Peptides Constrained to Type VI β-Turns. 2. Antiparallel β-Ladder Formation

Kyonghee Kim and Juris P. Germanas*

Department of Chemistry, University of Houston, Houston, Texas 77204-5641

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The preparation and characterization of an extensive series of bis-amino acid conjugates of a novel β-turn mimic are described. The conjugates were prepared by coupling amino acid residues to the amino and carboxyl groups of the mimic, which represented the central two residues of a peptide constrained to the type VI turn conformation. The resultant adducts had the capability to form either singly or doubly hydrogen-bonded conformations, which represented β-turn or antiparallel β-ladder structures, respectively. In the majority of the bis-amino acid conjugates of the *cis*-lactam **1** (or its enantiomer), only the interior hydrogen bond, characteristic of the singly hydrogen-bonded conformation, was present, according to NMR and IR spectra. When the lactam **1** or its enantiomer were coupled to L-Phe at its carboxyl group and *N*-AcGly at its amino group, the spectral properties indicated the presence of the doubly hydrogen-bonded form. The results are consistent with other workers' studies that demonstrated that aryl residues following Pro stabilize antiparallel type VI turn structures and that the propensity of an amino acid to adopt a β-conformation in proteins is highly dependent on context.

Introduction

Among the common peptide secondary structures, the β-sheet is the least well understood.[1] In contrast to α-helices and β-turns, which involve interactions between amino acids that are close together in sequence, the amino acids that interact in β-sheets are often located far apart in the primary structure. To investigate the thermodynamic and kinetic features of β-structures, model systems have been devised that enhance the probability of β-sheet formation by using specially designed templates that bring two polypeptide chains into close proximity. To this end, Kemp and co-workers prepared peptide−organic dye conjugates in which the dye moiety possessed an array of hydrogen-bond donors and acceptors that were complementary to those of a peptide in a β-strand.[2] Other workers, including Kelly,[3] Nowick,[4] and Gellman,[5] have used rigid or conformationally restricted β-turn analogs that position two polypeptide chains in a parallel or antiparallel manner to nucleate a β-ribbon structure. Ghadiri[6] has investigated the relative thermodynamic stabilities of antiparallel or parallel β-sheet structures using conformationally well-behaved cyclic octapeptides.

The formation of stable peptide secondary structures is a highly cooperative process.[1] The overall thermodynamic stability of a particular conformation results from the sum of a number of rather weak noncovalent interactions. The influence of a specific noncovalent interaction on the formation of a second interaction is thus an important determinant of peptide folding motifs. In the structure termed the β-ladder, or β-hairpin, a peptide adopts an antiparallel β-sheet structure in which the strands of the ladder are connected by a β-turn.[7] The β-ladder represents a potential model system for protein β-structure, since this motif involves amino acid interactions that are relatively local in nature. A "minimal" β-ladder contains two amino acids joined by a β-turn, as in structure **I** (Figure 1). Several reports have recently appeared describing β-ladder formation in simple peptides[8] and depsipeptides.[9]

We have recently prepared a bicyclic lactam that possessed high structural similarity to the central two residues of an unusual β turn, the type VI turn (**1**, Scheme 1).[10] We have also shown that the rigid framework of the lactam ring system positioned pendant carboxamide and carbamate functional groups in a geometry to allow the formation of a stable intramolecular hydrogen bond analogous to the *i*, *i* + 3 hydrogen bond of β-turns.[11] Interest next focused on whether a doubly hydrogen-bonded minimal β-ladder would form when two amino acids were attached to the pendant amino and carboxyl groups of lactam **1**, as in structure **III** (Figure 1). The formation of the doubly hydrogen-bonded structure **III** was expected to be less favorable than formation of the singly bonded structure **II**, since adoption of conformation **III** would require the simultaneous restriction of rotation about six single bonds, as opposed to the

* To whom correspondence should be addressed. Tel.: (713) 743-3300. Fax: (713) 743-2709. E-mail: germanas@uh.edu.

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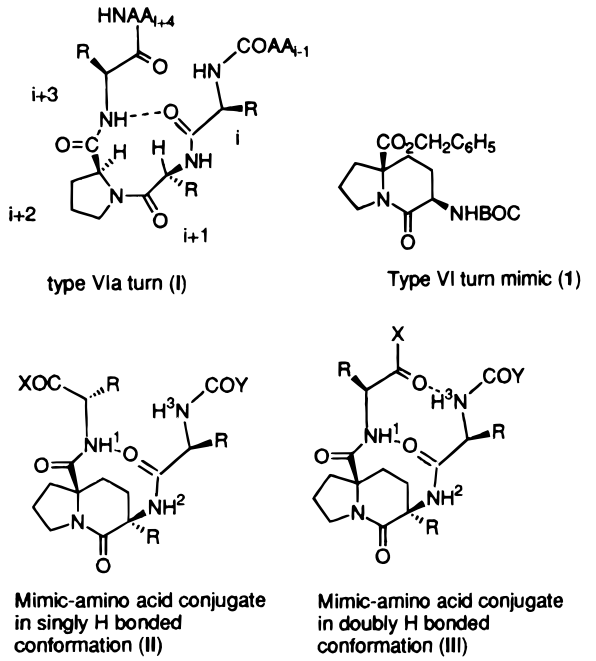

Figure 1. Conformations of peptide and bicyclic lactam conjugates.

two bonds restricted upon formation of structure **II**.[1] Herein we report that select bis-amino acid−bicyclic lactam conjugates adopt a minimal β-ladder secondary structure in solution and that the terminal hydrogen bond is significantly less stable than the internal bond.

Results

Synthesis. Bis-amino acid conjugates of the bicyclic lactam **1** were obtained by attaching amino acids to its amino and carboxyl groups using peptide-coupling procedures (Scheme 1).[12] The preparation and characterization of the mono-amino acid conjugates **2** were described previously.[11] *N*-BOC amino acids were attached to the amino function of compounds **2** using BOP-mediated coupling[13] after trifluoroacetic acid treatment, affording bis-amino acid lactam conjugates **3a** and **3b**. Alternatively, reaction with *N*-acetylamino acid anhydrides furnished the *N*-acetylamino acid conjugates **3c**−**f**. In a similar manner were the *trans*-lactam bis-amino acid hybrids **7** obtained from the *trans*-lactam monoamino acid adducts **6**. The amino acids used in the coupling reactions represented various structural types: two amino acids (Phe and Thr) were specifically chosen because of their propensity to appear in β-sheet regions of crystallographically characterized proteins.[14]

The C-terminal ester group of bis-amino acid conjugate **3g** was converted to the methylamide by the two-step sequence shown (Scheme 1). The properties of this compound would elucidate the effect on secondary structure formation of varying the hydrogen-bond-accepting strength of the C-terminal carbonyl group of the conjugates.

The bicyclic lactam−amino acid conjugates **4** were obtained from condensation of L-amino acids with the enantiomer of lactam **1** (Scheme 1). These structures contained lactams that represented "mirror images" of the type VI turn.[15] The conformational properties of these molecules would provide insight into the relative stabilities of β-ladders connected by "mirror image" and native turns.

NMR and IR Studies. The conformational features of the lactam bis-amino acid conjugates were illuminated by their ^{1}H NMR spectral features. Relevant parameters from the amide regions of the conjugates' NMR spectra are summarized in Tables 1 and 2. Site-specific assignments of the amide hydrogens were made with the aid of COSY spectra. Since the chemical shifts of the amide hydrogens of all the conjugates were sensitive to concentration and temperature, the parameters cited in the tables were obtained from samples at concentrations (about 1.5 mM) and temperatures (rt) at which aggregation was not significant.

A striking feature of the NMR spectra of conjugates **3** and **4** was the large chemical shift for the C-terminal amide hydrogens (Table 1). The chemical shift was only slightly sensitive to the nature of the amino acid from which it was derived. The analogous hydrogens of the *trans*-lactam isomers **7**, however, resonated at substantially higher field (Table 1). The deshielding of the carboxamide hydrogens of the *cis*-mimic conjugates **3** and **4** suggested that they were involved in hydrogen-bonding interactions. Previous studies had shown that a strong intramolecular hydrogen bond was present between the carboxamide hydrogen NH^1 and the carbamate carbonyl oxygen in the monoamino acid lactam hybrids **2**, but not in the *trans* isomers **6** (Figure 2).[11] Thus it was unsurprising that an analogous hydrogen bond would also form in the bis-amino acid conjugated *cis*-mimetics, as in structure **II** (Figure 1).

Further evidence for the intramolecular hydrogen bond of structure **II** in the *cis*-lactam conjugates **3** and **4** came from analysis of the temperature dependence of the chemical shift of the NH^1 hydrogens (Table 2). 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.[15] The absolute values of the temperature dependencies for the NH^1 hydrogens for all of the *cis*-mimic derivatives **3** and **4** were small and were consistent with amide hydrogens involved in strong intramolecular hydrogen-bonding interactions ($0 > \Delta\delta/\Delta T > -3.0$ ppb/K).[15] For the *trans* mimic isomers **7**, the analogous amide hydrogens displayed substantially larger temperature gradients, indicative of solvent-exposed NH's. Interestingly, neither the chemical shift nor the temperature dependence for the NH^1 hydrogen was sensitive to the absolute stereochemistry of the lactam moiety when chiral amino acids were coupled to the *cis*-mimic or its enantiomer.

Whereas the spectral characteristics of the NH^1 hydrogens established the presence of a strong intramolecular hydrogen bond involving the angular carboxamide hydrogen in all of the *cis*-lactam amino acid conjugates, greater variations in the NMR spectral properties for the *N*-terminal amide hydrogen NH^3 were found. For example, the chemical shifts of the *N*-terminal amide hydrogens varied between 5.0 and 5.9 ppm for carbam-

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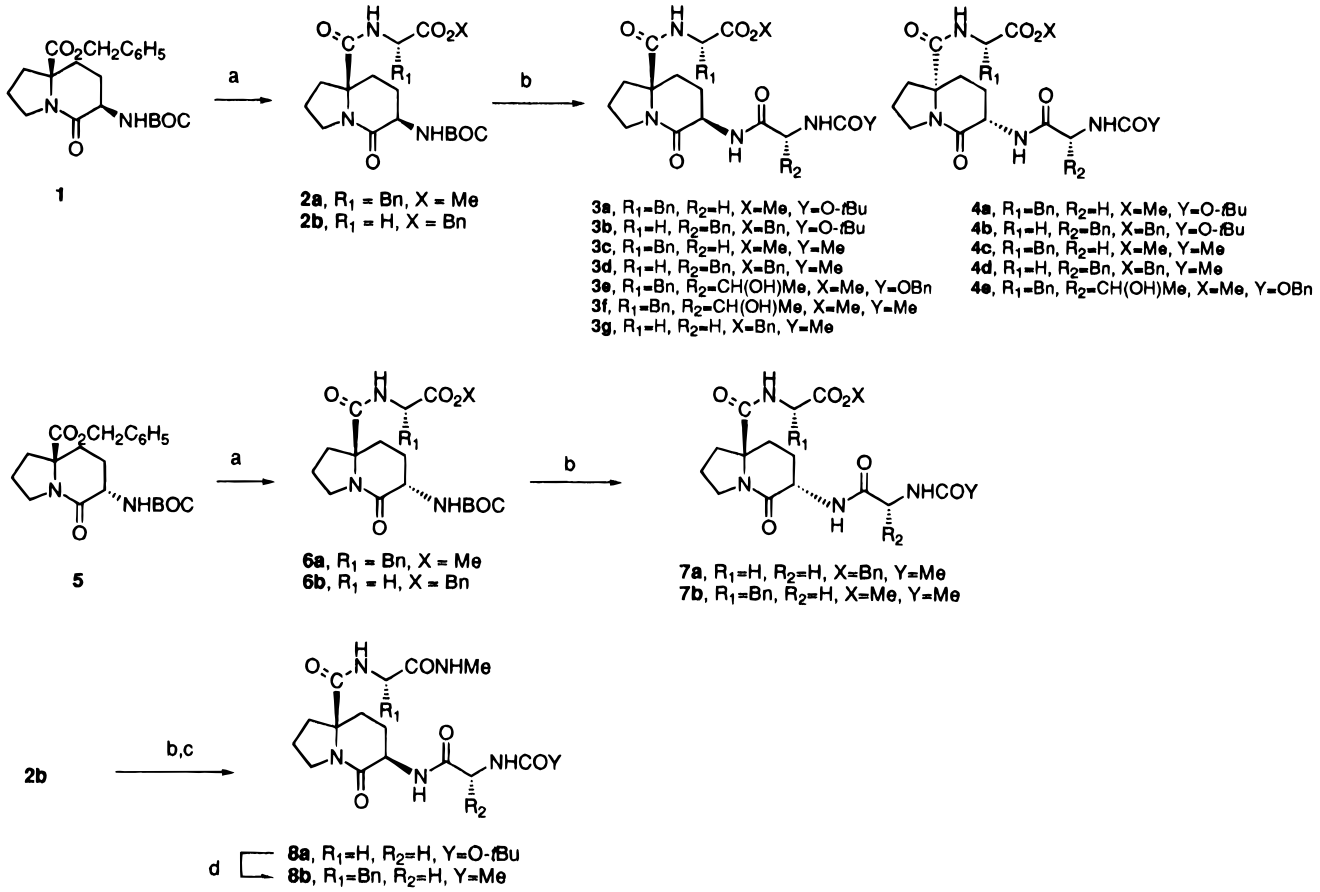
Scheme 1[a]



[a] Key: (a) (i) H_2, Pd−C; (ii) $H_2NCH(R)CO_2Me$, BOP; (b) (i) CF_3CO_2H, CH_2Cl_2; (ii) $[BOCNHCH(R)CO]_2O$, THF, or $AcNHCH(R)CO_2H$, BOP, DMF; (c) H_2, Pd−C; $MeNH_2$, BOP; (d) CF_3CO_2H, CH_2Cl_2; Ac_2O.

Table 1. ^{1}H NMR Chemical Shifts and Coupling Constants of Amide Hydrogens of Bis-amino Acid Conjugates[a]

compd	stereochemistry[c]	R_1	R_2	X	Y	δ NH$^{1\,d}$ (ppm)	JNH$^{1\,d}$ (Hz)	δNH$^{2\,d}$ (ppm)	JNH$^{2\,d}$ (Hz)	δNH$^{3\,d}$ (ppm)	JNH$^{3\,d}$ (Hz)
cis[b]											
3a	(6*R*,8a*R*)	CH_2Ph	H	OMe	O*t*-Bu	8.1	8.1	7.4	6.6	5.3	5.7, 5.4
3b	(6*R*,8a*R*)	H	CH_2Ph	OBn	O*t*-Bu	8.2	6.0, 6.0	6.9	*e*	5.0	6.0
3c	(6*R*,8a*R*)	CH_2Ph	H	OMe	Me	8.0	8.4	7.2	*e*	6.5	5.7, 5.7
3d	(6*R*,8a*R*)	H	CH_2Ph	OBn	Me	8.1	5.7, 5.7	7.3	*e*	6.4	8.1
3e	(6*R*,8a*R*)	CH_2Ph	CH(OH)Me	OMe	OBn	8.2	8.4	7.4	*e*	5.6	8.4
3f	(6*S*,8a*S*)	CH_2Ph	CH(OH)Me	OMe	Me	7.9	8.4	7.1	6.9	6.3	8.7
3g	(6*R*,8a*R*)	H	H	OBn	Me	8.3	6.0, 5.7	8.1	7.2	7.0	5.1, 5.1
4a	(6*S*,8a*S*)	CH_2Ph	H	OMe	O*t*-Bu	7.7	7.5	6.9	*e*	5.3	*e*
4b	(6*S*,8a*S*)	H	CH_2Ph	OBn	O*t*-Bu	8.3	5.4, 5.4	6.9	*e*	5.2	8.7
4c	(6*S*,8a*S*)	CH_2Ph	H	OMe	Me	7.7	8.4	6.8	6.9	6.5	6.3, 5.1
4d	(6*S*,8a*S*)	H	CH_2Ph	OCH_2Ph	Me	8.1	5.7, 5.7	7.3	*e*	6.2	8.1
4e	(6*S*,8a*S*)	CH_2Ph	CH(OH)Me	OMe	OBn	7.9	8.7	7.0	*e*	5.7	8.4
trans[b]											
7a	(6*S*,8a*R*)	H	H	OBn	Me	7.5	*e*	7.3	*e*	6.6	4.8, 4.8
7b	(6*S*,8a*R*)	CH_2Ph	H	OMe	Me	7.5	7.1	7.4	5.7	6.8	4.8, 4.8
cis[b]											
8b	(6*R*,8a*R*)	H	H	NHMe	Me	7.8	5.1, 6.4	7.7	*e*	6.7	7.2, 5.7

[a] Sample concentrations were 1−2 mM in $CDCl_3$, 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.

ates and between 6.2 and 7.0 for the *N*-acetyl derivatives (Table 1). Unlike those of the angular carboxamide NH^1, the chemical shifts of the *N*-acyl hydrogens NH^3 of the bis-amino acid-conjugated lactams, in general, did not appear in a region characteristic for hydrogen-bonded amides.

Considerable variation in the chemical shift temperature dependencies ($\Delta\delta/\Delta T$) for the *N*-terminal amide hydrogens NH^3 of conjugates **3** and **4** was also found (Table 2). The NH^3 chemical shift temperature gradients for several of the *cis*-mimic conjugates, as well as for the *trans*-mimic conjugate **7**, were sizable, indicating that these protons were predominantly solvent exposed. No easily discernible trend between the nature of the *N*-terminal amino acid and its $\Delta\delta/\Delta T$ value was found. For example, the temperature dependencies for bis-amino acid conjugates involving a Thr NH^3 were uniformly large; this result was surprising because Thr was ex-

Table 2. ^{1}H NMR Spectral Parameters of Bis-amino Acid Lactam Conjugates in DMSO-d_6[a]

compd	stereochemistry[c]	R_1	R_2	X	Y	$\Delta\delta/\Delta T$ NH^1 [d] (ppp/K)	$\Delta\delta/\Delta T$ NH^2 [d] (ppp/K)	$\Delta\delta/\Delta T$ NH^3 [d] (ppp/K)
cis[b]								
3a	(6*R*,8a*R*)	CH_2Ph	H	OMe	O-*t*-Bu	−2.6	−3.1	−5.3
3b	(6*R*,8a*R*)	H	CH_2Ph	OBn	O-*t*-Bu	−2.9	−6.1	−6.4
3c	(6*R*,8a*R*)	CH_2Ph	H	OMe	Me	−2.8	−4.6	−4.0
3d	(6*R*,8a*R*)	H	CH_2Ph	OBn	Me	−2.7	−5.2	−5.3
3e	(6*R*,8a*R*)	CH_2Ph	CH(OH)Me	OMe	OBn	−3.2	−4.5	−6.6
3f	(6*S*,8a*S*)	CH_2Ph	CH(OH)Me	OMe	Me	−4.1	−4.5	−6.5
3g	(6*R*,8a*R*)	H	H	OBn	Me	−2.5	−4.0	−4.9
4a	(6*S*,8a*S*)	CH_2Ph	H	OMe	O-*t*-Bu	−2.9	−4.1	−5.9
4b	(6*S*,8a*S*)	H	CH_2Ph	OBn	O-*t*-Bu	−3.2	−2.9	−5.5
4c	(6*S*,8a*S*)	CH_2Ph	H	OMe	Me	−2.6	−4.5	−3.3
4d	(6*S*,8a*S*)	H	CH_2Ph	OBn	Me	−2.8	−5.1	−5.5
4e	(6*S*,8a*S*)	CH_2Ph	CH(OH)Me	OMe	OBn	−3.1	−4.2	−6.0
trans[b]								
7b	(6*S*,8a*R*)	CH_2Ph	H	OMe	Me	−9.4	−6.3	−4.8
cis[b]								
8b	(6*R*,8a*R*)	H	H	NHMe	Me	−2.7	−4.0	−3.3

[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] Designations as defined in Figure 2.

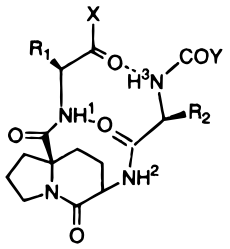


Figure 2. NH numberings in lactam−amino acid conjugates.

pected to particularly stabilize β conformations.[14] Similarly, the presence of an *N*-terminal Phe residue in the structure did not appear to consistently affect the value of the temperature dependence.

An intriguing correlation between conjugate structure and NH^3 chemical shift temperature dependence was observed, however, in those conjugates that possessed an N-terminal Gly residue. For example, the *N*-acyl hydrogen NH^3 of the bis-amino acid conjugate **4c** displayed a temperature dependence of −3.3 ppb/K for its chemical shift in DMSO, indicating a substantial degree of shielding from solvent (Table 2). Replacement of the *N*-terminal acyl group with a less effective hydrogen bond-donating *N*-terminal carbamoyl function, as in conjugate **4a**, resulted in a $\Delta\delta/\Delta T$ value of −5.9 ppb/K for NH^3, suggesting solvent exposure. Similarly, an NH^3 temperature dependence of −4.0 ppb/K for isomeric *N*-acyl conjugate **3c** changed to −5.3 ppb/K in the corresponding *N*-carbamoyl derivative **3a**. In contrast to these trends, conjugates that possessed the same amino acid compositions as compounds **3c** and **4c**, but reversed sequences, displayed much different behavior. For example, the NH^3 temperature dependences for the *N*-acyl/*N*-carbamoyl pair **4b**/**4d** were the same (−5.5 ppb/K) and those for the pair **3b**/**3d** were minimally different (−6.4 and −5.3, respectively), reflecting similar degrees of solvent exposure for the NH^3 hydrogens in all of these compounds.

Thus, the NH^3 chemical shift temperature dependencies suggested that the doubly hydrogen-bonded structure **III** (Figure 1) was appreciably populated for conjugates **3c** and **4c**, which had strong hydrogen bond donating *N*-terminal AcGly residues and C-terminal Phe methyl ester groups. The conjugate **3g**, which possessed *N*-terminal AcGly and C-terminal Gly benzyl ester moieties, displayed a $\Delta\delta/\Delta T$ value of −4.9 ppb/K for its NH^3 proton. Converting the C-terminal Gly benzyl ester function of **3g** to a Gly methyl amide group, as in compound **8b**, resulted in change of the NH^3 $\Delta\delta/\Delta T$ value to −3.3 ppb/K, consistent with greater population of conformer **III**. In sum, the degree of shielding of the NH^3 protons of the bis-amino acid conjugates depended on both the type of hydrogen bond accepting and donating groups of the terminal amino acids, as well as the sequence of the conjugate.

Further information regarding the conformational properties of the bis-amino acid hybrids was available from analysis of the NH-CαH coupling constants of the amino acid moieties. The value of $J_{NH-C\alpha H}$ is dependent on the torsion angle ϕ; for amino acids in the antiparallel β conformation, the value of J is usually 8−10 Hz.[17] According to analysis of the coupling constants (Table 1), there appeared to be no correlation between the value of $J_{NH-C\alpha H}$ and other spectroscopic properties of the conjugates, with all values falling between 7.2 and 10 Hz.

Better insight into the global conformation of specific conjugates was obtained from NOESY spectra. For instance, strong NOE's were observed between the NH^1 amide proton and the $H_{7\beta}$ and $H_{3\beta}$ protons of the bicyclic lactam system of the *cis*-lactam bis-amino acid conjugates **4c**, **3e**, and **4e** in $CDCl_3$ (Figure 3). These specific interactions, also noted in the spectra of monoamino acid conjugates **2**,[11] were diagnostic for the presence of the internal *i*, *i* + 3 hydrogen bond. The NOESY spectrum of compound **4c**, which possessed a low NH^3 chemical shift temperature dependence, also revealed crosspeaks indicative of a single conformer about the ψ bond of the Gly residue. A strong sequential CH_i-NH_{i+1} NOE was observed between *one* of the Gly CαH's and the NH^2 proton of the lactam moiety (Figure 3). Such crosspeaks are characteristic of amino acids in antiparallel β sheet conformations and indicated the presence of an extended conformation about the ψ bond of the Gly residue.[17] The conjugate **4c**, as well as the conjugate **8b**, also displayed very different chemical shifts and NH−CαH coupling constants for the diastereotopic Gly methylene hydrogens. This unique feature reflected a strongly anisotropic environment for the Gly CαH's. In contrast, the chemical shifts and coupling constants for the Gly CαH's of the

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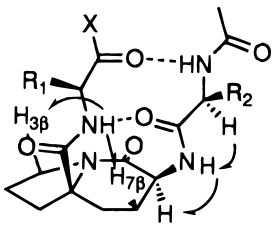

Figure 3. Graphical representation of observable NOE's in conjugate **4c**.

isomeric hybrid **4d** were similar due to statistical averaging of values for several stable conformations.

To complement the NMR studies, the hydrogen-bonding properties of the bis-amino acid conjugates were also investigated by IR spectroscopy. At moderate (20−30 mM) concentrations in CH_2Cl_2 all the conjugates displayed bands attributable to both hydrogen-bonded (3200−3300 cm^{-1}) and non-hydrogen-bonded (3400 to 3500 cm^{-1}) NH stretching vibrations.[18] To distinguish intramolecular from intermolecular hydrogen bonding in the conjugates, the dependence of the intensities of the individual IR bands on concentration was determined. As with the monoamino acid conjugates **6**, bis-amino acid conjugates incorporating the *trans*-lactam **5** displayed only bands for non-hydrogen-bonded NH stretches at concentrations below 10 mM. For the *cis*-lactam conjugates **3** and **4**, however, the hydrogen-bonded stretches persisted even at the lowest concentrations (2 mM) and must therefore have arisen from intramolecular interaction.

In the bis-amino acid conjugates **3** and **4**, two pairs of amides were capable of forming intramolecular hydrogen bonds. Because NMR spectroscopy established that the internal i, $i + 3$ hydrogen bond existed in all *cis*-lactam bis-amino acid hybrids, and IR spectra of the monoamino acid conjugates of the *cis*-lactam **1** displayed hydrogen-bonded NH stretches at low concentrations, a portion of the low-wavenumber NH stretch was assumed to be due the internal hydrogen bond in the *cis*-lactam derivatives **3** and **4**. The state of hydrogen bonding of the second amide pair was assessed by the relative intensities of the two NH stretching bands for each compound. The spectra of compounds **3c** and **4c**, which displayed low chemical shift temperature dependencies for their NH[3] protons, revealed relatively more intense bands for hydrogen-bonded NH stretches relative to non-hydrogen-bonded stretches (Figure 4). In contrast, the isomeric conjugate **3d** displayed a weaker hydrogen-bonded NH stretch relative to that of **3c** at all concentrations. The spectra of the other bis-amino acid conjugates appeared similar to that of **3d**. These results indicate that both amide pairs of conjugates **3c** and **4c** were involved in intramolecular hydrogen-bonding interactions, while only the internal interaction was present for the rest of the conjugates.

Discussion

The spectroscopic properties of the bis-amino acid conjugates of the type VI turn mimic **1** supported the presence of the internal intramolecular hydrogen bond, as in the β-turn conformation (**II**, Figure 1) for all of the derivatives **3** and **4**. The adoption of the doubly hydrogen-bonded conformation, as in structure **III**, was expected to be entropically less favorable relative to formation of conformation **II**. It was not surprising, then, that the β-ladder conformation **III** was absent in the majority of the conjugates studied, even when β-sheet stabilizing amino acids were present.

Interestingly, the spectroscopic properties supported the presence of the β-ladder conformation for select conjugates that possessed N-terminal AcGly residues (compounds **3c**, **4c**, and **8b**). Conjugates **3c** and **4c** incorporated C-terminal Phe residues; Dyson and co-workers had previously shown that peptides containing the sequence ArProAr, where Ar was Phe or Tyr, adopted type VI β-turn conformations in solution.[19] The noncovalent interactions that stabilize the type VI conformation in the peptides likely also contribute to the stability of conformation **III** in the conjugates **3c** and **4c**. The analogs **3c** and **4c** as well as **8b** contained Gly residues at the *N*-terminus. Thus, a possible origin of the stability of the β-ladder of these conjugates was the unique flexibility of Gly or the small steric features of Gly relative to other amino acids (Figure 5).

Compound **4c** possessed the stereochemistry of a "mirror image" β-turn. Gellman,[9] as well as Imperiali,[20] have noted that the innate twisting preferences of β-turns and β-strands are identical when a "mirror image" turn is present. Such an effect may be applicable to the case at hand, explaining the greater stability of the hairpin conformation of conjugate **4c** over **3c**. The β-ladder conformation was not present, however, in the other conjugates with "mirror image" turns (compounds **4a**−**4b**, **4d**−**4e**).

Other investigators have delved into structure-conformation relationships involving compounds containing the minimal number of residues (4) necessary to form a β-ladder structure.[18,21] Gellman and co-workers[9] discovered that a "mirror image" turn enhanced the stability of a β-hairpin conformation in a particular depsipeptide sequence; the dependence of conformation on amino acid sequence in these structures was also addressed. Kemp and co-workers[21] described the NMR spectroscopic features of a series of peptide−pyridinone hybrids that were capable of forming β-ladders. The absence of sequence-specific resonance assignments for these compounds made conformational analysis difficult. The present work represents the first exhaustive study of the conformational properties of β-turn mimic−amino acid conjugates capable of forming a minimal β-ladder. The strong sensitivity of the conjugate conformation on sequence reported here is therefore a novel result. Further studies into the origin of the unique conformational properties of the mirror image conjugates are under investigation.

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

(18) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164−1166.

(19) Yao, J.; Fehrer, V. A.; Espejo, B. F.; Reymond, M. T.; Wright, P. E.; Dyson, H. J. *J. Mol. Biol.* **1994**, *243*, 736−753.

(20) Struthers, M. D.; Cheng, R. P.; Imperiali, B. *Science* **1996**, *271*, 342−345.

(21) Kemp, D. S.; McNamara, P. *Tetrahedron Lett.* **1982**, *37*, 3761−3764.

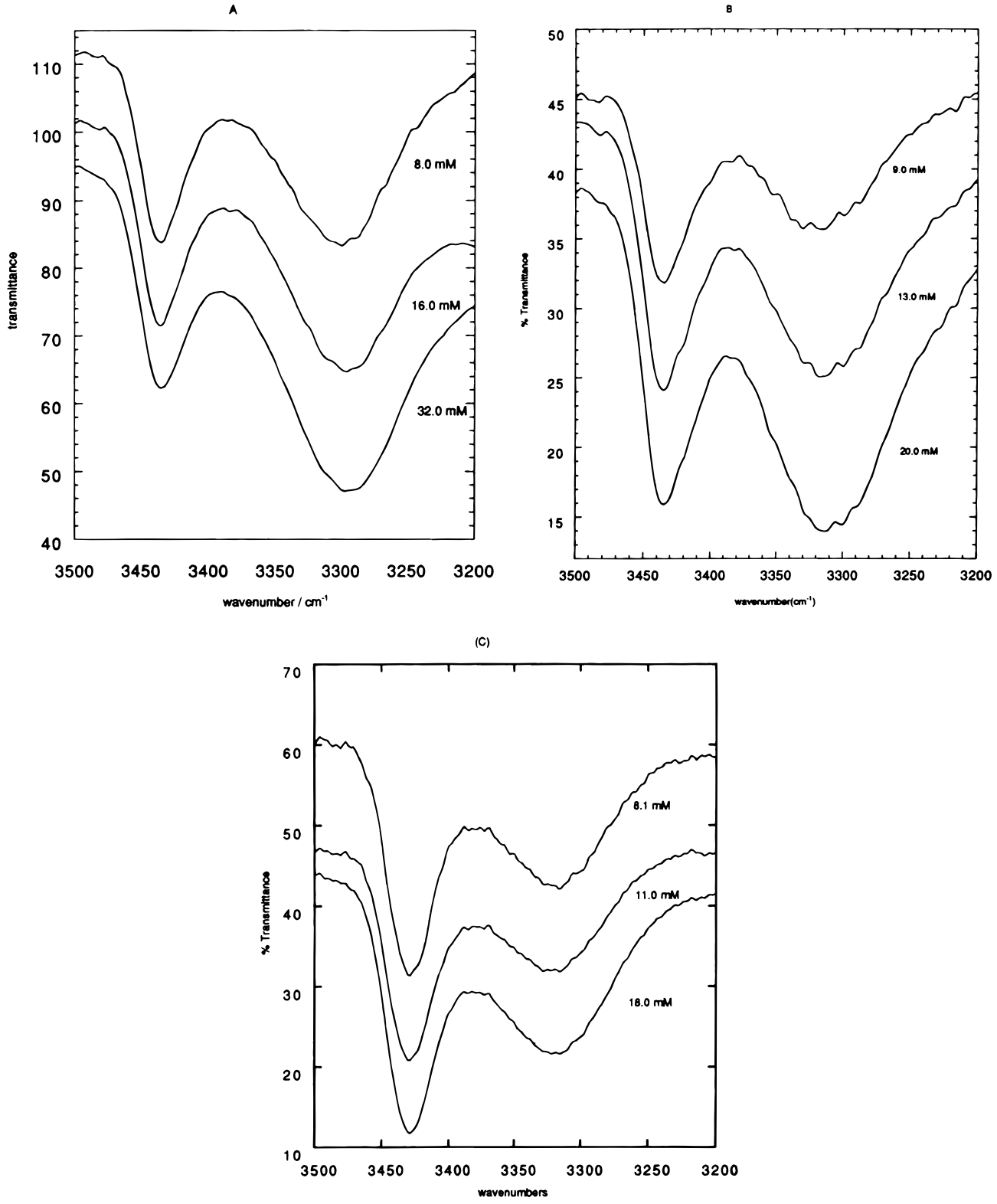

Figure 4. NH stretch region of the IR spectrum of select lactam−amino acid conjugates at different concentrations in CH_2Cl_2: (a) conjugate **3c**; (b) conjugate **4c**; (c) conjugate **4d**.

magnesium. Reactions were carried out under a dry nitrogen atmosphere in glassware that had been flame or ovendried (T > 100 °C) overnight.

^1H 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 dependencies 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. Reversed-phase liquid chromatography (RPLC) was performed using a PepRC column with a gradient of water−acetonitrile−0.1% trifluoroacetic acid.

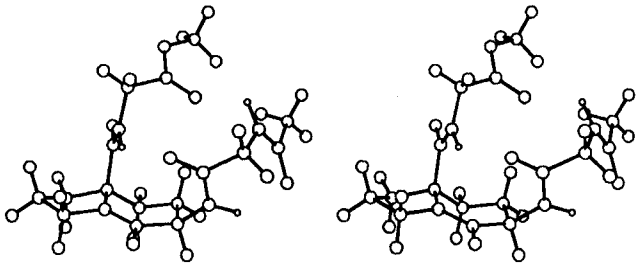

Figure 5. Stereoview of the molecular mechanics lowest energy conformation of **7a**, showing the doubly hydrogen-bonded conformation with absence of destabilizing steric interactions.

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

General Procedure for Elongation of Bicylic Lactam Amino Acid Conjugates with Anhydrides of *N*-BOC-amino Acids (3a−3b, 4a−4b). To a solution of peptide **2**[11] (0.53 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (1 mL, 5.9 mmol), after which the solution was allowed to stir 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). Meanwhile, the anhydride of the *N*-BOC-amino acid (2 equiv) was dissolved in THF (5 mL) and cooled with an ice bath. To the anhydride solution was added the amine salt solution dropwise. After 2 h, the reaction mixture was warmed to rt and quenched with water. The resulting mixture was partitioned between aqueous 0.1 N HCl solution and $CHCl_3$. The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure to give a colorless oil of the crude peptide. Column chromatography eluting with hexane:ethyl acetate: diethyl ether:ethyl alcohol (1:7:6:0.5) afforded the pure peptide **3a−3b**, which was homogeneous according to RPLC.

The (6*S*,8a*S*)-diastereomers **4a−4b** were obtained by reaction of amino acids with the racemic lactam of **1**. The individual diastereomers were obtained after separation by column chromatography, eluting with a solvent mixture of 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−*L*-phenylalanine methyl ester (3a): yield 56%; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 6.6 Hz, 1H), 7.36−7.18 (m, 5H), 5.39 (dd, J = 5.7, 5.4 Hz, 1H), 4.70 (ddd, J = 11.9, 8.1, 4.2 Hz, 1H), 3.94 (dd, J = 17.1, 5.7 Hz, 1H), 3.79 (m, 3H), 3.72 (s, 3H), 3.54 (m, 2H), 3.32 (dd, J = 4.5, 14.1 Hz), 3.06 (m, 2H), 2.56 (dd, J = 12.0, 6.6 Hz, 1H), 2.41 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.38 (m, 1H), 2.02 (m, 1H), 1.80−1.52 (m, 2H), 1.44 (s, 9H), 1.02 (m, 1H); ^{13} C NMR ($CDCl_3$) δ 172.9, 171.9, 169.7, 167.8, 156.0, 137.1, 129.3, 129.0, 128.5, 126.8, 80.2, 71.1, 54.2, 52.3, 51.5, 45.7, 37.8, 36.6, 37.0, 31.7, 28.3, 25.0, 20.5.

(6*R*,8a*R*)-*N*-[(*tert*-Butoxycarbonyl)-*L*-phenylalanyl]-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (3b): yield 50%; ^{1}H NMR ($CDCl_3$) δ 8.20 (br s, 1H), 7.38−7.23 (m, 10H), 6.90 (br s, 1H), 5.15 (s, 2H), 4.96 (d, J = 6.0 Hz, 1H), 4.32 (m, 1H), 4.06 (dd, J = 14.4, 6.0 Hz, 2H), 3.69 (m, 2H), 3.52 (m, 1H), 3.07 (m, 2H), 2.70 (dd, J = 12.0, 6.6 Hz, 1H), 2.48 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.12 (m, 1H), 1.82 (m, 3H), 1.52 (m, 1H), 1.37 (s, 9H), 0.82 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.5, 171.5, 169.4, 167.3, 136.7, 135.0, 129.6, 129.5, 128.6, 128.3, 126.9, 71.1, 67.0, 51.6, 45.9, 41.7, 38.3, 31.0, 28.3, 25.0, 22.6, 20.8, 14.9.

(6*S*,8a*S*)-*N*-[(*tert*-Butoxycarbonyl)glycyl]-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (4a): yield 56%; ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 7.5 Hz, 1H), 7.28−7.15 (m, 5H), 6.95 (br s, 1H), 5.26 (br s, 1H), 4.93 (m, 1H), 3.84 (dd, J = 17.1, 5.7 Hz, 1H), 3.73 (m, 3H), 3.70 (s, 3H), 3.45 (m, 1H), 3.33 (dd, J = 14.4, 5.4 Hz, 1H), 3.12 (m, 2H), 2.67 (dd, J = 11.4, 6.0 Hz, 1H), 2.38 (m, 1H), 1.87−1.53 (m, 4H), 1.44 (s, 9H), 0.87 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.5, 172.2, 169.6, 168.9, 137.5, 128.7, 127.3, 126.5, 71.5, 53.7, 52.5, 51.7, 45.2, 38.2, 37.3, 32.0, 28.7, 25.0, 20.7.

(6*S*,8a*S*)-*N*-[(*tert*-Butoxycarbonyl)-*L*-phenylalanyl]-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (4b): yield 50%; ^{1}H NMR ($CDCl_3$) δ 8.27 (br s, 1H), 7.38−7.20 (m, 10H), 6.90 (br s, 1H), 5.17 (br s, 1H), 5.15 (s, 2H), 4.32 (m, 1H), 4.06 (d, J = 5.4, 5.4 Hz, 2H), 3.69−3.45 (m, 3H), 2.98 (d, J = 6.0 Hz, 2H), 2.70 (dd, J = 12.0, 6.6 Hz, 1H), 2.45 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.12 (m, 1H), 1.82−1.52 (m, 4H), 1.37 (s, 9H), 0.82 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.5, 171.5, 169.4, 167.3, 135.2, 129.5, 128.5, 126.8, 71.1, 67.1, 51.6, 45.9, 42.2, 41.7, 38.2, 36.8, 31.5, 28.2, 25.1, 20.8.

General Procedure for Preparation of Bis-amino Acid Conjugates from Monoamino Acid Conjugates and *N*-Acetylamino Acids (3c−3g, 4c−4e, 7a−7b). The monoamino acid conjugate **2** or **6**[11] (0.5 mmol) was treated with 30% TFA/CH_2Cl_2 solution (10 mL) at rt. After 2 h, the solvent was removed under reduced pressure to give a yellowish oil. Meanwhile, BOP (0.32 g, 0.70 mmol) and the *N*-acylamino acid (3 equiv) were dissolved in anhydrous DMF and stirred for 30 min. The crude amine was dissolved in DMF (5mL) and Et_3N (0.54 mL, 3 mmol) and transferred into the solution of the activated *N*-acylamino acid and stirred for 5 h. DMF was removed under reduced pressure, and the crude mixture was suspended in $CHCl_3$. The solution was washed with saturated $NaHCO_3$ and water, dried with $MgSO_4$, filtered, and evaporated under reduced pressure to afford 0.2 g of crude peptide. Column chromatography on silica, eluting with a mixture of $CHCl_3$:MeOH (9:1), afforded 0.11 g of pure peptides **3a−3g**, or **7a−7b**, homogeneous by RPLC.

The (6*S*,8a*S*)-diastereomers **4c−f** were obtained by reaction of amino acids with the racemic lactam of **1**. The individual diastereomers were obtained after separation by column chromatography, eluting with a solvent mixture of hexane: ethyl acetate:diethyl ether:ethyl alcohol (1:7:6:0.5).

(6*R*,8a*R*)-*N*-(Acetyglycyl)-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (3c): yield 58%; ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 1H), 7.31−7.23 (m, 6H), 6.49 (dd, J = 5.7, 5.7 Hz, 1H), 4.76 (ddd, J = 12.9, 8.7, 4.5 Hz, 1H), 4.06 (dd, J = 16.8, 5.7 Hz, 1H), 3.87 (m, 2H), 3.73 (s, 3H), 3.59 (m, 1H), 3.32 (dd, J = 13.8, 4.2 Hz, 1H), 3.07 (m, 2H), 2.54 (dd, J = 11.7, 6.3 Hz, 1H), 2.43 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.21 (m, 1H), 2.05 (s, 3H), 1.87−1.40 (m, 4H), 1.05 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 172.8, 172.1, 170.9, 169.2, 168.1, 137.1, 128.9, 128.4, 128.3, 126.8, 71.1, 54.0, 52.3, 51.3, 45.7, 42.9, 37.9, 36.6, 31.0, 25.0, 22.9, 20.4; HR FAB MS calcd for $C_{23}H_{31}N_4O_6$ 459.2243, found 459.2235.

(6*R*,8a*R*)-*N*-(Acetyl-*L*-phenylalanyl)-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (3d): yield 53%; ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 6.0, 5.7 Hz, 1H), 7.34−7.05 (m, 11H), 6.39 (d, J = 8.1 Hz, 1H), 5.08 (s, 2H), 4.63 (m, 1H), 4.00 (dd, J = 9.6, 6.0 Hz, 1H), 3.90 (dd, J = 9.6, 5.7 Hz, 1H), 3.69 (m, 1H), 3.60−3.37 (m, 3H), 2.89 (dd, J = 14.0, 7.2 Hz, 1H), 2.72 (dd, J = 11.7, 6.0 Hz, 1H), 2.38 (m, 1H), 2.00 (m, 1H), 1.87 (s, 3H), 1.80 (m, 2H), 1.58 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.8, 172.2, 171.0, 169.3, 168.1, 136.7, 128.7, 128.1, 126.8, 71.5, 67.0, 54.3, 52.3, 51.3, 45.7, 42.9, 37.8, 36.0, 31.2, 25.0, 22.8, 20.5.

(6*R*,8a*R*)-*N*-[(Benzyloxycarbonyl)-*L*-threonyl]-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (3e): yield 45%; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8.4 Hz, 1H), 7.40−7.17 (m, 11H), 5.59 (d, J = 8.4 Hz, 1H), 5.18 (s, 2H), 4.76 (ddd, J = 11.4, 8.7, 4.5 Hz, 1H), 4.50 (m, 1H), 4.26 (d, J = 8.4 Hz, 1H), 3.78 (m, 1H), 3.67 (s, 3H), 3.58 (m, 2H), 3.26 (dd, J = 13.8, 4.5 Hz, 1H), 3.00 (m, 2H), 2.74 (dd, J = 11.7, 6.3 Hz, 1H), 2.45 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.21 (m, 1H), 1.98 (m, 1H), 1.78−1.42 (m, 2H), 1.23 (d, J = 6.3 Hz, 3H), 1.00 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.9, 172.9 (overlap), 171.0, 167.4, 156.8, 136.7, 136.0, 128.9, 128.6, 128.3, 128.1, 126.9, 71.1, 67.4, 66.9, 59.2, 53.9, 52.4, 51.8, 45.7, 37.8, 36.8, 31.0, 25.3, 20.5, 18.2.

(6*R*,8a*R*)-*N*-[Acetyl-*L*-(*O*-acetylthreonyl)]-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (3f): yield 68%; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 1H), 7.38−7.20 (m, 5H), 7.05 (d, J = 6.9 Hz, 1H), 6.31 (d, J = 8.7 Hz, 1H), 5.46 (m, 1H), 4.76 (ddd, J = 11.4, 8.7, 4.5 Hz, 1H), 4.59 (dd, J = 8.7, 5.4 Hz, 1H), 3.84 (ddd, J = 11.4, 7.2, 7.2 Hz, 1H), 3.73 (s, 3H), 3.54 (m, 2H), 3.27 (dd, J = 14.1, 4.5 Hz, 2H),

3.03 (m, 2H), 2.54 (dd, J = 11.7, 6.3 Hz, 1H), 2.45 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.21 (m, 1H), 1.98 (m, 2H), 1.78−1.42 (m, 3H), 1.23 (d, J = 6.0 Hz, 3H) 1.12 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.9, 172.3, 170.6, 170.2, 168.8, 167.2, 136.9, 128.9, 128.5, 126.8, 71.0, 69.7, 56.4, 53.9, 52.3, 51.5, 45.7, 37.8, 31.0, 25.0, 23.2, 21.1, 20.5, 16.6.

(6*R*,8a*R*)-*N*-(Acetylglycyl)-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (3g): yield 54%; ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, J = 6.0, 5.7 Hz, 1H), 8.04 (d, J = 7.2 Hz,1H), 7.40−7.33 (m, 5H), 6.92 (dd, J = 5.1, 5.1 Hz, 1H), 5.11 (s, 2H), 4.07 (m, 3H), 3.84 (ddd, J = 11.4, 7.2, 7.2 Hz, 1H), 3.75 (dd, J = 14.0, 5.1 Hz, 1H), 3.48 (m, 2H), 2.74 (dd, J = 12.0, 5.4 Hz, 1H), 2.45 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.11 (m, 1H), 1.98 (s, 3H), 1.89−1.76 (m, 3H), 1.52−1.65 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 173.2, 170.9, 169.4, 168.2, 168.1, 135.2,128.4, 128.5, 128.3, 71.2, 67.0, 51.2, 45.9, 42.8, 41.7, 38.2, 30.9, 24.9, 22.9, 20.8.

(6*S*,8a*R*)-*N*-(Acetylglycyl)-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (4c): yield 65%; ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 8.4 Hz, 1H), 7.27−7.14 (m, 5H), 6.81 (d, J = 6.9 Hz, 1H), 6.45 (dd, J = 6.3, 5.1 Hz, 1H), 4.93 (m, 1H), 4.03 (dd, J = 16.8, 6.3 Hz, 1H), 3.84 (dd, J = 16.5, 5.1 Hz, 1H), 3.72 (s, 3H), 3.68 (m, 2H), 3.49 (m, 1H), 3.33 (dd, J = 5.7, 14.7 Hz, 1H), 3.10 (dd, J = 9.9, 14.7 Hz, 1H), 2.67 (dd, J = 6.3, 11.7 Hz, 1H), 2.33 (m, 1H), 2.03 (s, 3H), 1.87−1.52 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.8, 169.4, 168.3, 168.1, 136.8, 128.9, 128.4, 126.8, 126.7, 71.2, 54.1, 53.2, 52.2, 51.5, 45.7, 42.9, 38.5, 36.7, 30.4, 24.5, 22.9, 20.7; HR FAB MS calcd for $C_{23}H_{31}N_4O_6$ 459.2243, found 459.2233.

(6*S*,8a*S*)-*N*-(Acetyl-*L*-phenylalanyl)-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (4d): yield 53%; ^{1}H NMR ($CDCl_3$) δ 8.04 (dd, J = 6.0, 5.7 Hz, 1H), 7.34−7.05 (m, 11H), 6.20 (d, J = 8.1 Hz, 1H), 5.08 (s, 2H), 4.60 (m, 1H), 4.0 (dd, J = 9.6, 6.0 Hz, 1H), 3.82 (m, 2H), 3.60−3.37 (m, 2H), 2.96 (d, J = 6.9 Hz, 2H), 2.72 (dd, J = 11.5, 5.8 Hz, 1H), 2.38 (m, 1H), 2.01 (m, 1H), 1.87 (s, 3H), 1.80 (m, 3H), 1.58 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 173.5, 173.3, 171.7, 171.6, 168.2, 136.5, 129.4, 128.3, 126.5, 71.5, 68.7, 54.4, 50.5, 48.7, 46.2, 43.2, 40.5, 35.0, 29.6, 26.2, 20.5.

(6*S*,8a*S*)-N-[(Benzyloxycarbonyl)-*L*-threonyl]-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (4e): yield 68%; ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 8.7 Hz, 1H), 7.80−7.65 (m, 10H), 7.02 (d, J = 7.3 Hz, 1H), 5.72 (d, J = 8.4 Hz, 1H), 5.20 (s, 2H), 4.98 (m, 1H), 4.38 (d, J = 6.9 Hz, 1H), 4.06 (dd, J = 8.7, 7.8 Hz, 1H), 3.75 (s, 3H), 3.54 (m, 2H), 3.30 (m, 2H), 3.10 (d, J = 6.0 Hz, 2H), 2.72 (dd, J = 6.3, 11.7 Hz, 1H), 2.40 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 1.65−1.25 (m, 5H), 1.20 (d, J = 6.3 Hz, 3H), 0.82 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.2, 172.2 (overlap), 171.8, 168.3, 156.6, 136.7, 136.0, 128.9, 128.5, 128.4, 128.0, 126.7, 71.20, 67.4, 67.2, 60.2, 53.6, 52.5, 52.2, 45.7, 38.4, 36.6, 30.5, 24.3, 20.7, 18.5.

(6*R*,8a*S*)-*N*-(Acetylglycinyl)-6-amino-8a-carboxyindolizin-5-one−glycine benzyl ester (7a): yield 55%; ^{1}H NMR ($CDCl_3$) δ 7.43 (dd, J = 5.4, 5.4 Hz, 1H), 7.35−7.29 (m, 6H), 6.57 (dd, J = 4.8, 4.8 Hz, 1H), 5.19 (s, 2H), 4.29 (dd, J = 7.5, 13.2 Hz, 1H), 4.08 (d, J = 5.7 Hz, 2H), 4.03 (dd, J = 5.4, 16.8 Hz, 1H), 3.87 (dd, J = 5.4, 16.8 Hz, 1H), 3.72 (m, 1H), 3.51 (m, 1H), 2.59−2.42 (m, 4H), 2.00 (s, 3H), 1.87−1.72 (m, 4H), 1.52 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.8, 170.9, 170.5, 170.1, 169.6, 135.5, 128.9, 128.1, 128.0, 70.6, 67.6, 49.4, 47.1, 43.3, 41.8, 39.6, 30.7, 26.6, 23.3, 21.8.

(6*S*,8a*R*)-*N*-(Acetylglycyl)-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (7b): yield 70%; ^{1}H NMR ($CDCl_3$) δ 7.48 (d, J = 7.1 Hz, 1H), 7.38 (d, J = 5.7 Hz, 1H), 7.27−7.18 (m, 5H), 6.79 (dd, J = 4.8, 4.8 Hz, 1H), 4.92 (ddd, J = 4.8, 4.8 14.7 Hz, 1H), 4.24 (m, 2H), 3.89 (dd, J = 16.5, 4.8 Hz, 1H), 3.74 (s, 3H), 3.68 (m, 1H), 3.30 (dd, J = 4.8, 14.1 Hz, 1H), 3.25 (m, 1H), 2.98 (m, 1H), 2.54−2.39 (m, 2H), 2.27 (m, 1H), 2.01 (s, 3H), 1.73−1.45 (m, 4H), 1.05 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.0, 173.1, 170.4, 169.9, 168.9, 136.7, 128.4, 128.8, 127.0, 69.8, 52.8, 53.0, 49.5, 46.5, 42.4, 39.3, 37.2, 30.3, 26., 23.1, 20.5; HR FAB MS calcd for $C_{16}H_{24}N_4O_6$ 368.1696, found 368.1686.

(6*R*,8a*S*)-*N*-(Acetylglycyl)-6-amino-8a-carboxyindolizin-5-one−*L*-phenylalanine methyl ester (7c): yield 70%; ^{1}H NMR ($CDCl_3$) δ 7.38−7.21 (m, 4H), 7.13 (d, J = 7.2 Hz, 1H), 6.92 (d, J = 5.7 Hz, 1H), 6.47 (d, J = 8.7 Hz, 1H), 6.38 (br s, 1H), 4.87 (ddd, J = 11.7, 5.4, 5.4 Hz, 1H), 3.99 (dd, J = 5.1, 5.1 Hz, 2H), 3.77 (s, 3H), 3.70 (m, 1H), 3.42 (m, 1H), 3.23 (dd, J = 13.8, 5.7 Hz, 1H), 3.00 (dd, J = 13.8, 5.7Hz, 1H), 2.46 (m, 1H), 2.24 (ddd, J = 13.5, 6.3, 6.3 Hz, 1H), 2.19 (m, 1H), 2.05 (s, 3H), 1.82−1.63 (m, 5H), 1.42 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.6, 172.1, 170.4, 169.8, 168.8, 136.0, 128.9, 128.8, 127.3, 70.4, 53.1, 52.5, 48.8, 46.4, 42.9, 38.7, 37.3, 29.8, 26.6, 22.1, 21.0.

(6a*R*,8a*R*)-*N*-[(*tert*-Butoxycarbonyl)glycyl]-6-amino-8a-carboxyindolizin-5-one−Glycine Methyl Amide (8a). Compound **3a** (20mg, 0.04 mmol) was dissolved in EtOH (5 mL) along with 10% palladium on activated carbon (5 mg). The reaction was stirred under a hydrogen atmosphere at rt until no starting material was observed. Catalyst was removed by filtration, and EtOH was evaporated under reduced pressure to afford a colorless oil of the acid of **3a**. This intermediate was dissolved in DMF and BOP added to activate the acid group (1.1 equiv); after cooling to −10 °C, gaseous methyl amine was passed into this solution. The reaction flask was sealed securely, warmed to room temperature, and stirred for 5 h. DMF was evaporated *in vacuo* to give 8 mg of crude methylamide. Colum chromatography on silica gel eluting with $CHCl_3$:MeOH (9:1) afforded the methylamide as a colorless oil: yield 47%; ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 6.0, 6.0 Hz, 1H), 7.69 (br s, 1H), 6.70 (br s, 1H), 5.68 (br s, 1H), 4.1 (dd, J = 12.0, 6.0 Hz, 1H), 3.89 (m, 2H), 3.73−3.60 (m, 2H), 2.76 (d, J = 5.1 Hz, 3H), 2.66 (m, 2H), 2.03 (m, 3H), 1.80 (m, 1H), 1.73−1.64 (m, 4H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.5, 170.8, 169.7, 168.6, 156.0, 80.0, 71.2, 51.4, 46.2, 43.8, 38.5, 31.3, 28.3, 26.1, 25.1, 21.0.

(6a*R*,8a*R*)-*N*-(Acetylglycyl)-6-amino-8a-carboxyindolizin-5-one−Glycine Methylamine (8b). To a solution of the previously prepared methylamide (8 mg, 0.02 mmol) in CH_2Cl_2 was added trifluoroacetic acid (0.02 mL, 0.2 mmol), and the resulting solution was stirred at rt. After 2 h, the solvent was removed under vacuum, and the mixture was transferred to a solution of acetic anhydride (0.01 mL, 0.1 mmol) and triethylamine (0.03 mL, 0.3 mmol) in DMF (5 mL). Stirring was continued for 5 h at rt. The reaction was concentrated, and the products were separated on silica gel, eluting with $CHCl_3$:MeOH (9:1) to afford **8** as a colorless oil: yield 70%; ^{1}H NMR ($CDCl_3$) δ 7.81 (dd, J = 5.4, 5.4 Hz, 1H), 7.54 (d, J = 6.6 Hz, 1H), 6.54 (dd, J = 7.2, 5.7 Hz, 1H), 6.32 (br s, 1H), 4.08 (dd, J = 16.2, 7.2 Hz, 1H), 4.02 (dd, J = -16.2, 5.7 Hz, 1H), 3.97 (ddd, J = 11.4, 7.2, 7.2 Hz, 1H), 3.89 (d, J = 5.7 Hz, 1H), 3.85 (m, 2H), 3.73 (dd, J = 16.5, 7.2 Hz, 1H), 3.57 (m, 1H), 2.71 (dd, J = 6.3, 11.7 Hz, 1H), 2.68 (d, J = 5.1 Hz, 3H), 2.60 (ddd, J = 13.8, 3.3, 3.3 Hz, 1H), 2.04 (s, 3H), 1.89 (m, 2H), 1.78−1.58 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 175.9, 173.8, 171.9, 171.8, 170.7, 72.7, 52.4, 47.4, 44.0, 43.7, 39.3, 32.2, 26.3, 22.6, 21.9, 11.7.

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Supporting Information Available: ^{1}H NMR spectra of **1**−**8**, ^{13}C spectra of **1**−**8**, and COSY and NOESY spectra of **4c** (16 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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