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Transformation of Pro-Leu-Gly-NH_2 Peptidomimetic Positive Allosteric Modulators of the Dopamine D_2 Receptor into Negative Modulators

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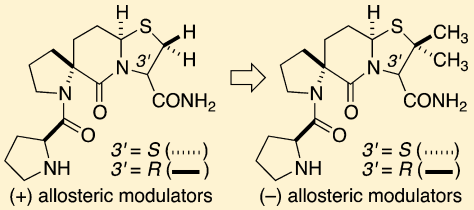
Supporting Information

ABSTRACT: The synthesis of dimethyl derivatives of 5.6.5 spiro bicyclic lactam Pro-Leu-Gly-NH_2 peptidomimetics was carried out to test the hypothesis that by placing methyl groups on the β-methylene carbon of the thiazolidine ring steric bulk would be introduced into the topological space that the β-methylene carbon is believed to occupy in the negative allosteric modulators of the dopamine D_2 receptor. With such a modification, a positive allosteric modulator would be converted into a negative allosteric modulator. This hypothesis was shown to be correct as **3a** and **4a** where found to be negative allosteric modulators, whereas their unmethylated derivatives were positive allosteric modulators of the dopamine D_2 receptor.



KEYWORDS: *Allosteric modulation, dopamine D_2 receptor, Pro-Leu-Gly-NH_2, spiro bicyclic lactam, peptidomimetic*

The endogenous neuropeptide L-prolyl-L-leucyl-glycinamide (PLG) is a positive allosteric modulator of dopamine receptors.[1−3] PLG and its peptidomimetics show selectivity toward the D_{2S}, D_{2L}, and D_4 dopamine receptor subtypes.[1,4] They do not exhibit activity at the D_1 dopamine receptor or the closely related adrenergic receptors.[1,4] PLG and its peptidomimetics exert their positive modulatory effects by increasing the affinity of the high-affinity state of the dopamine receptor for orthosteric agonists and by increasing the ratio of the dopamine receptor in the high-affinity state, which is coupled to G-proteins.[1,4] In vivo, PLG and its peptidomimetics enhance both amphetamine and apomorphine-dependent rotational behavior in 6-hydroxydopamine-lesioned rats,[5−7] prevent and reverse drug-induced dopamine receptor supersensitivity caused by dopamine receptor antagonists,[8,9] and inhibit neuroleptic drug-induced vacuous chewing in rat models of tardive dyskinesia.[7,10]

The synthesis of conformationally constrained analogues of PLG led to highly active PLG peptidomimetics such as **1a** (Figure 1), which possesses the very rigid 5.6.5 spiro bicyclic lactam type II β-turn mimic.[11] These studies provided strong support for the hypothesis that the bioactive conformation of PLG is a type II β-turn. However, the dopamine receptor modulating activity of triproline Pro-Pro-Pro-NH_2 did not fit this hypothesis, since it cannot exist in a type II β-turn.[12] We hypothesized that Pro-Pro-Pro-NH_2 was adopting a different conformation that placed the important pharmacophore groups in relatively the same topographical space as did the PLG peptidomimetics designed to mimic a type II β-turn. The synthesis of Pro-Pro-Pro-NH_2 peptidomimetic **2a** possessing a highly constrained 5.6.5 spiro bicyclic lactam scaffold designed to mimic a polyproline II helix was carried out to test this hypothesis.[13]

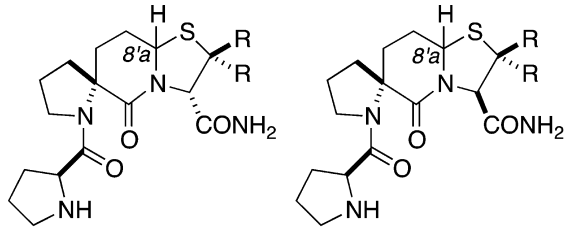


Figure 1. PLG peptidomimetics based upon the 5.6.5 spiro bicyclic scaffold.

Peptidomimetics **1a** and **2a** were found to be positive allosteric modulators of the dopamine receptor, thus providing support for the concept that conformationally different peptidomimetics are capable of serving as allosteric modulators of the dopamine receptor so long as these conformationally constrained scaffolds place key pharmacophores in the correct region of space. In a novel finding, however, the bridgehead epimers of **1a** and **2a**, peptidomimetics **1b** and **2b**, respectively, were found

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to be negative modulators of the dopamine receptor.[13] Since the structural difference between **1a** and **1b** and between **2a** and **2b** is the chirality of the bridgehead carbon atom, we postulated that the difference in bridgehead chirality has an effect on the conformational nature of the thiazolidine portion of the spiro bicyclic lactam scaffold that in turn translated into different conformational changes at the PLG allosteric site upon binding thereby bringing about different modulatory effects. In particular, modeling studies suggested that the pucker of the thiazolidine ring in the scaffolds of **1b** and **2b** caused the β-methylene carbon to jut into a different area of topological space than that found in **1a** and **2a** (Figure 2A).

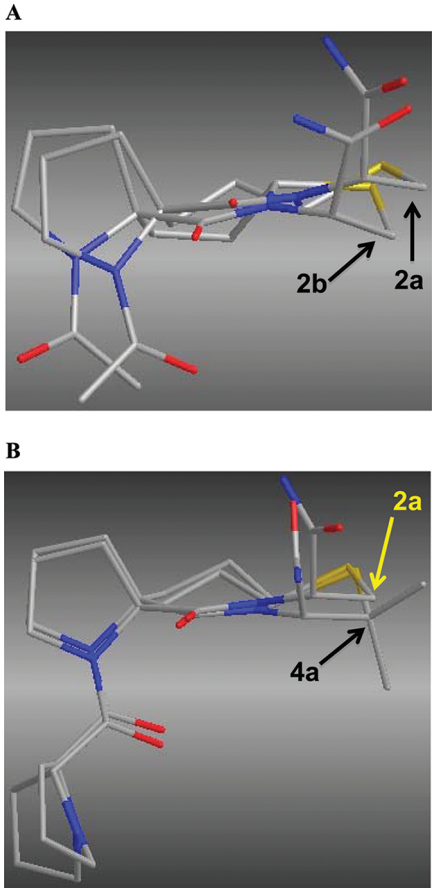

Figure 2. (A) Overlay of the 5.6.5 spiro bicyclic lactam scaffolds of **2a** and **2b**. (B) Overlay of **2a** and **4a**. The lowest energy conformers were obtained with the Maestro 9.1 Suite developed by Schrodinger. In particular, the OPLS force field was selected in *Macromodel*, which was initially used to conduct the conformational analysis. This was followed by conducting an *ab initio* QM energy optimization with *Jaguar*. The optimizations were performed on each of the lowest energy conformers obtained using *Macromodel*. All calculations were performed on the structurally intact PLG peptidomimetic; any structural features missing were removed for the purpose of clarity.

In this study, we have synthesized the dimethyl derivatives of **1a**, **1b**, **2a**, and **2b**, compounds **3a**, **3b**, **4a**, and **4b**, respectively, to test the above hypothesis. By placing methyl groups on the β-methylene carbon of the thiazolidine ring, we have attempted to place steric bulk in the topological space that the β-methylene carbon occupies in its different pucker conformations (Figure 2B). We hypothesize that this type of substitution will convert a positive allosteric modulator into a negative allosteric modulator.

RESULTS AND DISCUSSION

Syntheses. The synthesis of the 5.6.5. spiro bicyclic lactam PLG peptidomimetics **3a**, **3b**, **4a**, and **4b** were carried out as outlined in Schemes 1 and 2. α-Substituted proline **5** served as the starting material for the synthesis of these peptidomimetics. This compound was synthesized as described previously by us.[11,13,14] Compound **5** was condensed in separate reactions with the hydrochloride salts of L- and D-penicillamine methyl esters to give the respective thiazolidines **6a** and **6b** as diastereoisomeric mixtures. The immediate lactamization of **6a** and **6b** in the presence of 2-chloro-1-methyl pyridinium iodide (CMPI) gave the corresponding pairs of 5.6.5-spiro bicyclic thiazolidines scaffolds **7a**/**7b** and **8a**/**8b** in overall yields of 63% and 57%, respectively. For the condensation/lactamization sequence with the L-penicillamine derivative, a 3:1 ratio of the two C-8a′ epimers **7a** and **7b** was obtained with the major diastereoisomer **7a** having the *R* configuration at C-8′a (bridgehead hydrogen down). In contrast, in the reaction sequence with D-penicillamine-OMe, a 1:1 ratio of the diastereoisomers **8a** and **8b** was obtained. These diastereoisomeric ratios directly mirrored the diastereoselectivities observed previously with condensations carried out with D- and L-cysteine-OMe.[11,13] The absolute and relative stereochemical configuration about the bridgehead carbons (C-8′a) for each diastereoisomer was established by 1-D NOE experiments, as well as by single molecule X-ray structure analysis for three of the four diastereoisomers (see Supporting Information for structures of compounds **7b**, **8a**, and **8b**).[15]

All four diastereoisomers derived from the penicillamines, **7a**, **7b**, **8a**, and **8b**, were separately N-deprotected in the presence of TFA in anhydrous CH_2Cl_2 and subsequently coupled to Boc-L-Pro-OH in the presence of HATU as an activating agent to afford **9a**, **9b**, **10a**, and **10b**, respectively. We initially tried to convert **9b** to the carboxamide via ammonolysis, but this was unsuccessful as complete recovery of the starting material was obtained even when the ammonolysis was carried out under forcing conditions. Since our previous experience with the L-cysteine-OMe derivative did not present such problems,[13] we suspect that the added steric bulk of the 2′-*gem*-dimethyl groups hinders the nucleophilic attack on the ester carbonyl. We therefore decided to use the ester hydrolysis/ammonia coupling protocol that had been successfully used before.[13] Ester hydrolysis of **9b** proceeded smoothly under standard conditions without any epimerization. The subsequent coupling of the acid with ammonia resulted in a 20−25% yield of the carboxamide **11**. Despite the poor yield, the final N-deprotection was conducted with TFA/CH_2Cl_2 to give the trifluoroacetate salt of **4b** in quantitative yield.

Unfortunately, diastereoisomer **9a** was recalcitrant toward ester hydrolysis under standard conditions and required almost 10 equiv of LiOH, refluxing conditions, and prolonged reaction times for complete conversion. Under these forced conditions, almost complete epimerization at the C-3′ position (as determined by NOE analysis) was observed. Although epimerization under ester hydrolysis had not been observed for the previously synthesized des-dimethyl derivative, there is precedent for C-3′ epimerization under forced ammonolysis conditions.[13] We postulate that the pseudoaxial positioning of the methoxycarbonyl moiety in **9a**, in addition to the C-2′-*gem*-dimethyl groups, hinders ester hydrolysis and reinforces the epimerization under the harsh conditions. The carboxylic acid obtained was subjected to amidation via coupling with ammonia with HATU to give carboxamide **12**. For diastereoisomer **10a**, ester

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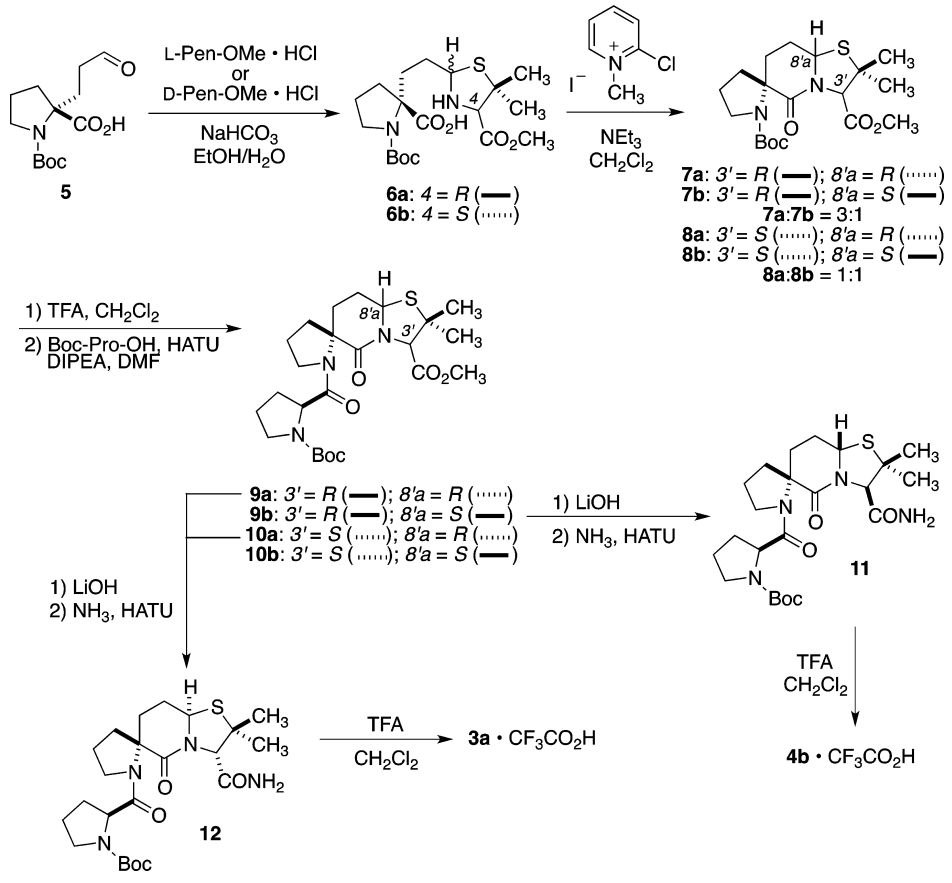
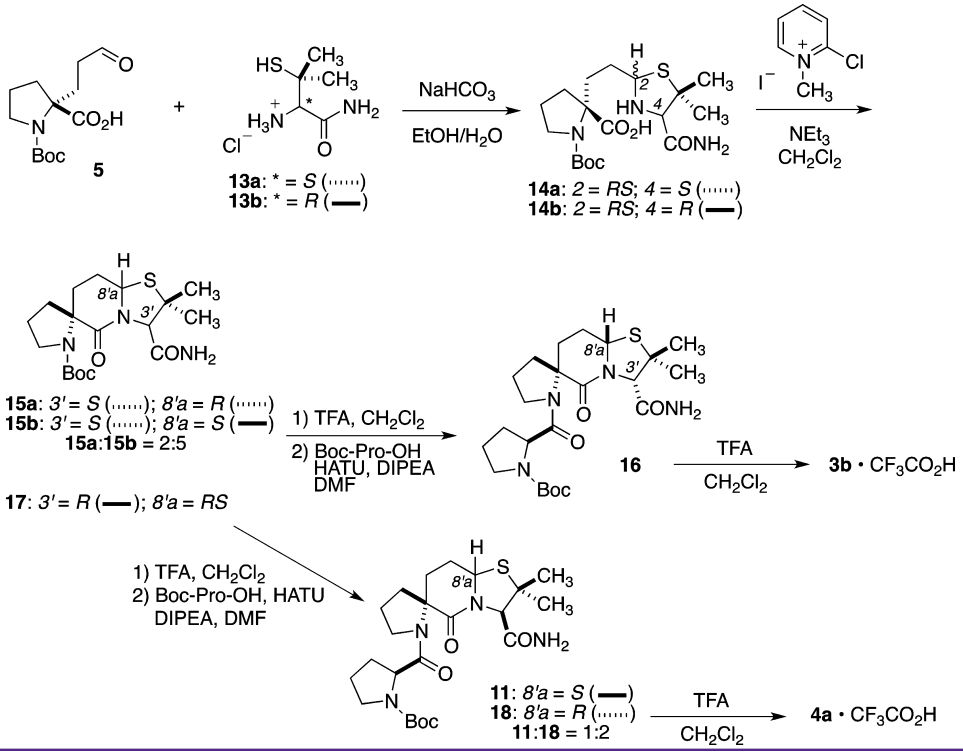
Scheme 1



Scheme 2



hydrolysis was quite facile with carboxylic acid formation complete and without epimerization within 6−8 h with 3 equiv of LiOH.[11] The carboxylic acid was coupled with ammonia to form carboxamide **12**. Removal of the Boc-group

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from **12** with TFA/CH_2Cl_2 gave the corresponding TFA salt of **3a** as a crystalline white solid.

In light of the epimerization issues encountered above, we tried to introduce the carboxamide moiety earlier within the synthetic scheme by attempting to carry out the ammonolysis on the diastereoisomeric thiazolidine mixture **6a**/**6b**. Unfortunately, the ammonolysis reaction was unsuccessful even under forced reaction conditions. We next attempted utilization of either the D- or L-penicillamine carboxamide in the condensation/lactamization sequence. An attempt to directly synthesize D-penicillamine carboxamide by ammonolysis of D-Pen-OMe in anhydrous MeOH failed. However, by masking the amine and thiol functionalities through the synthesis of the C-2 phenyl thiazolidine derivatives[16,17] D- and L-penicillamine were converted to D- and L-penicillamine carboxamide, **13a** and **13b**, respectively (see Supporting Information for the synthetic details).

Compounds **13a** and **13b** were utilized in the formation of the 5.6.5-spiro bicyclic lactam peptidomimetics **3b** and **4a**, respectively, as depicted in Scheme 2. The condensation of **13a** with **5** provided **14a** as a mixture of diastereoisomers. Mukaiyama's reagent, 2-chloro-1-methylpyridinium iodide, facilitated the cyclization of these diastereoisomeric thiazolidines to give a mixture of diastereoisomers **15a** and **15b** in a 2:5 ratio and in an overall yield of 71%. These diastereoisomers were separable by column chromatography and a crystal structure was obtained for **15b** (see Supporting Information for structure).[15] Isomer **15b** was treated with TFA to remove the *tert*-butoxycarbonyl group and the resulting amine was coupled with Boc-L-Pro-OH in the presence of HATU and DIPEA to give **16**. N-Deprotection of **16** gave **3b** as its trifluoroacetate salt.

In a similar manner, **13b** was condensed with **5** to give **14b**, the cyclization of which with Mukaiyama's reagent gave an inseparable mixture of diastereoisomers **17**. The removal of the *tert*-butoxycarbonyl group from **17** with TFA and the subsequent coupling of the amine to Boc-L-Pro-OH with HATU and DIPEA yielded diastereoisomers **11** and **18** in a 1:2 ratio, which were separated by silica gel column chromatography. The stereochemistry at the bridgehead carbon (C-8′a) of the bicyclic thiazolidine lactams was assigned on the basis of extensive NOE difference experiments. Removal of the *tert*-butoxycarbonyl protecting group from **18** with TFA/CH_2Cl_2 provided the desired PLG peptidomimetic **4a** as its trifluoroacetate salt.

Pharmacological Studies. The PLG peptidomimetics **3a**, **3b**, **4a**, and **4b** were evaluated for their ability to increase or decrease the binding of tritiated *N*-propylnorapomorphine ([^{3}H]NPA) on bovine striatal dopamine D_2 receptors. The percentage change in total specific [^{3}H]NPA binding was measured across various concentrations (10^{-5}–10^{-10} M) of each peptidomimetic. The results are depicted in Figure 3. Peptidomimetics **3b** and **4b** showed negative modulatory effects in their ability to affect [^{3}H]NPA binding to isolated dopamine D_2 receptors, but at no concentrations were the effects significant. In contrast, **3a** and **4a** significantly decreased agonist binding to dopamine D_2 receptors, displaying a dose-dependent effect. Peptidomimetic **3a** produced its maximum effect at 10^{-6} M where it decreased the binding of [^{3}H]NPA by 14.8 ± 1.9%. Peptidomimetic **4a** showed significant effects at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M with the maximal effect exhibited at 10^{-5} M. At this concentration, the binding of [^{3}H]NPA to isolated dopamine D_2 receptors was decreased by 15.4 ± 2.0%.

Concentration response curves were generated to obtain EC_{50} values for [^{3}H]NPA at bovine striatal dopamine D_2 receptors in the presence and absence of peptidomimetics **3a** and **4a**. These results are summarized in Table 1. Under control conditions, the EC_{50} for [^{3}H]NPA binding was 0.41 ± 0.055 nM. When **3a**, at a concentration of 10^{-6} M, was added to the dopamine receptor preparation, the EC_{50} of [^{3}H]NPA increased

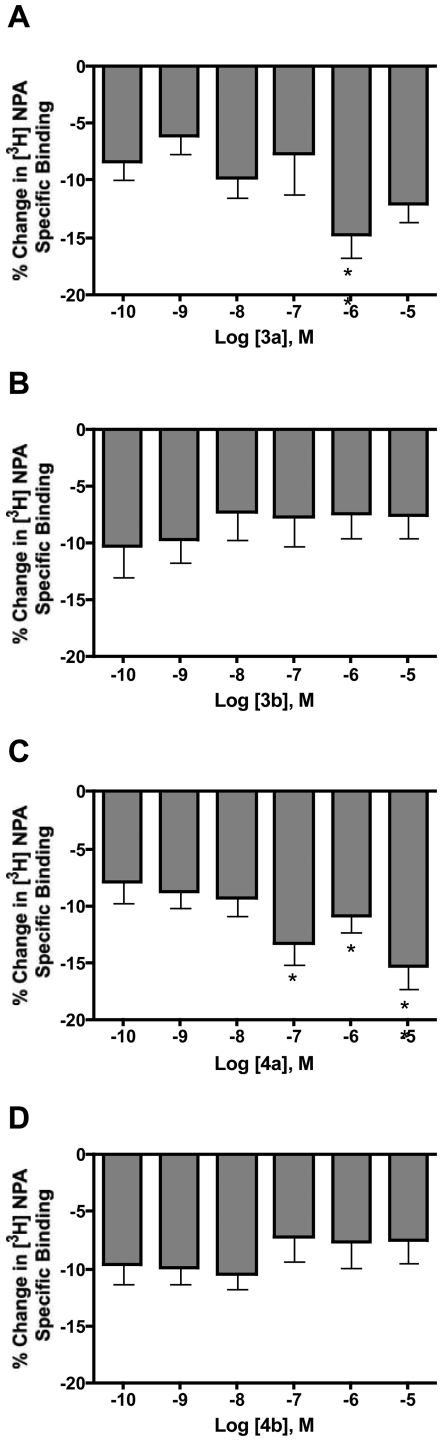

Figure 3. Modulation of [^{3}H]NPA binding to bovine striatal dopamine D_2 receptors by PLG peptidomimetics **3a**, **3b**, **4a**, and **4b**. Data represents the percent increase in specific [^{3}H]NPA binding relative to the control value when the indicated concentration of peptidomimetic was added directly to the assay buffer. Results are the mean ± SEM of at least six 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.

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Table 1. Effect of 3a and 4a on [^{3}H]NPA's EC_{50} Value at Dopamine D_2 Receptors[a]

treatment (conc.)	EC_{50} [^{3}H]NPA (nM)	EC_{50} fold-shift
control (H_2O)	0.41 ± 0.055	
3a (1 μM)	1.12 ± 0.35[b]	2.7
4a (10 μM)	1.15 ± 0.37[b]	2.8

[a]The results are the mean of at least four separate experiments each carried out in triplicate. The data were analyzed by a one way analysis of variance followed by an unpaired *t* test. [b]$p < 0.01$ compared to control.

significantly to 1.12 ± 0.35 nM. This represented a 2.7-fold shift in the EC_{50} value. A similar effect was seen with 10 μM **4a**. In this case, the EC_{50} of [^{3}H]NPA was increased to a value of 1.15 ± 0.37 nM, which represented 2.8-fold shift. Thus, both **3a** and **4a** decreased the affinity of the agonist NPA to dopamine D_2 receptors.

On the basis of the difference in allosteric modulatory activity of the dopamine receptor between the peptidomimetics **1a** and **2a** and **1b** and **2b**, we postulated that the difference in bridgehead chirality between these pair of compounds has an effect on the puckering conformations of the thiazolidine ring of the spiro bicyclic lactam scaffold wherein moieties like the β-methylene carbon jut into a different area of topological space in **1b** and **2b** than that seen in **1a** and **2a**. This in turn translates into different conformational changes at the PLG allosteric site upon binding, thereby bringing about opposite modulatory effects.

In this study, the dimethyl derivatives of **1a**, **1b**, **2a**, and **2b**, compounds **3a**, **3b**, **4a**, and **4b**, respectively, were synthesized to test this hypothesis. It was felt that by placing methyl groups on the β-methylene carbon of the thiazolidine ring of the positive modulators **1a** and **2a** steric bulk would be introduced into the topological space that the β-methylene carbon was believed to occupy in the negative allosteric modulators **1b** and **2b**. If our hypothesis was correct, we expected that this type of substitution would convert the positive allosteric modulators into negative allosteric modulators. This is what was observed. The dimethyl derivatives of the positive allosteric modulators **1a** and **2a**, peptidomimetics **3a** and **4a**, became a negative modulators of the dopamine receptor. This indicates that the introduction of dimethyl groups into the structure of the positive modulators is able to mimic the structural/conformational characteristics of the unsubstituted negative modulators in terms of interacting with the allosteric binding site.

In contrast, when dimethyl groups are placed on the thiazolidine β-methylene carbon of the dopamine receptor negative allosteric modulators **1b** and **2b** to give peptidomimetics **3b** and **4b**, the resulting compounds retain their negative modulatory activity, but only at a weak level. A comparison of **2b** and **4b**, for example, shows that the thiazolidine β-methylene carbons of these two compounds overlay quite well. This means that the methyl groups of **4b** occupy topographical space beyond that occupied by the thiazolidine β-methylene carbon of **2b**. We postulate that the steric bulk of the methyl groups in this case creates structural/conformational changes at the allosteric site that are less conducive to negatively modulating the dopamine receptor.

In conclusion, this study demonstrates that small structural changes in these spiro bicyclic dopamine receptor modulators can bring about major changes in modulatory activity of the compounds. We are presently investigating whether both methyl groups are responsible for these dramatic effects through the synthesis of the monosubstituted derivatives of **3a**, **3b**, **4a**, and **4b**.

METHODS

Compound Purity. The purity of compounds **3a**, **3b**, **4a**, and **4b** was >95% as determined by HPLC.

Methyl [3′*R*-(3′β,6′α,8′aα)]-1-(*tert*-Butyloxycarbonyl)-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]-piperidine]-3′-carboxylate (7a) and Methyl [3′*R*-(3′β,6′α,8′aβ)]-1-(*tert*-Butyloxycarbonyl)-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (7b). To a chilled (ice/water/salt) solution of the α-substituted proline derivative 5 (1.9 g, 7.0 mmol) in 1:1 95% EtOH/H_2O was added solid $NaHCO_3$ (588 mg, 7.0 mmol). L-Penicillamine methyl ester hydrochloride (1.4 g, 7.0 mmol) was then added as a solid in one portion. The suspension eventually formed a mostly clear solution, the pH of which was adjusted to 6.5−7.0 with sat. $NaHCO_3$ solution. It was then stirred overnight under ambient conditions. The next morning EtOH was removed in vacuo and the pale pink-colored aqueous solution was acidified to pH 4−5. The resulting emulsion was extracted using a 3:1 mixture of Et_2O/EtOAc (3×). The organic layers were pooled together, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a pale pink oil that turned into a pink-colored foam when placed under vacuum. The crude mixture of diastereoisomers **6a** (2.1 g, 70% yield) was directly subjected to the lactam formation reaction below.

The diastereomeric thiazolidine mixture **6a** (2.1 g, 5.0 mmol) was dissolved in 150 mL anhydrous CH_2Cl_2. Triethylamine (1.7 mL, 12.5 mmol) was added in one portion to the solution followed by CMPI (1.5 g, 6.0 mmol). A bright yellow-colored suspension was obtained. This suspension was heated at reflux for 17 h and at the end of this time a dark amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give an orange-colored syrup that was purified by flash chromatography (gradient elution: 10−66% EtOAc in hexanes; very slow increments in polarity and using a column diameter that would be used for a 7−8 g reaction scale). The two diastereoisomers, **7a** and **7b**, were obtained in a 3:1 ratio and in an overall yield of 63%.

7a. Oil (900 mg); TLC *Rf* 0.40 (EtOAc/hexanes, 1:1); $[\alpha]_D$ +13.7 (*c* 0.75, CH_2Cl_2). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond. ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.11 (dd, J_1 = 11.2 Hz, J_2 = 3.4 Hz, 0.4H, 8′a-C*H*), 4.97 (dd, J_1 = 11.4 Hz, J_2 = 3.4 Hz, 0.6H, 8′a-C*H*), 4.37 and 4.31 (s, 1H, 3′-C*H*), 3.68 and 3.67 (s, 3H, OCH_3), 3.57−3.40 (m, 2H, 5-CH_2), 2.61 (ddd, J_1 = J_2 = 13.7 Hz, J_3 = 3.1 Hz, 0.4H, 3-CH_2), 2.37 (m, ddd, J_1 = J_2 = 13.7 Hz, J_3 = 2.6 Hz, 0.6H, 7′-CH_2), 2.29−2.14 (m, 2H, 8′-CH_2, 3-CH_2), 2.02−1.71 (m, 5H, 8′-CH_2, 7′-CH_2, 3-CH_2, 4-CH_2), 1.61 and 1.57 (s, 3H, 2′-C(CH_3)$_2$), 1.38 (s, 9H, C(CH_3)$_3$), 1.34 and 1.32 (s, 3H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC) δ 171.6 and 171.4 (lactam C═O), 170.0 and 169.8 (ester C═O), 153.9 and 153.3 (carbamate C═O), 80.1 and 79.5 (C(CH_3)$_3$), 72.2 and 71.8 (3′-*C*), 64.8 and 64.7 (8′a-*C*), 62.3 and 62.0 (2′-*C*), 52.1 and 51.9 (OCH_3), 51.3 and 51.2 (6′-*C*), 48.7 and 48.4 (5-*C*), 39.6 and 38.8 (8′-*C*), 33.1 and 32.3 (7′-*C*), 32.2 and 31.9 (2′-C(CH_3)$_2$), 28.7and 28.6 (C(CH_3)$_3$), 27.2 and 27.1 (3-*C*), 24.6 and 24.5 (2′-C(CH_3)$_2$), 23.4 and 22.8 (4-*C*); ESI HRMS *m*/*z* 421.1781 $[M + Na]^+$, ($C_{19}H_{30}N_2O_5S$ + Na^+) requires 421.1768.

7b. White solid (300 mg); suitable crystals for an X-ray structure[15] were obtained after crystallization from hexanes and EtOAc; mp 160−162 °C (dec); TLC *Rf* 0.42 (EtOAc/hexanes, 1:1); $[\alpha]_D$ −198.5 (*c* 0.4, CH_2Cl_2). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond. ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.08 (dt, J_1 = 10.2 Hz, J_2 = 3.1 Hz, 1H, 8′a-C*H*), 4.75 and 4.63 (s, 1H, 3′-C*H*), 3.72 and 3.71 (s, 3H, OCH_3), 3.64−3.38 (m, 2H, 5-CH_2), 2.58−1.75 (m, 8H, 7′-CH_2, 8′-CH_2, 3-CH_2, 4-CH_2), 1.60 and 1.59 (s, 3H, 2′-C(CH_3)$_2$), 1.41 (app. s, 12H, C(CH_3)$_3$, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 171.2 and 170.8 (lactam C═O), 169.2 and 169.0 (ester C═O), 154.4 and 154.0 (carbamate C═O), 80.4 and 79.3 (C(CH_3)$_3$), 72.1 and 71.8

(3′-*C*), 63.9 and 63.5 (2′-*C*), 62.7 and 62.3 (8′a-*C*), 52.2 and 52.1 (6′-*C*), 51.1 (OCH_3), 48.6 and 48.4 (5-*C*), 43.2 and 42.2 (3-*C*), 36.0 and 35.8 (7′-*C*), 28.9, 28.8, 28.7, 28.6, 28.5 ($C(CH_3)_3$, 2′-$C(CH_3)_2$), 27.8 and 26.9 (8′-*C*), 22.9 and 22.4 (4-*C*); ESI HRMS *m*/*z* 421.1755 $[M + Na]^+$, ($C_{19}H_{30}N_2O_5S + Na^+$) requires 421.1768.

Methyl [3′*S*-(3′*α*,6′*α*,8′a*α*)]-1-(*tert*-Butyloxycarbonyl)-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]-piperidine]-3′-carboxylate (8a) and Methyl [3′*S*-(3′*α*,6′*α*,8′a*β*)]-1-(*tert*-Butyloxycarbonyl)-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (8b). For the synthesis of the diastereoisomeric mixture **6b**, compound **5** was treated with commercially available D-penicillamine methyl ester hydrochloride in the same manner as that described above for synthesis of **6a**. The crude diastereomeric thiazolidine mixture **6b** (1.9 g, 4.7 mmol) was then treated in the same manner as that describe above for the compound **6a**. The two diastereoisomers **8a** and **8b** were obtained in a 1:1 ratio and in an overall yield of 57%.

8a. Yield 500 mg; suitable crystals for an X-ray structure[15] were obtained after crystallization from hexanes and EtOAc; mp 166−167 °C (dec); TLC *Rf* 0.34 (EtOAc/hexanes, 1:1); $[\alpha]_D$ +138.4 (*c* 0.5, CH_2Cl_2). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond. ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) *δ* 5.32 (dd, J_1 = 10.8 Hz, J_2 = 3.9 Hz, 0.4H, 8′a-C*H*), 5.23 (dd, J_1 = 11.0 Hz, J_2 = 4.2 Hz, 0.6H, 8′a-C*H*), 4.61 and 4.57 (s, 1H, 3′-C*H*), 3.76 and 3.71 (s, 3H, OCH_3), 3.68−3.44 (m, 2H, 5-CH_2), 2.79−2.64 (m, 1H, 7′-CH_2), 2.32−2.20 (m, 2H, 8′-CH_2, 3-CH_2), 2.04−1.71 (m, 5H, 8′-CH_2, 7′-CH_2, 3-CH_2, 4-CH_2), 1.59 and 1.56 (s, 3H, 2′-$C(CH_3)_2$), 1.49−1.42 (m, 12H, 2′-$C(CH_3)_2$, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC assignment) *δ* 171.7 and 171.0 (lactam *C*═O), 169.0 and 168.7 (ester *C*═O), 153.6 and 153.5 (carbamate *C*═O), 80.5 and 79.3 ($C(CH_3)_3$), 72.3 and 72.1 (3′-*C*), 64.6 and 64.5 (2′-$C(CH_3)_2$), 62.4 and 62.3 (8′a-*C*), 51.9 and 51.8 (OCH_3), 51.4 and 51.1 (6′-*C*), 48.6 (5-*C*), 41.0 and 38.9 (3-*C*), 32.5 and 31.5 (7′-*C*), 30.3 and 30.0 (2′-$C(CH_3)_2$, 28.9, 28.5, 28.2, 27.8 (8′-*C* and $C(CH_3)_3$), 27.1 and 26.6 (4-*C*); ESI HRMS *m*/*z* 421.1792 $[M + Na]^+$, ($C_{19}H_{30}N_2O_5S + Na^+$) requires 421.1768.

8b. Yield 500 mg; suitable crystals for an X-ray structure[15] were obtained after crystallization from hexanes and EtOAc; mp 163−164 °C (dec); TLC *Rf* 0.26 (EtOAc/hexanes, 1:1); $[\alpha]_D$ −130.5 (*c* 0.5, CH_2Cl_2). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond. ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) *δ* 4.93 (dd, J_1 = 11.1 Hz, J_2 = 2.1 Hz, 1H, 8′a-C*H*), 4.27 and 4.26 (s, 1H, 3′-C*H*), 3.74 and 3.69 (s, 3H, OCH_3), 3.67−3.55 (m, 1H, 5-CH_2), 3.52−3.39 (m, 1H, 5-CH_2), 2.68−2.57 (m, 1.2H, 7′-CH_2), 2.36−2.25 (m, 0.8H, 8′-CH_2), 2.19−1.74 (m, 6H, 8′-CH_2, 7′-CH_2, 3-CH_2, 4-CH_2), 1.59 (s, 3H, 2′-$C(CH_3)_2$), 1.48−1.38 (m, 12H, 2′-$C(CH_3)_2$, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC assignment) *δ* 172.3 and 171.8 (lactam *C*═O), 169.8 and 169.7 (ester *C*═O), 154.4 and 154.1 (carbamate *C*═O), 80.5 and 79.1 ($C(CH_3)_3$), 73.5 and 73.4 (3′-*C*), 72.4 and 72.3 (6′-*C*), 63.9 and 63.6 (8′a-*C*), 61.5 and 61.47 (2′-$C(CH_3)_2$), 52.0 and 51.9 (O−CH_3), 48.3 (5-*C*), 44.6 and 42.9 (3-*C*), 36.7 (7′-*C*), 31.8 and 31.7 (2′-$C(CH_3)_2$), 28.9 and 28.5 (8′-*C*), 28.3 and 28.28 ($C(CH_3)_3$), 23.5 and 23.4 (2′-$C(CH_3)_2$), 22.7 and 22.0 (4-*C*); ESI HRMS *m*/*z* 421.1778 $[M + Na]^+$, ($C_{19}H_{30}N_2O_5S + Na^+$) requires 421.1768.

Methyl [3′*R*-(3′*β*,6′*α*,8′a*α*)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (9a). A solution of the spiro bicyclic compound **7a** (500 mg, 1.3 mmol) in 11 mL of anhydrous CH_2Cl_2 was cooled to 0 °C in an ice bath. Trifluoroacetic acid (3.8 mL, 50 mmol) was then added dropwise to this solution which was then stirred at 0 °C until removal of the Boc-group was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed up to rt, and the volatiles removed by rotary evaporation. The residue was redissolved in CH_2Cl_2 and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow-colored residue was dried to a constant weight for 5−6 h under vacuum.

A solution of Boc-L-proline (437 mg, 2.0 mmol) in 5 mL of DMF was cooled to −5 °C in an ice/salt bath. DIPEA (0.6 mL, 3.4 mmol) was added dropwise to this cooled solution followed by HATU (772 mg, 2.0 mmol). The clear solution was stirred for 30 min after which a solution of the spirocycle free amine in 5 mL of DMF [obtained by neutralizing the TFA salt (700 mg, 1.7 mmol) with DIPEA (0.6 mL, 3.4 mmol)] was added to the active ester. The yellow-colored solution was warmed to rt and then stirred under Ar for 36 h. At the end of this time, DMF and other volatiles were removed in vacuo. The residue was dissolved in 80 mL of EtOAc, and this solution was sequentially washed with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a yellow-colored residue which was purified by flash chromatography (gradient elution: 0−6% MeOH/CH_2Cl_2 in discrete steps) to give a clear oil weighing 492 mg (79% yield). TLC *Rf* 0.47 (CH_2Cl_2/MeOH, 20:1); $[\alpha]_D$ −33 (*c* 1.11, $CHCl_3$). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) *δ* 5.17 (dt, 1H, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 8′a-C*H*), 4.44 (dd, 0.5H, J_1 = 7.8 Hz, J_2 = 3.4 Hz, Pro *α*-C*H*), 4.40 and 4.39 (s, 1H, 3′-C*H*), 4.32 (dd, 0.5H, J_1 = 8.5 Hz, J_2 = 4.0 Hz, Pro *α*-C*H*), 3.89−3.85 (m, 0.5H, Pro *δ*-CH_2), 3.78−3.67 (m, 0.5H, Pro *δ*-CH_2), 3.71 (s, 3H, OCH_3), 3.60−3.49 (m, 2H, Pro *δ*-CH_2, 5-CH_2), 3.46−3.33 (m, 1H, 5-CH_2), 2.74−2.66 (m, 1H, 7′-CH_2), 2.29−1.84 (m, 9H, 8′-CH_2, Pro *β*-CH_2, Pro *γ*-CH_2, 3-CH_2, 4-CH_2), 1.83−1.76 (m, 2H, 4-CH_2, 7′-CH_2), 1.65 and 1.64 (s, 3H, 2′-$C(CH_3)_2$), 1.43 and 1.41 (s, 9H, $C(CH_3)_3$), 1.36 and 1.35 (s, 3H, 2′-$C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) *δ* 171.3 and 170.8 (lactam *C*═O), 170.7 and 170.6 (amide *C*═O), 170.1 (ester *C*═O), 154.6 and 153.7 (carbamate *C*═O), 79.5 and 79.4 ($C(CH_3)_3$), 71.9 and 71.8 (3′-*C*), 66.1 and 66.0 (6′-*C*), 62.0 and 61.9 (8′a-*C*), 58.1 and 58.0 (Pro *α*-*C*), 52.0 (OCH_3), 51.3 and 51.2 (2′-*C*), 48.4 and 48.3 (Pro *δ*-*C*), 47.2 and 46.9 (5-*C*), 37.7 and 37.6 (8′-*C*), 32.3 and 32.2 (2′-$C(CH_3)_2$), 31.8 and 31.5 (7′-*C*), 29.7 and 28.7 (3-*C*), 28.6 and 28.5 ($C(CH_3)_3$), 27.4 and 27.3 (Pro *β*-*C*), 24.6 and 24.5 (2′-$C(CH_3)_2$), 24.5, 24.3, 24.2, and 23.8 (Pro *γ*-*C*, 4-*C*); ESI HRMS *m*/*z* 518.2312 $[M + Na]^+$, ($C_{24}H_{38}N_3O_6S + Na^+$) requires 518.2295.

Methyl [3′*R*-(3′*β*,6′*α*,8′a*β*)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (9b). The same procedure used above to make **9a** was used to convert spiro bicyclic compound **7b** (115 mg, 0.3 mmol) to 110 mg (77%) of **9b** as a clear oil: TLC *Rf* 0.55 (CH_2Cl_2/MeOH, 20:1); $[\alpha]_D$ −132 (*c* 3.02, $CHCl_3$). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) *δ* 5.03 (dt, 1H, 8′a-C*H*), 4.69 and 4.67 (s, 1H, 3′-C*H*), 4.38 (dd, 0.5H, J_1 = 7.9 Hz, J_2 = 3.5 Hz, Pro *α*-C*H*), 4.30 (dd, 0.5H, J_1 = 8.4 Hz, J_2 = 3.6 Hz, Pro *α*-C*H*), 3.85−3.79 (m, 0.5H, Pro *δ*-CH_2), 3.68 and 3.67 (s, 3H, OCH_3), 3.68−3.64 (m, 0.5H, Pro *δ*-CH_2), 3.58−3.46 (m, 2H, Pro *δ*-CH_2, 5-CH_2), 3.41−3.28 (m, 1H, 5-CH_2), 2.56−2.42 (m, 2H, 8′-CH_2, 7′-CH_2), 2.28−1.82 (m, 9H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro *β*-CH_2, Pro *γ*-CH_2, 4-CH_2), 1.79−1.69 (m, 1H, 4-CH_2), 1.56 and 1.55 (s, 3H, 2′-$C(CH_3)_2$), 1.39−1.34 (m, 12H, 2′-$C(CH_3)_2$, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC) *δ* 171.2 and 170.9 (lactam *C*═O), 170.5 and 170.2 (amide *C*═O), 169.13 and 169.11 (ester *C*═O), 154.6 and 153.7 (carbamate *C*═O), 79.3 and 79.2 ($C(CH_3)_3$), 71.9 and 71.8 (3′-*C*), 64.9 and 64.8 (6′-*C*), 62.9 and 62.8 (8′a-*C*), 58.1 and 58.0 (Pro *α*-*C*), 52.1 and 52.0 (OCH_3), 50.9 and 50.7 (2′-*C*), 48.1 and 48.0 (Pro *δ*-*C*), 47.1 and 46.8 (5-*C*), 41.5 and 41.4 (3-*C*), 36.5 and 36.3 (8′-*C*), 29.7 (Pro *β*-*C*), 28.9, 28.8, 28.7, and 28.6 (7′-*C*, 2′-$C(CH_3)_2$), 28.5 ($C(CH_3)_3$), 26.8 and 26.7 (2′-$C(CH_3)_2$), 24.4, 23.69, 23.68, 23.6 (Pro *γ*-*C*, 4-*C*); ESI HRMS *m*/*z* 518.2298 $[M + Na]^+$, ($C_{24}H_{37}N_3O_6S + Na^+$) requires 518.2295.

Methyl [3′*S*-(3′*α*,6′*α*,8′a*α*)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (10a). The same procedure used above to make **9a** was used to convert spiro bicyclic compound **8a** (380 mg, 0.9 mmol) to 380 mg (81%) of **10a** as white solid: mp 140−142 °C; TLC *Rf* 0.35 (CH_2Cl_2/MeOH, 20:1); $[\alpha]_D$ +57.9 (*c* 0.63, $CHCl_3$). ^1H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) *δ* 5.33 (dt, 1H, 8′a-C*H*), 4.52 (s, 1H, 3′-C*H*), 4.47 (dd, 0.5H, J_1 = 7.5 Hz, J_2 = 3.4 Hz, Pro *α*-C*H*), 4.33 (dd, 0.5H, J_1 = 8.0 Hz, J_2 = 3.2 Hz, Pro *α*-C*H*), 3.88 (dt, 0.5H, J_1 = 7.7 Hz,

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J_2 = 3.1 Hz, Pro δ-CH_2), 3.74 (dt, 0.5H, J_1 = 8.9 Hz, J_2 = 3.1 Hz, Pro δ- CH_2), 3.69 (s, 3H, OCH_3), 3.61−3.50 (m, 2H, Pro δ-CH_2, 5-CH_2), 3.41−3.29 (m, 1H, 5-CH_2), 2.88−2.74 (m, 1H, 7′-CH_2), 2.27−1.69 (m, 11H, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 3-CH_2, 4-CH_2), 1.57 and 1.56 (s, 3H, 2′-C(CH_3)$_2$), 1.45−1.44 (m, 3H, 2′-C(CH_3)$_2$), 1.42 and 1.39 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC) δ 171.0 and 170.6 (lactam *C*═O), 170.5 and 170.3 (amide *C*═O), 169.1 and 169.0 (ester *C*═O), 154.6 and 153.7 (carbamate *C*═O), 79.4 and 79.3 (*C*(CH_3)$_3$), 72.5 and 72.4 (3′-*C*), 66.1 and 66.0 (6′-*C*), 62.4 and 62.3 (8′a-*C*), 58.0 and 57.9 (Pro α-*C*), 51.9 and 51.8 (OCH_3), 51.4 and 51.3 (2′-*C*), 48.4 and 48.3 (Pro δ-*C*), 47.0 and 46.8 (5-*C*), 37.94 and 37.93 (8′-*C*), 31.0 and 30.8 (3-*C*), 30.3 and 30.2 (2′-C(CH_3)$_2$), 29.7 (Pro β-*C*), 28.7, 28.6, 28.5, and 28.3 (7′-*C*, C(CH_3)$_3$), 27.13 and 27.10 (2′-C(CH_3)$_2$), 24.3, 24.1, and 23.5 (Pro γ-*C*, 4-*C*); ESI HRMS *m*/*z* 518.2293 [M + Na]$^+$ ($C_{24}H_{38}N_3O_6S$ + Na^+) requires 518.2295.

Methyl [3′*S*-(3′α,6′α,8′aβ)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro-[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxylate (10b). The same procedure used above to make **9a** was used to convert spiro bicyclic compound **8b** (380 mg, 0.9 mmol) to 400 mg (84%) of **10b** as a white solid: mp 162−163 °C; TLC *Rf* 0.40 (CH_2Cl_2/MeOH, 20:1); [α]$_D$ −125 (*c* 0.95, $CHCl_3$). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 4.96−4.91 (m, 1H, 8′a-C*H*), 4.45 (app. t, 0.5H, Pro α-C*H*), 4.32 (dd, 0.5H, J_1 = 7.9 Hz, J_2 = 3.0 Hz, Pro α-C*H*), 4.21 and 4.20 (s, 1H, 3′-C*H*), 3.87−3.81 (m, 0.5H, Pro δ-CH_2), 3.72−3.65 (ovlp. m, 0.5H, Pro δ-CH_2), 3.683 and 3.681 (s, 3H, OCH_3), 3.62−3.52 (m, 2H, Pro δ-CH_2, 5-CH_2), 3.41−3.29 (m, 1H, 5-CH_2), 2.67−2.57 (m, 2H, 8′-CH_2, 7′-CH_2), 2.19−1.73 (m, 10H, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 3-CH_2, 4-CH_2), 1.59 and 1.58 (s, 3H, 2′-C(CH_3)$_2$), 1.43−1.40 (s, 9H, C(CH_3)$_3$), 1.37 and 1.36 (s, 3H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 171.7 and 170.4 (lactam *C*═O), 171.0 and 170.8 (amide *C*═O), 169.8 and 169.7 (ester *C*═O), 154.7 and 153.8 (carbamate *C*═O), 79.4 and 79.2 (*C*(CH_3)$_3$), 73.7 and 73.6 (3′-*C*), 64.9 and 64.8 (6′-*C*), 62.6 and 62.5 (8′a-*C*), 58.2 (Pro α-*C*), 52.1 and 51.9 (2′-*C*), 51.04 and 51.01 (OCH_3), 47.9 (Pro δ-*C*), 47.1 and 46.9 (5-*C*), 42.2 and 42.1 (3-*C*), 37.2 and 37.1 (8′-*C*), 32.2 and 31.1 (2′-C(CH_3)$_2$), 29.7 (Pro β-*C*), 28.9 and 28.7 (7′-*C*), 28.6 and 28.5 (C(CH_3)$_3$), 24.2, 23.8, and 23.6 (Pro γ-*C*, 2′-C(CH_3)$_2$), 23.6 and 23.5 (4-*C*); ESI HRMS *m*/*z* 518.2293 [M + Na]$^+$, ($C_{24}H_{38}N_3O_6S$ + Na^+) requires 518.2295.

[3′*R*-(3′β,6′α,8′aβ)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (11). The spirobicyclic methyl ester **9b** (430 mg, 0.86 mmol) was dissolved in 9 mL of THF. LiOH (81.6 mg, 3.4 mmol) dissolved in 3 mL of H_2O was added to the above solution, and the reaction was stirred under Ar for 17 h at which time analysis by TLC indicated complete consumption of the starting material. THF was removed by rotary evaporation. The remaining aqueous residue was diluted with water while the pH was adjusted to 12−14 with 1 N NaOH. The solution was washed with 50 mL of EtOAc. The pH of the aqueous layer was then adjusted to 2−3 with dilute HCl, and the solution washed with EtOAc (2 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a colorless oil which converted to a foamlike solid under high vacuum. The pure carboxylic acid weighed 413 mg (quantitative yield). ^{1}H NMR analysis indicated that the product was a single diastereoisomer and that the ester hydrolysis had taken place without any epimerization/racemization of any stereo centers in the molecule. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (br. s, 1H, COOH), 5.06 (dt, J_1 = 12.6 Hz, J_2 = 2.9 Hz, 1H, 8′a-C*H*), 4.69 and 4.66 (s, 1H, 3′-C*H*), 4.41 (dd, J_1 = 7.7 Hz, J_2 = 3.5 Hz, 0.5H, Pro α-*C*), 4.32 (dd, J_1 = 8.3 Hz, J_2 = 4 Hz, 0.5H, Pro α-C*H*), 3.88−3.83 (m, 0.5H, Pro δ-CH_2), 3.71−3.66 (m, 0.5H, Pro δ-CH_2), 3.61−3.30 (m, 3H, Pro δ-CH_2, 5-CH_2), 2.60−2.40 (m, 2H, 8′-CH_2, 7′-CH_2), 2.30−1.85 (m, 9H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 4-CH_2), 1.80−1.70 (m, 1H, 4-CH_2), 1.60 and 1.59 (s, 3H, 2′-C(CH_3)$_2$), 1.47 and 1.46 (s, 3H, 2′-C(CH_3)$_2$), 1.40 and 1.38 (m, 9H, C(CH_3)$_3$).

A solution of the carboxylic acid (413 mg, 0.86 mmol) in 3 mL of DMF was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.3 mL, 1.72 mmol) and HATU (392 mg, 1.03 mmol). The reaction was stirred for 20 min at this temperature under Ar. The solution was then cooled to −78 °C. In a cooled measuring cylinder, 2−3 mL of anhydrous ammonia was condensed into 5 mL of anhydrous CH_2Cl_2. This solution was added to the previous solution of the active ester. A canary yellow-colored solution was formed instantly. A glass adapter fitted with a balloon was attached to the reaction flask. The reaction was stirred at −78 °C for 7 h and then warmed to rt. The next day, another 2 mL portion of liquid ammonia was added and the reaction stirred for another 17 h. At the end of this time, the reaction mixture was diluted with 50 mL of CH_2Cl_2 and filtered to remove the yellow-colored precipitate. The almost colorless filtrate was concentrated and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on a Combiflash Retrieve system with a prepacked Luknova 40 g SiO_2 cartridge employing a gradient elution of 0−15% MeOH/CH_2Cl_2 at a flow rate of 25 mL/min to give a cloudy colorless oil. The precipitated tetramethyl urea (TMU) was removed by filtering the product through a 0.45 μm membrane filter disk to give 80 mg (20% yield) of pure **11**. TLC *Rf* 0.35 (CH_2Cl_2/MeOH, 10:1); [α]$_D$ −162.4 (*c* 1.15, $CHCl_3$). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 6.28 (app. br t, 1H, N*H*), 5.95 (app. br d, 1H, N*H*), 5.11 (dt, 1H, 8′a-C*H*), 4.56 and 4.54 (s, 1H, 3′-C*H*), 4.41 (m, 0.5H, Pro α-C*H*), 4.30 (m, 0.5H, Pro α-C*H*), 3.86−3.80 (m, 0.5H, Pro δ-CH_2), 3.71−3.61 (m, 0.5H, Pro δ-CH_2), 3.55−3.45 (m, 2H, Pro δ-CH_2, 5-CH_2), 3.43−3.30 (m, 1H, 5-CH_2), 2.55−2.40 (m, 2H, 8′-CH_2, 7′-CH_2), 2.30−1.85 (m, 9H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 4-CH_2), 1.77−1.70 (m, 1H, 4-CH_2), 1.57, 1.47 (s, 3H, 2′-C(CH_3)$_2$), 1.40 and 1.37 (m, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 171.3 and 170.9 (lactam *C*═O), 170.9, 170.5, 170.2 (amide *C*═O), 154.6 and 153.8 (carbamate *C*═O), 79.5 and 79.3 (*C*(CH_3)$_3$), 72.9 and 72.8 (3′-*C*), 65.0 and 64.9 (6′-*C*), 63.3 and 63.1 (8′a-*C*), 58.2 and 58.1 (Pro α-*C*), 50.8 and 50.6 (2′-*C*), 48.1 and 48.0 (Pro δ-*C*), 47.1 and 46.9 (5-*C*), 41.3 and 41.2 (3-*C*), 36.3 and 36.1 (8′-*C*), 29.7 (Pro β-*C*), 29.1, 29.0, 28.8, 28.7, 28.5, and 28.4 (2′-C(CH_3)$_2$, 7′-*C*, C(CH_3)$_3$), 26.5 (2′-C(CH_3)$_2$), 24.5, 23.9, 23.7 (Pro γ-*C*, 4-*C*); ESI HRMS *m*/*z* 503.2317 [M + Na]$^+$, ($C_{23}H_{36}N_4O_5S$ + Na^+) requires 503.2299.

[3′*R*-(3′β,6′α,8′aβ)]-1-[[2″(*S*)-Pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]-piperidine]-3′-carboxamide Trifluoroacetate Salt (4b·CF_3CO_2H). The spiro-bicyclic carboxamide **11** (50 mg, 0.1 mmol) was dissolved in 2 mL of anhydrous CH_2Cl_2, and the solution cooled to 0 °C. Trifluoroacetic acid (0.4 mL, 5.1 mmol) was added dropwise to this solution which was then stirred at this temperature for 1 h and then allowed to warm to room temperature until the reaction was complete. The pale yellow-colored solution was concentrated to give an oil which was repeatedly triturated with a CH_2Cl_2/diethyl ether mixture and concentrated until a crystalline white-colored solid was obtained. The solid was dried well under high vacuum to give the final compound **4b** weighing 49 mg (quantitative yield). A solution of the material (1.6 mg/mL in 4:1 acetonitrile−water) was diluted (2× by volume) with 0.05% ammonium formate buffer solution and 10−20 μL was subjected to HPLC analysis at 214 nm on a Varian Pursuit C18 column (3 μm particle size, 100 mm × 2 mm dimension) using a gradient of 10−90% acetonitrile in 0.05% ammonium formate buffer solution. A retention time of 4.24 min was obtained in a 10 min run, and purity was 96%. mp 260 °C (dec); TLC *Rf* 0.75 (iPrOH/NH_4OH, 4:1); [α]$_D$ −163 (*c* 0.5, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, gCOSY assignment) δ 5.23 (dd, J_1 = 10 Hz, J_2 = 3.6 Hz, 1H, 8′a-C*H*), 4.52 (s, 1H, 3′-C*H*), 4.52−4.66 (m, 1H, Pro α-C*H*), 3.76−3.71 (m, 1H, Pro δ-CH_2), 3.57−3.51 (m, 1H, Pro δ-CH_2), 3.40−3.25 (m, 2H, 5-CH_2), 2.50−2.34 (m, 3H, 8′-CH_2, 7′-CH_2, 3-CH_2), 2.26−1.99 (m, 9H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 4-CH_2), 1.59, 1.50 (s, 3H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, CD_3OD, gHMQC and HMBC assignment) δ 170.6, 169.8, 165.8 (amide *C*═O),

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74.0 (3′-C), 66.7 (6′-C), 63.9 (8′a-C), 60.4 (Pro α-C), 51.6 (2′-C), 48.7 (Pro δ-C, overlapped by residual solvent peaks, gHMQC assignment), 47.7 (5-C), 41.9 (3-C), 36.5 (Pro β-C), 29.7 (2′-C(CH_3)$_2$), 29.3 (8′-C), 29.2 (7′-C), 27.4 (2′-C(CH_3)$_2$), 25.5, 25.2 (Pro γ-C, 4-C); ESI HRMS m/z 381.1961 $[M + H]^+$, ($C_{18}H_{29}N_4O_3S + H^+$) requires 381.1955.

[3′*S*-(3′α,6′α,8′aα)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (12). The spirobicyclic methyl ester **10a** (370 mg, 0.75 mmol) was hydrolyzed to its carboxylic acid in the same manner as described above for **9b** in the experimental procedure for **11**. The carboxylic acid was obtained in a quantitative yield (360 mg). ^{1}H NMR analysis indicated that the carboxylic acid was a single diastereoisomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (br. s, 1H, COOH), 5.23 (dt, J_1 = 10.4 Hz, J_2 = 3.2 Hz, 1H, 8′a-C*H*), 4.52 (s, 1H, 3′-C*H*), 4.41 (dd, J_1 = 7.6 Hz, J_2 = 2.8 Hz, 0.5H, Pro α-C), 4.31 (dd, J_1 = 8.0 Hz, J_2 = 3.6 Hz, 0.5H, Pro α-C*H*), 3.96 (t, J = 8.4 Hz, 0.5H, Pro δ-CH_2), 3.80 (t, J = 10.4 Hz, 0.5H, Pro δ-CH_2), 3.60−3.30 (m, 3H, Pro δ-CH_2, 5-CH_2), 2.51−1.67 (m, 12H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 4-CH_2), 1.80−1.70 (m, 1H, 4- CH_2), 1.64 (s, 3H, 2′-C(CH_3)$_2$), 1.54 (s, 3H, 2′-C(CH_3)$_2$), 1.41 and 1.39 (m, 9H, C(CH_3)$_3$).

The conversion of the carboxylic acid (262 mg, 0.54 mmol) to **12** was carried out in the same manner as described above for the synthesis of **11**. The product was purified by flash chromatography on a Combiflash Retrieve system with a prepacked Luknova 40 g SiO_2 cartridge employing a gradient elution of 0−6% MeOH/CH_2Cl_2 at a flow rate of 30 mL/min. The pure product was obtained as a colorless oil weighing 130 mg (50% yield). TLC *Rf* 0.55 (CH_2Cl_2/MeOH, 10:1); $[\alpha]_D$ +59.3 (*c* 0.5, $CHCl_3$). ^{1}H and ^{13}C NMR show the presence of two rotamers about the carbamate bond in a 1:1 ratio. ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 7.39 and 7.32 (br s, 1H, N*H*), 5.15−5.11 (m, 2H, 8′a-C*H*, N*H*), 4.43 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 0.5H, Pro α-C*H*), 4.36 (s, 1H, 3′-C*H*), 4.31 (dd, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 0.5H, Pro α-C*H*), 3.95 (t, 0.5H, Pro δ-CH_2), 3.79 (t, 0.5H, Pro δ-CH_2), 3.60−3.31 (m, 3H, Pro δ-CH_2, 5-CH_2), 2.59−2.47 (m, 1H, 3-CH_2), 2.36−2.22 (m, 2H, 8′-CH_2, 4-CH_2), 2.15−1.72 (m, 9H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro β-CH_2, Pro γ-CH_2, 4-CH_2), 1.66 (s, 3H, 2′-C(CH_3)$_2$), 1.54 (s, 3H, 2′-C(CH_3)$_2$), 1.41 and 1.39 (m, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 170.9, 170.5, 169.7, 169.5, 169.0, 168.8 (amide C═O), 153.3 and 152.3 (carbamate C═O), 79.7 and 79.6 (C(CH_3)$_3$), 73.3 (3′-C), 66.6 and 66.6 (6′-C), 62.4 and 62.3 (8′a-C), 58.2 (Pro α-C), 52.6 and 52.5 (2′-C), 48.9 (Pro δ-C), 47.5 and 47.2 (5-C), 37.3 (3-C), 31.3 and 31.1 (7′-C), 30.3 (Pro β-C), 29.6 and 29.5 (2′-C(CH_3)$_2$), 29.3 and 29.2 (8′-C, C(CH_3)$_3$), 27.7 and 27.6 (2′-C(CH_3)$_2$), 25.3, 25.0, 24.9, 24.5 (Pro γ-C, 4-C); ESI HRMS m/z 503.2317 $[M + Na]^+$, ($C_{23}H_{36}N_4O_5S$ + Na^+) requires 503.2299.

[3′*S*-(3′α,6′α,8′aα)]-1-[[2″(*S*)-Pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]-piperidine]-3′-carboxamide Trifluoroacetate Salt (3a·CF_3CO_2H). The spiro-bicyclic carboxamide **12** (84 mg, 0.17 mmol) was deprotected with the same procedure as described above for the formaton of **4b**. A white-colored solid was obtained in a yield of 71 mg (85%). A solution of this material (1.6 mg/mL in 4:1 acetonitrile−water) was diluted (2× by volume) with 0.05% ammonium formate buffer solution and 10−20 μL was subjected to HPLC analysis at 214 nm on a Varian Pursuit C18 column (3 μm particle size, 100 mm × 2 mm dimension) using a gradient 10−90% acetonitrile in 0.05% ammonium formate buffer solution. A retention time of 5.29 min was obtained in a 10 min run and purity was 99%. mp 260 °C (dec); TLC *Rf* 0.68 (iPrOH/NH_4OH, 5:1); $[\alpha]_D$ +80.4 (*c* 0.45, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, gCOSY assignment) δ 5.20 (dd, J_1 = 10.8 Hz, J_2 = 4.0 Hz, 1H, 8′a-C*H*), 4.87 (dd, J_1 = 8.8 Hz, J_2 = 5.2 Hz, 1H, Pro α-C*H*), 4.27 (s, 1H, 3′-C*H*), 3.76−3.72 (m, 1H, 5-CH_2), 3.53−3.47 (m, 1H, 5-CH_2), 3.33−3.22 (m, 2H, Pro δ-CH_2), 2.45−2.25 (m, 3H, 7′-CH_2, Pro β-CH_2, 8′-CH_2), 2.20−1.86 (m, 8H, 3-CH_2, 4-CH_2, Pro β-CH_2, Pro γ-CH_2, 7′-CH_2), 1.57, 1.49 (s, 3H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, CD_3OD, gHMQC and HMBC assignment) δ 171.1, 170.1, 166.3 (amide C═O), 74.4 (3′-C), 68.3 (6′-C), 63.4 (8′a-C), 60.3 (Pro α-C), 52.4 (2′-C), 49.5 (5-C, overlapped by residual solvent peaks, gHMQC assignment), 47.7 (Pro δ-C), 37.9 (3-C), 31.1 (7′-C), 29.9 (2′-C(CH_3)$_2$), 29.4 (Pro β-C), 28.1 (8′-C), 27.3 (2′-C(CH_3)$_2$), 25.5, 25.3 (Pro γ-C, 4-C); ESI HRMS m/z 381.1946 $[M + H]^+$, ($C_{18}H_{29}N_4O_3S + H^+$) requires 381.1955.

[3′*S*-(3′α,6′α,8′aα)]-1-(*tert*-Butyloxycarbonyl)-2,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (15a) and [3′*S*-(3′α,6′α,8′β)]-1-(*tert*-Butyloxycarbonyl)-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (15b). *N*-Boc-α-butenylproline (**5**, 1.65 g, 6.1 mmol) was dissolved in a 60 mL mixture of EtOH and H_2O (1:1). The solution was then cooled in an ice/salt bath. Sodium bicarbonate (504 mg, 6.0 mmol) was added to the solution followed by D-penicillaminecarboxamide·HCl (**13a**, 1.1 g, 6.0 mmol). The pH of the resulting solution was adjusted to 6.5 and the colorless solution was stirred for 17 h at room temperature under Ar. After this time period, EtOH was removed in vacuo and the resulting aqueous residue was washed with EtOAc (3 × 100 mL). The pH of the aqueous layer was adjusted to 4.0−4.5 between the washes to ensure maximum product precipitation. The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated to give the intermediate thiazolidine mixture of **14a** as a white colored foam weighing 2.2 g (91% yield). The diastereoisomeric thiazolidine mixture (2.2 g, 5.5 mmol) was dissolved in 165 mL of anhydrous CH_2Cl_2. Triethylamine (1.9 mL, 13.7 mmol) was added in one portion to the solution followed by CMPI (1.7 g, 6.6 mmol). A bright yellow-colored suspension was obtained. This suspension was refluxed for 17 h at the end of which an amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to an orange-colored syrup that was purified using flash chromatography on a Luknova 80 g SiO_2 gel prepacked column with a gradient elution of 40−100% EtOAc/hexanes followed by 1−5% MeOH/EtOAc. The two diastereoisomers, **15a** and **15b**, were obtained in a 2:5 ratio in a 71% yield.

15a. White foam (425 mg); mp 68−78 °C; TLC *Rf* 0.61 (MeOH/EtOAc, 2.5%); $[\alpha]_D$ +145.1 (*c* 1.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 7.47 (br.s, 1H, NH_2), 5.16 (dd, 1H, J_1 = 10.8 Hz, J_2 = 3.6 Hz, 8′a-C*H*), 5.07 (br. s, 1H, NH_2), 4.44 (s, 1H, 3′-C*H*), 3.55−3.44 (m, 2H, 5-CH_2), 2.45 (app. t, 1H, 7′-CH_2), 2.38−2.24 (m, 2H, 3-CH_2, 8′-CH_2), 2.02−1.74 (m, 5H, 4-CH_2, 3-CH_2, 7′-CH_2, 8′-CH_2), 1.63 (2′-C(CH_3)$_2$), 1.52 (2′-C(CH_3)$_2$), 1.33 (C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC assignment) δ 169.9, 169.8 (lactam and carboxamide C═O), 153.4 (carbamate C═O), 80.4 (C(CH_3)$_3$), 73.4 (3′-C), 65.2 (6′-C), 62.6 (8′a-C), 52.6 (2′-C(CH_3)$_2$), 48.9 (5-C), 38.4 (3-C), 32.2 (7′-C), 29.6 (2′-C(CH_3)$_2$), 29.4 (C(CH_3)$_3$), 27.8 (8′-C), 27.6 (2′-C(CH_3)$_2$), 24.4 (4-C); ESI HRMS m/z 406.1789 $[M + Na]^+$, ($C_{18}H_{29}N_3O_4S + Na^+$), requires 406.1771.

15b. White solid (1.1 g); suitable crystals for an X-ray structure[15] were obtained after crystallization from hexanes, EtOAc, and isopropyl alcohol; mp 238−241 °C; TLC *Rf* 0.38 (MeOH/EtOAc, 2.5%); $[\alpha]_D$ − 43.9 (*c* 1.09, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 7.18 (br s, 1H, NH_2), 5.09 (br s, 1H, NH_2), 4.92 (dd, 1H, J_1 = 11.2 Hz, J_2 = 2.4 Hz, 8a′-C*H*), 4.15 (s, 1H, 3′-C*H*), 3.57−3.51 (m, 1H, 5-CH_2), 3.45−3.39 (m, 1H, 5-CH_2), 2.77 (dddd or dq, 1H, J_1 = J_2 = J_3 = 15.6 Hz, J_4 = 4 Hz, 8′-CH_2), 2.45−2.33 (m, 2H, 7′-CH_2, 3-CH_2), 2.11−1.94 (m, 3H, 8′-CH_2, 7′-CH_2, 4-CH_2), 1.86−1.76 (m, 2H, 4-CH_2, 3-CH_2), 1.60 (s, 3H, 2′-C(CH_3)$_2$), 1.48 (s, 3H, 2′-C(CH_3)$_2$), 1.37 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 170.0, 169.2 (lactam and carboxamide C═O), 153.9 (carbamate C═O), 80.1 (C(CH_3)$_3$), 74.3 (3′-C), 62.9 (6′-C), 62.7 (8a′-C), 51.4 (2′-C(CH_3)$_2$), 48.9 (5-C), 42.9 (3-C), 38.9 (7′-C), 32.7 (2′-C(CH_3)$_2$), 29.3 (C(CH_3)$_3$), 27.8 (8′-C), 25.1 (2′-C(CH_3)$_2$), 24.7 (4-C); ESI HRMS m/z 406.1781 $[M + Na]^+$, ($C_{18}H_{29}N_3O_4S + Na^+$) requires 406.1771.

[3′*R*-(3′β,6′α,8′aα)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″-(*S*)-pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (18). *N*-Boc-α-butenylproline (**5**, 542 mg, 2 mmol) was dissolved in a 20 mL mixture of EtOH and H_2O (1:1). The solution was then cooled in an ice/salt bath. Sodium bicarbonate (168 mg, 2 mmol) was then added to the solution followed by L-penicillaminecarboxamide·HCl (**13b**, 386 mg, 2.1 mmol). The pH of the resulting solution was adjusted to 6.5, and the colorless solution was stirred for 17 h at room temperature under Ar.

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After this time period, EtOH was removed in vacuo and the resulting aqueous residue was washed with EtOAc (3 × 75 mL). The pH of the aqueous layer was adjusted to 4.0−4.5 between the washes to ensure maximum product precipitation. The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated to give the intermediate thiazolidine mixture of **14b** as a white colored foam weighing 682 mg (85% yield).

The diastereomeric thiazolidine mixture (682 mg, 1.69 mmol) was dissolved in 50 mL of anhydrous CH_2Cl_2. Triethylamine (0.592 mL, 4.25 mmol) was added in one portion to the solution followed by CMPI (516 mg, 2.02 mmol, 1.2 equiv). A bright yellow-colored suspension was obtained. This suspension was refluxed for 17 h at the end of which an amber-colored solution was obtained. The reaction mixture was washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to an orange-colored syrup. The mixture consisted of two diastereoisomers (**17**) that were obtained in a 1:2 ratio. These diastereoisomers were not separable on TLC, so the diastereoisomers were taken on the next reaction.

A solution of the diastereoisomeric spirocycle **17** (375 mg, 0.98 mmol) in 3 mL of anhydrous CH_2Cl_2 was cooled to 0 °C with an ice bath. Trifluoroacetic acid (3.7 mL, 49 mmol) was then added dropwise to this solution, which was then stirred at 0 °C until removal of the Boc-group was determined to be complete by TLC. This was usually between 1.5 and 2 h. The reaction was warmed to rt, and the volatiles removed by rotary evaporation. The residue was redissolved in CH_2Cl_2 and the solvent removed by evaporation twice until the excess TFA was removed. The pale yellow colored residue was dried to a constant weight for 5−6 h under vacuum.

A solution of Boc-L-proline (251 mg, 1.17 mmol) in 4 mL of DMF was cooled to −5 °C using an ice/salt bath. DIPEA (0.39 mL, 2.94 mmol) was added dropwise to this cooled solution followed by HATU (445 mg, 1.17 mmol). The clear solution was stirred for 30 min after which a solution of the diastereoisomeric spirocycle free amine in 4 mL of DMF, obtained by neutralizing the TFA salt (365 mg, 0.98 mmol) with DIPEA (0.393 mL, 2.94 mmol), was added to the active ester. The yellow colored solution was warmed to rt, and then it was stirred under Ar for 36 h. At the end of this time, DMF and other volatiles were removed in vacuo. The residue was dissolved in 120 mL of EtOAc, and this solution was sequentially washed with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give yellow-colored residue. The diastereoisomers were separated by silica gel flash chromatography with a gradient elution consisting of 40−70% acetone/ethyl acetate in discrete steps to give **11** and **18** in a 1:2 ratio.

18. NOESY studies were carried out to delineate the relative stereochemistry. Both the 3′-H and the 8′a-H protons were irradiated in separate experiments, and NOE enhancements were observed in each case. The experiments confirmed that the bridgehead proton was cis to the 3′-hydrogen of the thiazolidine portion of the molecule. ^1H and ^{13}C NMR showed the presence of two rotamers about the carbamate bond in a 1:1 ratio. mp 244 °C; TLC *Rf* 0.45 (acetone/ethyl acetate, 4:1); $[\alpha]_D$ −74.9 (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$, gCOSY assignment) δ 5.95 (d, 1H, *J* = 4.92, N*H*), 5.8 (br s, 1H, N*H*), 5.07−5.13 (m, 1H, 8′a-C*H*), 4.38 (m, 0.5H, Pro α-C*H*), 4.27 (m, 0.5H, Pro α-C*H*), 4.17 and 4.16 (s, 1H, 3′-C*H*), 3.86−3.80 (m, 0.5H, Pro 5-CH_2), 3.71−3.67 (m, 0.5H, Pro 5-CH_2), 3.53−3.44 (m, 2H, Pro δ-CH_2, 5-CH_2), 3.38−3.25 (m, 1H, δ-CH_2), 2.69−2.58 (m, 1H, 3-CH_2), 2.22−1.68 (m, 11H, 3-CH_2, 8′-CH_2, 7′-CH_2, Pro γ-CH_2, Pro β-CH_2, 4-CH_2), 1.57 (s, 3H, 2′-C(CH_3)$_2$), 1.39, 1.37, and 1.34 (s, 12H, 2′-C(CH_3)$_2$, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, gHMQC and HMBC assignment) δ 171.9 and 171.8 (lactam *C*═O), 171.2, 170.7, 170.4, 170.2 (amide *C*═O), 154.4 and 153.5 (carbamate *C*═O), 79.4 and 79.2 (*C*(CH$_3$)$_3$), 73.2 and 73.1 (3′-*C*), 65.9 and 65.8 (6′-*C*), 62.9 and 61.8 (8′a-C), 57.9 and 57.8 (Pro α-*C*), 51.2 and 51.2 (2′-*C*), 48.2 and 48.1 (5-C), 47.0 and 46.7 (Pro δ-*C*), 37.3 and 37.2 (8′-*C*), 32.0 and 31.9 (2′-C(*C*H$_3$)$_2$), 31.7, 31.5 (3′-C), 29.6, 29.3, 28.4, 28.3, 27.6 (Pro β-C, C(CH$_3$)$_3$, 7′-*C*), 24.2, 24.2, (2′-C(*C*H$_3$)$_2$), 23.9 (Pro γ-*C*), 23.5 (4-*C*); ESI HRMS *m*/*z* 503.2428 [M + Na]$^+$, ($C_{23}H_{36}N_4O_5S$ + Na$^+$) requires 503.2299.

[3′*R*-(3′β,6′α,8′aα)]-1-[[2″(*S*)-Pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide Trifluoroacetate Salt (4a·CF_3CO_2H). Spiro-bicyclic carboxamide **18** (66 mg, 0.1 mmol) was dissolved in 2 mL of anhydrous CH_2Cl_2, and the solution was cooled to 0 °C. Trifluoroacetic acid (0.4 mL, 5.1 mmol) was added dropwise to this solution, which was then stirred at this temperature for 1 h. The reaction was warmed to room temperature where it was stirred until the reaction was complete. The pale yellow-colored solution was concentrated to give a oil, which was repeatedly triturated with a CH_2Cl_2/diethyl ether mixture and concentrated until a white-colored solid was obtained. The solid was dried under high vacuum to give **4a** (49 mg) in a quantitative yield. The purity of the product was confirmed by HPLC with a Varian Pursuit C18 column (3 μm particle size, 100 mm × 2 mm dimension) and acetonitrile/H_2O (98:2) as the solvent system. A retention time of 4.24 min was obtained in a 30 min run and purity was 95%. mp 246 °C; TLC *Rf* 0.71 (iPrOH/NH_4OH, 5:1); $[\alpha]_D$ −23.9 (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CD_3OD, gCOSY assignment) δ 5.17 (dd, J_1 = 3.0, J_2 = 3.04 Hz, 1H, 8′a-C*H*), 4.53 (d, *J* = 2.76 Hz, 1H, Pro α-C*H*), 4.20 (s, 1H, 3′-C*H*), 3.82−3.77 (m, 1H, 5-CH_2), 3.58−3.48 (m, 1H, 5-CH_2), 3.40−3.33 (m, 2H, Pro δ-CH_2), 2.52−2.46 (m, 2H, 3′-CH_2, 8′-CH_2), 2.23−1.94 (m, 10H, 3-CH_2, 8′-CH_2, 4-CH_2, Pro β-CH_2, Pro γ-CH_2, 7′-CH_2), 1.64, 1.44 (s, 6H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, CD_3OD, gHMQC and HMBC assignment) δ 173.5, 171.7, 167.3 (amide C═O), 74.1 (3′-*C*), 68.1(6′-*C*), 63.4 (8′a-*C*), 60.5 (Pro α-*C*), 51.8 (2′-*C*), 49.0 (5-overlapped by residual solvent peaks, gHMQC assignment C), 47.5 (Pro δ-*C*), 38.4 (7′C), 32.3 (3-*C*), 32.0 (2′-C(*C*H$_3$)$_2$), 28.7(8′-C), 27.3 (Pro β-*C*), 25.1, 25.0 (Pro γ-*C*, 4-*C*), 24.5 (2′-C(*C*H$_3$)$_2$; ESI HRMS *m*/*z* 381.1874 [M+H]$^+$, ($C_{18}H_{29}N_4O_3S$ + H$^+$) requires 381.1955.

[3′*S*-(3′α,6′α,8′aβ)]-1-[[1″-(*tert*-Butyloxycarbonyl)-2″-(*S*)-pyrrolidinyl]carbonyl]- 2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]piperidine]-3′-carboxamide (16). A solution of the spirocycle **15b** (150 mg, 0.391 mmol) in 2 mL of anhydrous CH_2Cl_2 was converted to **16** by the same procedure used to convert **17** to **18**. The product was purified by flash chromatography with a gradient elution consisting of 0−5% MeOH/CH_2Cl_2 in discrete steps to give a lightly pink-colored foam of 108 mg (58% yield). TLC *Rf* 0.58 (MeOH/CH_2Cl_2, 1:9); $[\alpha]_D$ 24.5 (*c* 1.0, CH_3OH). NMR spectra indicated the presence of two rotameric forms of the molecule about the carbamate bond in a 1:1 ratio. ^1H NMR (400 MHz, $CDCl_3$, gCOSY) δ 7.36 and 7.28 (s, 1H, carboxamide N*H*), 5.16 (d. 1H, J = 7.9 Hz, carboxamide N*H*), 5.09 (dd, 1H, J_1 = 3.64, 3.76, 8′a-C*H*), 4.38 (dd, 0.5H, *J* = 1.92, 2.52 Hz, Pro α-C*H*), 4.33 (s, 1H, 3′-C*H*), 4.28 (dd, 0.5H, *J* = 2.28, 3.08 Hz, Pro α-C*H*), 3.91 (t, 0.4H, *J* = 3.69, 3.92 Hz, Pro δ-C*H*), 3.74 (dd, 0.6H, *J* = 2.8, 8.48 Hz, Pro δ-C*H*), 3.56−3.27 (m, 3H, Pro δ-C*H*, 5-CH_2), 2.49 (m, 1H, 8′-C*H*), 2.33−1.79 (m, 11H, 7′-CH_2, 8′-CH_2, Pro β-CH_2, Pro γ-CH_2, 3-CH_2, 4-CH_2), 1.62 (s, 3H, 2′-C(CH_3)$_2$), 1.51 (s, 3H, 2′-C(CH_3)$_2$), 1.38 and 1.35 (s, 9H, Boc-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, HMQC) δ 172.3, 171.9, 171.2, 172.0, 170.4, and 170.2 (amide C═O), 154.5 and 153.5 (Boc C═O), 79.6 and 79.5 (Boc C(CH$_3$)$_3$), 73.1 (3′-C), 66.39, 66.37 (8′a-C), 62.05, 62.01 (6′-*C*), 57.8 (Pro α-C), 52.10 and 51.99 (2′-C), 48.3 (Pro -δC), 46.9 and 46.6 (Pro −5C), 36.6 (3-C), 30.5 and 30.2 (8′-*C*), 29.4 (Pro β-*C*), 28.8, 28.6, 28.4, 28.3 (2′-C(CH$_3$)$_2$, Boc C(CH$_3$)$_3$, 26.8, 26.6 (2′-C(CH$_3$)$_2$), 24.6, 24.5, 23.5 (Pro γ-*C* and 4-C); ESI HRMS *m*/*z* 503.2333 [M + Na]$^+$, ($C_{23}H_{36}N_4O_5S$ + Na$^+$) requires 503.2299.

[3′*S*-(3′α,6′α,8′β)]-1-[[2″(*S*)-Pyrrolidinyl]carbonyl]-2′,2′-dimethyl-5′-oxospiro[pyrrolidine-2,6′-thiazolidino[3,2-*a*]-piperidine]-3′-carboxamide Trifluoroacetate Salt (3b). The spiro bicyclic carboxamide **16** (84 mg, 0.17 mmol) was deprotected in the same manner as described above for the conversion of **18** to **4a**. The product was obtained in a yield of 81 mg (97%). The purity of the product was confirmed by HPLC with a Varian Pursuit C18 column (3 μm particle size, 100 mm × 2 mm dimension) with acetonitrile/H_2O (98:2) as the solvent system. A retention time of 4.2 min was obtained in a 30 min run and the purity was 99%. mp 237 °C (dec); TLC *Rf* 0.74 (iPrOH/NH_4OH, 4:1); $[\alpha]_D$ −56.9 (*c* 0.49, CH_3OH); ^1H NMR (400 MHz, CD_3OD, gCOSY assignment) δ 5.15 (dd, J_1 = 2.64, J_2 = 2.28 Hz, 1H, 8′a-C*H*), 4.54 (dd, J_1 = 5.13, J_2 = 5.92 Hz, 1H, Pro

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α-CH), 4.11 (s, 1H, 3′-CH), 3.81−3.76 (m, 1H, 5-CH_2), 3.59−3.53 (m, 1H, 5-CH_2), 3.43−3.40 (m, 2H, Pro δ-CH_2), 2.63−2.41 (m, 3H, 7′-CH_2, 8′-CH_2), 2.20−1.86 (m, 10H, 3-CH_2, 4-CH_2, Pro γ-CH_2, Pro β-CH_2, 7′-CH_2), 1.65, 1.44 (s, 6H, 2′-C(CH_3)$_2$); ^{13}C NMR (100 MHz, CD_3OD, gHMQC and HMBC assignment) δ 173.6, 171.6, 168.0 (amide C═O), 75.3 (3′-C), 66.5 (6′-C), 63.0 (8′a-C), 60.5 (Pro α-C), 52.0(2′-C), 48.3 (5-C overlapped by residual solvent peaks, gHMQC assignment), 47.7 (Pro δ-C), 42.3 (7′-C), 37.7 (3-C), 32.0 (2′-C(CH_3)$_2$), 29.0 (Pro β-C), 28.6 (8′-C), 25.18, 25.12 (Pro γ-C, 4-C), 24.3 (2′-C(CH_3)$_2$); ESI HRMS m/z 381.1966 [M + H]$^+$, ($C_{18}H_{29}N_4O_3S$ + H^+) requires 381.1955.

Dopamine D_2 Receptor Binding Assay. The effect of compounds **3a**, **3b**, **4a**, and **4b** on [^{3}H]NPA binding in striatal membranes was analyzed using receptor binding studies. Bovine striatal membranes were prepared as previously described by Kazmi et al.,[18] where freshly dissected calf bovine striatum was homogenized and centrifuged in buffer to isolate cell membranes. [^{3}H]NPA was synthesized through a custom order from Vitrax Radiochemicals (Placentia, CA) and was provided with a specific activity of 60 Ci/mmol. Receptor modulatory assays were performed in a similar manner to Srivastava et al.,[4] wherein 100 μg of striatal membrane were prepared and incubated with the compounds at concentrations ranging from 10^{-5} to 10^{-10} M in the presence of [^{3}H]NPA. The reaction was incubated in a total 500 μL volume of assay buffer (50 mM Tris-HCl, 5 mM KCl, 4 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, and 120 mM NaCl (pH 7.4)). Reaction mixtures were incubated for 1 h at 25 °C and subsequently subjected to rapid filtration. Radioactivity-bound filter disks were placed in plastic scintillation vials containing 4 mL of scintillation fluid, equilibrated for at least 12 h, and counted in a Beckman LS5000 liquid scintillation counter (model LS5 KTA). Specific binding was defined as the difference between the radioactivity bound in the absence and presence of 10 μM sulpiride (Sigma).

Concentration response curves with varying concentrations of [^{3}H]NPA were generated under the same conditions as described above for the modulatory assay. As described previously, 100 μg of striatal membranes were prepared and incubated with compounds **3a** and **4a** at effective concentrations of 1 and 10 μM, respectively. The reaction was incubated in a total 500 μL volume of assay buffer (pH 7.4) with concentrations of [^{3}H]NPA ranging from 10^{-9} to 10^{-15} M. Reaction mixtures were incubated for 1 h at 25 °C and subsequently subjected to rapid filtration. Radioactivity-bound filter disks were placed in plastic scintillation vials containing 4 mL of scintillation fluid, equilibrated for at least 12 h, and counted in a Beckman LS5000 liquid scintillation counter. Specific binding was measured in the absence and presence of 10 μM sulpiride.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic scheme and experimental details of the synthesis of D- and L-penicillamine carboxamides **13a** and **13b**. ORTEP drawing of the X-ray crystal structure of compounds **7b**, **8a**, **8b**, **15b**, and **S2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S.B. and S.M. carried out the synthesis, purification, and characterization of the allosteric modulators. R.D. and J.M. carried out the pharmacological evaluation of the allosteric modulators. R.K.M. directed the pharmacological work. R.L.J. directed the synthetic work and wrote the paper based upon drafts written by S.B. and S.M.

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Notes

The authors declare no competing financial interest.

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