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Allosteric Modulation of the Dopamine D₂ Receptor by Pro-Leu-Gly-NH₂ Peptidomimetics Constrained in Either a Polyproline II Helix or a Type II β -Turn Conformation

Bhooma Raghavan[†], Kevin J. Skoblenick[‡], Swapna Bhagwanth[†], Niran Argintaru[‡], Ram K. Mishra[‡], and Rodney L. Johnson^{†,*}

Department of Medicinal Chemistry, University of Minnesota, 308 Harvard St. SE, Minneapolis, MN, 55455 and Department of Psychiatry and Behavioral Neuroscience, McMaster University, 1200 Main St. W., Hamilton, Ontario L8N 3Z5, Canada. johns022@umn.edu

Abstract

Type II β -turn mimics and polyproline II helix mimics based upon diastereoisomeric 5.6.5 spiro bicyclic scaffolds have provided two pairs of Pro-Leu-Gly-NH₂ (PLG) peptidomimetics with contrasting dopamine receptor modulating activities. Compounds **1a** and **3a** were found to be positive allosteric modulators of the dopamine receptor, while the corresponding diastereoisomeric compounds **1b** and **3b** provided the first PLG peptidomimetics with the ability to decrease the binding of agonists to the dopamine receptor. The positive allosteric modulating activity of **3a** supported the hypothesis that a polyproline II helix conformation is the bioactive conformation for the PLG analogue Pro-Pro-Pro-NH₂. The results also show that a change in the bridgehead chirality of the 5.6.5 scaffold brings about opposite effects in terms of the modulation of the dopamine receptor.

Introduction

L-Prolyl-L-leucyl-glycinamide (PLG^a), an endogenous neuropeptide, allosterically modulates the dopamine D₂ and D₄ receptor subtypes.^{1–3} PLG increases the affinity of the high-affinity state of the dopamine receptor for agonists and it increases the ratio of the dopamine receptor in the high-affinity state, which is coupled to G-proteins.^{1,4} PLG also prevents and reverses drug-induced dopamine receptor super sensitivity caused by dopamine receptor antagonists.^{5–7}

Structure activity studies wherein the ϕ and ψ angles of PLG are constrained suggest that the bioactive conformation of PLG is a type II β -turn.^{8,9} Peptidomimetic **1a** (Figure 1), which possesses the highly rigid 5.6.5 spiro bicyclic lactam type II β -turn mimic, was found to be significantly more potent than PLG in the [³H]spiroperidol/*N*-propylnorapomorphine (NPA) competition binding assay and in the 6-hydroxydopamine lesioned model of Parkinson's disease.⁹ This PLG peptidomimetic also increased, to a more significant degree than PLG, the ratio of dopamine receptors in the high-affinity state. The results with this highly constrained

*Corresponding author. Phone: +1-612-624-7997. Fax: +1-612-624-0139. E-mail: johns022@umn.edu.

[†]University of Minnesota

[‡]McMaster University

Supporting information Available: ORTEP drawing of the X-ray crystal structure of 7a. This material is available free of charge over the internet at <http://pubs.acs.org>.

^aAbbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; NPA, *N*-propylnorapomorphine; PLG, L-prolyl-L-leucyl-glycinamide; PPI, polyproline I; PPII, polyproline II.

PLG peptidomimetic provided strong support for the hypothesis that the bioactive conformation of PLG is a type II β -turn.

Interestingly, the triproline compound Pro-Pro-Pro-NH₂ (**2**, Figure 1), also showed activity in increasing apomorphine induced rotations in the 6-hydroxydopamine lesioned rat and in increasing the ratio of dopamine receptors in the high affinity state.¹⁰ Since **2** cannot exist as a type II β -turn, its activity either calls into question the hypothesis that the bioactive conformation of PLG is a type II β -turn or it implies that **2** can assume a different conformation that still places the important pharmacophore groups in relatively the same topographical space as do the PLG peptidomimetics designed to mimic a type II β -turn. In this paper, we report the synthesis of Pro-Pro-Pro-NH₂ peptidomimetics **3a** and **3b** (Figure 1) possessing the highly constrained 5.6.5 spiro bicyclic lactam scaffold designed to mimic a polyproline II helix. This gave a pair of compounds with the ability to either enhance or decrease the binding of the agonist NPA to dopamine receptors depending upon the chirality of the scaffold bridgehead carbon. In light of the differential activities of **3a** and **3b**, the bridgehead epimer of **1a**, **1b** (Figure 1), was also synthesized and evaluated.

Design Rationale

It is well established that a string of L-prolines in a polypeptide can assume either a polyproline I (PPI)- or a polyproline II (PPII) helix.¹¹ In a PPI helix conformation, the Pro-Pro amide bonds are all-cis, whereas in the more predominant PPII helix conformation all-trans amide bonds exist. The PPII helix is a left-handed helix with three residues per turn. An overlay of Ac-Pro-Pro-NH₂ in a PPII helix conformation and the 5.6.5 type II β -turn spiro bicyclic scaffold of **1a** (Figure 2A) shows that there is a good overlay of the backbone atoms and that the carboxamide NH₂ moieties of both project in the same relative area of space. However, the two carbonyl groups of the carboxamide moieties are oriented in opposite directions. If one compares the torsion angles of a PPII helix with those of the 5.6.5 spiro bicyclic scaffold of **1a** (Table 1) one sees that the ϕ_2 and ψ_2 angles are similar between the two, while the respective ϕ_3 angles have about the same magnitude, but are opposite in sign. Thus, a molecule with the opposite stereochemistry at the spiro bicyclic scaffold carbon possessing the carboxamide moiety would give a PPII helix mimic. This is illustrated by the overlays of Ac-Pro-Pro-NH₂ in a PPII helix conformation with the 5.6.5 spiro bicyclic frameworks of the proposed polyproline II helix PLG peptidomimetics **3a** and **3b** (Figure 2B and 2C). Previously, Witter et al.¹² reported on the use of the spiro bicyclic scaffold as a potential mimic of a polyproline II helix.

Synthesis

Proline aldehyde derivative **4** served as the starting material for the synthesis of **3a** and **3b** (Scheme 1). We previously reported the synthesis of this material through a modification of Seebach's oxazolidinone methodology.^{9,13} The debenzoylation of **4** posed some problems initially, as the use of solvents like benzene or EtOAc resulted in incomplete debenzoylation, while the use of MeOH resulted in internal acetal formation. However, when the debenzoylation of **4** was carried out in 95% EtOH with 10% Pd/C as the catalyst, complete debenzoylation was observed and acetal formation was minimized if the reaction was worked up immediately and the product carried directly onto the next reaction. Prolonged exposure of **5** to the alcohol solvent resulted in acetal formation. Addition of a base like Et₃N to the reaction further ensured prevention of the acid-catalyzed acetal formation during debenzoylation.

Carboxy aldehyde **5** was then condensed with either L-Cys-OMe-HCl or D-Cys-OMe-HCl to give a diastereomeric mixture of thiazolidines **6a** or **6b**, respectively. Mukaiyama's reagent, 2-chloro-1-methyl pyridinium iodide, facilitated the cyclization of thiazolidines **6a** to give a

mixture of diastereoisomers **7a** and **7b** in a 2:1 ratio. These diastereoisomers were separable by column chromatography. Isomer **7a** was crystallized from Et₂O/EtOAc and its absolute stereochemistry at the bridgehead position was determined by X-ray crystallography to have the *R* configuration (see Figure 1 in Supporting Information).¹⁴ Likewise, cyclization of **6b** with Mukaiyama's reagent gave the diastereoisomers **8a** and **8b**,⁹ which were separated through column chromatography, in a 1:1 ratio.

Spiro bicyclic ester **7a** was treated with HCl/dioxane to remove the *tert*-butoxycarbonyl group and the resulting hydrochloride salt was initially coupled to Boc-L-Pro-OH using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/1-hydroxybenzotriazole (EDC/HOBt) to give **9a** in about a 50% yield. Replacing EDC/HOBt with Mukaiyama's reagent increased the yield of **9a** to 70% (Scheme 2). The conversion of ester **9a** to its corresponding amide posed a problem, however. Treatment of **9a** with a methanolic solution of ammonia at room temperature overnight did not provide the desired amide **12a**. When the reaction mixture was heated to 90 °C in a sealed vessel amidation resulted, but so did epimerization at the carbon possessing the carboxamide moiety thereby giving the known epimeric spiro bicyclic lactam amide **10**.⁹ The epimerization was slow because even after two days only 50% of the starting material had been converted to amide **10**. In order to resolve this problem, **9a** was first hydrolyzed to acid **11** with LiOH in THF/H₂O. The coupling of **11** with ammonia via activation with EDC/HOBt resulted in the formation of amide **12a**. Removal of the *tert*-butoxycarbonyl protecting group from **12a** with HCl/dioxane provided the desired polyproline PLG peptidomimetic **3a** as its hydrochloride salt.

Spiro bicyclic ester **7b** was coupled to Boc-L-Pro-OH with the aid of 2-chloro-1-methyl pyridinium iodide to give **9b** (Scheme 3). In contrast with **9a**, the ammonolysis of diastereoisomer **9b** proceeded smoothly in methanolic ammonia solution at room temperature to give **12b** in a 77% yield after purification. The final product, **3b** • HCl, was obtained by removal of the *tert*-butoxycarbonyl group with HCl/dioxane. The assignment of the bridgehead stereochemistry of **3a** and **3b** was based upon the X-ray crystal structures of the corresponding precursors **12a** and **12b** (Figures 3)¹⁴ The purity of **3a** and **3b** was confirmed with HPLC.

The synthesis of **1b** was achieved from **8b** as outlined in Scheme 4. Treatment of **8b** with trifluoroacetic acid to remove the *N-tert*-butoxycarbonyl group gave the trifluoroacetate salt, which was coupled to Boc-L-Pro-OH using *O*-(7-azabenzotriazol-1-yl)-*N,N,N'*-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent. This provided **13** in a 79% yield. Initially, ammonolysis of **13** was tried with a saturated solution of ammonia in dichloromethane/methanol first at room temperature then followed by heating to 55 °C for 2 days (in the presence of 10 mol% DMAP on the third day). These conditions only resulted in partial conversion of **13** to a 1:1 mixture of carboxamides **14** and **12b**. This difficulty in ammonolysis was analogous to that seen with **9a**, another isomer where the bridgehead hydrogen and carboxamide group are in a *trans* relationship. We therefore hydrolyzed the methyl ester and then coupled the resulting acid to ammonia using HATU as the coupling reagent. Although complete conversion was obtained, a 1:1 mixture of **14** and **12b** was obtained once again, indicating epimerization at the 3' position. It was determined by further experimentation that this competing reaction occurred during the ester hydrolysis step. Attempting ester hydrolysis under cold conditions (–5 °C to 0 °C) only served to reduce the rate of the main hydrolysis reaction. Diastereoisomers **12b** and **14** were separated through column chromatography. Removal of the *tert*-butoxycarbonyl protecting group from **14** with TFA/CH₂Cl₂ provided the desired PLG peptidomimetic **1b** as its trifluoroacetate salt, the purity of which was confirmed by HPLC analysis.

Compounds **1a**, **1b**, **3a** and **3b** were tested in a tritiated *N*-propylnorapomorphine ([³H]NPA) binding assay with bovine striatal dopamine D₂ receptors.¹ Each PLG peptidomimetic, at

various concentrations, was incubated with the membrane preparation containing [^3H]NPA and the percent change in [^3H]NPA binding was measured. The results are depicted in Figure 4A–D for **1a**, **1b**, **3a** and **3b**, respectively. Both compound **1a** and **3a** behaved like PLG and typical PLG peptidomimetics, wherein they enhanced the binding of [^3H]NPA to isolated dopamine D₂ receptors and they exhibited bell-shaped dose response curves. The maximum effect for **1a** was at a concentration of 10^{-8} M where it significantly enhanced the binding of [^3H]NPA binding by $14.6 \pm 3.9\%$. In contrast, PLG peptidomimetic **3a** maximally enhanced [^3H]NPA binding by $21.4 \pm 6.1\%$ at a concentration of 10^{-7} M.

Interestingly, the diastereoisomers of **1a** and **3a**, spiro bicyclic compounds **1b** and **3b**, respectively, decreased the binding of [^3H]NPA to isolated dopamine D₂ receptors. Spiro bicyclic derivative **1b** decreased the binding of [^3H]NPA to dopamine D₂ receptors by $11.5 \pm 3.1\%$ at a concentration of 10^{-8} M, while **3b**, at a concentration of 10^{-6} M, decreased the binding of [^3H]NPA to dopamine D₂ receptors by $9.2 \pm 3.4\%$. Like the positive allosteric modulators, both **1b** and **3b** possessed bell-shaped dose response curves.

The spiro bicyclic PLG peptidomimetics **1a**, **1b**, **3a** and **3b** were also evaluated for their ability to displace [^{125}I]**15** (Figure 5) in a competition binding assay. PLG peptidomimetic **15** and its derivatives were previously shown to be positive modulators of the dopamine receptor and it was developed as a radioligand for the PLG allosteric modulatory site on the dopamine D₂ receptor.³ The results depicted in Figure 6 show that all four spiro bicyclic PLG peptidomimetics are able to displace [^{125}I]**15** from its binding site.

Discussion

An earlier study showed that Pro-Pro-Pro-NH₂ (**2**) possessed significant activity in enhancing the binding of NPA, a dopamine receptor agonist, to the dopamine D₂ receptor.¹⁰ Also, we previously showed and have confirmed in the present study that the 5.6.5 spiro bicyclic peptidomimetic of PLG, **1a**, possesses significant activity in enhancing the binding of the dopamine receptor agonist, NPA, to the dopamine D₂ receptor.⁹ Since the highly constrained 5.6.5 spiro bicyclic scaffold of **1a** has been shown to be an excellent mimic of a type II β -turn⁹ and since Pro-Pro-Pro-NH₂ (**2**) cannot exist in a type II β -turn, we postulated that **2** could assume a conformation that puts the pharmacophore groups required for dopamine receptor modulating activity in positions similar to those occupied by the corresponding pharmacophore groups of PLG peptidomimetics when they are in a type II β -turn conformation. Molecular modeling suggested that a polyproline II helix conformation might be one such conformation, as the overlay of a polyproline II helix with the 5.6.5 spiro bicyclic type II β -turn scaffold showed that the backbone atoms and the carboxamide NH₂ groups occupy the same relative area of space (Figure 2A). The only difference between the two was that the carbonyl groups were projected in the opposite directions.

A highly constrained 5.6.5 spiro bicyclic scaffold that could mimic a polyproline II helix was designed and incorporated into the Pro-Pro-Pro-NH₂ structure to give peptidomimetics **3a** and **3b** to test our hypothesis. The dihedral angles obtained from the X-ray analysis of **12a** and **12b** indicated that the synthesized molecules possessing such a 5.6.5 spiro bicyclic scaffold form a polyproline II helix conformation (Table 1). Furthermore, the PLG-like biological activity profile of **3a** indicated that Pro-Pro-Pro-NH₂ constrained in a polyproline II helix conformation is capable of activating the allosteric binding site with which PLG and its peptidomimetics interact. Previous work by us¹³ has shown that constraining Pro-Pro-Pro-NH₂ and Pro-Leu-Pro-NH₂ in a type VI β -turn conformation also leads to molecules with the ability to allosterically modulate dopamine receptors in a positive manner. Although the polyproline II helix and type VI β -turn conformations possess different ψ_3 and ω torsion angles, their other torsion angles are similar and both place the NH₂ group of the carboxamide moiety

in the same relative topological space that the NH₂ group occupies in PLG peptidomimetics constrained in a type II β -turn conformation.

The ability of **1b** and **3b** to decrease the binding of the dopamine receptor agonist NPA to dopamine D₂ receptors represents a novel finding with respect to PLG peptidomimetics. However, this is not the first report of the negative modulation of the dopamine receptor, since homocysteine recently has been shown to reduce the affinity of the dopamine D₂ receptor for agonists.¹⁵ The fact that **1b** and **3b**, like **1a** and **3a**, are able to displace [¹²⁵I]**15** in a competition binding assay suggests that **1b** and **3b** are interacting with the same PLG allosteric modulatory site on the dopamine D₂ receptor with which **1a** and **3a** interact.³ However, in contrast to **1a** and **3a**, they have a negative effect on dopamine receptor agonist binding. The structural basis behind the activity of **1b** and **3b** is not known at present. Clearly the structural difference between **1a** and **1b** and between **3a** and **3b** is the chirality of the bridgehead carbon atom. This difference in bridgehead chirality has an effect on the possible conformations of the spiro bicyclic scaffolds, particularly the bicyclic thiazolidines-lactam portion. For example, modeling studies suggest that isomer **3b** can exist in two conformations differing in the pucker of the lactam ring, which consequently affects the pucker of the thiazolidine ring and therefore the positions of the β -methylene group and the carboxamide moiety. Such conformations are not seen in modeling of **3a**. We postulate that such conformational differences between **3a** and **3b** and also between **1a** and **1b** may produce different conformational changes at the PLG allosteric binding site upon binding thereby causing different conformational effects at the orthosteric site to which NPA binds. The design and synthesis of conformationally constrained analogues of such conformations are underway to test this hypothesis.

In summary, type II β -turn mimics and polyproline II helix mimics based upon diastereoisomeric 5.6.5 spiro bicyclic scaffolds have provided two pairs of PLG peptidomimetics with contrasting dopamine receptor modulating activities. Compounds **1a** and **3a** were found to be positive allosteric modulators of the dopamine receptor, while the corresponding diastereoisomeric compounds **1b** and **3b** provided the first PLG peptidomimetics with the ability to decrease the binding of the dopamine receptor agonist NPA to dopamine D₂ receptors. The activity of **3a** supports our hypothesis that a polyproline II helix conformation is the bioactive conformation for the PLG analogue Pro-Pro-Pro-NH₂. These results also provide additional support for the concept that conformationally different molecules are able to serve as allosteric modulators of the dopamine receptor as long as these conformations place key pharmacophores in the correct region of space. Finally, that a change in the bridgehead chirality of the 5.6.5 scaffold brings about opposite effects in terms of the modulation of the dopamine receptor will make these peptidomimetics important ligands in future studies aimed at delineating the molecular mechanism behind the allosteric modulation of the dopamine receptor.

Experimental Section

Compound Purity

The purity of compounds **1b**, **3a**, and **3b** is >98%.

Methyl [3' R-(3' β ,6' α ,8' $\alpha\alpha$)]-1-(*tert*-Butoxycarbonyl)-5'-oxospiro[pyrrolidine-2,6'-thiazolidino [3,2-*a*]piperidine]-3'-carboxylate (7a) and Methyl [3' R-(3' β ,6' α ,8' β)]-1-(*tert*-Butoxycarbonyl)-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (7b)

Aldehyde benzyl ester **4** (3.3 g, 9.1 mmol) was dissolved in 95% EtOH in a Parr bottle purged with argon. An equivalent of Et₃N (1.3 mL, 9.1 mmol) and 10% wt Pd/C (200 mg) were added and the reaction was hydrogenated on a Parr shaker at 50 psi for 2 h. The reaction mixture was

filtered and the Pd residue rinsed with EtOAc. The reaction mixture was concentrated and then redissolved in CH₂Cl₂. This solution was filtered through an Acrodisc to remove any residual catalyst. The filtrate was concentrated under vacuum. The residue was dissolved in toluene, which was then removed under vacuum. This process was repeated with toluene and then done with CH₂Cl₂ to give **5** as a white foam, which was then carried on to the next reaction. Minimum contact with EtOH is essential to prevent acetal formation.

Carboxy aldehyde **5** was dissolved in 1:1 EtOH/H₂O (30 mL). The reaction mixture was cooled to -15 °C and NaHCO₃ (764 mg, 9.1 mmol) was added. The mixture was stirred for 15 min and to this mixture was added L-Cys-OMe-HCl in one portion. The pH of the reaction was adjusted to around 7 with NaHCO₃. The reaction was stirred overnight. The next day, the reaction mixture was concentrated to remove EtOH, the pH of the aqueous layer was adjusted to 6 with 1N HCl and extracted with EtOAc (2 × 50 mL). NaCl was added to the aqueous layer and the solution extracted with CHCl₃ (2 × 50 mL). The pH was always adjusted to 6 between the extractions. The organic extracts were combined and washed with NaCl solution, dried over MgSO₄ and concentrated under vacuum. This reaction gave a 1:1 mixture of diastereomeric thiazolidines (**6**) as a white foam in 80 % crude yield. No further purification was carried out.

The thiazolidine diastereomers were dissolved in dry CH₂Cl₂ (200 mL). 2-Chloro-1-methyl pyridinium iodide (Mukaiyama's reagent, 1.91 g, 7.3mmol) and Et₃N (2.54 mL, 18.3 mmol) were added to the solution and the reaction mixture was heated at reflux overnight. The reaction mixture was cooled and then extracted with 1M citric acid, water, NaHCO₃, water and brine. The organic layer was dried over MgSO₄ and concentrated under vacuum to give an orange oil consisting of diastereomers with the stereochemistry varying at the bridgehead carbon. The crude material was purified by column chromatography using 1:1 to 1:2 hexanes/EtOAc. The diastereomers **7a** and **7b** were obtained in the ratio of 2:1 in a 70 % yield. Diastereomer **7a** was crystallized from EtOAc/Et₂O and the stereochemistry at the bridgehead was determined by X-ray crystallography.

7a

mp 131–132 °C; [α]_D -40 (c 0.95, CHCl₃); TLC *R*_f 0.38 (EtOAc/hexanes, 2:1); ¹H and ¹³C NMR show the presence of two rotamers about the carbamate bond in a ratio 1.5:1. ¹H NMR (300 MHz, CDCl₃) δ 5.0 (dd, 0.5 H, *J* = 3.3 and 11.3 Hz, 8'a-CH); 4.77–4.86 (m, 1.5 H, 3'-CH and 8'a-CH), 3.74 and 3.76 (s, 3 H, OCH₃), 3.29–3.58 (m, 3 H, 5-CH₂, 2'-CH₂), 3.13 (dd, 1H, *J* = 12.75 and 20.25 Hz, 2'-CH₂), 2.56–2.66 (m, 1 H, 8'-CH₂), 2.21–2.41 (m, 2 H, 3-CH₂ and 7'-CH₂), 1.77–2.04 (m, 5 H, 4-CH₂, 8'-CH₂, 3-CH₂, 7'-CH₂), 1.40 and 1.42 (s, 9 H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 171.4, 171.2, 170.3, and 169.9 (CO₂CH₃ and 5'-C=O), 153.4 and 154.1 (Boc C=O), 79.8 and 80.3 (Boc C(CH₃)₃), 64.9 and 65.1 (6'-C), 64.0 and 64.2 (8'a-C), 61.7 and 61.9 (3'-C), 52.8 and 52.9 (OCH₃), 48.6 and 48.9 (5-C), 39.0 and 39.8 (3-C), 33.3 and 33.4 (2'-C), 32.6 and 33.7 (8'-C), 28.8 and 28.9 (Boc CH₃), 27.4 and 27.5 (7' C), 23.1 and 23.8 (4-C); ESI HRMS *m/z* 393.1460 [M+Na]⁺, (C₁₇H₂₆N₂O₅S + Na⁺) requires 393.1460.

7b

(oil); [α]_D -154.5 (c 1.42, CHCl₃); TLC *R*_f 0.39 (EtOAc/hexanes, 2:1); ¹H and ¹³C NMR show the presence of two rotamers about the carbamate bond. ¹H NMR (300 MHz, CDCl₃) δ 5.29–5.35 (m, 1H, 3'-CH), 4.90 (t, 1H, *J* = 6.45 Hz, 8'a-CH), 3.74 (s, 3H, OCH₃), 3.41–3.69 (m, 2H, 5-CH₂), 3.29–3.34 (m, 1H, 2'-CH₂), 3.15–3.19 (m, 1H, 2'-CH₂), 2.56–2.72 (m, 1H, 8'-CH₂), 2.18–2.35 (m, 2H, 3-CH₂ and 7'-CH₂), 1.76–1.97 (m, 5H, 4-CH₂, 8'-CH₂, 3-CH₂, 7'-CH₂), 1.42 (s, 9H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 22.7 and 23.2 (4-C), 26.6 and 28 (7' C), 29 (Boc CH₃), 32.3 and 32.3 (2'-C), 33.8 (8'-C), 41.2 and 42.2 (3-C), 48.6 and 48.8 (5-C), 53.0 (OCH₃), 61.2 and 61.8 (3'-C), 61.2 (8'a-C), 64.1 and 64.4 (6'-C), 79.6 and 80.6 (Boc C=O),

154.0 and 154.3 (Boc C=O), 170.8, 170.9, 171 (CO₂CH₃ and 5'-C=O); ESI HRMS *m/z* 393.1460 [M+Na]⁺, (C₁₇H₂₆N₂O₅S + Na⁺) requires 393.1460.

Methyl [3' *R*-(3' β,6' α,8' α)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (9a)

Spiro bicyclic ester **7a** (500 mg, 1.35 mmol) was dissolved in 5 mL 4N HCl/dioxane. The reaction was stirred under argon for 1 h and then it was concentrated under vacuum. The residue twice was dissolved in CH₂Cl₂ and then stripped of solvent. The white foam that was obtained was stored under vacuum for a couple of hours before being carried on to the next step without purification.

Boc-L-Pro-OH (580 mg, 2.7 mmol) was dissolved in CH₂Cl₂ under argon in a flask cooled in an ice-bath. N-Methyl morpholine (1.04 mL, 9.45 mmol) was added to the reaction, followed by 2-chloro-1-methyl pyridinium iodide (709 mg, 2.7 mmol). The reaction mixture was stirred for 15 min followed by addition of the spiro bicyclic ester hydrochloride. The reaction then was stirred overnight. The next day a catalytic amount of 4-DMAP (10 mg) was added and the reaction was stirred for another 2 h. EtOAc (75 mL) was then added to the reaction mixture and this solution was washed with water, 1M citric acid solution, NaHCO₃, water and brine. The organic layer was then dried over Na₂SO₄ and concentrated under vacuum to give the crude product which was purified in a 70% yield by silica gel column chromatography (6.5 × 3 cm) using EtOAc/MeOH (20:1) as the eluent. mp 123–125 °C; [α]_D –87.2 (c 1, ¹H and ¹³C NMR showed the presence of two rotamers CHCl₃); TLC *R_f* 0.63 (CH₂Cl₂/MeOH, 10:1); about the carbamate bond in a ratio around 1:1. ¹H NMR (300 MHz, CDCl₃) δ 5.00 (dt, 1H, *J* = 3 and 10.8 Hz, 8'-CH), 4.80 (dd, 1 H, *J* = 4.2 and 7.5 Hz, 3'-CH), 4.41 (dd, *J* = 3 and 8.4 Hz, 0.5 H, Pro αCH), 4.31 (dd, *J* = 3.3 and 8.1 Hz, 0.5 H, Pro α-CH), 3.80–3.86 (m, 0.5H, Pro δ-CH₂), 3.69 (s, 3H, OCH₃), 3.28–3.59 (m, 4.5H, 2'-CH₂, 5-CH₂, and Pro δ-CH₂), 3.08 (dd, 1H, *J* = 4.2 and 12.6 Hz, 2'-CH₂), 2.53–2.68 (m, 1H, 8'-CH₂), 1.70–2.34 (m, 11 H, 8'-CH₂, Pro γ-CH₂, Pro β-CH₂, 4-CH₂, 3-CH₂, 7-CH₂), 1.37 and 1.41 (s, 9 H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 171.3, 171, 170.5, 170.3, and 170.2 (C=O), 153.7 and 154.5 (Boc C=O), 79.5 and 79.6 (Boc C(CH₃)₃), 66.3 (6'-C), 63.8 (8' a-C), 61.7 (3'-C), 58.3 (Pro α-C), 52.8 (OCH₃), 47, 47.2, 48.4 and 48.5 (Pro δ-C and 5-C), 37.7 and 37.8 (3-C), 33.2 (2'-C), 32 and 32.2 (8'-C), 29.7 (Pro β-C), 28.7 and 28.8 (Boc CH₃), 27.5 (7'-C), 23.9, 24.5 and 24.6 (Pro γ-C and 4-C), ESI HRMS *m/z* 490.1997 [M+Na]⁺, (C₂₂H₃₃N₃O₆S + Na⁺) requires 490.1988.

Methyl [3' *R*-(3' β,6' α,8' α)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (9b)

Spiro bicyclic ester **7b** (440 mg, 1.2 mmol) was treated in the same manner as that described above for the conversion of **7a** into **9a**. The product **9b** still showed traces of impurities despite chromatographic purification. TLC *R_f* 0.72 (CH₂Cl₂/MeOH, 10:1); ¹H and ¹³C NMR show the presence of two rotamers about the carbamate bond in a ratio around 1:1. ¹H NMR (300 MHz, CDCl₃) δ 5.29 (dd, 1 H, *J* = 7.05 and 12.75 Hz, 3'-CH), 4.87 (td, 1 H, *J* = 3.6 and 8.7 Hz, 8' a-CH), 4.43 (dd, *J* = 3 and 7.8 Hz, 0.5 H, Pro α-CH), 4.33 (dd, 0.5 H, *J* = 3.45 and 8.25 Hz, Pro α-CH), 3.81–3.86 (m, 0.5 H, Pro δ-CH₂), 3.72 and 3.73 (s, 3H, OCH₃), 3.27–3.69 (m, 4.5 H, 2'-CH₂, 5-CH₂, and Pro δ-CH₂), 3.12 (dq, *J* = 3.6 and 5.1 Hz, 1H, 2'-CH₂), 2.59–2.67 (m, 1H, 8'-CH₂), 1.71–2.50 (m, 11H, 8'-CH₂, Pro γ-CH₂, Pro β-CH₂, 4-CH₂, 3-CH₂, 7-CH₂), 1.40 and 1.42 (s, 9H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 171.3, 171, 170.8, 170.3, and 170.1 (C=O), 153.8 and 154.6 (Boc C=O), 79.4 and 79.6 (Boc C(CH₃)₃), 65.3 (6'-C), 62.1 and 62.3 (8' a-C), 61.9 and 62.0 (3'-C), 58.4 (Pro α-C), 52.9 (OCH₃), 47.1, 47.3 and 48.2 (Pro δ-C and 5-C), 40.8 and 41 (3-C), 34.5 and 34.8 (8' a-C), 31.9 and 32.0 (2'-C), 29.0 and 29.9 (Pro β-C), 28.8 (Boc CH₃), 28.3 (7'-C), 23.9, 24.1 and 24.7 (Pro γ-C and 4-C); ESI HRMS *m/z* 490.2003 [M+Na]⁺, (C₂₂H₃₃N₃O₆S + Na⁺) requires 490.1988.

[3' *R*-(3' β ,6' α ,8' α)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro [pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (12a)

Spiro bicyclic compound **9a** (320 mg, 0.68 mmol) was dissolved in 10 mL THF/H₂O (3:1). LiOH (49 mg, 2.18 mmol) was added and the reaction was stirred overnight. The next day the reaction was concentrated under vacuum and the aqueous layer was extracted with Et₂O (5 mL). The aqueous layer was then acidified to pH 3 with citric acid whereupon it was extracted with CH₂Cl₂ (3 $\times$ 50 mL). The organic layers were combined and washed with brine and dried over MgSO₄. After removal of the solvent under reduced pressure, the spiro bicyclic free acid **11** was obtained as a solid (250 mg).

Carboxylic acid **11** (250 mg, 0.55 mmol) was stirred with HOBt (76 mg, 0.55 mmol) in CH₂Cl₂ at -78°C under nitrogen. EDC-HCl (105 mg, 0.55 mmol) was added to this mixture and the reaction was stirred for 15 min. The reaction was warmed to room temperature till the solution cleared and then it was recooled. A solution of NH₃ in CH₂Cl₂ was added to this reaction mixture and the reaction was stirred at -78°C for 2 h and then stirred overnight at room temperature. The next day the reaction was concentrated under vacuum to remove the excess ammonia. The reaction mixture was then partitioned between CH₂Cl₂ and water. The CH₂Cl₂ layer was washed with NaHCO₃ solution, water and brine, dried over MgSO₄ and concentrated under vacuum. The residue was purified on a normal phase Redisep column (40 g) on a Combiflash Retrieve system to give 100 mg of product. The product was crystallized from isopropyl alcohol to give white crystals suitable for X-ray studies. mp 221°C (dec); TLC *R_f* 0.56 (CH₂Cl₂/MeOH 5:1); [α]_D -95.8 (*c* 0.72, CHCl₃).

1D NOESY studies were carried out to delineate the relative stereochemistry by NMR. Both the 3'-H and the 8' α -H protons were irradiated in separate experiments and NOE enhancements observed in each case. The experiments confirmed that the bridgehead proton was *cis* to the α -proton of the thiazolidine ring. ¹H and ¹³C NMR showed the presence of two rotamers about the carbamate bond in a 1:1 ratio. ¹H NMR (300 MHz, CDCl₃) δ 6.46 (br s, 1 H, CONH₂), 5.74 (br s, 1 H, CONH₂), 4.99 (dt, 1 H, *J* = 3 and 10.8 Hz, 8' α -CH), 4.69–4.70 (apparent doublet, 1 H, 3'-CH), 4.42 (dd, 0.5 H, *J* = 3 and 8.4 Hz, Pro α CH), 4.32 (dd, 0.5 H, *J* = 3.3 and 8.1 Hz, Pro α -CH), 3.84–3.89 (m, 0.5 H, Pro δ -CH₂), 3.73–3.78 (m, 0.5 H, Pro δ -CH₂), 3.20–3.59 (m, 5 H, 2'-CH₂, 5-CH₂, and Pro δ -CH₂), 2.48–2.62 (m, 1 H, 8'-CH₂), 1.75–2.32 (m, 11 H, 8'-CH₂, Pro γ -CH₂, Pro β -CH₂, 4-CH₂, 3-CH₂, 7-CH₂), 1.38 and 1.41 (s, 9 H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 172.5, 172.3, 171.5, 171.4, 171.1, 170.8 (C=O), 153.7 and 154.5 (Boc C=O), 79.5 and 79.7 (Boc C(CH₃)₃), 66.5 (6'-C), 64.5 and 64.6 (8' α -C), 62.8 and 62.9 (3'-C), 58.2 (Pro α -C), 47, 47.2, 48.3 and 48.4 (Pro δ -C and 5-C), 37.7 and 37.8 (3-C), 32.9 and 33.0 (2'-C), 32.2 and 32.4 (8'-C), 29.7 (Pro β -C), 28.7 and 28.8 (Boc CH₃), 27.6 (7'-C), 23.9, 24.6 and 24.7 (Pro γ -C and 4-C); ESI HRMS *m/z* 475.1996 [M+Na]⁺, (C₂₁H₃₂N₄O₅S + Na⁺) requires 475.1991; Anal (C₂₁H₃₂N₄O₅S) C, H, N, S.

[3' *R*-(3' β ,6' α ,8' α)]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-5'-oxospiro [pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (12b)

Spiro bicyclic methyl ester **9b** (200 mg, 0.43 mmol) was dissolved in a saturated solution of methanolic ammonia that had been cooled to -78°C in a flask fitted with a balloon. The reaction was stirred overnight as it was allowed to warm to room temperature. The solvent was removed under vacuum and the residue was twice dissolved in CH₂Cl₂ and the solvent then removed under reduced pressure. The reaction went in quantitative yield. If needed, the product can be purified on a Combiflash column by eluting with EtOAc, followed by CH₂Cl₂/EtOAc/MeOH (20:3:5) and then finally by CH₂Cl₂/MeOH (5:1). The product was crystallized slowly from isopropyl alcohol to give 150 mg of a white crystalline solid. The stereochemistry of this molecule was confirmed through X-ray studies. mp 232°C (dec); TLC *R_f* 0.56 (CH₂Cl₂/MeOH, 5:1); [α]_D -200.5 (*c* 1.08, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 6.69 and 6.83 (br s, 1 H,

CONH₂), 5.51 (br s, 1 H, CONH₂), 5.02–5.09 (dd, 1 H, $J = 7.2$ and 4.2 Hz, 3'-CH), 4.78–4.86 (m, 1 H, 8' a-CH), 4.42–4.45 (dd, $J = 3$ and 7.8 Hz, 0.5 H, Pro α -CH), 4.31–4.35 (dd, $J = 4.2$ and 9.6 Hz, 0.5 H, Pro α -CH), 3.32–3.88 (m, 5 H, 2'-CH₂, 5-CH₂, and Pro δ -CH₂), 3.18–3.29 (m, 1 H, 2'-CH₂), 2.70–2.81 (m, 1 H, 8'-CH₂), 1.73–2.45 (m, 11 H, 8'-CH₂, Pro γ -CH₂, Pro β -CH₂, 4-CH₂, 3-CH₂, 7-CH₂), 1.40 and 1.43 (s, 9 H, Boc CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 172.0, 171.9, 171.6, 171.4, 171.1, and 170.9 (C=O), 153.8 and 154.6 (Boc C=O), 79.5 and 79.7 (Boc C(CH₃)₃), 65.5 and 65.6 (6'-C), 62.7 and 62.8 (8' a-C), 62.1 (3'-C), 58.4 and 58.5 (Pro α -C), 47.1, 47.4, 48.2 and 48.4 (Pro δ -C and 5-C), 39.7 and 39.4 (3-C), 32.4 and 32.5 (8'-C), 31.4 and 30.9 (2'-C), 29.9 (Pro β -C), 28.7 and 28.8 (Boc CH₃), 26.0 and 26.5 (7'-C), 24.0, 24.2 and 24.7 (Pro γ -C and 4-C); ESI HRMS m/z 475.2004 [M+Na]⁺, (C₁₂H₃₂N₄O₅S + Na⁺) requires 475.1991; Anal (C₂₁H₃₂N₄O₅S) C, H, N, S.

[3 'R-(3 'β,6 'α,8 'αα)]-1-[2(S)-Pyrrolidinylcarbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino [3,2-a]piperidine]-3'-carboxamide Hydrochloride (3a•HCl)

To the spirocycle amide **12a** (70 mg, 0.15 mmol) in a flask under argon was added 5 mL 4N HCl/dioxane. The reaction was stirred for 1 h. A white precipitate formed as the reaction progressed. On removal of the solvent under vacuum, a white solid was obtained. The white solid was suspended twice in CH₂Cl₂ and then concentrated under vacuum to obtain a white solid. Addition of ether to the flask followed by mildly boiling the solid in ether and then decanting the ether layer led to removal of impurities in the product. This procedure had to be repeated 4–5 times to ensure complete removal of the impurities and this resulted in 15 mg of pure product as a white solid. The purity of the product was confirmed by HPLC using 250 mm × 10 mm Phenomenex LunaPrep C18 column with acetonitrile/H₂O (99:1) and acetonitrile/MeOH (99:1) as the eluting systems. The product possessed a t_R of 6.96 min and 7.25 min, respectively, in the two systems. TLC R_f 0.25 (2-propanol/NH₄OH, 5:1); [α]_D –118.4 (c 0.57, CH₃OH). ¹H NMR (300 MHz, CD₃OD) δ 5.01 (d, 1H, $J = 10.5$ Hz, 8' a-CH), 4.60 (d, 1H, $J = 7.2$, 3'-CH), 4.53 (m, 1H, Pro α -CH), 3.73–3.80 (m, 1H, Pro δ -CH₂), 3.43–3.53 (m, 2H, Pro δ -CH₂, 2'-CH₂), 3.29–3.42 (m, 2H, 5-CH), 3.10 (d, 1H, $J = 12$ Hz, 2'-CH), 2.44–2.50 (m, 2H, 8'-CH₂, 3-CH₂), 1.94–2.30 (m, 10H, Pro β -CH₂, Pro γ -CH₂, 7'-CH₂, 8'-CH₂, 3-CH₂, 4-CH₂); ¹³C NMR (75 MHz, CD₃OD) δ 174.2, 171.5, 167.2 (C=O); 68.2 (6'-C), 65.4 (8' a-C), 63.9 (3'-C), 60.5 (Pro α -C), 49.6 (Pro δ -CH₂), 47.7 (5-C), 38.5 (3-C), 34.7 (2'-C), 32.1 (8'-C), 28.9 (Pro β -CH₂), 27.3 (7'-C), 25.2, 25.1 (4-C and Pro γ -C); ESI HRMS m/z 353.1647 [M]⁺; (C₁₆H₂₅N₄O₃S)⁺ requires 353.1642.

[3 'R-(3 'β,6 'α,8 'αβ)]-1-[2(S)-Pyrrolidinylcarbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino [3,2-a]piperidine]-3'-carboxamide Hydrochloride (3b•HCl)

Spiro bicyclic amide **12b** (70 mg, 0.15 mmol) was treated in the same manner as described above for the preparation of **3a** to give 20 mg of pure **3b** as a white solid. The purity of the product was confirmed by HPLC using a 250 mm × 10 mm Phenomenex LunaPrep C18 column with acetonitrile/H₂O (99:1) and acetonitrile/MeOH (99:1) as the eluting systems. The product possessed a $t_R = 7.20$ min and 7.08 min, respectively, in the two systems. TLC R_f 0.34 (2-propanol/NH₄OH, 5:1); [α]_D –174.5 (c 0.31, CH₃OH); ¹H NMR (300 MHz, CD₃OD) δ 5.01 (dd, 1H, $J = 4.5$ and 8.4 Hz, 8' a-CH), 4.88–4.95 (m, 1H, 3'-CH), 4.49–4.52 (m, 1H, Pro α -CH), 3.73–3.80 (m, 1H, Pro δ -CH₂), 3.52–3.60 (m, 1H, Pro δ -CH₂), 3.31–3.42 (m, 3H, 2'-C, 5-CH), 3.15 (dd, 1H, $J = 7.5$ and 11.7 Hz, 2'-CH), 1.96–2.59 (m, 12 H, Pro β -CH₂, Pro γ -CH₂, 7'-CH₂, 8'-CH₂, 3-CH₂, 4-CH₂); ¹³C NMR (75 MHz, CD₃OD) δ 174.3, 171.7, and 167.5 (C=O), 67.3 (6'-C), 64.8 and 64.5 (3'-C and 8' a-C), 60.7 (Pro α -C), 49.3 (Pro δ -C), 47.7 (5-C), 40.9 (3-C), 34.4 (8'-C), 33.4 (2'-C), 29.1 (Pro β -CH₂), 27.5 (7'-C), 25.2, 24.9 (Pro γ -C and 4-C); ESI HRMS m/z 353.1640 [M]⁺; (C₁₆H₂₅N₄O₃S)⁺ requires 353.1642.

Methyl [3'S-(3'α,6'α,8'αβ)]-1-[[1-(*tert*-Butoxycarbonyl)-2(S)-pyrrolidinyl]carbonyl]-5' oxospiro [pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxylate (13)

Trifluoroacetic acid (2.5 mL, 34 mmol) was added to an ice-cooled anhydrous dichloromethane solution of the spirocycle (313 mg, 0.85 mmol, 1.0 equiv.). The final concentration of TFA in dichloromethane was 20–25%. The reaction was monitored by thin layer chromatography (TLC) using 25% ethyl acetate in hexanes. After 45–60 minutes, the starting material was consumed completely. Solvent and excess TFA were removed *in vacuo*. The pale-yellow colored oil was used in the next step without further purification.

Boc-L-proline (220 mg, 1.0 mmol, 1.2 equiv.) was dissolved in anhydrous DMF (5 mL). The solution was cooled in an ice-salt bath, followed by the addition of DIPEA (0.2 mL, 1.3 mmol) and HATU (388 mg, 1.0 mmol). The solution was stirred under argon at this temperature for 30 min. In another 10 mL flask, the deprotected amine from the first step was suspended in anhydrous DMF (5 mL) and the trifluoroacetate salt neutralized with DIPEA (0.2 mL, 1.3 mmol). The clear solution obtained was added to the activated acid. The mixture was stirred under argon for 20 h after which the solvent was removed *in vacuo*. The residue was dissolved in EtOAc and washed sequentially with 10% citric acid, saturated NaHCO₃, and brine solutions. The organic layer was dried over anhydrous MgSO₄, filtered and concentrated *in vacuo* to give a yellow-colored oil which was purified by flash chromatography starting with 100% CH₂Cl₂ and increasing the polarity in discrete steps to 4% MeOH in CH₂Cl₂ to give the purified compound as a colorless oil in 79% yield. TLC *R_f* 0.65 (CH₂Cl₂/MeOH, 10:1); [α]_D –40.7 (*c* 0.43, CDCl₃); ¹H and ¹³C NMR spectra indicate presence of two rotameric forms of the molecule about the carbamate bond in a 1:1 ratio. ¹H NMR (300 MHz, CDCl₃) δ 4.80–4.74 (m, 1H, 8' a-CH), 4.68–4.65 (m, 1H, 3'-CH), 4.43 (*app* t, 0.5 H, *J* = 4.8, 9.9 Hz, Pro α-CH), 4.30 (dd, 0.5H, *J* = 1.2, 5.7 Hz, Pro α-CH), 3.86–3.78 (AB qt. 0.5 H, *J* = 9, 15 Hz, Pro δ-CH₂), 3.74–3.49 (m, 2.5H, Pro δ-CH₂, 5-CH₂), 3.67 (s, OCH₃), 3.39–3.26 (m, 2H, Pro δ-CH₂, 2'-CH₂), 3.15–3.09 (dd, 1H, *J* = 4.5, 12.6 Hz, 2'-CH₂), 2.68–2.52 (m, 2H, 8' CH₂), 2.12–1.84 (m, 9H, Pro β-CH₂, Pro γ-CH₂, 3-CH₂, 4-CH₂, 7'-CH₂), 1.80–1.70 (m, 1H, 7'-CH₂), 1.39 and 1.37 (s, 9H, Boc-CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 171.4, 171.1, 170.8, and 170.6 (amide and ester C=O), 154.7 and 153.9 (Boc C=O), 79.5 and 79.3 (Boc C(CH₃)₃), 65.0 and 64.9 (6'-C), 63.6, 63.5, 63.4 (8' a-C and 3'-C), 58.3 (Pro α-C), 52.6 and 52.5 (OCH₃), 48.1 (Pro δ-C), 47.2 and 46.9 (5-C), 42.0 and 41.9 (3-C), 37.0 (8'-C), 33.1 (2'-C), 29.8 (Pro β-C), 28.9 and 28.8 (7'-C), 28.7 and 28.6 (Boc CH₃), 24.3, 23.8 and 23.8, 23.6 (Pro γ-C and 4-C); ESI HRMS *m/z* 490.1978 [M+Na]⁺, (C₂₂H₃₃N₃O₆S+ Na⁺) requires 490.1988.

[3'S-(3'α,6'α,8'αβ)]-1-[[1-(*tert*-Butoxycarbonyl)-2(S)-pyrrolidinyl]carbonyl]-5'-oxospiro [pyrrolidine-2,6'-thiazolidino[3,2-*a*]piperidine]-3'-carboxamide (14)

A solution of the ester (240 mg, 0.5 mmol) in 3:1 THF-water was cooled to –5 °C in an ice-salt bath. Lithium hydroxide (37 mg, 1.5 mmol) was added at this temperature. The final molarity of LiOH was 0.5 M. The reaction was gradually warmed to room temperature. Ester hydrolysis was determined to be complete in less than 4 hours by thin layer chromatography. Two distinct spots were observed by TLC, indicative of possible epimerization of the thiazolidine stereocenter. The mixture of carboxylic acids was used in the subsequent coupling step.

The carboxylic acid obtained (211 mg, 0.5 mmol) was dissolved in anhydrous DMF (3 mL) in the presence of DIPEA (0.12 mL, 0.8 mmol, 1.6 equiv.) and the solution cooled to –5 °C in an ice-salt bath. HATU (179 mg, 0.5 mmol) was added to the solution which was then warmed to room temperature for 20 minutes. The pale-yellow colored solution obtained was cooled to –78 °C and a saturated solution of ammonia in anhydrous CH₂Cl₂ was added (8–10 mL). The solution turned into a bright canary yellow color. An empty balloon was fitted onto the flask via an adaptor and the reaction warmed to room temperature over 1 h. The reaction mixture

was stirred at ambient temperature for 24 h, following which an additional amount of ammonia in CH_2Cl_2 (3–4 mL) was added. Following another 24 h, the solvent and excess ammonia were removed *in vacuo*. The residue was dissolved in EtOAc and washed sequentially with 10% citric acid, saturated NaHCO_3 , and brine solutions. The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to give a pale yellow oil which was purified by flash chromatography using gradient elution (100% CH_2Cl_2 first and increasing to 20 % MeOH in CH_2Cl_2) to give an overall 85% yield of diastereomeric carboxamides (1:1 ratio) differing in stereochemistry about the 3' carbon.

TLC R_f 0.49 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 10:1); $[\alpha]_D -35.8$ (c 0.81, CDCl_3); ^1H and ^{13}C NMR spectra indicate presence of two rotameric forms of the molecule about the carbamate bond in a 1:1 ratio. ^1H NMR (300 MHz, CDCl_3 , gCOSY) δ 7.49 and 7.59 (s, 1H, carboxamide NH), 5.36 (br. s, 1H, carboxamide NH), 4.81–4.75 (m, 2H, 8' a-CH and 3'-CH), 4.45–4.41 (dd, 0.5H, $J = 3.0$ and 8.1 Hz, Pro α -CH), 4.37–4.33 (dd, 0.5H, $J = 3.0$ and 8.7 Hz, Pro α -CH), 3.98–3.91 (ddd, 0.5H, $J = 3.9$, 8.1, and 9.3 Hz, Pro δ -CH), 3.79–3.72 (ddd, 0.5H, $J = 3.0$, 7.8, and 8.7 Hz, Pro δ -CH), 3.62–3.22 (m, 5H, Pro δ -CH, 5- CH_2 , 2'- CH_2), 2.69–2.39 (m, 1H, 8'-CH), 2.33–1.79 (m, 11H, Pro β - CH_2 , Pro γ - CH_2 , 3- CH_2 , 4- CH_2 , 7'- CH_2 , 8'-CH), 1.43 and 1.41 (s, 9H, Boc- CH_3); ^{13}C NMR (75 MHz, CDCl_3 , HMQC) δ 172.5, 172.3, 172.2, 172.1, 170.5, and 170.2 (amide C=O), 154.8 and 153.9 (Boc C=O), 79.7 and 79.5 (Boc C(CH_3)₃), 64.5, 64.4, 64.1, 63.9 (6'-C, 8' a-C and 3'-C), 58.1 and 58.0 (Pro α -C), 47.96 and 47.94 (Pro δ -C), 47.1 and 46.9 (5-C), 41.2 and 41.1 (3-C), 38.1 and 38.0 (8'-C), 33.9 and 33.8 (2'-C), 29.5 (Pro β -C), 28.7 and 28.6 (Boc C(CH_3)₃), 28.1 and 27.8 (7'-C), 24.6, 24.5, 23.5 (Pro γ -C and 4-C); ESI HRMS m/z 475.1987 $[\text{M}+\text{Na}]^+$, ($\text{C}_{21}\text{H}_{32}\text{N}_4\text{O}_5\text{S} + \text{Na}^+$) requires 475.1991.

[3' S-(3' α ,6' α ,8' α β)]-1-[2(S)-Pyrrolidinylcarbonyl]-5'-oxospiro[pyrrolidine-2,6'-thiazolidino [3,2-a]piperidine]-3'-carboxamide Hydrochloride (1b)

The Boc-protected molecule (50 mg, 0.1 mmol) was dissolved in 1–2 mL anhydrous CH_2Cl_2 and cooled to -5°C using an ice-salt bath. Trifluoroacetic acid (0.2 mL, 2.2 mmol) was added drop-wise to this solution, which was stirred at that temperature for 45–60 minutes. The starting material was completely consumed at this point (as determined by thin layer chromatography). Solvent and excess TFA were removed *in vacuo*. The colorless oil obtained was suspended in CH_2Cl_2 and solvent removed in the rotary evaporator. This process was repeated several times until a semi-solid material was obtained. This material was triturated with ether and ether decanted to remove any soluble impurities. A white foam/semi-solid weighing 45 mg was obtained, indicating quantitative yield. A solution of the molecule in 4:1 acetonitrile-water was subjected to HPLC analysis on a Supelco Ascentis® C18 column (5 μm particle size, 250 mm $\times$ 10 mm dimension), using an isocratic 98:2 acetonitrile/0.1% TFA aq. solution. A retention time of 3.75 min. was obtained (100% compared to a blank composed of 4:1 acetonitrile-water); TLC R_f 0.53 (2-propanol/ NH_4OH , 4:1); $[\alpha]_D -11.5$ (c 0.13, CD_3OD); ^1H NMR (300 MHz, CDCl_3) δ 4.89–4.84 (dd, 1H, $J = 2.4$ and 10.8 Hz, 8' a-CH), 4.56 (d, 1H, $J = 6.6$ Hz, 3'-CH), 4.47–4.42 (m, 1H, Pro α -CH), 3.72–3.65 (m, 1H, Pro δ - CH_2), 3.49–3.41 (m, 1H, Pro δ - CH_2), 3.34–3.14 (m, 4H, 2'- CH_2 , 5- CH_2), 2.43–2.26 (m, 3H, 7'-CH, 8'- CH_2), 2.15–1.86 (m, 9H, Pro β - CH_2 , Pro γ - CH_2 , 3- CH_2 , 4- CH_2 , 7'-CH); ^{13}C NMR (75 MHz, CDCl_3 , HMQC) δ 174.8, 171.6, 168.5 (amide C=O), 66.7 (6'-C), 65.9, 65.1 (8' a-C and 3'-C), 60.5 (Pro α -C), 49.2 (Pro δ -C), 47.8 (5-C), 41.9 (3-C), 38.0 (8'-C), 34.3 (2-C), 29.1 (Pro β -C), 28.7 (7'-C), 25.3, 25.0 (Pro γ -C and 4-C); ESI HRMS m/z 353.1627 $[\text{M}+\text{H}]^+$, ($\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_3\text{S} + \text{H}^+$) requires 353.1642.

Dopamine D₂ Receptor Binding Assay

The effect of compounds 1a, 1b, 3a, and 3b on [^3H]NPA binding in striatal membranes was analyzed using receptor binding studies. Tritiated NPA was synthesized through a custom order with Perkin-Elmer (Boston, MA) and provided with a specific activity of 43.1 Ci/mmol.

Binding assays were carried out as previously described,^{1,4} wherein 100 µg of striatal membranes was prepared and incubated with the compounds at concentrations indicated in the presence of [³H]NPA. The mixture was incubated in a total 500 µL volume of assay buffer (50 mM Tris-HCl, 5 mM KCl, 4 mM MgCl₂, 1 mM ethylenediaminetetraacetic acid (EDTA) and 120 mM NaCl (pH 7.6)) for 1 h at 25 °C and the incubation then terminated by rapid filtration. Radioactivity-bound filter disks were placed in plastic scintillation vials containing 5 mL scintillation fluid and were counted in a Beckman LS5000 liquid scintillation counter (Model LS5 KTA). Specific binding was defined as the difference between the radioactivity bound in absence and presence of 10 mM dopamine.

Radiolabeling of **15** with [¹²⁵I] and Competitive Receptor Binding Assay

To iodinate **15** two iodo beads (Pierce, Rockford, IL) were washed with 1 mL of phosphate buffer saline for 5 min twice. One mg of the non-iodinated precursor of **15**³ was incubated with the iodo beads and 10 µL (1 mCi) of Na¹²⁵I for 15 min at room temperature with periodic shaking. The mixture was then transferred to the pre-equilibrated column (a C18 (18%) column (Canadian Life Science, Peterborough, ON) was equilibrated twice with 5 mL of MeOH and twice with 5 mL of distilled water). The column was washed five times with 1 mL of distilled water to remove unbound iodine, then washed with 1 mL of 25%, 50% and 100% methanol sequentially with each fraction being collected for incorporation analysis. Flow was collected and 2 µL of each collection was counted for radioactivity. Specific activity of the material was 20 Ci/mmol.

Competition binding assays were performed in triplicates using [¹²⁵I]**15**. Striatal membranes (100 µg) were incubated with 10 µM of the peptidomimetics (**1a**, **1b**, **3a**, or **3b**) and 0.1 µM of [¹²⁵I]**15**. The mixture was incubated at 4 °C for 8 h prior to rapid filtration and counting radioactivity (as described above). Specific binding was defined as the difference between the radioactivity bound in absence and presence of 10 mM PLG.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Structures of Pro-Leu-Gly-NH₂ (PLG), Pro-Pro-Pro-NH₂ (2), and PLG peptidomimetics with 5.6.5 spiro bicyclic scaffolds designed to mimic either a type II β -turn (1a and 1b) or a polyproline II helix (3a and 3b).

Figure 2. Overlay of Ac-Pro-Pro-NH₂ in a PPII conformation (grey) with: (A) the 5.6.5 spiro bicyclic scaffold of **1a** (black); (B) the 5.6.5 spiro bicyclic scaffold of **3a** (black); (C) the 5.6.5 spiro bicyclic scaffold of **3b** (black).

A **B**

Figure 3.
ORTEP drawings of the X-ray crystal structures of **12a** (A) and **12b** (B).

Figure 4.

Modulation of [³H]NPA binding to dopamine D₂ receptors by PLG peptidomimetics 1a (A), 1b (B), **3a** (C), and **3b** (D). Data represent the percent change in specific [³H]NPA binding relative to the control value when the indicated concentration of peptidomimetic was added directly to the assay buffer. Results are the mean ± SEM of five separate experiments carried out in triplicate. The data were analyzed by a one way analysis of variance followed by a Dunnett's post-hoc test. *Significantly different (*p*<0.05) from the control value. **Significantly different (*p*<0.01) from the control value.

Figure 5. PLG peptidomimetic employed as the radioligand in the competition binding assay for the PLG binding site on the dopamine D₂ receptor.

Figure 6.

Displacement of [125 I]15 from the PLG allosteric modulatory site on the dopamine D₂ receptor by analogues **1a**, **1b**, **3a**, and **3b**. Data represent the percent displacement of [125 I]15 from its binding site on bovine striatal membranes. Values graphed are relative to controls in which maximum specific binding was calculated as the amount of [125 I]15 bound in the presence and absence of 10 mM PLG. Results are the mean $\pm$ SEM of five separate experiments carried out in triplicate. The data were analyzed by a one way analysis of variance followed by a Dunnett's post-hoc test. *Significantly different ($p < 0.05$) from the control value.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Table 1

Comparison of the torsion angles of 5.6.5 spiro bicyclic scaffolds with those of a polyproline II helix.

System	ϕ_2	ψ_2	ϕ_3	ψ_3
Polyproline II helix	-80	155	-80	155
1a Spiro bicyclic scaffold ^a	-56	133.7	97.3	-19.9
1b Spiro bicyclic scaffold ^b	-54	159	41	-82
3a Spiro bicyclic scaffold ^c	-48.5	136.2	-64.3	160.5
3b Spiro bicyclic scaffold ^d	-50.0	148.4	-82.8	162.8

^aData obtained from ref. ⁹ and is based upon a X-ray structure of this scaffold.

^bValues were calculated using the Amber89 force field in MOE to do the optimizations with 0.001 kcal/mol Angstrom as the gradient. The relatively large difference in ϕ_3 between **1a** and **1b** is likely due to the difference in puckering of the thiazolidine ring brought about by the difference in bridgehead stereochemistry. The ψ_3 torsion angle is not constrained in these scaffolds and thus is expected to vary.

^cValues are from the X-ray structure of the precursor to **3a**, compound **12a**.

^dValues are from the X-ray structure of the precursor to **3b**, compound **12b**.