Peptides Constrained to Type VI β-Turns. 1. Evidence for an Exceptionally Stable Intramolecular Hydrogen Bond

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The synthesis and conformational analysis of conjugates of amino acids with a type VI β-turn dipeptide mimic (**1**) is described. The mimic possessed high structural similarity to the central two residues of the turn and was constrained from rotation about two of the four single bonds. Coupling of amino acids to the carboxyl group of the mimic afforded conjugates that were capable of forming intramolecular hydrogen bonds. In nonpolar solvents, IR and NMR spectra of the conjugates indicated that the amide hydrogen of the amino acid residue was hydrogen bonded to the carbonyl group of the N-terminal carbamate functionality, as in typical β-turns. In the hydrogen-bonding solvent DMSO, the intramolecular hydrogen bond was still present, according to the temperature dependence of the chemical shift. The presence of the *i*, *i* + 3 hydrogen bond in the conjugates of mimic **1** was substantiated by the spectral properties of conjugates of the isomeric mimic **3**, which showed no evidence for the presence of an intramolecular hydrogen bond. The results from these studies suggest that the intramolecularly hydrogen-bonded type VIa turn is an inherently more stable conformation for a peptide than the non-hydrogen-bonded type VIb conformation, in the absence of other structural constraints.

Introduction

Reverse turns play important roles in polypeptide function, acting as elements of structure as well as modulators of bioactivity.[1] Among the reverse turns found in proteins, the β turn is the most prevalent.[2] In this structure, four amino acids arrange themselves in a manner such that the polypeptide backbone undergoes a 180° change in its direction. Often, a hydrogen bond is formed between the carbonyl oxygen of the first, or *i* residue, and the amide hydrogen of the fourth, or *i* + 3 residue of the turn. Several types of β-turns are found in proteins, where distinct conformations for the individual amino acids result from different values of the intervening torsion angles.[2]

The type VI turn is a unique member of the β-turn family because it is the only turn that incorporates an *s-cis* peptide bond (Figure 1).[3] Natural type VI β-turns always contain a proline residue at the *i* + 2 position, since peptides incorporating this amino acid are the only ones that can exist substantially in the *s-cis* configuration. The type VI turn is often found in peptides and proteins containing the sequence ArProAr, where Ar represents an amino acid with an aryl side chain.[4] Type VI turns are subdivided into type VIa and VIb turns.[5] In the type VIa structures, an intramolecular hydrogen bond is formed between the *i* carbonyl oxygen and the *i* + 3 amide hydrogen, as in other β-turns; in the type VIb structures, the orientation of the torsion angle Ψ3 directs the C-terminus of the turn away from the N-terminus so that the hydrogen bonding groups are improperly aligned to interact (Figure 1).

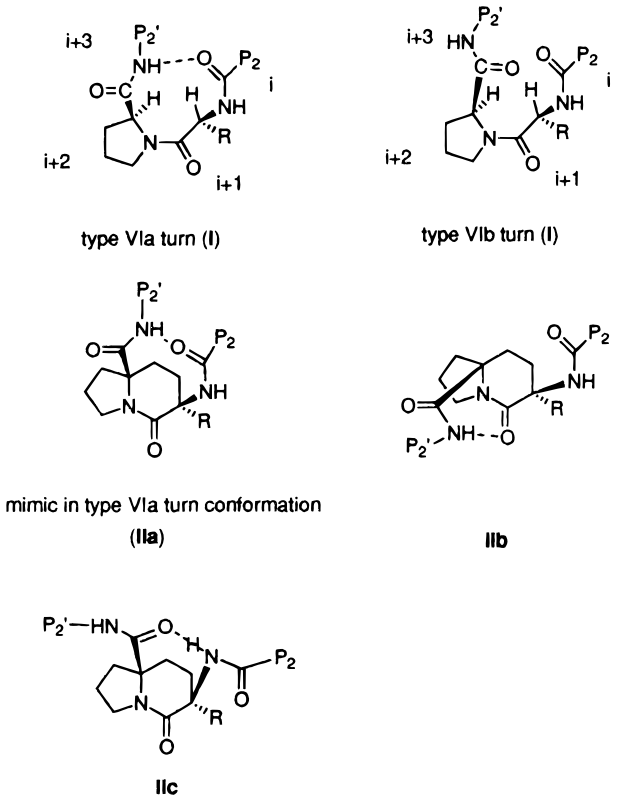

Figure 1. Hydrogen-bonded conformations available to peptides and bicyclic lactam amino acid conjugates.

Recently, we reported the design and synthesis of a series of bicyclic lactams (**1**) as mimics of the type VI turn (Scheme 1).[6] The lactams represented dipeptides of the formula XaaPro in which the torsion angles of the Ψ bond of the Xaa residue and the Φ and ω bonds of the Pro residue were fixed to the values found in type VI turns

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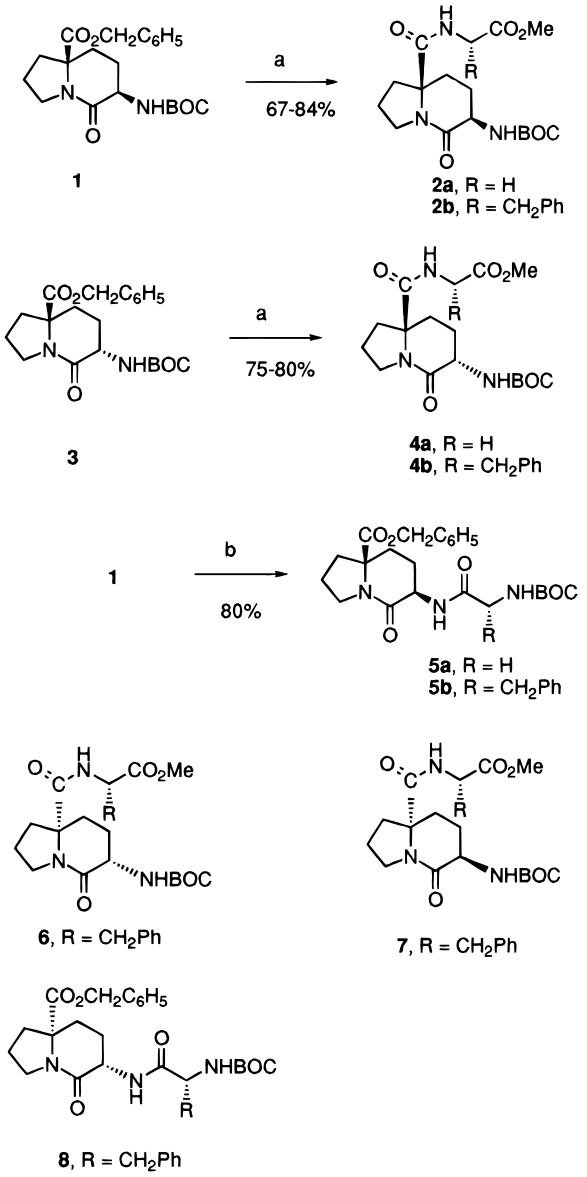
Scheme 1[a]



[a] Key: (a) (i) H_2, Pd−C; (ii) $H_2NCH(R)CO_2Me$, BOP; (b) (i) CF_3CO_2H, CH_2Cl_2; (ii) $BOCNHCH(R)CO_2H$.

(Figure 1). The two remaining bonds of the dipeptide, Φ2 and Ψ3, were unconstrained and were thus capable of assuming various conformations, including those characteristic of type VIa or VIb structures. Peptides incorporating lactam **1** would be capable of adopting intramolecular hydrogen-bonded conformations, as in structures **II** (Figure 1). The conformational properties of these compounds would afford the opportunity to investigate the effect of constraint of a peptide on its propensity to adopt a type VIa or alternative conformations. In this paper, we provide evidence that constraint of the central three dihedral angles of a peptide to the type VI turn conformation results in the formation of an exceptionally stable *i*, *i* + 3 intramolecular hydrogen bond as found in type VIa turns.

Results

Synthesis. The synthesis and structural characterization of the bicyclic lactam **1** was described previously.[6] Coupling of amino acids to the carboxyl group of lactam **1** would be carried out to introduce the amide hydrogen necessary for forming the *i*, *i* + 3 hydrogen bond, as in the 10-membered ring β-turn conformation **IIa**. Spectroscopic techniques would then be used to assess the hydrogen-bonding properties of the lactam−amino acid conjugates. In addition to structure **IIa**, other intramolecular hydrogen bonding motifs would be available to structure **II**, such as the *i*, *i* + 2 γ turn conformation **IIb**, as well as the eight-membered ring *i*, *i* +3 conformation **IIc**. To distinguish between these alternatives, bicyclic lactam-amino acid conjugates whose structures would allow only a subset of the alternative conformations to be attained were prepared and spectroscopically characterized.

The synthesis of the mimic−amino acid conjugates is shown in Scheme 1. Deprotection of the carboxyl group of lactam **1** was effected by hydrogenolysis of the benzyl ester function. Coupling of L-amino acid esters to the resultant acid was achieved with the aid of the BOP reagent,[7] affording peptides **2**. In an identical manner were L-amino acid esters attached to the carboxyl group of the isomeric *trans*-lactam **3** to provide peptides **4**. Peptides **5** were prepared by condensing *N*-protected amino acid anhydrides with the amino group of mimic **1**, obtained by acidolytic removal of the carbamate function (Scheme 1). Overall yields of conjugates were excellent (60−90%). No epimerization of the chiral center adjacent to the lactam carbonyl group in the coupling products was detected, according to NMR (>95% retention).

A second set of lactam−amino acid conjugates, peptides **6**−**8**, were prepared from L-amino acids and the enantiomers of the mimics **1** and **3** (Scheme 1). These compounds were diastereomers of conjugates **2b**, **4b**, and **5b**, respectively. Since the stereochemistry of the lactam moiety of peptide **6** was identical to that of a Gly-D-Pro dipeptide, it represented a "mirror image" β-turn.[8] The conformational properties of such a compound would provide insight into the stability of "mirror image" type VIa turns.

NMR Spectroscopy. Pertinent features of the NMR spectra of the mimic−amino acid conjugates are summarized in Tables 1 and 2. A particularly striking observation was the difference between the chemical shifts of the amide protons of the *cis* mimic conjugates **2** and **6** and those of the *trans* mimic isomers **4** and **7**. In $CDCl_3$, the chemical shifts of the carboxamide hydrogens (NH^1, Figure 2) of the *trans* mimic conjugates fell into a region characteristic for non-hydrogen-bonded amide NH's.[9] For the conjugates with the *cis*-lactam moiety (**2** and **6**), however, the chemical shifts of the analogous NH's appeared substantially downfield from this region. For instance, the carboxamide proton NH^1 of the *cis*-mimic conjugate **2b** resonated at 8.3 ppm, 2.0 ppm downfield from that for the *trans* isomer **4b**. Significantly deshielded amide protons often indicate that the nucleus is involved in a hydrogen bond.[9]

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Table 1. ^{1}H NMR Parameters for Mimic−Amino Acid Conjugates in $CDCl_3$[a]

compd	stereochemistry[c]	R	δ NH^{1} [d] (ppm)	δ NH^{2} [d] (ppm)	J_{NH^2} [d] (Hz)	δ NH^{3} [d] (ppm)
cis[b]						
1	(6*R*,8a*R*)			5.2	*e*	
2a	(6*R*,8a*R*)	H	8.3	5.4	6.9	
2b	(6*R*,8a*R*)	CH_2Ph	8.3	5.4	7.2	
6	(6*S*,8a*S*)	CH_2Ph	7.4	5.1	5.4	
trans[b]						
3	(6*S*,8a*R*)			5.6	*e*	
4a	(6*S*,8a*R*)	H	7.0	5.4	*e*	
4b	(6*S*,8a*R*)	CH_2Ph	6.3	5.4	4.5	
7	(6*R*,8a*S*)	CH_2Ph	6.3	5.4	4.8	
cis[b]						
5a	(6*R*,8a*R*)	H		6.5	4.8	5.3
5b	(6*R*,8a*R*)	CH_2Ph		6.5	4.8	5.0
8	(6*S*,8a*S*)	CH_2Ph		6.4	4.8	5.4

[a] Sample concentrations were 1−2 mM, sample temperature was rt. [b] Relationship between amino and carboxyl groups on six-membered ring of lactam. [c] Absolute stereochemistry of lactam moiety. [d] Designations as defined in Figure 2. [e] Not determined due to line broadening or obscuration by other resonances.

When DMSO-d_6 was used as the solvent for the NMR experiments, the chemical shifts for both the carboxamide and carbamate hydrogens of the *trans*-lactam conjugates **4** and **7** moved appreciably downfield (Table 2). Such behavior is typical for amides that experience a change in state of hydrogen bonding when placed into the strongly hydrogen bond accepting solvent DMSO.[10] The carboxamide proton chemical shifts for the corresponding *cis*-lactam isomers, on the other hand, were essentially identical to their values in $CDCl_3$, indicating that their hydrogen-bonding state had not been influenced by the change in solvent.

The temperature dependence of the chemical shifts ($\Delta\delta/\Delta T$) of amide hydrogens is often used to assess intramolecular hydrogen bonding in peptides. When an amide is involved in a stable intramolecular hydrogen bond, its hydrogen experiences only a small change in its chemical shift in DMSO upon variation of temperature.[11] For the *trans*-mimic conjugates **4** and **7**, both carbamate (NH^2) and carboxamide (NH^1) hydrogens displayed sizable values for the temperature gradients of their chemical shifts ($\Delta\delta/\Delta T < -4.0$ ppb/K, Table 2), indicating that these hydrogens were not involved in intramolecular hydrogen bonds. The temperature dependence of δ for the *cis*-mimic conjugates **2** and **6**, however, was uniformly small ($\Delta\delta/\Delta T \geq -3.0$ ppb/K, Table 2). These values fell within the range indicative of intramolecularly hydrogen-bonded NH's, according to the criterium of Kessler ($0 > \Delta\delta/\Delta T \geq -3.0$ ppb/K).[11]

Two-dimensional NMR spectroscopy provided further evidence for the presence of a stable conformation incorporating an intramolecular hydrogen bond in the *cis*-mimic conjugates. The NOESY spectrum of conjugate **2a** displayed crosspeaks between the resonances assigned to $H_{7\beta}$ and $H_{3\beta}$ of the lactam ring and the hydrogen atom of the angular carboxamide (Figure 3). Crosspeaks between the amide hydrogen and the H_1 or H_8 protons, however, were absent. These results were indicative of significant population of a stable conformer about the C_{8a}−CO bond, in which the amide hydrogen of the angular carboxamide (NH^1) was positioned over the six-membered lactam ring of the mimic.

Additional support for an *i*, *i* + 3 hydrogen bond in structures **2** and **6** came from the values of the H_6-carbamate hydrogen coupling constants of the conjugates. All conformations of the conjugates described in this study are assumed to interconvert rapidly on the NMR time scale; as a result, the NMR-derived parameters are weighted averages of the values for the individual conformers.[12] In the *trans*-mimic conjugates **4** and **7**, as well as in conjugates **5** and **8**, where amino acids were coupled to the amino group of the *cis*-mimic **1**, the values of J_{H6-NH} were approximately 6 Hz, consistent with the presence of several populated rotamers about the C_6−N_{BOC} bond (Figure 2). For *cis*-lactam conjugates **2** and **6**, however, the value of J_{H6-NH} was over 8 Hz; the large value in these structures signified substantial population of a rotamer about the C_6−N_{BOC} bond with a dihedral angle between the N−H and C_6−H bonds close to 0 or 180°.[13] An intramolecular hydrogen bond between the carboxamide hydrogen and the carboxyl oxygen in the *cis*-mimic conjugates required restriction of the NH−C_6H torsion angle to approximately 0°.

IR Spectroscopy. The NH stretch regions of the IR spectra of the isomeric monoamino acid conjugates **4b** and **2b** in CH_2Cl_2 are shown in Figure 4. At moderate concentrations (15−30 mM) both compounds displayed bands attributable to hydrogen-bonded (3300−3350 cm^{-1}) and non-hydrogen-bonded (3400−3500 cm^{-1}) NH stretching vibrations.[14] At lower concentrations (<10 mM) only the band at 3420 cm^{-1} was visible in the spectrum of the *trans*-mimic conjugate **4b** (Figure 4a). The spectrum of the *cis*-mimic conjugate **2b** displayed hydrogen-bonded and free NH stretches at 3320 and 3455 cm^{-1}, respectively, at moderate concentrations. Upon dilution, the relative intensities of the two bands did not change appreciably; in fact, the hydrogen-bonded NH stretch was still intense at a concentration of 1 mM (Figure 4b).

Since the intensity of the 3350 cm^{-1} band for the *trans*-mimic conjugate **4b** was strongly concentration dependent, the hydrogen bonding observed at high concentrations of this compound was likely due to intermolecular interaction. The spectral properties of the *cis*-lactam isomer **2b**, however, were consistent with intramolecular hydrogen bonding that was unaffected by dilution.

Discussion

To assess the conformational propensities of peptides incorporating type VI turn mimics, the spectroscopic features of select amino acid adducts of lactams **1** and **3** were analyzed. The improper stereochemical relationship between the carboxyl and amino groups on the six-membered ring of the lactam moiety prevented the adoption of the type VIa β-turn conformation **IIa** by the *trans* lactam conjugates **4** and **7**; the γ-turn conformation **IIb**, however, was conceivable for both *cis*- and *trans*-lactam conjugates (Figure 1). The absence of intramolecular hydrogen bonding in *trans* mimic amino acid conjugates **4** and **7**, however, suggested instability of γ-turn **IIb** for the *trans* lactam addcuts. The NMR spectral properties of monoamino acid mimic conjugates **2** and **6** suggested that their carboxmide hydrogens (NH^1, Figure 2) were involved in a strong intramolecular

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Table 2. ^{1}H NMR Parameters for Lactam−Amino Acid Conjugates in DMSO-d_6[a]

compd	stereochemistry[c]	R	$\Delta\delta$ NH^1 [d,e] $CDCl_3$−DMSO (ppm)	$\Delta\delta/\Delta T$ NH^1 (ppb/K)	$\Delta\delta$ NH^2 [d,e] $CDCl_3$−DMSO (ppm)	$\Delta\delta/\Delta T$ NH^2 (ppb/K)
cis[b]						
1	(6*R*,8a*R*)					
2a	(6*R*,8a*R*)	H	−0.4	−3.0	−1.9	−5.3
2b	(6*R*,8a*R*)	CH_2Ph	−0.1	−2.7	−1.4	−6.0
2c	(6*S*,8a*S*)	CH_2Ph	−0.6	−3.0	−2.1	−5.2
trans[b]						
3	(6*S*,8a*R*)					
4a	(6*S*,8a*R*)	H	−1.3	−4.5	−1.3	−8.6
4b	(6*S*,8a*R*)	CH_2Ph	−2.1	−4.7	−1.6	−9.0
7	(6*R*,8a*S*)	CH_2Ph	−2.1	−4.0	−1.7	−9.0

[a] Sample concentrations were 1−2 mM. [b] Relationship between amino and carboxyl groups on six-membered ring of lactam. [c] Absolute stereochemistry of lactam moiety. [d] Chemical shift difference for hydrogen in $CDCl_3$ and DMSO-d_6. [e] Designations as defined in Figure 2.

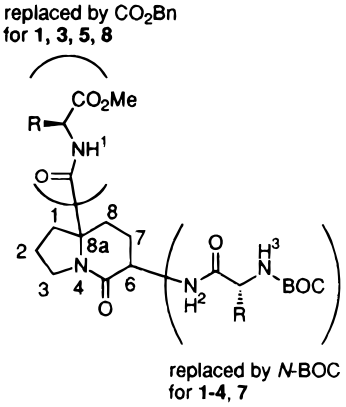
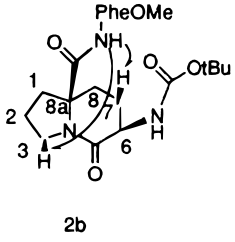


Figure 2. C and NH numberings in lactam−amino acid conjugates.



2b

Figure 3. Graphical representation of observable NOE's in conjugate **2b**.

hydrogen bond. Both the β-turn structure **IIa** and the γ-turn structure **IIb** would be consistent with these results (Figure 1). NOESY spectra and coupling constants, however, supported the β-turn conformation **IIa**. The absence of the γ-turn conformation **IIb** for the *trans*-lactam adducts **4** also indirectly discounted the presence of the γ-turn conformation for analogs **2** and **6** as well, since the formation of **IIb** was not dependent on the presence of an N-terminal hydrogen-bonding group.

According to the spectral properties, the β-turn conformation **IIa** was present in all C-terminal amino acid adducts of the *cis*-lactam carboxyl group, regardless of the amino acid coupled to the carboxyl function (**2a** vs **2b**) or the stereochemical relationship between the bicyclic lactam moiety and the pendant amino acid (**2b** vs **6**). Thus, the positioning of the carboxyl and amino groups on the six-membered ring of the lactam moiety of all adducts **2** and **6** was sufficient to induce formation of the type VIa turn conformation. The low NH chemical shift temperature dependence and other NMR properties of conjugates of **1** indicated that the intramolecularly

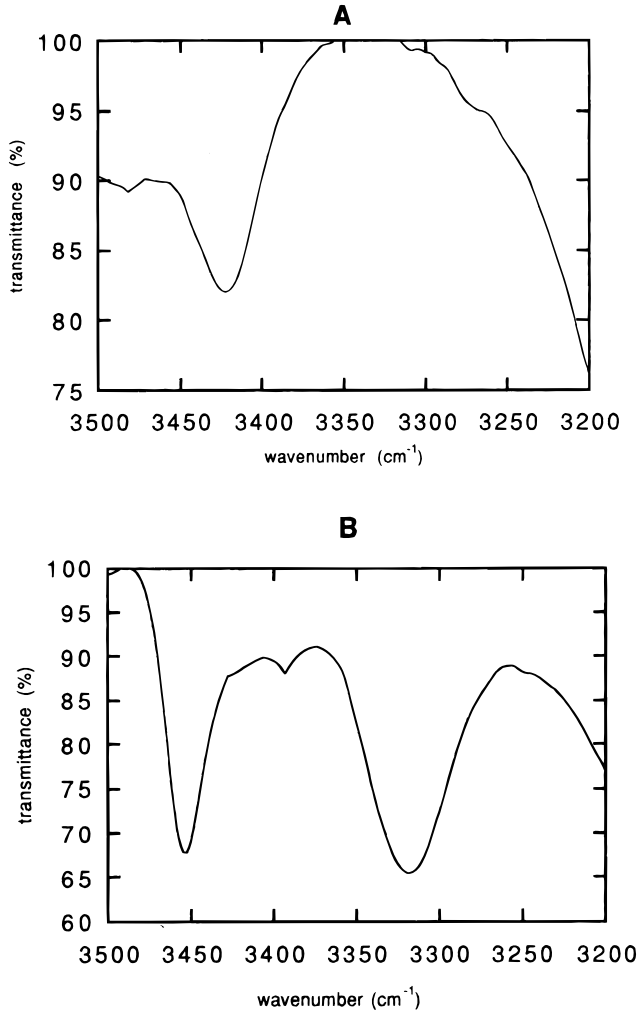


Figure 4. NH stretch region of IR spectrum of select lactam amino acid conjugates at 2 mM concentrations in CH_2Cl_2: (a) conjugate **4b**; (b) conjugate **2b**.

hydrogen-bonded conformation was highly populated in solution.

Several mimics of the central dipeptide of other β-turn types have been designed.[15] A number of these have shown the ability to adopt intramolecular hydrogen-bonded conformations analogous to **IIa**.[15c,e,h−l,m] In certain cases, the mimic was incorporated into a cyclic peptide,[15c,e,i] which restricted the number of possible conformations. In other cases, the mimics were analyzed in a non-hydrogen-bonding solvent.[15h,j] In a few cases the mimic was dissolved in polar solvent, and in many,

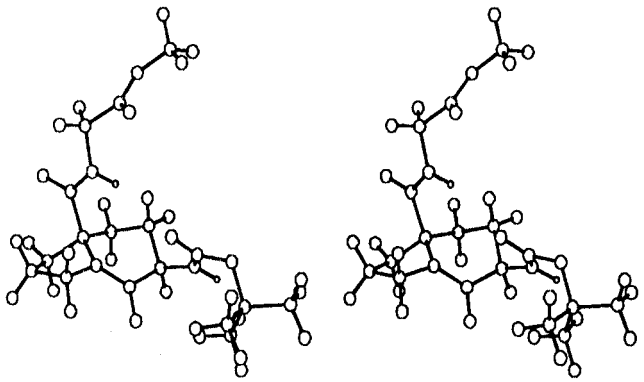


Figure 5. Stereoview of the molecular mechanics lowest energy conformation of **2a**.

but not all, of those instances the hydrogen-bonded conformation was largely absent.[15h] Stable β-sheets have been observed in aqueous solution when relatively long peptide chains were attached to a β-turn template; factors besides hydrogen bonding, however, likely contribute to the stability of the secondary structure in this case.[15j,m] The intramolecularly hydrogen-bonded conformation for the *cis*-mimic adducts **2** and **6**, on the other hand, persisted in DMSO. The stability of conformation **IIa** for such peptides should prove useful as a model for antiparallel β-sheets in polar solvents.

The exceptional stability of the 10-membered-ring hydrogen-bonded conformation **IIa** in compounds **2** and **6** likely arises from the unique structural features of mimic **1**. First, the lactam moiety of compound **1** possesses considerable structural homology to the analogous dipeptide from a typical type VI turn. Second, the mimic **1** incorporates a high degree of conformational constraint (three of five rotatable bonds restricted). Third, according to the NMR spectral properties and molecular mechanics calculations, the bicyclic lactam moiety of compound **1** is fairly rigid. Fourth, the length and geometry of the hydrogen bond appears to be close to ideal. The minimum energy structure of **2a**, calculated with the CHARMM force field, revealed a length of 2.75 Å for the hydrogen bond and a 120° angle between the CO_{BOC} and NH_{Gly} bonds (Figure 5). The ability of the *cis*-lactam **1** to enhance the stability of more complex peptide secondary structures, such as β-ladders, will be reported in due course.

Experimental Section

General Methods. Unless otherwise noted, all starting materials and solvents were obtained from commercial suppliers and used without further purification. Dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure directly before use. Tetrahydrofuran (THF) and ether (Et_2O) were distilled just prior to use from sodium/benzophenone, and dichloromethane (CH_2Cl_2) was distilled from P_2O_5. Benzene was distilled from sodium, and methanol was distilled from magnesium. Reactions were carried out under a dry nitrogen atmosphere in glassware that had been flame or oven dried (T > 100 °C) overnight.

^{1}H and ^{13}C NMR spectra were recorded at 300 or 600 MHz, using $(CH_3)_4Si$ as an internal standard. COSY and NOESY spectra were recorded with 2048 by 512 data points and were zero filled to 1K × 1K sizes. A mixing time of 350 ms was used for the NOESY spectra. Chemical shift temperature dependences were determined in DMSO-d_6 by varying the sample temperature between 278 and 328 K. At least five temperatures were determined for each sample, and the value of $\Delta\delta/\Delta T$ was obtained by a linear least-squares fit of the data.

Column chromatography was performed on silica gel (Merck reagents silica gel 60, 230−400 mesh ASTM). Thin-layer chromatography (TLC) was carried out on Analtech Uniplate silica gel plates with a 0.25 mm coating containing fluorescent indicator. Spots were visualized using ninhydrin, phosphomolybdic acid, iodine, or UV light.

Molecular mechanics calculations and theoretical and structural analyses were performed with the Quanta package using the CHARMM force field.

General Procedure for Conjugation of 1 (or 3) with Amino Acid Esters. A solution of ester **1** or **3** (0.6 g, 1.7 mmol) in EtOH (5 mL) containing 10% Pd/C (10 mg) was stirred under an atmosphere of hydrogen gas provided by a balloon fitted with a syringe needle. After 1 h, the solution was filtered and solvent removed under reduced pressure to afford the crude carboxylic acid (0.5 g, 100%). To a stirred solution of the carboxylic acid of compound **1** (or **3**) (0.5 g, 1.7 mmol) and BOP reagent (1.0 g, 2.3 mmol) in anhydrous DMF was added L-amino acid ester hydrochloride (3 equiv) followed by DIEA (1.9 mL, 10 mmol). After 5 h, the reaction mixture was concentrated under reduced pressure and extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ NaCl and then dried with $MgSO_4$. Excess DMF was evaporated under reduced pressure to afford the crude product of conjugated peptidomimetic. Column chromatography on silica eluting with a solvent mixture of hexane:ethyl acetate:diethyl ether:ethyl alcohol (1:5:4:0.5) afforded the peptide product, which was homogeneous by RPLC.

The (6*S*,8a*S*)-diastereomers of the conjugates were obtained by reaction of amino acid anhydrides with the racemic lactams **1** or **3**. The individual diastereomers were obtained after separation by column chromatography, eluting with a solvent mixture of hexane:ethyl acetate:diethyl ether:ethyl alcohol (1:5:4:0.5).

(6*R*,8a*R*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-one−glycine methyl ester (2a): yield 84%; ^{1}H NMR ($CDCl_3$) δ 8.23 (br s, 1H), 5.33 (d, J = 6.9 Hz, 1H), 4.01 (d, J = 7.2 Hz, 2H), 3.78 (m, 1H), 3.71 (s, 3H), 3.56 (m, 2H), 2.73 (dd, J = 6.0, 11.7 Hz, 1H), 2.50 (ddd, J = 3.3, 3.3, 13.8 Hz, 1H), 2.36 (m, 1H), 2.15−1.87 (m, 3H), 1.63−1.50 (m, 2H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.1, 170.6, 168.7, 156.1, 80.8, 71.7, 52.5, 52.6, 46.3, 41.9, 38.7, 31.7, 28.8, 26.6, 21.4; HR FAB MS calcd for $C_{17}H_{27}N_3O_6$ 369.1825, found 369.1829.

(6*R*,8a*R*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-one-L-phenylalanine methyl ester (2b): yield: 67%; ^{1}H NMR ($CDCl_3$) δ 8.28 (d, J = 8.4 Hz, 1H), 7.28−7.20 (m, 5H), 5.39 (d, J = 7.2 Hz, 1H), 4.68 (ddd, J = 3.9, 8.1, 12.0 Hz, 1H), 3.75 (s, 3H), 3.70 (m, 1H), 3.55 (m, 2H), 3.37 (dd, J = 4.2, 13.8 Hz, 1H), 2.97 (m, 1H), 2.57 (dd, J = 6.3, 11.7 Hz, 1H), 2.40 (ddd, J = 2.7, 2.7, 13.8 Hz, 1H), 1.98 (m, 1H), 1.73−1.52 (m, 2H), 1.48 (s, 9H), 1.42−1.23 (m, 2H), 1.05 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.6, 172.1, 168.5,156.0, 137.7, 129.7, 129.4, 127.3, 80.7, 71.5, 54.8, 52.6, 46.0, 38.3, 37.0, 31.6, 30.1, 28.9, 25.7, 21.1.

(6*R*,8a*S*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-one−glycine methyl ester (4a): yield 80%; ^{1}H NMR ($CDCl_3$) δ 6.72 (br s, 1H), 5.41 (d, J = 5.7 Hz, 1H), 4.10 (m, 2H), 3.96 (dd, J = 5.1, 17.7 Hz, 1H), 3.81 (m, 1H), 3.75 (s, 3H), 3.58 (m, 1H), 2.58−2.49 (m, 2H), 2.40 (m, 1H), 1.97−1.85

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(m, 5H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.1, 171.1, 170.4, 155.6, 79.78, 70.4, 52.4, 49.7, 46.73, 41.3, 39.2, 30.3, 28.35, 26.8, 21.5.

(6a*S*,8a*R*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-one−L-phenylalanine methyl ester (4b): yield 75%; ^{1}H NMR ($CDCl_3$) δ 7.30−7.21 (m, 3H), 7.14 (d, J = 6.6 Hz, 2H), 6.28 (d, J = 8.4 Hz, 1H), 5.42 (d, J = 4.5 Hz, 1H), 4.84 (ddd, J = 5.1, 8.7, 8.7 Hz, 1H), 4.07 (dd, J = 6.0, 12.6 Hz, 1H), 3.82 (s, 3H), 3.71 (m, 1H), 3.30 (dd, J = 5.4, 14.4 Hz, 1H), 3.19 (m, 1H), 2.96 (dd, J = 8.8, 14.1 Hz, 1H), 2.53−2.19 (m, 3H), 1.72−1.62 (m, 4H), 1.43 (s, 9H), 1.23 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.6, 170.4, 155.4, 136.1, 128.6, 128.7, 127.7, 79.6, 70.5, 53.0, 52.6, 49.4, 46.5, 38.8, 37.1, 30.2, 28.3, 26.7, 21.0.

(6*S*,8a*S*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-one−L-phenylalanine methyl ester (6): yield 67%; ^{1}H NMR ($CDCl_3$) δ 7.36 (d, J = 6.6 Hz, 1H), 7.21−7.11 (m, 5H), 5.06 (d, J = 5.4 Hz, 1H), 4.72 (dd, J = 7.8, 14.1 Hz, 1H), 3.73 (s, 3H), 3.65 (m, 1H), 3.54 (m, 1H), 3.46 (m, 1H), 3.27 (dd, J = 6.0, 14.1 Hz, 1H), 3.07 (dd, J = 8.4, 14.1 Hz, 1H), 2.60 (dd, J = 6.0, 11.7 Hz, 1H), 2.39 (ddd, J = 3.3, 3.3, 13.8 Hz, 1H), 1.89−1.76 (m, 2H), 1.39 (s, 9H), 1.65−1.52 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 172.3, 171.2, 168.2, 155.3, 136.0, 128.5, 128.2, 128.1, 126.6, 79.7, 70.8, 53.4, 51.8, 51.8, 45.4, 38.1, 36.7, 30.4, 28.0, 24.9, 20.4.

(6a*R*,8a*S*)-*N*-(*tert*-Butoxycarbonyl)-6-amino-8a-carboxyindolizin-5-onyl-*L*-phenylalanine methyl ester (7): yield 75%; ^{1}H NMR ($CDCl_3$) δ 7.30−7.21 (m, 3H), 7.10 (d, J = 6.6 Hz, 2H), 6.37 (d, J = 8.4 Hz, 1H), 5.37 (d, J = 4.8 Hz, 1H), 4.80 (ddd J = 5.7, 8.7, 8.7 Hz, 1H), 3.75 (s, 3H), 3.69 (m, 2H), 3.39 (m, 1H), 3.18 (dd, J = 5.4, 13.8 Hz, 1H), 3.03 (dd, J = 7.8, 13.8 Hz, 1H), 2.47 (m, 1H), 2.29 (m, 1H), 2.04 (m, 1H), 1.87−1.75 (m, 4H), 1.51 (m, 1H), 1.46 (s, 9H), 1.23 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.3, 172.1, 170.7, 155.9, 135.9, 129.6, 129.4,129.4, 127.9, 80.2, 70.7, 53.3, 52.9, 50.2, 46.9, 39.5, 37.9, 30.2, 28.8, 26.9, 21.6.

General Procedure for the Conjugation of 1 with Anhydrides of *N*-BOC-amino Acids. To a solution of ester **1** (0.21 g, 0.59 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (1 mL, 5.9 mmol) and the solution was stirred at rt. After 2 h, CH_2Cl_2 and trifluoroacetic acid were removed under reduced pressure to give a yellowish oil. The resultant amine salt was dissolved in THF (10 mL) and Et_3N (0.5 mL). The preformed anhydride of the *N*-BOC amino acid (2 equiv) was dissolved in THF (5 mL) and cooled with an ice bath. To the solution of the anhydride was added the amino acid salt solution dropwise and the mixture allowed to stir at 4 °C. After 2 h, the reaction mixture was warmed to rt and quenched with water. The resulting mixture was diluted with $CHCl_3$ and washed with 0.1 N HCl. The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure to give the amides as colorless oils. Individual diastereomers were separated by silica gel column chromatography, eluting with hexane:ethyl acetate:diethyl ether:ethyl alcohol (1:7:6:0.5).

The (6*S*,8a*S*)-diastereomers of the conjugates were obtained by reaction of amino acid anhydrides with the racemic lactam **1** followed by diastereomer separation by silica gel chromatography eluting with hexane:ethyl acetate:diethyl ether:ethyl alcohol (1:7:6:0.5).

(6*R*,8a*R*)-*N*-[(*tert*-Butoxycarbonyl)glycyl]-6-amino-8a-carboxyindolizin-5-one benzyl ester (5a): yield 80%; IR ($CHCl_3$), 3650, 2980, 1738, 1700, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.38−7.29 (m, 5H), 6.72 (d, J = 7.5 Hz, 1H), 5.18 (s, 2H), 5.15 (br s, 1H), 4.30 (ddd, J = 11.4, 6.3, 6.3 Hz, 1H), 3.90 (dd, J = 4.8, 14.4 Hz, 1H), 3.78 (dd, J = 5.7, 14.4 Hz, 1H), 3.62−3.52 (m, 2H), 2.54 (ddd, J = 14.1, 3.3, 3.3 Hz, 1H), 2.45 (m, 2H), 1.89 (m, 1H), 1.80−1.67 (m, 3H), 1.52 (m, 1H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.8, 169.9, 167.5, 154.3, 135.1, 128.7, 128.3, 80.0, 70.1, 67.6, 50.7, 45.1, 44.0, 37.5, 30.6, 28.3, 26.0, 20.6; HR FAB MS calcd for $C_{23}H_{32}N_3O_6$ 446.2291, found 446.2293.

(6*R*,8a*R*)-*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl-6-amino-8a-carboxyindolizin-5-one benzyl ester (5b): yield 80%; ^{1}H NMR ($CDCl_3$) δ 7.41−7.12 (m, 10H), 6.35 (d, J = 5.1 Hz, 1H), 5.17 (d, J = 14.8 Hz, 1H), 5.07 (d, J = 14.8 Hz, 1H), 5.00 (br s, 1H), 4.47 (m, 1H), 4.38 (m, 1H), 3.68−3.51 (m, 3H), 3.19 (dd, J = 8.4, 14.7 Hz, 1H), 3.02 (m, 1H), 2.62−2.40 m, 3H), 1.97−1.60 (m, 3H), 1.43 (s, 9H), 1.23 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.3, 171.9, 167.7, 137.2, 135.6, 129.7, 129.1, 128.9, 128.6, 127.2, 80.5, 70.6, 68.0, 56.3, 51.0, 45.5, 38.0, 31.0, 28.7, 26.3, 21.0.

(6*S*,8a*S*)-*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl-6-amino-8a-carboxyindolizin-5-one benzyl ester (8): yield 80%; 1H NMR ($CDCl_3$) δ 7.40−7.18 (m, 10H), 6.41 (br s, 1H), 5.17 (s, 2H), 4.95 (br s, 1H), 4.38 (m, 1H), 4.21 (m, 1H), 3.61−3.55 (m, 3H), 3.14 (dd, J = 8.4, 14.7 Hz, 1H), 3.06 (m, 1H), 2.51−2.42 (m, 3H), 1.91−1.57 (m, 3H), 1.39 (s, 9H), 1.23 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.3, 171.9, 167.7, 137.2, 135.6, 129.7, 129.1, 128.9, 128.6, 127.2, 80.5, 70.6, 68.0, 56.3, 51.0, 45.5, 38.0, 31.0, 28.7, 26.3, 21.0.

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Supporting Information Available: ^{1}H NMR spectra of **2a**, **4a**, **5a**,**b, 6**, and **8**, ^{13}C spectra of **2b**, **4b**, and **7**, and NOESY spectrum of **2a** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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