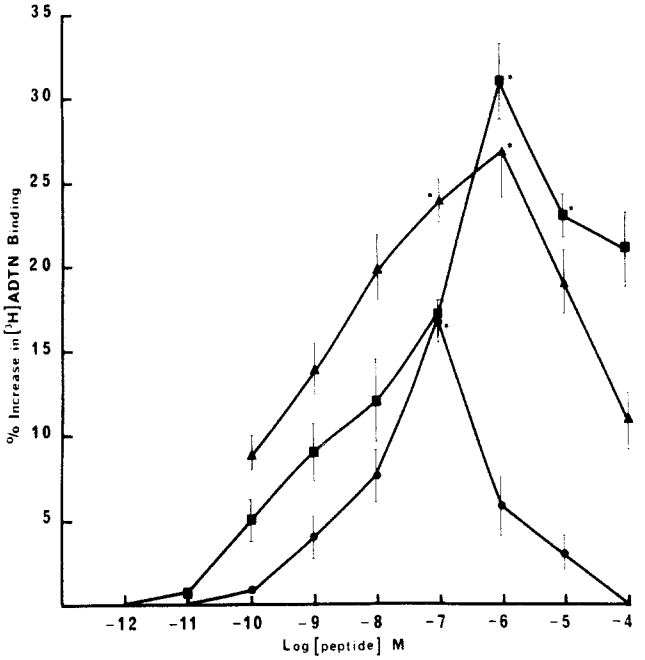


Figure 1. Stimulation of [^{3}H]ADTN binding to striatal membranes by PLG and its analogues **3** and **6**. Percent increase in specific [^{3}H]ADTN binding over the control value when the indicated concentration of PLG (1 (▲)), **3** (●) or **6** (■) dissolved in assay buffer was added directly to the assay mixture. Control tubes contained a corresponding volume of assay buffer. Results are the means ± SEM of 4–5 experiments carried out in triplicate. (*) Significantly different ($p < 0.05$) from control values.

and not to an increase in the number of dopamine receptors.[4]

Each analogue was tested for its effect on the binding of [^{3}H]ADTN to bovine striatal membrane dopamine receptors at doses ranging from 1 pM to 100 μM. Of the 10 PLG analogues evaluated in this study two analogues showed significant ability to enhance the binding of the dopamine receptor agonist ADTN to dopamine receptors. These two analogues were Pro-Ahx-Gly-NH_2 (**3**) and Pro-Phe-Gly-NH_2 (**6**). The dose–response curves for these two compounds are depicted in Figure 1. Like PLG, both of these compounds possessed a dose–response curve that was bell-shape in nature. Pro-Phe-Gly-NH_2 (**6**) produced its maximum effect at a concentration of 1 μM where it enhanced the binding of ADTN by 31%. Thus, this compound's profile is similar to that seen with PLG. In contrast, Pro-Ahx-Gly-NH_2 (**3**) produced its maximum effect at a concentration of 0.1 μM, but its percent enhancement of ADTN binding to dopamine receptors was only one-half that produced by Pro-Phe-Gly-NH_2. These results indicate that although **3** has a slightly greater affinity for the PLG receptor than does either PLG or **6**, it has a lower intrinsic activity than these two compounds. A possible explanation for these observations is that **3** may actually be a partial agonist. Although the PLG analogue Pro-Ile-Gly-NH_2 (**2**) was previously shown to block the tolerance to the pharmacological effects of morphine,[19] this compound was found to be inactive in our assay system. The difference in the activities seen with **2** is most likely due to the fact that totally different biological assay systems were employed.

Although the results of this study suggest that the side chain of the second amino acid residue of PLG may be either alkyl or aryl in nature, they also indicate that there are some fairly strict requirements for the type of alkyl or aryl side chain that is allowed. Of the various alkyl and aryl side chains examined in this study, only the butyl and benzyl side chains of analogues **3** and **6**, respectively, were capable of replacing the Leu isobutyl side chain of PLG. The placement of the electron-donating methoxy group or the electron-withdrawing nitro group in the para position of the aromatic ring of **6** had an adverse effect on this compound's dopamine receptor modulating activity, since the analogues Pro-Tyr(OMe)-Gly-NH_2 (**10**) and Pro-Phe-(4-NO_2)-Gly-NH_2 (**11**) were inactive. The fact that both types of aromatic substitutions led to inactive analogues suggests that steric effects may be coming into play in these cases. This hypothesis is supported by the inactivity of the analogue Pro-L-Hph-Gly-NH_2 (**8**), in which the benzyl side chain has been extended to the phenylethyl side chain.

Although adverse steric interactions may very well explain the inactivity of the analogues **8**, **10**, and **11**, it is unlikely that such an explanation can account for the inactivity seen with the analogues **2**, **4**, **5**, and **7**. Clearly, if there is a hydrophobic pocket on the PLG receptor with which the isobutyl side chain of PLG and the butyl and benzyl side chains of analogues **3** and **6** interact then the smaller alkyl side chains found in analogues **2**, **4**, and **5** and the phenyl group found in analogue **7** should be readily accommodated by such a pocket. It may be that the interaction of the alkyl or aryl side chain with a hydrophobic pocket on the PLG receptor is required for dopamine receptor modulation. Thus, while the isobutyl, butyl, and benzyl side chains are able to interact with the hydrophobic binding site, the shorter alkyl and aryl side chains, while accommodated in the pocket, are just too short for the necessary interaction to take place. If this is the case then these analogues might be potential antagonists of PLG. Studies are underway to test this possibility. Alternatively, the inactivity of analogues **2**, **4**, **5**, and **7** may be due to their inability to exist in a type II β-turn, the conformation that we have shown[22] through the use of conformationally constrained analogues is the likely biologically active conformation of PLG.

In summary, the Leu residue of PLG can be replaced with other alkyl or aryl amino acid residues to yield active analogues of PLG. However, the extent to which this residue can be varied does not appear to be as great as that for either the Pro or Gly-NH_2 residues.[2,3]

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K and are uncorrected. Specific

rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D line). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ± 0.4% of the theoretical values. ^{1}H NMR spectra were recorded on either a JEOL FX-90-MHz, an IBM 200-MHz, a Bruker 250-MHz, or a Nicolet Zeta 300-MHz spectrometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in $CDCl_3$ or DMSO-d_6 and to sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (TSP) in D_2O. ^{13}C NMR was performed on either a JEOL FX-90-MHz instrument at 22.5 MHz or an IBM instrument at 50 MHz. When DMSO-d_6 was used as solvent, it served as the internal standard at δ 39.5. FAB mass spectra were obtained on a VG 7070E-HF mass spectrometer. Column chromatography was performed with Silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF uniplates. Visualization was achieved with either UV, I_2, or ninhydrin spray. 2-[5,8-^{3}H]Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]ADTN) was obtained from New England Nuclear, Boston, MA.

General Procedure for the Synthesis of Dipeptide Esters 12a–e. A solution of either Boc-L-Ile, Boc-L-Ahx, Boc-L-Ape, Boc-L-Abu, or Boc-L-Phe (6.5 mmol) and *N*-methylmorpholine (0.71 mL, 6.5 mmol) in THF (25 mL) was cooled to −20 °C under nitrogen. Isobutyl chloroformate (0.85 mL, 6.5 mmol) was then added by syringe. After the reaction mixture was mixed for 15 min, it was transferred to a cooled (−20 °C) suspension of either Gly-OEt·HCl (0.91 g, 6.5 mmol) or Gly-OMe·HCl (0.82 g, 6.5 mmol) and NEt_3 (0.9 mL, 6.5 mmol) in THF. The reaction mixture was allowed to warm to 4 °C over the period of 1 h where it was stored overnight. The precipitate was filtered off and rinsed with cold THF. The combined filtrates were evaporated to give a white solid. This residue was dissolved in EtOAc (75 mL) and washed with 5% citric acid (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and H_2O (25 mL). The EtOAc layer was dried (Na_2SO_4) prior to being evaporated to dryness. The residue was purified by recrystallization or by flash chromatography. The physical properties for dipeptides **12a–e** are listed in Table I.

General Procedure for the Synthesis of Dipeptide Amides 12f–j. The required *tert*-butoxycarbonyl-protected amino acid (5.9 mmol) was dissolved in THF (15 mL) and *N*-methylmorpholine (0.6 g, 5.9 mmol) was added to the solution. The solution was cooled to −20 °C, after which time, a solution of isobutyl chloroformate (0.81 g, 5.9 mmol) in THF (5 mL) was introduced. This mixture was stirred at −20 °C for 10 min and then treated with a solution of Gly-NH_2·HCl (0.66 g, 5.9 mmol) and NEt_3 (0.6 g, 5.9 mmol) in 10 mL of DMF and 1 mL of H_2O. The solution was stirred at 25 °C for 18 h. The solvents were evaporated under reduced pressure, and the solid residue obtained was partitioned between 10% citric acid and EtOAc. The acidic aqueous layer was extracted two more times with EtOAc (2 × 20 mL) and the combined EtOAc extracts were washed with 1 M $NaHCO_3$ (30 mL) and saturated NaCl solution (30 mL). The EtOAc layer was dried over $MgSO_4$ and then evaporated to dryness under reduced pressure. The residue obtained was purified by silica gel column chromatography. The physical properties for dipeptides **12f–j** are listed in Table I.

General Procedure for the Synthesis of Tripeptide Esters 13a–e. Each of the protected dipeptides **12a–e** (4.4 mmol) in dioxane (5 mL) at room temperature was treated with 10 mL of a saturated solution of HCl in dioxane. After each reaction mixture was stirred for 2 h, the solvent was stripped off and the residue dissolved in absolute EtOH. The EtOH was removed by evaporation, and the process was repeated. The dipeptide ester hydrochloride salts obtained were dried under vacuum over NaOH pellets before they were used in the coupling reaction described below.

The mixed carbonic anhydride of Z-L-Pro (1.1 g, 4.4 mmol) was prepared in the usual manner in THF with *N*-methylmorpholine and isobutyl chloroformate. The cooled (−20 °C) suspension of the anhydride was added 15 min later to a cooled suspension (−20 °C) of dipeptide ester hydrochloride salt and NEt_3 (0.61 mL, 4.4 mmol) in THF (20 mL). The reaction mixture was allowed to warm to 4 °C where it was stored overnight. The precipitate was filtered off and rinsed with THF. The combined filtrates were evaporated to dryness, and the residue was partitioned between EtOAc (75 mL) and 5% citric acid (25 mL). The organic phase was washed with 5% citric acid (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and H_2O (25 mL) before it was dried over Na_2SO_4. The residue obtained after removal of the solvent was purified by recrystallization. The physical properties of the protected tripeptide esters **13a–e** are listed in Table II.

General Procedure for the Synthesis of the N-Protected Tripeptide Amides 14a–e. The protected tripeptide ester (**13a–e**, 2.7 mmol) was suspended in cold (0 °C) methanolic ammonia (20 mL). The reaction mixture became homogeneous upon warming to room temperature. The solution was stirred at room temperature for 36 h after which time the solvent and excess NH_3 were removed in vacuo and the residue purified by recrystallization. The physical properties of the protected tripeptide amides **14a–e** obtained are listed in Table II.

General Procedure for the Synthesis of the N-Protected Tripeptide Amides 14f–j. Protected dipeptide amide (**12f–j**, 4.2 mmol) was treated with 5 mL of 4 N HCl in dioxane. The mixture was stirred at room temperature for 1 h, after which time the dioxane and excess HCl were evaporated under reduced pressure. The dipeptide hydrochloride salts obtained were used without further purification in the coupling procedure described below.

A solution of Z-Pro-OH (1.05 g, 4.2 mmol) and *N*-methylmorpholine (0.42 g, 4.2 mmol) in THF (10 mL) was cooled to −20 °C. Isobutyl chloroformate (0.56 g, 4.2 mmol) was added to this solution. The solution was stirred at −20 °C for 10 min, after which time, a solution of the dipeptide amide hydrochloride salt (4.2 mmol) and NEt_3 (0.42 g, 4.2 mmol) in 10 mL of DMF and 1 mL of H_2O was added. The resultant solution was stirred at 25 °C for 18 h. At the end of this time period 10% citric acid was added to the solution and the mixture extracted with EtOAc (3 × 40 mL). The EtOAc extracts were combined and washed with H_2O (40 mL) and saturated NaCl solution (40 mL) before they were dried over $MgSO_4$. The solvent was removed in vacuo and the crude product obtained purified by recrystallization.

The same general methodology was used to prepare Boc-Pro-Phe(4NO_2)-Gly-NH_2 (**14j**) except that Boc-Pro-OH was used instead of Z-Pro-OH. The physical properties of the protected tripeptide amides **14f–j** obtained by the above procedures are listed in Table II.

General Procedure for the Synthesis of PLG Analogues 2–11. A solution of the N-protected tripeptide amide (**14a–i**, 2 mmol) in MeOH was degassed with nitrogen and this solution added to a flask containing 10% Pd/C (0.1 g). Hydrogen was bubbled into the stirred reaction mixture until TLC indicated that the reaction was complete. The mixture was filtered through a pad of Celite, and the filtrate was stripped of solvent under reduced pressure. The crude deprotected tripeptide obtained was purified either by recrystallization or by medium-pressure reverse-phase chromatography with a C-8 column.

Protected tripeptide **14j** was deprotected by treating with 4 N HCl in dioxane (5 mL) at 25 °C for 1 h. The dioxane and excess HCl were removed in vacuo to provide pure **11**.

The physical properties of compounds **2–11** are listed in Table III. The spectroscopic characteristics of these compounds are given below:

Pro-Ile-Gly-NH_2 (2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.93 (t, 3 H, Ile δ-CH_3), 0.95 (d, 3 H, J = 6.7 Hz, Ile γ'-CH_3), 1.06–1.31 and 1.44–1.59 (m, 2 H, Ile γ-CH_2), 1.67–2.23 (m, 5 H, Ile β-CH, Pro β,γ-CH_2), 2.88–3.13 (m, 2 H, Pro δ-CH_2), 3.72–3.78 (m, 1 H, Pro α-CH), 3.84 and 4.06 (2d, 1 H each, Gly α-CH_2), 4.14 (t, 1 H, Ile α-CH), 5.85 (br s, 1 H, $CONH_2$), 6.75 (br s, 1 H, $CONH_2$), 7.20 (t, 1 H, Gly NH), 8.26 (d, 1 H, Ile NH).

Pro-Ahx-Gly-NH_2 (3). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.89 (t, 3 H, Ahx CH_3), 1.08–1.58 (m, 4 H, Ahx γ,δ-CH_2), 1.69 (t, 2 H, Ahx β-CH_2), 1.79–2.21 (m, 4 H, Pro β,γ-CH_2), 2.84–3.08 (m, 2 H, Pro δ-CH_2), 3.71–3.74 (m, 1 H, Pro α-CH), 3.75–4.03 (m, 2 H, Gly α-CH_2), 4.08–4.25 (m, 1 H, Ahx α-CH), 5.80 (br s, 1 H, $CONH_2$), 6.73 (br s, 1 H, $CONH_2$), 7.18 (t, 1 H, Gly NH), 8.18 and 8.25 (2 d, 1 H, Ahx NH).

Pro-Ape-Gly-NH_2 (4). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.94 (t, 3 H, Ape δ-CH_3), 1.27–1.44 (m, 2 H, Ape γ-CH_2), 1.60–1.75 (m, 2 H, Ape β-CH_2), 1.77–1.90 (m, 2 H, Pro γ-CH_2), 2.05–2.31 (m, 2 H, Pro β-CH_2), 2.85–3.09 (m, 2 H, Pro δ-CH_2), 3.66–3.84 (dd

over m, 2 H, Gly α-CH_2 and Pro α-CH), 4.03 (dd, J = 6.2 and 20 Hz, 1 H, Gly α-CH_2), 4.27 (dd, 1 H, Ape α-CH), 6.14 and 6.88 (2 br s, 2 H, $CONH_2$), 7.56 (m, 1 H, Gly NH), 8.14 (d, 1 H, J = 6.9 Hz, Ape NH).

Pro-Abu-Gly-NH_2 (5). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.96 (t, 3 H, Abu γ-CH_3), 1.38–1.75 (m, 2 H, Abu β-CH_2), 1.79–2.22 (m, 4 H, Pro β,γ-CH_2), 2.87–3.03 (m, 2 H, Pro δ-CH_2), 3.71–3.90 (dd over m, 2 H, Gly α-CH_2 and Pro α-CH), 4.09 (dd, J = 6.6 and 10.3 Hz, 1 H, Gly α-CH_2), 4.19 (dd, 1 H, Abu α-CH), 5.85 and 6.76 (2 br s, 2 H, $CONH_2$), 7.30 (m, 1 H, Gly NH), 8.14 (d, 1 H, J = 6.8 Hz, Abu NH).

Pro-Phe-Gly-NH_2·HCl (6). ^{1}H NMR (300 MHz, D_2O): δ 1.95–2.08 (m, 3 H, Pro β-CH, γ-CH_2), 2.38–2.47 (m, 1 H, Pro β-CH), 3.12 (d, J = 7.9 Hz, 2 H, Phe β-CH_2), 3.34–3.44 (m, 2 H, Pro δ-CH_2), 3.80 (dd, J = 14.4 and 17.2 Hz, 2 H, Gly α-CH_2), 4.33–4.38 (m, 1 H, Pro α-CH), 4.63 (t, J = 7.9 Hz, 1 H, Phe α-CH), 7.29–7.43 (m, 5 H, Ar H's). TLC: R_f(1-propanol/NH_4OH, 4:1) = 0.42; R_f(1-butanol/HOAc/EtOAc/H_2O, 1:1:1:1) = 0.53.

Pro-Phg-Gly-NH_2 (7). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57–1.64 (m, 3 H, Pro β-CH, γ-CH_2), 1.89–1.92 (m, 1 H, Pro β-CH), 2.74–2.91 (m, 2 H, Pro δ-CH_2), 3.51–3.62 (m, 2 H, Gly α-CH_2), 3.74 (dd, J = 6.2 and 16.7 Hz, 1 H, Pro α-CH), 5.45 (d, J = 7.7 Hz, 1 H, Phg α-CH), 7.04 (s, 1 H, $CONH_2$), 7.26–7.39 (m, 6 H, Ar H's and $CONH_2$), 8.61–8.67 (m, 2 H, Phg and Gly CONH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 26.1 (Pro γ-C), 30.4 (Pro β-C), 42.0 (Gly α-C), 46.9 (Pro δ-C), 55.6 (Phg α-C), 60.2 (Pro α-C), 126.8, 127.7, 128.5, 139.3 (C_6H_5), 170.1, 170.6, 174.0 (C=O). FAB-MS: m/z 305 $[MH]^+$.

Pro-L-Hph-Gly-NH_2 (8). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.61–1.96 (m, 6 H, Pro β,γ-CH_2 and Hph β-CH_2), 2.53–2.70 (m, 2 H, CH_2Ph), 2.75–3.00 (m, 2 H, Pro δ-CH_2), 3.64–3.67 (m, 3 H, Pro α-CH and Gly α-CH_2), 4.32 (m, 1 H, Hph α-CH), 7.10–7.32 (m, 7 H, Ar H's and $CONH_2$), 8.31 (m, 2 H, Hph and Gly CONH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 25.9 (Pro γ-C), 30.6 (Pro β-C), 31.4 (Hph β-C), 34.5 (CH_2Ph), 42.3 (Gly α-C), 46.9 (Pro δ-C), 52.0 (Hph α-C), 60.4 (Pro α-C), 125.9, 128.3, 128.4, 141.6 (C_6H_5), 170.9, 171.6, 174.6 (C=O). FAB-MS: m/z 333 $[MH]^+$.

Pro-D-Hph-Gly-NH_2 (9). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.50–2.01 (m, 6 H, Pro β-CH_2, γ-CH_2 and Hph β-CH_2), 2.52–2.65 (m, 2 H, CH_2Ph), 2.75–2.90 (m, 2 H, Pro δ-CH_2), 3.56–3.66 (m, 2 H, Gly α-CH_2), 3.80–4.10 (m, 1 H, Pro α-CH), 4.25–4.35 (m, 1 H, Hph α-CH), 7.08–7.32 (m, 7 H, Ar H's and $CONH_2$), 8.19–8.30 (m, 2 H, CONH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 25.3 (Pro γ-C), 29.9 (Pro β-C), 30.8 (Hph β-C), 33.7 (CH_2Ph), 41.3 (Gly α-C), 46.2 (Pro δ-C), 51.6 (Hph α-C), 59.7 (Pro α-C), 125.4, 127.6, 141.0 (C_6H_5), 170.3, 171.0, 174.0 (C=O). FAB-MS: m/z 333 $[MH]^+$.

Pro-Tyr(OMe)-Gly-NH_2 (10). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.36–1.52 (m, 3 H, Pro β-CH, γ-CH_2), 1.78–1.82 (m, 1 H, Pro β-CH), 2.59–3.04 (m, 4 H, Pro δ-CH_2, Tyr(OCH_3) β-CH_2), 3.37–3.68 (m, 3 H, Gly α-CH_2, Pro α-CH), 3.71 (s, 3 H, OCH_3), 4.43–4.54 (m, 1 H, Tyr(OCH_3) α-CH), 6.80 (d, J = 8.6 Hz, 2 H, Tyr(OCH_3) 3,5-H's), 7.08 (d, J = 8.6 Hz, 3 H, Tyr(OCH_3) 2,6-H's and $CONH_2$), 7.29 (s, 1 H, $CONH_2$), 8.09 (d, 1 H, Tyr(OCH_3) NH), 8.33 (t, 1 H, Gly NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 25.8 (Pro γ-C), 30.3 (Pro β-C), 37.1 (Tyr β-C), 42.0 (Gly α-C), 46.7 (Pro δ-C), 53.3 (OCH_3), 55.1 (Tyr(OCH_3) α-C), 60.2 (Pro α-C), 113.6, 129.4, 130.4, 158.0 (C_6H_4), 170.9, 171.3, 174.4 (C=O). FAB-MS: m/z 349 $[MH]^+$.

Pro-Phe(4-NO_2)-Gly-NH_2·HCl (11). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.70–1.90 (m, 3 H, Pro β-CH and γ-CH_2), 2.20–2.40 (m, 1 H, Pro β-CH), 2.89–3.30 (m, 4 H, Pro δ-CH_2 and CH_2Ph), 3.67 (d, J = 5 Hz, 2 H, Gly α-CH), 4.11 (m, 1 H, Pro α-CH), 4.68 (m, 1 H, Phe(4-NO_2) α-CH), 7.13 and 7.37 (2 s, 2 H, $CONH_2$), 7.60 (d, J = 8.65 Hz, 2 H, Phe(4-NO_2) 2,6-H's), 8.11 (d, J = 8.65 Hz, 2 H, Phe(4-NO_2) 3,5-H's), 8.30–8.45 (br s, 1 H, Pro $^+$NH), 8.54 (t, J = 5 Hz, 1 H, Gly NH), 8.99 (d, J = 8 Hz, 1 H, Phe(4-NO_2)NH), 9.90–10.10 (br s, 1 H, Pro $^+$NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.6 (Pro γ-C), 29.7 (Pro β-C), 37.4 (CH_2Ph), 42.0 (Gly α-C), 45.6 (Pro δ-C), 54.2 (Phe(4-NO_2) α-C), 58.7 (Pro α-C), 123.4, 130.8, 146.2, 146.5 (C_6H_4), 168.3, 170.3, 170.7 (C=O).

[^{3}H]ADTN Binding Assay. The binding assay for [^{3}H]-ADTN was carried out as described earlier by us.[22] Freshly dissected bovine caudate was initially suspended in 40 volumes of 50 mM Tris HCl buffer containing 1 mM EDTA (pH 7.4 at 25 °C, Tris–EDTA buffer) and homogenized with a Polytron homogenizer for 20 s. The tissue homogenate was twice centrifuged at 27000g for 10 min in a refrigerated Sorvall centrifuge. The initial pellet was resuspended in fresh 50 mM Tris–EDTA buffer. The final pellet was suspended in 40 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) containing 0.1% ascorbic acid, 1 mM Mn_2Cl, 0.5 mM DTT, 0.4 mM PMSF, 0.1 mg/mL soybean trypsin inhibitor, and 0.1 mg/mL bacitracin (tissue resuspension buffer). The standard direct addition protocol consisted of 0.2 to 0.3 mg of protein of striatal membrane, 8 nM of [^{3}H]ADTN (specific activity equal to 24.4 Ci/mmol) and Tris-HCl buffer (pH 7.4) containing 0.1% ascorbic acid and 1 mM Mn_2Cl, with or without (control) different concentrations (10^{-12}–10^{-4} M) of the PLG analogues in a total incubation volume of 0.6 mL. Incubation was carried out in triplicate in a water shaker bath maintained at 37 °C for 10 min. The contents of each incubation tube was rapidly filtered under partial vacuum over Whatman GF/B filters. The filters were washed 4 times with 2.2 mL of ice-cold 50 mM Tris-HCl (pH 7.4). The filters were then placed in liquid scintillation vials, and 5 mL of scintillation A counting cocktail (Packard) was added. The vials were equilibrated for at least 6 h before being counted in a liquid scintillation counter. The specific binding of [^{3}H]ADTN was defined as the difference in binding occurring in the absence and presence of 1 μM d-butaclamol. The data were analyzed statistically by using the Student's t test.

Acknowledgment. This work was supported by grants from the National Institutes of Health (Grant No. NS 20036) and the Ontario Mental Health Foundation.

Supplementary Material Available: ^{1}H and ^{13}C NMR data for intermediates **12a–j**, and **13a–e**, and **14a–j** (5 pages). Ordering information is given on any current masthead page.