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iso-Lactam and Reduced Amide Analogues of the Peptidomimetic Dopamine Receptor Modulator 3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide

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Abstract—An analogue of the highly potent γ-lactam Pro-Leu-Gly-NH_2 peptidomimetic, 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl) amino]-2-oxo-1-pyrrolidineacetamide (**2**), 4(*R*)-[[2(*S*)-pyrrolidinylcarbonyl]amino]-2-oxo-1-pyrrolidineacetamide (**3**), in which the lactam carbonyl moiety has been placed in a different position with respect to the 3-amino group was synthesized. Also, a series of analogues of **2**, compounds **4**–**6**, were synthesized in which each of the amide bonds of **2** were systematically replaced with a reduced amide bond surrogate. The analogues were tested for their ability to enhance the binding of [^{3}H]*N*-propylnorapomorphine to dopamine receptors in a functional in vitro assay utilizing bovine striatal membranes. Peptidomimetic **3** was shown to be more potent than **2**, while **4** and **5** were significantly less effective than **2**. Peptidomimetic **6** had a pharmacological profile similar to that of **2**.

Introduction

L-Prolyl-L-leucyl-glycinamide (**1**, PLG) has the unique ability to modulate D_2 dopamine receptors within the central nervous system.[1–7] This neuropharmacological activity has prompted the synthesis of numerous peptide and peptidomimetic analogues of PLG.[8–16] One of the most potent PLG peptidomimetics to be made is the γ-lactam 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (**2**).[11] The pharmacological profile of **2** resembles that of PLG except that it is 100–1000× more potent. For example, both 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.[6,17] Like PLG, **2** is able to inhibit dopamine-stimulated adenylyl cyclase activity,[18] enhance *N*-propylnorapomorphine (NPA)-stimulated low K_m GTPase activity in rat striatal membranes,[18] potentiate the contralateral rotational behavior induced by apomorphine in the rat nigrostriatal 6-hydroxydopamine lesion model of Parkinson's disease,[19,20] protect C57 BL/6 mice against MPTP-induced dopaminergic degeneration,[21] and attenuate haloperidol-induced *c-fos* and Fos expression.[22]

Analogues of **2** in which the carbonyl moieties of the peptidomimetic have been modified were designed to determine the role that these moieties might play in the activity and potency of **2**. One such analogue was the iso-γ-lactam **3** in which the lactam carbonyl group found in **2** has been shifted from the 2-position to the 5-position of the lactam ring. It was felt that this modification would maintain the ability of the γ-lactam to function as a β-turn mimic, but would orient the lactam carbonyl in a different area of space. Analogues **4**–**6** represent the systematic replacement of each of the amide bonds in **2** with a reduced amide bond surrogate.

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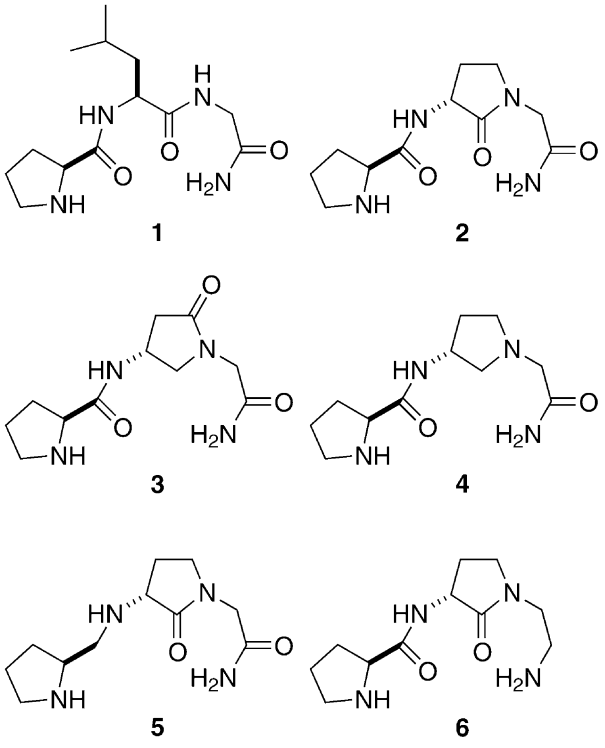
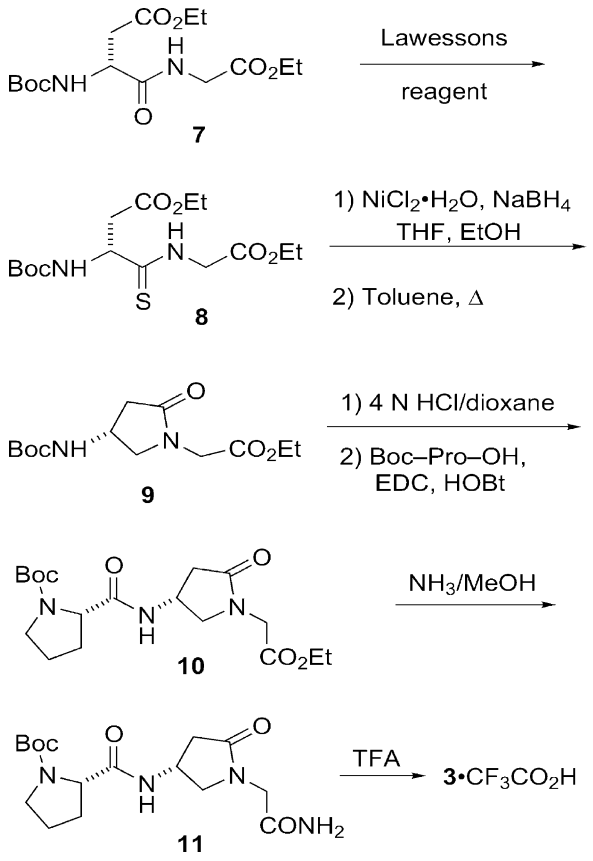


Results

Synthesis

Synthesis of the *iso*-γ-lactam **3** began with the dipeptide ethyl *N*-(*tert*-butoxycarbonyl)-β-ethyl-D-aspartyl-glycinate (**7**) as outlined in Scheme 1. Compound **7** was made by the same method as that reported for the synthesis of its enantiomer.[23] Treatment of **7** with Lawesson's reagent formed thiopeptide **8** in a 58% yield. Reductive desulfurization of **8** by the reaction of nickel borohydride, formed in situ from sodium borohydride and nickel chloride was accomplished by the procedure of Ede et al.[24] This procedure was immediately followed by a subsequent thermal closure in refluxing toluene to give iso-γ-lactam **9**. The *tert*-butoxycarbonyl group was removed from **9** and the product was coupled to Boc-L-proline to give **10**. This ester was converted to amide **11**. Trifluoroacetic acid deprotection of **11** gave the desired *iso*-γ-lactam PLG peptidomimetic **3** as its trifluoroacetate salt.



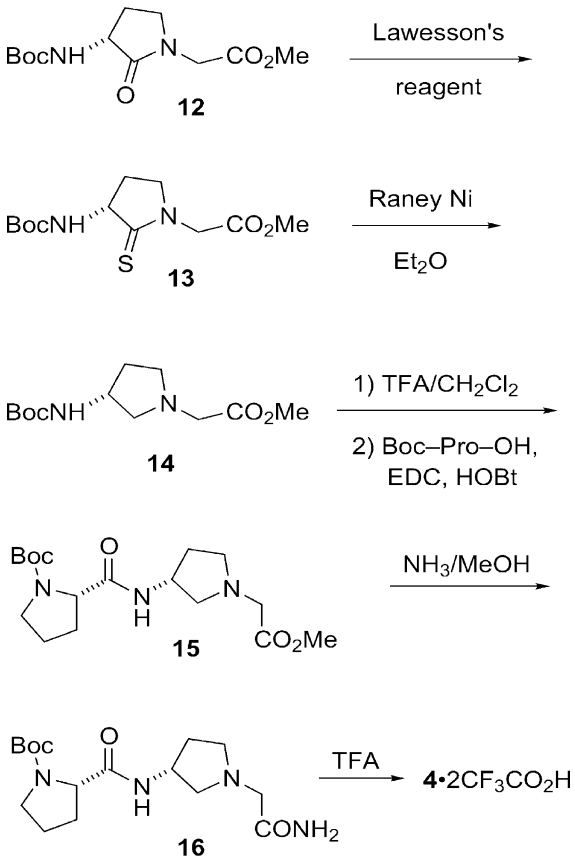
Scheme 1.

The synthesis of the amino pyrrolidinyl peptidomimetic **4** began with the known γ-lactam **12**[11] (Scheme 2). This material was converted to thiolactam **13** with Lawesson's reagent[25] in refluxing benzene. Desulfurization of **13** to give pyrrolidine **14** was accomplished overnight with Raney nickel in diethyl ether.[26] The *tert*-butoxycarbonyl group of **14** was removed with trifluoroacetic acid and the material that was obtained was coupled to Boc-L-proline using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) to give **15** in an excellent yield (86%). Interestingly, when 4 N HCl in dioxane was used instead of trifluoroacetic acid to remove the *tert*-butoxycarbonyl group from **14** the subsequent coupling reaction of the product with Boc-L-proline yielded only 22% of **15**. The difference in coupling efficiencies are suspected to be due to the differential solubility properties of the ditrifluoracetate salt versus the dihydrochloride salt in CH_2Cl_2. Aminolysis of methyl ester **15** with ammonia gave a 79% yield of amide **16**. Deprotection of **16** with trifluoroacetic acid followed by reverse phase chromatography gave peptidomimetic **4** as its ditrifluoroacetate salt.

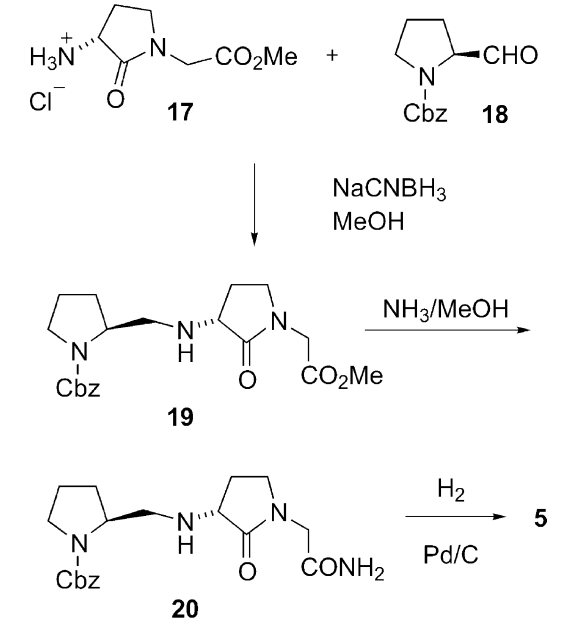
Synthesis of reduced amide peptidomimetic **5** was carried out as outlined in Scheme 3. 3-Amino-γ-lactam **17**, which was obtained through the deprotection of **12**,[11] and Cbz-L-prolinal (**18**), which was obtained through diisobutylaluminum hydride reduction of Cbz-L-Pro-OMe,[27] were reacted together in the presence of sodium cyanoborohydride to give **19**. This material was converted to primary amide **20**, which was then subjected to hydrogenolysis to give pyrrolidinyl γ-lactam **5**.

The synthesis of **6** (Scheme 4) began with the Boc-D-methionine amide **21**, which was obtained through the coupling of Boc-D-Met with mono benzyloxycarbonyl protected diaminoethane.[28] Formation of the γ-lactam **22** was accomplished by the methodology of Freidinger et al.[29,30] as modified by Yu et al.[11] Treatment of **22** with 4 N HCl in dioxane followed by EDC coupling of the product to Cbz-L-proline yielded two distinct products. Detailed 1D and 2D NMR analysis, combined with MS and elemental analysis identified these compounds to be the expected prolyl-lactam **23** and the bis-prolyl-lactam **24** in yields of 32 and 26%, respectively. It

appears that during the removal of the *tert*-butoxycarbonyl group from **22** some of the benzyloxycarbonyl protecting group also is being removed despite the fact that this protecting group is normally stable under these conditions. Hydrogenolysis of **23** in methanol with catalytic 10% palladium on carbon gave the desired peptidomimetic **6**.



Scheme 2.



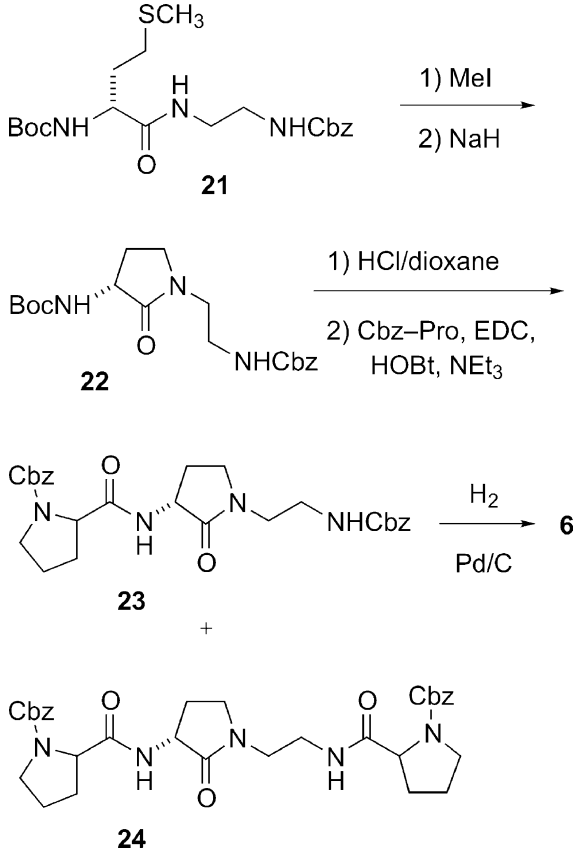
Scheme 3.

Pharmacology

iso-γ-Lactam peptidomimetic **3** and the reduced amide bond peptidomimetics **4–6** were evaluated in a functional in vitro assay utilizing bovine striatal membranes.[6,31] These analogues were tested for their ability to enhance the binding of [^{3}H]NPA to dopamine receptors at three concentrations: 1, 10, and 100 nM. The results, along with the results previously obtained for **2**,[32] are summarized in Figure 1. Peptidomimetics **3–6** all retained some ability to enhance the binding of [^{3}H]NPA to dopamine receptors isolated from bovine striatal membranes when compared to control preparations.

Peptidomimetics **2** and **3** at a concentration of 100 nM showed similar ability to enhance [^{3}H]NPA binding to dopamine receptors. At a concentration of 10 nM, **2** was more active than **3**, but at 1 nM **3** became significantly more active than **2**. Because of its enhanced activity at the 1 nM concentration, **3** also was evaluated at a concentration of 0.1 nM (data not shown). The results were that in going from 1 to 0.1 nM the percentage enhancement of [^{3}H]NPA binding dropped from 37 to 7%. Thus, the maximum effect of **3** occurred at 1 nM.

Like **2**, compounds **4** and **5** showed very low activity at a concentration of 0.1 nM. At concentrations of 10 nM and 100 nM, the activities of **4** and **5** were significantly lower than that observed for **2**. Peptidomimetic **6** showed an activity profile similar to that of **2**.



Scheme 4.

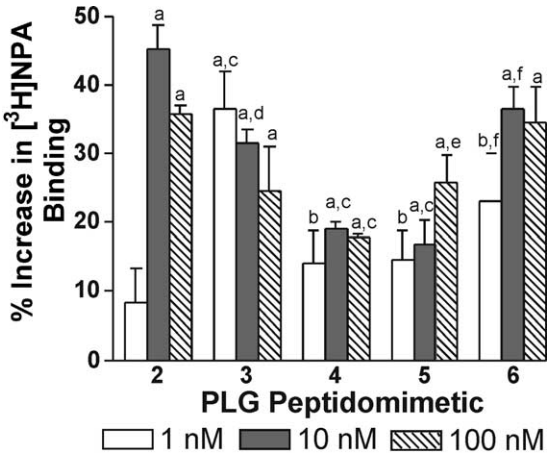


Figure 1. Stimulation of [^{3}H]NPA binding to bovine striatal membranes by the PLG peptidomimetics **2**–**6**. Data represent the percent increase in specific [^{3}H]NPA binding over the control value (the binding of [^{3}H]NPA in the absence of any PLG peptidomimetic) when the indicated concentration of peptidomimetic was added directly to the assay buffer. Data for **2** are from ref 32. Results are the means ± SEM of 3–4 separate experiments carried out in triplicate. [a]Significantly different ($p < 0.01$) from control value. [b]Significantly different ($p < 0.001$) from control value. [c]Significantly different ($p < 0.0001$) from the corresponding concentration of **2**. [d]Significantly different ($p < 0.001$) from the corresponding concentration of **2**. [e]Significantly different ($p < 0.01$) from the corresponding concentration of **2**. [f]Significantly different ($p < 0.05$) from the corresponding concentration of **2**.

Discussion

It was anticipated that the *iso*-γ-lactam constraint of peptidomimetic **3** would possess the potential to serve as a β-turn mimic in the same manner as the γ-lactam constraint in **2** does,[33] since previous studies with human growth hormone fragments that incorporated *iso*-γ-lactam and γ-lactam residues that are enantiomeric to those of this study showed the ability of both residues to assume a β-turn.[23,34] Thus, the difference between the *iso*-γ-lactam and γ-lactam constraints would be the position in space in which the carbonyl would be projected. The significant activity seen with **3** suggests that the lactam carbonyl in **2** is not an absolute requirement for the activity seen with **2**. Although at a concentartion of 10 nM **2** produced a greater enhancement of [^{3}H]NPA binding to dopamine receptors than **3**, the effect of **3** at 1 nM was significantly greater than that of **2** at 1 nM. The drop in the activity of **3** when going from 1 to 0.1 nM indicates that the maximal enhancement of [^{3}H]NPA binding for this peptidomimetic occurs at 1 nM, a concentration that is ten times lower than the concentration at which the maximum effect for **2** is seen.

The reduced amide bond analogues **4** and **5** are analogous to the reduced amide bond PLG analogues that have been synthesized previously: Pro-Leuψ[$NHCH_2$]Gly-NH_2 (**25**) and Proψ[$NHCH_2$]Leu-Gly-NH_2 (**26**), respectively.[35] The significant loss of activity seen with **4** could be due to the fact that in going from **2** to **4** the amide nitrogen atom in the lactam ring becomes an amine that would be protonated to a significant extent at physiological pH. Protonation could have an adverse impact on the binding of the PLG peptidomimetic to its recognition site. However, the fact that **25** previously had been shown to potentiate the effects of L-dopa in rats[35] would seem to argue against this possibility. Alternatively, the reduced activity seen with **4** may be the result of changes in conformation that are allowed by the more flexible pyrrolidinyl ring as compared to the lactam ring.

The decrease in the activity seen with **5** is consistent with the loss of activity seen with **26**, the corresponding reduced amide bond analogue of PLG. An NMR study on **26** showed that the compound did not exist in a β-turn because of the lack of the prolyl carbonyl needed to form the 10-membered hydrogen bonded system.[35] The reduced amide bond that has been incorporated in **5** also eliminates the carbonyl group that may help stabilize, through a 10-membered hydrogen bond, the postulated β-turn bioactive conformation of **2**.[11,33]

In previous structure–activity studies on PLG, the carboxamide moiety was shown to be necessary for the dopamine receptor modulating activity of the molecule as measured in the Dopa potentiation test and the oxotremorine antagonism test.[36] It was postulated that the carboxamide group was participating in important hydrogen bonding interactions. The retention of activity seen with **6** suggests that the reduced amide bond present in this analogue can substitute for the carboxamide moiety present in **2**. This may be because the amino group of **6**, whether it is in its protonated or unprotonated form possesses the ability to participate in hydrogen bonding interactions either intramolecularly or intermolecularly as does the amide NH_2 group of **2**.

Conclusion

In summary, this study has shown that replacing the γ-lactam moiety of **2** with the *iso*-γ-lactam constraint leads to a potent modulator of dopamine receptors, peptidomimetic **3**. The study also has shown that the amide bond involving the prolyl residue and the lactam amide bond are important structural features of **2**, since replacing these with reduced amide bond surrogates result in peptidomimetics with significantly lower activity. Only the C-terminal carboxamide group of **2** can be replaced with a reduced amide bond without a significant decrease in activity.

Experimental

General aspects

Thin-layer chromatography was performed on Analtech 250 μm silica gel GHLF Uniplates and were visualized either by UV, I_2, ninhydrin spray (amines), 2,6-dichlorophenol indophenol spray (acids), or 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, AZ, USA.

***N*-(*tert*-Butoxycarbonyl)-β-ethyl-D-aspartyl-ψ[CSNH]-glycine ethyl ester (8).** *N*-(*tert*-Butoxycarbonyl)-β-ethyl-D-aspartyl-glycine ethyl ester[23] (**7**, 17.2 g, 0.05 mol) was dissolved in 300 mL dry benzene and 10.0 g of Lawesson's reagent (0.025 mol) was added. The reaction was heated at reflux for 4 h, then it was cooled and concentrated in vacuo to a yellow oil. The oil was twice chromatographed on a 4×40 cm silica gel column eluting with 20% Et_2O in CH_2Cl_2 to yield 10.5 g (58%) of **8** as a yellow oil. $[\alpha]_D$ −12.9 (*c* 1.15, $CHCl_3$) (lit[23] $[\alpha]_D$ for the enantiomer of **8** was 7.5 in $CDCl_3$). ^1H NMR ($CDCl_3$) δ 1.22–1.31 (m, 6H), 1.44 (s, 9H), 2.87 (dd, 1H, *J* = 7.2 and 17.1 Hz), 3.19 (dd, 1H, *J* = 3.6 and 17.1 Hz), 4.14 (q, 2H, *J* = 6.9 Hz), 4.24 (q, 2H, *J* = 7.2 Hz), 4.34–4.36 (m, 2H), 4.80–4.86 (m, 1H), 5.79–5.82 (br d, 1H), 8.83–8.85 (br m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.3, 28.4, 39.6, 47.4, 56.6, 61.3, 62.0, 80.8, 155.4, 168.4, 171.7, 202.7; FAB MS (glycerol matrix) *m*/*z* 363 $[M+H]^+$. Anal. calcd for $C_{15}H_{26}N_2O_6S$: C, 49.71; H, 7.23; N, 7.73; S, 8.85. Found: C, 49.51; H, 6.97; N, 8.00; S, 8.63.

Ethyl 4(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetate (9). Thiopeptide **8** (10.4 g, 0.029 mol) was dissolved in 250 mL dry THF and 250 mL 100% EtOH. The solution was cooled to 0 °C. $NiCl_2{\cdot}6H_2O$ (54.6 g, 0.23 mol) was added to the solution which then turned emerald green. $NaBH_4$ (26.0 g, 0.67 mol) was added in portions over 15 min causing the solution to turn black, bubble and expel gas. The reaction was allowed to warm to room temperature where upon it was stirred for 30 min. The reaction was filtered through a pad of silica gel in a 4×10 cm column. The black residue on the silica gel was eluted with 500 mL EtOH, 500 mL THF, 500 mL Et_2O, and 500 mL EtOAc, successively. The organic layers were combined and concentrated in vacuo to give a black residue that was dissolved in CH_2Cl_2. The solution was washed with 200 mL of saturated $NaHCO_3$ solution. An emulsion was formed that was broken up by the addition of 150 mL of saturated EDTA solution. The organic layer was washed twice with saturated EDTA solution and once with H_2O, then dried ($MgSO_4$), filtered and concentrated in vacuo to a yellow oil. This oil in 100 mL toluene was heated at reflux for 4 days after which time the solvent was removed in vacuo, and the resulting oil chromatographed on a 4×40 cm silica gel column eluting with 25% EtOAc in hexanes followed by 60% EtOAc in hexanes. The total yield of **9** was 3.39 g (41%) as a white solid. Mp: 95–96 °C (lit[24] mp for the enantiomer of **9** was 71.5–72 °C); $[\alpha]_D$ 17.3 (*c* 1.24, $CHCl_3$) (lit[24] $[\alpha]_D$ for the enantiomer of **9** was −14.3 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.18 (t, 3H, *J* = 7.5 Hz), 1.33 (s, 9H), 2.27 (dd, 1H, *J* = 5.1 and 17.1 Hz), 2.64 (dd, 1H, *J* = 8.4 and17.1 Hz), 3.32 (dd, 1H, *J* = 3.6 and 9.6 Hz), 3.67 (dd, 1H, *J* = 6.0 and 9.6 Hz), 3.85 (d, 1H, *J* = 18.3 Hz), 4.02–4.10 (br d, 1H), 4.09 (q, 2H, *J* = 7.5 Hz), 4.19–4.26 (br m, 1H), 5.57 (d, 1H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 13.8, 28.1, 37.4, 43.4, 44.2, 54.2, 61.2, 79.4, 155.2, 168.3, 173.0. FAB MS (glycerol matrix) *m*/*z* 287 $[M+H]^+$. Anal. calcd for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.74; N, 9.78. Found: C, 54.54; H, 7.59; N, 9.81.

Ethyl 4(*R*)-[[1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinyl-carbonyl]amino]-2-oxo-1-pyrrolidineacetate (10). *iso*-Lactam **9** (0.14 g, 0.49 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. The residue was dissolved in CH_2Cl_2 and this solution was evaporated to dryness. This process was repeated twice and yielded a white foam. The hydrochloride salt was dried under vacuum overnight and the salt was dissolved in 40 mL dry CH_2Cl_2 and 5 mL dry DMF. $HOBt{\cdot}H_2O$ (0.08 g, 0.58 mmol) and Boc-L-Pro-OH (0.126 g, 0.58 mmol) were added and the stirred solution chilled to −78 °C. EDC·HCl (0.112 g, 0.58 mmol) was added to the mixture followed by Et_3N (0.16 mL, 1.17 mmol) and the reaction was allowed to warm to room temperature overnight. The reaction was further stirred under nitrogen at room temperature for 7 days. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. The reaction was washed with 10% citric acid solution, 1 M $NaHCO_3$ solution, and saturated NaCl solution successively. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to a yellow oil that was chromatographed on a 2×20 cm silica gel column eluted with 5% EtOH/CH_2Cl_2 to yield 0.10 g (55%) of **10** as a clear oil. $[\alpha]_D$ −50.5 (*c* 2.08, $CHCl_3$). ^1H NMR (DMSO, rotamers present) δ 1.18 (t, 3H, *J* = 7.2 Hz), 1.31 and 1.37 (s, 9H), 1.70–1.79 (m, 3H), 2.04–2.16 (m, 1H), 2.22 (dd, 1H, *J* = 8.4 and 16.8 Hz), 2.59 (dd, 1H, *J* = 8.4 and 16.8 Hz), 3.15–3.31 (m, 3H), 3.67 (dd, 1H, *J* = 7.5 and 9.6 Hz), 3.93–4.02 (m, 3H, Pro), 4.09 (q, 2H, *J* = 7.2 Hz), 4.31–4.35 (br m, 1H), 8.27 and 8.31 (d, 1H, *J* = 6.9 Hz); ^{13}C NMR (DMSO) δ 14.5, 23.6, 28.5, 31.5, 36.8, 42.9, 43.8, 47.0, 53.9, 60.1, 61.2, 78.9, 153.7, 169.1, 172.9, 173.1; FAB MS (*m*-nitrobenzylalcohol matrix) *m*/*z* 384 $[M+H]^+$. Anal. calcd for $C_{18}H_{29}N_3O_6$: C, 56.38; H, 7.62; N, 10.96. Found: C, 56.17; H, 7.63; N, 10.72.

4(*R*)-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-pyrrolidineacetamide (11). A solution of **10** (1.13 g, 2.95 mmol) in 50 mL of a concentrated solution of methanolic ammonia was stirred overnight. Solvent was removed in vacuo to yield a white foam that was chromatographed on a 4×20 cm silica gel column with 5% MeOH in CH_2Cl_2 as the eluting solvent. The product was isolated as a white solid (0.83 g, 79%). Crystallization was achieved from EtOAc/hexanes. Mp: 122–123 °C; $[\alpha]_D$ −20.0 (*c* 0.86, MeOH). ^1H NMR (DMSO, rotamers present) δ 1.32 and 1.38 (s, 9H), 1.71–1.77 (m, 3H), 2.07–2.11 (m, 1H), 2.16 (dd, 1H, *J* = 4.0 and 16.5 Hz), 2.60 (dd, 1H, *J* = 9.0 and 16.5 Hz), 3.12–3.17 (m, 1H), 3.23–3.28 (m, 1H), 3.34–3.38 (m, 1H), 3.65 (dd, 1H, *J* = 7.5 and 9.5 Hz), 3.74 (s, 2H), 3.96 (dd, 1H, *J* = 3.5 and 8.5 Hz), 4.33–4.35 (m, 1H), 7.13 (s, 1H), 7.37 (s, 1H), 8.25 and 8.30 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (DMSO) δ 23.2, 28.1, 31.0, 36.7, 42.1, 44.7, 46.5, 53.9, 59.7, 78.4, 153.2, 169.5, 172.3, 172.5; FAB MS (glycerol matrix) *m*/*z* 355 $[M+H]^+$. Anal. calcd for

$C_{16}H_{26}N_4O_5$: C, 54.22; H, 7.39; N, 15.81. Found: C, 54.10; H, 7.29; N, 15.76.

4(*R*)-[[2(*S*)-Pyrrolidinylcarbonyl]amino]-2-oxo-1-pyrrolidineacetamide trifluoroacetate (3·CF_3CO_2H). Boc-protected isolactam **11** (66 mg, 0.19 mmol) was dissolved in 20 mL dry CH_2Cl_2 and 286 μL TFA (3.7 mmol) was added. The solution was stirred at room temperature overnight and then the solvent was removed in vacuo. For a total of 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 the solution lyophilized to give 48 mg (70%) of **3** 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/acetonitrile with 0.1% TFA as the eluting solvent: $t_R = 2.2$ min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH and 0.1% TFA gave a $t_R = 3.6$ min. $[\alpha]_D$ 3.3 (*c* 0.57, MeOH). ^{1}H NMR (D_2O) δ 1.84–1.89 (m, 3H), 2.17–2.26 (buried m, 1H), 2.25 (dd, 1H, $J = 4.2$ and 17.7 Hz), 2.74 (dd, 1H, $J = 8.4$ and 17.7 Hz), 3.15–3.25 (m, 3H), 3.69 (dd, 1H, $J = 7.2$ and 10.8 Hz), 3.81 (d, 1H, $J = 17.1$), 3.88 (d, 1H, $J = 17.1$ Hz), 4.12 (t, 1H, $J = 7.2$ Hz), 4.33–4.38 (m, 1H); ^{13}C NMR (D_2O) δ 23.6, 29.5, 36.6, 43.3, 44.8, 46.3, 54.3, 59.6, 169.1, 172.3, 175.9; FAB HRMS (glycerol matrix) *m*/*z* 255.1461 ($C_{11}H_{18}N_4O_3 + H^+$ requires 255.1458).

Methyl 3(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]-2-thioxo-1-pyrrolidineacetate (13). Lactam **12**[11] (1.0 g, 3.67 mmol) was dissolved in 100 mL dry benzene and Lawesson's reagent (0.73 g, 1.8 mmol) was added. The reaction was heated at reflux for 4 h after which it was cooled to room temperature. Concentration of the reaction in vacuo yielded a yellow oil. The oil was chromatographed on 4×30 cm silica gel column eluting with CH_2Cl_2 followed by 20% Et_2O in CH_2Cl_2 to yield 0.6 g (58%) product. Mp: 79–81 °C; $[\alpha]_D$ 58.9 (*c* 1.41, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.90–1.98 (m, 1H), 2.83–2.84 (br m, 1H), 3.68 (t, 1H, $J = 9.5$ Hz), 3.77 (s, 3H), 3.78–3.82 (m, 1H), 4.30–4.44 (br m, 1H), 4.50 (d, 1H, $J = 17.5$ Hz), 4.63 (d, 1H, $J = 17.5$ Hz), 5.49 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.4, 30.0, 49.4, 52.2, 52.6, 61.7, 79.9, 155.6, 167.2, 201.9; FAB MS (*m*-nitrobenzyl-alcohol matrix) *m*/*z* 288 $[M]^+$ and 287 $[M\text{-}H]^-$.

Methyl 3(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]-1-pyrrolidineacetate (14). Thiolactam **13** (1.9 g, 6.5 mmol) was dissolved in 50 mL anhydrous Et_2O. Raney nickel (50% in H_2O, 10 g) was washed with 100% EtOH, and twice with Et_2O, carefully decanting the solution away from the catalyst each time. Et_2O (50 mL) was added to the Raney nickel and this slurry was added to the solution of **13**. The solution was vigorously stirred overnight upon which time the solvent was decanted away and the Raney nickel was washed with Et_2O. The organic layers were combined and filtered through a pad of wet Celite which was then washed with Et_2O. Concentration of the filtrate in vacuo yielded a clear oil that solidified to a white solid. Silica gel chromatography using a 4×40 cm column and eluting with 10% hexanes in EtOAc gave 1.36 g (80%) of product. mp: 59–60 °C; $[\alpha]_D$ 10.9 (*c* 0.51, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.66–1.67 (m, 1H), 2.26–2.30 (m, 1H), 2.47–2.48 (m, 1H), 2.69–2.71 (m, 1H), 2.76–2.79 (m, 1H), 2.96–2.97 (m, 1H), 3.35 (s, 2H), 3.73 (s, 3H), 4.20 (br m, 1H), 4.95 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.3, 32.7, 49.9, 51.6, 52.2, 55.8, 60.3, 79.2, 155.3, 170.8; FAB MS (glycerol matrix) *m*/*z* 259 $[M + H]^+$. Anal. calcd for $C_{12}H_{22}N_2O_4$: C, 55.80; H, 8.58; N, 10.84. Found: C, 56.00; H, 8.33; N, 10.59.

Methyl 3(*R*)-[[1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl]amino]-1-pyrrolidineacetate (15). Pyrrolidine **14** (1.26 g, 4.9 mmol) was dissolved in 100 mL dry CH_2Cl_2 and 5 mL TFA (65.3 mmol) was added to the solution. The solution was stirred under N_2 at room temperature for 5 h and then the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 and the solution was evaporated. This was done for a total of 4× to ultimately yield to a white solid. This solid was dried under vacuum overnight and then dissolved in 60 mL dry CH_2Cl_2 and 30 mL dry DMF. HOBt·HO (0.79 g, 5.8 mmol) and Boc-L-Pro-OH (1.26 g, 5.8 mmol) were added and the solution was cooled to −78 °C. EDC·HCl (1.12 g, 5.8 mmol) was added followed by 1.6 mL Et_3N (11.7 mmol). The reaction and workup was the same as that used in the synthesis of **10**. The oil obtained was chromatographed on a 4×40 cm silica gel column eluting with 5% MeOH/EtOAc to yield 1.49 g (86%) of **15** as a golden oil. A small sample was further purified on a 2×20 cm silica gel column eluting with 20% hexanes in EtOAc and 0.1% NH_4OH to yield analytically pure product. $[\alpha]_D$ −36.2 (*c* 1.62, MeOH). ^{1}H NMR (DMSO, rotamers present) δ 1.42 (s, 9H), 1.63–1.70 (m, 1H), 1.83–1.93 (m, 2H), 2.06 and 2.14 (br s, 2H), 2.19–2.26 (m, 1H), 2.52–2.57 (br m, 1H), 2.62–2.68 (br m, 1H), 2.82–2.90 (br m, 2H), 3.35 (s, 2H), 3.40–3.45 (br m, 2H), 3.70 (s, 3H), 4.15 and 4.21 (br s, 1H), 4.40–4.50 (br m, 1H), 6.78 and 7.27 (s, 1H); ^{13}C NMR (DMSO, 60 °C) δ 23.0, 28.0, 30.8, 31.2, 46.4, 48.0, 51.0, 51.6, 55.2, 58.9, 59.4, 78.2, 153.3, 170.6, 172.1; FAB MS (*m*-nitrobenzyl-alcohol matrix) *m*/*z* 356 $[M + H]^+$. Anal. calcd for $C_{17}H_{29}N_3O_5$: C, 57.45; H, 8.22; N, 11.82. Found: C, 57.19; H, 8.03; N, 12.05.

3(*R*)-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl]amino]-1-pyrrolidineacetamide (16). A solution of **15** (1.07 g, 3.0 mmol) in 40 mL concentrated methanolic ammonia was stirred for 2 days. The solvent was removed in vacuo and the oily residue that was obtained was dissolved in CH_2Cl_2 and then the solvent was removed. This procedure was carried out 3×. The foam that was obtained was chromatographed on a 2×30 cm silica gel column eluting with 10% MeOH in EtOAc to yield 0.86 g (84%) of product. An analytical sample was obtained through purification on a 1×20 cm silica gel column eluting with 10% MeOH in EtOAc and 0.1% NH_4OH. $[\alpha]_D$ −21.6 (*c* 3.46, MeOH). ^{1}H NMR ($CDCl_3$, 50°C) δ 1.44 (s, 9H), 1.60–1.67 (m, 1H), 1.85–1.95 (m, 3H), 2.23–2.27 (m, 2H), 2.48–2.52 (m, 1H), 2.58–2.61 (m, 1H), 2.70–2.80 (m, 1H), 2.88–2.95 (m, 1H), 3.14 (s, 2H), 3.34–3.40 (m, 2H), 4.16–4.23 (m, 1H), 4.35–4.45 (m, 1H), 5.77 (s, 1H), 6.91 (s, 1H); ^{13}C NMR ($CDCl_3$) δ

24.5, 28.4, 31.2, 32.5, 47.2, 48.8, 53.0, 58.4, 59.8, 60.7, 80.5, 156.7, 171.4, 173.3; FAB MS (*m*-nitrobenzylalcohol matrix) *m/z* 341 $[M+H]^+$. Anal. calcd for $C_{16}H_{28}N_4O_4 \cdot 0.5H_2O$: C, 55.00; H, 8.36; N, 16.03. Found: C, 55.22; H, 8.11; N, 16.09.

3(*R*)-[[2(*S*)-Pyrrolidinylcarbonyl]amino]-1-pyrrolidineacetamide ditrifluoroacetate (4·2CF_3CO_2H). Compound **16** (212 mg, 0.62 mmol) was deprotected in the same manner as that described for the synthesis of **3**. Purification on a 1×5 cm C-8 reverse-phase column eluting with 10% MeOH/acetonitrile and a 1×10 cm silica gel column eluting with 10% MeOH/$CHCl_3$ gave an off-white solid. The solid was dissolved in H_2O and lyophilized to give 68.4 mg (23%) of analytically pure **4** as a hygroscopic off-white solid. The product was shown to be pure by analytical HPLC analysis on a Waters Spherisorb® 4.6×150 mm analytical column. Product was eluted with 25% MeOH/$CHCl_3$ and 0.1% NH_4OH and had a t_R of 7.2 min. Further HPLC analysis on a LiChrosorb® RP-18 4.6×250 mm analytical column with 40% H_2O/acetonitrile and 0.1% TFA gave a t_R of 2.8 min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH and 0.1% TFA gave a t_R of 3.5 min. $[\alpha]_D$ −26.0 (*c* 0.50, MeOH). ^1H NMR (D_2O) δ 1.66–1.72 (m, 1H), 1.86–1.92 (m, 3H), 2.18–2.30 (m, 2H), 2.74–2.81 (m, 2H), 2.97–3.02 (m, 1H), 3.05 (dd, 1H, *J* = 7.0 and 10.5 Hz), 3.18–3.29 (m, 2H), 3.34 (d, 1H, *J* = 15.5 Hz), 3.45 (d, 1H, *J* = 15.5 Hz), 4.14–4.17 (m, 1H), 4.22–4.27 (m, 1H); ^{13}C NMR (D_2O) δ 23.7, 29.6, 30.1, 46.4, 49.0, 52.8, 56.5, 58.7, 59.6, 169.2, 172.7; CI HRMS *m/z* 241.1664 ($C_{11}H_{20}N_4O_2+H^+$ requires 241.1665).

Methyl 3(*R*)-[[1-(benzyloxycarbonyl)-2(*S*)-pyrrolidinylmethyl]amino]-2-oxo-1-pyrrolidineacetate (19). Cbz-Pro-OMe[27] (2.6 g, 9.9 mmol) was dissolved in 100 mL dry toluene and this solution was cooled to −78 °C. DIBAL-H (13.4 mL of 1.5 M toluene solution, 20.1 mmol) was added dropwise to this solution via an addition funnel. The chilled solution was stirred under N_2 for 3 h and then MeOH (30 mL) was added dropwise to quench the reaction. This was followed by 80 mL 1 N HCl. The reaction was allowed to warm to room temperature whereupon the layers were separated. The aqueous layer was extracted 4× with EtOAc and the organic layers were combined and washed with saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo (under 40 °C) to give 2(*S*)-*N*-(benzyloxycarbonyl)-pyrrolidine-2-carboxaldehyde (**18**, 2.2 g, 95%) that was used directly without purification in the next step.

Lactam **12**[11] (1.1 g, 4.0 mmol) was dissolved in 10 mL 4 N HCl/dioxane and the solution was stirred overnight. The solvent was removed in vacuo and the residue that was obtained was dissolved in CH_2Cl_2 and the solution stripped of solvent. This addition and evaporation of CH_2Cl_2 was done an additional 3×. The white solid of the deprotected lactam **17** that was obtained was dried under high vacuum overnight. Aldehyde **18** (2.2 g, 9.4 mmol) was dissolved in 50 mL dry MeOH and this solution was added to the flask containing deprotected lactam **17**. Molecular sieves (4 Å, 10 g) were added and the reaction was stirred at room temperature, under nitrogen, for 4 h. $NaCNBH_3$ (0.76 g, 12.1 mmol) was added in one portion and the reaction was stirred under N_2 for 6 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 4× with EtOAc. The organic layer was washed with H_2O and saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated in vacuo to give a yellow oil. This oil was chromatographed on a 2×40 cm silica gel column eluting with 10% MeOH in hexanes and 0.1% NH_4OH to yield 0.86 g (55%) of **19** as a clear oil. $[\alpha]_D$ −33.7 (*c* 2.11, MeOH). ^1H NMR ($CDCl_3$, rotamers present) δ 1.65–1.69 (m, 1H), 1.81–1.97 (m, 3H), 2.09–2.12 (m, 1H), 2.34–2.37 (m, 1H), 2.50 and 2.59 (t, 1H, *J* = 10.0 Hz), 2.87 and 3.02 (d, 1H, *J* = 8.5 Hz), 3.20–3.45 (m, 4H), 3.54–3.57 (t, 1H, *J* = 7.5 Hz), 3.72 (s, 3H), 3.87–3.97 (m, 2H), 4.19 (dd, 1H, *J* = 8.0 and 17.0 Hz), 5.07–5.19 (m, 2H), 7.27–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, rotamers present) δ 22.9 (23.8), 26.8, 28.9 (29.5), 44.2, 44.9, 46.7 (46.9), 49.6 (50.0), 52.2, 57.0 (57.6), 57.9, 66.6 (66.9), 127.8, 127.9, 128.0, 128.3, 128.5, 137.0, 155.1 (155.2), 169.0, 174.9; FAB MS (*m*-nitrobenzylalcohol matrix) *m/z* 390 $[M+H]^+$. Anal. calcd for $C_{20}H_{27}N_3O_5$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.48; H, 6.76; N, 10.57.

3(*R*)-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinylmethyl]-amino]-2-oxo-1-pyrrolidineacetamide (20). A solution of **19** (0.7 g, 1.8 mmol) in 40 mL concentrated methanolic ammonia was stirred overnight. The solvent was removed in vacuo to yield an oil that was chromatographed on a 2×40 cm silica gel column eluting with 10% MeOH and 0.1% NH_4OH in EtOAc to yield 0.59 g (86%) of product as a white solid. Mp 49–50 °C; $[\alpha]_D$ −28.7 (*c* 0.79, MeOH). ^1H NMR ($CDCl_3$, rotamers present) δ 1.61–1.65 (m, 1H), 1.78–1.95 (m, 3H), 2.02-2.09 (m, 1H), 2.30–2.40 (m, 1H), 2.54–2.58 and 2.47–2.51 (m, 1H), 2.81–2.83 and 2.93–2.96 (m, 1H), 3.24–3.27 (m, 1H), 3.34–3.43 (m, 3H), 3.50 (t, 1H, *J* = 8.5 Hz), 3.81–3.98 (m, 3H), 5.03–5.26 (m, 2H), 6.28 (s, 1H), 6.61 (s, 1H), 6.75 (s, 1H), 7.25–7.32 (m, 5H); ^{13}C NMR (DMSO, rotamers present) δ 22.3 (23.2), 26.4, 28.0 (28.7), 44.4, 45.0, 46.2 (46.6), 49.0 (49.1), 56.7 (57.5), 57.8, 65.6 (65.9), 127.4, 127.7, 128.4, 137.2, 154.1, 169.5, 174.1; FAB MS (*m*-nitrobenzylalcohol matrix) *m/z* 375 $[M+H]^+$. Anal. calcd for $C_{19}H_{26}N_4O_4$: C, 60.95; H, 7.00; N, 14.96. Found: C, 60.71; H, 7.02; N, 14.91.

3(*R*)-[[2(*S*)-Pyrrolidinylmethyl]amino]-2-oxo-1-pyrrolidineacetamide (5). Cbz-protected lactam **20** (80 mg, 0.21 mmol) was dissolved in 30 mL dry MeOH. Pd/C (100 mg) was added to a Parr hydrogenation flask and the mixture was shaken on a hydrogenator under 40 psi H_2 overnight. The solution was filtered and concentrated in vacuo. MeOH was added to the residue and the mixture was filtered through an ultrafiltration disk attached to a syringe. The solvent was removed in vacuo to yield a yellow oil which was chromatographed on a 1×20 cm silica gel column eluting with 33% MeOH/$CHCl_3$ and 0.1% NH_4OH. Further purification on a 1×5 cm column of C-8 reverse-phase silica gel eluting

with 10% MeOH/acetonitrile and a 1×10 cm silica gel column eluting with 10% MeOH/$CHCl_3$ gave an off-white solid. The solid was dissolved in H_2O and lyophilized to give 7.5 mg (15%) of analytically pure **5** as a hygroscopic off-white solid. HPLC analysis on a Waters Spherisorb® 4.6×150 mm analytical column gave a t_R of 1.9 min when eluted with 25% MeOH and 0.1% NH_4OH in $CHCl_3$. Further HPLC analysis on a LiChrosorb® RP-18 4.6×250 mm analytical column using 40% H_2O and 0.1% TFA in acetonitrile gave a t_R of 3.3 min and HPLC analysis on the RP-18 column eluting with 20% H_2O and 0.1% TFA in MeOH gave a t_R of 3.7 min. $[\alpha]_D$ 22.4 (*c* 0.42, MeOH). ^1H NMR (D_2O) δ 1.28–1.35 (m, 1H), 1.62–1.79 (m, 3H), 1.83–1.90 (m, 1H), 2.26–2.32 (m, 1H), 2.59 (dd, 1H, $J=6.0$ and 12.0 Hz), 2.65 (dd, 1H, $J=6.2$ and 12.0 Hz), 2.79–2.88 (m, 2H), 3.13–3.19 (m, 1H), 3.32 (dd, 2H, $J=5.0$ and 8.7 Hz), 3.52 (t, 1H, $J=8.5$ Hz), 3.88 (d, 1H, $J=17.2$ Hz), 3.92 (d, 1H, $J=17.2$ Hz); ^{13}C NMR (D_2O) δ 24.0, 25.0, 28.8, 45.3, 45.4, 45.7, 49.6, 57.9, 58.2, 172.4, 176.8; FAB HRMS (*m*-nitrobenzylalcohol matrix) *m*/*z* 241.1666 ($C_{11}H_{20}N_4O_2+H^+$ requires 241.1665).

N^1-[(*tert*-Butoxycarbonyl)-D-methionyl]-N^2-(benzyloxycarbonyl)-ethane-1,2-diamine (21). Boc-Met-OH (8.0 g, 32 mmol), was dissolved in 300 mL dry CH_2Cl_2 and 50 mL dry DMF. *N*-(Benzyloxycarbonyl)-ethane-1,2-diamine·HCl[28] (11.1 g, 48 mmol) and HOBt·H_2O (6.50 g, 48 mmol) were added and the solution was cooled to −78 °C. EDC·HCl (5.25 g, 27 mmol) and Et_3N (13.3 mL, 96 mmol) were then added and the reaction was allowed to warm to room temperature under N_2 overnight. The reaction was then stirred for 3 days. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. This solution was washed with saturated $NaHCO_3$ solution, 10% citric acid solution, H_2O, and saturated NaCl solution successively. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to a white solid that was chromatographed on a 4×30 cm silica gel column eluting with 10% MeOH in EtOAc. The material obtained from this chromatographic procedure was crystallized from EtOAc/hexanes to give 7.52 g of **21**. An additional 2.4 g of **21** was obtained by chromatography of the mother liquor on a 4×30 cm silica gel column eluting with a gradient of 500 mL 20% EtOAc in hexanes, 500 mL 50% EtOAc in hexanes, and 1 L 10% MeOH in EtOAc. The total yield of **21** was 9.9 g (73%). Mp 132–133 °C; $[\alpha]_D$ 12.5 (*c* 0.99, MeOH). ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.86–1.95 (m, 1H), 2.04–2.15 (m, 1H), 2.10 (s, 3H), 2.52–2.57 (m, 2H), 3.35–3.42 (m, 4H), 4.22–4.24 (m, 1H), 5.11 (s, 2H), 5.30 (d, 1H, $J=7.2$ Hz), 5.42–5.45 (br m, 1H), 6.81–6.84 (br m, 1H), 7.32–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 15.4, 28.4, 30.2, 31.6, 40.3, 40.8, 53.8, 66.9, 80.3, 128.2, 128.5, 136.4, 155.7, 157.0, 172.4; FAB MS (*m*-nitrobenzylalcohol matrix) *m*/*z* 426 $[M+H]^+$. Anal. calcd for $C_{20}H_{31}N_3O_5S$: C, 56.45; H, 7.34; N, 9.87; S, 7.53. Found: C, 56.62; H, 7.06; N, 10.11; S, 7.91.

***N*-[((1-Benzyloxycarbonyl)amino)ethyl]-3(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]-pyrrolidin-2-one (22).** Compound **21** (5.0 g, 11.8 mmol) was stirred at room temperature in excess neat MeI (30 mL) overnight after which the excess MeI was removed in vacuo. The resulting oil was dissolved in CH_2Cl_2 and the solvent removed in vacuo. This procedure was carried out 3× to give a yellow foam. The residue was dried under high vacuum overnight after which it was dissolved in 150 mL dry CH_2Cl_2 and 30 mL dry DMF. After the solution was cooled to 0 °C, NaH (60% in oil, 0.49 g, 12.3 mmol) was added in portions over 10 min. The reaction was stirred under N_2 at 0 °C for 3 h and then at room temperature for 2 days. Citric acid (1.40 g, 12.3 mmol) was added to quench the reaction and removal of the solvent in vacuo gave a yellow slurry. The slurry was partitioned between 10% citric acid solution and EtOAc. The aqueous layer was extracted 4× with EtOAc and the organic layers were combined and washed with 1 M $NaHCO_3$ solution followed by saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to a yellow oil that was chromatographed on a 4×40 cm silica gel column eluting with 10% MeOH in EtOAc to yield 2.35 g (53%) of product as a white powder. Mp 147–149 °C; $[\alpha]_D$ 14.8 (*c* 0.94, MeOH). ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.82–1.93 (m, 1H), 2.48–2.55 (m, 1H), 3.33–3.60 (m, 6H), 4.07–4.12 (m, 1H), 5.08–5.13 (m, 2H), 5.25–5.28 (br m, 1H), 5.54–5.56 (br m, 1H), 7.29–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 27.8, 28.3, 38.6 43.3, 44.5, 52.5, 66.7, 80.0, 128.1, 128.5, 136.6, 155.8, 156.8, 173.2; FAB MS (*m*-nitrobenzylalcohol matrix) *m*/*z* 378 $[M+H]^+$. Anal. calcd for $C_{19}H_{27}N_3O_5$: C, 60.46; H, 7.21; N, 11.13. Found: C, 60.50; H, 6.95; N, 10.87.

***N*-[((1-Benzyloxycarbonyl)amino)ethyl]-3(*R*)-[[1-(benzyloxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl]amino]-pyrrolidin-2-one (23).** Lactam **22** (1.57 g, 4.16 mmol) was treated with 20 mL 4 N HCl/dioxane for 5 h. The solvent was removed in vacuo from the cloudy white mixture and the residue was dissolved in CH_2Cl_2 which was then removed in vacuo. The salt was dried under high vacuum overnight and then dissolved in 100 mL dry CH_2Cl_2 and 60 mL dry DMF. HOBt·H_2O (0.67 g, 5 mmol) and Cbz-L-Pro-OH (1.24 g, 5 mmol) were added and the solution was cooled to −78 °C. EDC·HCl (0.96 g, 5 mmol) was added followed by 1.4 mL Et_3N (10 mmol) and the reaction was allowed to warm to room temperature overnight. The reaction was further stirred under nitrogen at room temperature for 3 days. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. The organic layer was washed with 1 M $NaHCO_3$ solution, 10% citric acid solution and saturated NaCl solution and then dried ($MgSO_4$), filtered and concentrated in vacuo to a yellow oil. This oil was chromatographed on a 3×40 cm silica gel column eluting with 10% MeOH/EtOAc to give 0.66 g (26%) of a side product that was determined to be *N*-[[1-(benzyloxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl]amino]ethyl]-3(*R*)-[[1-(benzyloxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl]amino]-pyrrolidin-2-one (**24**) [mp 75–77 °C; $[\alpha]_D$ −72.9 (*c* 1.23, MeOH); FAB MS (*m*-nitrobenzylalcohol matrix) *m*/*z* 606 $[M+H]^+$] and 0.68 g (32%) of the desired product **23** as a white foam. Mp 54–55 °C; $[\alpha]_D$ −54.1 (*c* 0.94, MeOH). ^1H NMR (DMSO-d_6, rotamers present) δ 1.66–1.80 (m, 4H), 2.06–2.18 (m, 2H), 3.07–3.11 (br m, 3H), 3.14–3.38 (m, 5H), 4.17–4.31 (m, 2H), 4.95–5.10 (m, 4H), 7.25–7.36 (m, 11H), 8.18 and 8.25 (d,

1H, $J=8.1$ Hz); ^{13}C NMR (DMSO-d_6, rotamers present) δ 23.4 (24.2), 26.2, 30.7 (31.7), 38.3 (43.0), 43.9, 467.0 (47.6), 50.6, 59.9 (60.4), 65.8 (66.2), 127.5, 127.8, 127.9, 128.2, 128.2, 128.7, 128.8, 137.5, 137.6, 154.3 (156.6), 172.1, 172.4 (172.7); FAB MS (m-nitrobenzyl-alcohol matrix) m/z 509 $[M+H]^+$. Anal. calcd for $C_{27}H_{32}N_4O_6$·MeOH: C, 62.21; H, 6.71; N, 10.36. Found: C, 62.54; H, 6.51; N, 10.72.

3(*R*)-[[2(*S*)-Pyrrolidinylcarbonyl]amino]-1-(aminoethyl)-pyrrolidin-2-one (6). Diprotected Cbz lactam **23** (190.4 mg, 0.37 mmol) was dissolved in 50 mL dry MeOH. Pd/C (100 mg) was added to a Parr hydrogenation flask and the flask was shaken on the hydrogenator under 60 psi H_2 overnight. The solution was filtered and concentrated in vacuo. The residue was dissolved in MeOH and then filtered through an ultrafiltration disk attached to a syringe. The solvent was removed in vacuo to yield a yellow oil which was chromatographed on a 1×20 cm silica gel column eluting with 25% MeOH and 0.1% NH_4OH in $CHCl_3$. Further purification on a 1×5 cm column of C-8 reverse-phase silica gel eluting with 10% MeOH/acetonitrile and a 0.5×3 cm silica gel column eluting with 20% MeOH/$CHCl_3$ gave an off-white solid. The material was dissolved in H_2O and lyophilized to give 32 mg (36%) of analytically pure product as a hygroscopic off-white solid. HPLC analysis using a Water Spherisorb® 4.6×150 mm analytical column gave a t_R of 4.7 min when the product was eluted with 25% MeOH and 0.1% NH_4OH in $CHCl_3$. HPLC analysis using a LiChrosorb® RP-18 4.6×250 mm analytical column using 40% H_2O and 0.1% TFA in acetonitrile gave a t_R of 2.9 min. HPLC analysis on the RP-18 column eluting with 20% H_2O/MeOH with 0.1% TFA gave a t_R of 4.0 min. $[\alpha]_D$ −5.6 (c 0.72, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.56–1.71 (m, 3H), 1.74–1.81 (m, 1H), 1.84–1.97 (m, 1H), 2.19–2.29 (m, 1H), 2.69 (t, 2H, $J=6.3$ Hz), 2.72–2.86 (m, 2H), 3.21 (t, 2H, $J=6.3$ Hz), 3.25–3.30 (m, 2H), 3.42 (br s, 2H), 3.50 (dd, 1H, $J=5.4$, 8.7 Hz), 4.31 (dd, 1H, $J=9.0$ and 18.0 Hz), 8.15 (d, 1H, $J=8.0$ Hz); ^{13}C NMR (DMSO-d_6) δ 25.7, 26.2, 30.4, 38.4, 43.5, 44.3, 46.7, 49.9, 60.2, 172.1, 174.6; CI HRMS m/z 241.1666 ($C_{11}H_{20}N_4O_2+H^+$ requires 241.1665).

Biological assay

The ability of the peptidomimetics **3–6** to enhance the binding of [^{3}H]*N*-propylnorapomorphine (NPA) to dopamine receptors was carried out in bovine striatal membranes, which were prepared as described previously.[6,31] The binding of [^{3}H]NPA to the striatal membranes was carried out in triplicate in 1.0 mL of assay buffer (pH 7.4, 50 mM Tris–HCl, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM PMSF, 100 μg/mL bacitracin, and 5 μg/mL soybean trypsin inhibitor) containing 1 nM of radioligand, the indicated concentrations of PLG peptidomimetics (either 1, 10, or 100 nM), and 150–200 μg of membrane protein. 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 through Whatman GF/B filters. The filters were washed with 3×5 mL of Tris–EDTA buffer (pH 7.4, 50 mM Tris–HCl, 1 mM EDTA) and the radioactivity was determined on a Beckman scintillation counter (Model 1780). The percent increase in NPA binding produced by compounds **3–6** was compared against the control value (the binding of [^{3}H]NPA in the absence of any PLG peptidomimetic) and against the activity of peptidomimetic **2**. All data were analyzed using the student t test in the GraphPad Prism (Version 2.01) software package.

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