7.1). The filters were then placed in liquid scintillation vials and 10 mL of PCS counting cocktail was added (Amersham Corporation, Chicago, IL). 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 using the Student's *t* test.

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Registry No. **1**, 2002-44-0; **2**, 65126-64-9; **3**, 103305-45-9; **3** (free base), 103305-58-4; **4**, 65166-58-7; **5**, 65126-62-7; **6**, 103305-46-0; **6** (free base), 103305-57-3; **7**, 103305-47-1; **7** (free base), 103305-56-2; **8**, 103305-48-2; **8** (free base), 103365-86-2; **9**, 103305-49-3; **9** (free base), 103365-87-3; **10**, 65126-63-8; **11**, 103305-50-6; **12**, 103320-93-0; **13**, 103305-51-7; **14**, 78058-41-0; **15**, 78058-42-1; Z-Pro-Leu, 1634-90-8; Z-Pro-Leu-D-Pro-NH_2, 103305-52-8; Boc-Pro-Leu-OH, 68385-28-4; Z-Pro-Leu-Aze-OMe, 103305-54-0; Z-Pro-Leu-Pip-NH_2, 103305-55-1; dopamine, 51-61-6; D-prolinamide, 62937-45-5; L-3,4-dehydroprolinamide hydrochloride, 64869-59-6; D-3,4-dehydroprolinamide, 103305-53-9; methyl L-azetidine-2-carboxylate hydrochloride, 69684-69-1; *N*-butoxycarbonyl-L-piperidine-2-carboxylic acid, 26250-84-0.

Notes

Synthesis of Pro-Leu-Gly-NH_2 Analogues Modified at the Prolyl Residue and Evaluation of Their Effects on the Receptor Binding Activity of the Central Dopamine Receptor Agonist, ADTN

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Several analogues of L-prolyl-L-leucylglycinamide (PLG) were synthesized wherein the prolyl residue was replaced with other heterocyclic amino acid residues. Among the analogues synthesized were D-Pro-Leu-Gly-NH_2 (**2**), <Glu-Leu-Gly-NH_2 (**3**), Thz-Leu-Gly-NH_2 (**4**), Pip-Leu-Gly-NH_2 (**5**), Aze-Leu-Gly-NH_2 (**6**), L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**7**), and D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**8**). These analogues were tested for their ability to enhance the binding of the agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene to central dopamine receptors. Analogues **2**, **3**, and **5**–**7** showed activity comparable to that of PLG, while the tripeptides **4** and **8** were found to be inactive. The results show that the N-terminal prolyl residue of PLG is not an essential requirement for this tripeptide's ability to modulate dopamine receptors.

The tripeptide L-prolyl-L-leucylglycinamide (**1**, PLG) has been shown to possess activity in a wide variety of in vivo and in vitro neuropharmacological assay systems.[1] In some of the first studies conducted, PLG was found to potentiate the behavioral effects of L-DOPA,[2] to antagonize the central and peripheral effects of oxotremorine,[3,4] and to potentiate the behavioral effects of apomorphine.[5] More recently, PLG has been shown to attenuate both morphine- and haloperidol-induced catalepsy.[6-8] PLG has also been shown to enhance the binding of [^{3}H]apomorphine to dopamine receptors by increasing the affinity of this ligand to these receptors.[7] In contrast to its effects on the binding of dopamine agonists (apomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN)) to dopamine receptors, PLG does not alter the binding of antagonists such as [^{3}H]spiroperidol. PLG does, however, prevent, as well as reverse, the development of dopamine receptor hypersensitivity in the striatum and mesolimbic areas of the brain that are induced by neuroleptic drugs such as haloperidol. This effect has been demonstrated through both biochemical[9-11] and behavioral[10,12] studies. The above pharmacological profile of PLG thus suggests that this tripeptide is capable of modulating dopamine receptors.[1,11]

In an effort to elucidate the structural and conformational requirements of PLG with respect to its ability to modulate dopamine receptor sensitivity, we have undertaken the synthesis of a number of series of analogues of PLG. In the present paper, we wish to report our efforts in modifying the prolyl residue of PLG. We have replaced the prolyl residue of PLG with the D-prolyl, L-pyroglutamyl, L-thiazolidine-4-carbonyl (Thz), L-piperidine-2-carbonyl (Pip), L-azetidine-2-carbonyl (Aze), and L- and D-3,4-dehydroprolyl ($\Delta^{3,4}$-Pro) residues to give the PLG analogues **2**–**8**, respectively. These particular substitutions have been carried out in order to determine the importance of the pyrrolidine ring system in the ability of PLG to

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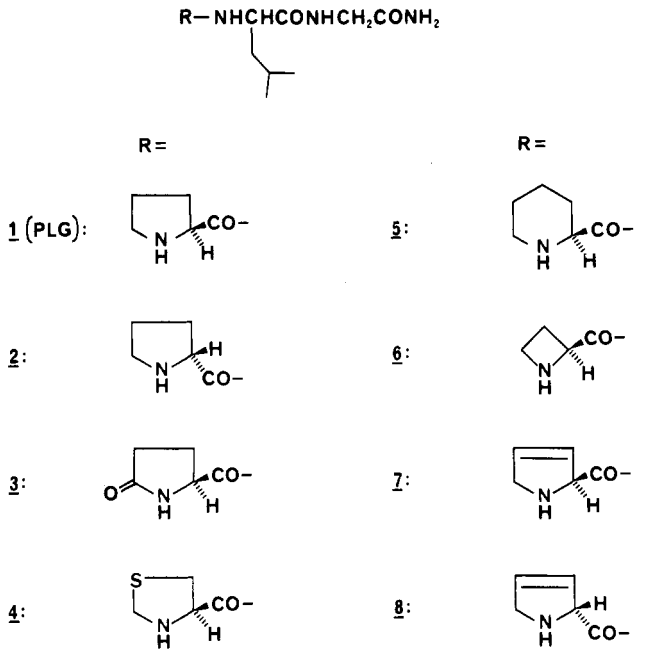
enhance the binding of the dopamine agonist ADTN to striatal dopamine receptors.



Results and Discussion

Chemical Syntheses. The synthesis of Thz-Leu-Gly-NH_2 (**4**) was carried out as described by us previously.[13] The analogues D-Pro-Leu-Gly-NH_2 (**2**), <Glu-Leu-Gly-NH_2 (**3**), and Pip-Leu-Gly-NH_2 (**5**) were synthesized by coupling either (*Z*)-D-Pro-OH, Z-<Glu-OH, or Boc-Pip-OH (**9**) with the dipeptide Leu-Gly-NH_2 using the mixed anhydride method,[14] and then deprotecting the resulting tripeptide using either hydrogenolysis (H_2, Pd/C) or acidolysis (HCl/dioxane). In the case of Aze-Leu-Gly-NH_2 (**6**), Boc-Aze-OH (**10**) was coupled to Leu-Gly-OEt to give Boc-Aze-Leu-Gly-OEt. This material was converted to the primary amide Boc-Aze-Leu-Gly-NH_2 (**11**) using NH_3/MeOH. Removal of the *tert*-butoxycarbonyl protecting group with HCl/dioxane yielded the desired analogue **6**.

The dehydroproline derivatives, Boc-D- and L-3,4-dehydroproline, were synthesized according to the procedures of Felix et al.[15] Both derivatives were coupled to Leu-Gly-NH_2·HCl using the mixed anhydride method to give the known Boc-L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2[15] and the new dehydropeptide Boc-D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**12**). Deprotection of these two peptides yielded the desired PLG analogues L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**7**) and D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**8**).

Binding Studies. PLG and its analogues were tested for their ability to enhance the binding of [^{3}H]ADTN to dopamine receptors isolated from fresh bovine caudate tissue. The [^{3}H]ADTN binding assay that was employed was analogous to that reported by Creese and Snyder.[16] The percent increase in the binding of [^{3}H]ADTN to dopamine receptors produced by PLG and its analogues **2–8** at three different doses (1, 10, 100 nM) is shown in Table I. Between the dose levels of 1 and 100 nM PLG increased the specific binding of [^{3}H]ADTN to dopamine receptors in a dose-dependent manner. The maximum effect was seen at a PLG dose level of 100 nM, at which point the specific binding of [^{3}H]ADTN to dopamine receptors was increased by 46%. This percentage increase was of the same magnitude as that obtained previously by Chiu et al.[7] using another dopamine receptor agonist, [^{3}H]apomorphine, as the ligand. This increase in binding has been shown to result from a decrease in the dissociation constant (i.e., increased affinity) of the dopamine receptor ligand not from an increase in the number of dopamine receptors.[7] Above a PLG dose level of 100 nM the percent enhancement of ADTN binding began to decrease. This type of bell-shaped dose–response relationship for PLG is well-known. For example, Bjorkman and Sievertsson[17] found such a dose–response relationship in PLG's antagonism of oxotremorine-induced tremor, and Chiu et al.[7] observed a bell-shaped curve for PLG's enhancement of the specific binding of the dopamine receptor agonist, [^{3}H]apomorphine, to dopamine receptors.

Analogues **2**, **3**, and **5–7**, in which the prolyl residue has been replaced with the D-prolyl, L-pyroglutamyl, L-piperidine-2-carbonyl, L-azetidine-2-carbonyl, and L-3,4-dehydroprolyl residues, respectively, also enhanced the binding of [^{3}H]ADTN to dopamine receptors in a dose-dependent manner. The percent increases produced by these analogues at the various doses were comparable to the increases produced by the parent compound PLG. Peptides **4** and **8**, in which the prolyl residue has been replaced with the thiazolidine-4-carbonyl and D-3,4-dehydroprolyl residues, respectively, on the other hand, failed to significantly increase the binding of [^{3}H]ADTN regardless of the dose employed.

The above results indicate that the pyrrolidine ring system is not essential for PLG's ability to enhance the binding of dopamine agonists to dopamine receptors, since both analogues **5** and **6**, which possess the higher and lower homologue ring systems of proline, respectively, show the same level of activity as PLG. Although the pyrrolidine ring system is not essential, it does appear that there are limits to the type of heterocyclic ring system that can be accommodated at this position of the molecule, since the analogue that possesses the thiazolidine ring system, analogue **4**, is inactive. The inactivity of **4** in the present in vitro assay system is consistent with the inactivity observed before for this analogue in the in vivo DOPA potentiation and oxotremorine antagonism tests.[13] Since the

Table I. Enhancement of the Binding of [^{3}H]ADTN to Dopamine Receptors by PLG Analogues

no.	compd	N^a	% enhancement of [^{3}H]ADTN binding, mean ± SEM 1 nM	10 nM	100 nM
1	Pro-Leu-Gly-NH_2	4	16.0 ± 3.0	28.0 ± 6.0[b]	46.2 ± 13.4[b]
2	D-Pro-Leu-Gly-NH_2	2	18.0[c]	25.0[c]	33.0[c]
3	<Glu-Leu-Gly-NH_2	6	13.5 ± 2.5	26.5 ± 4.5[b]	36.4 ± 9.8[b]
4	Thz-Leu-Gly-NH_2	2	7.0[c]	8.5[c]	9.5[c]
5	Pip-Leu-Gly-NH_2	3	17.0 ± 9.7	38.7 ± 6.8[b]	58.7 ± 9.3[b]
6	Aze-Leu-Gly-NH_2	4	19.0 ± 8.7	40.0 ± 6.3[b]	42.0 ± 7.3[b]
7	L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2	2	12.4[c]	40.0[c]	55.0[c]
8	D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2	5	9.5 ± 2.8	13.0 ± 3.9	14.4 ± 3.3

[a] Number of independent experiments with triplicate determinations. [b] Significantly different ($P < 0.05$) from basal level where no drug is present. [c] Statistical analysis not possible due to the limited number of experiments.

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thiazolidine ring system is comparable in steric size to the piperidine ring system, the inactivity of **4** is probably not due to steric factors. Rather, it may be that the sulfur atom with its two sets of lone-pair electrons may be involved in an unfavorable electronic interaction with the receptor.

The significant activity of **3** also indicates that the basic amino nitrogen of the pyrrolidine ring is not essential for PLG's ability to increase [^{3}H]ADTN binding to dopamine receptors, a finding that has also been observed in the oxotremorine test.[4,18] Surprisingly, the replacement of the L-prolyl residue of PLG with its enantiomer did not result in a significant reduction in activity. At first glance this would appear to suggest that the relative stereochemistry of the N-terminal residue of PLG is unimportant. However, this lack of stereochemical preference is not seen in analogues **7** and **8**, wherein the L- and D-3,4-dehydroprolyl residues were used, respectively. In this instance the analogue possessing the L-enantiomer is able to increase [^{3}H]ADTN binding, while the analogue with the D-enantiomer is essentially inactive. It may be that the higher conformational freedom of the proline ring as compared to the dehydroproline ring allows the nitrogen atom of the prolyl residue to interact with the receptor irrespective of whether or not it has the D or L configuration, while in the dehydroprolyl case only the L-isomer can assume the required conformation.

In summary, the present work shows that the prolyl residue of PLG can be replaced by a number of other heterocyclic amino acid residues without seriously altering the ability of the molecule to enhance the binding of dopamine agonists to their receptors. These results suggest that the prolyl residue of PLG is not playing a significant role in the modulation of dopamine receptors by this tripeptide.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt and are uncorrected. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. ^{1}H NMR spectra were obtained on either a JEOL FX 90-MHz or a Nicolet 300-MHz spectrometer. All intermediates and final compounds gave NMR spectra that were consistent with their proposed structures. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results are within ±0.4% of the theoretical values. Medium-pressure liquid chromatography (20–40 psi) was carried out on Silica Woelm (32–63 μm) obtained from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel FG uniplates. The following solvent systems were employed: (A) *n*-propanol/NH_4OH (4:1), (B) EtOH/H_2O/EtOAc (7:4:8), (C)EtOAc/MeOH/H_2O (16:4:1), and (D) CH_2Cl_2/EtOAc (1:1). Visualization was carried out with either I_2 or ninhydrin. (5,8-^{3}H)-2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]ADTN, specific activity = 24.4 Ci/mmol) was obtained from New England Nuclear, Boston, MA.

General Procedure for the Mixed Anhydride Coupling Reaction.[14] The protected amino acid derivative (1 equiv) was dissolved in THF (10 mL). To this solution was added *N*-methylmorpholine (1 equiv). The resulting solution was cooled to −20 °C and then treated with a solution of isobutylchloroformate (1 equiv) in THF (5 mL). The mixture was stirred at −20 °C for 10 min, after which time a solution of Leu-Gly-NH_2·HCl (1 equiv, mp 184.5–185.5 °C, $[\alpha]^{24}_D$ +41.4° (*c* 0.84, MeOH)) and NEt_3 (1 equiv) in a mixture of DMF and H_2O (9:1) was added all at once. The mixture was stirred at room temperature for 1 h and then stripped of solvent in vacuo. The residue was partitioned between either EtOAc or CH_2Cl_2 and 10% citric acid. The organic phase was washed with 1 M $NaHCO_3$ and saturated NaCl solution and then dried (Na_2SO_4).

General Procedure for the Removal of the Benzyloxycarbonyl Protecting Group. The protected peptide (1–2 mmol) was dissolved in MeOH (20 mL). This solution was added to a flask containing 5% Pd/C (100 mg) and 10% HCl (1.1 equiv; in the case of compound **3**, AcOH was used as the proton source). Hydrogen was bubbled into the stirred mixture until TLC suggested the absence of starting material. The mixture was then filtered through a pad of Celite and the filtrate stripped of solvent in vacuo.

General Procedure for the Removal of the *tert*-Butoxycarbonyl Protecting Group. To the *tert*-butoxycarbonyl protected peptide (1 mmol) was added 4 N HCl in dioxane (5–10 mL). The resulting solution was stirred at room temperature for 30–45 min. The excess reagent and solvent were removed under reduced pressure and the residue dried under vacuum over KOH, before being recrystallized.

D-Prolyl-L-leucylglycinamide·HCl (2). (*Z*)-D-Proline (0.5 g, 2 mmol) was coupled to Leu-Gly-NH_2·HCl (0.45 g, 2 mmol) by use of the mixed anhydride procedure. The residue that was obtained after the workup procedure was crystallized from EtOAc to give 0.51 g (61%) of (*Z*)-D-Pro-Leu-Gly-NH_2: mp 169–170 °C; $[\alpha]^{24}_D$ +8.8° (*c* 1.0, MeOH). A portion of this material (0.49 g) was deprotected by hydrogenolysis. The crude product was recrystallized from a mixture of Et_2O and isopropyl alcohol to give 320 mg (84%) of **2**: mp 190–191.5 °C; $[\alpha]^{24}_D$ +8.4° (*c* 1.0, MeOH); TLC, R_f (A) = 0.57; NMR (D_2O) δ 4.38–4.47 (m, 2 H, Pro and Leu α-CH), 3.93 (dd, 2 H, *J* = 7.35 and 18.0 Hz, Gly α-CH_2), 3.37–3.47 (m, 2 H, Pro δ-CH_2), 2.43–2.55 (m, 1 H, Pro β-CH), 2.03–2.15 (m, 3 H, Pro β-CH and γ-CH_2), 1.60–1.75 (m, 3 H, Leu β-CH_2 and γ-CH), 0.93 (dd, 6 H, Leu CH_3). Anal. ($C_{13}H_{25}N_4O_3Cl$) C, H, N.

L-Pyroglutamyl-L-leucylglycinamide (3). (*Z*)-L-<Glu (0.98 g, 3.72 mmol) was coupled to Leu-Gly-NH_2·HCl (0.83 g, 3.72 mmol) using the mixed anhydride method. The reaction product(*Z*)-<Glu-Leu-Gly-NH_2 precipitated from the reaction mixture. This material was collected and washed successively with 1 M $NaHCO_3$, H_2O, 10% HCl, and H_2O and then dried under vacuum over KOH. A yield of 1.18 g (76%) of (*Z*)-<Glu-Leu-Gly-NH was obtained: mp 201–202 °C; $[\alpha]^{25}_D$ −57.6° (*c* 1.04, MeOH). The benzyloxycarbonyl protecting group was removed from this material by hydrogenolysis. The crude product was recrystallized from EtOAc/MeOH to give 0.55 g (70%) of **3**: mp 180–181 °C; $[\alpha]^{24}_D$ −16.6° (*c* 1.04, MeOH) [lit.[4] mp 175–176 ° C,$[\alpha]_D$ −17° (*c* 1.0, MeOH)]; TLC, R_f(B) = 0.68.

***N*-(*tert*-Butoxycarbonyl)-L-piperidine-2-carboxylic Acid (9).** A solution of L-pipecolic acid (1.0 g, 7.7 mmol) in H_2O (15 mL) was cooled in an ice bath. To this solution was added 3.5 mL of 10% NaOH, followed by a solution of di-*tert*-butyldicarbonate in dioxane (20 mL). The reaction mixture was stirred at 5 °C for 6 h and then at room temperature overnight. The mixture was washed with Et_2O and the layers separated. The basic aqueous layer was acidified with 10% citric acid and then extracted with EtOAc (3 × 40 mL). The combined EtOAc extracts were washed with saturated NaCl solution and then dried (Na_2SO_4). Evaporation of the solvent in vacuo yielded a white solid, which was collected with the aid of hexane to give 1.16 g (65.7%) of **9**: mp 121–122 °C; $[\alpha]^{24}_D$ −45.1° (*c* 1.0, MeOH); $[\alpha]^{24}_D$ −67.2° (*c* 0.5, AcOH) [lit.[19] mp 110° C, $[\alpha]_D$ −35.3° (*c* 1.0, MeOH); lit.[20] mp 124 °C, $[\alpha]^{24}_D$ −56° (*c* 1.0, AcOH)]; TLC, R_f(C) = 0.57.

L-Piperidine-2-carbonyl-L-leucylglycinamide·HCl (5). Boc-L-piperidine-2-carboxylic acid (**9**, 0.33 g, 1.4 mmol) was coupled to Leu-Gly-NH_2·HCl (0.195 g, 1.4 mmol) using the mixed anhydride method. A crude yield of 0.43 g (76%) of the tripeptide Boc-Pip-Leu-Gly-NH_2 was obtained. This material was purified by medium-pressure liquid chromatography using a silica gel column (1.5 × 50 cm) with CH_2Cl_2/MeOH (9:1) as the eluting solvent. The pure protected tripeptide was obtained as a foam: $[\alpha]^{24}_D$ −45.6° (*c* 1.13, MeOH). The *tert*-butoxycarbonyl protecting group was removed from this material using HCl/dioxane. The

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deprotected material was recrystallized from a mixture of Et_2O and isopropyl alcohol to give 168 mg (67%) of product: mp 190.5–192 °C; $[\alpha]^{24}_D$ −15.9° (*c* 0.54, MeOH); TLC, R_f(A) = 0.71; NMR (D_2O) δ 4.39 (t, 1 H, *J* = 5.7 Hz, Leu α-CH), 3.85–3.9. (m with a dd at 3.92, *J* = 8.05 and 17.1 Hz, Pip α-CH and Gly α-CH_2), 3.48 (br d, 1 H, *J* = 12.5 Hz, Pip eq δ-CH), 3.06 (dt, 1 H, *J* = 2.97 and 12.5 Hz, Pip ax δ-CH) 2.24 (br d, 1 H, *J* = 11.8 Hz, Pip eq β-CH), 1.88–2.00 (m, 2 H, Pip CH's), 1.59–1.78 (m, 6 H, Pip CH's and Leu β-CH_2 and γ-CH), 0.935 (dd, 6 H, Leu CH_3). Anal. ($C_{14}H_{27}N_4O_3Cl$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-azetidine-2-carboxylic Acid Dicyclohexylammonium Salt (10).** L-Azetidine-2-carboxylic acid (0.5 g, 4.95 mmol) was reacted with di-*tert*-butyldicarbonate (1.2 g, 5 mmol) in the same manner as described above in the synthesis of **9**. Boc-L-Aze-OH was obtained as an oil that failed to crystallize in our hands. This material was mixed with an equivalent amount of dicyclohexylamine in Et_2O to yield 1.07 g (77%) of **10**: mp 156–157 °C; $[\alpha]^{24}_D$ −60.6° (*c* 1.17, MeOH). Anal. ($C_{21}H_{38}N_2O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-azetidine-2-carbonyl-L-leucylglycinamide (11).** Boc-Aze-OH·DCHA (1 g, 2.6 mmol) was converted to the free acid by partitioning between EtOAc and 10% citric acid. This material was coupled to Leu-Gly-OEt by use of the standard mixed anhydride procedure. The product Boc-Aze-Leu-Gly-OEt was obtained as an oil in a 96% yield: TLC, R_f(D) = 0.4. This material was treated directly with 50 mL of a solution of MeOH saturated with NH_3. The solution was stirred for 2 days, after which time TLC showed no starting material remaining. The NH_3 and MeOH were removed in vacuo, and the residue that remained was triturated with Et_2O/petroleum ether (bp 30–60 °C) and collected to give 0.77 g (83%) of **11**: mp 105–107 °C; $[\alpha]^{25}_D$ −119.5° (*c* 1.13, CH_2Cl_2). Anal. ($C_{17}H_{30}N_4O_5$) C, H, N.

L-Azetidine-2-carbonyl-L-leucylglycinamide·HCl (6). Peptide **11** (0.35 g, 0.9 mmol) was deprotected with HCl/dioxane as described above. The material was recrystallized from a mixture of MeOH and isopropyl alcohol to give 160 mg (55%) of **6**: mp 194–195 °C dec; $[\alpha]^{25}_D$ −70.4° (*c* 0.5, H_2O); TLC, R_F(A) = 0.38; NMR (Me_2SO-d_6) δ 8.64 (d, 1 H, *J* = 7.89 Hz, Leu NH), 8.24 (t, 1 H, *J* = 5.7 Hz, Gly NH), 7.24 (s, 1 H, 1° amide NH), 7.03 (s, 1 H, 1° amide NH), 4.88 (t, 1 H, *J* = 8.2 Hz, Aze α-CH), 4.37 (m, 1 H, Leu α-CH), 3.88–3.98 (m, 1 H, Aze γ-CH), 3.68–3.78 (m, 1 H, Aze γ-CH), 3.64 (d, 2 H, *J* = 5.7 Hz, Gly α-CH_2), 2.3–2.45 (m, 2 H, Aze β-CH_2), 1.56–1.78 (m, 1 H, Leu γ-CH), 1.44–1.55 (m, 2 H, Leu β-CH_2), 8.78 (dd, 6 H, Leu CH_3). Anal. ($C_{12}H_{23}N_4O_3Cl$) C, H, N.

L-(3,4-Dehydroprolyl)-L-leucylglycinamide·HCl (7). *N*-(*tert*-Butoxycarbonyl)-L-3,4-dehydroproline (4.06 g, 19 mmol) was prepared according to the procedure of Felix et al.[15] and then coupled with Leu-Gly-NH_2·HCl (4.25 g, 19 mmol) using the mixed anhydride method. A yield of 4.12 g (57%) of Boc-L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 was obtained: mp 108–112 °C; $[\alpha]^{24}_D$ −232.8° (*c* 1.0, MeOH) [lit.[15] mp 108.5–112 °C, $[\alpha]^{25}_D$ −222.55° (*c* 1.0, MeOH)].

The protected tripeptide was deprotected by using HCl/dioxane, and the resulting product was recrystallized from absolute EtOH/Et_2O to give 2.16 g (64%) of **7**: mp 201–205 °C; $[\alpha]^{24}_D$ −194.7° (*c* 1.01, MeOH) [lit.[15] mp 201–205.5 °C, $[\alpha]^{25}_D$ −195.36° (*c* 1, MeOH)]; TLC, R_f(A) = 0.67.

***N*-(*tert*-Butoxycarbonyl)-D-(3,4-dehydroprolyl)-L-leucylglycinamide (12).** *N*-(*tert*-Butoxycarbonyl)-D-3,4-dehydroproline[15] (0.4 g, 1.88 mmol) was coupled to Leu-Gly-NH_2·HCl (0.41 g, 1.88 mmol) using the mixed anhydride method described above. The crude product that was obtained from the workup procedure was recrystallized from a mixture of hexane/EtOAc/isopropyl alcohol to give 0.34 g (47%) of **12**: mp 199–201 °C; $[\alpha]^{24}_D$ +141.1° (c 1.0, MeOH). Anal. ($C_{18}H_{30}N_4O_5$) C, H, N.

D-(3,4-Dehydroprolyl)-L-leucylglycinamide·HCl (8). Boc-D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**12**, 0.32 g, 0.84 mmol) was deprotected by using the general procedure described above for the removal of the *tert*-butoxycarbonyl protecting group. The crude product was recrystallized from absolute EtOH/Et_2O to afford 228 mg (85%) of **8**: mp 201–201.5 °C; $[\alpha]^{24}_D$ +132.4° (*c* 0.5, MeOH); TLC, R_f(A) = 0.35; NMR(Me_2SO-d_6) δ 9.0 (d, 1 H, *J* = 8.3 Hz, Leu NH), 8.42 (t, 1 H, *J* = 5.8 Hz, Gly NH), 7.26 (s, 1 H, 1° amide NH), 7.04 (s, 1 H, 1° amide NH), 6.00 (dd, 1 H, *J* = 1.98 and 4.2 Hz, $\Delta^{3,4}$-Pro β-HC=), 5.91 (dd, 1 H, *J* = 2.0 and 4.2 Hz, $\Delta^{3,4}$-Pro γ HC=), 5.06 (br m, 1 H, $\Delta^{3,4}$-Pro α-CH), 4.4 (m, 1 H, Leu α-CH), 4.01 (dddd, 2 H $\Delta^{3,4}$-Pro δ-CH_2), 3,63 (dddd, 2 H, *J* = 6.12 and 10.85 Hz, Gly α-CH_2), 1.5–1.6 (m, 3 H, Leu β-CH_2 and γ-CH), 0.9 (dd, 6 H, *J* = 12.97 and 5.86 Hz, Leu CH_3). Anal. ($C_{13}H_{23}N_4O_3Cl$) C, H, N.

[^{3}H]ADTN Binding Assay. The binding assay for [^{3}H]ADTN was carried out by use of a modification of the method of Creese and Snyder.[16] Freshly dissected bovine caudate (purchased from the local slaughter house) was initially suspended in 50 vol of 50 mM Tris·HCl buffer (pH 7.7 at 25 °C) and homogenized with a Polytron homogenizer for 20 s. The tissue homogenate was twice centrifuged at 40000*g* for 10 min in a refrigerated Sorvall centrifuge. The initial pellet was resuspended in fresh Tris buffer. The final pellet was suspended in 50 mM Tris buffer (pH 7.1 at 25 °C) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 10 μM pargyline to give an approximate concentration of 10 mg of wet tissue/mL of incubation buffer. The standard assay consisted of 0.4–0.6 mg of protein of the brain homogenate, 8 nM of [^{3}H]ADTN (specific activity = 24.4 Ci/mmol), and the buffer with or without 1, 10, or 100 nM of PLG or the PLG analogue in a total incubation volume of 0.6 mL. Incubation was carried out in triplicate in a water shaker bath maintained at 37 °C. Upon termination of the 10-min incubation period, the contents of the incubation tubes were rapidly filtered under partial vacuum through Whatman GF/B filters. The filters were washed 4 times with 2.2 mL of ice-cold 50 mM Tris·HCl (pH 7.1). The filters were then placed in liquid scintillation vials containing 10 mL of PCS counting cocktail (Amersham Corporation, Chicago, IL). 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 in the presence of 1 μM (*d*)-butaclamol. The data were analyzed statistically using the Student's *t* test.

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Registry No. **1**, 2002-44-0; **2**, 103225-04-3; **2** (free base), 103225-11-2; **3**, 39705-61-8; **4**, 65416-51-5; **5**, 16295-99-1; **5** (free base), 90529-16-1; **6**, 103225-05-4; **6** (free base), 72287-62-8; **7**, 103225-06-5; **7** (free base), 64817-34-1; **8**, 103302-76-7; **8** (free base), 103302-77-8; **9**, 26250-84-0; **10**, 103239-76-5; **11**, 103225-07-6; **12**, 103225-08-7; ADTN, 53463-78-8; Z-D-Pro-OH, 6404-31-5; Leu-Gly-NH_2·HCl, 38173-66-9; Z-D-Pro-Leu-Gly-NH_2, 7672-21-1; Z-L-pGlu-OH, 32159-21-0; Z-pGlu-Leu-Gly-NH_2, 39705-62-9; L-Pip-OH, 3105-95-1; BOC-Pip-Leu-Gly-NH_2, 103225-09-8; L-Aze-OH, 2133-34-8; BOC-L-Aze-OH, 51077-14-6; Leu-Gly-OEt, 53375-57-8; BOC-Aze-Leu-Gly-OEt, 103225-10-1; BOC-L-$\Delta^{3,4}$-Pro-OH, 51154-06-4; BOC-L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2, 64817-33-0; BOC-D-$\Delta^{3,4}$-Pro-OH, 58617-29-1; di-*tert*-butyl dicarbonate, 24424-99-5; dopamine, 51-61-6.