Design, Synthesis, and Dopamine Receptor Modulating Activity of Spiro Bicyclic Peptidomimetics of L-Prolyl-L-leucyl-glycinamide

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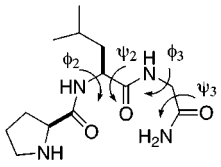
In the present study, the synthesis of the 5.5.6. and 5.6.5. spiro bicyclic lactam PLG peptidomimetics, compounds **3** and **4**, respectively, was undertaken. These peptidomimetics were designed to examine the following: (1) the effect that changing the size of the thiazolidine and lactam ring systems would have on the ability of these systems to mimic the type-II β-turn and (2) the effect that these structural perturbations would have on the ability of the peptidomimetics to modulate dopamine receptors. Through the use of the [^{3}H]spiroperidol/*N*-propylnorapomorphine (NPA) dopamine D_2 receptor competitive binding assay, **3** and **4**, at a concentration of 100 nM, decreased the dissociation constant of the high-affinity state of the dopamine receptor for the agonist. These effects were observed when either Gpp(NH)p was absent or present and they were comparable to those produced by PLG at a concentration of 1 μM. Peptidomimetics **3** and **4** also increased the percentage of D_2 receptors that existed in the high-affinity state. Even with Gpp(NH)p present, **3** and **4** were able to return the R_H/R_L ratios to values observed in the respective controls where Gpp(NH)p was absent. Furthermore, both peptidomimetics were able to attenuate the Gpp(NH)p-induced shift to the low-affinity state to a greater extent than PLG. Peptidomimetics **3** and **4** were evaluated in vivo as modulators of apomorphine-induced rotational behavior in the 6-hydroxydopamine-lesioned rat model of hemiparkinsonism, and each affected the rotational behavior in a bell-shaped dose-response relationship producing increases of 95 ± 31% (0.01 mg/kg, ip) and 88 ± 14% (0.001 mg/kg, ip), respectively. In comparison, the previously reported 5.5.5. spiro bicyclic lactam **2** increased rotational behavior by 25 ± 11% (0.01 mg/kg, ip).

L-Prolyl-L-leucyl-glycinamide (PLG, **1**) is an endogenously derived peptide that has been shown to modulate dopaminergic neurotransmission within the central nervous system. Through in vitro studies, PLG and its peptidomimetic analogues have been shown to increase the percentage of D_2 receptors that exist in the high-affinity state and to increase the affinity of the high-affinity state of the D_2 receptor for agonists.[1−5] They also have been shown to potentiate the contralateral rotational behavior induced by apomorphine in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway.[5−9]

In an attempt to elucidate the bioactive conformation of PLG, numerous conformationally constrained analogues of PLG have been synthesized and tested.[4,5,10−13] One such analogue, **2**, incorporated the highly rigid 5.5.5. spiro bicyclic system previously designed by us as a type-II β-turn mimic.[14] This analogue was found to possess the same ability as PLG with respect to enhancing the binding of [^{3}H]ADTN to dopamine receptors.[13] In the present study, the synthesis of the 5.5.6. and 5.6.5. spiro bicyclic lactam PLG peptidomimetics, compounds **3** and **4**, respectively, was undertaken. These peptidomimetics were designed to examine the effect that changing the size of the thiazolidine and lactam ring systems would have on the ability of these systems to mimic the type-II β-turn and the effect that these structural perturbations would have on the ability of the peptidomimetics to modulate dopamine receptors.



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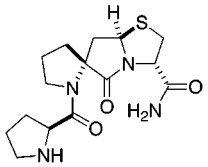

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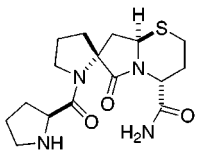

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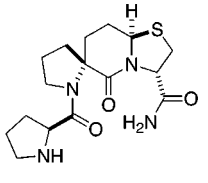

4

Synthesis

The synthesis of the 5.5.6. spiro bicyclic lactam PLG peptidomimetic **3** was carried out as outlined in Scheme 1. The synthesis started with the stereoselective allyl alkylation of L-Pro-OH by Seebach's method of self-

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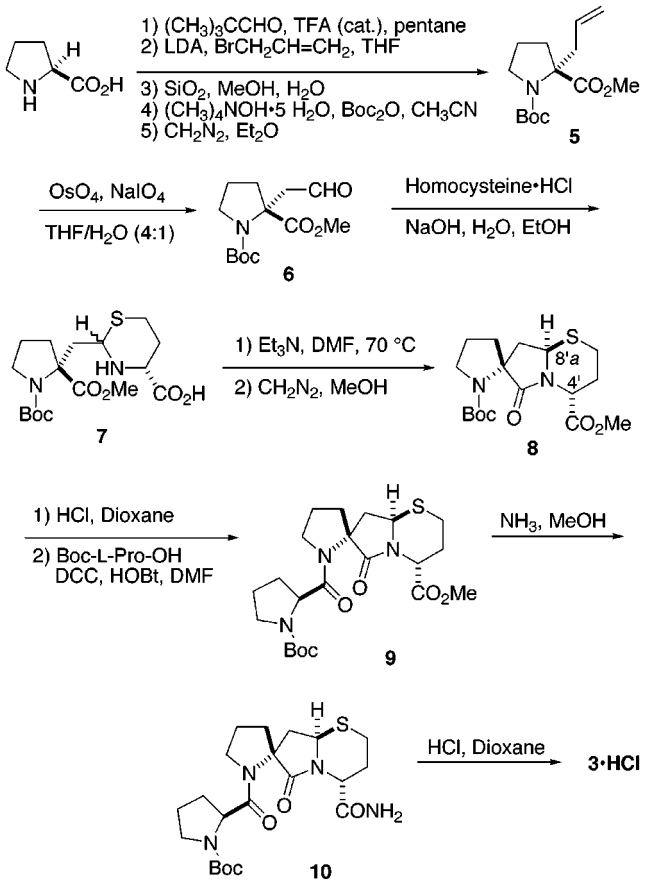
Scheme 1

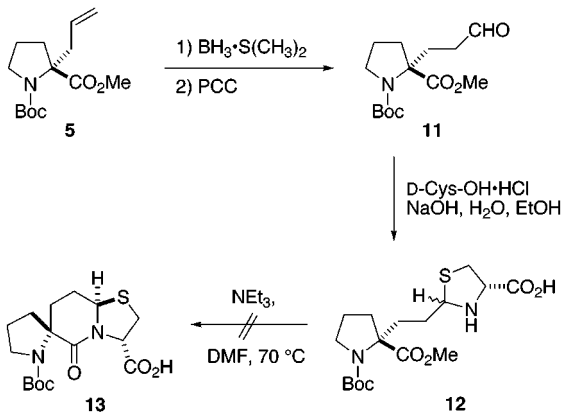


reproducing chirality.[15,16] The alkylated oxazolidinone thus formed was cleanly deprotected to (*R*)-α-allylproline under the mild conditions previously developed in our laboratory.[17] Amino protection of (*R*)-α-allylproline as the *N*-*tert*-butoxycarbamate was done as reported by Khalil et al.,[18] and this step was followed by esterification with diazomethane. Oxidative cleavage of the double bond of **5** with OsO_4 and $NaIO_4$ furnished the previously reported aldehyde **6**.[14]

Aldehyde **6** was condensed with D-homocysteine (prepared by the reduction of D-homocystine with PPh_3 in the presence of HCl as described by Overman et al.[19]) under the conditions reported previously by Genin and Johnson[14] to give a diastereoisomeric mixture of thiazinanes **7**. Conversion of **7** to spiro bicyclic lactam **8** was achieved by heating the diastereomeric mixture in DMF for 3 days followed by treatment of the crude spiro bicyclic carboxylic acid with diazomethane. Under the thermal reaction conditions, only the spiro bicyclic lactam diastereoisomer in which the C4′ and C8′*a* hydrogens are in an anti relationship was obtained. This result is in agreement with that seen with the 5.5.5 spiro bicyclic lactam system.[14] This stereochemical relationship was confirmed through the X-ray crystal structure of **8** (see Supporting Information).[20]

A number of routes were explored for the attachment of the prolyl residue to the deprotected derivative of **8**. In one route, **8** was converted to the primary amide with NH_3 in MeOH and the amino deprotected species then reacted with the pentafluorophenyl ester of Boc-L-proline. Although this method had proved useful in coupling other bicyclic lactams to Boc-L-proline,[12,21] it failed to produce any product in this case. In another attempt, the deprotected primary amide derivative of **8** was condensed with Boc-L-Pro-OH with excess PyBroP as the condensing reagent, since this reagent had been reported to give good results for the coupling of sterically demanding amino acids.[21,22] In our hands, only a modest yield (21%) of **10** was obtained when this reagent was used. The best results (81% yield) were obtained by deprotecting **8** with HCl in dioxane and coupling the deprotected amine with Boc-L-Pro-OH using excess dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) and a long reaction time (3 days). Ester **9** was smoothly converted to the primary amide **10**, which was deprotected to give PLG peptidomimetic **3** as a hygroscopic hydrochloride salt.

Scheme 2

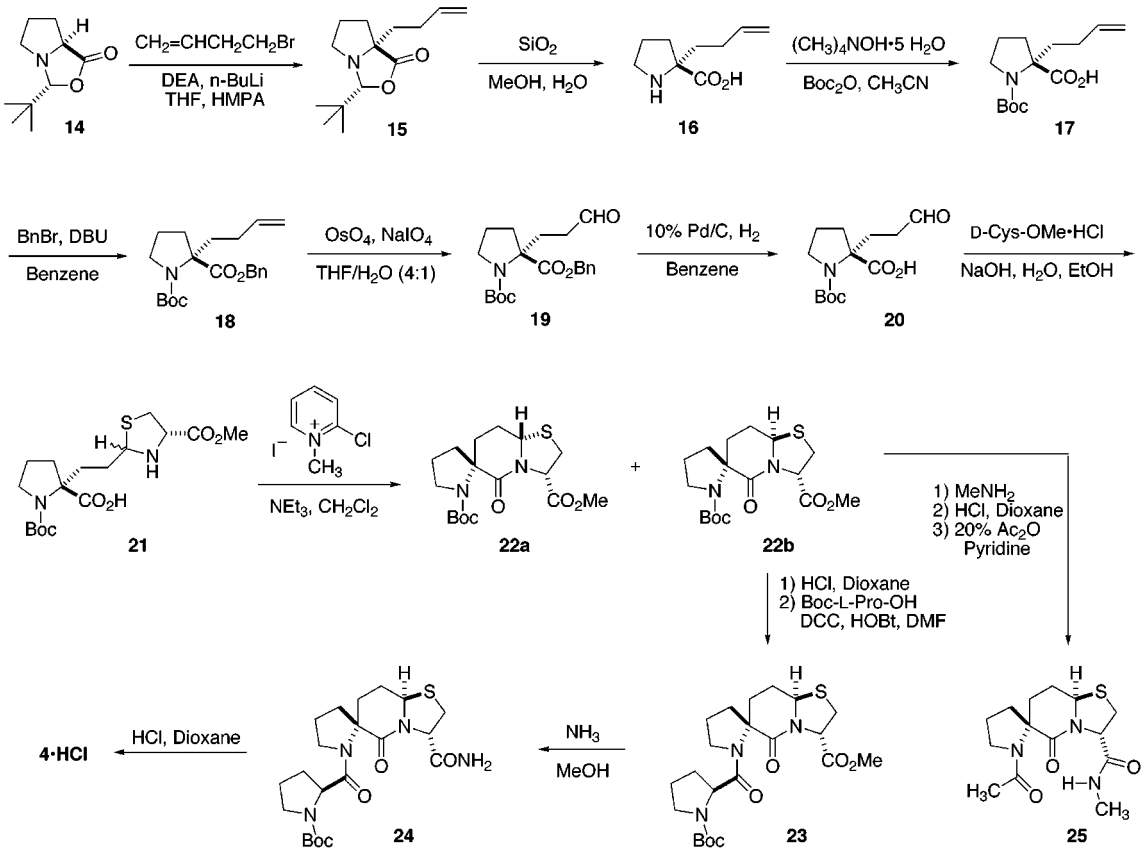


In building the 5.6.5. spiro bicyclic ring system, we initially envisioned applying the protocol of Brown et al.[23,24] for the oxidation of terminal olefins to their corresponding aldehydes to **5**. As outlined in Scheme 2, hydroboration of **5** followed by oxidation with pyridinium chlorochromate gave the desired aldehyde **11** in modest yields (25−30%). Condensation of this aldehyde with D-cysteine gave a diastereoisomeric mixture of thiazolidines (**12**). However, in contrast with the 5.5.6. spiro bicyclic system, formation of the lactam to give 5.6.5. spiro bicyclic system **13** did not take place under thermal reaction conditions.

To overcome the poor yields associated with the formation of **11** from **5** and to overcome the obstacle encountered in trying to form the 5.6.5. spiro bicyclic lactam ring system under thermal conditions, an alternate route to **4** was developed as outlined in Scheme 3. In a modification of Seebach's original procedure,[15] alkylation of oxazolidinone **14** with the relatively unactivated electrophile 4-bromo-1-butene was achieved in the presence of HMPA to give **15**.[25] This alkylated oxazolidinone was deprotected to **16** with the procedure of Genin et al.[17] This was followed by protection of the amino group under the conditions of Khalil et al.[18] Following the procedure of Ono et al.,[26] the carboxylic acid functionality of **17** was protected as the benzyl ester. Oxidative cleavage of **18** provided aldehyde **19** from which the benzyl ester was removed by palladium-catalyzed hydrogenolysis to give **20**. Condensation of this material with D-cysteine methyl ester hydrochloride afforded a mixture of epimeric thiazolidines (**21**).

In initial experiments, closure of **21** to the corresponding lactams was attempted with either DCC or

Scheme 3



PyBroP as the condensing agent. Both reactions resulted in complex mixtures that, after chromatography, gave very poor yields (<15%) of the desired product. Excellent results were ultimately achieved with the pyridinium-based condensing agent developed by Mukaiyama et al.[27] The epimeric lactams **22a** and **22b** were obtained in a 1:1 ratio (83% yield) after chromatographic separation. Delineation of the stereochemistry of these two isomers was accomplished by obtaining an X-ray crystal structure of **22b** (see Supporting Information).[20] Isomer **22b** was carried onto **4** by use of the same set of reaction conditions used to make **3**. This isomer also was converted to the model dipeptide isostere **25** by converting **22b** to the methylamide followed by acidolytic removal of the *tert*-butoxycarbonyl group and then acylation with acetic anhydride.

Pharmacological Studies

The novel spiro bicyclic lactam PLG peptidomimetics **3** and **4** were initially evaluated in a [^{3}H]spiroperidol/*N*-propylnorapomorphine (NPA) D_2 receptor competition binding assay.[2,5] The assay was carried out in either the presence or the absence of 5′-guanylylimidodiphosphate (Gpp(NH)p), a nonhydrolyzable analogue of GTP. The kinetic binding data obtained in such a study, along with that previously obtained for PLG, are summarized in Table 1. At a concentration of 100 nM, both **3** and **4** decreased the dissociation constant of the high-affinity state of the dopamine receptor for the agonist NPA, thereby increasing the affinity of the dopamine receptor for agonists. This effect was seen when either Gpp(NH)p was absent or present. In the presence of Gpp(NH)p, compound **3** decreased the agonist dissociation constant of the high-affinity receptor state from a control value of 0.122 nM to a value 0.064 nM. For **4**, the dissociation constant went from a control value of 0.196 nM to a value of 0.09 nM. No significant change in the dissociation constant of NPA for the low-affinity state of the receptor was observed. These effects on the dissociation constant were comparable to those produced by PLG at a concentration of 1 μM.

Peptidomimetics **3** and **4** also increased the percentage of D_2 receptors that existed in the high-affinity state, an effect that was seen both in the presence and absence of Gpp(NH)p. In the absence of Gpp(NH)p, treatment with **3** increased the ratio of the percentage of receptors in the high- and low-affinity states (R_H/R_L) from 0.92 to 1.77, while treatment with **4** resulted in an increase in R_H/R_L from 1.21 to 2.12. In the control experiment with Gpp(NH)p present, there is a significant decrease in the R_H/R_L ratio. The low-affinity state of the receptor becomes the predominant species. Both **3** and **4**, however, were able to attenuate significantly the Gpp(NH)p-induced shift to the low-affinity state. Treatment with **3** resulted in a change in the ratio from 0.17 to 1.12, while in the case of **4** the change in the ratio was from 0.2 to 1.22. Thus, whereas PLG partially reverses the Gpp(NH)p-induced shift to the low-affinity state, peptidomimetics **3** and **4** were able to return the R_H/R_L ratios to values observed in their respective controls where Gpp(NH)p was absent.

Peptidomimetics **3** and **4**, along with the previously reported spiro bicyclic lactam peptidomimetic **2**, also were evaluated in vivo as modulators of apomorphine-induced rotational behavior in the 6-hyroxydopamine-lesioned rat model of hemiparkinsonism.[5,8,9] In this

Table 1. Modulation of [^{3}H]Spiroperidol/*N*-Propylnorapomorphine Binding Competition by **3** and **4**[a]

experiment	binding parameters				
	K_H (nM)	K_L (nM)	R_H (%)	R_L (%)	R_H/R_L
control for **3**					
−Gpp(NH)p	0.071 ± 0.008	196 ± 16	52 ± 7	48 ± 3.2	1.08 ± 0.7
+Gpp(NH)p	0.122 ± 0.01	246 ± 18	15 ± 2	85 ± 5.8	0.17 ± 0.008
pretreatment with **3** (100 nM)					
−Gpp(NH)p	0.031 ± 0.002[b]	168 ± 12	64 ± 5[b]	36 ± 2.4	1.77 ± 0.52[b]
+Gpp(NH)p	0.064 ± 0.005[b]	202 ± 26	53 ± 4[b]	47 ± 3.5	1.12 ± 0.22[b]
control for **4**					
−Gpp(NH)p	0.067 ± 0.004	190 ± 17	53 ± 6	47 ± 3.5	1.13 ± 013
+Gpp(NH)p	0.196 ± 0.017	260 ± 19	17 ± 3	83 ± 6.7	0.2 ± 0.03
pretreatment with **4** (100 nM)					
−Gpp(NH)p	0.05 ± 0.002[b]	188 ± 22	68 ± 7[b]	32 ±4	2.12 ± 0.24[b]
+Gpp(NH)p	0.09 ± 0.006[b]	211 ± 24	55 ± 4[b]	45 ±6	1.22 ± 0.13[b]
control for **1**[d]					
−Gpp(NH)p	0.09 ± 0.005	73.2 ± 5.5	49 ± 5	51 ± 3	0.96 ± 0.05
+Gpp(NH)p	0.13 ± 0.01	60.0 ± 8.6	19 ± 2	82 ± 5	0.23 ± 0.01
pretreatment with **1** (1 μM)[d]					
−Gpp(NH)p	0.04 ± 0.002[c]	60.1 ± 5.4	61 ± 4[b]	41 ± 2	1.48 ± 0.06[c]
+Gpp(NH)p	0.06 ± 0.006[c]	78.0 ± 9.9	32 ± 3[c]	72 ± 6	0.44 ± 0.03[c]

[a] Competition data were obtained as described in ref 5. K_H and K_L represent the inhibitor constant (K_i) of agonist calculated for the high- and low-affinity components of the [^{3}H]spiroperidol binding, respectively. R_H and R_L are percentages of receptors in the high- and low-affinity form for the agonist, respectively. Values for each preparation are the mean ± SEM of three to four separate experiments with each experiment carried out in duplicate or triplicate. Concentration of Gpp(NH)p was 100 μM. [b,c] Statistical difference from the respective control group indicated as follows: (*b*) $p < 0.05$, (*c*) $p < 0.01$. [d] Data for **1** from ref 2.

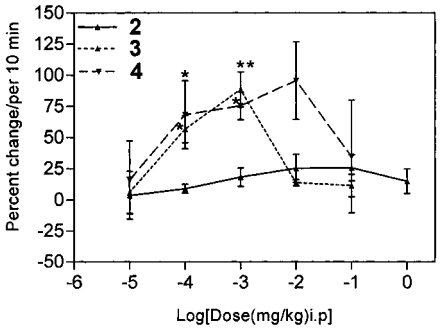


Figure 1. Effect of ip administered PLG peptidomimetics **2**, **3**, and **4** on contralateral behavior of ip administered apomorphine (0.25 mg/kg) in rats ($n = 4$) with unilateral lesions. Number of contralateral rotations in 10 min expressed as a percentage change from control rotational response. Dosage expressed as log (mg/kg) from −5 or 0.00001 mg/kg to 0 or 1 mg/kg. Each point represents the mean percent change ± SEM. Statistical significance: *$p < 0.05$, **$p < 0.005$.

model, a unilateral lesion is created in the substantia nigra of the rat with 6-hydroxydopamine, thereby leading to destruction of the dopaminergic efferents from the substantia nigra to the striatum. Loss of these neurons leads to a development of postsynaptic supersensitivity on the lesioned side which leads to a contralateral rotational response by the animal upon administration of a direct acting dopaminergic agonist like apomorphine.

The spiro bicyclic lactam PLG analogues were tested at a number of doses for their ability to modify the rotational response to apomorphine. The results are summarized in Figure 1. The peptidomimetics when administered with apomorphine were seen to affect the rotational behavior in a bell-shaped dose-response relationship. The maximal response to **2** occurred at 0.01 mg/kg, ip, resulting in a 25 ± 11% increase in contralateral rotations. Peptidomimetic **3** also had its maximum effect at 0.01 mg/kg, ip, but it produced an increase of 95 ± 31% in contralateral rotations. Compound **4**, on the other hand, produced a maximal change of 88 ± 14% at a dose of 0.001 mg/kg, ip.

Discussion

Both spiro bicyclic lactam peptidomimetics **3** and **4** were found in this study to exhibit a pharmacological profile similar to PLG in terms of their activity in the [^{3}H]spiroperidol/*N*-propylnorapomorphine D_2 receptor competition binding assay and the 6-hydroxydopamine-lesioned animal model of Parkinson's disease. In the former assay, **3** and **4** induced a change in the D_2 receptor *N*-propylnorapomorphine dissociation constant comparable to that induced by PLG. Both peptidomimetics, however, produced a greater shift from the low-affinity state to the high-affinity state of the dopamine D_2 receptor than did PLG.

The 5.5.6. and 5.6.5. spiro bicyclic lactam peptidomimetics, compounds **3** and **4**, respectively, were found to be much more effective in inducing apomorphine-induced rotational behavior in the 6-hydroxydopamine-lesioned animal model of Parkinson's disease than the 5.5.5. spiro bicyclic lactam peptidomimetic **2**. Furthermore, these two peptidomimetics are more active than PLG, as this peptide was shown previously to increase apomorphine-induced rotations 30% at a dose of 1.0 mg/kg, ip.[8] As with PLG, peptidomimetics **2**−**4** all produced bell-shaped dose-response curves. The underlying basis for such dose-response curves observed for PLG and its analogues is not known. Several models, however, have been proposed to account for such a phenomenon.[28]

The effectiveness of **3** and **4** with respect to PLG may be due to the ability of these spiro bicyclic lactams to mimic a type-II β-turn, which has been postulated to be the bioactive conformation of PLG.[10−13] A comparison of the torsion angles of the spiro bicyclic lactam systems with those for an ideal type-II β-turn are shown in Table 2. The values for the 5.5.5. system of **2** were obtained previously by computational methods.[14] Those for the 5.5.6. and 5.6.5. systems of **3** and **4** were obtained from the X-ray crystal structures of the respective amide derivatives **10** (Figure 2) and **25** (Figure 3).[20] Both **10** and **25** were found to exist in a type-II β-turn. A comparison of the three torsional angles (ϕ_2, ψ_2, and ϕ_3)

Table 2. Torsion Angle Comparisons of the Peptidomimetic Spiro Bicyclic Lactam Systems

system	ϕ_2	ψ_2	ϕ_3	ψ_3
5.5.5.[a]	−40.3	108.1	77.7	−16.7
5.5.6. (**9**)	−48.9	134.1	116.0	−29.8
5.6.5. (**21**)	−56	133.7	97.3	−19.9
ideal type-II β-turn[b]	−60 ± 30	120 ± 30	80 ± 30	0 ± 30

[a] Data for the 5.5.5. spiro bicyclic lactam system are from ref 14. [b] Values for the ideal type-II β-turn are from ref 29.

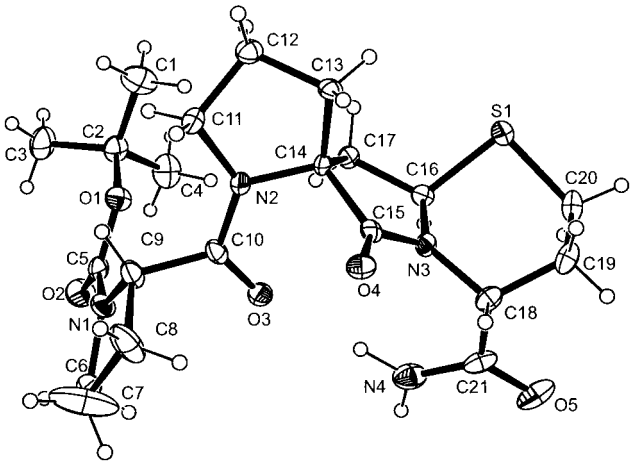


Figure 2. ORTEP representation of **10** at the 50% probability level with the crystallographic numbering system. Hydrogen atoms are drawn as spheres of arbitrary radius. A molecule of water was found to associate with the carboxamide functionality.

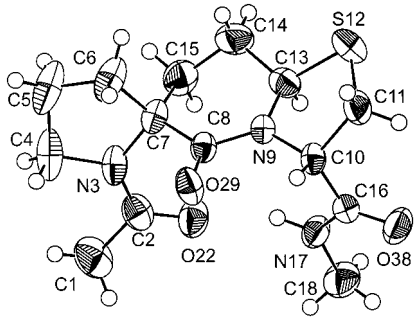


Figure 3. ORTEP representation of **25** at the 40% probability level with the crystallographic numbering system. Hydrogen atoms are drawn as spheres of arbitrary radius.

contrained by the 5.5.6. and 5.6.5. spiro bicyclic lactam systems found in **3** and **4**, respectively, shows the ϕ_2 and ψ_2 torsion angles have similar values. In contrast, the ψ_2 and ϕ_3 values for the 5.5.5. system are quite different from those seen for the 5.5.6 and 5.6.5. systems. The structural ramifications of these different ψ_2, and ϕ_3 torsion angles for the 5.5.5. system can be seen in the overlay of the three structures (Figure 4). There appear to be two main differences. One is the position of the lactam carbonyl where it is seen that that of the 5.5.5. system projects out from the ring in a different direction than do the carbonyls of the 5.5.6. and 5.6.5. spiro bicyclic systems. A second difference is the direction that the carboxamide moiety projects out from the spiro bicyclic systems. That of the 5.5.5. system projects in a significantly different direction than the carboxamide moieties of the 5.5.6. and 5.6.5. systems. Since the lactam carbonyl and carboxamide moiety are believed to be important pharmacophoric groups, this may

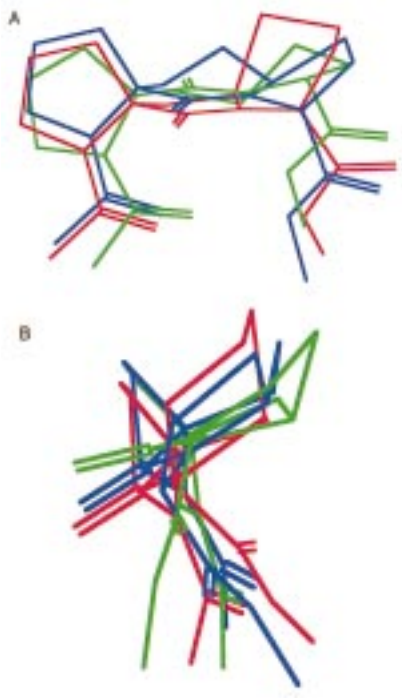


Figure 4. Overlay of the spiro bicyclic lactam ring systems of peptidomimetics **2** (green), **3**, (red), and **4** (blue) in which the N- and C-terminal ends of the ring systems have been substituted with the acetyl and *N*-methylcarboxamide moieties, respectively: A, frontal view; B, side view. The starting structures for the superimposition were obtained by computational methods in the case of **2**[14] and from X-ray structures in the case of **3** and **4**. The superimposition was done by calculating the best fit for all backbone atoms (10 atoms) using Insight II (release 95.0). Calculated RMS values were as follows: 5.6.5 and 5.5.6. = 0.26 Å; 5.5.5. and 5.5.6. = 0.59 Å; 5.5.5. and 5.6.5. = 0.47 Å.

explain the differences seen between peptidomimetic **2** and peptidomimetics **3** and **4**.

In summary, this study shows that changing the ring sizes of the spiro bicyclic lactam peptidomimetic systems does result in changes in the torsional angles which in turn produce changes in the pharmacological activity of the PLG peptidomimetics. These spiro bicyclic lactam systems should also prove to be valuable tools in studying the bioactive conformation of other biologically active peptides, since the ability to produce subtle differences in backbone torsional angles should be useful given the heterogeneous nature of β-turn secondary structures.

Experimental Section

General Aspects. Thin-layer chromatography was performed on Analtech 250 μm silica gel HLF Uniplates and were visualized by UV, I_2, ninhydrin spray (amines), 2,6-dichlorophenol indophenol spray (acids), and 2,4-dinitrophenylhydrazine (aldehydes). Chromatographic purification on silica gel (Merck, grade 60, 240−400 mesh, 60 Å) was done by flash or gravity methods and semipreparative HPLC was done with a Waters Associates 25 × 100 mm PrepPak cartridge. 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. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 300 and 75.5 MHz, respectively, with $CDCl_3$ as the internal reference for ^{1}H (δ 7.26) and ^{13}C (δ 77.06).

(2*RS*,4*R*)-2-[[2′-(*R*)-*N*-(*tert*-Butoxycarbonyl)-2′-(methoxycarbonyl)pyrrolidinyl]methyl]thiazinane-4-carboxylic Acid (7). D-Homocysteine·HCl (0.705 g, 4.13 mmol) was dissolved in H_2O (10 mL). NaOH (165 mg, 4.13 mEq) in H_2O (3.4 mL) was added followed by a solution of **6**[14] (1.12 g, 4.13 mmol) in EtOH (95%, 20 mL). The reaction mixture was stirred overnight at room temperature and then concentrated in vacuo to a white solid which was partitioned between H_2O (70 mL) and EtOAc (100 mL). The aqueous layer was extracted with additional portions of EtOAc (2 × 100 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was removed in vacuo to give 1.21 g (75%) of the diastereomeric mixture as a white solid. This material was used without further purification: mp 94−97 °C; FAB MS *m*/*z* 389 $[MH]^+$. Anal. ($C_{17}H_{28}N_2O_6S$) C, H, N, S.

Methyl [4′*R*-(4′α,7′α,8′aα)]-1-(*tert*-Butoxycarbonyl)tetrahydro-6′-oxospiro-[pyrrolidine-2,7′-(6′*H*)-pyrrolo[2,1-*b*]-thiazine]-4′-carboxylate (8). In an oven-dried 250 mL round-bottom flask, the thiazinane diastereomeric mixture **7** (1.19 g, 3.06 mmol) was dissolved in dry DMF (100 mL). To the flask was added NEt_3 (0.43 mL, 3.06 mol), and the solution was heated to 70 °C and stirred for 3 days under N_2. The solution turned a deep golden color with time. The DMF was removed in vacuo to give a dark yellow oil which was dissolved in MeOH (10 mL) and then cooled to 0 °C in an ice bath. CH_2N_2 in Et_2O was added in portions until the evolution of gas ceased. Excess CH_2N_2 and solvent were removed in vacuo. The resulting oil was dissolved in EtOAc (100 mL) and extracted with 10% citric acid (50 mL) and saturated NaCl solution and dried with $MgSO_4$. The solvent was removed in vacuo to give 1.1 g (97% crude yield) of a yellow oil which was chromatographed on a silica gel column using EtOAc/hexane (1:1) as eluent. The product was obtained as a colorless oil in a yield of 303 mg (27%). This oil was crystallized from Et_2O to give **8** as colorless crystals: mp 156−158 °C; $[\alpha]_D^{25}$ +126.1 (*c* 1.2, $CHCl_3$); ^1H NMR (presence of rotamers about the carbamate bond in a ratio of 3:2 observed, $CDCl_3$) 1.36 and 1.41 (s, 9 H, Boc CH_3), 1.71−2.05 (m, 4 H, 4-CH_2, 8′-CH_2, and 3′-CH_2), 2.13−2.43 (m, 3 H, 3-CH_2 and 3′-CH_2), 2.61 (dt, *J* = 14.6 and 3.0 Hz, 1 H, 2′-CH_2), 2.82 (dd, *J* = 13.4 and 8.5 Hz, 1 H, 8′-CH_2), 2.93−3.05 (m, 1 H, 2′-CH_2), 3.45−3.55 (m, 2 H, 5-CH_2), 3.72 and 3.76 (s, 3 H, OCH_3), 5.07−5.12 (m, 1 H, 4′-CH), 5.30 and 5.41 (d, *J* = 8.7, 1 H, 8′-CH); ^{13}C NMR ($CDCl_3$) δ 23.24 and 24.07 (4-C), 25.57 and 25.87 (3′-C), 27.63 and 28.00 (3-C), 29.01 and 29.19 (Boc CH_3), 37.22 and 37.55 (8′-C), 39.57 and 40.32 (2′-C), 48.29 and 48.42 (5-C), 51.91 and 51.99 (OCH_3), 52.90 and 53.23 (4′-C), 53.87 and 54 65 (8′a-C), 66.28 and 66.45 (7′-C), 80.30 and 81.25 (Boc C−O), 153.77 and 153.80 (Boc C═O), 171.00 (6′-C═O), 174.71 and 175.04 (CO_2CH_3); FAB MS *m*/*z* 371 (40%) $[MH]^+$, 271 (100%) $[M\text{-}(CH_3)_3COCO]^+$. Anal. ($C_{17}H_{26}N_2O_5S$) C, H, N, S.

Methyl [4′*R*-(4′α,7′α,8′aα)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]tetrahydro-6′-oxospiro[pyrrolidine-2,7′-(6′*H*)-pyrrolo[2,1-*b*]thiazine]-4′-carboxylate (9). Spiro bicyclic ester **8** (730 mg, 1.97 mmol) was deprotected in 4 N HCl in dioxane (10 mL) under Ar for 1 h. Solvent and excess HCl were removed in vacuo. Traces of HCl were removed with the aid of CH_2Cl_2 to give a white solid which was dissolved in DMF (40 mL). To that solution, Boc-L-Pro-OH (1.02 g, 4.73 mmol, 2.4 equiv), DCC (0.98 g, 4.73 mmol, 2.4 equiv), HOBt·H_2O (0.64 g, 4.73 mmol, 2.4 equiv), and Et_3N (0.27 mL, 1.97 mmol, 1 equiv) were added. The reaction was stirred at room temperature under Ar for 3 days. The DMF was removed in vacuo and the residue partitioned between H_2O (50 mL) and EtOAc (200 mL). The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with H_2O and brine and dried with $MgSO_4$, and the solvent was removed in vacuo. The crude product was isolated as an oil which was crystallized from EtOAc to give 644 mg of **9** as white needles. The mother liquor was chromatographed on silica gel (2% MeOH in EtOAc) affording an additional 100 mg of product (combined yield 81%): mp 216−217 °C; $[\alpha]_D^{25}$ +56.1 (*c* 0.26, $CHCl_3$), +25.6 (*c* 0.70, MeOH); ^1H NMR (presence of rotamers about the carbamate bond in approximately a 1:1 ratio observed, $CDCl_3$) δ 1.41 and 1.43 (s, 9 H, Boc CH_3), 1.62−2.21 (m, 10 H, Pro γ-CH_2, Pro β-CH_2, 4-CH_2, 3′-CH_2, and 3-CH_2), 2.36−2.41 (m, 1 H, 8′-CH_2), 2.57−2.66 (m, 1 H, 8′-CH_2), 2.96−3.13 (m, 2 H, 2′-CH_2), 3.32−3.62 and 3.94−4.01 (m, 4 H, 5-CH_2 and Pro δ-CH_2), 3.77 and 3.78 (s, 3 H, OCH_3), 4.35 and 4.46 (dd, *J* = 7.4 and 3.6 Hz, 1 H, Pro α-CH), 5.06 (d, *J* = 4.0 Hz, 1 H, 4′-CH), 5.37 (dd, *J* = 7.4, 6.0 Hz, 1 H, 8′a-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.23 and 25.00 (Pro γ-C), 24.69 (4-C), 25.74 (3′-C), 27.64 (3-C), 29.08 (Boc CH_3), 29.90 and 30.83 (Pro β-C), 37.09 (8′-C), 38.84 (2′-C), 47.40 and 47.57 (Pro δ-C), 48.32 (5-C), 51.92 (OCH_3), 52.97 and 53.00 (Pro α-C), 54.78 (4′-C), 58.34 (8′a-C), 67.36 (7′-C), 79.94 (Boc C−O), 154.2 and 156.2 (Boc C═O), 170.74 and 170.77 (CO_2CH_3), 171.5, 171.6, 173.7, and 173.9 (6′-C═O and Pro C═O); FAB MS *m*/*z* 468 $[MH]^+$. Anal. ($C_{22}H_{33}N_3O_6S$) C, H, N, S.

[4′*R*-(4′α,7′α,8′aα)]-1-[[(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]tetrahydro-6′-oxospiro[pyrrolidine-2,7′-(6′*H*)-pyrrolo[2,1-*b*]thiazine]-4′-carboxamide (10). Spiro bicyclic ester **9** (550 mg, 1.48 mmol) was treated with a concentrated solution of ammonia in MeOH (10 mL) at room temperature. After the mixture was stirred for 3 h, the solvent and excess ammonia were removed in vacuo to give a white foam which was chromatographed on a silica gel column using EtOAc/hexanes (1:1) as the eluting solvent. The product was isolated as a white foam which was crushed to a fine white powder (520 mg, 98%): mp 104−106 °C; $[\alpha]_D^{25}$ +107.0 (*c* 96, MeOH); ^1H NMR ($CDCl_3$) δ 1.41 (s, 9 H, Boc CH_3), 1.72−2.01 (m, 4 H, 4-CH_2, 3′-CH_2), 2.06−2.15 (m, 1 H, 3-CH_2), 2.32−2.41 (m, 1 H, 3-CH_2), 2.65 (dt, *J* = 14.0 and 3.7 Hz, 1 H, 2′-CH_2), 2.76−2.83 (m, 2 H, 8′-CH_2), 2.96 (dt, *J* = 14.0 and 2.4 Hz, 1 H, 2′-CH_2), 3.48 (dd, *J* = 7.8 and 4.8 Hz, 5-CH_2), 4.93 (dd, *J* = 5.4 and 1.8 Hz, 1 H, 4′-CH), 4.99 (dd, *J* = 9.2 and 3.2 Hz, 1 H, 8′a-CH), 5.39 (br s, 1 H, *cis*-$CONH_2$), 7.74 (br s, 1 H, *trans*-$CONH_2$); ^{13}C NMR ($CDCl_3$) δ 24.54 (4-C), 25.65 (3′-C), 26.70 (3-C), 29.08 (Boc CH_3), 38.51 and 39.70 (8′-C and 4′-CH_2), 48.29 (5-C), 52.69 (4′-C), 55.38 (8′a-C), 65.84 (7′-C), 81.43 (Boc C−O), 154.88 (Boc C═O), 171.72 and 173.80 ($CONH_2$ and 6′-C═O); FAB MS *m*/*z* 356 (10%) $[MH]^+$, 256 (100%) $[M\text{-}(CH_3)_3COCO]^+$. Anal. ($C_{16}H_{25}N_3O_4S$) C, H, N, S.

[4′*R*-(4′α,7′α,8′aα)]-1-[2(*S*)-Pyrrolidinylcarbonyl]tetrahydro-6′-oxospiro [pyrrolidine-2,7′-(5′*H*)-pyrrolo[2,1-*b*]thiazine]-4′-carboxamide Hydrochloride (3). Spiro bicyclic amide **10** (210 mg, 0.46 mmol) was deprotected in 4 N HCl in dioxane for 8 h under N_2. The solvent and excess HCl were removed in vacuo. The resulting residue was twice suspended in CH_2Cl_2 (5 mL) and then evaporated to a white solid. This material was lyophilized to give **3**·HCl as a hygroscopic white solid (180 mg, 100%): mp 139−140 °C; $[\alpha]_D^{25}$ +83.0 (*c* 1.45, MeOH); ^1H NMR (DMSO-d_6) δ 1.54−1.74 and 1.80−1.99 (m, 8 H, 4-CH_2, Pro γ-CH_2, 3-CH_2, and 3′-CH_2), 2.31−2.49 (m, 3 H, 8′-CH_2, Pro β-CH_2), 2.65−2.88 (m, 3 H, 8′-CH_2 and 2′-CH_2), 3.09−3.13 (m, 2 H, 5-CH_2), 3.38−3.44 (m, 1 H, Pro δ-CH_2), 3.74−3.79 (m, 1 H, Pro δ-CH_2), 4.42 (t, *J* = 7.6 Hz, 1 H, Pro α-CH), 4.62 (d, *J* = 4.9 Hz, 1 H, 4′-CH), 5.13 (d, *J* = 8.6 Hz, 1 H, 8′a-CH), 6.98 (br s, 1 H, *cis*-$CONH_2$), 7.53 (br s, 1 H, *trans*-$CONH_2$), 8.62 (br s, 1 H, $^+NH_2$), 10.12 (br s, 1 H, $^+NH_2$); ^{13}C NMR (DMSO-d_6) δ 23.41 and 23.77 (4-C and Pro γ-C), 25.03 and 25.47 (3′-C and 3-C), 27.77 (Pro β-C), 36.02 and 37.25 (8′-C and 2′-CH_2), 45.44 and 47.54 (Pro δ-C and 5-C), 51.14 (Pro α-C), 54.09 (4′-C), 58.09 (8′a-C), 66.62 (7′-C), 166.62, 170.39, and 171.53 ($CONH_2$, 6′-C═O, and Pro C═O); FAB HRMS *m*/*z* 353.1645 ($C_{16}H_{24}N_4O_3S$ + H^+ requires 353.1649). Anal. ($C_{16}H_{24}N_4O_3S$·HCl·$3H_2O$) C, H, N, S.

Methyl (*R*)-*tert*-Butoxycarbonyl-2-(3′-formylethyl)prolinate (11). To an oven-dried 500 mL round-bottom flask charged with 40 mL of distilled CH_2Cl_2 was transferred under N_2 a 2.0 M solution of borane−methyl sulfide complex in THF (1.55 mL, 3.1 mmol). Compound **5** (2.48 g, 9.22 mmol) was dissolved in 5 mL of dry CH_2Cl_2, and this solution was transferred dropwise to the reaction flask at room temperature. The solution was stirred under Ar for 1 h, after which time the mixture was concentrated in vacuo. The resulting trialkylborane was suspended in CH_2Cl_2 (10 mL) and then

transferred dropwise to a well-stirred suspension of pyridinium chlorocarbonate (3.9 g, 18.4 mmol) in CH_2Cl_2 (40 mL). After the addition was complete, the suspension was refluxed for 4 h with stirring. The reaction mixture was allowed to cool to room temperature, diluted with 100 mL of Et_2O, and filtered through a pad of silica gel. The filtrate was concentrated in vacuo, and the residue was subjected to silica gel chromatography (EtOAc/hexanes, 1:3) to give 0.65 g (25%) of product as a tan oil: $[\alpha]_D^{25}$ +24.3 (*c* 0.4, $CHCl_3$); FAB MS *m*/*z* 286 $[MH]^+$. Anal. ($C_{14}H_{23}NO_5$) C, H, N.

(2*RS*,4*S*)-2-[[2′(*R*)-*tert*-Butoxycarbonyl)-2′-(methoxycarbonyl)pyrrolidinyl]ethyl]thiazolidine-4-carboxylic Acid (12). To a solution of D-cysteine hydrochloride monohydrate (0.4 g, 2.29 mmol) dissolved in H_2O (5 mL) was added a solution of NaOH (92 mg, 2.29 mmol) in H_2O (1.9 mL). A solution of **5** (0.65 g, 2.29 mmol) in EtOH (5 mL) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with 15 mL of H_2O and then extracted with EtOAc (2 × 100 mL). The pH of the aqueous layer was adjusted to 5 with 1 N $NaHCO_3$, and then the aqueous layer was extracted with an additional portion of EtOAc (75 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was removed in vacuo to give 0.17 g (80%) of diastereoisomers as a white foam, which was used without further purification: FAB MS *m*/*z* 389 $[MH]^+$.

(2*R*,5*S*)-5-(3′-Butenyl)-2-*tert*-butyl-1-aza-3-oxabicyclo-[3.3.0]-octan-4-one (15). Freshly distilled diethylamine (28.4 mL, 0.274 mol) and THF (300 mL) were transferred via cannula to a dry 1 L round-bottom flask under Ar. The solution was cooled to −78 °C, and then a 1.6 M solution of *n*-BuLi in hexanes (158 mL, 0.253 mol) was added via a syringe while the reaction was stirred. After 10 min, a solution of freshly prepared oxazolidinone **14** in dry THF (150 mL) was transferred to the reaction vessel with the aid of a cannula, while maintaining the temperature at −78 °C. The resulting solution was stirred for 20 min and then HMPA (44 mL, 0.253 mol) was added. After an additional 30 min, 4-bromo-1-butene was added dropwise in 60 mL of THF over ca. 1 h. The reaction was stirred for 12 h at −76 °C and for 5 more hours at −40 °C (CH_3CN/dry ice bath). The reaction mixture was concentrated in vacuo to an oil which was partitioned between H_2O (250 mL) and CH_2Cl_2 (400 mL). The aqueous layer was washed with an additional portion of CH_2Cl_2 (400 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was removed to give an orange oil which was purified by Kugelrohr distillation (bp 90−120 °C, 0.2 mmHg) to give **15** as a clear oil (25.4 g, 59%). ^1H and ^{13}C NMR indicated the presence of about 13% HMPA. This material was used without further purification. ^1H NMR ($CDCl_3$) δ 0.88 (s, 9 H, $(CH_3)_3C$), 1.54−1.91 (m, 5 H, 7-CH_2, 6-CH_2, and C$H_2$$CH_2$CH=$CH_2$), 2.04−2.32 (m, 3 H, C$H_2$$CH_2$CH=$CH_2$, and CH_2CH_2CH=CH_2), 2.80−3.03 (m, 2 H, 8-CH_2), 4.22 (s, 1 H, 2-CH), 4.92−5.05 (m, 2 H, CH=CH_2), 5.74−5.87 (m, 1 H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 24.94 (C(CH_3)$_3$), 25.46 (7-C), 28.79 ($CH_2$$CH_2$CH=$CH_2$), 36.34, 37.04, and 37.53 (CH_2CH_2CH=CH_2, *C*(CH_3)$_3$, and 6-C), 58.09 (8-C), 72.31 (5-C), 105.71 (2-C), 115.35 (CH=CH_2), 138.67 (*C*H=CH_2), 179.01 (4-C).

(*S*)-2-(3′-Butenyl)proline (16). Oxazolidinone **15** (21.2 g, 89.7 mmol) was dissolved in 220 mL of MeOH/H_2O (6:1). Silica gel (22 g, 200−400 mesh, 60 Å) was added to that solution, and the resulting suspension was stirred at room temperature for 48 h. The reaction mixture was filtered on a fritted Buchner funnel, and the filtrate was evaporated in vacuo to a yellowish solid which was triturated with copious amounts of Et_2O to give 14.3 g (94%) of the pure free amino acid **16** as a white solid: mp 281−282 °C (dec); $[\alpha]_D^{25}$ −73.6 (*c* 0.88, MeOH). ^1H NMR (DMSO-d_6) δ 1.5−2.1 (m, 7 H, β-CH_2, γ-CH_2, C$H_2$$CH_2$-CH=$CH_2$, and CH_2CH_2CH=CH_2), 2.19−2.24 (m, 1 H, CH_2CH_2-CH=CH_2), 2.93−3.15 (m, 1 H, δ-CH_2), 3.16−3.23 (m, 1 H, δ-CH_2), 4.89−4.99 (m, 2 H, CH=CH_2), 5.67−5.82 (m, 1 H, CH=CH_2), 8.34 (br s, 1 H, $^+NH_2$), 8.74 (br s, 1 H, $^+NH_2$); ^{13}C NMR (75 MHz, $CDCl_3$ with trace CD_3OD) δ 24.20 (γ-CH_2), 30.04 (CH_2CH_2CH=CH_2), 35.85 and 36.47 (CH_2CH_2CH=CH_2 and β-C), 46.21 (δ-C), 74.51 (α-C), 115.97 (CH=CH_2), 137.40 (*C*H=CH_2), 175.29 (C=O); FAB MS *m*/*z* 170 $[MH]^+$, 339 $[2M + H]^+$. Anal. ($C_9H_{15}NO_2$) C, H, N.

(*S*)-*N*-*tert*-Butoxycarbonyl-2-(3′-butenyl)proline (17). (*R*)-2-(3′-Butenyl)proline (**16**, 4.0 g, 23.6 mmol) and tetramethylammonium hydroxide pentahydrate (4.28 g, 23.6 mmol) were added to CH_3CN (100 mL) which had been freshly distilled from CaH_2. The mixture was stirred at room temperature until a solution was formed. Boc_2O (7.73 g, 35.4 mmol) was then added, and stirring was continued for 2 days. On the third day, another 0.5 equiv of Boc_2O (2.6 g, 11.8 mmol) was added, and the mixture was stirred for another day. The CH_3CN was removed in vacuo, and the residue was partitioned between H_2O and Et_2O. The aqueous layer was washed with an additional portion of Et_2O and then acidified with solid citric acid to pH 3−4. The aqueous solution was extracted 3 times with EtOAc. The combined organic extracts were washed with H_2O and dried with $MgSO_4$, and the EtOAc was removed in vacuo to give **17** as a white solid (6.2 g, 98%), which was recrystallized from hot Et_2O: mp 90−91 °C; $[\alpha]_D^{25}$ +362 (*c* 2.13, MeOH). ^1H and ^{13}C NMR show the presence of 2 rotamers about the carbamate bond in a ratio of 3:2: ^1H NMR ($CDCl_3$) δ 1.42 and 1.49 (s, 9 H, Boc CH_3), 1.7−2.3 (m, 7 H, β-CH_2, γ-CH_2, and C$H_2$$CH_2$CH=$CH_2$), 2.70−2.78 (m, 1 H, CH_2CH_2-CH=CH_2), 3.26−3.78 (m, 2 H, δ-CH_2), 4.94−5.06 (m, 2 H, CH=CH_2), 5.70−5.86 (m, 1 H, CH=CH_2), 11.05 (br s, 1 H, COOH); ^{13}C NMR ($CDCl_3$) δ 23.35 (γ-C), 28.56, 29.02 (Boc CH_3), 34.20, 34.66, 35.74, 38.15, and 38.20 (β-C and CH_2CH_2-CH=CH_2), 49.22, 49.95 (δ-C), 70.92 (α-C), 82.73 (Boc C−O), 115.31, 115.83 (CH=CH_2), 137.87, 138.69 (*C*H=CH_2), 157.73 (Boc C=O), 175.39 (COOH); FAB MS *m*/*z* 270 $[MH]^+$, 214 $[M\text{-}(CH_3)_3C]^+$. Anal. ($C_{14}H_{23}NO_4$) C, H, N.

(*S*)-*N*-*tert*-Butoxycarbonyl-2-(3′-butenyl)proline Benzyl Ester (18). By use of the procedure of Ono et al.[26] **17** (4.3 g, 16.0 mmol) was dissolved in benzene (60 mL). 1,8-Diazabicyclo[5.4.0]undec-7-ene (2.43 mL, 16.0 mmol) was added followed by a solution of benzyl bromide (2.3 mL, 19.2 mmol) in benzene (5 mL). A white precipitate appeared after ca. 10 min. The mixture was refluxed, and reaction progress was monitored by TLC. After 4.5 h, additional benzyl bromide was added (0.57 mL, 4.8 mmol), and the reaction was refluxed for 4 more hours. The mixture was cooled to room temperature, and the precipitate was removed by filtration with suction. The white solid was washed with EtOAc. The combined filtrate and EtOAc wash were extracted with H_2O (50 mL), 10% citric acid (70 mL), 1 N $NaHCO_3$ (50 mL), and again with H_2O (50 mL). The organic layer was dried with $MgSO_4$, and the solvent was removed in vacuo to give a pale yellow oil which was subjected to silica gel chromatography (EtOAc/hexanes, 1:10). The pure product was isolated as a clear oil in 93% yield (5.27 g): $[\alpha]_D^{25}$ +8.4 (*c* 1.1, $CHCl_3$); TLC R_f = 0.21 (EtOAc/hexanes, 1:10). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a ratio of 2:1: ^1H NMR ($CDCl_3$) δ 1.30 and 1.38 (s, 9 H, Boc CH_3), 1.73−2.08 (m, 7 H, β-CH_2, γ-CH_2, and C$H_2$$CH_2$CH=$CH_2$), 2.22−2.50 (m, 1 H, CH_2CH_2-CH=CH_2), 3.27−3.74 (m, 2 H, δ-CH_2), 4.88−5.18 (m, 4 H, CH=CH_2 and OCH_2Ph), 5.70−5.83 (m, 1 H, CH=CH_2), 7.24−7.32 (m, 5 H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.71 and 23.86 (γ-C), 28.56, 28.88, 29.03, 33.91, 34.84, 36.78, and 38.06 (CH_2CH_2CH=CH_2, Boc CH_3, and β-C), 49.25 (δ-C), 67.26, 67.35, 67.95, and 68.42 (α-C and OCH_2Ph), 80.02 and 80.63 (Boc C−O), 115.03 and 115.29 (CH=CH_2), 128.56, 128.60, 128.72, 128.86, 129.04, 129.22, 136.39, and 136.76 (Ph), 138.75, 139.11 (*C*H=CH_2), 154.35 and 154.57 (Boc C=O), 175.01 and 175.14 (*C*O_2Bn); FAB MS *m*/*z* 360 $[MH]^+$; 304 $[M\text{-}(CH_3)_3C]^+$; 260 $[M\text{-}(CH_3)_3COCO]^+$. Anal.($C_{21}H_{29}NO_4$) C, H, N.

(*S*)-*N*-*tert*-Butoxycarbonyl-2-(3′-formylethyl)proline Benzyl Ester (19). Alkene **18** (5.2 g, 14.46 mmol) was dissolved in THF/H_2O (250 mL, 4:1). To that solution OsO_4 (5 mol %, 184 mg, 0.723 mmol) was added while the reaction was stirred under Ar. After 5 min, $NaIO_4$ (7.73 g, 36.15 mmol) was added in several portions. The reaction mixture changed in color from black to lavender-purple upon addition of $NaIO_4$. The reaction was stirred overnight at room temperature, and

then the mixture was gravity-filtered to remove a white precipitate. The clear filtrate was concentrated in vacuo to about $^1/_4$ of its volume, and then it was extracted with EtOAc (2 × 200 mL). The organic layer was dried ($MgSO_4$), and the solvent was removed under vacuum to give a brown oil. This oil was chromatographed on a silica gel column using EtOAc/hexanes (1:1 → 3:1) as the eluent to give 4.3 g (82%) of the clean product as pale yellow oil: $[\alpha]_D^{25}$ +11.5 (*c* 1.7, $CHCl_3$). ^{1}H and ^{13}C NMR show the presence of 2 rotamers about the carbamate bond in a ratio of 1:1. ^{1}H NMR ($CDCl_3$) δ 1.32 and 1.38 (s, 9 H, Boc CH_3), 1.7−2.3 (m, 5 H, β-CH_2, γ-CH_2, and CH_2CH_2CHO), 2.3−2.72 (m, 3 H, CH_2CH_2CHO and β-CH_2), 3.27−3.75 (m, 2 H, δ-CH_2), 5.02−5.22 (m, 2 H, OCH_2Ph), 7.27−7.34 (m, 5 H, Ph), 9.67 and 9.76 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 22.26 and 22.87 (γ-C), 27.26 and 27.33 (CH_2CH_2-CHO), 27.93 and 27.99 (Boc CH_3), 36.12 and 37.07 (β-C), 38.68 and 39.02 (CH_2CH_2CHO), 48.18 and 48.26 (δ-C), 66.60 and 66.65 (OCH_2Ph), 66.70 and 67.12 (α-C), 79.69 and 80.30 (Boc C−O), 127.73, 127.83, 128.07, 128.17, 128.34. 135.16, and 135.53 (Ph), 153.35 and 154.03 (Boc C═O), 173.51 and 174.03 (*C*O_2Bn), 200.99 and 202.59 (CHO); FAB MS *m*/*z* 362 $[MH]^+$. Anal. ($C_{20}H_{27}NO_5$) C, H, N.

(*R*)-*N*-*tert*-Butoxycarbonyl-2-(3′-formylethyl)proline (20). Compound **19** (1.95 g, 5.4 mmol) was dissolved in benzene (25 mL) in an Ar-purged pressure bottle. To that solution, 10% Pd/C (200 mg) catalyst was added, and the mixture was placed on a Parr shaker under 35 psi of H_2 for 34 h. The mixture was gravity-filtered, and the filtrate was passed through a 0.45 μm PTFE filter disk. Concentration of the resulting clear solution under vacuum gave 1.36 g (93%) of **20** as a white foam, which was used without further purification: $[\alpha]_D^{25}$ −4.6 (*c* 0.87, MeOH); $[\alpha]_{365}^{25}$ −26.4 (*c* 0.87, MeOH). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a ratio of 1:1: ^{1}H NMR ($CDCl_3$) δ 1.28 and 1.30 (s, 9 H, Boc CH_3), 1.68−1.93 (m, 3 H, γ-CH_2 and CH_2CH_2CHO), 2.07−2.40 (m, 5 H, CH_2CH_2CHO, CH_2CH_2CHO, and β-CH_2), 3.2−3.6 (m, 2 H, δ-CH_2), 9.62 and 9.78 (m, 2 H, CHO and COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.12 and 23.03 (γ-C), 27.69 and 28.07 (CH_2-CH_2CHO), 28.88 and 28.98 (Boc CH_3), 37.03 and 37.97 (β-C), 39.3 (CH_2CH_2CHO), 49.02 and 49.20 (δ-C), 67.37 and 67.98 (α-C), 81.18 and 81.64 (Boc C−O), 154.48 and 155.48 (Boc C═O), 177.95 and 179.20 (*C*O_2H), 203.84 (CHO); FAB MS *m*/*z* 272 $[MH]^+$. Anal. ($C_{13}H_{21}NO_5$) C, H, N.

(2*RS*,4*S*)-2-[[2′-(*R*)-*N*-(*tert*-Butoxycarbonyl)-2′-carboxypyrrolidinyl]ethyl]thiazolidine-4-carboxylic Acid Methyl Ester (21). Aldehyde **20** (1.3 g, 4.79 mmol) was dissolved in 95% EtOH (20 mL), and the solution cooled to −15 °C in an ice/H_2O/NaCl bath. A solution of $NaHCO_3$ (374 mg, 4.45 mmol) in 20 mL of H_2O was added while the reaction was stirred. To the resulting mixture was added D-cysteine methyl ester hydrochloride (763 mg, 4.45 mmol) in one portion. The pH of the solution was adjusted to ca. 7 using $NaHCO_3$, and the reaction was allowed to warm to room temperature. After 16 h, the solvents were removed under vacuum, and the residue was redissolved in H_2O (25 mL). The solution was extracted with EtOAc (100 mL). The pH of the aqueous layer was readjusted to ca. 6 using 1 N HCl, and then the aqueous layer was extracted with EtOAc (100 mL). To facilitate partitioning of the thiazolidine mixture into organic solvents, NaCl was added to the aqueous layer followed by extractions into $CHCl_3$. This process was repeated 3 times with adjustment of the pH to 6 between extractions. The organic layers were combined and dried with $MgSO_4$. Removal of the solvent under vacuum gave 1.8 g (97%) of the diastereomeric mixture **21** as a white foam: FAB MS *m*/*z* 389 $[MH]^+$, 333 $[M-(CH_3)_3C]^+$. Anal. ($C_{17}H_{28}N_2O_6S$) C, H, N, S.

Methyl [3′*S*-(3′α,6′α,8′a*β*)]-1-(*tert*-Butoxycarbonyl)-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (22a) and Methyl [3′*S*-(3′α,6′α,8′aα)]-1-(*tert*-Butoxycarbonyl)-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (22b). To a solution of the thiazolidine mixture **21** (1.6 g, 4.12 mmol) in freshly distilled CH_2Cl_2 (300 mL) was added 2-chloro-1-methylpyridinium iodide (1.13 g, 4.44 mmol). This was followed by addition of NEt_3 (1.2 mL, 8.88 mmol) in 20 mL of CH_2Cl_2. The resulting solution was refluxed for 6 h. The reaction mixture was allowed to cool, and then was extracted with 10% citric acid, 1 N $NaHCO_3$, and brine. Drying with $MgSO_4$ and subsequent removal of the solvents under vacuum gave 1.5 g of a mixture of two epimers with respect to the bridgehead carbon (8′a-C) in a ratio of 1:1. These epimers were readily separated by silica gel chromatography (EtOAc/hexanes, 1:1 → 3:1). Of the two epimers, **22b**, which has the higher R_f value on TLC, was recrystallized from MeOH/EtOAc to give white needles. The bridgehead stereochemistry was tentatively assigned based on NOE experiments and subsequently confirmed by X-ray structure determination of **22b**.

22a: Obtained 0.63 g (42%) of a clear oil; $[\alpha]_D^{25}$ +11.5 (*c* 0.61, $CHCl_3$); $[\alpha]_{365}^{25}$ +33.1 (c 0.61, $CHCl_3$); TLC R_f = 0.27 (EtOAc/hexanes, 3:1), 0.38 (CH_2Cl_2/MeOH, 20:1). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a ratio of ca. 1:1: ^{1}H NMR ($CDCl_3$, 328 K) δ 1.44 and 1.49 (s, 9 H, Boc CH_3), 1.69−2.35 (m, 7 H, 4-CH_2, 7′-CH_2, 8′-CH_2, and 3-CH_2), 2.69 (ddd, J = 13.4, 7.3, and 2.4 Hz, 1 H, 8′-CH_2), 3.08−3.17 (m, 1 H, 2′-CH_2), 3.33−3.62 (m, 3 H, 2′-CH_2 and 5-CH_2), 3.73 (s, 3 H, OCH_3), 4.62−4.73 (m, 1 H, 3′-CH) 4.80 (dd, J = 11.0 and 2.4 Hz, 8′a-CH); ^{1}H NMR (CD_3CN) δ 1.42 and 1.43 (s, 9 H, Boc CH_3), 1.70−1.84 (m, 2 H, 4-CH_2), 1.92−2.15 (m, 5 H, 7′-CH_2, 8′-CH_2, and 3-CH_2), 2.50−2.57 (m, 1 H, 8′-CH_2), 3.08 (d, J = 12.2 Hz, 1 H, 2′-CH_2), 3.36−3.46 (m, 3 H, 2′-CH_2 and 5-CH_2), 3.63 and 3.67 (s, 3 H, OCH_3), 4.56 (d, J = 7.3 Hz, 3′-CH), 4.84−4.88 (m, 1 H, 8′a-CH); ^{13}C NMR (CD_3CN) δ 22.47 and 23.16 (4-C), 28.17 and 28.40 (Boc CH_3), 28.52 and 28.95 (7′-C), 33.22, 33.32, 35.77, and 36.42 (3-C and 8′-C), 42.22 and 44.00 (2′-C), 48.79 and 48.84 (5-C), 52.25 and 52.51 (OCH_3), 62.60, 63.02, and 63.28 (3′-C and 8′a-C), 64.62 and 64.78 (6′-C), 79.21 and 80.12 (Boc C−O), 154.68 (Boc C═O), 170.69 and 171.67 (*C*O_2CH_3 and 5′-C═O); FAB MS *m*/*z* 371 $[MH]^+$, 315 $[M-(CH_3)_3C]^+$, 271 $[M-(CH_3)_3COCO]^+$. Anal. ($C_{17}H_{26}N_2O_5S$) C, H, N.

22b: Obtained 0.62 g (41%) of a white solid; mp 181.5−182.0 °C; $[\alpha]_D^{25}$ +139.1 (*c* 0.78, $CHCl_3$); TLC R_f = 0.49 (EtOAc/hexanes, 3:1); 0.62 (CH_2Cl_2/MeOH, 20:1). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a ratio of ca. 1:1. ^{1}H NMR ($CDCl_3$) δ 1.43 and 1.49 (s, 9 H, Boc CH_3), 1.72−2.04 (m, 5 H, 4-CH_2, 8′-CH_2, and 3-CH_2), 2.18−2.35 (m, 2 H, 3-CH_2 and 7′-CH_2), 2.67−2.80 (m, 1 H, 7′-CH_2), 3.12−3.33 (m, 2 H, 2′-CH_2), 3.48−3.70 (m, 2 H, 5-CH_2), 3.74 and 3.76 (s, 3 H, OCH_3), 4.91−5.11 (m, 2 H, 3′-CH and 8′a-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.17 and 24.17 (4-C), 28.56, 28.84, 29.05, and 29.11 (7′-C and Boc CH_3), 32.38, 32.49, 32.98, and 33.26 (3-C and 8′-C), 39.34 and 41.68 (2′-C), 49.17 and 49.33 (5-C), 53.05 and 53.17 (OCH_3), 62.88, 63.05, 63.09, and 63.25 (3′-C and 8′a-C), 65.26 and 65.40 (6′-C), 80.07 and 81.30 (Boc C−O), 154.26 and 154.30 (Boc C═O), 171.13, 171.29, 171.85, and 172.17 (*C*O_2CH_3 and 5′-C═O); FAB MS *m*/*z* 371 $[MH]^+$, 315 $[M-(CH_3)_3C]^+$, 271 $[M-(CH_3)_3COCO]^+$. Anal. ($C_{17}H_{26}N_2O_5S$) C, H, N, S.

Methyl [3′*S*-(3′α,6′α,8′aα)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (23). Spiro bicyclic ester **22b** (520 mg, 1.59 mmol) was deprotected in 4 N HCl in dioxane under Ar for 3 h. Solvent and excess HCl were removed in vacuo. Trace HCl was removed with the aid of CH_2Cl_2 to give a white solid which was dissolved in DMF (40 mL). To that solution were added Boc-L-Pro-OH (1.37 g, 6.37 mmol), DCC (1.31 g, 6.37 mmol), HOBt·H_2O (0.80 g, 6.37 mmol), and Et_3N (0.22 mL, 1.59 mmol), and the reaction was stirred at room temperature under Ar for 3 days. The DMF was removed in vacuo, and the residue was dissolved in EtOAc (120 mL). The solution was filtered to remove undissolved material. The filtrate was successively washed with 50 mL of each of the following solutions: 1 N $NaHCO_3$, H_2O, 10% citric acid, 1 N $NaHCO_3$, H_2O, and brine. The organic layer was dried with $MgSO_4$ and evaporated in vacuo to give a crude oil which was chromatographed on a 3.5 × 44 cm silica gel column using 2% MeOH in EtOAc solution as the eluent. The product was isolated as a white foam in 76% yield (500 mg). For

analysis, a sample of **23** was further purified on an HPLC column (Waters radial compression module, 25 × 10 cm Prep-Pak) using CH_2Cl_2/MeOH (30:1) as the mobile phase. The product had a $t_R = 10.2$ min: $[\alpha]_D^{25}$ +58.0 (*c* 0.55, $CHCl_3$); TLC $R_f = 0.64$ (CH_2Cl_2/MeOH, 10:1); 0.28 (2% MeOH in EtOAc). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a ratio of ca. 1:1: ^{1}H NMR ($CDCl_3$) δ 1.39 and 1.42 (s, 9 H, Boc CH_3), 1.70−2.32 (m, 11 H, 8′-CH_2, Pro γ-CH_2, Pro β-CH_2, 4-CH_2, 3-CH_2, 7′-CH_2), 2.72−2.87 (m, 1 H, 7′-CH_2), 3.14−3.92 (m, 6 H, 2′-CH_2, 5-CH_2, and Pro δ-CH_2), 3.71 (s, 3 H, OCH_3), 4.33 (dd, J = 7.8 and 3.0 Hz, 0.5 H, Pro α-CH), 4.47 (dd, J = 7.2 and 3.6 Hz, 0.5 H, Pro α-CH), 4.90−4.97 (m, 1 H, 3′-CH), 5.07−5.13 (m, 1 H, 8′a-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.97, 24.54, and 24.94 (Pro γ-C and 4-C), 28.75 and 28.79 (7′-C), 29.08 and 29.16 (Boc CH_3), 30.12 (Pro β-C), 31.74, 31.85, and 32.31 (3-C and 8′-C), 38.24 and 38.27 (2′-C), 47.31, 47.50,48.82, and 48.86 (Pro δ-C and 5-C), 53.00 and 53.08 (OCH_3), 58.52 (Pro α-C), 62.78, 62.92, and 62.97 (3′-C and 8′a-C), 66.61 and 66.71 (6′-C), 79.85 and 79.99 (Boc C−O), 154.27 and 155.12 (Boc C═O), 170.93, 171.05, 171.19, 171.60, 173.46, and 173.95 (C═O); FAB HRMS *m/z* 468.2172 $[MH]^+$ ($C_{22}H_{33}N_3O_6S$ + H^+ requires 468.2170).

[3′*S*-(3′α,6′α,8′aα)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (24). Compound **23** (475 mg, 1.0 mmol) was treated with a solution of concentrated NH_3 in MeOH (10 mL) at 0 °C. After 5 min, the reaction was allowed to warm to room temperature, and stirring was continued until starting material disappeared by TLC. After 10 h, the solvent and excess NH_3 were removed in vacuo, and the resulting residue was chromatographed on a silica gel column (2 × 40 cm, CH_2Cl_2/MeOH, 20:1) to give 440 mg (96%) of the product as a white foam. For analysis, a sample of **24** was further purified on an HPLC column (Waters radial compression module, 25 × 10 cm Prep-Pak) using CH_2Cl_2/MeOH (20:1) as the mobile phase. The product had a t_R = 11.2 min: $[\alpha]_D^{25}$ +36.0 (*c* 0.78, $CHCl_3$); TLC $R_f = 0.29$ (CH_2Cl_2/MeOH, 20:1). ^{1}H and ^{13}C NMR show the presence of 2 rotamers about the carbamate bond in a ratio of ca. 1:1: ^{1}H NMR ($CDCl_3$) δ 1.40 and 1.43 (s, 9 H, Boc CH_3), 1.71−2.35 (m, 11 H, 8′-CH_2, Pro γ-CH_2, Pro β-CH_2, 4-CH_2, 3-CH_2, 7′-CH_2), 2.40−2.55 (m, 1 H, 7′-CH_2), 3.27 (dd, J = 11.0 and 7.8 Hz, 1 H, 2′-CH_2), 3.33−3.64 (m, 4 H, 2′-CH_2, 5-CH_2, and Pro δ-CH_2), 3.82 (t, J = 8.0 Hz, 0.5 H, Pro δ-CH_2), 3.98 (t, J = 7.4 Hz, 0.5 H, Pro δ-CH_2), 4.35 (dd, J = 8.6 and 3.0 Hz, 0.5 H, Pro α-CH), 4.46 (dd, J = 7.8 and 2.4 Hz, 0.5 H, Pro α-CH), 4.92 (dd, J = 10.8 and 3.6 Hz, 1 H, 3′-CH), 5.27−5.30 (m, 2 H, *cis*-NH and 8′a-CH) 7.70 and 7.77 (s, 1 H, *trans*-NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.19, 24.87, and 24.95 (Pro γ-C and 4-C), 29.05 and 29.11 (Boc CH_3), 29.18, 29.24, 30.04, 30.35, 31.54, 31.58, and 31.69 (Pro β-C, 7′-C, 3-C, and 8′-C), 37.03 (2′-C), 47.34, 47.63, and 48.91 (Pro δ-C and 5-C), 58.48 (Pro α-C), 61.42, 61.50, 63.02, and 63.05 (3′-C and 8′a-C), 66.99 (6′-C), 80.19 and 80.35 (Boc C−O), 154.27 and 155.20 (Boc C═O), 170.38, 170.67, 172.19, 172.42, 172.84 (C═O); FAB HRMS *m/z* 453.2196 ($C_{21}H_{32}N_4O_5S$ + H^+ requires 453.2174). Anal. ($C_{21}H_{42}N_4O_5S$) C, H, N, S.

[3′*S*-(3′α,6′α,8′aα)]-1-[2(*S*)-Pyrrolidinylcarbonyl]-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide Hydrochloride (4·HCl). Spiro bicyclic amide **20** (268 mg, 0.59 mmol) was deprotected in 4 N HCl in dioxane for 8 h under N_2. The solvent and excess HCl were removed in vacuo. The resulting residue was twice suspended in CH_2Cl_2 (5 mL) and then evaporated to a white solid. This material was lyophilized to give **4**·HCl as a hygroscopic white solid (215 mg, 93%): mp 158−159 °C; $[\alpha]_D^{25}$ +55.3 (*c* 0.47, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.67−2.05 and 2.21−2.29 (m, 12 H, 3-CH_2, 4-CH_2, Pro β-CH_2, Pro γ-CH_2, 7′-CH_2, and 8′a-CH_2), 3.12−3.13 (m, 4 H, 5-CH_2 and 2′-CH_2), 3.38−3.44 (m, 1 H, Pro δ-CH_2), 3.81−3.86 (m, 1 H, Pro δ-CH_2), 4.47 (m, 1 H, Pro α-CH), 4.80 (dd, J = 10.99 and 4.9 Hz, 1 H, 3′-CH), 4.95 (dd, J = 7.33 and 4.88 Hz, 1 H, 8′a-CH), 6.92 (s, 1 H, *cis*-$CONH_2$), 7.35 (s, 1 H, *trans*-$CONH_2$), 8.66 (br s, 1 H, $^+NH_2$), 10.23 (br s, 1 H, $^+NH_2$); ^{13}C NMR (DMSO-d_6) δ 23.08 and 23.61 (4-C and Pro γ-C), 27.52, 27.77, 29.97, and 30.44 (Pro β-C, 8′-C, 7′-C, and 3-C), 36.06 (2′-CH_2), 45.49 and 48.15 (Pro δ-C and 5-C), 57.86 (Pro α-C), 60.94 and 62.25 (3′-C and 8′a-C), 66.34 (6′-C), 166.82, 168.94, and 170.54 ($CONH_2$, 6′-C═O, and Pro C═O); FAB HRMS *m/z* 353.1671 ($C_{16}H_{24}N_4O_3S$ + H^+ requires 353.1649). Anal. ($C_{16}H_{24}N_4O_3S$·HCl·1.5H_2O): C, H, N, S, Cl.

[3′*S*-(3′α,6′α,8′aα)]-1-Acetyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-*N*-methylcarboxamide (25). A concentrated solution of methylamine in MeOH was added to **22b** (143 mg, 0.386 mmol) in a round-bottom flask equipped with a magnetic stirrer. The reaction vessel was sealed, and the reaction was stirred at room temperature for 7 h. The mixture was concentrated under vacuum to give a white foam which was chromatographed on a 2.5 × 33 cm silica gel column with EtOAc/hexanes (3:1) as the eluting solvents. The pure product was obtained as a clear oil (132 mg, 92%) which was crystallized from Et_2O to give [3′*S*-(3′α,6′α,8′aα)]-1-(*tert*-butoxycarbonyl)-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-*N*-methylcarboxamide as white needles: mp 153−154 °C; $[\alpha]_D^{25}$ +142.4° (*c* 0.79, MeOH; FAB MS *m/z* 370 $[MH]^+$. Anal. ($C_{17}H_{27}N_2O_4S$) C, H, N, S.

The above carboxamide (83 mg, 0.225 mmol) was dissolved in 4 N HCl in dioxane (3 mL), after which the reaction was stirred for 4 h. The solvent and excess HCl were removed in vacuo, and fresh HCl in dioxane (3 mL) was added. After another 2 h of stirring, the mixture was concentrated under vacuum and the resulting hydrochloride salt was dissolved in pyridine (10 mL). Acetic anhydride (2 mL) was added under N_2, and the mixture was stirred overnight. The reaction mixture was evaporated under vacuum, and residual pyridine was twice azeotroped with MeOH (5 mL). The resulting crude product was loaded on a 2.5 × 33 cm silica gel column and eluted with CH_2Cl_2/MeOH (20:1), affording 61 mg (87%) of the product as a clear oil. Crystallization from EtOAc/hexanes gave **25** as white needles: mp 153−154 °C; $[\alpha]_D^{25}$ +195.8 (*c* 0.92, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.64−1.80 (m, 2 H, 8′-CH_2), 1.90−2.15 (m, 3 H, 4-CH_2 and 3-CH_2), 2.02 (s, 3 H, $COCH_3$), 2.20−2.40 (m, 3 H, 3-CH_2 and 7′-CH_2), 2.75, d, J = 3.6 Hz, 3 H, NCH_3), 3.20 (dd, J = 11.1 and 7.5 Hz, 1 H, pro *R* 2′-CH_2), 3.50 (dd, J = 11.1 and 3.9 Hz, 1 H, pro *S* 2′-CH_2), 3.61 (dd, J = 9.2 and 4.3 Hz, 2 H, 5-CH_2), 4.81 (dd, J = 9.6 and 3.6 Hz, 1 H 8a′-CH), 5.30 (dd, J = 7.5 and 3.7 Hz, 3′-CH), 7.73 br s, 1 H, NH; ^{13}C NMR ($CDCl_3$) δ 23.07, ($COCH_3$), 24.02 (4-C), 26.59 (NCH_3), 28.67, 30.77, and 31.00 (7′-C, 3-C, and 8′-C), 36.81 (2′-C), 49.46 (5-C), 60.60 and 62.61 (3′-C and 8a′-C), 65.59 (6′-C), 169.20, 169.54, and 169.97 (C═O); FAB MS *m/z* 312 $[MH]^+$. Anal. ($C_{14}H_{21}N_3O_3S$) C, H, N, S.

X-ray Diffraction. Colorless prisms of **8** were grown from Et_2O, while colorless plates of **10** were grown from MeOH/hexanes. Colorless needles of **22b** and **25** were grown from MeOH/EtOAc and EtOAc/hexanes, respectively. All measurements were made on a Rigaku AFC6S diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å) as described previously.[5]

Pharmacological Assays. The [^{3}H]spiroperidol/*N*-propylnorapomorphine (NPA) dopamine D_2 receptor competition binding assay was carried out as described previously.[2,5] The procedures used to measure the apomorphine-induced rotational behavior in the 6-hyroxydopamine-lesioned rat model of hemiparkinsonism were the same as that described by us in previous work.[5,8,9]

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Supporting Information Available: X-ray crystallographic data including tables of positional parameters, bond distances, and bond angles for **8**, **10**, **22b**, and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chiu, S.; Paulose, C. S.; Mishra, R. K. Effect of L-Prolyl-L-Leucyl-Glycinamide (PLG) on Neuroleptic-Induced Catalepsy and Dopamine/Neuroleptic Receptor Bindings. *Peptides* **1981**, *2*, 105−111.

(2) Srivastava, L. K.; Bajwa, S. B.; Johnson, R. L.; Mishra, R. K. Interaction of L-Prolyl-L-Leucyl Glycinamide with Dopamine D_2 Receptor: Evidence for Modulation of Agonist Affinity States in Bovine Striatal Membranes. *J. Neurochem.* **1988**, *50*, 960−967.

(3) Mishra, R. K.; Srivastava, L. K.; Johnson, R. L. Modulation of High-Affinity CNS Dopamine D_2 Receptor by L-Pro-L-Leu-Glycinamide (PLG) Analogue 3(*R*)-(N-L-Prolylamino)-2-oxo-1-pyrrolidineacetamide. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **1990**, *14*, 821−827.

(4) Baures, P. W.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, Synthesis, X-ray Analysis, and Dopamine Receptor-Modulating Activity of Mimics of the "C5" Hydrogen-Bonded Conformation in the Peptidomimetic 2-Oxo-3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide. *J. Med. Chem.* **1994**, *37*, 3677−3683.

(5) Baures, P. W.; Ojala, W. H.; Costain, W. J.; Ott, M. C.; Pradhan, A.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, Synthesis, and Dopamine Receptor-Modulating Activity of Diketopiperazine Peptidomimetics of L-Prolyl-L-leucylglycinamide. *J. Med. Chem.* **1997**, *40*, 3594−3600.

(6) Kostrzewa, R. M.; Kastin, A. J.; Sobrain, S. K. Potentiation of Apomorphine Action in Rats by L-Prolyl-L-leucyl-glycinamide. *Pharmacol. Biochem. Behav.* **1978**, *9*, 375−378.

(7) Smith, J. R.; Morgan, M. The Effects of PLG on Drug Induced Rotation in Rats. *Gen. Pharmacol.* **1982**, *13*, 203−207.

(8) Ott, M. C.; Mishra, R. K.; Johnson, R. L. Modulation of Dopaminergic Neurotransmission in the 6-Hydroxydopamine Lesioned Rotational Model by Peptidomimetic Analogues of L-Prolyl-L-leucyl-glycinamide. *Brain Res.* **1996**, *737*, 287−291.

(9) Mishra, R. K.; Marcotte, E. R.; Chugh, A.; Barlas, C.; Whan, D.; Johnson, R. L. Modulation of Dopamine Receptor Agonist-Induced Rotational Behaviour in 6-OHDA-Lesioned Rats by a Peptidomimetic Analogue of Pro-Leu-Gly-NH_2 (PLG). *Peptides* **1997**, *18*, 1209−1217.

(10) Yu, K.-L.; Rajakumar, G.; Srivastava, L. K.; Mishra, R. K.; Johnson, R. L. Dopamine Receptor Modulation by Conformationally Constrained Analogues of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1988**, *31*, 1430−1436.

(11) Sreenivasan, U.; Mishra, R. K.; Johnson, R. L. Synthesis and Dopamine Receptor Modulating Activity of Lactam Conformationally Constrained Analogues of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 256−263.

(12) Subasinghe, N. L.; Bontems, R. J.; McIntee, E.; Mishra, R. K.; Johnson, R. L. Bicyclic Thiazolidine Lactam Peptidomimetics of the Dopamine Receptor Modulating Peptide Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 2356−2361.

(13) Genin, M. J.; Mishra, R. K.; Johnson, R. L. Dopamine Receptor Modulation by a Highly Rigid Spiro Bicyclic Peptidomimetic of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 3481−3483.

(14) Genin, M. J.; Johnson, R. L. Design, Synthesis, and Conformational Analysis of a Novel Spiro-Bicyclic System as a Type II β-Turn Peptidomimetic. *J. Am. Chem. Soc.* **1992**, *114*, 8778−8783.

(15) Seebach, D.; Boes, M.; Naef, R.; Schweizer, B. Alkylation of Amino Acids without Loss of the Optical Activity: Preparation of α-Substituted Proline Derivatives. A Case of Self-Reproduction of Chirality. *J. Am. Chem. Soc.* **1983**, *105*, 5390−5398.

(16) Beck, A. K.; Blank, S.; Job, K.; Seebach, D.; Sommerfeld, T. Synthesis of (*S*)-2-Methylproline: A General Method for the Preparation of α-Branched Amino Acids. *Org. Synth.* **1994**, *72*, 62−73.

(17) Genin, M. J.; Baures, P. W.; Johnson, R. L. An Improved Method of Oxazolidinone Hydrolysis in the Asymmetric Synthesis of α-Alkylprolines. *Tetrahedron Lett.* **1994**, *35*, 4967−4968.

(18) Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. An Efficient and High Yield Method for the *N*-*tert*-Butoxycarbonyl Protection of Sterically Hindered Amino Acids. *Tetrahedron Lett.* **1996**, ***37***, 3441−3444.

(19) Overman, L. E.; Smool, J.; Overman, J. D. The Reduction of Aryl Disulfides with Triphenylphosphine. *Synthesis* **1974**, 59−60.

(20) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(21) Spencer, J. R.; Antonenko, V. V.; Delaet, G. J.; Goodman, M. Comparative Study of Methods to Couple Hindered Peptides. *Int. J. Peptide Protein Res.* **1992**, *40*, 282−293.

(22) Coste, J.; Frerot, E.; Jouin, P. Coupling *N*-Methylated Amino Acids Using PyBroP and PyCloP Halogenphosphonium Salts: Mechanism and Fields of Application. *J. Org. Chem.* **1994**, *59*, 2437−2446.

(23) Rao, C. G.; Kulkarni, S. U.; Brown, H. C. Oxidation of Organoboranes Containing Primary Alkyl Groups with Pyridinium Chlorochromate. A Direct Synthesis of Aldehydes from Terminal Alkenes. *J. Organomet. Chem.* **1979**, *172*, C20−C22.

(24) Brown, H. C.; Kulkarni, S. U.; and Rao, C. G. Highly Selective Conversion of Terminal Olefins into Aldehydes. *Synthesis* **1980**, 151−153.

(25) Williams, R. M.; Lee, B. H.; Miller, M. M.; Anderson, O. P. Synthesis and X-ray Crystal Structure Determination of 1,3-Bridged β-Lactams: Novel, Anti-Bredt β-Lactams. *J. Am. Chem. Soc.* **1989**, *111*, 1073−1081.

(26) Ono, N.; Yamada, T.; Saito, T.; Tanaka, K.; Kaji, A. A Convenient Procedure for Esterification of Carboxylic Acids. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2401−2404.

(27) Mukaiyama, T.; Usui, M.; Saigo, K. Facile Synthesis of Lactones. *Chem. Lett.* **1976**, 49−50.

(28) Pliska, V. Models to Explain Dose-response Relationships that Exhibit a Downturn Phase. *Trends Pharmacol. Sci.* **1994**, *15*, 178−181.

(29) Chou, P. Y.; Fasman, G. D. β-Turns in Peptides. *J. Mol. Biol.* **1977**, *115*, 135−175.

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