Synthesis and Dopamine Receptor Modulating Activity of 3-Substituted γ-Lactam Peptidomimetics of L-Prolyl-L-leucyl-glycinamide

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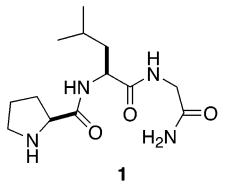
Received October 4, 2002

γ-Lactam peptidomimetic **2** of Pro-Leu-Gly-NH_2 (PLG) was substituted at the 3-position with isobutyl, butyl, and benzyl moieties to give the PLG peptidomimetics **3**−**5**, respectively. These compounds were synthesized to test the hypothesis that attaching a hydrophobic moiety to the lactam ring to mimic the isobutyl side chain of the leucyl residue of PLG would increase the dopamine receptor modulating activity of such peptidomimetics. These peptidomimetics were tested for their ability to enhance the binding of [^{3}H]-*N*-propylnorapomorphine to dopamine receptors isolated from bovine striatal membranes. The rank order of effectiveness of the 3-substituent was benzyl > *n*-butyl > isobutyl > H.

Introduction

The tripeptide L-prolyl-L-leucyl-glycinamide (**1**, PLG) has the unique ability to modulate D_2 dopamine receptors within the CNS.[1−7] Numerous peptide analogues and peptidomimetics of PLG have been synthesized and tested.[8−16] One of the most potent PLG peptidomimetics to be made is the γ-lactam **2**.[11] PLG and **2** induce an increase in agonist binding to the D_2 dopamine receptor by increasing the affinity of the receptor for agonists and by increasing the proportion of D_2 receptors existing in the high affinity state.[5,17] Both **1** and **2** also have been shown to inhibit dopamine-stimulated adenylyl cyclase activity and to enhance *N*-propylnorapomorphine (NPA)-stimulated low K_m GTPase activity in rat striatal membranes.[18]

In the rat nigrostriatal 6-hydroxydopamine lesion model of Parkinson's disease,[19] **1** and **2** have been shown to potentiate the contralateral rotational behavior induced by apomorphine.[2,5,20,21] These two compounds also protect C57 BL/6 mice against MPTP-induced dopaminergic degeneration[22] and they attenuate haloperidol-induced *c-fos* and Fos expression.[23]



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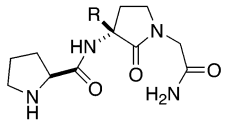
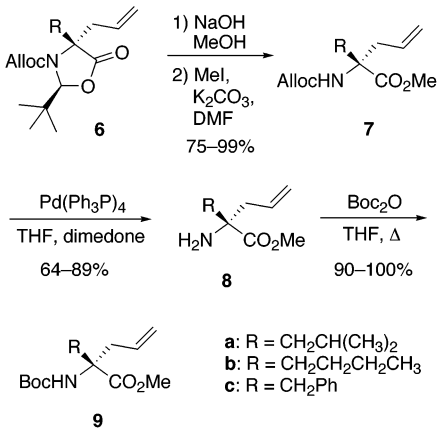


2: R = H
3: R = $CH_2CH(CH_3)_2$
4: R = $(CH_2)_3CH_3$
5: R = CH_2Ph

Scheme 1



In the original design of **2**, the isobutyl side chain that would correspond to the leucyl side chain was left off the molecule for synthetic ease. Early SAR studies with linear tripeptide analogues of **1** modified at the leucine position showed that a hydrophobic residue at this position was important for activity.[10] Thus, we postulated that incorporation of a hydrophobic side chain into the structure of **2** would enhance the activity of this peptidomimetic. To test this hypothesis, the synthesis of the 3-isobutyl γ-lactam peptidomimetic **3** was undertaken. In addition, the 3-butyl and 3-benzyl derivatives also were synthesized to give peptidomimetics **4** and **5**, respectively. These latter two analogues were made since SAR studies with linear tripeptide analogues of **1** showed that replacing the leucyl residue with either the norleucine or phenylalanine residues provided analogues equal in potency and efficacy to that of PLG.

Synthesis

The synthesis of the 3-substituted γ-lactam PLG peptidomimetics began with the asymmetric synthesis of the α-allylated oxazolidinones **6a**−**c** through methodology reported previously.[24,25] In each case as shown in Scheme 1, basic hydrolysis of the oxazolidinone afforded an acid that was immediately converted to its methyl ester (**7a**−**c**). Since the allyloxycarbonyl (Alloc) protecting group was expected to be a problem with

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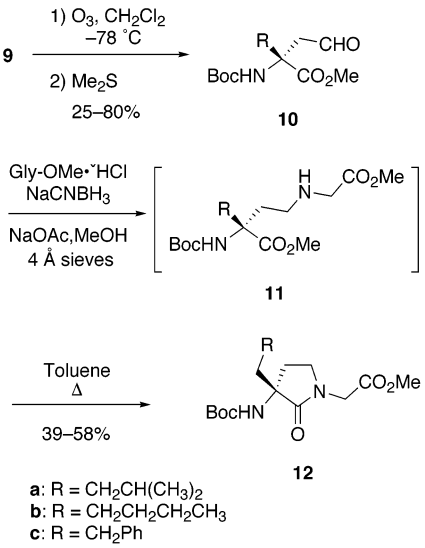
10.1021/jm020441o CCC: $25.00
Published on Web 01/18/2003

Scheme 2



some of the subsequent reaction conditions, it was removed from **7a**−**c** with tetrakis(triphenylphosphine)-palladium(0) and dimedone to give the free amines **8a**−**c**. Protection of the amines with Boc_2O in THF under reflux conditions gave the α-allyl derivatives of the amino acids leucine, norleucine, and phenylalanine, compounds **9a**−**c**, respectively.

Conversion of the α-allyl amino acids **9a**−**c** to the corresponding 3-substituted γ-lactams **12a**−**c** is outlined in Scheme 2. Cleavage of the terminal alkene in **9a**−**c** by ozonolysis gave moderate to very good yields of aldehydes **10a**−**c**. Alternatively, the use of osmium tetroxide and sodium periodate in this instance gave yields that generally were quite poor. The aldehydes obtained after silica gel chromatography were treated with glycine methyl ester hydrochloride under reductive amination conditions with sodium cyanoborohydride, sodium acetate, and activated 4 Å molecular sieves in methanol. It was found that a portion of the amine formed in this reductive amination process immediately cyclized to the desired 3-substituted γ-lactam. Thus the amines **11a**−**c** were not isolated, but rather the crude mixture of products was heated under reflux conditions for 2 days to give the desired γ-lactams **12a**−**c**.

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) coupling of the hydrochloride salt of the γ-lactams with Boc-L-Pro-OH occurred in excellent yields in a mixture of DMF/CH_2Cl_2 with triethylamine and 1-hydroxybenzotriazole (HOBt) (Scheme 3). Aminolysis with ammonia gave tripeptide amides **14a**−**c** in moderate yields after crystallization from ethyl acetate/hexanes. Removal of the *tert*-butoxycarbonyl group with trifluoracetic acid in methanol gave final products **3**−**5** as very hygroscopic trifluoracetate salts.

Pharmacology

The substituted γ-lactam PLG peptidomimetics **3**−**5** were evaluated in a functional in vitro assay utilizing bovine striatal membranes.[6,26] These analogues along with **2** were tested for their ability to enhance the binding of [^{3}H]NPA at three concentrations: 1 nM, 10 nM, and 100 nM. The results are shown in Figure 1. At a concentration of 100 nM, the percent increase in NPA

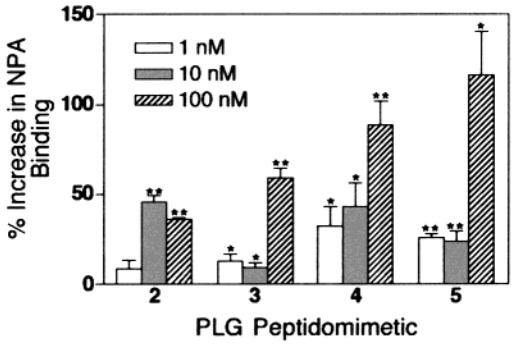
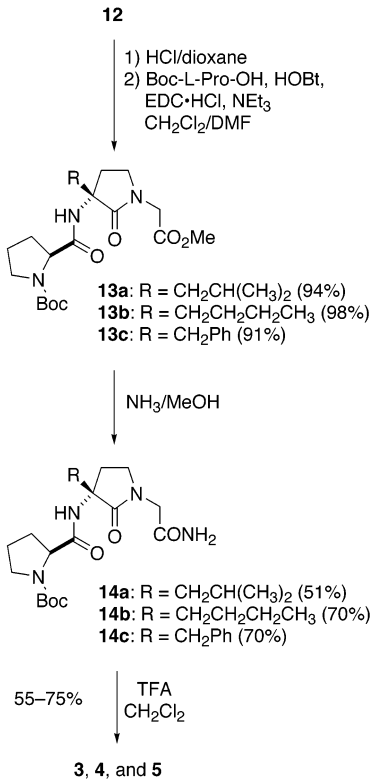


Figure 1. Stimulation of [^{3}H]NPA binding to bovine striatal membranes by the PLG peptidomimetics **2**−**5**. Data represent the percent increase in specific [^{3}H]NPA binding over the control value when the indicated concentration of peptidomimetic was added directly to the assay buffer. Results are the means ± SEM of 3−4 separate experiments carried out in triplicate. * Significantly different ($p < 0.01$) from control value; ** significantly different ($p < 0.001$) from control value.

Scheme 3



binding for **2**−**5** was as follows: **2**, 36 ± 1%; **3**, 59 ± 5%; **4**, 89 ± 13%; **5**, 116 ± 24%. At this concentration, the general trend was clearly one in which the ability of a compound to enhance the binding of the dopamine receptor agonist to dopamine receptors was increased by the presence of a lipophilic α-substituent. The rank order of effectiveness was the benzyl > *n*-butyl > isobutyl > H.

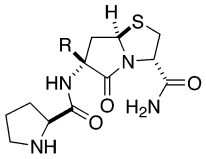
Discussion

Earlier work on the structure−activity relationships of PLG showed that replacing the leucyl isobutyl side chain with smaller alkyl moieties gave PLG analogues that possessed little or no ability to enhance the binding of dopamine receptor agonists such as ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) to dopam-

ine receptors. However, the PLG analogues possessing either a butyl or benzyl group in place of the isobutyl side chain had about the same activity as PLG in enhancing the binding of ADTN to dopamine receptors.[10]

The incorporation of conformational constraints into the PLG structure such as the lactam moiety in **2** and the bicyclic thiazolidine lactam moiety in **15** yielded PLG peptidomimetics that were more active than PLG itself in modulating dopamine receptors even though these peptidomimetics lacked the isobutyl side chain found in PLG.[11,13] We postulated that the placement of lipophilic side chains on the lactam and bicyclic thiazolidine lactam scaffolds would enhance the activity of these PLG peptidomimetics by virtue of their ability to access the lipophilic binding pocket with which the leucyl isobutyl side chain interacts.

Previously, we reported on the dopamine receptor modulating activity of the substituted bicyclic lactams **16**−**18**.[25] These PLG peptidomimetics were evaluated in the rat nigrostriatal 6-hydroxydopamine lesion rotational model. Compound **16** maximally enhanced the rotations produced by apomorphine by 44% at a dose of 1 μg/kg ip. For compound **17**, the maximal percent increase in rotations was 56% at a dose of 0.1 μg/kg ip. while for **18** it was 30% at 1 μg/kg ip. In comparison, the unsubstituted bicyclic lactam **15** only produced a 23% increase in rotations at a dose of 0.1 μg/kg ip. The results of this previous study showed that incorporation of a lipophilic substituent on the bicyclic thiazolidine lactam scaffold enhanced the dopamine receptor modulating activity of the PLG peptidomimetics.



15: R = H
16: R = $CH_2CH(CH_3)_2$
17: R = $(CH_2)_3CH_3$
18: R = CH_2Ph

The present study demonstrates that the dopamine receptor modulating activity of the lactam PLG peptidomimetics also is enhanced by the placement of a lipophilic substituent off the lactam scaffold. Interestingly, in this case, the benzyl derivative provided the greatest enhancement followed by the *n*-butyl and isobutyl derivatives. This is a different rank order than that seen with the bicyclic lactam scaffold where it was *n*-butyl > isobutyl > benzyl. Although this difference could be due to the fact that a different assay system was used in the bicyclic lactam series than that in the lactam series, there is ample experience in the literature that previously demonstrates a good correlation between the activity in these two model systems.[16,20] It seems more likely that either we are seeing differences in transport into the brain coming into play for the bicyclic thiazolidine lactams or that the different scaffolds induce a subtle change in the interaction of the lipophilic side chain with the PLG binding site.

Experimental Section

General Aspects. Thin-layer chromatography was performed on Analtech 250 μm silica gel GHLF Uniplates which were visualized by UV, I_2, ninhydrin spray (amines), 2,6-dichlorophenol indophenol spray (acids), and 2,4-dinitrophenylhydrazine (aldehydes). Chromatographic purification on silica gel (Merck, grade 60, 240−400 mesh, 60 Å) was done by flash or gravity methods. Optical rotations were measured on a Rudolph Research Autopol III polarimeter at the 589 nm Na D-Line. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M−H−W Laboratories, Phoenix, Arizona.

(*R*)-*N*-Allyloxycarbonyl-α-allyl-leucine Methyl Ester (7a). Allyl oxazolidinone **6a**[25] (11.9 g, 36.8 mmol) was dissolved in 184 mL of 1 N NaOH and 185 mL of MeOH and the solution was heated at reflux for 2 days. The solution was concentrated in vacuo and the residue partitioned between H_2O and EtOAc. $NaHSO_4$ (10% solution) was added to the aqueous solution until the pH = 1, and then the solution was extracted with EtOAc (6×). The organic layer was washed with H_2O followed by saturated NaCl solution and then dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to give the free acid as a yellow oil.

The acid was dissolved in 50 mL of DMF and 10 g of K_2CO_3 was added. The reaction was cooled to 0 °C and MeI (5.3 mL, 85 mmol) was added dropwise. The reaction was stirred at 0 °C under Ar for 1.5 h and then at room temperature for 4 h. Solvent was removed from the reaction via high vacuum and the residue dissolved in H_2O and extracted with EtOAc (5×). The organic layer was washed with H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution successively and then dried ($MgSO_4$), filtered, and concentrated in vacuo to give a yellow oil. Silica gel chromatography (4 × 40 cm) with 5% EtOAc/hexanes as the eluting solvent yielded 9.88 g (99%) of **7a** as a clear oil. $[\alpha]_D$ −5.6 (*c* 1.70, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3 H, J = 7.2 Hz), 0.89 (d, 3 H, J = 6.0 Hz), 1.54−1.70 (m, 2 H), 2.33−2.44 (m, 2 H), 3.11 (dd, 1 H, J = 7.5, 13.5 Hz), 3.74 (s, 3 H), 4.53 (d, 2 H, J = 6.0 Hz), 5.00−5.06 (m, 2 H), 5.18−5.33 (m, 2 H), 5.62−5.50 (m, 1 H), 5.84−5.97 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 22.4, 23.7, 24.5, 40.7, 43.7, 52.5, 63.5, 65.0, 117.4, 118.8, 132.2, 132.9, 153.8, 174.4. FAB MS (glycerol matrix) *m*/*z*: 270 $[M + H]^+$. Anal. ($C_{14}H_{23}NO_4$) C, H, N.

(*R*)-*N*-Allyloxycarbonyl-α-allyl-norleucine Methyl Ester (7b). Allyl oxazolidinone **6b**[25] (7.29 g, 22.5 mmol) was converted to **7b** by the same procedure as described above for **7a**. The product was obtained as a clear oil in a yield 4.56 g (75%). $[\alpha]_D$ 7.4 (*c* 1.81, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.1 Hz), 0.90−1.00 (m, 1 H), 1.20−1.33 (m, 3 H), 1.69−1.78 (m, 1 H), 2.23−2.30 (br t, 1 H), 2.48 (dd, 1 H, J = 7.4, 14.1 Hz), 3.05 (dd, 1 H, J = 7.4, 13.8 Hz), 3.73 (s, 3H), 4.51 (d, 2 H, J = 5.1 Hz), 5.01−5.06 (m, 2 H), 5.16−5.31 (m, 2 H), 5.52−5.66 (m, 1 H), 5.83−5.95 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 26.3, 35.1, 39.8, 52.8, 64.1, 65.2, 117.4, 118.9, 132.5, 133.0, 154.0, 173.9. FAB MS (glycerol matrix) *m*/*z*: 270 $[M + H]^+$. Anal. ($C_{14}H_{23}NO_4$) C, H, N.

***(S)*-*N*-Allyloxycarbonyl-α-allyl-phenylalanine Methyl Ester (7c).** Allyl oxazolidinone **6c**[24] (9.64 g, 27 mmol) was converted to **7c** by the same procedure as described above for **7a**. The product was obtained as a clear oil in a yield 7.55 g (92%). $[\alpha]_D$ 7.3 (*c* 1.81, MeOH). ^{1}H NMR ($CDCl_3$) δ 2.64 (dd, 1 H, J = 7.5, 13.5 Hz), 3.14 (d, 1 H, J = 13.5 Hz), 3.25 (dd, 1 H, J = 6.6, 13.8 Hz), 3.63 (d, 1 H, J = 16.2 Hz), 3.76 (s, 3 H), 4.54−4.67 (m, 2 H), 5.14−5.09 (m, 2 H), 5.23−5.36 (m, 2 H), 5.58−5.72 (m, 2 H), 5.88−6.01 (m, 1H), 7.02−7.29 (m, 5 H); ^{13}C NMR (CD_3CN) δ 40.5, 41.3, 53.5, 65.7, 66.1, 117.9, 120.1, 128.3, 129.6, 131.4, 133.7, 134.8, 137.6, 155.6, 174.0. FAB MS (glycerol matrix) *m*/*z*: 304 $[M + H]^+$. Anal. ($C_{17}H_{21}NO_4$) C, H, N.

(*R*)-α-Allyl-leucine Methyl Ester (8a). Methyl ester **7a** (2.70 g, 10 mmol) was dissolved in 50 mL of dry THF. Dimedone (8.43 g, 60 mmol) and 0.092 g of $Pd(PPh_3)_4$ (0.8%) were added, and the solution was stirred under N_2 overnight. The solution was diluted with Et_2O and extracted with 1 N HCl (6 × 75 mL). The acid solution was made basic by the addition of solid K_2CO_3 until the solution reached a pH of 12. Extraction of the basic aqueous solution with EtOAc (4 × 100

mL) was followed by washing the organic layer with saturated $NaHCO_3$ solution and saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to a yellow oil. Kugelrohr distillation at reduced pressure yielded 1.19 g (64%) of **8a** as a clear oil. $[\alpha]_D$ 41.3 (*c* 1.02, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.72 (d, 3 H, J = 6.0 Hz), 0.84 (d, 3 H, J = 6.0 Hz), 1.40−1.48 (m, 1 H), 1.56 (s, 2 H), 1.60−1.70 (m, 2 H), 2.11 (dd, 1 H, J = 8.4, 13.5 Hz), 2.44 (dd, 1 H, J = 6.0, 13.5 Hz), 3.60 (s, 3 H), 5.03 (d, 2 H), 5.62−5.49 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.2, 24.8, 25.2, 46.3, 49.2, 52.4, 60.8, 120.1, 133.2, 178.4. FAB MS (glycerol matrix) *m/z*: 186 $[M + H]^+$. Anal. ($C_{10}H_{19}NO_2$) C, H, N.

(*R*)-α-Allyl-norleucine Methyl Ester (8b). Methyl ester **7b** (4.05 g, 15 mmol) was deprotected under the same conditions as described above for **8a**. Kugelrohr distillation at 65 °C under reduced pressure yielded 2.39 g (86%) of **8b** as a clear oil. $[\alpha]_D$ 20.3 (*c* 1.38, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.2 Hz), 1.03−1.14 (m, 1 H), 1.22−1.37 (m, 3 H), 1.52 (dt, 1 H, J = 4.5, 12.6 Hz), 1.68−1.78 (m, 3 H), 2.23 (dd, 1 H, J = 8.4, 13.5 Hz), 2.54 (dd, 1 H, J = 6.0, 13.2 Hz), 3.69 (s, 3 H), 5.09−5.14 (m, 2 H), 5.60−5.73 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 23.1, 26.3, 39.9, 44.4, 52.2, 61.0, 119.6, 132.9, 177.5. FAB MS (glycerol matrix) *m/z*: 186 $[M + H]^+$. Anal. ($C_{10}H_{19}NO_2$) C, H, N.

***(S)*-α-Allyl-phenylalanine Methyl Ester (8c).** Methyl ester **7c** (7.10 g, 23.4 mmol) was deprotected under the same conditions as described above for **8a**. Kugelrohr distillation at 100−102 °C at 0.1 mmHg yielded 4.59 g (89%) of **8c** as a clear oil. $[\alpha]_D$ 4.8 (*c* 1.68, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 2 H), 2.28 (dd, 1 H, J = 8.4, 13.2 Hz), 2.68 (dd, 1 H, J = 7.2, 13.2 Hz), 2.75 (d, 1 H, J = 13.5 Hz), 3.14 (d, 1 H, J = 13.5 Hz), 3.65 (s, 3 H), 5.10−5.17 (m, 2 H), 5.61−5.75 (m, 1 H), 7.10−7.26 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 44.1, 45.5, 51.5, 61.5, 119.2, 126.6, 128.0, 129.5, 132.2, 135.8, 176.0. FAB MS (glycerol matrix) *m/z*: 220 $[M + H]^+$. Anal. ($C_{13}H_{17}NO_2$) C, H, N.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-α-allyl-leucine Methyl Ester (9a). Amine methyl ester **8a** (4.80 g, 18.4 mmol) was dissolved in 125 mL of dry THF. Boc_2O (4.84 g, 18.4 mmol) was added, and the solution was refluxed under Ar for 2 days. H_2O (50 mL) was added along with 50 mg of DMAP to catalyze any excess Boc_2O breakdown, and the solution was stirred for 1 day. The solution was extracted with EtOAc (5×), and then the organic layer was washed successively with 10% $NaHSO_4$ solution, saturated $NaHCO_3$ solution, and saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield a clear oil, which was chromatographed on a 4 × 40 cm silica gel column. Elution with 3% EtOAc in hexanes gave a quantitative yield of **9a** as a clear oil. $[\alpha]_D$ −6.2 (*c* 1.02, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.74 (d, 3 H, J = 7.2 Hz), 0.87 (d, 3 H, J = 6.3 Hz), 1.40 (s, 9 H), 1.53−1.65 (m, 2 H), 2.28−2.40 (m, 2 H), 3.08 (dd, 1 H, J = 7.2, 13.5 Hz), 3.70 (s, 3 H), 4.98−5.03 (m, 2 H), 5.51−5.60 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 22.7, 23.6, 24.4, 28.3, 40.7, 43.8, 52.1, 63.3, 78.9, 118.4, 132.5, 153.7, 174.3. FAB MS (glycerol matrix) *m/z*: 286 $[M + H]^+$. Anal. ($C_{15}H_{27}NO_4$) C, H, N.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-α-allyl-norleucine Methyl Ester (9b). Amine methyl ester **8b** (2.04 g, 11 mmol) was protected with the *tert*-butoxycarbonyl group in the same manner as described for **9a** to yield 2.85 g (91%) of **9b** as a white solid. $[\alpha]_D$ 7.5 (*c* 0.94, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.2 Hz), 0.97−1.07 (m, 1 H), 1.26−1.34 (m, 3 H), 1.43 (s, 9 H), 1.68−1.78 (m, 1 H), 2.21 (br t, 1 H, J = 11.7 Hz), 2.51 (dd, 1 H, J = 7.2, 13.5 Hz), 2.96−3.02 (m, 1 H), 3.73 (s, 3 H), 5.03−5.07 (m, 2 H), 5.37 (br s, 1 H), 5.56−5.69 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 26.3, 28.5, 35.1, 39.8, 52.7, 63.8, 79.3, 118.8, 132.8, 154.0, 174.2. FAB MS (glycerol matrix) *m/z*: 286 $[M + H]^+$. Anal. ($C_{15}H_{27}NO_4$) C, H, N.

***(S)*-*N*-(*tert*-Butoxycarbonyl)-α-allyl-phenylalanine Methyl Ester (9c).** Amine methyl ester **8c** (4.29 g, 19.6 mmol) was protected with the *tert*-butoxycarbonyl group in the same manner as described for **9a** to give a quantitative yield of **9c** as a clear oil. $[\alpha]_D$ 7.6 (*c* 0.85, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9 H), 2.60 (dd, 1H, J = 7.2, 13.2 Hz), 3.13 (d, 1 H, J = 13.5 Hz), 3.22 (dd, 1 H, J = 6.3, 13.5 Hz), 3.62 (d, 1 H, J = 14.7 Hz), 3.75 (s, 3 H), 5.09−5.15 (m, 2 H), 5.35 (s, 1 H), 5.61−5.70 (m, 1 H), 7.06−7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 28.3, 39.8, 40.6, 52.4, 64.8, 79.1, 118.9, 126.8, 128.1, 129.7, 132.3, 136.3, 153.9, 172.9. FAB MS (glycerol matrix) *m/z*: 320 $[M + H]^+$. Anal. ($C_{18}H_{25}NO_4$) C, H, N.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-α-formylmethyl-leucine Methyl Ester (10a). α-Allyl amino acid **9a** (6.73 g, 23.6 mmol) was dissolved in 150 mL of dry CH_2Cl_2, and MeOH (1 mL) was added. The solution was cooled to −78 °C, and ozone was bubbled into the solution until a blue color persisted. Nitrogen was bubbled into the solution, and then dimethyl sulfide (5.2 mL, 0.7 mol) was added dropwise via syringe to quench the reaction. The solution initially was stirred at −78 °C and then allowed to warm to room-temperature overnight. Concentration in vacuo of the solution provided a clear oil that was chromatographed twice on a 4 × 10 cm silica gel column using 5→20% EtOAc/hexane as the eluent. Final yield was 4.02 g (59%) of **10a** as a clear oil that was used immediately in the next reaction. $[\alpha]_D$ 42.3 (*c* 0.62, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.79 (d, 3 H, J = 6.0 Hz), 0.89 (d, 3 H, J = 6.0 Hz), 1.40 (s, 9 H), 1.48−1.61 (m, 2 H), 2.31−2.35 (m, 1 H), 2.88 (d, 1 H, J = 18.3 Hz), 3.63−3.72 (m, 1 H), 3.75 (s, 3 H), 5.78 (s, 1 H), 9.62 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.2, 23.6, 23.8, 28.2, 43.9, 49.7, 52.8, 59.3, 79.6, 153.9, 173.6, 199.44.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-α-formylmethyl-norleucine Methyl Ester(10b). α-Allyl amino acid **9b** (2.74 g, 9.60 mmol) was oxidatively cleaved by the same procedure used for **10a**. The product was obtained as a clear oil (0.7 g, 25%) after chromatographic purification on a 4 × 10 cm silica gel column with 10% EtOAc/hexane as the eluent. $[\alpha]_D$ 4.5 (*c* 1.47, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H, J = 7.2 Hz), 1.00−1.09 (m, 1 H), 1.18−1.30 (m, 3 H), 1.39 (s, 9 H), 1.59−1.69 (m, 1 H), 2.16−2.25 (m, 1 H), 2.93 (d, 1 H, J = 17.1 Hz), 3.50 (d, 1 H, J = 16.8 Hz), 3.73 (s, 3 H), 5.56 (s, 1 H), 9.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.9, 26.1, 28.8, 36.5, 49.5, 53.3, 60.6, 80.4, 154.7, 173.7, 199.8.

(R)-*N*-(*tert*-butoxycarbonyl)-α-formylmethyl-phenylalanine Methyl Ester (10c). α-Allyl amino acid **9c** (1.03 g, 3.22 mmol) was oxidatively cleaved by the same procedure used for **10a**. The desired product was obtained as a clear oil (0.83 g, 80%) that was used immediately in the next reaction. $[\alpha]_D$ −4.2 (*c* 1.24, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 2.97 (d, 1 H, J = 9.2 Hz), 3.06 (d, 1 H, J = 9.2 Hz), 3.61 (d, 1 H, J = 13.5 Hz), 3.73 (s, 3 H), 3.78−3.87 (m, 1 H), 5.57 (s, 1 H), 6.99−7.28 (m, 5 H), 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.0, 41.1, 48.4, 52.4, 60.6, 79.4, 127.0, 128.0, 129.5, 134.6, 153.9, 171.9, 198.8.

Methyl 3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-3-(2-methylpropyl)-2-oxo-1-pyrrolidineacetate (12a). Aldehyde **10a** (2.42 g, 8.4 mmol) was dissolved in 34 mL of dry MeOH, and the solution was stirred at room temperature under nitrogen. Glycine methyl ester hydrochloride (1.06 g, 8.4 mmol), sodium acetate (2.07 g, 25.6 mmol), and 9 g of 4 Å molecular sieves were added, and the reaction was stirred at room temperature for 1.5 h. Sodium cyanoborohydride (1.06 g, 16.8 mmol) was added, and the reaction was stirred for 2 days. The reaction was acidified with 10% citric acid solution to pH 4 and then made basic (pH 10) by the addition of saturated $NaHCO_3$ solution. The aqueous solution was extracted 6 × with EtOAc. The organic layer was washed 2× with saturated NaCl solution, then dried ($MgSO_4$), filtered, and concentrated in vacuo to a yellow oil. This oil was dissolved in toluene and heated at reflux for 2 days. The solvent was removed, and the yellow oil was chromatographed on a 3 × 45 cm silica gel column using 25% EtOAc/hexanes as the eluent to give 1.09 g (39%) of **12a** as a clear oil. $[\alpha]_D$ −24.9 (*c* 1.10, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.79 (d, 3 H, J = 7.5 Hz), 0.84 (d, 3 H, J = 6.3 Hz), 1.30 (s, 9 H), 1.46−1.52 (m, 1 H), 1.65−1.76 (m, 2 H), 2.26−2.33 (m, 2 H), 3.20 (dt, 1 H, J = 2.5, 8.6 Hz), 3.32−3.41 (m, 1 H), 3.60 (s, 3 H), 3.66 (d, 1 H, J = 17.1 Hz), 4.24 (d, 1 H, J = 17.1 Hz), 5.05 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.8, 24.1, 24.7, 28.4, 32.7, 43.1, 44.3, 44.7, 52.2, 60.1, 79.4, 154.6, 168.8, 174.9. FAB MS (glycerol matrix) *m/z*: 329 $[M + H]^+$. Anal. ($C_{16}H_{28}N_2O_5$) C, H, N.

Methyl 3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-3-butyl-2-oxo-1-pyrrolidineacetate (12b). Aldehyde **10b** (0.70 g, 2.43 mmol) was converted to lactam **12b** by the same method as that described above for **12a**. The product was obtained as a clear oil (0.31 g, 39%) after chromatography on a 3 × 45 cm silica gel column using 10% EtOAc/hexanes followed by 60% EtOAc/hexanes as the eluents. $[\alpha]_D$ −40.9 (*c* 0.71, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.5 Hz), 1.20−1.33 (m, 4 H), 1.37 (s, 9 H), 1.58−1.81 (m, 2 H), 2.25−2.32 (m, 1 H), 2.39−2.50 (m, 1 H), 3.22−3.31 (m, 1 H), 3.46−3.78 (m, 1 H), 3.67 (s, 3 H), 3.67−3.73 (buried d, 1 H), 4.34 (d, 1 H, J = 16.8 Hz), 5.03 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 23.0, 25.5, 28.5, 31.1, 35.8, 44.4, 44.7, 52.3, 60.2, 79.6, 154.7, 169.0, 174.9. FAB MS (glycerol matrix) *m*/*z*: 329 $[M + H]^+$. Anal. ($C_{16}H_{28}N_2O_5$) C, H, N.

Methyl 3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-3-benzyl-2-oxo-1-pyrrolidineacetate (12c). Aldehyde **10c** (0.261 g, 0.8 mmol) was converted to lactam **12c** by the same method as that described above for **12a**. The product was obtained as a clear oil (0.17 g, 58%) after chromatography on a 3 × 45 cm silica gel column using 20% EtOAc/hexanes as the eluent. $[\alpha]_D$ 22.6 (*c* 0.71, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 2.42−2.50 (m, 1 H), 2.54−2.61 (m, 1 H), 2.69−2.78 (m, 1 H), 3.11 (s, 2 H), 3.20 (t, 1 H, J = 9.2 Hz), 3.71 (s, 3 H), 3.90 (d, 1 H, J = 17.1 Hz), 3.99 (d, 1 H, J = 17.1 Hz), 5.19 (s, 1 H), 7.16−7.29 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 28.2, 31.3, 41.2, 44.0, 44.5, 52.1, 60.6, 79.5, 126.9, 128.1, 130.1, 135.1, 154.5, 168.5, 174.0. FAB MS (glycerol matrix) *m*/*z*: 363 $[M + H]^+$. Anal. ($C_{19}H_{26}N_2O_5$) C, H, N.

Methyl 3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-(2-methylpropyl)-2-oxo-1-pyrrolidineacetate (13a). Lactam **12a** (0.78 g, 2.37 mmol) was treated with excess HCl (4 N in dioxane, 10 mL) overnight at room temperature. The excess HCl and dioxane were removed in vacuo, and for a total of three times the residue was dissolved in CH_2Cl_2 and the solution evaporated to dryness. The hydrochloride salt was dried under vacuum for 3 h and then dissolved in 30 mL of dry CH_2Cl_2 and 10 mL of dry DMF. HOBt·H_2O (0.39 g, 2.85 mmol) and Boc-Pro-OH (0.61 g, 2.85 mmol) were added, and the solution was cooled to −78 °C. Triethylamine (0.8 mL, 5.7 mmol) and EDC·HCl (0.55 g, 2.85 mmol) were added, and the reaction was allowed to warm to room-temperature overnight. The reaction was stirred under nitrogen at room temperature for 3 days. Solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. This solution was washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to a yellow oil. This oil was chromatographed on a 3 × 45 cm silica gel column that was eluted with 5% MeOH/EtOAc. The product was obtained in a yield of 0.95 g (94%) as a clear oil. $[\alpha]_D$ −92.3 (*c* 1.34, MeOH). ^{1}H NMR (DMSO-d_6) δ 0.85 (d, 6 H, J = 7.2 Hz), 1.33 (s, 9 H), 1.53−1.59 (m, 2 H), 1.70−1.81 (m, 4 H), 1.97−2.01 (br m, 1 H), 2.09−2.17 (m, 1 H), 2.35−2.26 (br m, 1 H), 3.14−3.39 (m, 4 H), 3.61 (s, 3 H), 3.80 (d, 1 H, J = 17.1 Hz), 4.10−4.13 (m, 1 H), 4.20 (d, 1 H, J = 18.3 Hz), 7.42 (s, 1 H); ^{13}C NMR (DMSO−d_6) δ 23.5, 23.8, 24.2, 24.8, 28.5, 30.4, 31.2, 44.1, 44.2, 44.4, 47.0, 52.3, 59.4, 60.1, 78.8, 153.7, 169.5, 172.4, 173.8. FAB MS (glycerol matrix) *m*/*z*: 426 $[M + H]^+$. Anal. ($C_{21}H_{35}N_3O_6$) C, H, N.

Methyl 3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-butyl-2-oxo-1-pyrrolidineacetate (13b). Lactam **12b** (0.29 g, 0.88 mmol) was converted to the title compound by the same procedures that were used above for **13a**. The material was obtained as a clear oil in a yield of 0.37 g (98%). $[\alpha]_D$ −91.2 (*c* 1.22, MeOH). ^{1}H NMR (DMSO-d_6) δ 0.78 (t, 3 H, J = 7.4 Hz), 1.16−1.27 (m, 4 H), 1.36 (s, 9 H), 1.57−1.90 (m, 5 H), 2.07−2.14 (br m, 1 H), 2.23−2.39 (m, 2 H), 3.23−3.42 (m, 4 H), 3.61 (s, 3 H), 3.61−3.67 (buried d, 1 H), 4.09−4.15 (br m, 1 H), 4.29 (d, 1 H, J = 17.1 Hz), 7.85 (s, 1 H); ^{13}C NMR (DMSO-d_6)) δ 14.3, 23.0, 23.5, 25.0, 28.5, 29.2, 31.3, 36.0, 44.1, 44.4, 47.0, 52.3, 59.1, 60.1, 78.7, 153.6, 169.6, 172.6, 173.9. FAB MS (glycerol matrix) *m*/*z*: 426 $[M + H]^+$. Anal. ($C_{21}H_{35}N_3O_6$) C, H, N.

Methyl 3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-benzyl-2-oxo-1-pyrrolidineacetate (13c). Lactam **12c** (0.84 g, 2.32 mmol) was converted to the title compound by the same procedures that were used above for **13a**. The material was obtained as a clear oil (0.97 g, 91%) after chromatography on a 3 × 45 cm silica gel column that was eluted with 10% MeOH/EtOAc. $[\alpha]_D$ 37.7 (*c* 1.01, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9 H), 1.82−1.86 (m, 2 H), 2.00−2.05 (br m, 1 H), 2.16−2.20 (br m, 1 H), 2.36−2.47 (m, 1 H), 2.62−2.69 (m, 1 H), 2.74−2.82 (m, 1 H), 3.09−3.26 (m, 3 H), 3.38−3.42 (m, 2 H), 3.72 (s, 3 H), 3.98 (s, 2 H), 4.20−4.27 (br m, 1 H), 7.18−7.32 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 23.1, 28.0, 28.1, 30.8, 40.9, 43.6, 44.1, 46.5, 51.9, 59.0, 60.6, 78.4, 126.9, 128.1, 130.1, 135.1, 153.2, 168.8, 172.2, 173.1. FAB MS (glycerol matrix) *m*/*z*: 460 $[M + H]^+$. Anal. ($C_{24}H_{33}N_3O_6$) C, H, N.

3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-(2-methylpropyl)-2-oxo-1-pyrrolidineacetamide (14a). Methyl ester **13a** (0.94 g, 2.21 mmol) was placed in 50 mL of a concentrated solution of methanolic ammonia overnight. Solvent was removed in vacuo, and for a total of three times the oily residue was dissolved in CH_2Cl_2 and then evaporated to dryness. The oil was chromatographed on a 3 cm × 45 cm silica gel column with 10% MeOH/EtOAc as the eluting solvent to yield a white solid. Crystallizaton from EtOAc/hexanes afforded 0.46 g (51%) of product. mp 171−172 °C; $[\alpha]_D$ −35.0 (*c* 0.98, MeOH). ^{1}H NMR (DMSO-d_6, rotamers present) δ 0.82 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 7.0 Hz), 1.30 and 1.34 (s, 9 H), 1.51−1.61 (m, 2 H), 1.70−1.75 (m, 4 H), 2.05−2.07 (m, 1 H), 2.14−2.18 (m, 1 H), 2.29−2.35 (m, 1 H), 3.21−3.54 (m, 4 H), 3.53 (d, 1 H, J = 17.0 Hz), 3.87 (d, 1 H, J = 16.5 Hz), 4.10−4.15 (m, 1 H), 7.18 (s, 1 H), 7.28 (s, 1 H), 7.94 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 23.0, 23.2, 23.3, 24.6, 28.0, 29.3, 30.8, 44.0, 44.4, 45.9, 46.6, 58.7, 59.7, 78.4, 153.2, 169.5, 172.8, 173.0. FAB MS (glycerol matrix) *m*/*z*: 411 $[M + H]^+$. Anal. ($C_{20}H_{34}N_4O_5$) C, H, N.

Methyl 3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-butyl-2-oxo-1-pyrrolidineacetamide (14b). Methyl ester **13b** (0.28 g, 0.66 mmol) was converted to the amide by the same procedure as that used to make **14a**. Crystallizaton from EtOAc/hexanes afforded 0.19 g (70%) of product. mp 174−175 °C; $[\alpha]_D$ −35.2 (*c* 0.84, MeOH). ^{1}H NMR (DMSO-d_6, rotamers present) δ 0.86 (t, 3 H, J = 7.5 Hz), 1.16−1.30 (m, 4 H), 1.33 (s, 9 H), 1.54−1.58 (m, 2 H), 1.68−1.77 (m, 3 H), 2.05−2.11 (m, 2 H), 2.27−2.30 (m, 1 H), 3.23−3.33 (m, 4 H), 3.53 (d, 1 H, J = 17.0 Hz), 3.87 (d, 1 H, J = 17.0 Hz), 4.09−4.11 (m, 1 H), 7.21 (s, 1 H), 7.30 (s, 1 H), 8.15 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 13.8, 22.5, 23.1, 24.5, 28.0, 28.6, 30.8, 35.9, 43.9, 45.8, 46.4, 58.6, 59.8, 78.4, 153.1, 169.6, 172.9, 173.0. FAB MS (glycerol matrix) *m*/*z*: 411 $[M + H]^+$. Anal. ($C_{20}H_{34}N_4O_5$) C, H, N.

Methyl 3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2*(S)*-pyrrolidinyl]carbonyl]amino]-3-benzyl-2-oxo-1-pyrrolidineacetamide (14c). Methyl ester **13c** (0.50 g, 1.09 mmol) was converted to the amide by the same procedure as that used to make **14a**. Crystallizaton from EtOAc/hexanes afforded 0.30 g (62%) of product. mp 239−240 °C; $[\alpha]_D$ 20.2 (*c* 0.91, MeOH). ^{1}H NMR (DMSO-d_6, rotamers present) δ 1.33 and 1.40 (s, 9 H), 1.65−1.85 (m, 3 H), 2.10−2.38 (m, 4 H), 2.88−2.97 (m, 2 H), 3.06−3.15 (m, 2 H), 3.29−3.34 (m, 2 H), 3.90 (d, 1 H, J = 16.8 Hz), 4.13−4.16 (m, 1 H), 7.18−7.29 (m, 7 H), 8.33 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 23.1, 28.0, 28.1, 30.9, 41.3, 43.8, 45.8, 46.6, 58.9, 60.8, 78.6, 127.1, 128.1, 130.1, 134.6, 159.2, 169.4, 172.6, 172.9. FAB MS (glycerol matrix) *m*/*z*: 445 $[M + H]^+$. Anal. ($C_{23}H_{32}N_4O_5$) C, H, N.

3(*R*)-[[[2*(S)*-Pyrrolidinyl]carbonyl]amino]-3-(2-methylpropyl)-2-oxo-1-pyrrolidineacetamide Trifluoroacetate (3·CF_3CO_2H). Protected lactam **14a** (93.7 mg, 0.23 mmol) was dissolved in 20 mL of dry CH_2Cl_2 and 349 μL TFA (4.56 mmol) was added to this solution. The solution was stirred at room-temperature overnight, and then the solvent was removed in vacuo. For three times, the residue was dissolved in CH_2Cl_2 and the solvent then evaporated. The clear oil which was obtained was chromatographed on a 1 × 20 cm silica gel

column eluting with 10% MeOH/$CHCl_3$ to give a white solid. The solid was dissolved in H_2O, and then the solution was lyophilized to give 52.8 mg (55%) of product as a hygroscopic white solid. The product was shown to be pure by analytical HPLC analysis on a LiChrosorb RP-18 4.6 × 250 mm analytical column using 40% H_2O/CH_3CN with 0.1% TFA as the eluting solvent: t_R 3.0 min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH and 0.1% TFA gave a t_R 3.7 min. $[\alpha]_D$ 14.2 (*c* 0.76, MeOH). ^{1}H NMR (D_2O) δ 0.60 (d, 3 H, J = 6.5 Hz), 0.68 (d, 3 H, J = 6.5 Hz), 1.40 (dd, 1 H, J = 7.5, 13.0 Hz), 1.48−1.55 (m, 2 H), 1.74−1.83 (m, 3 H), 2.07−2.11 (m, 1 H), 2.15−2.23 (m, 2 H), 3.09−3.18 (m, 2 H), 3.25−3.37 (m, 2 H), 3.59 (d, 1 H, J = 17.0 Hz), 3.93 (d, 1 H, J = 17.5 Hz), 4.10 (t, 1 H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 22.2, 22.9, 23.5, 23.9, 29.4, 29.7, 44.0, 45.0, 45.8, 46.3, 59.3, 61.5, 168.4, 172.3, 175.6. FAB HRMS (*m*-nitrobenzyl alcohol matrix) *m*/*z*: 311.2090 ($C_{15}H_{26}N_4O_3$ + H^+ requires 311.2084).

3(*R*)-[[[2(*S*)-Pyrrolidinyl]carbonyl]amino]-3-butyl-2-oxo-1-pyrrolidineacetamide Trifluoroacetate (4·CF_3CO_2H). The *tert*-butoxycarbonyl group of **14b** (33.7 mg, 0.082 mmol) was removed by the same procedure as that described above for **3**. A yield of 26.2 mg (75%) was obtained. The product was shown to be pure by analytical HPLC analysis on a LiChrosorb RP-18 4.6 × 250 mm analytical column using 40% H_2O/CH_3CN with 0.1% TFA as the eluting solvent: t_R 3.0 min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH and 0.1% TFA gave a t_R 3.8 min. $[\alpha]_D$ 3.0 (*c* 0.57, MeOH). ^{1}H NMR (D_2O) δ 0.67 (t, 3 H, J = 7.5 Hz), 0.99−1.05 (m, 1 H), 1.08−1.21 (m, 3 H), 1.54 (dt, 1 H, J = 4.5, 12.5 Hz), 1.61 (dt, 1 H, J = 4.5, 12.4 Hz), 1.80−1.92 (m, 3 H), 2.11 (ddd, 1 H, J = 2.5, 8.5, 13.5 Hz), 2.22−2.28 (m, 2 H), 3.14−3.24 (m, 2 H), 3.30 (dd, 1 H, J = 8.5, 18.5 Hz), 3.39 (dt, 1 H, J = 2.0, 10.0 Hz), 3.69 (d, 1 H, J = 17.0 Hz), 3.96 (d, 1 H, J = 17.5 Hz), 4.15 (dd, 1 H, J = 7.0, 8.5 Hz); ^{13}C NMR (D_2O) δ 13.0, 22.1, 23.6, 24.3, 29.0, 29.9, 35.6, 45.1, 45.8, 46.4, 59.4, 61.8, 168.7, 172.4, 175.7. FAB HRMS (*m*-nitrobenzyl alcohol matrix) *m*/*z*: 311.2092 ($C_{15}H_{26}N_4O_3$ + H^+ requires 311.2084).

3(*R*)-[[[2(*S*)-Pyrrolidinyl]carbonyl]amino]-3-benzyl-2-oxo-1-pyrrolidineacetamide Trifluoroacetate (5·CF_3CO_2H). The *tert*-butoxycarbonyl group of **14c** (47.2 mg, 0.11 mmol) was removed by the same procedure as that described above for **3**. A yield of 27.6 mg (57%) was obtained. The product was shown to be pure by analytical HPLC analysis on a LiChrosorb RP-18 4.6 × 250 mm analytical column using 40% H_2O/CH_3CN with 0.1% TFA as the eluting solvent: t_R 3.3 min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH and 0.1% TFA gave a t_R 3.2 min. $[\alpha]_D$ 9.3 (*c* 0.45, MeOH). ^{1}H NMR (D_2O) δ 1.79−1.96 (m, 4 H), 2.20−2.28 (m, 3 H), 2.82 (d, 1 H, J = 13.5 Hz), 2.88 (d, 1 H, J = 13.5 Hz), 2.96−3.00 (m, 1 H), 3.12 (d, 1 H, J = 17.2), 3.13−3.20 (m, 2 H), 3.83 (d, 1 H, J = 17.0 Hz), 4.17 (t, 1 H, J = 7.5 Hz), 7.01−7.13 (m, 5 H); ^{13}C NMR (D_2O) δ 23.6, 28.3, 29.8, 41.1, 44.6, 45.7, 46.3, 59.4, 62.5, 127.7, 128.5, 129.8, 132.9, 168.5, 172.1, 174.9. FAB HRMS (*m*-nitrobenzyl alcohol matrix) *m*/*z*: 345.1922 ($C_{18}H_{24}N_4O_3$ + H^+ requires 345.1927).

Membrane Preparation. Bovine striatal membranes were prepared essentially as described previously.[6,26] The tissues were homogenized in 10 volumes of 0.25 M sucrose in a Potter-Elvehjem homogenizer and centrifuged at 1000*g* for 10 min. The supernatant was saved, and the pellet was resuspended in 10 volumes of 0.25 M sucrose and centrifuged as described above. The two supernatants were pooled and centrifuged at 17000*g* for 20 min. The resulting pellet was resuspended in 50 mM Tris-HCL buffer containing 1 mM EDTA (pH 7.4, Tris-EDTA buffer). The concentration of protein was determined using the Bradford method,[27] and the membrane preparation was stored at −70 °C in 1 mL aliquots. On the day of use, the membrane preparation was thawed and diluted with Tris-EDTA buffer containing 5 mM $MgCl_2$, 0.1 mM dithiothreitol (DTT), 0.1 mM PMSF, 100 μg/mL bacitracin, and 5 μg/mL soybean trypsin inhibitor (assay buffer) before being used for the binding assays.

Binding Assays. The binding of [^{3}H]*N*-propylnorapomorphine (NPA) to the membrane preparation was assayed in triplicate in 1.0 mL of assay buffer containing the indicated concentrations of radioligand and PLG analogues, and 150−200 μg of membrane protein to start the reaction. Incubation of the membranes with ligands was carried out at 25 °C for 60 min in darkness. At the end of the incubation period, the bound and free ligands were separated by vacuum filtration on Whatman GF/B filters. The filters were washed with 3 × 5 mL of Tris-EDTA buffer, and the radioactivity was determined on a Beckman scintillation counter (Model 1780). The percent increase in NPA binding for compounds **2**−**5** at the concentrations of 1, 10, and 100 nM was as follows: 1 nM: **2**, 8 ± 5%; **3**, 12 ± 4%; **4**, 32 ± 11%; **5**, 26 ± 2%; 10 nM: **2**, 45 ± 3%; **3**, 9 ± 3%; **4**, 43 ± 13%; **5**, 24 ± 6%; 100 nM: **2**, 36 ± 1%; **3**, 59 ± 5%; **4**, 89 ± 13%; **5**, 116 ± 24%.

Acknowledgment. This work was supported in part by a NIH grant (NS20036) to R.L.J.

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JM020441O