Synthesis and Dopamine Receptor Modulating Activity of Lactam Conformationally Constrained Analogues of Pro-Leu-Gly-NH_2

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A series of analogues of the potent analogue of Pro-Leu-Gly-NH_2 (PLG), 2-oxo-3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide (**2**) were synthesized in which the (*R*)-γ-lactam residue of **2** was replaced with a (*R*)-β-lactam, (*R*)-aminosuccinimide, (*R*)-cycloseryl, (*R*)-δ-lactam, (*R*)-ϵ-lactam, or (*S*)-ϵ-lactam residue to give analogues **3**–**8**, respectively. These substitutions were made so as to vary the ψ_2 torsion angle. The analogues were tested for their ability to enhance the binding of the dopamine receptor agonist ADTN to the dopamine receptor. Analogues **3**–**6** and **8** exhibited dose–response curves that were bell-shaped in nature with the maximum effect occurring at a concentration of 1 μM. Analogue **7** was inactive. Analogues **3** and **4** were found to be as effective as PLG, while analogues **5**, **6**, and **8** appeared to be more effective than PLG in terms of enhancing the binding of ADTN to dopamine receptors. The activity of analogues **3**–**6** and **8** with their ψ_2 angles in the vicinity of that observed in a type II β-turn is consistent with the hypothesis that this type of turn is the bioactive conformation of PLG.

L-Prolyl-L-leucylglycinamide (PLG, **1**) is an endogenous tripeptide which has a direct modulatory effect on dopamine receptors in the central nervous system. Its pharmacological and clinical effects have been reviewed by Mishra et al.[1,2] PLG and its analogues selectively enhance the binding of dopamine receptor agonists such as 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN),[3–5] apomorphine,[6–8] and *n*-propylnorapomorphine[9,10] to dopamine receptors. This PLG-induced increase in the affinity of dopamine receptor agonists is associated with an increase in the number of high-affinity agonist binding sites.[10] PLG-treated dopamine receptors also show an increased resistance to inhibition of agonist binding by guanine nucleotides. PLG is thus proposed to act by modulating the affinity states of the dopamine receptor, possibly by enhancing the interaction between G-proteins and the dopamine receptor.[10] The modulation of dopamine receptors by PLG is also seen in the ability of this peptide to down-regulate supersensitized dopamine receptors produced by the chronic administration of neuroleptic drugs.[11,12]

Conformational analysis of PLG by NMR[13] and X-ray[14] spectroscopic methods indicates that this tripeptide can adopt a type II β-turn both in solution and in the crystalline state wherein the β-turn is stabilized by a hydrogen bond between the trans primary carboxamide hydrogen and the prolyl carbonyl oxygen (Figure 1). Computational methods also indicate that the type II β-turn is one of the low-energy conformations for PLG.[15] In order to determine whether this conformation may be the bioactive conformation of PLG, we previously incorporated the γ-lactam β-turn mimic developed by Freidinger et al.[16,17] into the structure of PLG.[18] The resulting (*R*)-γ-lactam analogue of PLG, 2-oxo-3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide (**2**), was found to be 10 000 times more potent than PLG in enhancing the binding of [^{3}H]-ADTN to dopamine receptors[18] and 1000 times more potent than PLG in preventing the GTP-induced conversion of the high-affinity state of the D_2-dopamine receptor to its low-affinity state.[19] These findings led us to postulate that the bioactive conformation of PLG is a type II β-turn conformation.

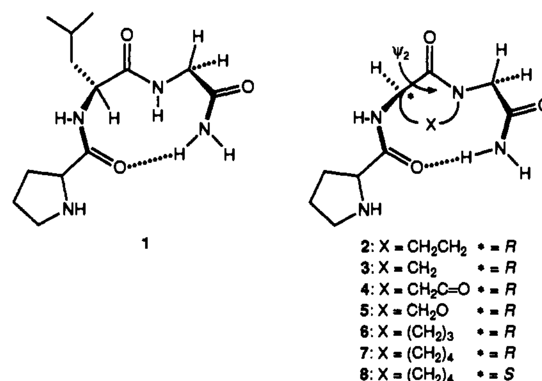

Figure 1. Illustration of the type II β-turn of Pro-Leu-Gly-NH_2 (**1**) and the proposed relationship of the lactam analogues **2**–**8** to this conformation.

In order to more fully explore the structural basis of the high potency of **2** and to better define the bioactive conformation of PLG, a series of analogues of **2**, compounds **3**–**8** (Figure 1), have been synthesized in which the γ-lactam residue present in **2** has been replaced with different sized or functionalized lactam rings so as to vary the ψ_2 torsion angle. These analogues have been tested for their ability to enhance the binding of the dopamine receptor agonist ADTN to the dopamine receptor.

Syntheses

The β-, δ-, and ϵ-lactam PLG analogues, compounds **3** and **6**–**8**, were synthesized as outlined in Scheme I. In all four cases the corresponding lactam dipeptide mimic was synthesized first and then coupled with an appropriately protected proline residue. Compound **9** served as the starting material for the synthesis of the β-lactam PLG analogue **3**. This material was made by coupling the 4,5-diphenyl-4-oxazolin-2-one derivative of D-serine[20] with Gly-OMe using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). The conversion of **9** to the

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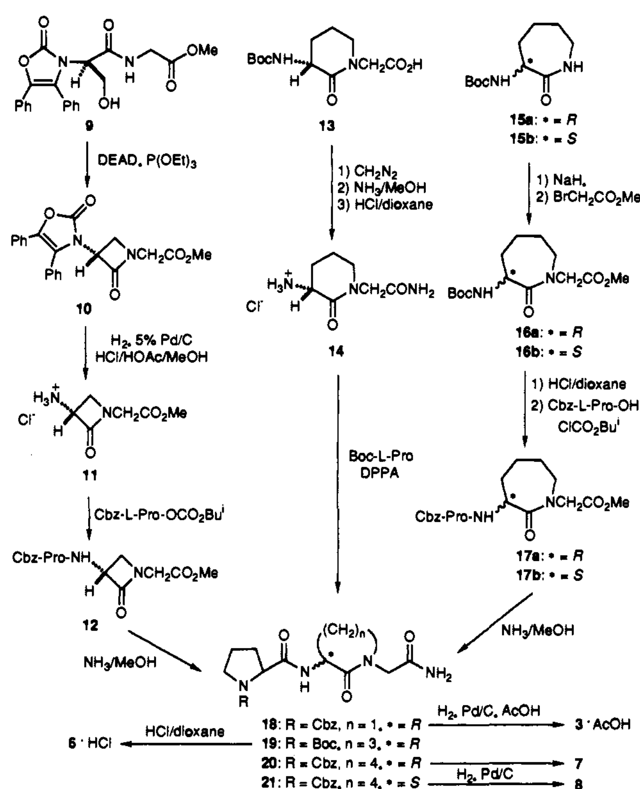
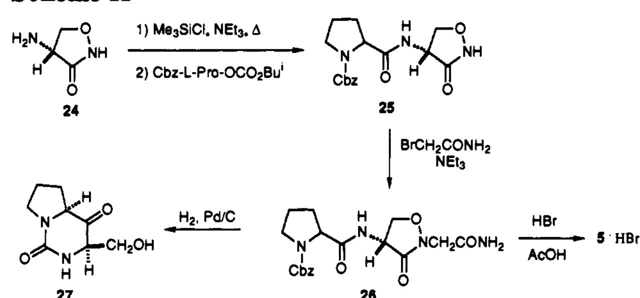
Scheme I



Scheme II



protected β-lactam dipeptide mimic 10 was based on the methodology developed by Townsend et al.[21] in their synthesis of the nocardicins and employed a modified Mitsunobu reaction involving triethyl phosphite and diethyl azodicarboxylate (DEAD). Townsend et al.[22] have shown that epimerization does not occur at the serine α-carbon under the Mitsunobu cyclization reaction conditions employed, but that epimerization of the C-terminal amino acid can occur. Since the C-terminal residue is glycine in 9, this was not a problem in the synthesis of β-lactam 10. Deprotection of the amino group of 10 by catalytic hydrogenation afforded the desired β-lactam dipeptide mimic 11. This material was coupled to Cbz-L-Pro-OH by the mixed anhydride method with isobutyl chloroformate. The resulting ester 12 was converted to primary amide 18 with NH_3 in MeOH. The benzyloxycarbonyl protecting group was removed from 18 by hydrogenolysis to give 3 in the form of its acetate salt.

The (*R*)-δ-lactam PLG analogue 6 was synthesized by the same procedure we used previously for the synthesis of the diastereomeric (*S*)-δ-lactam PLG analogue.[18] In this route the protected δ-lactam dipeptide mimic 13 was synthesized by the method of Freidinger et al.[23] This material was converted to its methyl ester with diazomethane. The ester was then transformed to the primary amide with NH_3/MeOH and the amide deprotected with HCl to give 14. Compound 14 was coupled to Boc-L-Pro-OH with diphenyl phosphorazidate (DPPA) to give the protected δ-lactam PLG analogue 19. Removal of the *tert*-butoxycarbonyl protecting group from 19 gave the desired δ-lactam PLG analogue 6 as its HCl salt.

The (*R*)- and (*S*)-ϵ-lactam PLG analogues 7 and 8 were synthesized from commercially available (*R*)- and (*S*)-3-amino-ϵ-caprolactam, respectively, by first protecting the amino group of each isomer with the *tert*-butoxycarbonyl group to give 15a and b. The lactam nitrogens of 15a and b were alkylated with methyl bromoacetate to give the corresponding protected ϵ-lactam dipeptide mimics 16a and b. These two compounds were deprotected with HCl and the resultant amino esters were coupled to Cbz-L-Pro-OH via the mixed anhydride method to give 17a and b, which were in turn converted to the primary amides 20 and 21, respectively. Removal of the benzyloxycarbonyl group from 20 and 21 by hydrogenolysis provided the desired (*R*)- and (*S*)-ϵ-lactam PLG analogues 7 and 8.

The (*R*)-aminosuccinimide PLG analogue 4 was synthesized by the same method that Schon and Kisfaludy[24] employed for the synthesis of the diastereomeric compound 3(*S*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-1-succinimideacetamide. N-Protected dipeptide Cbz-D-Asp(OBu^t)-Gly-NH_2 (22), obtained by coupling Gly-NH_2 to Cbz-D-Asp(OBu^t)-OH with DCC/*N*-hydroxysuccinimide (HOSu), was deprotected by hydrogenolysis to give D-Asp(OBu^t)-Gly-NH_2. This material was coupled to Boc-L-Pro-OSu to give Boc-Pro-D-Asp(OBu^t)-Gly-NH_2 (23). Treatment of 23 with a saturated solution of HCl in AcOH led to the removal of the *tert*-butoxycarbonyl and *tert*-butyl protecting groups and brought about cyclization between the amino group of the glycyl residue and the β-carboxyl group of the D-aspartyl residue to give 4-HCl in a 57% yield.

Our initial synthetic approach to the cycloserine PLG analogue 5 involved protection of the primary amino group of (*R*)-cycloserine followed by alkylation of the isoxazolidine NH with methyl bromoacetate. This approach, however, proved quite problematic since amino protection of (*R*)-cycloserine with the benzyloxycarbonyl group gave in addition to the desired Cbz-(*R*)-cycloserine a fair amount of material in which the isoxazolidine NH was also protected. Furthermore, alkylation of Cbz-(*R*)-cycloserine with methyl bromoacetate gave a complex mixture of products with only small amounts of the desired alkylated species. Ultimately, the synthetic sequence depicted in Scheme II provided us with the desired cycloserine PLG analogue 5. In this approach (*R*)-cycloserine (24) was coupled with Cbz-L-Pro-OH using the trimethylsilyl chloride/mixed anhydride method[25] whereby the amino and isoxazolidine nitrogens of (*R*)-cycloserine are first silylated and the bis-silylated derivative then coupled to the mixed anhydride formed between Cbz-L-Pro-OH and isobutyl chloroformate. The resultant dipeptide 25 was alkylated directly with bromoacetamide to obtain the protected tripeptide amide 26. Deprotection of 26 by catalytic hydrogenolysis, even under acidic conditions, gave diketopiperazine cyclo(L-Pro-D-Ser) (27) as the major product. The formation of 27 most likely involves the initial hydrogenolysis of the prolyl benzyloxycarbonyl group, followed by attack of the prolyl imino nitrogen on the relatively labile hydroxamide bond to give a diketopiperazine. Subsequent cleavage of the N–O bond of this diketopiperazine derivative under the reductive reaction conditions yields 27. The desired product, 5, was

Table I. Percent Enhancement of [^{3}H]ADTN Binding to Dopamine Receptors by PLG Lactam Analogues 3–8[a]

	% increase ± SEM						
concn (M)	PLG[b]	2[c]	3	4	5	6	8
10^{-12}		2.2 ± 0.9	1.5 ± 0.4	4.0 ± 0.5	2.0 ± 0.05	2 ± 0.02	
10^{-11}		12.0 ± 2.28		5.5 ± 0.8	2.0 ± 0.07	4 ± 0.05	8 ± 1.5
10^{-10}		24.4 ± 0.87[d]	2.5 ± 0.9	3.3 ± 0.2	2.5 ± 0.04	3 ± 0.04	15 ± 1.8
10^{-9}		23.0 ± 1.22[d]	3.9 ± 0.8	4.6 ± 0.18	6.8 ± 1.2	4 ± 0.1	30 ± 3.2[e]
10^{-8}		9.2 ± 1.9	5.0 ± 1.8	13.5 ± 1.6	14.0 ± 1.8	11 ± 2	35 ± 4.6[e]
10^{-7}		5.6 ± 1.2	8.0 ± 2	42.0 ± 5[e]	30.0 ± 4.7[e]	16 ± 4	38 ± 6.7[e]
10^{-6}	27 ± 4.8[e]	2.8 ± 0.86	27.0 ± 6.2[e]	38.0 ± 6[e]	54.0 ± 7.6[f]	43 ± 7[f]	61 ± 9.2[f]
10^{-5}		1.6 ± 0.6	22.0 ± 3.8[e]	33.0 ± 4[e]	37.0 ± 3.9[e]	18 ± 2	27 ± 4.0[e]
10^{-4}		1.4 ± 0.4	9.5 ± 1.7	13.0 ± 2	7.0 ± 1.1		17 ± 1.0

[a] Each value is an average ± SEM of three or four separate experiments each carried out in triplicate. Analogue 7 showed no activity in the assay. [b] In these experiments PLG was used for comparison purposes and as such was tested only at the concentration at which it produces its maximum effect. Complete dose–response curves for PLG can be found in refs 5 and 18. [c] Data are from ref 18. [d] Significantly different ($p < 0.001$) from control membranes. [e] Significantly different ($p < 0.05$) from control membranes. [f] Significantly different ($p < 0.01$) from control membranes.

eventually obtained by deprotection of **26** with HBr in acetic acid and was determined to be 95.8% pure by reverse-phase HPLC.

^{1}H and ^{13}C NMR spectra at room temperature of the protected lactam peptide intermediates **18–21**, **25**, and **26** showed multiple peaks for several of the hydrogen and carbon resonances, thus suggesting the presence of rotational isomers about the prolyl carbamate bond.[26,27] These multiple resonances in the NMR spectra of these intermediates disappeared when the temperature was raised, thereby confirming the presence of rotational isomers as opposed to diastereoisomers.

Pharmacological Studies

The assay used to determine the ability of the conformationally constrained lactam analogues **3–8** to modulate dopamine receptors has been described in detail in our previous structure–activity relationship studies on PLG.[3-5,18] This in vitro assay involves the measurement of the ability of a compound to enhance the binding of the dopamine receptor agonist [^{3}H]ADTN to dopamine receptors obtained from bovine caudate membranes. In this dopamine receptor modulation assay PLG possesses a bell-shaped dose–response curve with the maximum effect occurring at a concentration of 1 μM. At this concentration PLG typically enhances the binding of ADTN by 25–40%.

In the present study compounds **3–8** were tested in the assay described above at doses ranging from 1 pM to 100 μM. The results obtained are shown in Table I. Also shown in this table for comparison are the results for PLG at 1 μM and the results obtained previously for the potent γ-lactam PLG analogue **2**.[18] Of the six new conformationally constrained analogues of PLG synthesized in this study, all but one were found to enhance the binding of ADTN to dopamine receptors. Analogue **7** was the only analogue that did not show any activity. The active lactam analogues, compounds **3–6** and **8**, all exhibited dose–response curves that resembled the dose–response curve for PLG in that they were bell-shaped in nature with the maximum effect occurring at a concentration of 1 μM. The activities of **3–6** and **8**, however, differed in two respects from the activity of the (*R*)-γ-lactam PLG analogue **2**. First of all, none of the conformationally constrained lactam analogues synthesized in this study possessed the potency of γ-lactam analogue **2**. Secondly, the active compounds **3–6** and **8** did not have to be preincubated with ADTN and the striatal membrane preparation in order for their activity to be observed, as required for **2**.

Discussion

The molecular mechanism behind the enhancement of ADTN binding to dopamine receptors by PLG has been shown to possibly involve two factors. Firstly, PLG increases the affinity of dopamine receptor agonists to the dopamine receptor.[6] Secondly, PLG partially prevents the GTP-induced conversion of the high-affinity state of the dopamine D_2 receptor to the low-affinity state.[10,19] Although most of the mechanism of action studies have been carried out with PLG, it is assumed that the numerous PLG analogues which have been shown to modulate dopamine receptors are also acting by similar mechanisms of action.

The lactam analogues **3–8** were synthesized in order to further explore the bioactive conformation of PLG. Previously, we had proposed that the bioactive conformation of PLG is a type II β-turn.[18] A type II β-turn is characterized by ϕ_2, ψ_2, ϕ_3, and ψ_3 angles of −60°, +120°, +80°, and 0°, respectively.[28] This bioactive conformation was postulated based upon the activity of the (*R*)-γ-lactam analogue **2** in which the Leu-Gly amide bond is fixed in the trans position and the ψ_2 torsion angle has a value of +141.9°.[29] Different types of lactams were utilized in this study, since by varying the size and nature of the lactam ring it was known that the magnitude of the ψ_2 torsion angle could be varied. The predicted values for the ψ_2 angle of the lactam analogues **3–8** were based on values previously reported in the literature for compounds containing analogous lactam constraints. Thus, for analogue **3** the ψ_2 angle was expected to have values between 120 and 132°.[30] In the case of the two γ-lactam analogues **4** and **5**, the (*R*)-aminosuccinimide analogue **4** was expected to have a ψ_2 very close to 120°,[31] while the (*R*)-cycloseryl analogue **5** was expected to possess a ψ_2 angle similar to that observed in **2**. Conformational analysis of the more flexible δ-lactam constraints indicated that the two low-energy conformations of (*R*)-δ-lactam analogue **6** should possess ψ_2 angles around 108° and 135°.[16,32] For the ε-lactams, the (R)-ε-lactam in analogue **7** should possess a ψ_2 torsion angle around −168°, while the (*S*)-ε-lactam in analogue **8** should possess a ψ_2 angle around +168°.[16,32,33]

The results show that those analogues that possess lactam constraints with a positive ψ_2 torsional angle retained the ability to enhance the binding of ADTN to dopamine receptors. Only the (*R*)-ε-lactam containing analogue **7** with a predicted ψ_2 torsion angle around −168° was found to be inactive in this series of PLG analogues. This result is consistent with the inactivity observed previously for the (*S*)-δ-lactam analogue of PLG.[18] A

crystal structure of a derivative of this (*S*)-δ-lactam analogue has shown that this lactam constraint possesses a ψ_2 angle of −155.6°.[34] Although the activities of analogues **3–6** and **8** indicate that there is a fair amount of flexibility allowed in terms of the lactam restraint, the results also show that the size and nature of the lactam constraint can bring about subtle differences in the effectiveness of the analogues. For example, while analogues **3** and **4** are about as effective as PLG in terms of enhancing the binding of ADTN to dopamine receptors, analogues **5**, **6**, and **8** appear to be more effective than PLG. In fact, analogue **8** with the relatively large (*S*)-ε-lactam constraint enhances the binding of ADTN to dopamine receptors about twice as much as does PLG.

As pointed out above, none of the lactam conformationally constrained PLG analogues made in this study were as potent as the γ-lactam analogue **2** and none required preincubation for their activity to be seen. These results would seem to indicate that the potency, as well as the preincubation requirement, of **2** is not solely due to the ψ_2 torsion angle being constrained to a value around that found in a type II β-turn since several of the lactam PLG analogues made in this study possess ψ_2 torsion angles of similar magnitude to that found in **2**. In addition, NMR solution studies have shown that some of these analogues behave like PLG and **2** in terms of the effect of temperature and solvent composition on the chemical shift of the trans Gly-NH$_2$ proton.[35] We initially postulated that a partitioning phenomenon might be responsible for the preincubation requirement of **2**.[18] However, the close structural similarity between several of the lactam analogues of this study and **2** would seem to argue against this explanation. Metabolism of **2** has also been suggested as a possible explanation for its unique behavior in this assay system. Since one of the obvious ways in which **2** could be broken down involves enzymatic cleavage of the amide bond between the prolyl residue and the lactam dipeptide mimic, 2-oxo-3(*R*)-amino-1-pyrrolidineacetamide, we have examined the latter residue in the ADTN assay. This compound did not show any activity, however. Thus, at this point in time it is not clear why **2** behaves so differently from the structurally related lactams examined in this study. Nevertheless, the activity of analogues **3–6** and **8** with their ψ_2 angles constrained to values found in a type II β-turn is consistent with our hypothesis that this type of turn is the bioactive conformation of PLG.

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 MHW Laboratories, Phoenix, AZ. ^{1}H-NMR spectra were recorded on one of the following instruments: a Bruker AC at 200 MHz, a Bruker AC at 300 MHz, a Nicolet Zeta at 300 MHz, or a GE Omega at 300 MHz. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in CDCl$_3$ or DMSO-d_6. ^{13}C-NMR spectra were performed on one of the following instruments: a Bruker AC200 at 50 MHz, a Bruker AC300 at 75 MHz, or a Nicolet Zeta 300 at 75 MHz. When either DMSO-d_6 or CDCl$_3$ was used as solvent, they served as the internal standard at δ 39.5 and 77.0 ppm, respectively. FAB mass spectra were obtained on a VG 7070E-HF mass spectrometer. Medium-pressure liquid chromatography (MPLC) 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 HLF Uniplates. Visualization was achieved with either UV or I$_2$. HPLC was performed using a Spectraphysics HPLC system on a 30-cm Spherisorb C8 analytical column. Amino acids were purchased from the Sigma Chemical Co. and 4 N HCl in dioxane was obtained from the Pierce Co. All other chemicals were obtained from the Aldrich Chemical Co.

Methyl [[2(*R*)-(2-Oxo-4,5-diphenyl-3(2*H*)-oxazolyl)-3-hydroxypropanoyl]amino]acetate (9). The dicyclohexylammonium salt of the 4,5-diphenyl-4-oxazolin-2-one derivative of D-serine[20] (1.03 g, 2 mmol) and Gly-OMe·HCl (0.26 g, 2 mmol) were dissolved in DMF/CH$_2$Cl$_2$ (2 mL/20 mL). The cloudy solution was cooled in an ice bath and HOBt (0.31 g, 2.29 mmol) was added followed by DCC (0.48 g, 2.32 mmol). The reaction mixture was stirred at 4 °C for 2 h and at room temperature overnight. The mixture was chilled in an ice bath, filtered, and evaporated under vacuum. The residue obtained was dissolved in EtOAc and washed successively with H$_2$O, 10% NaHCO$_3$, and 10% citric acid. The organic phase was dried over MgSO$_4$, filtered, and evaporated to give a brown oil. MPLC (EtOAc/hexanes, 3:2) of the oil provided 0.57 g (70.8%) of **9** as a white foam: $[\alpha]^{25}_D$ −5.9° (*c* 1.1, CHCl$_3$). Anal. (C$_{21}$H$_{20}$N$_2$O$_6$) C, H, N.

Methyl 3(*R*)-(2-Oxo-4,5-diphenyl-3(2H)-oxazolyl)-2-oxo-1-azetidineacetate (10). Compound **9** (0.93 g, 2.35 mmol) was dissolved in 40 mL of dry THF with stirring, under N$_2$. Triethyl phosphite (0.44 mL, 2.59 mmol) and diethyl azodicarboxylate (0.43 mL, 2.58 mmol) were added, and the mixture was stirred at room temperature for 3.5 h. The solvent was removed in vacuo and the resultant oil was dissolved in a mixture of EtOAc/hexanes (1:1) and stored in the freezer overnight. The yellow-brown solid which formed was removed by filtration and the filtrate was concentrated to yield a yellow oil. This oil was purified by MPLC (EtOAc/hexanes, 3:2) to provide 0.75 g (84.5%) of **10** as a clear foam: $[\alpha]^{25}_D$ +33.7° (*c* 0.60, CHCl$_3$); FAB MS *m/z* 357 (M − H)$^-$. Anal. (C$_{21}$H$_{18}$N$_2$O$_5$·H$_2$O) C, H, N.

Methyl 3(*R*)-Amino-2-oxo-1-azetidineacetate Hydrochloride (11). Compound **10** (0.63 g, 1.67 mmol) was dissolved in a mixture of EtOAc/MeOH/AcOH (25 mL/10 mL/10 mL) in a Parr bottle. The bottle was flushed with Ar for 5 min and then 1 N HCl (1.77 mL) and 5% Pd/C (0.315 g) were added. The mixture was hydrogenated under 40 psi H$_2$ for 48 h. Filtration of the mixture through Celite followed by evaporation under vacuum and azeotropic removal of the AcOH with CCl$_4$ gave a solid, which was partitioned between EtOAc and H$_2$O. The aqueous phase was dried in vacuo to afford 0.32 g (98.5%) of the product as a light yellow foam, which was used without further purification: TLC R_f (nPrOH/NH$_4$OH, 4:1) = 0.23; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (br s, 3 H, $^+$NH$_3$), 4.52–4.64 (m, 1 H, 3-CH), 4.26 (d, 1 H, *J* = 17.8 Hz, CH$_2$CO$_2$), 3.99 (d, 1 H, *J* = 17.8 Hz, CH$_2$CO$_2$), 3.68 (s, 3 H, OMe), 3.56–3.80 (m, 1 H, 4-CH$_2$), 3.46 (dd, 1 H, *J* = 2.3 and 6.8 Hz, 4-CH$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.3 (CO$_2$Me), 163.0 (CON), 54.5 (3-C), 52.2 (OMe), 45.5 (4-C), 42.9 (CH$_2$CO$_2$); FAB MS *m/z* 159 (M − Cl)$^+$.

Methyl 3(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-azetidineacetate (12). Cbz-L-Pro-OH (0.56 g, 2.49 mmol) was dissolved in 6 mL of dry THF and cooled to −20 °C in a dry ice/CCl$_4$ bath, under N$_2$. *N*-Methylmorpholine (0.35 mL, 2.49 mmol) and isobutyl chloroformate (0.32 mL, 2.49 mmol) were added to the above solution, and stirring was continued at −20 °C for 20 min. Compound **11** (0.44 g, 2.26 mmol) and *N*-methylmorpholine (0.33 mL, 2.26 mmol) in THF/DMF/H$_2$O (6 mL/10 mL/2 mL) were added slowly to the mixed anhydride, and the mixture was stirred at −20 °C for 6 h and then at room temperature for 12 h. The solvent was removed in vacuo and the yellow residue obtained was dissolved in EtOAc. The solution was washed with 10% citric acid and saturated NaHCO$_3$, dried over MgSO$_4$, filtered, and evaporated to give 0.66 g of a yellow oil. MPLC (CHCl$_3$/MeOH, 9:1) yielded 0.29 g (32.7%) of the ester as a white foam: $[\alpha]^{25}_D$ −82.8° (*c* 0.90, CHCl$_3$); FAB MS *m/z* 390 (M + H)$^+$. Anal. (C$_{19}$H$_{23}$N$_3$O$_6$) C, H, N.

3(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-azetidineacetamide (18). Ester **12** (0.25 g, 0.643 mmol) was dissolved in 15 mL of methanolic ammonia and this solution stirred in an ice bath for 4 h. The solvent and NH$_3$ were removed in vacuo, and the foam obtained was purified by MPLC (CHCl$_3$/MeOH, 9:1) to give 0.19 g (78.9%) of **18** as a

white, hygroscopic foam: $[\alpha]^{25}{}_D$ −60.0° (c 0.96, MeOH); FAB MS m/z 375 $(M + H)^+$. Anal. ($C_{18}H_{22}N_4O_5{\cdot}0.25H_2O$) C, H, N.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-azetidineacetamide Acetic Acid (3·AcOH). A solution of 18 (0.19 g, 0.507 mmol) in MeOH (10 mL) was hydrogenated under 40 psi of H_2 in a Parr hydrogenator in the presence of AcOH (0.032 mL, 0.558 mmol) and 10% Pd/C (0.02 g) for 3 h. The mixture was filtered through Celite and the filtrate was concentrated to give 0.16 g of a clear glassy residue. This material was dissolved in H_2O (4 mL) and lyophilized to provide 0.138 g (90.6%) of the product as a hygroscopic foam: $[\alpha]^{25}{}_D$ +13.2° (c 0.92, MeOH); TLC R_f (nPrOH/NH_4OH, 4:1) = 0.49, R_f (nBuOH/H_2O/AcOH, 4:1:1) = 0.10; ^1H NMR (300 MHz, DMSO-d_6) δ 8.92 (d, 1 H, J = 8.2 Hz, CONH), 7.59 (s, 1 H, trans $CONH_2$), 7.33 (s, 1 H, cis $CONH_2$), 4.80–4.94 (m, 1 H, 3-CH), 3.93 (d, 1 H, J = 17.3 Hz, CH_2CON), 3.56 (d, 1 H, J = 17.3 Hz, CH_2CON), 3.74–3.81 (m, 1 H, 4-CH_2), 3.55 (t, 1 H, J = 5.3 Hz, Pro α-CH), 3.34 (dd, 1 H, J = 2.5 and 5.0 Hz, 4-CH_2), 2.86–3.08 (m, 2 H, Pro δ-CH), 2.01–2.19 (m, 1 H, Pro β-CH_2), 1.91 (s, 3 H, CH_3CO_2), 1.65–1.80 (m, 3 H, Pro β,γ-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.8 (CONH), 172.1 ($CONH_2$), 169.3 (CH_3CO_2), 166.6 (CON), 59.6 (Pro α-C), 56.3 (3-C), 47.7, 46.3 (Pro δ-C and 4-C), 44.4 (CH_2CONH_2), 30.1 (Pro β-C), 24.9 (Pro γ-C), 21.1 (CH_3CO_2); FAB MS m/z 241 $(M-OAc)^+$. Anal. ($C_{12}H_{20}N_4O_5{\cdot}2H_2O$) C, H, N.

3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-piperidineacetic Acid (13). N^{α}-(*tert*-Butoxycarbonyl)-N^{δ}-(carboxymethyl)-D-ornithine (1 g, 3.45 mmol) was heated, with stirring, in DMF (32 mL) at 55 °C for 2 h. The solvent was removed under reduced pressure and the yellow oil obtained was dissolved in EtOAc and chilled. The resultant precipitate was removed by filtration and the filtrate was evaporated in vacuo to an oil, which when mixed with EtOAc/hexanes provided 0.9 g (95.9%) of 12 as a white solid: mp 113–114.5 °C; $[\alpha]^{25}{}_D$ +25.9° (c 0.90, MeOH) [lit.[23] mp 113–116 °C; $[\alpha]^{25}{}_D$ −22.14° (c 1.0, MeOH) for the (S)-isomer]. Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

3(*R*)-Amino-2-oxo-1-piperidineacetamide Hydrochloride (14). Compound 13 (0.85 g, 3.13 mmol) was dissolved in 10 mL of MeOH and stirred with an excess of CH_2N_2 in Et_2O at room temperature for 1 h. The solvent was removed under vacuum and the resultant yellow oil was dissolved in methanolic ammonia (25 mL). The solution was stirred for 2 days at room temperature. Evaporation of the solvent in vacuo afforded 0.84 g (99%) of 3(*R*)-[*N*-*tert*-butoxycarbonyl)amino]-2-oxo-1-piperidineacetamide as a white solid: mp 161.5–163 °C; $[\alpha]^{25}{}_D$ +43.7° (c 1.25, MeOH) [lit.[18] mp 163.5–164 °C; $[\alpha]^{25}{}_D$ −36.5° (c 1.0, MeOH) for the (S)-isomer].

The above primary amide (0.40 g, 1.47 mmol) was dissolved in 4 N HCl/dioxane (10 mL) and the solution stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure and the solid obtained was mixed with MeOH and dried to afford 0.287 g (94%) of 14 as a pale yellow solid: mp 244–245 °C; $[\alpha]^{25}{}_D$ −3.09° (c 1.1, MeOH) [lit.[18] mp 249 °C; $[\alpha]^{25}{}_D$ +3.07° (c 1.01, MeOH) for the (S)-isomer]; ^1H NMR (200 MHz, DMSO-d_6) δ 8.50 (s, 3 H, $^+NH_3$), 7.53 (s, 1 H, trans $CONH_2$), 7.19 (s, 1 H, cis $CONH_2$), 3.80–4.02 (m, 1 H, 3-CH), 3.91 (s, 2 H, CH_2CO), 3.24–3.50 (m, 2 H, 6-CH_2), 2.10–2.36 (m, 1 H, 4-CH_2), 1.76–2.10 (m, 3 H, 4,5-CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 169.5, 166.5 (CON and $CONH_2$), 49.6 (3-C), 49.4 (CH_2CO), 48.4 (6-C), 25.2 (4-C), 20.1 (5-C).

3(*R*)-[[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-piperidineacetamide (19). Compound 14 (0.276 g, 1.33 mmol) was suspended in DMF (6 mL), with stirring, under N_2. Boc-L-Pro-OH (0.286 g, 1.33 mmol) was added, followed by NEt_3 (0.41 mL, 2.94 mmol). The mixture was cooled in an ice bath and diphenylphosphorazidate (0.32 mL, 1.47 mmol) was added. The reaction mixture as stirred for 3 days at 4 °C and then at room temperature for 1 day. The solvent was removed in vacuo and the oil obtained was purified by MPLC (EtOAc/MeOH, 9:1) to give 0.38 g (77%) of 19 as a white hygroscopic foam: $[\alpha]^{25}{}_D$ −45.3° (c 0.88, MeOH); FAB MS m/z 369 $(M + H)^+$. Anal. ($C_{17}H_{28}N_4O_5{\cdot}H_2O$) C, H, N.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-piperidineacetamide Hydrochloride (6·HCl). Cold 4 N HCl/dioxane (4 mL) was added to 19 (0.05 g, 0.136 mmol) in a round-bottom flask and the mixture was stirred at room temperature for 1 h. The mixture was then mixed with cold Et_2O and filtered. The solid obtained was dried in vacuo, dissolved in water (5 mL), and lyophilized to provide 0.02 g (48.8%) of 6·HCl as a white foam: $[\alpha]^{25}{}_D$ + 7.06° (c 1.02, MeOH); TLC R_f (nPrOH/NH_4OH, 4:1) = 0.35, R_f (nBuOH/H_2O/AcOH 4:1:1) = 0.12; ^1H NMR (300 MHz, DMSO-d_6) δ 10.14 (br s, 1 H, Pro $^+NH_2$), 8.99 (d, 1 H, J = 8.0 Hz, CONH), 8.55 (br s, 1 H, Pro $^+NH_2$), 7.37 (s, 1 H, trans $CONH_2$), 7.12 (s, 1 H, cis $CONH_2$), 4.24–4.35 (m, 1 H, 3-CH), 4.11–4.24 (m, 1 H, Pro α-CH), 3.86 (s, 2 H, CH_2CO), 3.08–3.54 (m, 4 H, 6-CH_2 and Pro δ-CH_2), 2.20–2.40 (m, 1 H, Pro β-CH_2), 1.93–2.11 (m, 1 H, 4-CH_2), 1.60–1.93 (m, 6 H, 4,5-CH_2 and Pro β,γ-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.9, 168.0 (CON, CONH and $CONH_2$), 58.7 (Pro α-C), 49.8 (CH_2CO), 49.4 (3-C), 48.5 (6-C), 45.6 (Pro δ-C), 29.7 (Pro β-C), 27.5 (4-C), 23.6 (Pro γ-C), 20.5 (5-C); FAB MS m/z 269 $(M - Cl)^+$. Anal. ($C_{12}H_{21}N_4O_3Cl{\cdot}1.5H_2O$) C, H, N.

3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxoperhydroazepine (15a). A solution of di-*tert*-butyl dicarbonate (6.95 g, 31.9 mmol) in H_2O/dioxane (60 mL/100 mL) was added to a solution of 3(*R*)-amino-2-oxoperhydroazepine (4.08 g, 31.9 mmol) and NEt_3 (3.26 g, 32 mmol) in 30 mL of H_2O. The yellow mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo, and the resultant slurry partitioned between 125 mL of EtOAc and 125 mL of 10% citric acid. The aqueous phase was washed once with EtOAc, and the organic phases were combined, dried over $MgSO_4$, filtered, and evaporated to afford 6.22 g (85.6%) of a pale yellow solid: mp 150–151 °C; $[\alpha]^{25}{}_D$ −39.2° (c 1.2, $CHCl_3$); FAB MS m/z 229 $(M + H)^+$. Anal. ($C_{11}H_{20}N_2O_3$) C, H, N.

3(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxoperhydroazepine (15b). This material was synthesized from 3(*S*)-amino-2-oxoperhydroazepine in a manner similar to that for compound 15a: mp 152–153 °C; $[\alpha]^{25}{}_D$ +39.0° (c 1.0, $CHCl_3$); FAB MS m/z 229 $(M + H)^+$. Anal. ($C_{11}H_{20}N_2O_3$) C, H, N.

Methyl 3(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-perhydroazepineacetate (16a). Compound 15a (4.5 g, 19.74 mmol) was dissolved in 120 mL of THF and the solution placed in a dry three-necked flask under a stream of N_2. The solution was cooled in an ice bath and NaH (50% w/w oil dispersion, 1.9 g 39.48 mmol) was added. After the solution was stirred for 30 min, methyl bromoacetate (6.04 g, 39.48 mmol) was added through a syringe. The mixture was stirred at 4 °C for 4 h and then at room temperature for 12 h. The reaction mixture was diluted with EtOAc and quenched with H_2O. The volatile solvents were evaporated, and the residue was partitioned between 50 mL each of EtOAc and H_2O. The aqueous phase was washed once with EtOAc (50 mL) and the organic phases were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo to an oil. MPLC (EtOAc/hexanes, 3:2) of the oil afforded 5.44 g (91.8%) of 16a as a clear oil: $[\alpha]^{25}{}_D$ = −22.8° (c 0.143, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.91 (br d, 1 H, J = 6.6 Hz, OCONH), 4.42 (dd, 1 H, J = 6.1 and 10.5 Hz, 3-CH), 4.16 (s, 2 H, CH_2CO_2), 3.72 (s, 1 H, OCH_3), 3.61–3.76 (m, 1 H, 7-CH_2), 3.16 (dd, 1 H, J = 4.8 and 15.3 Hz, 7-CH_2), 1.40–2.08 (m, 6 H, 4,5,6-CH_2), 1.42 (s, 9 H, Me_3C); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.5 (CON), 169.7 (CO_2Me), 155.0 (OCON), 79.4 (Me_3C), 53.5 (3-C), 52.2 (OCH_3), 50.7 (CH_2CO_2 and 7-CH_2), 32.6 (4-C), 28.5 ($(CH_3)_3C$), 27.1 and 28.0 (5,6-C); FAB MS m/z 301 $(M + H)^+$. Anal. ($C_{14}H_{24}N_2O_5$) C, H, N.

Methyl 3(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-1-perhydroazepineacetate (16b). This methyl ester was obtained from 15b by the same method that was used for the synthesis of its enantiomer 16a: $[\alpha]^{25}{}_D$ = +25.3° (c 0.95, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.90 (br d, J = 6.6 Hz, 1 H, OCONH), 4.42 (dd, 1 H, J = 6.2 and 10.6 Hz, 3-CH), 4.16 (s, 2 H, CH_2CO_2Me), 3.73 (s, 1 H, OCH_3), 3.61–3.73 (m, 1 H, 7-CH_2), 3.18 (dd, 1 H, J = 5.0 and 15.3 Hz, 7-CH_2), 1.30–2.20 (m, 6 H, 4,5,6-CH_2), 1.43 (s, 9 H, Me_3C); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8 ($CONCH_2$), 169.7 (CO_2Me), 155.2 (OCON), 79.4 (Me_3C), 53.5 (3-C), 52.2 (OCH_3), 50.7 (CH_2CO_2Me and 7-CH_2), 32.4 (4-C), 28.4 ($(CH_3)_3C$), 27.0 and 28.0 (5,6-C).

Methyl 3(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-perhydroazepineacetate (17a). Lactam ester 16a (0.25 g, 0.83 mmol) was stirred in 4 N HCl/dioxane (2 mL) in an ice bath for 1.5 h. The solvent was removed in vacuo and the product, methyl 3(*R*)-amino-2-oxo-1-perhydroazepineacetate hydrochloride, which was obtained as a white solid (0.195 g, 98.9%), was carried on to the next reaction without

further purification: mp 225–226 °C; $[\alpha]^{25}_D$ −7.5° (c, 0.8, MeOH); TLC R_f (EtOAc) = 0.30, R_f (nPrOH/NH_4OH, 4:1) = 0.69; FAB MS *m/z* 201 (M − Cl)$^+$.

Cbz-L-Pro-OH (0.16 g, 0.64 mmol) was dissolved in THF (5 mL) and the solution was cooled in a dry ice/CCl_4 bath to −20 °C, with stirring, under a stream of N_2. NEt_3 (0.09 mL, 0.64 mmol) was added, followed by isobutyl chloroformate (0.08 mL, 0.64 mmol). After stirring of the reaction mixture for 20 min, a mixture of methyl 3(*R*)-amino-2-oxo-1-perhydroazepineacetate hydrochloride (0.137 g, 0.58 mmol) and NEt_3 (0.08 mL, 0.58 mmol) in DMF (5 mL) was added. Stirring was continued at −20 °C for 2 h and at room temperature for another 2 h. The solvent was removed in vacuo and the residue was partitioned between EtOAc and H_2O. The organic phase was washed successively with 10% citric acid, saturated $NaHCO_3$ and H_2O, dried over $MgSO_4$, filtered, and stripped of solvent in vacuo to give a clear oil (0.25 g). The pure product was obtained as a white foam (0.14 g, 55.9%) after MPLC (EtOAc): $[\alpha]^{25}_D$ −61.4° (c 0.89, $CHCl_3$); FAB MS *m/z* 432 (M + H)$^+$. Anal. ($C_{22}H_{29}N_3O_6$) C, H, N.

Methyl 3(*S*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-perhydroazepineacetate (17b). Lactam ester **16b** was deprotected with 4 N HCl/dioxane to give methyl 3(*S*)-amino-2-oxo-1-perhydroazepineacetate hydrochloride: mp 225–226 °C; $[\alpha]^{25}_D$ +8.5° (c, 0.75, MeOH); TLC R_f (nBuOH/H_2O/AcOH 4:1/:1) = 0.31, R_f (nPrOH/NH_4OH 4:1) = 0.70; FAB MS *m/z* 201 (M − Cl)$^+$. This material was coupled to Cbz-Pro-OH using the mixed anhydride procedure described above for **17a** to give **17b**: $[\alpha]^{25}_D$ −48.1° (c 1.06, $CHCl_3$); FAB MS *m/z* 432 (M + H)$^+$. Anal. ($C_{22}H_{29}N_3O_6$) C, H, N.

3(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-perhydroazepineacetamide (20). Ester **17a** (1.03 g, 2.39 mmol) was stirred in methanolic ammonia (100 mL) for 2.5 days at room temperature. The solvent was evaporated under reduced pressure to give 0.98 g (98.6%) of **20** as a white foam: $[\alpha]^{25}_D$ −68.4° (c, 1.0, $CHCl_3$); FAB MS *m/z* 417 (M + H)$^+$, 415 (M − H)$^-$. Anal. ($C_{21}H_{28}N_4O_5$) C, H, N.

3(*S*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-perhydroazepineacetamide (21). This material was obtained from **17b** in the same manner as that described above for **20**: $[\alpha]^{25}_D$ −45.6° (c 1.06, $CHCl_3$); FAB MS *m/z* 417 (M + H)$^+$. Anal. ($C_{21}H_{28}N_4O_5 \cdot 0.5H_2O$) C, H, N.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-perhydroazepineacetamide (7). Compound **20** (0.199 g, 0.48 mmol) in 10 mL of MeOH was hydrogenated for 3 h at room temperature and pressure in the presence of 5% Pd/C (0.02 g). Filtration of the reaction mixture through Celite followed by removal of solvent in vacuo afforded a white solid (0.19 g) which when recrystallized from $CHCl_3/Et_2O$ gave 0.131 g (97.2%) of **7** as a white solid: mp 165–166 °C; $[\alpha]^{25}_D$ −7.96° (c 0.88, MeOH); TLC R_f (nPrOH/NH_4OH, 4:1) = 0.37; ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1 H, *J* = 7.4 Hz, CONH), 7.35 (s, 1 H, trans $CONH_2$) and 7.03 (s, 1 H, cis $CONH_2$), 4.53 (dd, 1 H, *J* = 6.4 and 10.3 Hz, 3-CH_2), 4.11 (d, 1 H, *J* = 16.5 Hz, CH_2CONH_2), 3.73 (d, 1 H, *J* = 16.5 Hz, CH_2CONH_2), 3.54–3.67 (m, 2 H, Pro α-CH and 7-CH_2), 3.09–3.26 (m, 1 H, 7-CH_2), 2.93 (br s, 1 H, Pro NH), 2.88 (dt, 1 H, *J* = 6.4 and 10.2 Hz, Pro δ-CH_2), 2.70 (dt, 1 H, *J* = 6.4 and 10.1 Hz, Pro δ-CH_2), 1.36–1.71 (m, 10 H, Pro β-CH_2, Pro γ-CH_2, and 4,5,6-CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4 (CON), 172.7 (CONH), 170.2 ($CONH_2$), 60.2 (Pro α-C), 51.2 (3-C), 51.1 (CH_2CONH_2), 49.2 (7-C), 46.7 (Pro δ-C), 31.4 (4-C), 30.1 (Pro β-C), 27.5, 26.4 (5,6-C), 25.9 (Pro γ-CH_2); FAB MS *m/z* 283 (M + H)$^+$. Anal. ($C_{13}H_{22}N_4O_3$) C, H, N.

3(*S*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-perhydroazepineacetamide (8). Compound **21** (0.199 g, 0.48 mmol) in 10 mL of MeOH was hydrogenated for 3 h at room temperature and pressure in the presence of 5% Pd/C (0.02 g). Filtration of the reaction mixture through Celite followed by removal of solvent in vacuo afforded a white solid (0.19 g) which when recrystallized from $CHCl_3/Et_2O$ gave 0.131 g (97.2%) of **21** as a white solid: mp = 151–152 °C; $[\alpha]^{25}_D$ −26.1° (c 1.44, MeOH); TLC R_f (nPrOH/NH_4OH 4:1) = 0.56, R_f (nBuOH/H_2O/HOAc 4:1:1) = 0.12; ^1H NMR (200 MHz, DMSO-d_6) δ 8.53 (d, 1 H, *J* = 6.4 Hz, CONH), 7.37 (s, 1 H, trans $CONH_2$) and 7.06 (s, 1 H, cis $CONH_2$), 4.51 (dd, 1 H, *J* = 6.3 and 10.3 Hz, 3-CH_2), 4.10 (d, 1 H, *J* = 16.5 Hz, CH_2CONH_2), 3.74 (d, 1 H, *J* = 16.5 Hz, CH_2CONH_2), 3.63 (m, Pro α-CH), 3.53 (dd, 1 H, *J* = 5.0 and 9.0 Hz, 7-CH_2), 3.12–3.27 (m, 1 H, 7-CH_2), 3.02 (br s, 1 H, Pro NH), 2.91 (dt, 1 H, *J* = 6.5 and 10.1 Hz, Pro δ-CH_2), 2.71 (dt, 1 H, *J* = 6.3 and 10.1 Hz, Pro δ-CH_2), 1.20–2.10 (m, 10 H, Pro β-CH_2, Pro γ-CH_2 and 4,5,6-CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 173.6 (CON), 172.9 (CONH), 170.4 ($CONH_2$), 60.4 (Pro α-C), 51.32 (CH_2CONH_2), 51.26 (3-C), 49.5 (7-C), 46.9 (Pro δ-C), 31.7 (4-C), 30.6 (Pro β-C), 27.6, 26.6 (5,6-C), 26.0 (Pro γ-C); FAB MS *m/z* 283 (M + H)$^+$. Anal. ($C_{13}H_{22}N_4O_3 \cdot 0.5H_2O$) C, H; N: calcd, 19.23, found 18.66.

D-(Benzyloxycarbonyl)-β-*tert*-butylaspartylglycinamide (22). Cbz-D-Asp(OBu^t)-OH (2 g, 6.19 mmol) was dissolved in dry DMF (20 mL), under a stream of N_2. Gly-NH_2·HCl (0.75 g, 6.8 mmol) and NEt_3 (0.95 mL, 6.8 mmol) were added, and the mixture was cooled in an ice bath. *N*-Hydroxysuccinimide (0.78 g, 6.8 mmol) was added, followed by DCC (1.84 g, 8.92 mmol). The reaction mixture was stirred for 3 h at 4 °C and then allowed to warm to room temperature over a 3-h period. The mixture was then stored in a refrigerator overnight. The mixture was stripped of DMF and the residue then placed in EtOAc. The suspension was filtered and the filtrate was washed with 10% citric acid followed by saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered, and stripped of solvent to give a white solid which when crystallized from Et_2O gave 2.1 g (96.6%) of **22**: mp 130.5–131 °C; $[\alpha]^{25}_D$ +13.8° (c 0.8, EtOH) [lit.[24] $[\alpha]^{25}_D$ −13.2° (EtOH) for the L-isomer].

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-D-β-*tert*-butylaspartylglycinamide (23).** A mixture of **22** (2.0 g, 5.28 mmol) and 5% Pd/C (0.47 g) in MeOH (60 mL) was hydrogenated at 30 psi for 70 min. The mixture was filtered through Celite and the filtrate was dried in vacuo to give 1.28 g (99%) of D-β-*tert*-butylaspartylglycinamide as a white foam. This material was used in the following reaction without further purification.

D-β-*tert*-Butylaspartylglycinamide (0.17 g, 0.69 mmol) and Boc-L-Pro-OSu (0.216 g, 0.69 mmol) were dissolved in 5 mL of DMF and stirred at room temperature for 24 h. The solvent was removed in vacuo and the resultant oil was partitioned between EtOAc (20 mL) and H_2O (10 mL). The organic phase was washed twice with H_2O, dried over $MgSO_4$, filtered, and evaporated to a white foam. MPLC ($CHCl_3$/MeOH, 9:1) afforded 0.22 g (70.9%) of **23** as a white solid: mp 108–110 °C; $[\alpha]^{25}_D$ +2.7° (c 0.74, EtOH); FAB MS *m/z* 443 (M + H)$^+$. Anal. ($C_{20}H_{34}N_4O_7$) C, H, N.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-1-succinimideacetamide Hydrochloride (4·HCl). Boc-L-Pro-D-Asp(OBu^t)-Gly-NH_2 (**23**, 0.83 g, 2.19 mmol) was dissolved in 25 mL of a freshly prepared saturated solution of HCl in AcOH. The mixture was stirred for 3.5 days at room temperature. The solvent was removed in vacuo, with CCl_4 used to azeotropically remove the AcOH. A light yellow syrup was obtained. This was taken up in water and the solution was treated with activated charcoal, filtered through glass wool and lyophilized to give 0.56 g of a clear, sticky glass. All attempts to crystallize this material from various solvents failed. The glassy material was dissolved in water and the solution treated with activated charcoal, filtered, and lyophilized to give 0.383 g (57.4%) of a white foam: $[\alpha]^{25}_D$ +11.0° (c 1.2, H_2O); TLC R_f (EtOAc/pyridine/AcOH/H_2O, 37:20:6:11) = 0.29; ^1H NMR (300 MHz, DMSO-d_6) δ 9.56 (d, 1 H, *J* = 7.5 Hz, CONH), 9.40 (br s, 1 H, Pro NH), 7.55 (s, 1 H, trans $CONH_2$), 7.26 (s, 1 H, cis $CONH_2$), 4.70 (AB q, 1 H, *J* = 8.0 and 13.0 Hz, Asu α-CH), 4.15–4.29 (m, 1 H, Pro α-CH), 3.96 (AB q, 2 H, *J* = 17.0 and 24.4 Hz, CH_2CONH_2), 3.05–3.55 (m, 5 H, Pro NH, Pro δ-CH_2, Asu β-CH_2), 2.69 (dd, 1 H, *J* = 4.8 and 17.6 Hz, Asu β-CH_2), 2.14–2.40 (m, 1 H, Pro β-CH_2), 1.68–2.05 (m, 3 H, Pro β-CH_2 and Pro γ-CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 175.0 (CONH), 174.3 ($CONH_2$), 168.9 (CONCO), 167.3 (CONCO), 58.5 (Pro α-C), 48.6 (Asu α-C), 45.5 (Pro δ-C), 40.6 (CH_2CONH_2), 34.9 (Asu β-C), 29.4 (Pro β-C), 23.5 (Pro γ-C); FAB MS *m/z* 269 (M − Cl)$^+$. Anal. ($C_{11}H_{17}N_4O_4Cl \cdot 2H_2O$) C, H, N.

4(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-3-oxo-2-isoxazolidine (25). (*R*)-Cycloserine (**24**, 0.306 g, 3 mmol) was suspended in CH_2Cl_2 (15 mL) with stirring, under N_2. Chlorotrimethylsilane (1.14 mL, 9 mmol) was added and the mixture was heated briefly to reflux. The heat source was removed and NEt_3 (1.26 mL, 9 mmol) was added. The mixture was then refluxed, with vigorous stirring, for 10 min. After a further 10 min of stirring the reaction mixture without heat, the white slurry was transferred via a cannula to a reaction vessel containing the mixed anhydride between Cbz-L-Pro-OH

(0.747 g, 3 mmol) and isobutyl chloroformate (0.39 mL, 4.42 mmol) that was formed in THF (15 mL) at −25 °C. The reaction mixture was stirred at −25 °C for 30 min and then at room temperature for 15 h. The solvent was removed in vacuo and the residue was partitioned between EtOAc and 10% citric acid. The organic phase was dried over $MgSO_4$, filtered, and concentrated to yield 1.39 g of a yellow foam. The purified product was obtained by MPLC (EtOAc/hexanes, 9:1) as a white solid in a yield of 0.71 g (71%): mp 151–152 °C; $[\alpha]^{25}{}_D$ −98.8° (c 0.85, $CHCl_3$); FAB MS *m/z* 334 (M + H)$^+$. Anal. ($C_{16}H_{19}N_3O_5$) C, H, N.

4(*R*)-[[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]amino]-3-oxo-2-isoxazolidineacetamide (26). Isoxazolidine **25** (0.97 g, 2.91 mmol) was dissolved in dry THF (10 mL) with stirring, under N_2. NEt_3 (0.45 mL, 3.2 mmol) was added, followed by a solution of bromoacetamide (0.52 g, 3.77 mmol) in 5 mL of THF. The mixture was stirred at room temperature for 20 h and then concentrated in vacuo to a white foam. MPLC (EtOAc/MeOH, 9:1) of the foam gave 0.725 g (63.8%) of **26** as a white solid: mp 106–107 °C; $[\alpha]^{25}{}_D$ −57.9° (c 1.01, MeOH); FAB MS *m/z* 391 (M + H)$^+$. Anal. ($C_{18}H_{22}N_4O_6$) C, H, N.

Cyclo(L-Pro-D-Ser) (27). Isoxazolidineacetamide **26** (0.35 g, 0.9 mmol) was dissolved in MeOH (10 mL) and hydrogenated for 14 h under 40 psi of H_2 in the presence of 10% Pd/C (0.035 g) and AcOH (0.056 mL, 0.99 mmol). The mixture was filtered through Celite and the filtrate was dried under reduced pressure. The residue was taken up in MeOH and the solid obtained upon filtration was dried in vacuo to provide 0.072 g of diketopiperazine **27**: mp 296–270 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 8.08 (br s, 1 H, CONH), 5.29 (t, 1 H, J = 4.9 and 5.5 Hz, OH), 4.11 (dd, 1 H, J = 6.8, 7.3 and 10.7 Hz, Ser α-CH), 3.60–3.81 (m, 2 H, Pro α-CH and Ser β-CH_2), 3.23–3.55 (m, 3 H, Pro δ-CH_2 and Ser β-CH_2), 2.04–2.23 (m, 1 H, Pro β-CH_2), 1.60–1.98 (m, 3 H, Pro β,γ-CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 169.4, 165.0 (CONH), 63.7 (Ser β-C), 59.7, 58.4 (Pro α-C and Ser α-C), 45.0 (Pro δ-C), 28.9 (Pro β-C), 21.6 (Pro γ-C). Anal. ($C_8H_{12}N_2O_3$) C, H, N.

4(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-3-oxo-2-isoxazolidineacetamide Hydrobromide (5·HBr). Isoxazolidineacetamide **26** (0.685 g, 1.76 mmol) was stirred in 10 mL of a solution of HBr in AcOH (34.75% w/w) at room temperature for 15 min. Et_2O (80 mL) was added and the mixture was chilled in a dry ice bath. More Et_2O (200 mL in total) was added and the flocculent white precipitate that formed was collected in a sintered-glass funnel under vacuum and then dried in vacuo to give 0.627 g of **5·HBr** as a white solid: mp 118–120 °C; $[\alpha]^{25}{}_D$ −2.9° (c 1.20, MeOH); TLC R_f (nBuOH/H_2O/AcOH, 4:1:1) = 0.09, R_f (EtOAc/pyridine/AcOH/H_2O, 37:20:6:11) = 0.11; ^1H NMR (300 MHz, DMSO-d_6) δ 9.28 (br s, 1 H, Pro $^+NH_2$), 9.22 (d, 1 H, J = 8.6 Hz, CONH), 8.69 (br s, 1 H, Pro $^+NH_2$), 7.54 (s, 1 H, trans $CONH_2$), 7.34 (s, 1 H, cis $CONH_2$), 4.94 (AB q, 1 H, J = 8.5, 9.2 and 17.4 Hz, 4-CH), 4.59 (t, 1 H, J = 7.9 and 9.2 Hz, 5-CH_2), 4.18–4.39 (m, 1 H, Pro α-CH), 4.21 (d, 1 H, J = 17.1 Hz, CH_2CONH_2), 4.02 (t, 1 H, J = 9.8 Hz, 5-CH_2), 3.98 (d, 1 H, J = 15.9 Hz, CH_2CONH_2), 3.08–3.29 (m, 2 H, Pro δ-CH_2), 2.18–2.37 (m, 1 H, Pro β-CH_2), 1.71–2.24 (m, 3 H, Pro β,γ-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.7, 168.6, 167.2 ($CONH_2$, CONH, CONO), 69.9 (5-C), 58.9 (Pro α-C), 51.3 (4-C), 48.5 (CH_2CONH_2), 45.7 (Pro δ-C), 29.6 (Pro β-C), 23.5 (Pro γ-C); FAB MS *m/z* 257 (M − Br)$^+$. Anal. ($C_{10}H_{17}N_4O_4Br$) C, H; N: calcd, 16.62; found, 15.30. HPLC analysis (C8, CH_3CN/0.1% aqueous TFA (9:1), 0.5 mL/min) indicated the product had a purity of 95.8% and a t_R = 9.76 min.

[^3H]ADTN Binding Assay. The detailed protocol for measuring the enhancement of binding of ADTN to dopamine receptors has been described earlier by us.[5,18] In short, striatal synaptosomal membranes from bovine caudate along with [^3H]-ADTN are incubated with or without (control) different concentrations (10^{-12}–10^{-4} M) of peptide analogue. Incubation is carried out in triplicate at 37 °C for 10 min. The mixtures are filtered and the filters counted on a liquid scintillation counter. In the present experiment the total binding was 3000 dpm and nonspecific binding was equal to 1200 dpm. The nonspecific binding is defined as the amount of [^3H]ADTN remaining bound in the presence of 10 μM (+)-butaclamol. The statistical significance of the data at various doses was determined using Student's *t*-test. The unpaired *t*-test was performed on the actual disintegrations/minute obtained at each dose level before converting them to percent enhancement. Values of p less than 0.05 are considered significantly different from the control.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of intermediates **9, 10, 12, 15a, 15b, 17a, 17b, 18, 19, 20, 21, 25, 26** (4 pages). Ordering information is given on any current masthead page.

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