Allosteric Modulation of the Dopamine Receptor by Conformationally Constrained Type VI β-Turn Peptidomimetics of Pro-Leu-Gly-NH_2

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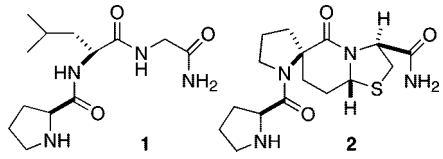
Received July 24, 2007

A peptidomimetic of Pro-Leu-Pro-NH_2, **7**, possessing an indolizidinone type VI β-turn mimic was synthesized via improved high-yielding protocols for the preparation and Cbz protection of α-allylproline. Bicyclic peptidomimetic **7** and spirobicylic peptidomimetic **8** enhanced the binding of [^{3}H]*N*-propylnorapomorphine to dopamine receptors indicating that a type VI β-turn is a possible bioactive conformation of the homochiral Pro-Leu-Pro-NH_2 and Pro-Pro-Pro-NH_2 analogues of Pro-Leu-Gly-NH_2 at the dopamine receptor allosteric regulatory site.

Introduction

The neuropeptide prolyl-leucyl-glycinamide (**1**, PLG) has been shown to modulate dopaminergic neurotransmission as illustrated by its ability to enhance amphetamine- and apomorphine-dependent rotational behavior in 6-hydroxydopamine-lesioned rats.[1–3] Evidence supports the hypothesis that PLG acts as an allosteric modulator of the dopamine receptor.[4] PLG and its peptidomimetics have been shown to increase the binding affinity of agonists to the high-affinity state of the dopamine receptor and to shift the ratio of high- and low-affinity states of the dopamine receptor in favor of the G-protein-coupled high-affinity state.[5,6] The actions of PLG appear to be specific toward dopamine receptors because PLG does not interact with other aminergic receptors such as α-adrenergic,[5,7] GABA-ergic,[8] or serotonergic receptors.[9] Furthermore, studies carried out in cell lines transfected with human dopamine receptor subtypes have shown that PLG and a PLG peptidomimetic enhance agonist binding to the D_{2S}, D_{2L}, and D_4 subtypes, whereas the D_1 and D_3 subtypes are unaffected.[7]



Investigations in our laboratory have centered on the synthesis and evaluation of conformationally constrained analogues of PLG designed to test the hypothesis that the bioactive conformation of PLG at the allosteric site is a type II β-turn conformation.[10] This approach led to the successful development of numerous PLG peptidomimetics,[11–13] one example of which was the highly rigid spiro bicyclic analogue **2**.[14] These peptidomimetics supported the proposition that the bioactive conformation of PLG is a type II β-turn.

In other studies, a set of PLG analogues possessing a more flexible framework was examined in which prolyl residues were substituted for the leucyl and glycinamide residues of PLG.[15,16] These analogues consisted of Pro-Leu-Pro-NH_2 (**3**), Pro-Leu-D-Pro-NH_2 (**4**), Pro-Pro-Pro-NH_2 (**5**), and Pro-Pro-D-Pro-NH_2 (**6**). Because of the effect that the proline pyrrolidine ring has on the φ torsion angle, those PLG analogues that contained a D-prolyl residue in place of the glycinamide residue are able to attain a type II β-turn, while those possessing a L-prolyl residue at the C-terminal end are not able to attain such a conformation.

Surprisingly, when these prolyl PLG analogues were evaluated in in vitro and in vivo assays for dopamine receptor modulation activity, the homochiral set of analogues were found to be as active or even more active, depending on the assay, than the heterochiral set of analogues.[15,16] The activity of the homochiral set of prolyl PLG analogues, which could not adopt a type II β-turn, was inconsistent with the hypothesis, strongly supported by the good activity of the highly constrained PLG peptidomimetics like **2**, that the bioactive conformation of PLG is a type II β-turn. To explain this inconsistency, we speculated that the homochiral prolyl PLG analogues are able to adopt a conformation other than a type II β-turn that places the key pharmacophores in the same relative topological space in which these same key pharmacophores exist when PLG assumes a type II β-turn conformation.

In considering potential conformations that the prolyl homochiral peptides could assume, we noted that the presence of a prolyl residue in the $i + 2$ position introduces the possibility of a *cis*-amide bond with a resulting type VI β-turn conformation.[17] A comparison of this turn with the type II β-turn reveals several similarities, like the key amide NH_2 group projecting in the same relative space in both turn types (Figure 1). The major topological difference between the two turn conformations is a nearly 109° difference in the projection of the carboxamide carbonyl groups. This suggested to us the possibility that the homochiral peptides **3** and **5** might assume a type VI β-turn when interacting with the PLG allosteric binding site. To test this hypothesis, the design, synthesis, and evaluation of analogues of **3** and **5** constrained in a type VI β-turn conformation were undertaken. Although the restriction of the ω dihedral angle to a value of 0° can be achieved in a number of ways, we viewed the indolizidinone approach of Germanas[18] as the most apt, since the two-carbon bridge between the α-carbons of the $i + 1$ and $i + 2$ residues of the turn provides minimal steric deviation. Incorporation of this mimic into the prolyl tripeptides **3** and **5**

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10.1021/jm070895r CCC: $37.00
Published on Web 12/01/2007

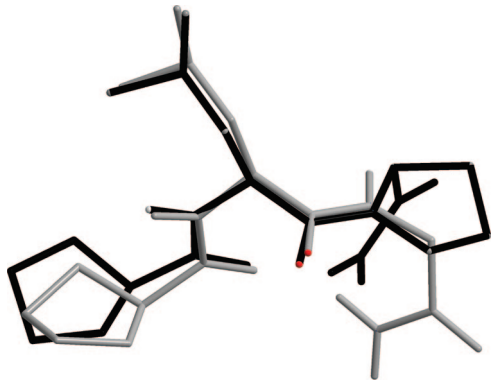


Figure 1. Pro-Leu-Gly-NH_2 (**1**, PLG) in a type-II β-turn conformation (gray) overlaid with Pro-Leu-Pro-NH_2 (**3**) in a type-VI β-turn conformation.

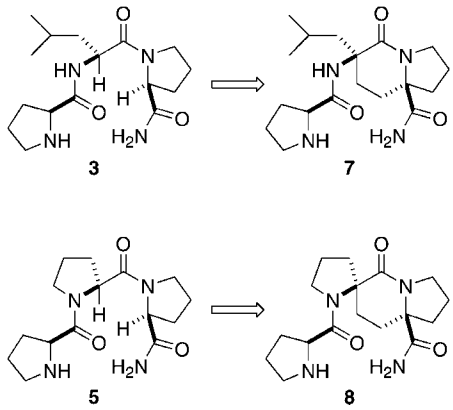


Figure 2. Type-VI β-turn mimics of Pro-Leu-Pro-NH_2 (**3**) and Pro-Pro-Pro-NH_2 (**5**), compounds **7** and **8**, respectively.

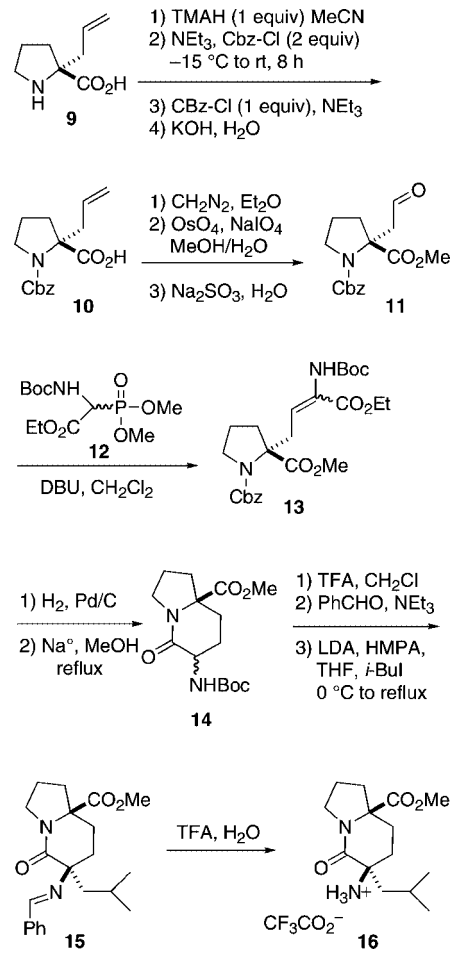
afforded the bicyclic peptidomimetic **7** and the spirobicyclic peptidomimetic **8**, respectively (Figure 2).

Chemistry

For the synthesis of **7**, we followed Germanas' general route to the indolizidinone framework[18] but made several improvements and modifications. The key starting material for the indolizidinone synthesis was (*R*)-α-allyl proline (**9**, Scheme 1), of which substantial quantities were required. This amino acid is traditionally prepared through Seebach's "self-regeneration of chirality" approach where the acid-catalyzed condensation of proline with 6 equiv of pivalaldehyde in pentane with continuous removal of water yields a highly moisture-sensitive oxazolidinone.[19] Alkylation of the lithium enolate of this oxazolidinone with allyl bromide yields a stable allylated oxazolidinone, which when hydrolyzed provides **9**. There are several shortcomings to this approach including the high cost of pivalaldehyde, the large excess of pivalaldehyde required, and the duration of the condensation reaction (typically 3–4 days). Modifications made to the condensation and hydrolysis reaction steps of this sequence provided **9** in overall yields of 70–80% on a 20 g scale (see Supporting Information).

Cbz protection of **9** has been a low-yielding process, as documented in the past. Repeated additions of Cbz-Cl under Schotten−Baumann conditions over 3 days produce excellent yields of **9** on a small scale,[20] but we found this approach to be not scalable. We previously reported a procedure for the Boc protection of **9** that involved its solubilization in acetonitrile by forming a salt with tetramethylammonium hydroxide (TMAH).[21] Application of these same conditions to the Cbz

Scheme 1

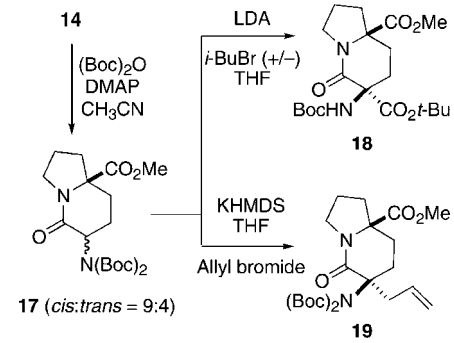


protection of **9** led to rapid decomposition of Cbz-Cl and a low yield (∼20%) of **10**. However, we found that the reaction of the tetramethylammonium salt of **9** with 2 equiv of Cbz-Cl in the presence of 1 equiv of Et_3N led to the formation of a stable mixed-anhydride, which provided **10** in 60% yield upon saponification. The reaction could be made quantitative by the addition of another equivalent of Cbz-Cl, followed by saponification with aqueous KOH. This quantitative Cbz protection was reproducible on a scale as large as 15 g and suggests that this protocol may be potentially useful for many such substrates where steric hindrance around the nitrogen prevents efficient Cbz protection.

Treatment of **10** with diazomethane followed by oxidative cleavage of the terminal olefin of the resulting ester provided aldehyde **11**. The subsequent olefination of **11** required the glycine anion equivalent **12**. Traditionally, this phosphonate is prepared through an Arbuzov reaction between trimethyl phosphite and a Cbz-protected α-bromoglycine ester, followed by replacement of the Cbz group with a Boc group.[22] We used an alternative method in which a photochemical electrophilic bromination of Boc-Gly-OEt by NBS[23] was carried out in hexanes instead of the typical hazardous and expensive CCl_4 solvent to generate an α-bromoglycine intermediate that could be converted to **12** through an Arbuzov reaction.

In our hands, attempts at affecting the olefination between the lithium enolate of **12** and aldehyde **11** in THF led to low yields of the desired alkene **13**. Fortunately, a protocol by Zhang, et al.[24] that uses a DBU enolate in CH_2Cl_2 at room temperature worked well in this case and gave alkene **13** in excellent yield with the *Z*-isomer predominating.

Scheme 2



Reduction of the olefin and hydrogenolysis of the Cbz group of **13** was accomplished simultaneously with H_2 over Pd/C. Heating the resulting product for 5–6 h at reflux in MeOH in the presence of sodium metal resulted in complete cyclization to give **14** in a 2:1 ratio of trans to cis isomers. After removal of the Boc group from **14** by TFA, the free amine was reacted with benzaldehyde to form a Schiff base. Initial attempts at alkylating the lithium enolate of the Schiff base with isobutyl bromide in THF at temperatures from −78 °C to the reflux temperature were met with failure, even though the enolate was found to be quite stable to heat. The use of isobutyl iodide in place of the bromide, solvation of the enolate by HMPA, and heating the reaction to reflux in THF, on the other hand, resulted in a complete reaction within about 20 min to give the alkylated product **15** as a single diastereoisomer. Breakdown of the Schiff base with aqueous TFA provided bicyclic amine **16**.

We also explored the use of bis-Boc protection in place of the Schiff base for the alkylation reaction (Scheme 2). When the 2:1 trans/cis diastereoisomeric mixture of lactam **14** was treated with 10 equiv of Boc_2O and DMAP in CH_3CN, a diastereoisomeric mixture of the bis-Boc lactam **17** was obtained in an 88% yield. Surprisingly, the coupling constants of the lactam α-proton in this diastereoisomeric mixture (8.1 and 10.2 Hz for the major isomer and 9.9 and 3.6 Hz for the minor isomer) indicated that the trans/cis ratio was now 4:9. The precise mechanism for this inversion is not known, although it is known that epimerization does takes place when indolizidinones of this relative stereochemistry are exposed to alkoxide. On the basis of the observation that DMAP causes a vigorous bubbling of CO_2 when added to a solution of Boc_2O, we believe that the transient *tert*-butoxide liberated in this process may be responsible for the deprotonation of the α-proton. Reprotonation from the less sterically hindered *si* face leads to the preferential formation of a *cis*-lactam.

Deprotonation of diastereoisomeric mixture **17** by LDA followed by addition of isobutyl iodide led to the formation of lactam **18** instead of the expected alkylated product, a net N → C shift. This product formed even without isobutyl iodide being present. An NOE between the carbamate NH and the methyl ester hydrogens served to support the expected *R* configuration about the newly formed quaternary carbon. In contrast, deprotonation of **17** with KHMDS in the presence of 2 equiv of allyl bromide afforded a single diastereoisomer of the α-allylated bicyclic lactam **19**. An NOE between the *tert*-butyl hydrogens of the Boc group and the hydrogens of the methyl ester suggested that the allylation had occurred trans to the methyl ester function.

The coupling reaction of **16** with Boc-proline to give **21** was sluggish with reagents that produce relatively stable esters (e.g., HOBt ester of Boc-proline via treatment with 1-(3-dimethylaminopropyl)-3-ethylcarbodimide (EDC)), as well as with the mixed anhydride reagent isobutyl chloroformate in the presence or absence of DMAP. The main byproduct in these reactions was the diketopiperazine **20** (Scheme 3). We observed an identical result during the synthesis of spirobicyclic lactam **8**.[25] The coupling reagents bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) and 1,3-dicyclohexylcarbodiimide (DCC) were comparable to EDC. On the other hand, the coupling behavior of 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent, CMPI) was radically different depending on whether DMAP was present. With CMPI alone, the yield of **21** was only ∼30% after 12 h. When DMAP was combined with CMPI, an exothermic reaction was observed that yielded **21** in a 60–75% yield within 1–2 h, averting formation of **20**.

The direct amidation of **21** to give the primary carboxamide derivative **23** was not attainable by the following conditions: heating a solution of **21** in methanolic ammonia at 100 °C in a sealed stainless steel vessel, heating a solution of **21** in methanolic ammonia in the presence of DMAP, NaCN, or LiI, and Wienreb's amidation with NH_3 in the presence of an equivalent of trimethylalane. However, **21** could be hydrolyzed quite easily to the acid **22** with LiOH in a mixture of THF and HMPA at reflux. EDC-mediated coupling of the free carboxylic acid **22** with ammonia then afforded carboxamide **23**. Subsequent removal of the Boc protecting group from **23** gave the target molecule **7** as its HCl salt.

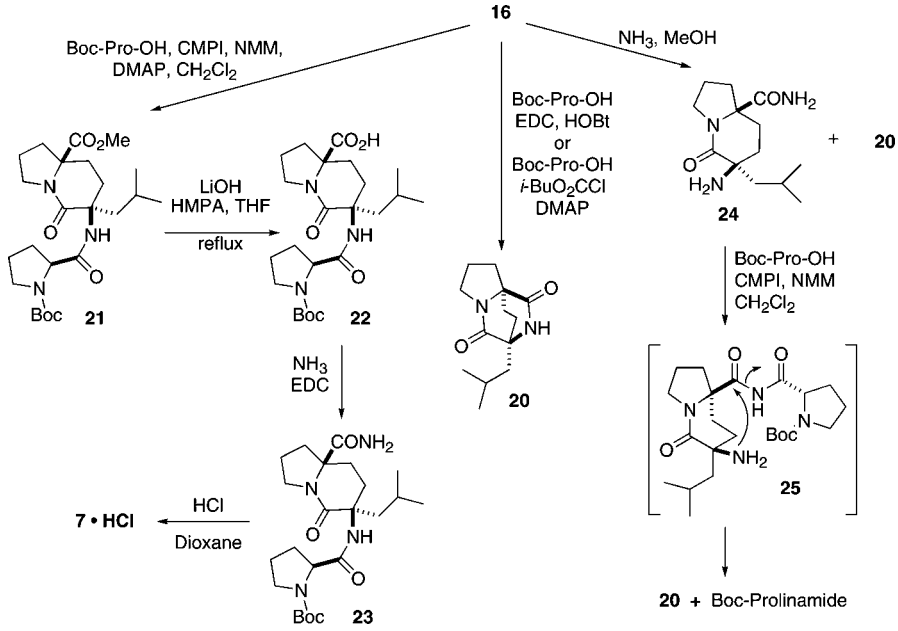
We also attempted to incorporate the primary carboxamide function prior to the coupling stage. While we could form carboxamide **24** upon treating **16** with methanolic ammonia, diketopiperazine **20** also formed. Attempts to couple **24** to Boc-Pro-OH with CMPI failed. Instead, diketopiperazine **20** and Boc-prolinamide were isolated, suggesting that the primary amide function of **24** initially gets acylated, thereby forming **25**. Nucleophilic attack of the free amine on the imide carbonyl then ejects Boc-prolinamide and forms diketopiperazine **20**.

In $CDCl_3$, the primary carboxamide hydrogens of **23** appeared in a typical hydrogen-bonded pattern with chemical shifts of 5.7 and 8.3 ppm. Further support for a hydrogen bond was obtained by the addition of CD_3OD to the NMR sample of **23**, which led to complete exchange of the non-H-bonded, upfield hydrogen while the H-bonded downfield hydrogen required 30 min for complete exchange. Similar behavior was seen with a solution of **7** in CD_3OD, in which case the downfield carboxamide hydrogen resonance at 7.87 ppm diminishes more slowly than the upfield carboxamide hydrogen resonance at 7.42 ppm. Such behavior was observed previously with **8** and its Boc-protected precursor.[25] The results for **7** and **8** are consistent with a hydrogen bond between the carboxamide NH and the prolyl carbonyl group that one would expect in a type VIa β-turn conformation.

Pharmacology

Bicyclic peptidomimetic **7** and spirobicyclic peptidomimetic **8**, the synthesis of which was carried out as reported by us previously,[25] were examined for their ability to potentiate the binding of tritiated *N*-propylnorapomorphine ([^{3}H]NPA) to bovine striatal membranes.[5,26] The results are shown in Figure 3. Compound **7** exhibited a bell-shaped dose response curve that is typical of the allosteric modulation of the dopamine receptor by PLG and its analogues. Statistically significant increases were observed at the following concentrations of **7**: 10 nM, 100 nM, and 1 μM. The maximum effect for **7** was 19.5 ± 3.75% at 100 nM. For spirobicyclic **8**, a bell-shaped dose response curve was also seen but only at 10 nM was a significant increase in [^{3}H]NPA binding observed (11.4 ±

Scheme 3



3.3%). At 100 nM, PLG peptidomimetic **2** and the triproline analogue **5** enhance NPA binding to dopamine receptors by 38.2 ± 11.9% and 34.0 ± 13.1%, respectively.

Discussion

The greater activity of bicyclic peptidomimetic **7** compared to the spirobicyclic peptidomimetic **8** may be because the greater rigidity of the spirobicyclic scaffold prevents the optimal interaction of **8** with the allosteric binding site. It also may be that the isobutyl side chain of **7** interacts more effectively with the hydrophobic pocket in the allosteric binding site than does the propylene-bridging unit of **8**. Previous work has shown that PLG analogues and peptidomimetics with a hydrophobic moiety placed in this topological region possess enhanced dopamine receptor modulating activity.[26–28]

The activity of **7** and **8** supports the hypothesis that the homochiral prolyl peptides **3** and **5** may assume a type VI β-turn as their bioactive conformation and that such a conformation is able to initiate a modulatory response because it can present the necessary topological arrangement of important pharmacophore moieties in a manner similar to that of PLG peptidomimetics that assume a type II β-turn bioactive conformation. In this regard, the data indicate that the amide NH_2 group may be a more important pharmacophore group than the carboxamide carbonyl group in activating the D_2 receptor allosteric modulatory site, as the major difference between a type II β-turn conformation and a type VI β-turn conformation is the nearly 109° difference in orientation of the carboxamide carbonyl groups. The binding conformation of the homochiral prolyl PLG analogues **3** and **5** perhaps possesses a greater capability of projecting key pharmacophores in the correct topological space when compared to the heterochiral prolyl analogues **4** and **6** that are relatively unconstrained when compared to the active highly rigid type-II β-turn PLG peptidomimetics, such as **2**. Clearly, in terms of allosteric modulation of the dopamine receptor, more than one secondary structure is able to project pharmacophores in an orientation that satisfies the pharmacophore requirements for bioactivity.

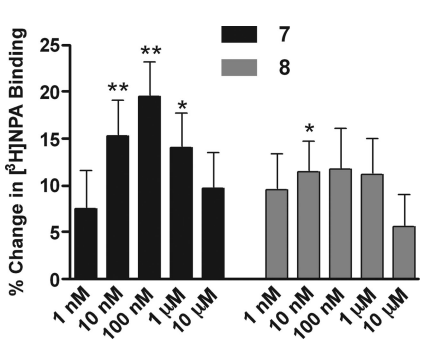


Figure 3. Enhancement of [^{3}H]NPA binding to bovine striatal membranes by **7** and **8**. Data represent the percent increase in specific [^{3}H]NPA binding over the control value when the indicated concentration of compound was added directly to the assay buffer. Results are the mean ± SEM of three to four separate experiments carried out in triplicate. The data were analyzed by a one way analysis of variance followed by Dunnett's posthoc test: (*) significantly different ($p < 0.01$) from control value; (**) significantly different ($p < 0.001$) from control value.

Experimental Section

(6*S*,8a*R*,2′*S*)-6-*N*-(*tert*-Butoxycarbonyl-2′-pyrrolidinylcarbonyl)amino-6-(2-methylpropyl)-8a-carboxamidoindolizidin-5-one (23). To the crude carboxylic acid **22** (480 mg, 1.38 mmol) were added CH_2Cl_2 (5 mL), DMF (5 mL), and HATU (279 mg, 1.05 equiv). The mixture was stirred for 15 min. The flask was evacuated (vacuum needle line) for 1 min, and excess NH_3 was introduced through a balloon. This resulted in an exothermic reaction and the precipitation of a canary-yellow solid. The mixture was stirred for 24 h, diluted with CH_2Cl_2 (50 mL), and filtered. The filtrate was concentrated under reduced pressure, and the resulting residue was mixed with xylenes (50 mL). The solvent was removed, and the crude yellow oil that was obtained was dissolved in EtOAc. This solution was loaded onto a plug of silica gel (30 g) with the aid of EtOAc. The plug was washed with ether (300 mL), which removed most of the HMPA, and then eluted with EtOAc (100 mL), EtOAc/MeOH (20:1, 100 mL), EtOAc/MeOH (15:1, 200 mL), and EtOAc/MeOH (10:1, 100 mL). The product was found in the last 80 mL of the eluent. The relevant fractions were concentrated under reduced pressure to yield a white foam that was triturated with Et_2O and then dissolved in EtOAc/CH_2Cl_2/Et_2O (1:1:40). The solution was allowed to stand uncovered to give a white powder that was filtered to give **23** (430 mg, 87%). $[\alpha]_D$ −43.6 (*c* 1.0, $CDCl_3$); R_f = 0.4 (EtOAc/MeOH, 9:1); ^{1}H NMR

($CDCl_3$, 300 MHz, rotamers present about the N−CO_2t-Bu bond) δ 8.26 (s, 1H, $CONH_2$), 7.68 and 6.43 (2s, 1H, CONH), 5.68 (s, 1H, $CONH_2$), 4.26 (br s, Pro α-CH), 3.73–3.29 (m, 4H, δ-CH_2 and 3-CH_2), 2.56 (dd, $J_1 = 11.7$ Hz, $J_2 = 6.0$ Hz, 1H, β-CH_2), 2.35–1.60 (m, 14H, Pro β-CH_2, 1-CH_2, Pro γ-CH_2, 2-CH_2, 7-CH_2, 8-CH_2, $CH_2CH(CH_3)_2$), 1.45 (s, 9H, *t*-Bu), 0.97 (dd, $J_1 = 12$ Hz, $J_2 = 7.2$ Hz, 6H, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz, rotamers present about the N−CO_2t-Bu bond) δ 176.2 ($CONH_2$), 171.2 (CONH), 170.0 (5-C), 80.8 ($C(CH_3)_3$), 71.0 (8a-C), 60.7, 59.8 (Pro α-C), 58.6 (6-C), 47.4 (Pro δ-C or 3-C), 45.9 ($CH_2CH(CH_3)_2$), 45.0 (Pro δ-C or 3-C), 38.4 (1-C), 31.2 and 30.0 (Pro β-C), 30.3 (7-C), 29.3 (8-C), 28.1 (*t*-Bu), 27.5 (Pro γ-C), 24.9 ($CH_2CH(CH_3)_2$), 24.3 ($CH_2CH(CH_3)_2$), 21.4 (8-C). ESI-HRMS *m/z* 472.2752 $(M + Na)^+$, $C_{23}H_{38}N_4O_5 + Na^+$ requires 472.2740. Anal. ($C_{23}H_{38}N_4O_5$) C, H, N.

(6*S*,8a*R*)-6-*N*-(2′-Pyrrolidinylcarbonyl)amino-6-(2-methylpropyl)-8a-carboxamidoindolizidine-5-one Hydrochloride (7·HCl). To **23** (100 mg, 0.22 mmol) was added 4 N HCl in dioxane (5 mL) under a stream of argon. A white solid precipitated from the solution within 15–20 s. The suspension was stirred for an extra 15 min, and a vacuum line was inserted in the septum to remove excess HCl. The residue was then evaporated under reduced pressure to afford a white gum, which was dissolved in CH_2Cl_2, and the solvent was then removed (5×). The resulting white gum was dissolved in CH_2Cl_2/MeOH (10:1), and this solution was poured into ether, upon which a white precipitate formed. The solvents were decanted and the residue was dried for 2 days under high vacuum to yield 74 mg of **1** as a hygroscopic white solid. $R_f = 0.7$ (EtOH/NH_4OH, 4:1); $[\alpha]_D$ −24.8 (*c* 0.5, CD_3OD); ^{1}H NMR (300 MHz, CD_3OD, acquired within 5 min of mixing) δ 7.87 and 7.42 (2s, 2H, $CONH_2$, these resonances disappear within 30 min of mixing), 4.31 (app dd, $J_1 = 6.2$ Hz, $J_2 = 6.6$ Hz, 1H, Pro α-CH), 3.67–3.29 (m, 4H, Pro δ-CH_2 and 3-CH_2), 2.67 (dd, $J_1 = 5.7$ Hz, $J_2 = 12.0$ Hz, 1H, Pro β-CH_2), 2.46–1.66 (m, 14H, Pro β-CH_2, Pro γ-CH_2, 2-CH_2, 1-CH_2, $CH_2CH(CH_3)_2$, 7-CH_2, 8-CH_2), 0.98 (dd, $J_1 = 11.4$ Hz, $J_2 = 6.3$ Hz, 6H, $CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.5 ($CONH_2$), 170.1 (CONH), 173.6 (5-C), 70.1 (8a-C), 61.2 (Pro α-C), 58.6 (6-C), 48.0 (Pro δ-C or 3-C), 44.0 ($CH_2CH(CH_3)_2$), 43.8 (Pro δ-C or 3-C), 38.1 (1-C), 32.1 (Pro β-C), 31.4 (7-C), 29.3 (8-C), 24.9 (Pro γ-C), 24.5 ($CH(CH_3)_2$), 24.3 ($CH(CH_3)_2$), 21.4 (8-C). ESI-HRMS *m/z* 351.2358 $(M)^+$, $(C_{18}H_{31}N_4O_3)^+$ requires 351.2364. Anal. ($C_{18}H_{31}ClN_4O_3 \cdot {}^1/_3H_2O$) C, H, N.

Acknowledgment. This work was supported in part by NIH grant NS20036 to R.L.J. We thank Dr. David Rusterholz for helpful discussions on the proline−pivaldehyde condensation reaction.

Supporting Information Available: Experimental details for the synthesis of **9**–**14** and **16**–**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM070895R