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Pyrroloisoquinoline-Based Tetrapeptide Analogues Mimicking Reverse-Turn Secondary Structures

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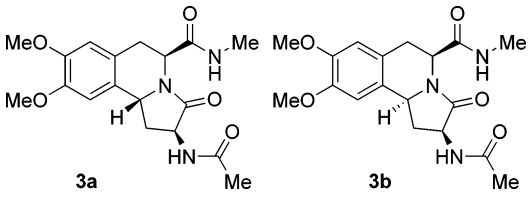
Received July 20, 2007



New pyrroloisoquinoline-based tetrapeptides were synthesized in enantiomerically pure form, and their conformational features were studied by NMR, IR, and molecular-modeling techniques. The presence of a reverse turn was observed in both structures, with the C1 stereochemistry playing a central role in determining stable conformations. In particular, all of the analyses led to the conclusion that a type II′ β-turn is mostly stabilized in tetrapeptide mimic **3a**, while a typical inverse γ-turn geometry is revealed for the diastereoisomer **3b**.

The β-turn motif, a segment of four amino acid residues that reverse the direction of peptide chains, is an important structural feature of proteins, playing relevant roles in several recognition events. A general approach in the synthesis of peptidomimetic compounds involves the use of nonpeptide building blocks, which enforce or stabilize a particular type of β-turn, when inserted into a peptide chain. In this respect, a variety of conformationally restricted compounds, mostly lactams, have been proposed as dipeptide mimetic replacements for the $i + 1$ and $i + 2$ residues of β-turns, in some cases affording peptidomimetics with enhanced activity or metabolic stability, compared to the native peptide. In our ongoing studies on Tic-based peptidomimetics[1] (Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid), we designed the 2-amino-8,9-dimethoxy-3-oxo-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline-5-carboxylate system **1** (Figure 1) as a potential Tyr-Ala dipeptide mimic rigidified through the formation of the Tic core and of the fused pyrrolidin-2-one ring. The particular interest in this heterocyclic framework is supported by the known ability of certain bicyclic lactams, structurally related to the hexahydroindolizidino moiety, to mimic the central dipeptide core of

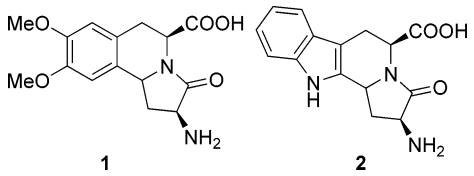


FIGURE 1. Indolizin-9-one-based scaffolds.

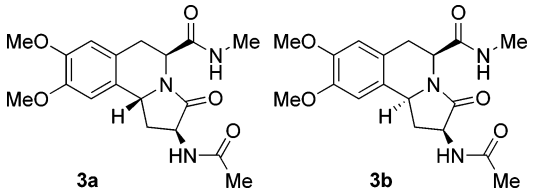


FIGURE 2. Tetrapeptide mimics Ac-AHPIC-NHMe **3a** and **3b**.

β-turns.[2] In particular, indolizin-9-one-based amino acids have been synthesized in all possible stereoisomers and employed in structure−activity studies of various peptides.[3]

The presence in **1** of an aromatic ring, which can be considered as the side chain of the $i + 2$ residue, might represent an additional advantage in order to mimic β-turns having aromatic or hydrophobic amino acids in the third residue. To the best of our knowledge, the only synthesis of a pyrroloisoquinoline ring system bearing an amine substituent was recently reported by Meldal et al.[4] by means of a solid-phase procedure. Closely related 2-amino-3-oxohexahydroindolizino[8,7-*b*]indole carboxylic acid **2** has been proposed as a dipeptide surrogate of type II′ β-turn[5] and recently employed for the synthesis of potential inhibitors of zinc metalloproteinases[6] and of potent and selective CCK1 receptor antagonists.[7]

Herein, we report the synthesis of enantiopure tetrapeptide mimics **3a** and **3b** (Ac-AHPIC-NHMe, AHPIC = 2-amino-8,9-dimethoxy-3-oxo-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline-5-carboxylic acid) (Figure 2) and their conformational analysis by molecular modeling calculations,[8] ^{1}H NMR,[9] and FT IR.[10] This study indicated that both the diastereisomeric Ac-

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(3) Indolizidin-9-one amino acids: (a) Cluzeau, J.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 1504−1512. (b) Lamazzi, C.; Carbonnel, S.; Calinaud, P.; Troin, Y. *Heterocycles* **2003**, *60*, 1447−1456. (c) Shimizu, M.; Nemoto, H.; Kakuda, H.; Takahata, H. *Heterocycles* **2003**, *59*, 245−255. (d) Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 7463−7471. (e) De La Figuera, N.; Rosas, I.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R. *J. Chem. Soc., Chem. Commun.* **1994**, 613.

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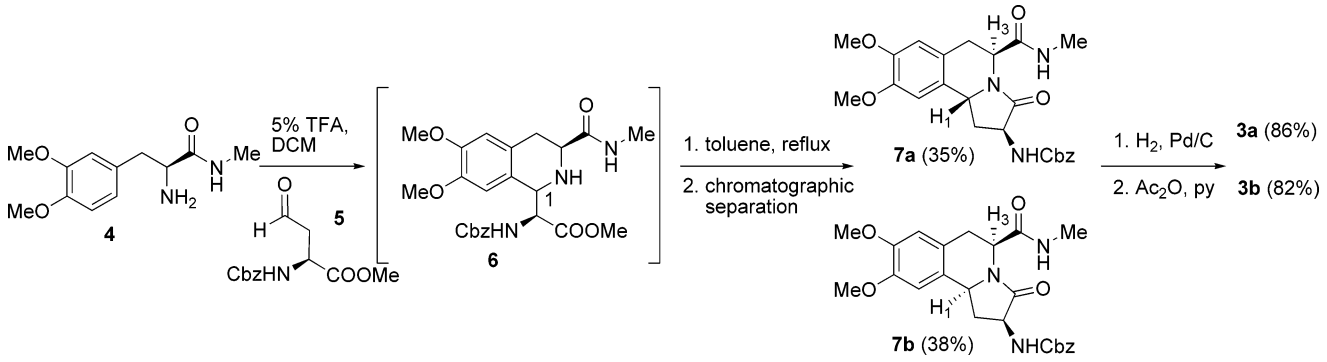
10.1021/jo701581j CCC: $37.00
Published on Web 11/09/2007

SCHEME 1. Synthesis of 3a and 3b



AHPIC-NHMe **3a** and **3b** have good propensity to adopt reverse turn-like conformations. In particular, the structure **3a** shows a strong preference to form a type II′ β-turn, while **3b** seems to be stabilized as an inverse γ-turn.

Synthesis. Pictet−Spengler condensation (5% TFA, CH_2Cl_2) between the L-DOPA derivative **4**[1] and the aldehyde **5**[11] afforded the tetrahydroisoquinoline **6** in a 1:1 diastereoisomeric mixture at C1, as determined by ^{1}H NMR (Scheme 1). The crude compound **6** was then subjected to thermal intramolecular lactamization (toluene, reflux) to give, after chromatographic separation, hexahydropyrroloisoquinolines **7a** and **7b** in a 1:1 ratio. Thorough inspection of 1D and 2D NMR spectra permitted the complete assignment of all protons and carbons chemical shift in both diastereoisomers. The determination of the configuration at the new stereogenic center C1 was deduced by 2D ^{1}H NOESY NMR and assigned as *R* in **7a** and *S* in **7b**.[12] Removal of Cbz protecting group (H_2, Pd/C) in compounds **7a** and **7b**, followed by acetylation of the amino group, afforded the final products **3a** and **3b**.

Conformational Analysis. The reverse turn mimicry ability of the two AHPIC scaffolds **3a** and **3b** was evaluated by computing[13] and analyzing different geometric parameters (Figure 3). The $C\alpha_1$-$C\alpha_4$ interatomic distance (dα) and the virtual torsion angle β, defined by $C_1-C\alpha_2-C\alpha_3-N_4$, were measured.[14,15] The presence of hydrogen bonds involved in 10-membered (C_1O-HN_4) and in 7-membered (C_2O-HN_4) rings was estimated by means of the "hydrogen bonds" function implemented in the Spartan software.[16] The computational procedure consisted of an unconstrained Monte Carlo/Energy Minimization conformational search using the molecular mechanics MMFF94 force field[17] in vacuo. For each diastereoisomer, 255 conformers were generated and only conformations within 6 kcal/mol of the global minimum were kept. Results are reported in Table 1 as percentages of conformers which meet the requirements for a reverse turn.

Compounds **3a** and **3b** show moderate percentages of conformers satisfying the reverse turn requirements. In particular, compound **3a**, bearing a H-1, H-3 trans relative configuration, seems to be slightly favored in assuming a reverse turn conformation, with 64% of conformers having dα < 7 Å. Analysis of intramolecular hydrogen bond shows some tendency for both **3a** and **3b** to form 10- and 7-membered rings, with a preference for the latter ones. The calculated backbone geometries are reported in Table 2. The φ and ψ backbone torsion angles in residue $i + 1$ and $i + 2$ of β-turns (φ_2, ψ_2, φ_3, and ψ_3, see Figure 3) or in residue $i + 1$ of γ-turns (φ_3 and ψ_3, see Figure 3) define the specific β-turn and γ-turn type.[18] The lowest energy conformer for **3a** (conf 1) has a good reverse turn propensity (dα = 5.41 Å, $\beta = -0.46°$), and the presence of a 10-membered ring hydrogen bond specifies a β-turn geometry.

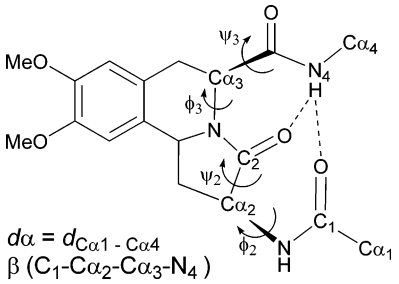


FIGURE 3. Definition of parameters to characterize reverse-turn propensity of AHPIC systems.

TABLE 1. Mc/EM Conformational Analysis for AHPIC Structures 3a and 3b

compd	no. of conf <6 kcal/mol	% d*a* < 7 Å[a]	% |β| < 30°[a]	% |β| < 60°[a]	% C_1O-HN_4 H bond[a]	% C_2O-HN_4 H bond[a]
3a	50	64 (32)	52 (26)	86 (43)	12 (6)	34 (17)
3b	47	45 (21)	83 (39)	100 (47)	4 (2)	38 (18)

[a] Results are reported as percentage of conformers which meet the requirement. The occurrence numbers are given in parentheses.

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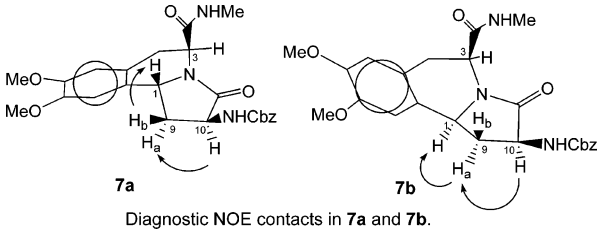
(11) Garrard, E. A.; Borman, E. C.; Cook, B. N.; Pike, E. J.; Alberg, D. G. *Org. Lett.* **2000**, *23*, 3639 - 3642.

(12) In compound **7a**, NOE contacts between H-1 and H-9b and between H-10 and H-9a were present, while for **7b** NOE interaction of H-9a with both H-1 and H-10 were observed.



(13) *Spartan'06*; Wavefunction, Inc.: Irvine, CA.

(14) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467−3478.

TABLE 2. Characteristics of Low-Energy Conformers Calculated for 3a and 3b

conf no.		ΔE^a	da[b]	βc	$\varphi_2{}^c$	$\psi_2{}^c$	$\varphi_3{}^c$	$\psi_3{}^c$	C_1O-HN_4 dist[d]	C_2O-HN_4 dist[e]
3a	1	0.00	5.41	−0.46	62.22	−120.81	−102.09	32.52	2.08	3.14
	9	0.89	6.46	35.76	−174.07	−114.50	−81.68	65.29	6.16	2.07
3b	1	0.00	7.43	18.46	−173.71	−143.17	−72.85	45.22	6.26	1.89
	15	2.51	5.47	−15.59	60.61	−137.67	−75.55	−15.88	2.12	3.14

[a] Energies are reported in kcal/mol. ΔE refers to the lowest energy conformer. [b] The distances are measured in Å. [c] Torsion angles are reported in deg. [d] 10-membered ring. Distances are measured in Å. [e] 7-membered ring. Distances are measured in Å.

TABLE 3. Similarity Analysis of Ac-AHPIC-NHMe 3a and 3b with Standard-Type β-Turns[a]

		β-turn type					
conf no.		I	I′	II	II′	III	III′
3a	1	0.88	0.84	0.82	0.97	0.86	0.83
3b	1	0.72	0.66	0.71	0.75	0.71	0.67
	15	0.86	0.85	0.78	0.97	0.86	0.82

[a] Results are reported as scores; see ref 19.

Analysis of the torsion angles suggests a type-II′ β-turn classification. The first γ-turn is found in conformer 9 which lies 0.89 kcal/mol above the minimum. The torsion angles values are indicative of an inverse γ-turn. In the lowest energy conformer of **3b**, the formation of a 7-membered ring hydrogen bond C_2O-HN_4 is favored, and the torsion angles values suggest an inverse γ-turn geometry. The first well-defined type-II′ β-turn is found in conformer 15 at 2.51 kcal/mol above the minimum.

In summary, computational data suggest **3a** as a good candidate to mimic a reverse turn, with a preference for a type-II′ β-turn geometry; in diastereoisomer **3b**, a typical inverse γ-turn geometry is revealed, characterized by the presence of a 7-membered ring hydrogen bond. An analysis was performed to evaluate and quantify the similarity of **3a** and **3b** to standard type β-turns by superimposing the atoms of the amide backbone. Results are reported as scores,[19] for which a value of 1 means a perfect similarity (Table 3). This analysis confirms the type II′ β-turn assignment for the lowest energy conformer of **3a** and for the conformer 15 of **3b**, both with a score of 0.97. On the other hand, the lowest energy conformer of **3b** has low scores for any β-turn type. The lowest energy conformers for **3a** and **3b**, superimposed with standard type II′ β-turns, are shown in Figure 4.

Spectroscopic NMR and IR Analyses. In order to evaluate the presence of intramolecular hydrogen bonds between the termini of the turn regions, the tetrapeptide mimics Ac-AHPIC-NHMe **3a** and **3b** were investigated by mean of ^{1}H NMR[9] and FT-IR[10] spectroscopy. To avoid strong hydrogen-bonding competition with the solvent, ^{1}H NMR spectra were recorded in the weakly polar solvent $CDCl_3$.[20,21] All analyses were performed on 3.0 mM $CDCl_3$ solutions to ensure the absence of significant aggregation. The temperature dependence ($\Delta\delta/\Delta T$) of the ^{1}H NMR chemical shifts of the amide protons has been evaluated. This is a well-established procedure to investigate whether amide protons are involved in intramolecular hydrogen bonds.[22]

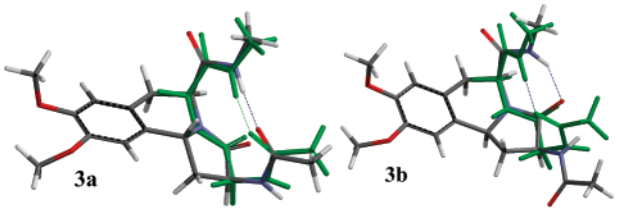


FIGURE 4. Lowest energy conformers obtained by Monte Carlo calculations. Superimposition with standard type II′ β-turns (in green) is shown.

TABLE 4. Spectroscopic Data of Tetrapeptide Mimics Ac-AHPIC-NHMe 3a and 3b

		(δ $CDCl_3$)[a]	(Δd/ΔT)[b]	IR absorption bands[c]
3a	NHMe	7.51	−4.5	3453, 3352
	NHAc	6.52	−5.5	
3b	NHMe	6.90	−4.4	3458, 3370
	NHAc	6.29	−4.0	

[a] δ are measured in ppm. [b] ppb/K. [c] Absorptions are reported in cm^{-1}.

In $CDCl_3$ solution, the N*H*Me resonates at 7.51 ppm in **3a** and at 6.90 ppm in **3b**, that is at lower fields with respect to N*H*Ac, appearing at 6.52 ppm in **3a** and 6.29 ppm in **3b** (Table 4). These data suggest the participation of N*H*Me amide hydrogen in the formation of intramolecular hydrogen bonds for **3a**, while compound **3b** represents a borderline situation. The temperature-dependent chemical shift changes ($\Delta\delta$ N*H*Me /ΔT) of −4.5 (**3a**) and −4.4 ppb/K (**3b**) suggest that N*H*Me is involved in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state.

In addition, observation of the N−H stretching region of the IR spectra allows us to distinguish hydrogen-bonded from non-hydrogen-bonded states. The IR spectrum of a $CHCl_3$ 3 mM solution of **3a** exhibits a strong band at 3352 cm^{-1} (hydrogen-bonded state) together with a weaker signal at 3453 cm^{-1} (non-hydrogen-bonded state). Under the same conditions, **3b** shows

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(16) Hydrogen bonds are defined as non-bonded contacts between a nitrogen or oxygen and an hydrogen attached to nitrogen or oxygen, separated by a distance ranging from 1.6 to 2.1 Å and making an X−H−Y (X, Y = N, O) angle >120°.

(17) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.

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(19) Scores are reported as obtained by the similarity analysis function implemented in the Spartan '06 software. The score is defined as [(1 − R2)/*N*], where R2 is the rms distance between template and molecule centers and *N* is the number of similarity centers.

(20) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048−7050.

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(22) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389−400.

an extensive absorption band at 3370 cm^{-1} for a hydrogen-bonded NH stretching and a band at 3458 cm^{-1} for a non-hydrogen-bonded NH stretching.

All of these experimental observations agree with predictions from modeling, according to which both the Ac-AHPIC-NHMe **3a** and **3b** show a propensity to form intramolecular hydrogen bonds.

In this paper, we have described the synthesis of new enantiomerically pure tetrapeptide mimics. Both MM calculations and spectroscopic NMR and IR investigations support the conclusion that the AHPIC-based scaffolds **3a** and **3b** are good reverse turns. In particular, the 1,3-trans relative configuration greatly enhances the tendency of **3a** to adopt a β-turn conformation, allowing the formation of a hydrogen-bonded 10-membered ring. A type II′ β-turn can be postulated for **3a** in its lowest energy state, based on analysis of the torsion angles and similarity with standard β-turns.

Choosing appropriate protecting groups, should allow to introduce reverse turn inducers such as **1** into selected linear or cyclic peptides, giving rise to peptidomimetics which bear a constrained Tyr-Ala dipeptide mimic as central core of β- or γ-turn secondary structures.

Experimental Section

Compound **4** was prepared according to ref 1.

Compound **5** was prepared according to ref 11.

7a and 7b. Compound **4** (320 mg, 1.34 mmol) was dissolved in a 5% TFA/CH_2Cl_2 solution (15 mL) and cooled to 0 °C with an ice bath. A solution of **5** (355 mg, 1.34 mmol) in a 5% TFA/CH_2Cl_2 solution (5 mL) was then slowly added. After the addition, the ice bath was removed and the reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue oil was rinsed with saturated $NaHCO_3$ aq solution (20 mL) until pH 8 and then extracted with dichloromethane (3 × 20 mL). The organic phase was then dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was dissolved in dry toluene and refluxed under nitrogen atmosphere for 48 h. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (ethyl acetate/methanol, 93:7) affording **7a** (212 mg, 35% yield) and **7b** (230 mg, 38% yield) as oils.

7a: R_f = 0.4 (methanol/ethyl acetate, 1:9); $[\alpha]^{25}_D$ = 34.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (m, 6H), 6.70 (s, 1H), 6.54 (s, 1H), 5.95 (d, *J* = 7.7 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 5.04 (br t, *J* = 6.6 Hz, 1H), 4.96 (d, *J* = 6.1 Hz, 1H), 4.01 (m, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.56 (d, *J* = 15.9 Hz, 1H), 3.02 (dd, *J* = 15.9, 7.5 Hz, 1H), 2.80 (d, *J* = 4.5 Hz, 3H), 2.65 (m, 1H), 2.44 (m, 1H); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 171.3, 169.3, 156.6, 148.3, 135.9, 128.6−128.0 (5C), 127.6, 124.0, 111.8, 107.5, 67.2, 56.1, 55.9, 53.3, 52.8, 51.0, 50.9, 34.4, 28.3, 26.5; HRMS *m*/*z* calcd 453.1900, found 453.1897. Anal. Calcd for $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27; O, 21.17. Found: C, 63.50; H, 6.03; N, 9.25.

7b: R_f = 0.27 (methanol/ethyl acetate, 1:9); $[\alpha]^{25}_D$ = −48.8 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 5H), 6.94 (br q, *J* = 4.6 Hz, 1H), 6.83 (s, 1H), 6.65 (s, 1H), 5.79 (d, *J* = 5.3 Hz, 1H), 5.10 (s, 2H), 4.66 (br d, *J* = 6.2 Hz, 1H), 4.50 (dd, *J* = 10.6, 6.1 Hz, 1H), 4.24 (m, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.50 (d, *J* = 14.7 Hz, 1H), 3.00 (m, 2H), 2.60 (m, 1H), 2.58 (d, *J* = 4.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 171.4, 170.0, 156.1, 148.7, 148.1, 135.9, 128.6−128.1(5C), 126.7, 111.8, 106.6, 67.2 (2C), 56.1, 56.0, 54.0, 53.7, 52.6, 31.5, 31.7, 26.5; HRMS *m*/*z* calcd 453.1900, found 453.1896. Anal. Calcd for $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27; O, 21.17. Found: C, 63.59; H, 6.08; N, 9.31.

3a. Compound **7a** (200 mg, 0.44 mmol) was dissolved in methanol (10 mL), and 10% Pd/C (20 mg, 10% w/w) was added. The reaction mixture was then placed in hydrogen atmosphere (1 atm) and stirred at room temperature overnight. The mixture was filtered over Celite, and the solvent was evaporated under reduced pressure, affording 140 mg of the *N*-Cbz deprotected product as an oil. The crude product was dissolved in dry pyridine (5 mL) and cooled to 0 °C with an ice bath. DMAP (27 mg, 0.22 mmol) and acetic anhydride (51 μL, 0.53 mmol) were then added. The cooling bath was removed, and the mixture was stirred at room temperature overnight. After removal of the solvent, the residue was treated with 5% aq H_3PO_4 and then extracted with dichloromethane (3 × 20 mL). The organic phase was then dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified by flash chromatography (ethyl acetate/methanol, 93:7) affording **3a** (135 mg, 86% yield): R_f = 0.18 (methanol/ethyl acetate, 1:9); $[\alpha]^{25}_D$ = 19.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (d, *J* = 7.0 Hz, 1H), 7.65 (br q, *J* = 4.3 Hz, 1H), 6.67 (s, 1H), 6.48 (s, 1H), 5.06 (br t, *J* = 7.7 Hz, 1H), 4.91 (d, *J* = 7.7 Hz, 1H), 3.89 (m, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.53 (d, *J* = 16.7 Hz, 1H), 3.03 (dd, *J* = 16.7, 7.7 Hz, 1H), 2.80 (d, *J* = 4.5 Hz, 3H), 2.62 (m, 1H), 2.35 (m, 1H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 171.6, 171.3, 169.4, 148.3, 148.2, 127.7, 123.8, 111.7, 107.4, 56.1, 55.9, 53.7, 52.8, 50.8, 34.2, 28.4, 26.6, 22.7; IR (3 mM $CHCl_3$ solution) 3453, 3352, 3008, 1695, 1672, 1545, 1516, 1434, 1258, 1237, 1126, 1047, 912, 806, 736, 705, 681 cm^{-1}; HRMS *m*/*z* calcd 361.1638, found 361.1636. Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63; O, 22.14. Found: C, 59.86; H, 6.43; N, 11.65.

3b. The same procedure as for preparation of **3a** was followed (82% yield from **7b**): R_f = 0.12 (methanol/ethyl acetate, 1:9); $[\alpha]^{25}_D$ = −12.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (m, 2H), 6.96 (s, 1H), 6.73 (s, 1H), 4.62 (d, *J* = 6.4 Hz, 1H), 4.53 (m, 1H), 4.21 (m, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.43 (dd, *J* = 14.8, 4.0 Hz, 1H), 2.98 (m, 2H), 2.66 (m, 1H), 2.58 (d, *J* = 4.7 Hz, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 172.5, 171.8, 170.9, 149.6, 149.1, 135.8, 127.3, 112.8, 107.8, 56.9, 56.8, 54.3, 54.2, 53.7, 32.4, 31.9, 27.0, 23.6; IR (3 mM $CHCl_3$ solution) 3458, 3370, 3020, 1703, 1668, 1549, 1513, 1466, 1422, 1359, 1324, 1280, 1197, 1119, 809, 705 cm^{-1}; HRMS *m*/*z* calcd 361.1638 found 361.1640. Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63; O, 22.14. Found: C, 59.85; H, 6.45; N, 11.60.

Supporting Information Available: General procedures for the synthesis of compounds, ^{1}H NMR and ^{13}C NMR spectra of **7a**, **7b**, **3a**, and **3b**, VT ^{1}H NMR data for **3a** and **3b**, IR spectra of the NH region for **3a** and **3b**, and computational data from conformational analysis for **3a** and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO701581J