Dopamine Receptor Modulation by Conformationally Constrained Analogues of Pro-Leu-Gly-NH_2

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Two series of conformationally constrained analogues of Pro-Leu-Gly-NH_2 (PLG) have been synthesized. In one series of analogues, the Leu-Gly-NH_2 dipeptide segment of PLG was replaced with the γ-lactam residues 3(*S*)- and 3(*R*)-amino-2-oxopyrrolidineacetamide and the δ-lactam residue 3(*S*)-amino-2-oxopiperidineacetamide. The corresponding γ-lactam analogues of <Glu-Leu-Gly-NH_2 were also synthesized. In a second series of analogues, the glycinamide residue of PLG was replaced with the 2-ketopiperazine, 3(*S*)-amino-2-pyrrolidone, and 3(*S*)-amino-2-piperidone residues. The above 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. Of the conformationally constrained analogues of PLG synthesized in this study, only the γ-lactam analogue 3(*R*)-(*N*-L-prolylamino)-2-oxo-1-pyrrolidineacetamide (**3**) was found to possess significant activity. This analogue was 10 000 times more active than PLG, under preincubation conditions. It significantly enhanced the binding of ADTN at concentrations of 10^{-9} and 10^{-10} M.

The tripeptide L-prolyl-L-leucylglycinamide (PLG, 1) has been shown to possess a variety of pharmacological activities in the central nervous system.[1] Animal studies have revealed that PLG is capable of potentiating the behavioral effects of L-Dopa[2] and apomorphine,[3] reversing the tremors induced by oxotremorine,[4,5] antagonizing neuroleptic-induced catalepsy,[6] and attenuating puromycin-induced amnesia.[7] In addition, it has been shown that PLG selectively enhances the affinity of dopamine agonists (ADTN,[8] apomorphine,[6,9] and *n*-propylnorapomorphine[10]) to dopamine receptors. It is also able to desensitize neuroleptic drug (haloperidol) induced supersensitivity of dopamine receptors.[11] The above neuropharmacological and biochemical studies suggest that PLG is exerting its effects on the CNS through the modulation of dopaminergic receptors in the central nervous system.[1]

Although there have been several structure–activity relationship (SAR) studies carried out on PLG,[8,12-14] little is known about what is the biologically active conformation of PLG. In an attempt to determine the solution and solid-state conformation of PLG, numerous spectroscopic studies have been carried out on PLG. Proton NMR studies in dimethyl sulfoxide[15] and X-ray structural analysis[16] have shown that PLG exists in a type II β-bend conformation with a 4 → 1 intramolecular hydrogen bond between the carbonyl oxygen of the proline residue and the trans carboxamide proton of the glycinamide residue. Solid state ^{13}C NMR[17] and laser Raman[18] spectroscopic studies of PLG also support a β-bend conformation as do theoretical calculations with classical potential functions.[19] Circular dichroism spectroscopy[20,21] and ^{13}C NMR spin–lattice relaxation measurements,[22,23] on the other hand, have suggested that PLG is a very conformationally flexible molecule in aqueous systems. Conformational energy analysis[24] using ECEPP (Empirical Conformation Energy Program for Peptides) has also demonstrated that PLG can exist in a variety of conformations in solution, the predominant structures being bend conformations.

Although these physical studies provide information about the conformation of PLG either in solution or in the solid state, they do not provide any indication about the conformation that PLG is in when it interacts with its receptor. In an attempt to gain some information about the biologically active conformation of PLG, we have synthesized two series of conformationally constrained analogues of PLG. In one series of analogues, we have utilized the γ- and δ-lactam residues developed by Freidinger et al.[25,26] to serve as mimics of the β- and γ-bend

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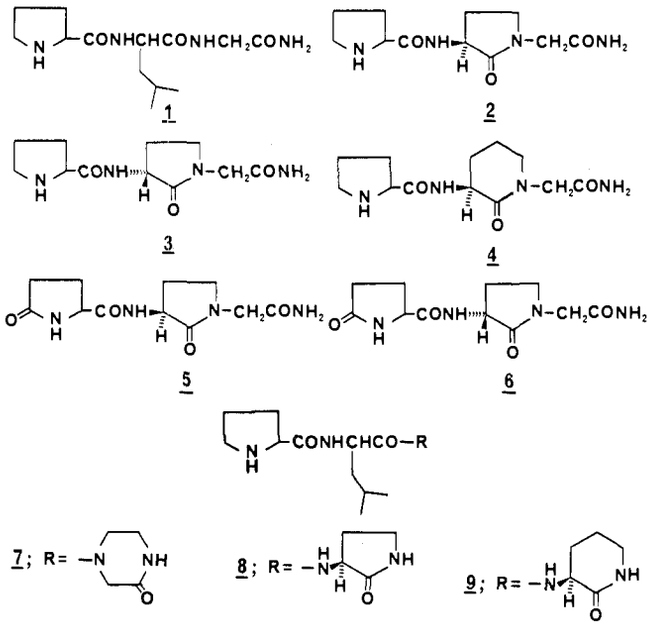
conformations, respectively. Replacing the Leu-Gly-NH_2 dipeptide segment of PLG with the 3(*S*)-amino-2-oxopyrrolidineacetamide, 3(*R*)-amino-2-oxopyrrolidineacetamide, and 3(*S*)-amino-2-oxopiperidineacetamide residues yielded the PLG analogues **2–4**, respectively. Since earlier SAR studies[13,14] showed that the pyroglutamyl residue could replace the prolyl residue of PLG without a loss of biological activity, the corresponding pyroglutamyl γ-lactam PLG analogues **5** and **6** were also synthesized and investigated.

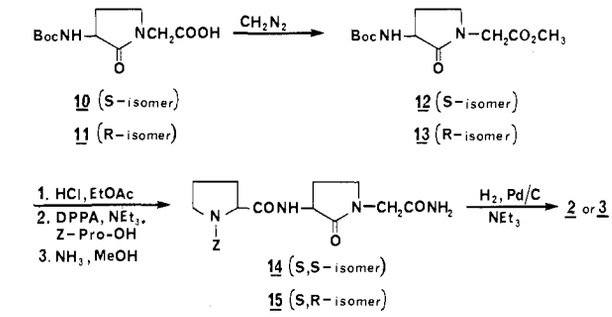


In a second series of conformationally constrained analogues of PLG, the glycinamide residue was replaced with the 2-ketopiperazine, 3(*S*)-amino-2-pyrrolidone, and 3(*S*)-amino-2-piperidone residues to give the PLG analogues **7–9**, respectively. These analogues were designed to represent extended conformations of PLG. It was reasoned that because an intramolecular hydrogen bond between the C=O of the proline residue and the NH of the heterocyclic residue of these analogues could not be formed, the formation of a β-bend type of conformation would be precluded. In this paper we report the synthesis of these conformationally constrained analogues of PLG and the ability of these PLG analogues to enhance the binding of the dopamine receptor agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to striatal dopamine receptors.

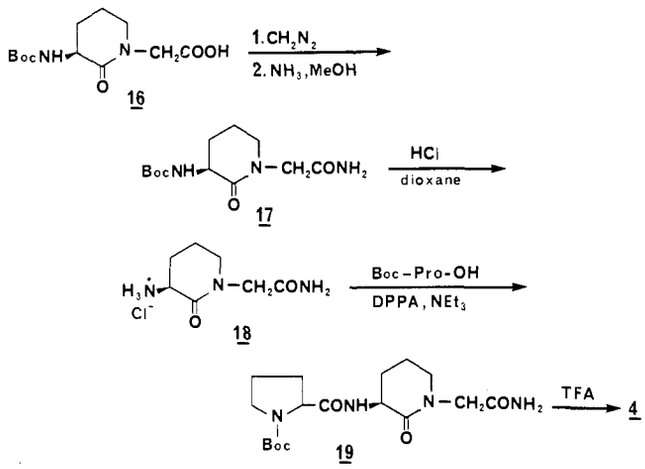
Results and Discussion

Chemical Syntheses. The synthesis of the γ-lactam PLG analogues **2** and **3** utilized the *S* and *R* isomers of *tert*-butoxycarbonyl-protected 3-amino-2-oxo-1-pyrrolidineacetic acid (**10** and **11**), respectively. These two precursors were prepared from the corresponding isomer of Boc-Met-Gly-OCH_3 by using a combination of the methods of Freidinger et al.[26] and Prochazka et al.[27] The two acids, **10** and **11**, were converted into their respective methyl esters **12** and **13** with diazomethane. Initially, these esters were converted into the corresponding amides, which were then deprotected and coupled with Boc-L-Pro-OH with diphenyl phosphorazidate (DPPA).[28] Deprotection

Scheme I



Scheme II



of the *tert*-butoxycarbonyl-protected derivatives of **2** and **3** with trifluoroacetic acid afforded the trifluoroacetate salts of **2** and **3**. This route worked reasonably well in the case of compound **2** but not in the case of compound **3** where the coupling reaction gave a very low yield of the protected tripeptide analogue, and the trifluoroacetate salt of **3** was not amenable to purification. Therefore, an alternate route to **2** and **3** that employed the sequence of reactions shown in Scheme I was utilized.

The esters **12** and **13** were each deprotected by using EtOAc saturated with HCl, and the deprotected products were then coupled to Z-L-Pro-OH with DPPA. The resulting esters, which were obtained as oils, were then converted into their corresponding primary amides **14** and **15** with methanolic ammonia. Hydrogenolysis of **14** and **15** over Pd/C in the presence of NEt_3[29] provided the γ-lactam analogues **2** and **3**, respectively, in good yields. The pyroglutamyl γ-lactam analogues **5** and **6** were obtained by using the same method as that outlined in Scheme I, except that L-pyroglutamic acid was used instead of Z-L-Pro-OH.

The route used to synthesize the δ-lactam PLG analogue, compound **4**, is depicted in Scheme II. The *tert*-butoxycarbonyl derivative of 3(*S*)-amino-2-oxo-1-piperidineacetic acid, compound **16**, was synthesized from N^{α}-Boc-N^{δ}-Z-L-Orn-OH by using the method of Friedinger et al.[26] This lactam acid was transformed into its primary amide derivative **17** via its methyl ester. The protected lactam amide was deprotected to give **18**, which was then coupled to Boc-L-Pro-OH with DPPA to give the protected δ-lactam tripeptide analogue **19**. Removal of the *tert*-butoxycarbonyl protecting group from **19** with trifluoroacetic acid

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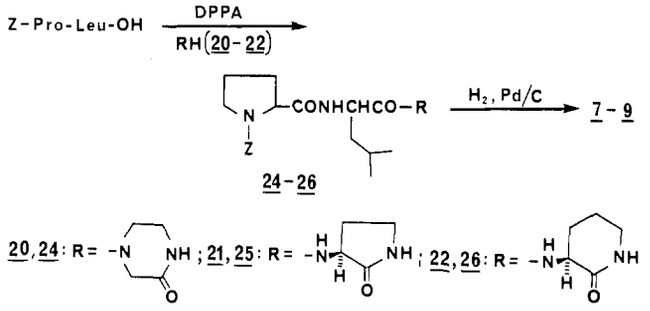
Scheme III



afforded the trifluoroacetate salt of the δ-lactam PLG analogue **4**.

The route used for the synthesis of the constrained glycinamide PLG analogues **7–9** is outlined in Scheme III. The required precursors 2-ketopiperazine (**20**), 3(*S*)-amino-2-pyrrolidone (**21**), and 3(*S*)-amino-2-piperidone (**22**) were obtained in the following manner. Compound **20** was prepared by condensing ethyl chloroacetate with ethylenediamine according to the procedure of Aspinall.[30] Lactam **21** was prepared by using the procedure of Wilkinson[31] wherein L-2,4-diaminobutyric acid was converted into its ethyl ester, and this intermediate was then cyclized in the presence of EtONa. This lactam was unstable and hence was used in the next reaction without extensive purification. Lactam **22** was synthesized from N^{α}-Boc-N^{δ}-Z-L-Orn-OCH_3. The Z protecting group of this starting material was removed by catalytic hydrogenolysis in a mixture of acetic acid and methanol. Intramolecular acylation of the deprotected material was carried out in DMF with Na_2CO_3 and gave 3(*S*)-[(*tert*-butyoxycarbonyl)amino]-2-piperidone (**23**). Removal of the protecting group of **23** with 4 N HCl in dioxane gave lactam **22**. The three precursors **20**, **21**, and **22** were each coupled to Z-L-Pro-L-Leu-OH with DPPA to give the protected tripeptides **24–26**, respectively. DPPA was chosen as the coupling reagent in these cases because it is less likely to cause racemization of the C-terminal amino acid in segmental peptide coupling reactions.[32] Finally, catalytic hydrogenolysis of the protected tripeptides **24–26** yielded the corresponding PLG analogues **7–9**.

Pharmacological Studies. The conformationally constrained analogues of PLG, compounds **2–9**, were tested for their ability to enhance the binding of [^{3}H]ADTN to dopamine receptors prepared from bovine caudate membranes. This assay, which has been described previously by us,[8,14] has been used to measure the ability of PLG and its analogues to modulate dopamine receptors. In the present study two different experimental protocols were employed. In one case PLG and its conformationally constrained analogues **2–9** were incubated directly with [^{3}H]ADTN and the striatal membrane preparation. In the second case these compounds were preincubated with the striatal membranes at 4 °C for 1 h prior to the addition of the [^{3}H]ADTN.

PLG was found to enhance the binding of ADTN to dopamine receptors under both direct and preincubation conditions. In both cases a bell-shaped dose–response curve was obtained with the maximum effect seen at a concentration of PLG equal to 10^{-6} M (Figure 1). At this concentration PLG enhanced the binding of ADTN by 23.2% under direct addition conditions and by 26.6% under preincubation conditions. Similar bell-shaped

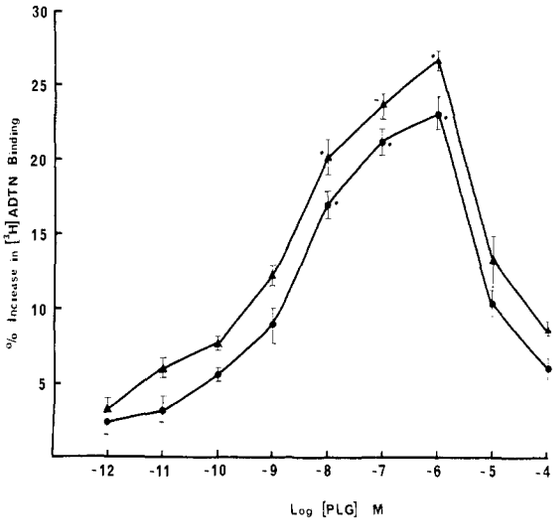


Figure 1. Stimulation of [^{3}H]ADTN binding to striatal membranes by PLG. Percent increase in specific [^{3}H]ADTN binding over the control value when the indicated concentration of PLG was added directly to the assay buffer (●). Percent increase in specific [^{3}H]ADTN binding in membranes preincubated with indicated concentrations of PLG, as compared to control membranes, which were incubated similarly but without PLG (▲). Results are the means ± SEM of five experiments carried out in triplicate. (*) Significantly different ($p < 0.001$) from control values.

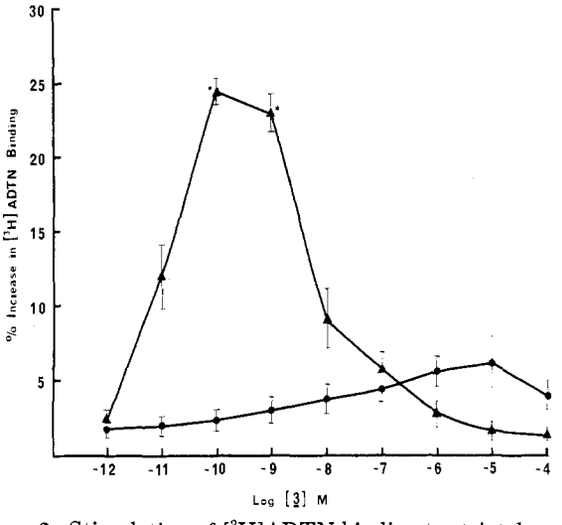


Figure 2. Stimulation of [^{3}H]ADTN binding to striatal membranes by **3**. Percent increase in specific [^{3}H]ADTN binding over the control value when the indicated concentration of PLG was added directly to the assay buffer (●). Percent increase in specific [^{3}H]ADTN binding in membranes preincubated with indicated concentrations of PLG, as compared to control membranes, which were incubated similarly but without PLG, (▲). Results are the means ± SEM of five experiments carried out in triplicate. (*) Significantly different ($p < 0.001$) from control values.

dose–response curves for PLG have been observed with the dopamine receptor agonists apomorphine[6] and *n*-propylnorapomorphine.[33]

Of the eight conformationally constrained analogues evaluated in this study, only compound **3**, which contains the (*R*)-γ-lactam residue, was found to behave like PLG and significantly enhance the binding of the dopamine agonist ADTN to dopamine receptors. Unlike PLG, however, the effects of **3** were only observed under preincubation conditions (Figure 2). Under these conditions

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(32) Shiori, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1974**, *22*, 859.
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3 gave a bell-shaped dose–response curve with significant enhancement of ADTN binding occurring at concentrations equal to 10^{-9} and 10^{-10} M. At these two concentrations the percent enhancement of ADTN binding was 23.0% and 24.4%, respectively. Under the direct assay conditions, a very slight increase in the percent of ADTN binding was observed for 3 at 10^{-5} and 10^{-6} M, but this was not significant.

Since earlier SAR studies[13,14] showed that the pyroglutamyl residue could replace the prolyl residue of PLG without a loss of biological activity, the corresponding pyroglutamyl γ-lactam PLG analogues, compounds 5 and 6, were synthesized and tested. Of these two analogues, the analogue that contained the (*R*)-γ-lactam residue, compound 6, was found to produce a small enhancement in the binding of ADTN to dopamine receptors. At a peak concentration of 10^{-5} M, the percent increase was 21 ± 7.2% under direct addition conditions and 15 ± 4.0% under preincubation conditions. However, these percent enhancements were not found to be statistically significant.

The above data show that of the PLG analogues in which the γ-lactam moiety has been incorporated at the position of the leucine residue, only those analogues that contain the *R* isomer of this lactam residue, compounds 3 and 6, enhance the binding of ADTN to dopamine receptors. Since the *R* form of the γ-lactam residue is purported to mimic a type II β-bend while the *S* form mimics a type II′ β-bend,[25] the results obtained in this study would suggest that the biologically active conformation of PLG may be one resembling a type II β-bend. This is consistent with the results of some of the physical studies that have indicated that the preferred conformation of PLG is a type II β-bend.[15,16] The fact that analogues 7–9, which possess constrained glycinamide residues and which were designed to mimic extended conformations of PLG, are inactive supports such a conclusion.

Under the preincubation conditions employed in this study, 3 was found to be 10 000 times more potent that PLG in enhancing the binding of ADTN to dopamine receptors. A similar potency difference between 3 and PLG has been observed in the attenuation by these two compounds of 5′-guanylyl imidodiphosphate induced inhibition of high-affinity binding of the selective D_2 receptor agonist, *n*-propylnorapomorphine.[34] Since the dopamine D_2 receptor has been implicated in such neurological disorders as schizophrenia, tardive dyskinesia, and Parkinson's disease, compounds like 3, which can modulate the dopamine receptor, may find use in the treatment of such disorders.[35]

The reason for the activity of 3 only being observed under preincubation conditions is not known at this time. One possible explanation may be that under these conditions 3 is metabolized and that it is a metabolite that gives rise to the potency seen with 3. It is also possible that we are dealing with a partitioning phenomenon. The chemical behavior of 3 suggests that it is a more polar compound than PLG. For example, on TLC (silica gel) 3 has R_f values of 0.35 and 0.13 in 1-propanol/NH_4OH (5:1) and $CHCl_3$/MeOH/H_2O (14:6:1), respectively. The corresponding R_f values for PLG (1), on the other hand, are 0.47 and 0.30, respectively. It may be that in the in vitro preparation that is employed in this study PLG and its analogues have to transverse some lipid barrier in order to reach their site of action. Thus, because of its more polar nature, it takes a longer time for sufficient concentrations of 3 to be reached. Further studies are currently under way to test these possibilities.

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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. ^1H NMR spectra were recorded on either a JEOL FX-90-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 spectroscopy was performed on the JEOL FX-90-MHz instrument at 25 MHz. When DMSO-d_6 was used as solvent, it worked as the internal standard at δ 39.5. When D_2O was used, dioxane (δ 64.5) was added as the external standard. 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 with use of the following solvent systems: (A) CH_2Cl_2/MeOH (20:1), (B) $CHCl_3$/MeOH (10:1), (C) 1-propanol/NH_4OH (5:1), (D) $CHCl_3$/MeOH/H_2O (14:6:1). Visualization was done with either UV, I_2, or ninhydrin spray. 2-[5,8-$^3H_2$]Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^3H]ADTN) was obtained from New England Nuclear, Boston, MA.

3(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic Acid (10). A combined method from the reports of Freidinger et al.[26] and Prochazka et al.[27] was used. The sulfonium iodide salt of Boc-L-Met-Gly-OCH_3, which was obtained by reacting Boc-L-Met-Gly-OCH_3 (5.7 g, 17.9 mmol) with methyl iodide, was dissolved in 20 mL of dry DMF under N_2. After this solution was cooled in an ice bath, it was treated with NaH (0.84 g, 36.7 mmol). The resulting mixture was stirred at 4 °C for 5 min and then at room temperature for 15 min. The reaction mixture was quenched with 100 mL of water and then stirred for 1 h before the solvent was removed in vacuo. The residue was mixed with 50 mL of 10% citric acid and then extracted with EtOAc (150 mL × 4). The combined extracts were washed with water (50 mL) and saturated NaCl aqueous solution and then dried over $MgSO_4$. After evaporation of the organic phase, the crude solid was recrystallized from MeOH/Et_2O to yield 2.4 g (53%) of 10: mp 172 °C dec; $[\alpha]_D$ −30.9° (*c* 1.12, MeOH). [Lit.[26] mp 171–172 °C dec; $[\alpha]_D$ −31.2° (*c* 1.0, MeOH)].

3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic Acid (11). The synthesis of this material was carried out by using the same method as that described above for 10, except that Boc-D-Met-Gly-OCH_3 served as the starting material. A 64% yield was obtained: mp 174 °C dec; $[\alpha]_D$ +29.7° (*c* 1.02, MeOH).

3(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic Acid Methyl Ester (12). A solution of the free acid 10 (0.73 g, 2.83 mmol) in methanol (50 mL) was cooled in an ice bath, treated with a slight molar excess of an ether solution of CH_2N_2, and stirred at room temperature for 2 h. Evaporation of the solvent and excess CH_2N_2 from the reaction mixture gave a residue, which was crystallized from Et_2O/petroleum ether (bp 30–60 °C) to yield 0.72 g (93%) of product: mp 84–86 °C; $[\alpha]_D$ −23.2° (*c* 1.0, MeOH); TLC R_f (A) 0.62; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H, Boc CH_3), 1.90–2.05 (m, 1 H, 4-CH), 2.60–2.75 (m, 1 H, 4-CH), 3.35–3.55 (m, 2 H, 5-CH_2), 3.74 (s, 3 H, OCH_3), 4.00 (d, *J* = 17.6 Hz, 1 H, CH_2CO_2), 4.18 (d, *J* = 17.6 Hz, 1 H, CH_2CO_2), 4.25–4.35 (m, 1 H, 3-CH), 5.08 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 25.52 (4-C), 28.07 (Boc CH_3), 43.45 (5-C), 43.94 (CH_2CO_2Me), 50.93 (3-C), 51.36 (OCH_3), 77.80 (Boc C—O), 154.90 (Boc C=O), 168.66 (COOMe) 172.18 (2-C=O). Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic Acid Methyl Ester (13). The same method used to make 12 was employed: mp 89–91 °C; $[\alpha]_D$ +24.0° (*c* 1.09, MeOH).

3(*S*)-[[*N*-(Benzyloxycarbonyl)-L-prolyl]amino]-2-oxo-1-pyrrolidineacetamide (14). Lactam ester 12 (1 g, 3.67 mmol)

was deprotected by the addition of 4 N HCl in dioxane (5 mL) at 0 °C for 20 min. The solvent and excess HCl were removed in vacuo. The residue and Z-L-Pro-OH (832 mg, 3.34 mmol) were mixed in dry DMF (20 mL), and this solution was cooled in a salt–ice bath. DPPA (1.10 g, 3.67 mmol) and Et_3N (1.02 mL, 7.34 mmol) were then added. The reaction mixture was stirred at 0 °C for 2 days and at room temperature overnight. After evaporation of the DMF, the residue was partitioned between EtOAc (120 mL) and 10% citric acid (40 mL). The EtOAc layer was washed with 1 N $NaHCO_3$, water, and saturated NaCl solution (40 mL of each) and then dried with $MgSO_4$. The solvent was evaporated under reduced pressure, and the resultant residue was passed through a silica gel column with CH_2Cl_2/MeOH (20:1) as the eluting solvent. The crude product obtained from the chromatography step was treated with 80 mL of methanolic ammonia solution and stirred at room temperature for 12 h. Evaporation of the MeOH and NH_3 and then crystallization of the residue from MeOH/ether yielded 572 mg (40%) of the product: mp 188–189 °C; $[\alpha]_D$ −79.5° (*c* 1.24, MeOH); TLC R_f (B) 0.38; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65–1.95 (m, 4 H, 4-CH and Pro β-CH and γ-CH_2), 2.00–2.35 (m, 2 H, 4-CH and Pro β-CH), 3.3–3.5 (m, 4 H, 5-CH_2 and Pro δ-CH_2), 3.72 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 3.82 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 4.15–4.25 (m, 1 H, 3-CH), 4.39 (dd, *J* = 9.0 and 8.8 Hz, 1 H, Pro α-CH), 4.90–5.14 (m, 2 H, cis and trans rotamers, OCH_2), 7.18 (s, 1 H, cis $CONH_2$), 7.32 and 7.36 (s, 5 H, trans and cis rotamers, Ph), 7.38 (s, 1 H, trans $CONH_2$), 8.26 and 8.34 (d, 1 H, cis and trans rotamers, 2:3, 3-NH); ^{13}C NMR (DMSO-d_6) δ 23.08 (Pro γ-C), 25.30 (4-C), 30.50 (Pro β-C), 44.00 (5-C), 45.46 (CH_2CONH_2), 46.60 (Pro δ-C), 49.63 (3-C), 59.49 (Pro α-C), 65.67 (OCH_2), 127.00, 127.22, 127.92, 136.75 (Ph), 153.76 (*Z*-C=O), 169.15 ($CONH_2$), 171.65, 172.02 (Pro and 2-C=O). Anal. ($C_{19}H_{24}N_4O_5$) C, H, N.

3(*R*)-[[*N*-(Benzyloxycarbonyl)-L-prolyl]amino]-2-oxo-1-pyrrolidineacetamide (15). Lactam ester **13** was carried through the same sequence of reactions that its enantiomer **12** was carried through. Pure **15** was obtained in a 53% yield: mp 168–170 °C; $[\alpha]_D$ −57.6° (*c* 1.22, MeOH); TLC R_f (B) 0.35; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70–1.90 (m, 4 H, 3-CH and Pro β-CH, γ-CH_2), 2.00–2.35 (m, 2 H, 4-CH and Pro β-CH), 3.3–3.5 (m, 4 H, 5-CH_2 and Pro δ-CH_2), 3.76 (s, 2 H, CH_2CONH_2), 4.15–4.25 (m, 2 H, 3-CH and Pro α-CH) 4.95–5.12 (m, 2 H, trans and cis rotamers, OCH_2), 7.20 (s, 1 H, cis $CONH_2$), 7.32 and 7.36, (s, 5 H, trans and cis rotamers, Ph), 7.38 (s, 1 H, trans $CONH_2$), 8.32 and 8.38 (d, *J* = 7.8, 1 H, cis and trans rotamers, 1:1.6, 3-NH); ^{13}C NMR δ 22.95 (Pro γ-C), 25.17 (4-C), 30.42 (Pro β-C), 44.10 (5-C), 45.54 (CH_2CONH_2), 46.60 (Pro δ-C), 49.85 (3-C), 59.49 (Pro α-C), 65.61 (OCH_2), 126.94, 127.24, 127.95, 136.75 (Ph), 153.76 (Ph—C=O), 169.12 ($CONH_2$), 171.46, 171.94 (Pro and 2-C=O). Anal. ($C_{19}H_{24}N_4O_5$) C, H, N.

3(*S*)-(*N*-L-Prolylamino)-2-oxo-1-pyrrolidineacetamide (2). Compound **14** (0.45 g, 1.16 mmol) was dissolved in MeOH (10 mL), and to this solution were added Et_3N (0.16 mL, 1.16 mmol) and 10% Pd/C (50 mg). Hydrogenolysis was carried out on a Parr apparatus at H_2 = 40 psi. The resulting solution was filtered through a pad of Celite, and the filtrate was stripped of solvent in vacuo. The material obtained was crystallized from MeOH/Et_2O to yield 109 mg (40%) of **2**: mp 199–200 °C; $[\alpha]_D$ −104.5° (*c* 1.01, MeOH); TLC R_f (C) 0.43; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53–1.74 (m, 3 H, Pro γ-CH_2 and β-CH), 1.75–2.00 (m, 2 H, 4-CH and Pro β-CH), 2.24–2.35 (m, 1 H, 4-CH), 2.70–2.90 (m, 2 H, Pro δ-CH_2), 3.20–3.40 (m, 2 H, 5-CH_2), 3.54 (dd, *J* = 8.7 and 9.0 Hz, 1 H, Pro α-CH), 3.69 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 3.80 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 4.34 (dd, *J* = 9.2 and 9.1 Hz, 1 H, 3-CH), 7.20 (br s, 1 H, cis $CONH_2$), 7.40 (br s, 1 H, trans $CONH_2$), 8.26 (d, *J* = 8.1 Hz, 1 H, 3-NH); ^{13}C NMR (DMSO-d_6) δ 25.72 (4-C), 25.91 (Pro γ-C), 30.46 (Pro β-C), 44.29 (5-C), 45.68 (CH_2CO), 46.67 (Pro δ-C), 49.53 (3-C), 60.11 (Pro α-C), 169.48 ($CONH_2$), 172.10, 174.78 (Pro and 2-C=O). Anal. ($C_{11}H_{18}N_4O_3$) C, H, N.

3(*R*)-(*N*-L-Prolylamino)-2-oxo-1-pyrrolidineacetamide (3). Compound **3** was prepared from the protected tripeptide **15** (0.64 g, 1.65 mmol) by using the same method as that described above for **2**. The product was crystallized from EtOH/Et_2O to give 210 mg (50%) of **3**: mp 184.5–186 °C; $[\alpha]_D$ +16.1° (*c* 1.02, MeOH). TLC R_f (C) 0.35, R_f (D) 0.13; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55–1.75 (m, 3 H, Pro γ-CH_2 and β-CH), 1.80–2.00 (m, 2 H, 4-CH and Pro β-CH), 2.22–2.38 (m, 1 H, 4-CH), 2.7–2.9 (m, 2 H, Pro δ-CH_2), 3.25–3.4 (m, 2 H, 5-CH_2), 3.52 (dd, *J* = 8.6 and 9.0 Hz, 1 H, Pro α-CH), 3.67 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 3.81 (d, *J* = 16.7 Hz, 1 H, CH_2CO), 4.33 (dd, *J* = 9.1 and 9.2 Hz, 1 H, 3-CH), 7.21 (br s, 1 H, cis $CONH_2$), 7.40 (br s, 1 H, trans $CONH_2$), 8.29 (d, *J* = 8.2 Hz, 1 H, 3-NH); ^{13}C NMR (DMSO-d_6) δ 25.70 and 25.74 (4-C and Pro γ-C), 44.29 (5-C), 45.72 (CH_2CO), 46.65 (Pro δ-C), 49.53 (3-C), 60.13 (Pro α-C), 169.52 ($CONH_2$), 172.08 (2-C=O), 174.83 (Pro C=O). Anal. ($C_{11}H_{18}N_4O_3$) C, H, N.

3(*S*)-(*N*-L-Pyroglutamylamino)-2-oxo-1-pyrrolidineacetamide (5). Protected lactam ester **12** (1.34, 4.92 mmol) was stirred with 4 N HCl in dioxane (8 mL) at 0 °C for 20 min. The reaction mixture was evaporated in vacuo to give the deprotected lactam. To this material was added a solution of L-pyroglutamic acid (635 mg, 4.92 mmol) in dry DMF (10 mL). The mixture was cooled in a salt–ice bath after which time DPPA (1.5 g, 5.41 mmol) and Et_3N (1.37 mL, 9.84 mmol) were added. The reaction mixture was stirred at 0 °C for 36 h and then at room temperature for 6 h. The mixture was filtered, and the resultant filtrate was stripped of solvent in vacuo to give a crude product, which was chromatographed on a silica gel column (100 g) with a mixture of $CHCl_3$ and MeOH (10:1) as an eluent. The isolated solid product was recrystallized from MeOH/acetone/Et_2O to yield 685 mg (49%) of 3(*S*)-(*N*-L-pyroglutamylamino)-2-oxo-1-pyrrolidineacetic acid methyl ester: mp 165–167 °C; $[\alpha]_D$ −37.9° (*c* 1.13, MeOH); TLC R_f (B) 0.34. Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

The above ester (305 mg, 1.08 mmol) was dissolved in an excess of methanolic ammonia solution (20 mL). The solution was stirred at room temperature for 2 days after which time the solvent and excess NH_3 were evaporated. A white solid was obtained, which was crystallized from MeOH to yield 150 mg (52%) of the desired product. A second crop yielded an additional 110 mg to give a total yield of 260 mg (70%): mp 264 °C dec; $[\alpha]_D$ −61.1° (*c* 1.10, H_2O); TLC R_f (D) 0.45; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75–2.00 (m, 2 H, 4-CH and <Glu β-CH), 2.00–2.30 (m, 3 H, <Glu β-CH, γ-CH_2), 2.25–2.40 (m, 1 H, 4-CH_2), 3.25–3.40 (m, 2 H, 5-CH_2), 4.00–4.08 (m, 1 H, 3-CH), 4.35–4.46 (m, 1 H, <Glu α-CH), 7.20 (br s, 1 H, cis $CONH_2$), 7.36 (br s, 1 H, trans $CONH_2$), 7.86 (s, 1 H, <Glu NH), 8.39 (br d, *J* = 8.1 Hz, 3-NH); ^{13}C NMR (D_2O) δ 25.63, 25.95 (4-C and <Glu β-C), 30.02 (<Glu γ-C), 46.43 (5-C), 46.78 (CH_2CO), 52.07 (3-C), 57.81 (<Glu α-C), 173.19 ($CONH_2$), 175.27, 175.70 (<Glu α-C=O and 2-C=O), 182.94 (<Glu, δ-C=O). Anal. ($C_{11}H_{16}N_4O_4$) C, H, N.

3(*R*)-(*N*-Pyroglutamylamino)-2-oxo-1-pyrrolidineacetamide (6). This compound was synthesized from **13** by using the same procedure described above for **5**. The desired product was obtained by recrystallization of the crude product with EtOH/H_2O: yield 72%; mp 271 °C dec; $[\alpha]_D$ +43.7° (*c* 1.02, H_2O). TLC R_f (D) 0.48; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.90 (m, 2 H, <Glu γ-CH_2), 2.00–2.38 (m, 4 H, <Glu β-CH_2 and 4-CH_2), 3.30–3.40 (m, 2 H, 5-CH_2), 3.74 (d, *J* = 15.3 Hz, 1 H, CH_2CO), 3.80 (d, *J* = 15.3 Hz, 1 H, CH_2CO), 7.20 (s, 1 H, cis $CONH_2$), 7.37 (s, 1 H, trans $CONH_2$), 7.88 (br s, 1 H, <Glu NH), 8.39 (br d, *J* = 8.1 Hz, 1 H, 3-NH); ^{13}C NMR (D_2O) δ 25.71, 25.98 (4-C and <Glu β-C), 30.04 (<Glu γ-C), 46.43 (5-C), 46.81 (CH_2CO), 52.04 (3-C), 57.81 (<Glu α-C), 173.13 ($CONH_2$), 175.19, 175.62 (<Glu α-C=O and 2-C=O), 182.88 (<Glu δ-C=O). Anal. ($C_{11}H_{16}N_4O_4$) C, H, N.

3(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-piperidineacetic Acid (16). This compound was synthesized from N^{α}-Boc-N^{δ}-Z-L-Orn-OH by using the procedure of Freidinger et al.:[26] mp 117–119 °C; $[\alpha]_D$ −20.8° (*c* 0.85, MeOH) [lit.[26] mp 113–116 °C; $[\alpha]_D$ −22.14° (*c* 0.998, MeOH)].

(3*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-piperidineacetamide (17). To a methanolic solution of **16** (0.9 g, 3.3 mmol) at 0 °C was added a slight molar excess of CH_2N_2 in ether. The mixture was stirred at room temperature for 0.5 h. The excess CH_2N_2 and methanol were removed by evaporation, and the resultant residue was treated with an excess of ammonia in MeOH. This solution was stirred at room temperature for 2 days. The solvent was removed under reduced pressure, and the residue was crystallized from a mixture of MeOH/Et_2O to give 0.75 g (83%) of the product: mp 163.5–164 °C; $[\alpha]_D$ −36.5° (*c* 1.0, MeOH); TLC R_f (B) 0.47; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9 H, Boc CH_3), 1.70–2.10 (m, 3 H, 5-CH_2 and 4-CH), 2.20–2.35 (m, 1 H, 4-CH), 3.25–3.35 (m, 1 H, 6-CH), 3.50–3.65 (m, 1 H,

6-CH), 3.54 (d, J = 15.8 Hz, 1 H, CH_2CO), 3.80–3.90 (m, 1 H, 3-CH), 4.48 (d, J = 15.8, 1 H, CH_2CO), 5.50 (m, 2 H, cis $CONH_2$ and 3-NH), 6.82 (br s, 1 H, trans $CONH_2$); ^{13}C NMR (DMSO-d_6) δ 20.32 (5-C), 27.58, 28.12 (Boc CH_3 and 4-C), 48.08 (6-C), 49.68 (CH_2CO), 50.60 (3-C), 77.70 (Boc C—O), 155.11 (Boc C=O), 168.91 ($CONH_2$), 169.72 (2-C=O). Anal. ($C_{12}H_{21}N_3O_4$) C, H, N.

3(*S*)-Amino-2-oxo-1-piperidineacetamide Hydrochloride (18). Compound **17** (200 mg, 0.74 mmol) was treated with 4 N HCl in dioxane (2 mL). The mixture was stirred at room temperature for 2 h and stripped of dioxane and HCl in vacuo. The residue, which was subsequently obtained, was crystallized from MeOH/Et_2O to give 125 mg (82%) of **18**: mp 249 °C dec; $[\alpha]_D$ +4.91° (c 1.12, H_2O); TLC R_f (C) 0.33; ^1H NMR (300 MHz, D_2O) δ 1.90–2.18 (m, 3 H, 4-CH and 5-CH_2), 2.34–2.90 (m, 1 H, 4-CH), 3.40–3.58 (m, 2 H, 6-CH_2), 4.13 and 4.00–4.18 (s, over m, 3 H, CH_2CO and 3-CH); ^{13}C NMR (D_2O) δ 20.56 (5-C), 25.52 (4-C), 49.98 (2-CH_2), 49.98, 50.97 (CH_2CONH_2, 3-C and 6-C), 168.55 ($CONH_2$), 173.29 (2-C=O). Anal. ($C_7H_{14}N_3O_2Cl$) C, H, N.

3(*S*)-[[*N*-(*tert*-Butoxycarbonyl)-L-prolyl]amino]-2-oxo-1-piperidineacetamide (19). Boc-L-Pro-OH (1.37 g, 6.36 mmol) and lactam **18** (1.32 g, 6.36 mmol) were dissolved in DMF (10 mL), and the resulting solution was cooled to 0 °C. DPPA (1.93 g, 7 mmol) and NEt_3 (1.95 mL, 14 mmol) were added. The reaction mixture was stirred at 0 °C for 24 h and then at room temperature for 20 h. The reaction mixture was filtered and stripped of DMF under reduced pressure. The residue was chromatographed on a low pressure silica gel column (100 g) with a mixture of EtOAc/MeOH (10:1) as the eluting solvent. The fractions containing the desired product were combined and evaporated to dryness. The residue obtained was crystallized from MeOH/Et_2O to yield 1.52 g (67%) of **19**: mp 209 °C dec; $[\alpha]_D$ −71.7° (c 0.85, MeOH); TLC R_f (B) 0.39; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9 H, Boc CH_3), 1.60–2.30 (m, 8 H, Pro β-CH_2, γ-CH_2 and 4-CH_2, 5-CH_2), 3.30–3.70 (m, 6 H, 6-CH_2, Pro δ-CH_2, and CH_2CO), 3.85–3.95 (m, 1 H, 3-CH), 4.1–4.3 (m, 1 H, Pro α-CH), 4.70–4.80 (m, 1 H, 3-NH), 5.45–5.55 (m, 1 H, cis $CONH_2$), 7.00–7.15 (m, 1 H, trans $CONH_2$); ^{13}C NMR (DMSO-d_6) δ 20.34 (5-C), 22.97 (Pro γ-C), 27.44, 27.71, 27.98 (Boc CH_3 and 4-C), 30.26 (Pro β-C), 46.32 (Pro δ-C), 48.06 (6-C), 49.20 (CH_2CO), 49.55 (3-C), 59.49 (Pro α-C), 78.26 (Boc C—O), 153.28 (Boc C=O), 168.53 ($CONH_2$), 169.70 (2-C=O), 171.91 (Pro C=O). Anal. ($C_{17}H_{28}N_4O_5$) C, H, N.

3(*S*)-(*N*-L-Prolylamino)-2-oxo-1-piperidineacetamide Trifluoroacetic Acid (4). Tripeptide **19** (1 g, 2.71 mmol) was treated with an excess of CF_3COOH (5 mL) at room temperature for 20 min. The excess CF_3COOH was removed in vacuo, and the resultant residue was dried under vacuum over NaOH overnight. Crystallization from isopropyl alcohol and Et_2O gave 595 mg (57%) of hygroscopic product: $[\alpha]_D$ −72.3° (c 1.0, MeOH). TLC R_f (C) 0.43; ^1H NMR (300 MHz, DMSO-d_6) δ 1.65–1.80 (m, 1 H, 5-CH), 1.9–2.1 (m, 4 H, 5-CH, 4-CH_2, and Pro β-CH), 2.2–2.4 (m, 1 H, Pro β-CH), 3.1–3.4 (m, 4 H, Pro δ-CH_2 and 6-CH_2), 3.80 (d, J = 16.4 Hz, 1 H, CH_2CO), 3.89 (d, J = 16.4 Hz, 1 H, CH_2CO), 4.1–4.3 (m, 1 H, Pro α-CH), 4.32–4.46 (m, 1 H, 3-CH), 7.10 (s, 1 H, cis $CONH_2$), 7.36 (s, 1 H, trans $CONH_2$), 8.56 (br s, 1 H, Pro NH), 8.75 (d, J = 8.3 Hz, 2 H, 3-NH), 9.52 (br s, 1 H, Pro NH); ^{13}C NMR (DMSO-d_6) δ 20.40 (5-C), 23.16 (Pro γ-C), 27.04 (4-C), 29.26 (Pro β-C), 45.57 (δ-C), 48.08 (6-C), 49.47 (3-C and CH_2CO), 58.81 (Pro α-C), 167.77, 167.99 (Pro and 2-C=O), 169.58 ($CONH_2$). Anal. ($C_{14}H_{21}N_4O_5F_3 \cdot H_2O$) C, H, N.

(Benzyloxycarbonyl)-L-prolyl-L-leucyl-2-oxopiperazine (24). 2-Ketopiperazine (**20**) was obtained as light yellow crystals from the condensation of ethyl chloroacetate with ethylenediamine as described by Aspinall:[30] mp 134–136.5 °C (Lit.[30] mp 136 °C). Z-Pro-Leu-OH (1.81 g, 5 mmol) and **20** (0.5 g, 5 mmol) were disolved in 10 mL of dry DMF. After the solution was cooled to 0 °C with an ice bath, diphenyl phosphorazidate (1.3 mL, 6 mmol) and NEt_3 (0.83 mL, 6 mmol) were added. The reaction mixture was stirred at 0 °C for 2 days and at room temperature for 12 h. The solvent was evaporated, and the residue was partitioned between EtOAc (150 mL) and 10% citric acid (50 mL). The EtOAc layer was washed successively with 1 N $NaHCO_3$, water, and saturated NaCl solution (50 mL of each) and dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was purified by medium-pressure chromatography (silica gel; EtOAc/MeOH, 20:3). The product collected was triturated with petroleum ether (bp 30–60 °C) to give 1.15 g (52%) of a white solid: mp 65–70 °C; $[\alpha]_D$ −90.3° (c 1.51, MeOH); TLC R_f (B) 0.56. The NMR spectrum of **24** showed the existence of a pair of rotamers with a ratio 3:4 due to the isomerization about the Z-Pro carbamate bond: ^1H NMR (300 MHz, DMSO-d_6) δ 0.76 and 0.87 (d, J = 6.0 Hz and d, J = 6.2 Hz, 6 H, cis and trans rotamer, Leu δ-CH_3), 1.3–1.7 (m, 3 H, Leu β-CH_2 and γ-CH), 1.70–1.86 (m, 3 H, Pro β-CH and γ-CH_2), 2.0–2.2 (m, 1 H, Pro β-CH), 3.1–3.3 (m, 2 H, Pro δ-CH_2), 3.3–3.5 (m, 2 H, 6-CH_2), 3.50–3.77 (m, 2 H, 5-CH_2), 3.78–4.15 (m, 2 H, 3-CH_2), 4.20–4.32 (m, 1 H, Pro α-CH), 4.55–4.75 (m, 1 H, Leu α-CH), 4.88–5.10 (m, 2 H, cis and trans rotamers, OCH_2), 7.25–7.40 (m, 5 H, cis and trans rotamers, Ph), 8.09 and 8.12 (s, 1 H, cis and trans rotamers, 1-NH), 8.24 and 8.31 (d, J = 7.8 and d, J = 8.2 Hz, 1 H, cis and trans rotamers, 3-NH). Anal. ($C_{23}H_{32}N_4O_5$) C, H, N.

L-Prolyl-L-leucyl-2-oxopiperazine Hydrochloride (7). Compound **24** (1.02 g, 2.30 mmol) was dissolved in 4 mL of MeOH. To this solution were added concentrated HCl (0.21 mL, 2.5 mmol) and 10% Pd/C (100 mg) under N_2. Hydrogenolysis was carried out on Parr apparatus for 1 h at H_2 = 40 psi. The filtrate was evaporated, and the residue was crystallized from isopropyl alcohol to give 549 mg (69%) of the desired product: mp 276 °C dec; $[\alpha]_D$ −48.9° (c 1.13, MeOH); TLC R_f (C) 0.69; ^1H NMR (300 MHz, DMSO-d_6) δ 0.90 (d, J = 6.3 Hz, 6 H, Leu δ-CH_3), 1.40–1.75 (m, 3 H, Leu β-CH_2 and γ-CH), 1.75–1.92 (m, 3 H, Pro β-CH and γ-CH_2), 2.2–2.4 (m, 1 H, Pro β-CH), 3.15–3.25 (m, 3 H, Pro δ-CH_2 and 6-CH), 3.50–3.75 (m, 3 H, 5-CH_2 and 6-CH), 3.83 (d, J = 17.0 Hz, 1 H, 3-CH), 4.02 (d, J = 17.0 Hz, 1 H, 3-CH), 4.14–4.25 (m, 1 H, Pro α-CH), 4.61–4.77 (m, 1 H, Leu α-CH), 8.19 (br d, J = 11.8 Hz, 1 H, 1-NH), 8.51 (br s, 1 H, Pro NH), 8.2 (d, J = 7.5 Hz, 1 H, Leu NH), 10.32 (br s, 1 H, Pro NH). Anal. ($C_{15}H_{27}N_4O_3Cl$) C, H, N.

3(*S*)-[*N*-[(Benzyloxycarbonyl)-L-prolyl-L-leucyl]-amino]-2-oxopyrrolidine (25). 3(*S*)-Amino-2-oxopyrrolidine (**21**; 110 mg, 1.1 mmol) prepared by using the procedure of Wilkinson[31] was coupled to Z-L-Pro-L-Leu-OH (362 mg, 1 mmol) by using the same method as that described above for the preparation of **24**. The product was crystallized from a mixture of CH_2Cl_2 and Et_2O to afford 231 mg (54%) of material: mp 178–179 °C; $[\alpha]_D$ −103.6° (c 1.34, MeOH); TLC R_f (A) 0.29. The NMR spectrum indicated a pair of rotamers in a ratio of 3:4 due to isomerism about the Z-Pro carbamate bond. ^1H NMR (300 MHz, DMSO-d_6) δ 0.76 (dd, J = 6.4 and 6.5 Hz, trans rotamer, Leu δ-CH_3), 0.86 (dd, J = 6.4 and 6.5 Hz, cis rotamer, Leu δ-CH_3), 1.3–1.9 (m, 7 H, Leu β-CH_2 and γ-CH, Pro β-CH and γ-CH_2, 4-CH), 2.0–2.2 (m, 1 H, 4-CH), 2.20–2.35 (m, 1 H, Pro β-CH), 3.1–3.2 (m, 2 H, Pro δ-CH_2), 3.3–3.5 (m, 2 H, 5-CH_2), 4.20–4.34 (m, 3 H, 3-CH, Pro α-CH, Leu α-CH), 5.03 and 5.07 (dd, J = 13.1 Hz and dd, J = 12.7 Hz, 2 H, trans and cis rotamers, OCH_2), 7.25–7.40 (m, 5 H, Ph), 7.81 (br s, 1 H, 1-NH), 7.98–8.07 (br d, 3-NH and 2 dd, cis and trans rotamers Leu NH). Anal. ($C_{23}H_{32}N_4O_5$) C, H, N.

3(*S*)-[*N*-(L-Prolyl-L-leucyl)amino]-2-oxopyrrolidine Hydrochloride (8). Protected tripeptide **25** (160 mg, 0.36 mmol) was hydrogenolyzed by the same method as described for compound **24**. The residue obtained was triturated with Et_2O to yield 111 mg (86%) of product: mp 132 °C dec; $[\alpha]_D$ −79.2° (c 1.0, MeOH); TLC R_f (C) 0.56; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (d, J = 6.8 Hz, 3 H, Leu δ-CH_3), 0.91 (d, J = 6.7 Hz, 3 H, Leu δ-CH_3), 1.45–1.60 (m, 2 H, Leu β-CH_2), 1.6–1.75 (m, 1 H, Leu δ-CH), 1.75–1.90 (m, 4 H, 4-CH and Pro β-CH, γ-CH_2), 2.2–2.4 (m, 2 H, 4-CH, Pro β-CH), 3.1–3.28 (m, 4 H, 5-CH_2 and Pro δ-CH_2), 4.15–4.25 (m, 1 H, 3-CH), 4.25–4.40 (m, 2 H, Pro α-CH and Leu α-CH), 7.84 (s, 1 H, 1-NH), 8.25 (d, J = 8.3 Hz, 1 H, 3-NH), 8.48 (br s, 1 H, Pro NH), 8.70 (d, J = 8.1 Hz, 1 H, Leu NH). Anal. ($C_{15}H_{27}N_4O_3Cl \cdot {}^3/_4H_2O$) C, H, N.

3(*S*)-[(Butoxycarbonyl)amino]-2-oxopiperidine (23). To a solution of N^{α}-Boc-N^{δ}-Z-ornithine methyl ester (2.49 g, 6.55 mmol) in MeOH (5 mL) were added AcOH (0.45 mL, 7.86 mmol) and 10% Pd/C (250 mg). Hydrogenolysis was performed on a Parr apparatus at H_2 = 40 psi for 1 h. The catalyst was removed by filtration. The filtrate was evaporated and dried in vacuo. Dry DMF (100 mL) and Na_2CO_3 (3.47 g, 32.75 mmol) were added to the resulting residue. The reaction mixture was stirred at room temperature for 2 days and then filtered. The filtrate was stripped of solvent in vacuo, and the resulting residue was partitioned between EtOAc (120 mL) and 10% citric acid (40 mL). The organic layer was washed with water (40 mL) and saturated NaCl

aqueous solution (40 mL) and dried over $MgSO_4$. The solvent was removed, and the residue was purified through medium-pressure chromatography (CH_2Cl_2/MeOH, 20:1). The product collected was crystallized from CH_2Cl_2/petroleum ether (bp 30–60 °C) to give 1.22 g (87%) of material: mp 101–103 °C; $[\alpha]_D$ −10.6° (c 1.22, MeOH); TLC R_f (A) 0.45; ^1H NMR (90 MHz, $CDCl_3$) δ 1.45 (s, 9 H, Boc CH_3), 1.40–2.08 (m, 3 H, 5-CH_2, 4-CH), 2.48–2.64 (m, 1 H, 4-CH), 3.20–3.44 (m, 2 H, 6-CH_2), 3.88–4.16 (m, 1 H, 3-CH), 6.10 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 20.86 (5-C), 27.63 (4-C), 27.97 (Boc CH_3), 40.67 (6-C), 50.38 (3-C), 77.55 (Boc C—O), 155.09 (Boc C=O), 169.91 (2-C=O). Anal. ($C_{10}H_{18}N_2O_3$) C, H, N.

3(*S*)-[*N*-[(Benzyloxycarbonyl)-L-prolyl-L-leucyl]amino]-2-oxopiperidine (26). Protected lactam **23** (1.21 g, 5.65 mmol) was treated with EtOAc saturated with HCl (20 mL) at 0 °C for 1 h. The solvent was evaporated, and the solid residue that remained behind was washed with Et_2O and dried over NaOH under vacuum to give 839 mg (99%) of 3(*S*)-amino-2-oxopiperidine (**22**) as its hydrochloride salt. This material (800 mg, 5.31 mmol) was coupled to Z-Pro-Leu-OH (1.93 g, 5.31 mmol) in the same manner as described above for the synthesis of **24**. A solution of the crude product in a small amount of EtOAc when mixed with water yielded a precipitate. This precipitate when collected and dried under vacuum yielded 1.44 g (55%) of the desired product **26**: mp 165–166 °C; $[\alpha]_D$ −87.1° (c 1.23, MeOH). TLC R_f (A) 0.38. The NMR spectrum indicated a pair of rotamers in a ratio of 3:4 due to isomerism about the Z-Pro carbamate bond: ^1H NMR (300 MHz, DMSO-d_6) δ 0.75 (dd, J = 6.3 and 6.4 Hz, trans rotamer Leu δ-CH_3), 0.86 (dd, J = 6.4 and 6.5 Hz, cis rotamer Leu δ-CH_3), 1.38–1.60 (m, 3 H, Leu β-CH_2 and γ-CH), 1.6–2.0 (m, 7 H, 3-CH_2, 4-CH_2 and Pro β-CH, γ-CH_2), 2.02–2.22 (m, 1 H, Pro β-CH), 3.08–3.15 (m, 2 H, Pro δ-CH_2), 3.3–3.5 (m, 2 H, 6-CH_2), 4.05–4.17 (m, 1 H, 3-CH), 4.20–4.35 (m, 2 H, Pro α-CH and Leu α-CH), 4.80–5.12 (m, 2 H, cis and trans rotamers OCH_2), 7.27–7.40 (m, 5 H, Ph), 7.58 (br s, 1-NH), 7.82 and 7.88 (br d, J = 7.9 Hz, and br d, J = 7.8 Hz, 1 H, cis and trans rotamers Leu NH), 8.02 and 8.06 (br d, J = 7.8 Hz, and br d, J = 8.4 Hz, cis and trans rotamers, 3-NH). Anal. ($C_{24}H_{34}N_4O_5$) C, H, N.

3(*S*)-[*N*-(L-Prolyl-L-leucyl)amino]-2-oxopiperidine Hydrochloride (9). This material was prepared by hydrogenolyzing the protected tripeptide **26** (1 g, 2.13 mmol) and triturating the residue obtained with Et_2O. A yield of 755 mg (94%) of a white solid was obtained: mp 148–150 °C; $[\alpha]_D$ −58.9° (c 1.0, MeOH); TLC R_f (C) 0.59; ^1H NMR (300 MHz, DMSO-d_6) δ .89 (dd, J = 6.6 Hz, 6 H, Leu δ-CH_3), 1.45–2.00 (m, 9 H, Leu β-CH_2, γ-CH, Pro β-CH, γ-CH_2, and 4-CH_2, 5-CH_2), 2.2–2.4 (m, 1 H, Pro β-CH), 3.18–3.30 (m, 4 H, 6-CH_2 and Pro δ-CH_2), 4.08–4.25 (m, 2 H, 3-CH and Pro α-CH), 4.31 (dd, J = 7.3 and 7.7 Hz, Leu α-CH), 7.61 (s, 1 H, 1-NH), 8.12 (d, J = 8.0 Hz, 1 H, 3-NH), 8.49 (br s, 1 H, Pro NH), 8.76 (d, J = 8.8 Hz, 1 H, Leu NH), 10.17 (br s, 1 H, Pro NH); ^{13}C NMR (DMSO-d_6) δ 20.91 (5-C), 21.47, 23.03 (Leu δ-C), 23.44 (Pro γ-C), 24.09 (Leu γ-C), 27.41 (4-C), 29.71 (Pro β-C), 40.76 and 40.87 (6-C and Leu β-C), 45.48 (Pro δ-C), 48.86 (Leu α-C), 51.68 (3-C), 58.50 (Pro α-C), 167.96, 169.52, 171.10 (Pro, Leu, 2-C=O). Anal. ($C_{16}H_{29}N_4O_3Cl \cdot H_2Oe$ C, H, N.

[^{3}H]ADTN Binding Assay. The binding assay for [^{3}H]ADTN was carried out as described earlier by us.[8] 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 (20 s). The tissue homogenate was twice centrifuged at 27000*g* 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 0.2–0.3 mg of protein of the brain homogenate, 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 PLG or one 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 were rapidly filtered under partial vacuum over Whatman GF/B filters. The filters were washed four 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 butaclamol-*d*. The data were analyzed statistically by using the Student's *t* test.

For the preincubation experiments, the striatal membrane preparation, was incubated with various concentrations (10^{-12}–10^{-4}M) of PLG or one of the PLG analogues for 1 h at 4 °C and then centrifuged at 27000*g* for 10 min. The pellet was washed and resuspended in the original volume of the tissue resuspension buffer. The binding assay for [^{3}H]ADTN was carried out as described above. Control membranes were preincubated in the same way but without the presence of PLG or a PLG analogue.

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Registry No. **2**, 106732-52-9; **3**, 114200-31-6; **4**·TFA, 114200-32-7; **5**, 114200-33-8; **6**, 114200-34-9; **7**·HCl, 114200-35-0; **8**·HCl, 114200-36-1; **9**·HCl, 114200-37-2; **10**, 79839-26-2; **11**, 78444-90-3; **12**, 114200-38-3; **13**, 114200-39-4; **14**, 114200-40-7; **15**, 114200-41-8; **16**, 74411-97-5; **17**, 114200-42-9; **18**, 114200-43-0; **19**, 106732-56-3; **20**, 5625-67-2; **21**, 4128-00-1; **22**, 34294-79-6; **23**, 92235-39-7; **24**, 114200-44-1; **25**, 114200-45-2; **26**, 114200-46-3; BOC-Met-Gly-OMe, 68164-11-4; BOC-NH-L-CH($CH_2CH_2S^+Me_2$)CO-Gly-OMe·I^-, 79839-23-9; BOC-D-Met-Gly-OMe, 70668-86-9; Z-Pro-OH, 1148-11-4; H-pGlu-OH, 98-79-3; BOC-OHN(Z)-OH, 2480-93-5; BOC-Pro-OH, 15761-39-4; Z-Pro-Leu-OH, 1634-90-8; BOC-Orn(Z)-OMe, 2480-95-7; $ClCH_2CO_2Et$, 105-39-5; $H_2NCH_2CH_2NH_2$, 107-15-3; 3(*S*)-(*N*-L-pyroglutamylamino)-2-oxo-1-pyrrolidineacetic acid methyl ester, 114200-47-4; dopamine, 51-61-6.