Rationally Designed Bicyclic Lactams Control Different Turn Motifs and Folding Patterns in Hexapeptide Mimics

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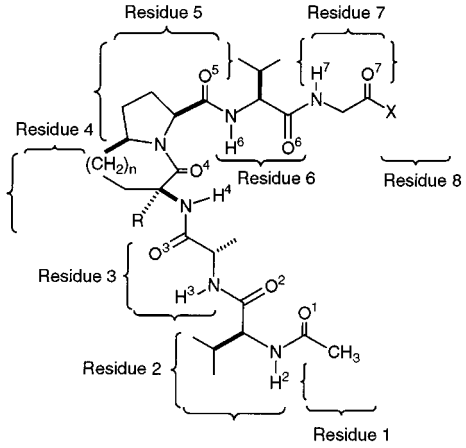
Conformational analysis of *N*-acetylated hexapeptide mimics incorporating a bicyclic lactam (**1**–**4**) was carried out by a combination of ^{1}H-NMR spectroscopy, IR spectroscopy, and computer modeling. The nature of the bicyclic lactam determines the turn motifs and the folding patterns of these constrained peptides. The (5,6)-bicyclic lactam derivatives **1** and **2**, characterized by a type-II′ β-turn (C=O^3···H^6–N), are very compact intramolecularly H-bonded structures. The (5,7)-bicyclic lactam derivative **3**, characterized by an inverse γ-turn (C=O^4···H^6–N), is a quite flexible "tweezer-like" structure.

Introduction

Synthesis of artificial proteins requires the design and construction of stable mimics for the secondary structural elements. In this context, a number of initiators for α-helix formation,[1] conformationally constrained analogues of the various turns,[2] and β-sheet (or β-hairpin) nucleators have been reported.[3]

In this communication, we report the conformational analysis of *N*-acetylated hexapeptide mimics incorporating a bicyclic lactam (**1**–**4**, Figure 1),[4] which was carried out by a combination of ^{1}H-NMR spectroscopy,[5] IR spectroscopy, and computer modeling.[6–10] We show that intramolecular hydrogen bonding provides a principal driving force for turn formation,[11] and that the nature of the bicyclic lactam determines the turn motifs and the folding patterns of these constrained peptides.



1; n = 1, X = OMe, R = CH_2Ph
2; n = 1, X = NH^8CH_2Ph, R = CH_2Ph
3; n = 2, X = OMe, R = H
4; n = 2, X = NH^8CH_2Ph, R = H

Figure 1. Numbering system for *N*-acetylated hexapeptide mimics (**1**–**4**)

Results and Discussion

The amide protons of **1** and **2** display no concentration dependence, which suggests that these hexapeptide mimics do not aggregate in the concentration range examined.[5] Moreover, the temperature coefficients show a linear relationship between chemical shift and temperature in 2.0 mM chloroform solutions.[5] The low temperature coefficients[2i,12,13] ($\Delta\delta NH/\Delta T$ = –1.5/0.0 [ppb/K]) of the amide protons H^6 (δNH = 7.37) and H^7 (δNH = 7.27) of compound **1**, the small chemical shift changes on addition of methanol ($\Delta\delta NH$ = 0.09/0.02), and the relatively slow exchange rates with CD_3OD (60/20 min) indicate that these protons (Figure 2, Table 1) are locked in an intramolecularly H-bonded state. Protons H^3 and H^4 {δNH = 7.54/7.28, instant./10 min exchange rates with CD_3OD, high temperature coefficients (–8.0/–2.7 [ppb/K])} are in equilibrium between a non-H-bonded and a H-bonded state, while proton H^2 (δNH = 6.08, $\Delta\delta NH/\Delta T$ = –2.1 [ppb/K], on addition of methanol: $\Delta\delta NH$ = 0.38, fast exchange rate with CD_3OD) is clearly in a non-H-bonded state.[14]

An analysis of intramolecular hydrogen bond parameters[15] and backbone torsion angles[16] in molecular mechanics conformational minima of *N*-acetylated hexapeptide mimics **1** and **2** indicates that the (5,6)-bicyclic lac-

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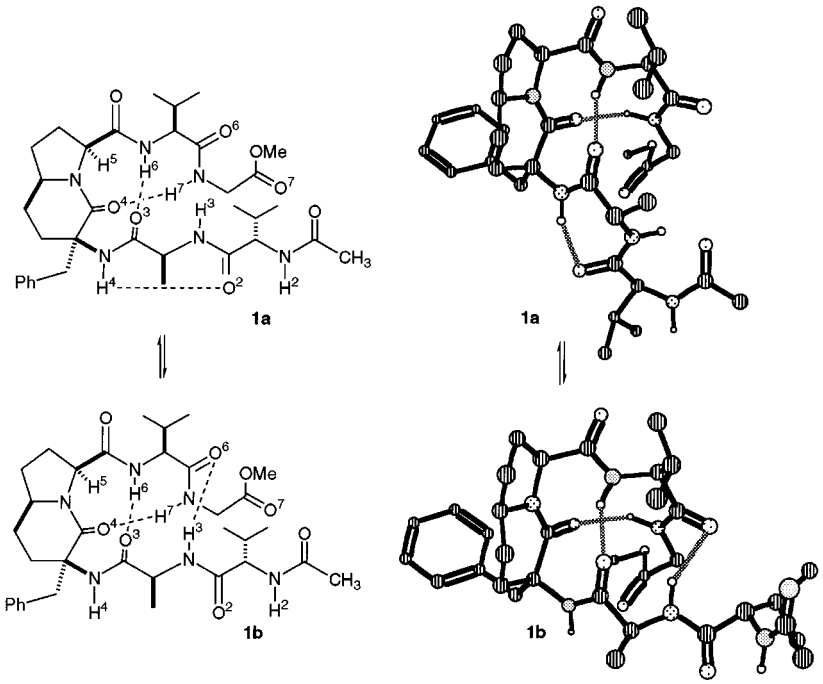

Figure 2. Preferred intramolecular hydrogen-bonding patterns proposed for the *N*-acetylated hexapeptide mimic **1** (left drawings; hydrogen bonds are indicated with dotted lines); lowest energy molecular mechanics conformers featuring the proposed hydrogen-bonding patterns (right drawings; for clarity all hydrogen atoms, except those attached to nitrogen, have been omitted)

Table 1. ^{1}H-NMR parameters for amide protons of *N*-acetylated hexapeptide mimics **1**–**3**

	N–H	Chem. shift [ppm][a]	^3J [Hz] NH–C_αH	$\Delta\delta$NH/ΔT [ppb/K][b]	$\Delta\delta$NH ($CDCl_3/CH_3OH$)[c]	N–H/N–D exchange rate with CD_3OD[d]
1	H^2	6.08	8.0	–2.1	0.38	instant.
	H^3	7.54	7.5	–8.0	0.12	instant.
	H^4	7.28	–	–2.7	0.11	10 min
	H^6	7.37	9.0	–1.5	0.09	60 min
	H^7	7.27	5.0	0.0	0.02	20 min
	H^8	–	–	–	–	–
2	H^2	6.38	6.5	–4.8	0.82	1 h
	H^3	7.73	7.0	–3.2	0.11	3 h
	H^4	7.36	–	–3.6	0.12	4 h
	H^6	7.20	5.5	–1.5	0.25	24 h
	H^7	7.60	6.3	0.0	–0.30	18 h
	H^8	7.77	6.3	–1.2	–0.22	17 h
3	H^2	6.17	7.8	–6.0	0.67	instant.
	H^3	6.54	7.8	–5.0	0.96	instant.
	H^4	7.37	6.8	–5.5	0.21	instant.
	H^6	7.27	7.8	–4.0	0.19	18 min
	H^7	6.58	5.8	–4.0	0.67	instant.
	H^8	–	–	–	–	–

[a] For all compounds described, NMR experiments show that the N–H proton chemical shifts are independent of concentration at 300 K at or below 2.0 mM, therefore all experiments were conducted using 1.0–2.0 mM $CDCl_3$ solutions. – [b] The temperature coefficients were determined with 1.0–2.0 mM $CDCl_3$ solutions between 240 and 300 K (where a linear dependence was observed for **1** and **2**) and between 260 and 300 K (where a linear dependence was observed for **3**). – [c] Change in chemical shift on changing the solvent from $CDCl_3$ to $CDCl_3/CH_3OH$ (4:1). – [d] N–H/N–D exchange rate for 2.0 mM solutions in $CDCl_3/CD_3OD$ (4:1).

tam effectively induces a β-turn conformation. In all the conformers within 3 kcal/mol of the global minimum of these peptide mimics, proton H^6 forms a 10-membered ring H-bond with $C{=}O^3$ of alanine within a type II′ β-turn.[17] In addition, the preferred intramolecular hydrogen-bonding pattern of methyl ester **1** is characterized by the presence of a second consecutive β-turn (10-membered ring H-bond between H^7 and the lactam $C{=}O^4$ within a type I β-turn). Proton H^3 may be involved in a 14-membered ring H-bond with $C{=}O^6$ while H^4 may form a seven-membered ring H-bond with $C{=}O^2$; the two possibilities are mutually exclusive. All the above results suggest that compound **1** has the folding pattern outlined in Figure 2. Long-range NOEs between CH^5 and the N–H proton H^7, and between Ala–CH_3 and the N–H proton H^6 were observed (NOESY studies). While the first interaction is diagnostic for the presence of

the H-bond between H^7 and $C{=}O^4$, the second one is compatible only with calculated structures of type **1a**.

In compound **2** the benzyl amide mimics the incorporation of one additional amino acid. The low temperature coefficients ($\Delta\delta NH/\Delta T$ = –1.5/0.0 [ppb/K]) of the amide protons H^6, H^7, and H^8 (δNH = 7.20/7.77), the small or even negative chemical-shift changes on addition of methanol ($\Delta\delta NH$ = 0.25/–0.30), and the very slow exchange rates with CD_3OD (17/24 h) indicate that these protons (Figure 3, Table 1) are locked in an intramolecularly H-bonded state. Protons H^3 and H^4 {δNH = 7.73/7.36, 3/4 h exchange rates with CD_3OD, relatively high temperature coefficients (–3.2/–3.6 [ppb/K])} are in equilibrium between a non-H-bonded and a H-bonded state, while proton H^2 (δNH = 6.38, $\Delta\delta NH/\Delta T$ = –4.8 [ppb/K], $\Delta\delta NH$ on addition of methanol = 0.82, 1 h exchange rate with CD_3OD) is clearly in a non-H-bonded state.[14]

For compound **3** [(5,7)-bicyclic lactam derivative] (Figure 4 , Table 1) the temperature coefficients were calculated considering only the 300–260 K temperature range where the temperature dependence of the chemical shifts proved to be linear; at lower temperatures significant intermolecular aggregation occurs. Protons H^6 and H^4 [δNH = 7.27/7.37, 18 min/instant. exchange rates with CD_3OD, high temperature coefficients (–4.0/–5.5 [ppb/K]), relatively small chemical shift changes on addition of methanol ($\Delta\delta NH$ = 0.19/0.21)] are in equilibrium between a non-H-bonded and a H-bonded state, while protons H^2, H^3, and H^7 (δNH = 6.17/6.58, $\Delta\delta NH/\Delta T$ = –4.0/–6.0 [ppb/K], $\Delta\delta NH$ on addition of methanol = 0.67/0.96, fast exchange rate with CD_3OD) are clearly in a non-H-bonded state.[14]

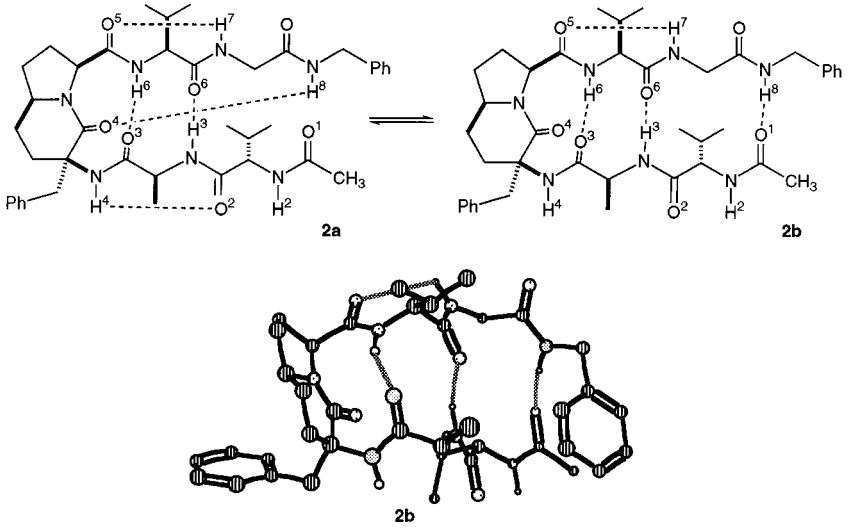

Figure 3. Preferred intramolecular hydrogen-bonding patterns proposed for the *N*-acetylated hexapeptide mimic **2** (top drawings; hydrogen bonds are indicated with dotted lines); lowest energy molecular mechanics conformer featuring the hydrogen-bonding pattern of type **2b** (bottom drawing; for clarity all hydrogen atoms, except those attached to nitrogen, have been omitted)

The slow N–H/N–D rates of exchange suggest that **2** adopts a remarkably stable intramolecularly hydrogen-bonded structure in chloroform. Disruption of this structure by methanol results in an *upfield shift* for amide protons H^7 and H^8 (from δ = 7.60 and 7.77 to 7.30 and 7.55, respectively), showing that the intramolecular hydrogen bonds involving those two amide protons are stronger than the intermolecular H-bonds with methanol. A highly preferred intramolecular H-bond pattern was identified among the calculated structures.[6–10] In addition to the 10-membered ring H-bond formed between H^6 and $C{=}O^3$ of alanine (type II′ β-turn), H^3 forms a 14-membered ring H-bond with $C{=}O^6$ of valine, H^8 a 13-membered ring H-bond with the lactam $C{=}O^4$, while H^7 and H^4 are involved in 7-membered ring H-bonds (inverse γ-turns)[18] with $C{=}O^5$ of proline and $C{=}O^2$ of valine, respectively (**2a**, Figure 3). Alternatively, H^8 may form a 22-membered ring H-bond with $C{=}O^1$ within a β-hairpin conformation (**2b**, Figure 3). A long range NOE between $CO–CH_3$ and the N–H proton H^8 was observed (NOESY studies), which is compatible only with calculated structures of type **2b**.

A comparison of the N–H stretch region IR data for (5,7)-bicyclic lactam derivative **3** and (5,6)-bicyclic lactam derivatives **1** and **2** (2.0 mM in $CHCl_3$) indicates that there is a greater extent of intramolecular hydrogen bonding in **1** and **2** than in **3**, which is consistent with a greater folding propensity of **1** and **2**. Although each hexapeptide mimic displays a complex spectrum in this region, the larger amount of C=O···H–N hydrogen bonding in **1** and **2** is readily apparent from the dominant band at 3328 cm^{-1} (weak in **3**, at 3320 cm^{-1}), compared to the weak band at 3425 cm^{-1} (dominant in **3**, at 3420 cm^{-1}).

High conformational flexibility and absence of strongly preferred intramolecular H-bond interactions emerge from the modeling studies of methyl ester **3**, in accordance with the ^{1}H-NMR temperature coefficients observed for all the N-H protons of this compound (Table 1) and with the IR data. Computational results suggest that the protons involved in equilibria between non-hydrogen bonded and hy-

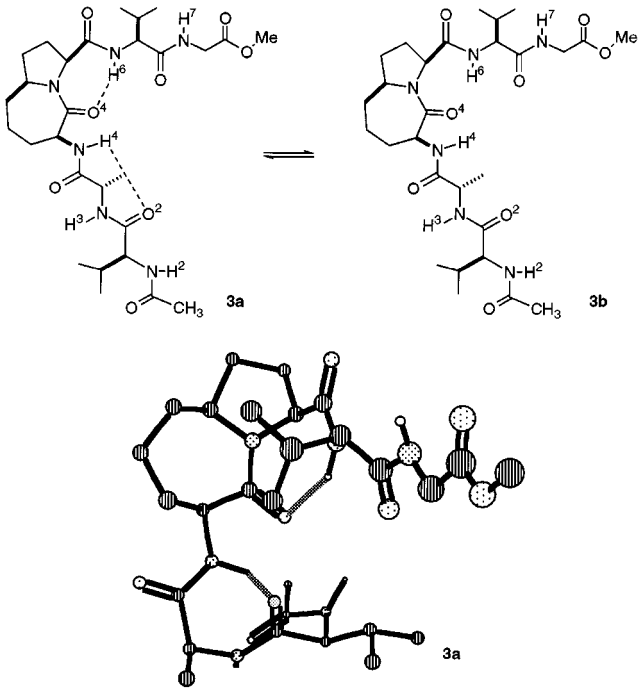

Figure 4. Preferred intramolecular hydrogen-bonding patterns proposed for the *N*-acetylated hexapeptide mimic **3** (top drawings; hydrogen bonds are indicated with dotted lines); lowest energy molecular mechanics conformer featuring the hydrogen-bonding pattern of type **3a** (bottom drawing; for clarity all hydrogen atoms, except those attached to nitrogen, have been omitted)

drogen bonded states are H^6 (7-membered ring H-bond with the lactam $C{=}O^4$ within an inverse γ-turn) and H^4 (7-membered ring H-bond with $C{=}O^2$ of valine) (Figure 4).[18]

For compound **4** [(5,7)-bicyclic lactam derivative], the temperature coefficients for all the N–H protons showed a nonlinear dependence in the interval 300–240 K, indicating a significant intermolecular aggregation. Compounds **3** and **4** are more prone to aggregation than are **1** and **2**: This difference provides indirect evidence that **1** and **2** are intramolecularly hydrogen bonded to a greater extent, and therefore less available for intermolecular hydrogen bonding than are **3** and **4**.[3o]

The main conclusion which can be drawn by the comparison of the folding patterns of constrained peptides **1–3** is that the conformational behavior can be efficiently controlled by the nature of the turn-inducing element, as for the smaller *N*-acetylated tetrapeptide mimics,[11] based on the different dihedral angles of the bicyclic scaffolds. The (5,6)-bicyclic lactam derivatives **1** and **2**, characterized by a type-II′ β-turn ($C{=}O^3\cdots H^6$–N), are very compact intramolecularly H-bonded structures (Figure 2 and Figure 3), while the (5,7)-bicyclic lactam derivative **3**, characterized by an inverse γ-turn ($C{=}O^4\cdots H^6$–N), is a quite flexible, ''tweezer-like'' structure (Figure 4).

Acknowledgments

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[1] [1a] D. S. Kemp, T. P. Curran, J. G. Boyd, T. J. Allen, *J. Org. Chem.* **1991**, *56*, 6683–6697. – [1b] W. M. Wolf, M. Stasiak, M. T. Leplawy, A. Bianco, F. Formaggio, M. Crisma, C. Toniolo, *J. Am. Chem. Soc.* **1998**, *120*, 11558–11566, and references therein.

[2] [2a] A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303–1326; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244–1267. – [2b] G. L. Olson, D. R. Bolin, M. P. Bonner, M. Bös, C. M. Cook, D. C. Fry, B. J. Graves, M. Hatada, D. E. Hill, M. Kahn, V. S. Madison, V. K. Rusiecky, R. Sarabu, J. Sepinwall, G. P. Vincent, M. E. Voss, *J. Med. Chem.* **1993**, *36*, 3039–3049. – [2c] R. M. J. Liskamp, *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1–19. – [2d] K. Burgess, K.-K. Ho, *J. Am. Chem. Soc.* **1994**, *116*, 799–800. – [2e] U. Slomczynska, D. K. Chalmers, F. Cornille, M. L. Smythe, D. D. Beusen, K. D. Moeller, G. R. Marshall, *J. Org. Chem.* **1996**, *61*, 1198–1204. – [2f] R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461–7472. – [2g] S. Hanessian, G. McNaughton-Smith, H.-G. Lombart, W. D. Lubell, *Tetrahedron* **1997**, *38*, 12789–12854. – [2h] Y. Kuroda, H. Ueda, H. Nozawa, H. Ogoshi, *Tetrahedron Lett.* **1997**, *38*, 7901–7904. – [2i] I. G. Jones, W. Jones, M. North, *J. Org. Chem.* **1998**, *63*, 1505–1513. – [2j] B. Schmidt, C. Kuhn, *Synlett* **1998**, 1240–1242.

[3] For references after 1996, see: [3a] E. M. Smith, D. L. Holmes, A. J. Shaka, J. S. Nowick, *J. Org. Chem.* **1997**, *62*, 7906–7907. – [3b] J. S. Nowick, M. Pairish, I. Q. Lee, D. L. Holmes, J. W. Ziller, *J. Am. Chem. Soc.* **1997**, *119*, 5413–5420. – [3c] D. L. Holmes, E. M. Smith, J. S. Nowick, *J. Am. Chem. Soc.* **1997**, *119*, 7655–7669. – [3d] K. Kyonghee, J. P. Germanas, *J. Org. Chem.* **1997**, *62*, 2847–2852. – [3e] K. Kyonghee, J. P. Germanas, *J. Org. Chem.* **1997**, *62*, 2853–2860. – [3f] C. Strässler, A. Linden, H. Heimgartner, *Helvetica Chim. Acta* **1997**, *80*, 1528–1551. – [3g] T. S. Haque, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 2303–2304. – [3h] S. Krauthäuser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719–11720. – [3i] T. Kayashi, T. Asai, H. Ogoshi, *Tetrahedron Lett.* **1997**, *38*, 3039–3042. – [3j] S. Hanessian, H. Yang, *Tetrahedron Lett.* **1997**, *38*, 3155–3158. – [3k] B. E. Fink, P. R. Kym, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344. – [3l] Y. Jun Chung, L. A. Christianson, H. E. Stanger, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 10555–10556. – [3m] D. Ranganathan, V. Haridas, S. Kurur, A. Thomas, K.P. Madhusudanam, R. Nagaraj, A. C. Kunwar, A. V. S. Sarma, I. L. Karle, *J. Am. Chem. Soc.* **1998**, *120*, 8448–8460. – [3n] M. J. Soth, J. S. Nowick, *J. Org. Chem.* **1999**, *64*, 276–281. – [3o] R. R. Gardner, G.-B. Liang, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 1806–1816.

[4] The synthesis of compounds **1–4** is reported in: C. Gennari, A. Mielgo, D. Potenza, C. Scolastico, U. Piarulli, L. Manzoni, *Eur. J. Org. Chem.* **1999**, 379–388.

[5] The conformational studies were carried out in a relatively nonpolar solvent (chloroform) which does not provide strong hydrogen bonding competition. $CDCl_3$ solutions of compounds **1–4** at concentrations of 0.5–20 mM were used for assessing intermolecular aggregation, while 1–2 mM solutions were employed for all conformational analyses. One-dimensional ^{1}H-NMR spectra for determining temperature coefficients were obtained at 240–300 K with increments of 10 K. Complete proton resonance assignments were made with the aid of COSY experiments.

[6] Structures **1–3** were subjected to an extensive, unconstrained Monte Carlo/Energy Minimization (MC/EM) conformational search (ref.[7]) within the framework of MacroModel (ref.[8]) version 5.5, using the MacroModel implementations of the AMBER all atom force field (ref.[9]) and the implicit chloroform GB/SA solvation model; ref.[10] The search protocol was identical to that employed in the study of shorter sequences incorporating reverse-turn mimetic bicyclic lactams; see ref.[11]

[7] G. Chang, W. C. Guida, W. C. Still, *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.

[8] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[9] S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comput. Chem.* **1986**, *7*, 230–252.
[10] W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
[11] L. Belvisi, C. Gennari, A. Mielgo, D. Potenza, C. Scolastico, *Eur. J. Org. Chem.* **1999**, 389–400.
[12] G. B. Liang, C. J. Rito, S. H. Gellman, *J. Am. Chem. Soc.* **1992**, *114*, 4440–4442.
[13] [13a] E. S. Stevens, N. Sugawara, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050. – [13b] S. H. Gellman, B. R. Adams, *Tetrahedron Lett.* **1989**, *30*, 3381–3384. – [13c] S. H. Gellman, G. P. Dado, G.-B. Liang, B. R. Adams, *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173.
[14] According to the analysis of the coupling constants for compounds **1–4** (Table 1), there appeared to be no correlation between the value of ^3J(NH–C_αH) and the other spectroscopic properties, with all values falling between 5.0 and 9.0 Hz (refs.[3b,3e]).
[15] According to the criteria set in ref.[11], it was assumed that a hydrogen bond is formed when the distance between the hydrogen of the donor and the acceptor is smaller than 2.5 Å (NH···O distance), the N–H···O bond angle is greater than 120°, and the H···C=O angle is greater than 90°.
[16] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Prot. Chem.* **1985**, *37*, 1–109.
[17] The type of β-turn is strictly correlated to the configuration of C-α carbons at position $i + 1$ and $i + 2$ (ref.[16]).
[18] It should be noted that calculations tend to overestimate the relative stability of γ-turn conformations relative to other turn types.

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