Conformational Preferences of Peptides Containing Reverse-Turn Mimetic Bicyclic Lactams: Inverse γ-Turns versus Type-II′ β-Turns – Insights into β-Hairpin Stability

Laura Belvisi,[a] Cesare Gennari,*[a,b] Antonia Mielgo,[a] Donatella Potenza,[a] and Carlo Scolastico*[a,b]

Keywords: Reverse-turn mimics / γ-Turns / β-Turns / β-Hairpins / NMR spectroscopy

The solid-phase synthesis and characterization of a series of peptides (**3**–**9**), containing reverse-turn mimetic bicyclic lactams (**1a**, **1b**), was reported in the preceding paper. The bicyclic lactams (**1a**, **1b**) possess high structural similarity to the two central residues of a β-turn. The conformational preferences of the constrained peptides have been investigated by NMR spectroscopy and IR spectroscopy. Our experimental results have been complemented by computer modelling studies and show that the constrained peptides (**3**–**9**) form an inverse γ-turn or a type-II′ β-turn through intramolecular hydrogen bonding, depending on the nature of the reverse-turn mimic. In *N*-acetylated tetrapeptide mimics incorporating the two different bicyclic lactams (**a** series and **b** series), H^5 is available for either a γ-turn (7-membered ring with the carbonyl group of the bicyclic lactam) or a β-turn (10-membered ring with the carbonyl group of residue 2), as shown in Figures 7 and 9. The **a** series incorporating the (5,7)-bicyclic lactam predominantly induces the γ-turn conformation, while the **b** series incorporating the (5,6)-bicyclic lactam can promote either a γ-turn or a β-turn conformation, with the β-turn usually being preferred and with varying degrees of β-hairpin formation.

Introduction

Reverse-turns are common motifs in protein structures and have often been postulated as recognition elements. A turn is defined as a site where the polypeptide chain reverses its overall direction. The terms γ- and β-turn have more restricted definitions and describe turns of three or four consecutive residues, respectively. These turns may or may not be stabilized by an intramolecular hydrogen bond. In γ-turns, the C=O of the first residue (*i*) may be hydrogen-bonded to the NH of the third residue (*i* + 2), giving rise to a seven-membered ring. In β-turns, the C=O of the first residue (*i*) may be hydrogen-bonded to the NH of the fourth residue (*i* + 3), forming a ten-membered ring (Figure 1). The terms "open" β- or γ-turns are used for situations where no intraturn hydrogen bond is present. The further classification into specific β-turn or γ-turn classes is based upon the geometry of the peptide backbone, as described by the φ and ψ backbone torsion angles in residues *i* + 1 and *i* + 2 (β-turn) or in residue *i* + 1 (γ-turn).[1]

β-Hairpins are also widely occurring secondary structural elements in proteins consisting of two adjacent strands of antiparallel β-sheet and a connecting loop. Cross-strand hydrogen bonding usually stabilizes β-hairpin structures. The shortest common loop involves two residues, in which case the loop and the two adjacent residues constitute a β-turn. A tetrapeptide can adopt a "minimal β-hairpin" conformation, defined by the presence of 10- and 14-membered ring N−H···O=C hydrogen bonds, as illustrated in Figure 1. However, simply enforcing a β-turn conformation across a dipeptide loop is not sufficient to induce β-hairpin folding. The majority of β-turns in folded proteins adopt type-I and type-II conformations, but these "common" turns are rare in two-residue β-hairpins. Turns that serve as two-residue β-hairpin loops tend to be of types I′ and II′, where the prime indicates that the φ and ψ torsion angles of the

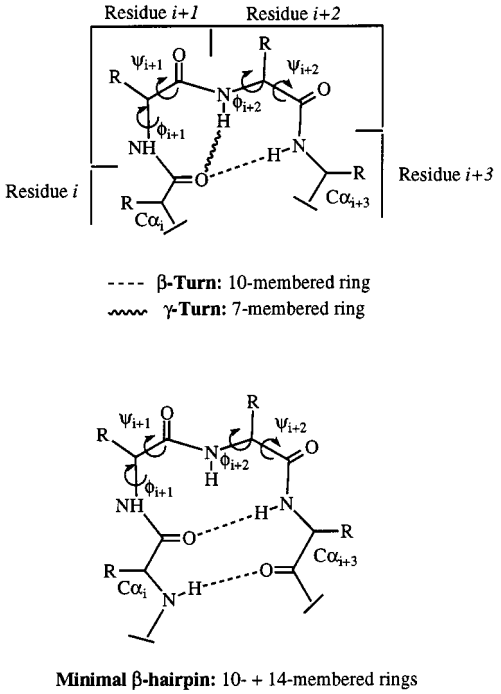


Figure 1. Structures of the γ-turn, β-turn, and minimal β-hairpin

[a] Dipartimento di Chimica Organica e Industriale, Universitá degli Studi di Milano,
[b] Centro CNR per lo Studio delle Sostanze Organiche Naturali, via G. Venezian 21, I-20133 Milano, Italy
Fax: (internat.) + 39-02/236-4369
E-mail: cesare@iumchx.chimorg.unimi.it

  1434−193X/99/0202−0389 $ 17.50+.50/0

central residues are opposite to those in the corresponding common turns.[2a] Sibanda and Thornton have rationalized the correlation between these turns and β-hairpins by noting that the natural twist of these rare turns (but not of the common type-I and-II turns) is compatible with the natural twist between two strands of antiparallel β-sheet made up from L-amino acids.[2b]

De novo design of synthetic protein structures requires the design and construction of stable mimics for the secondary structural elements. In this context, a number of initiators for α-helix formation,[3] conformationally constrained analogues of the various turns,[4] and β-sheet (or β-hairpin) nucleators have been reported.[5,6]

Reverse-turn mimics are generally cyclic or bicyclic compounds, which, by virtue of their covalent geometry, force a peptide chain to fold back upon itself.[4e] In the previous paper, we described the solid-phase synthesis of peptides **3**−**9** (Figure 3) incorporating the bicyclic lactams **1a** and **1b** (Figure 2).[7] These lactams can be viewed as X−Pro dipeptide mimics and might be used as synthetic replacements for the $i + 1$ and $i + 2$ elements of the four consecutive residues of β-turn motifs. The conformational preferences of the constrained peptides **3**−**9** thus offer the opportunity of investigating in detail the secondary structures (γ- vs. β-turns), and in particular the propensity to adopt an antiparallel β-sheet conformation (β-hairpin). We report herein on a conformational analysis of these peptides in chloroform solution, which has been carried out by a combination of ^{1}H-NMR spectroscopy, IR spectroscopy, and computer modelling. In these constrained peptides, intramolecular hydrogen bonding provides the principal driving force for β-turn and β-hairpin formation. For this reason, the conformational studies were carried out in a relatively non-polar solvent (chloroform), which does not provide strong hydrogen-bonding competition and mimics the hydrophobic core of a protein.[8] The influences of the bicyclic lactam, of the amino acid sequence, and of the type of carboxy terminal group on the preferred folding pattern are discussed.

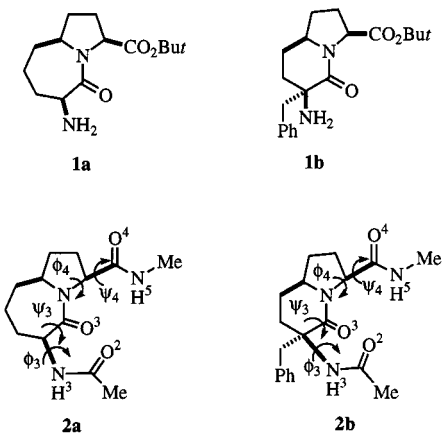
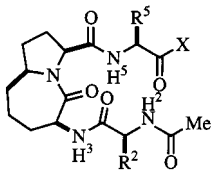
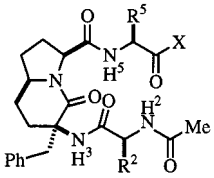


Figure 2. Reverse-turn mimetic bicyclic lactams **1a** and **1b** and *N*-acetyl-*N*′-methylamide dipeptide mimics **2a** and **2b**



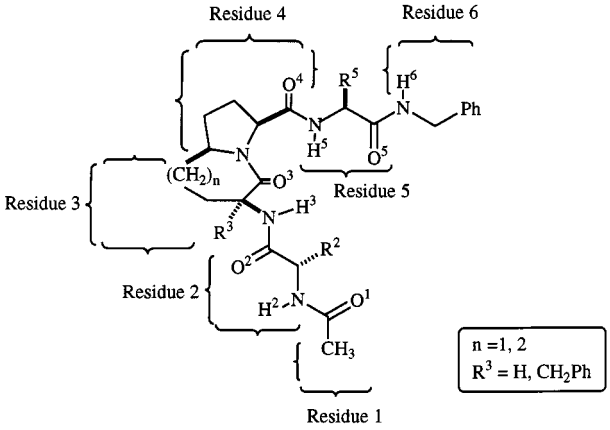
3a X = OMe, R^5 = H, R^2 = CH_2Ph
4a X = OMe, R^5 = *i*Pr, R^2 = CH_2Ph
5a X = OMe, R^5 = CH_2Ph, R^2 = H
6a X = OMe, R^5 = *i*Pr, R^2 = H
7a X = OMe, R^5 = *i*Pr, R^2 = Me
8a X = NH^6Bn, R^5 = *i*Pr, R^2 = CH_2Ph



3b X = OMe, R^5 = H, R^2 = CH_2Ph
7b X = OMe, R^5 = *i*Pr, R^2 = Me
9b X = NH^6Bn, R^5 = *i*Pr, R^2 = Me

Figure 3. *N*-Acetylated tetrapeptide mimics **3**−**9**

For ease of discussion of the preferred conformations of the peptide mimics (**3**−**9**), the residues were numbered starting from the *N*-terminal position in the longer peptide derivatives (**8a**, **9b**) (Figure 4). In this way, the acetyl group mimics the carbonyl group of the first residue (residue 1) and the benzyl amide group mimics the amino group of the last residue (residue 6). The bicyclic lactams mimic two amino acids (residues 3 and 4). The number adopted for each residue was also assigned to the respective amide NH proton, carbonyl group, Cα carbon atom and R substituent.



8a n = 2, R^2 = Bn, R^3 = H, R^5 = *i*Pr
9b n = 1, R^2 = Me, R^3 = Bn, R^5 = *i*Pr

Figure 4. Numbering system of the peptide mimics

Molecular Modelling of the Reverse-Turn Mimetic Core

Molecular modelling studies on the dipeptide mimics were undertaken to investigate the ability of the designed bicyclic scaffolds to adopt reverse-turn conformations. Computational studies were performed on the *N*-acetyl-*N′*-methylamide dipeptide analogues **2a** and **2b** (Figure 2). Each structure was subjected to an extensive, unconstrained Monte Carlo/Energy Minimization (MC/EM) conformational search by molecular mechanics methods in chloroform as implicitly represented by a solvation model (see Computational Methods, Experimental Section). The turn propensity of the minimum energy conformations was assessed by computing and analyzing different geometrical parameters: the $C\alpha_i-C\alpha_{i+3}$ distance, the ϕ and ψ backbone torsion angles in residues $i + 1$ and $i + 2$ (residues 3 and 4 for compounds **2a** and **2b**), the virtual torsion angle β, and parameters indicative of hydrogen bonding (distance between the carbonyl oxygen and the amide hydrogen atom, and directional requirements).

A β-turn has usually been defined as any tetrapeptide sequence occurring in a non-helical region, in which the $C\alpha_i-C\alpha_{i+3}$ distance is less than or equal to 7 Å.[1] This criterion is often used to define the presence of a reverse-turn.

The ϕ and ψ backbone torsion angles in residues $i + 1$ and $i + 2$ (β-turn) or in residue $i + 1$ (γ-turn) represent the basis for classification into specific β-turn and γ-turn types. Accordingly, assignment of a low-energy conformation to a particular turn type was made, where possible, on the basis of the ideal ϕ and ψ torsion angles (± 30°) reported by Rose et al.[1]

The virtual torsion angle β ($C\alpha_i-C\alpha_{i+1}-C\alpha_{i+2}-N_{i+3}$) is another parameter that has been defined based on topographical analysis of β-turns. This angle provides a complete description of the spatial relationship between the entry and exit peptide bonds, as well as the relative orientations of the intervening side-chains, for any β-turn. This description should facilitate the development and application of novel conformationally constrained peptide mimetic compounds, for which a classification based on peptide backbone geometry may be entirely irrelevant. The virtual torsion angle β has been found to vary from approximately −100° through 0° to +100°, with a general preference for small positive values, for a database of 146 β-turns extracted from the literature.[9a] The range 0 ± 30° is usually taken to indicate a tight reverse-turn.[4d,9b]

With regard to the intramolecular hydrogen-bond parameters, stringent criteria were set, assuming that a hydrogen bond is formed only when the distance between acceptor and donor is less than 2.5 Å, the N−H···O bond angle is larger than 120°, and the H···O=C angle is larger than 90°. These values are taken to indicate significant interactions between the donor and acceptor groups.

The principal types of backbone geometries calculated for compounds **2a** and **2b** are given in Tables 1 and 2. A summary of the reverse-turn mimetic properties exhibited by the calculated structures of compounds **2a** and **2b** is given in Table 3. The turn propensity was quantitatively assessed by computing the percentage values of the previously defined parameters. The numbering system described above and depicted in Figures 2 and 4 was adopted for these dipeptide mimics.

Table 1. Characteristics of low-energy conformers (MC/EM, AMBER*, $CHCl_3$ GB/SA) calculated for dipeptide mimic **2a**[a]

Conf.	ΔE	ϕ_3	ψ_3	ϕ_4	ψ_4	β[b]	$C\alpha^2-C\alpha^5$ distance	NH^5-O^3 dist. (7-membd. ring)	NH^5-O^2 dist. (10-membd. ring)	NH^3-O^4 dist. (8-membd. ring)
inverse γ-turn	–	–	–	−70/−85	60/70	–	–	–	–	–
1	0.00	−166.3	174.1	−86.3	63.0	−78.1	8.11	1.88	6.46	6.41
3	1.94	−166.5	171.4	−44.6	119.8	−41.9	8.12	3.17	7.90	5.00
4	2.57	−167.7	−179.4	−58.0	−24.9	−81.6	7.67	2.88	6.15	5.71

[a] The relative energies are in kcal/mol, the torsion angles in°, and the distances in Å. – [b] The virtual torsion angle is defined by C^2, $C\alpha^3$, $C\alpha^4$, and N^5.

Table 2. Characteristics of low-energy conformers (MC/EM, AMBER*, $CHCl_3$ GB/SA) calculated for dipeptide mimic **2b**[a]

Conf.	ΔE	ϕ_3	ψ_3	ϕ_4	ψ_4	β[b]	$C\alpha^2-C\alpha^5$ distance	NH^5-O^3 dist (7-membd. ring)	NH^5-O^2 dist (10-membd. ring)	NH^3-O^4 dist (8-membd. ring)
inverse γ-turn	–	–	–	−70/−85	60/70	–	–	–	–	–
type-II′ β-turn	–	60	−120	−80	0	–	–	–	–	–
1	0.00	44.7	−123.4	−64.8	−15.9	3.0	4.90	2.84	1.86	6.98
2	0.34	173.6	−99.5	−47.1	152.7	44.7	5.91	3.90	7.86	2.04
3	0.98	44.5	−130.2	−63.2	65.5	24.4	4.75	1.78	3.10	6.69
4	1.91	174.9	−161.3	−62.4	68.9	−5.5	6.79	1.80	6.24	5.72

[a] The relative energies are in kcal/mol, the torsion angles in°, and the distances in Å. – [b] The virtual torsion angle is defined by C^2, $C\alpha^3$, $C\alpha^4$, and N^5.

Table 3. Quantitative characterization of the reverse-turn-forming ability of the conformers (MC/EM, AMBER*, $CHCl_3$ GB/SA) calculated for **2a** and **2b**[a]

Compd.	no. of conf. < 6 kcal/mol	% *d* $C\alpha^2-C\alpha^5$ < 7 Å	% β < 30°	% β < 60°	% H-bond NH5···O3 (7-membd. ring)	% H-bond NH5···O2 (10-membd. ring)	% H-bond NH3···O4 (8-membd. ring)
2a	15	0	0	60	53	0	0
2b	37	84	60	95	32	19	22

[a] % *d* ($C\alpha^2-C\alpha^5$) is the percentage of all conformers for which the distance between $C\alpha^2$ and $C\alpha^5$ is < 7 Å;% β is the percentage of all conformers in which the virtual torsion angle β (absolute value) is < 30° (or 60°);% H bond is the percentage of all conformers in which H···O distance < 2.5 Å, N−H···O bond angle > 120°, and H···O=C angle > 90°.

On the basis of their backbone torsion angles, none of the conformations (within 6 kcal/mol of the lowest energy conformer) found for compound **2a** could be assigned to standard β-turn types (Table 1). The lowest energy conformer could be considered as featuring an inverse γ-turn centered at the Pro residue of the bicyclic lactam. In this conformer, the value of the NH^5-O^3 distance, as well as the directional parameters (not shown), lend support for the intramolecular hydrogen-bond characteristics of a γ-turn, which forms a seven-membered ring.

A similar analysis of compound **2b** shows that the lowest energy conformer (Table 2) accurately mimics a type-II′ β-turn, the second lowest energy conformer does not mimic a known turn type, the third minimum mimics an inverse γ-turn/distorted type-II′ β-turn, and the fourth features an inverse γ-turn. The distances between the amide hydrogen and the carbonyl oxygen atoms, presented in Table 2, and the directional parameters (not shown), indicate significant interactions and appropriate hydrogen-bonds characteristic of the turn types assigned as above.

It is worth noting that conformers fulfilling the geometric requirements for the formation of seven- or ten-membered rings possess φ and ψ backbone torsion angles within 30° of the γ- or β-turn ideal values (see, for example, the values reported in Tables 1 and 2). The relationship also holds in the opposite sense: Whenever torsion angles in calculated structures deviate less than 30° from the ideal values, optimal hydrogen-bond parameters are observed.

The results of the computational studies on the dipeptide mimics reveal that the (5,6)-fused lactam of type **1b** is a better turn inducer than the (5,7)-bicyclic scaffold **1a**. The (5,6)-system should be able to induce either a type-II′ β-turn or an inverse γ-turn conformation, while the (5,7)-bicyclic skeleton should be able to constrain the peptide sequence only in an inverse γ-turn geometry.

In order to elucidate the consequences of incorporating the bicyclic lactams into peptides, conformational analyses on tetrapeptide analogues of these systems (compounds **3**−**9**) were performed and their turn-inducing potential was investigated. Analysis of intramolecular hydrogen-bonding patterns in these peptide mimics was also undertaken to gain insight into what extent the designed peptidomimetic scaffolds fulfill the geometrical requirements set out for a β-sheet nucleator. Computational studies using Monte Carlo conformational searches in an implicit chloroform solvation model were used to complement the ^{1}H-NMR data and were helpful with regard to the interpretation of the experimental results (vide infra).

Conformational Preferences of the Constrained Peptides: An Overview

The ^{1}H-NMR chemical shifts of the amide protons, the amide proton−deuterium exchange rates, the Δδ(NH) upon addition of a solvent able to compete for the formation of hydrogen bonds, the temperature dependence of the amide proton chemical shifts [Δδ(NH)/Δ*T*], and IR spectroscopy have been used to ascertain which amide protons are involved in intramolecular hydrogen bonds. Since the ^{1}H-NMR and IR data of the amide hydrogen atoms are very sensitive to temperature and concentration, the data reported herein were all obtained from samples at 300 K and at concentrations of 1−2 mM,[10] at which aggregation is not significant.

Insights into hydrogen bonding can be gained from the chemical shifts of the amide NHs: Signals of protons that are hydrogen-bonded, either intramolecularly or to the solvent, appear downfield of those that are not hydrogen-bonded. In solvents that do not form strong hydrogen bonds (e.g. $CDCl_3$), significantly deshielded amide protons often indicate that they are involved in hydrogen bonding.[11]

Amide protons that are involved in hydrogen bonding within a secondary or tertiary structural element (α-helix, β-sheet, reverse-turns) exchange much more slowly with the solvent than those exposed to the bulk solvent. Amide proton−deuterium exchange rates permit the identification of amide protons that are involved in intramolecular hydrogen bonding. The Δδ(NH) upon addition of a solvent able to compete for the formation of H bonds (MeOH, DMSO) can also provide information regarding the possible participation of an amide proton in a stable intramolecular H bond.

Equilibration between hydrogen-bonded and non-hydrogen-bonded states for a given amide proton is almost always fast on the NMR timescale, and thus observed amide proton chemical shifts are weighted averages of the chemical shifts of the contributing states. IR spectroscopy is, in principle, more useful for detecting amide hydrogen-bonding equilibria, as the much shorter timescale of IR measurements permits the observation of distinct N−H stretching

absorptions for equilibrating hydrogen-bonded and non-hydrogen-bonded states.[12]

The temperature dependence of the ^{1}H-NMR chemical shifts of amide protons also reflects their hydrogen-bonding state. In $CDCl_3$, different ways of interpreting these data have been proposed. According to some authors, peptide NH protons that are either non-hydrogen-bonded or locked in a hydrogen-bonded conformation exhibit a small temperature dependence [$\Delta\delta(NH)/\Delta T$], while protons that participate in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state exhibit a large temperature dependence. Temperature dependence is typically 0 to −3 ppb/K in the former case and −4 to −8 ppb/K in the latter.[10,13,14] However, other authors consider that NH protons with a temperature coefficient [$\Delta\delta(NH)/\Delta T$] smaller in absolute value than −2.6 ppb/K are intramolecularly hydrogen-bonded, irrespective of the chemical shift value or of other parameters, while protons with a temperature coefficient larger in absolute value than −2.6 ppb/K are non-H-bonded.[15]

In our opinion, a more correct overview of the hydrogen-bonded state of an amide proton can be obtained considering all its ^{1}H-NMR parameters [chemical shift, temperature coefficient, proton−deuterium exchange rates, and $\Delta\delta(NH)$ upon addition of a competitive solvent]. Accordingly, three different types of amide protons can be considered when studying the conformational preferences of small peptides in chloroform solution: (a) Strongly hydrogen-bonded amide protons: protons with a low temperature coefficient (−2.6 ppb/K or smaller in absolute value), a high chemical shift value (usually $\delta \geq 7.0$) in comparison with corresponding protons in reference compounds, and a small $\Delta\delta(NH)$ upon addition of a competitive solvent ($\Delta\delta \leq 0.2$ ppm in absolute value); (b) non-hydrogen-bonded amide protons: protons with a low temperature coefficient (−2.6 ppb/K or smaller in absolute value), a low chemical shift value (usually $\delta \leq 7.0$) in comparison with corresponding protons in reference compounds, and a high $\Delta\delta(NH)$ upon addition of a competitive solvent ($\Delta\delta \geq 0.2$ ppm in absolute value); (c) amide protons in equilibrium between hydrogen-bonded and non-hydrogen-bonded states: because of the slow timescale of the NMR experiments (milliseconds to seconds) relative to the timescale of peptide conformational interconversions, the NMR data can reflect a conformational equilibrium pattern. This would be the case for protons with a large temperature dependence (larger than −2.6 or −3 ppb/K in absolute value).

For the following discussion, the ^{1}H-NMR spectral resonances of the peptide mimics were assigned by a combination of decoupling and NOE studies. In particular, the NH resonances were assigned to H^2−H^6 on the basis of the distinct coupling patterns of the α-protons (α-protons of glycine, alanine, valine, and phenylalanine exhibit an ABX pattern, quintuplet, triplet or doublet of doublets, and a ddd system, respectively).

Conformational Preferences of the Constrained Peptides: *N*-Acetylated Tetrapeptide Mimics (Methyl Esters)

Initially, a series of compounds consisting of two amino acid groups attached to the (5,7)- and (5,6)-bicyclic lactams was studied (compounds **3a**, **4a**, **5a**, **6a**, **7a**, **3b**, and **7b**, Figure 3). These tetrapeptide mimics contain glycine, alanine, valine and phenylalanine residues in the top and bottom halves.

Bicyclic lactams **10a** and **12a**, which have only one peptidic NH, and dipeptide mimic **14** (Figure 5) were used as reference compounds for the (5,7)-bicyclic lactam derivatives (**3a**, **4a**, **5a**, **6a**, and **7a**). The ^{1}H-NMR parameters for the amide protons of the *N*-acetylated tetrapeptide mimics methyl esters **3a**−**7a**, as well as of the reference compounds **10a** and **12a**, are collected in Table 4.

In $CDCl_3$ solution, the proton H^a of dipeptide **14** exhibits a chemical shift of $\delta = 6.06$ within the concentration-independent limit. For compound **10a**, the NH^3 signal appears at $\delta = 6.18$ (Table 4). These chemical shifts are characteristic of peptide backbone protons that are not involved in hydrogen bonding. In contrast, the chemical shift of H^5 in the model compound **12a** ($\delta = 7.62$) indicates a high degree of intramolecular hydrogen bonding [$\Delta\delta(NH)/\Delta T = -1.0$]. This implies that compound **12a** has a high tendency to adopt a γ-turn conformation (seven-membered ring hydrogen bond) across the proline residue.

For the *N*-acetylated tetrapeptide mimics (methyl esters) **3a**, **4a**, **5a**, **6a**, and **7a**, the ^{1}H-NMR resonances of amide H^5 and H^3 appear downfield (chemical shifts between $\delta =$

Table 4. ^{1}H-NMR parameters in $CDCl_3$ of amide hydrogen atoms of *N*-acetylated tetrapeptide mimics (methyl esters) **3a**−**7a** and of reference compounds **10a** and **12a**[a,b]

Compd.	δ H^2	δ H^3	δ H^5	$\Delta\delta(NH)/\Delta T$ H^2	$\Delta\delta(NH)/\Delta T$ H^3	$\Delta\delta(NH)/\Delta T$ H^5
3a	5.90	7.20	7.20	−	−	−
4a	5.97	7.27	7.33	−4.6	[c]	−3.6
5a	6.14	7.08	7.14	−3.2	−2.7	−1.6
6a	6.13	7.17	7.32	−3.2	−2.8	−2.5
7a	6.15	7.21	7.36	−5.3	−4.3	−2.8
10a	−	6.18	−	−	−	−
12a	−	−	7.62	−	−	−1.0

[a] For all compounds described, NMR experiments showed the NH proton chemical shifts to be independent of concentration at 300 K at or below 2.0 mM, and therefore all experiments were conducted using 1.0−2.0 mM $CDCl_3$ solutions. − [b] All the temperature coefficients were determined with 1.0−2.0 mM $CDCl_3$ solutions between 240 and 300 K. − [c] Not determined due to overlap with other resonances.

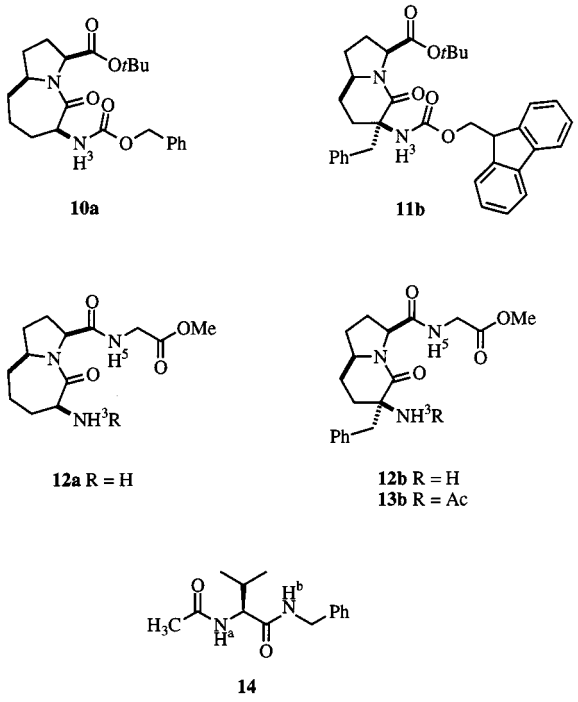


Figure 5. Reference compounds for NMR experiments

7.14 and δ = 7.36 for H^5 and between δ = 7.08 and δ = 7.27 for H^3) compared with the resonances of the corresponding reference compounds [H^5 signal shifted downfield by 1.08−1.30 ppm relative to that of the corresponding N−H^a in **14** (δ = 6.06), and H^3 by 0.90−1.09 ppm relative to that of the corresponding N−H^3 in **10a**]. However, it is worth noting that resonances of amide H^5 for the *N*-acetylated tetrapeptide mimics (methyl esters) appear slightly upfield compared to the resonance of H^5 in reference compound **12a**. The Δδ(NH)/Δ*T* values fall between −2.7 and −4.3 ppb/K for H^3 and between −1.6 and −3.6 ppb/K for H^5. All these data suggest that H^5 can be classified as an intramolecularly hydrogen-bonded proton, while H^3 is probably involved in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state.

In contrast, H^2 exhibits a chemical shift between δ = 5.90 and δ = 6.15, and has a temperature coefficient [Δδ(NH)/Δ*T*] between −3.2 and −5.3 ppb/K. These data suggest that this proton is also involved in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state, with the latter being favoured.

Figure 6 shows the temperature dependence of the chemical shifts of the amide protons in compounds **5a** and **6a** in $CDCl_3$ (all samples 2.0 mM).

In determining the preferred intramolecular hydrogen-bonding pattern of these derivatives, the behaviour of H^3 is particularly revealing. In fact, this proton can participate in a hydrogen bond (11-membered-ring hydrogen bond) only if H^5 experiences a γ-turn and not a β-turn.

In the infrared spectra of compounds **3a**, **4a**, and **5a,** the hydrogen-bonded NH stretching vibrations appear as broad bands at 3379 and 3288 cm^{-1}, while the non-hydrogen-bonded NH stretching vibrations appear as a narrower band at 3420 cm^{-1}. The former bands suggest the presence of two different hydrogen-bonded NH′s, one to an amide

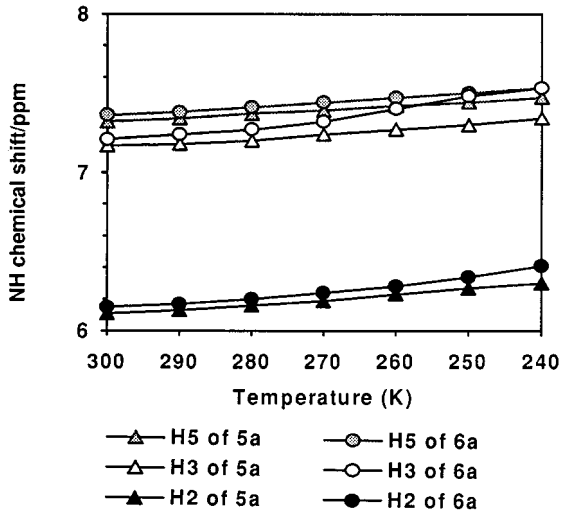


Figure 6. NH proton NMR chemical shifts for tetrapeptide methyl esters **5a** and **6a** as a function of temperature; data were obtained for 2.0 mM $CDCl_3$ solutions

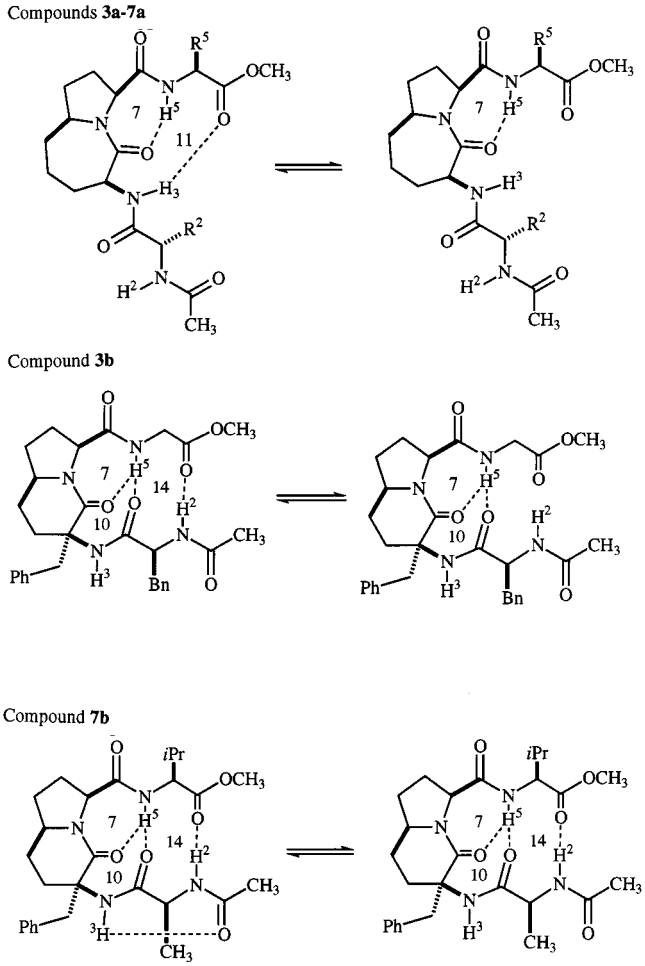


Figure 7. Preferred intramolecular hydrogen-bonding patterns proposed for the *N*-acetylated tetrapeptide mimics (methyl esters) on the basis of the spectroscopic data

carbonyl group (3288 cm^{-1}) and the other to an ester carbonyl group (3379 cm^{-1}). Only protons H^2 and H^3 can be involved in a hydrogen bond to the ester carbonyl group ($C=O^5$). As the ^{1}H-NMR data suggest that H^2 is non-hydrogen-bonded, the IR data can only be explained by assuming an equilibrium involving an 11-membered ring hydrogen bond between H^3 and the ester carbonyl group ($C=O^5$). Consequently, with H^3 involved in this hydrogen bond, H^5 cannot form a β-turn and is intramolecularly hydrogen-bonded to the carbonyl group of the bicyclic lactam ($C=O^3$), giving rise to a 7-membered ring hydrogen bond (γ-turn), as shown in the upper part of Figure 7.

The magnitude of the hydrogen-bonded NH band in the IR spectrum also reflects the position of the conformational equilibrium and provides independent corroboration of the NMR data. Comparison of compounds **3a**, **4a**, **5a**, **6a**, and **7a** is particularly interesting as it allows investigation of the influence of the various amino acids on the propensity for intramolecular hydrogen-bond formation. Generally speaking, the NH parameters are only slightly sensitive to the nature of the amino acids in the flanked chains and the folding pattern seems to be dictated only by the bicyclic lactam.

In order to study the tetrapeptide mimics incorporating the (5,6)-bicyclic lactam (**b** series), compounds **3b** and **7b** (*N*-acetylated, methyl esters) were investigated (Figure 3, Table 5).

For this series, compounds **11b**, **12b**, and **13b** were taken as reference controls (Figure 5). In compound **11b**, the NH^3 signal appears at δ = 6.15. This chemical shift is characteristic of a peptide backbone proton that is not involved in hydrogen bonding. In $CDCl_3$ solution, the model compound **12b** can adopt a γ-turn conformation (seven-membered-ring hydrogen bond across the proline residue); in fact the chemical shift of H^5 (δ = 7.18) and the $\Delta\delta(NH)/\Delta T$ value (0.0 ppb/K) indicate a high degree of intramolecular hydrogen bonding, although the H^5 signal is shifted upfield with respect to that of the (5,7)-analogue **12a** (δ = 7.62). In order to rationalize the tendency to adopt γ- or β-turn conformations in these derivatives, compound **13b** was studied. In **13b**, the resonance of H^5 appears at δ = 7.58 [$\Delta\delta(NH)/\Delta T$ = −3.3 ppb/K] while that of H^3 appears at δ = 6.23 with $\Delta\delta(NH)/\Delta T$ = −1.6 ppb/K. The latter figures are characteristic of a peptide backbone proton that is not involved in hydrogen bonding, while the former data indicate that H^5 can equilibrate between two hydrogen-bonded conformations (γ- and β-turn across the proline residue).

Compound **3b**, with glycine and phenylalanine residues in the the top and bottom halves of the tetrapeptide, respectively, gave the following ^{1}H-NMR parameters for H^5: δ = 7.71 (shifted downfield by 0.53 ppm relative to the corresponding H^5 in **12b**) and $\Delta\delta(NH)/\Delta T$ = −2.2 ppb/K. The addition of [D_6]DMSO (500 equiv.) to a $CDCl_3$ solution resulted in a slight downfield shift (Δδ = 0.03 ppm). These observations are consistent with the participation of this proton in a stable intramolecular H bond. On the other hand, the H^2 signal appears at δ = 6.42 (compared with δ = 5.90−6.15 for the **a** series), the $\Delta\delta(NH)/\Delta T$ value is −6.4 ppb/K, and the δ(NH) (addition of [D_6]DMSO to $CDCl_3$) is 0.2 ppm. These data suggest that H^2 is probably involved in an equilibrium between hydrogen-bonded and non-hydrogen-bonded states.

The H^3 signal appears at δ = 6.75 (compared with δ = 6.15 for control **11b**) and is shifted upfield by 0.33−0.52 ppm relative to that of the corresponding H^3 in the **a** series. The $\Delta\delta(NH)/\Delta T$ coefficient is −1.4 ppb/K and the δ(NH) (addition of [D_6]DMSO to $CDCl_3$) is 0.3 ppm. These data are consistent with a non-hydrogen-bonding situation.

Figure 8 shows the temperature dependence of the chemical shifts of the amide protons in compounds **3b** and **7b** in $CDCl_3$ (all samples 2.0 mM).

The IR spectrum of a 2.0 mM sample of compound **3b** in $CHCl_3$ at room temperature shows two absorptions of similar intensity, at 3300 and 3361 cm^{-1}. These two frequencies suggest the presence of two different hydrogen-bonded NH′s, one to an amide carbonyl group (3300 cm^{-1}) and the other to an ester carbonyl group (3361 cm^{-1}). The non-hydrogen-bonded NH stretching vibrations appear at 3421 cm^{-1}.

All the above data indicate that compound **3b** undergoes some β-hairpin formation: H^5 forms a strong hydrogen bond with either the $C=O^2$ of phenylalanine (10-membered ring) or the $C=O^3$ of the bicyclic lactam (7-membered ring), while H^2 is involved in a hydrogen-bonding equilibrium with $C=O^5$ of glycine (14-membered ring) (Figure 7). The simultaneous interaction of H^5 with both $C=O^2$ and $C=O^3$ is unlikely, as three-center H bonds are known to be energetically less favourable than two-center H bonds.[13d]

The situation for **7b** (R^5 = *i*Pr, R^2 = Me) is different, and shows that in the **b** series there is a distinct dependence

Table 5. ^{1}H-NMR parameters in $CDCl_3$ of amide hydrogen atoms of *N*-acetylated tetrapeptide mimics (methyl esters) **3b**, **7b**, and of reference compounds **11b**, **12b**, **13b**[a,b]

Compd.	δ H^2	δ H^3	δ H^5	$\Delta\delta(NH)/\Delta T$ H^2	$\Delta\delta(NH)/\Delta T$ H^3	$\Delta\delta(NH)/\Delta T$ H^5
3b	6.42	6.75	7.71	−6.4	−1.4	−2.2
7b	7.11	7.11	7.18	−1.1	−6.8	−2.6
11b	−	6.15	−	−	−	−
12b	−	−	7.18	−	−	0.0
13b	−	6.23	7.58	−	−1.6	−3.3

[a] For all compounds described, NMR experiments showed the NH proton chemical shifts to be independent of concentration at 300 K at or below 2.0 mM, and therefore all experiments were conducted using 1.0−2.0 mM $CDCl_3$ solutions. − [b] All the temperature coefficients were determined with 1.0−2.0 mM $CDCl_3$ solutions between 240 and 300 K.

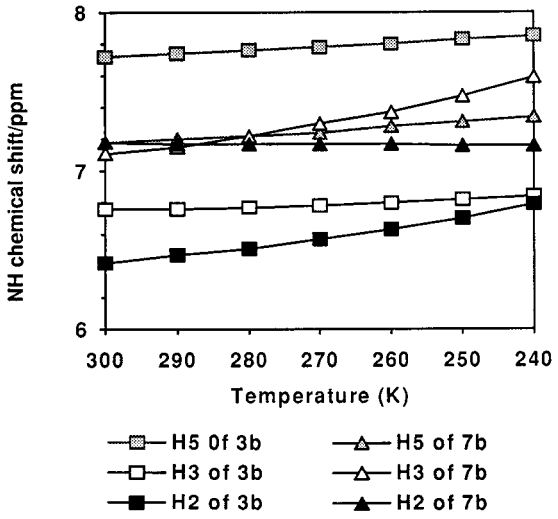


Figure 8. NH proton NMR chemical shifts for tetrapeptides **3b** and **7b** as a function of temperature; data were obtained for 2.0 mM $CDCl_3$ solutions

of the conformational preferences on the nature of the amino acids in the flanked chains. The chemical shift values of H^2, H^3, H^5 (shown in Table 5) are similar, but the temperature coefficients [$\Delta\delta(NH)/\Delta T$] reveal markedly lower temperature dependences for protons H^2 and H^5, which are likely to be intramolecularly hydrogen-bonded. The remaining proton H^3 shows a relatively large temperature dependence (−6.8 ppb/K), suggesting that it is an amide proton in equilibrium between hydrogen-bonded and non-hydrogen-bonded states.

A possible interpretation of these data is that compound **7b** undergoes considerable β-hairpin formation, forming intramolecular hydrogen bonds with both H^2 and H^5 (10- and 14-membered ring or 7- and 14-membered ring hydrogen bonds), while H^3 is in equilibrium between a bonded (7-membered ring hydrogen bond) and a non-bonded state, as shown in the lower part of Figure 7.

In *N*-acetylated tetrapeptide mimics (methyl esters) incorporating a bicyclic lactam (**a** series and **b** series), H^5 is available for either a γ-turn (7-membered ring with the carbonyl group of the bicyclic lactam) or a β-turn (10-membered ring with the carbonyl group of residue 2), as shown in Figure 7. All the above data indicate that the **a** series incorporating the (5,7)-bicyclic lactam predominantly induces the γ-turn conformation, while the **b** series incorporating the (5,6)-bicyclic lactam can promote either a γ-turn or a β-turn conformation, with varying degrees of β-hairpin formation (14-membered ring involving N−H^2 and C=O^5). CD_3OD addition experiments showed a very fast H−D exchange for all the NH′s of the tetrapeptide mimics of both the **a** and **b** series, indicating that in these small peptide mimics even intramolecularly hydrogen-bonded protons are rapidly accessible to the solvent.

Conformational Preferences of the Constrained Peptides: *N*-Acetylated Tetrapeptide Mimics (Benzyl Amides)

The behaviour of *N*-acetylated tetrapeptide mimics (benzyl amides) (**a** and **b** series) reflects the trends seen with the corresponding methyl esters. In particular, we studied tetrapeptide mimics **8a** (R^5 = *i*Pr, R^2 = Bn) and **9b** (R^5 = *i*Pr, R^2 = Me) (Figure 3, Table 6).

The ^{1}H-NMR spectrum of **8a** ($CDCl_3$ solution) exhibits chemical shifts of δ = 5.94 and δ = 6.34 for H^2 and H^6, respectively, which are characteristic of peptide backbone NH′s not involved in hydrogen bonding. A single signal at δ = 7.28 can be assigned to both H^5 and H^3. The latter proton (H^3) is shifted downfield compared to the corresponding reference (Δδ = 1.1 ppm relative to **10a**), while H^5 is shifted upfield (Δδ = 0.34 ppm relative to **12a**). For all the N−H protons (H^2, H^3, H^5, H^6), the temperature coefficients range from −5.0 to −5.2 ppb/K, typical of equilibria between non-hydrogen-bonded and hydrogen-bonded states. The above data suggest an equilibrium between a non-hydrogen-bonded conformation and a γ-turn conformation with intramolecular hydrogen bonds involving both H^5 and H^3 (7- and 11-membered-ring hydrogen bonds) (Figure 9).

Figure 10 shows the NH proton NMR chemical shifts of compounds **8a** and **9b** as a function of temperature.

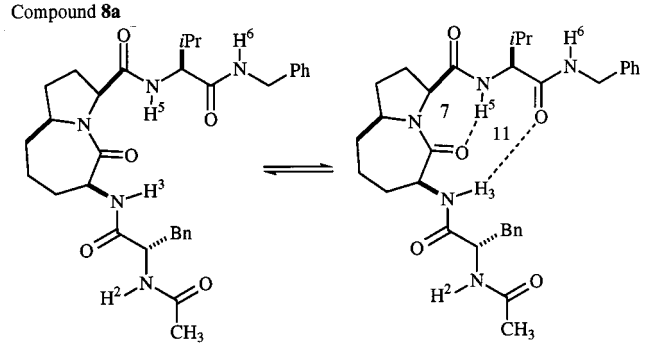
For benzyl amide **9b**, the ^{1}H-NMR parameters are completely different (Table 6). This compound exhibits H^5 and H^2 signals further downfield (δ = 7.51 and 7.19, respectively) compared to those of the corresponding protons of the (5,7)-bicyclic lactam derivative **8a** (δ = 0.23 and 1.25) and of the corresponding methyl ester **7b** (δ = 0.33 and 0.08). The temperature coefficients of H^5 and H^2 in compound **9b** (−1.4 and −1.8 ppb/K, respectively) are characteristic of protons involved in strong intramolecular hydrogen bonding. The signal due to H^3 is obscured by those of the aromatic protons, while H^6 [δ = 6.87, $\Delta\delta(NH)/\Delta T$ = −1.6 ppb/K] is probably hydrogen-bonded to the carbonyl group of the bicyclic lactam (C=O^3). The above NMR data

Table 6. ^{1}H-NMR chemical shifts (ppm) in $CDCl_3$ of amide hydrogen atoms of *N*-acetylated tetrapeptide mimic **8a** incorporating the (5,7)-bicyclic lactam and of tetrapeptide mimic **9b** incorporating the (5,6-Bn)-bicyclic lactam[a,b]

Compd.	δ H^2	δ H^3	δ H^5	δ H^6	$\Delta\delta(NH)/\Delta T$ H^2	$\Delta\delta(NH)/\Delta T$ H^3	$\Delta\delta(NH)/\Delta T$ H^5	$\Delta\delta(NH)/\Delta T$ H^6
8a	5.94	7.28	7.28	6.34	−5.0	−5.2	−5.2	−5.0
9b	7.19	[c]	7.51	6.87	−1.8	[c]	−1.4	−1.6

[a] For all compounds described, NMR experiments showed the NH proton chemical shifts to be independent of concentration at 300 K at or below 2.0 mM, and therefore all experiments were conducted using 1.0−2.0 mM $CDCl_3$ solutions. − [b] All the temperature coefficients were determined with 1.0−2.0 mM $CDCl_3$ solutions between 240 and 300K. − [c] Not determined due to overlap with other resonances.

Compound **8a**



Compound **9b**

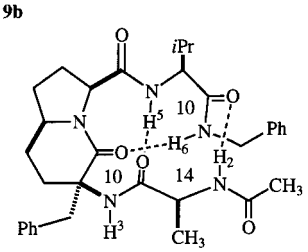


Figure 9. Preferred intramolecular hydrogen-bonding patterns proposed for the *N*-acetylated tetrapeptide mimics (benzyl amides) on the basis of the spectroscopic data

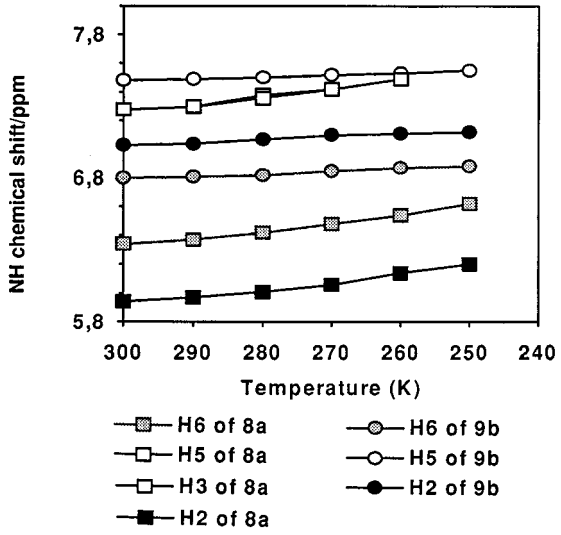


Figure 10. NH proton NMR chemical shifts of tetrapeptides **8a** and **9b** as a function of temperature; data were obtained for 1.0 mM $CDCl_3$ solutions

indicate that compound **9b** undergoes considerable β-hairpin formation (H^5 forms a 10-membered-ring hydrogen bond with $C{=}O^2$ of alanine, H^2 a 14-membered-ring hydrogen bond with $C{=}O^5$ of valine) with an additional β-turn involving H^6 and $C{=}O^3$ of the bicyclic lactam (10-membered ring hydrogen bond) (Figure 9). This type of compact folding is typical of longer sequences [*N*-acetylated hexapeptide mimics containing the (5,6)-bicyclic lactam, **b** series], which are presently under investigation, and the conformational properties of which will be reported in due course.

Computational Studies

N-Acetylated Tetrapeptide Mimics (Methyl Esters)

Computational studies designed to elucidate the conformational preferences of the *N*-acetylated tetrapeptide mimics (methyl esters) were performed on compounds **3a** and **7b**. A summary of the results from the Monte Carlo conformational searches is given in Table 7.

Table 7. Analysis of intramolecular hydrogen-bond interactions in the calculated structures (MC/EM, AMBER*, $CHCl_3$ GB /SA) for the *N*-acetylated tetrapeptide mimics **3a** and **7b**[a]

	3a	**7b**
no. of conformers < 3 kcal/mol	36	30
% β ($C^1{-}C\alpha^2{-}C\alpha^3{-}N^4$) < 30°	3%	13%
% β ($C^2{-}C\alpha^3{-}C\alpha^4{-}N^5$) < 30°	11%	80%
% β ($C^3{-}C\alpha^4{-}C\alpha^5{-}$OMe) < 30°	56%	23%
% H bond NH5···O3 (7-membd. ring)	75% (0.0)[b]	13% (1.0)
% H bond NH5···O2 (10-membd. ring)	0% (> 3.0)	53% (0.0)
% H bond NH3···O5 (11-membd. ring)	3 + 14[c]% (0.0)	27% (1.5)
% H bond NH3···O1 (7-membd. ring)	3% (1.5)	40% (0.0)
% H bond NH2···O5 (14-membd. ring)	8% (2.5)	63% (0.0)

[a] % β is the percentage of all conformers in which the virtual torsion angle β (absolute value) is < 30°; % H bond is the percentage of all conformers in which H···O distance < 2.5 A, N−H···O bond angle > 120°, and H···O=C angle > 90°. − [b] The number in parentheses indicates the relative energy [kcal/mol] of the lowest energy conformer exhibiting this H bond. − [c] Percentage of conformers in which 2.5 A < H···O distance < 4 A, N−H···O bond angle > 120°, and H···O=C angle > 90°.

The data refer to the conformers within 3 kcal/mol of the lowest energy conformation, but similar results are obtained when all the conformers found by the Monte Carlo search are considered (energy cut-off = 6 kcal/mol). Among all the intramolecular hydrogen-bond interactions that could be defined in *N*-acetylated tetrapeptide methyl esters, only those meeting the stringent criteria set out previously for an H bond (see the above Molecular Modelling Section) are reported.

These data indicate that the H^5 proton is often intramolecularly hydrogen-bonded and that it follows the same pattern as observed in the dipeptide mimics. In the (5,7)-fused derivative **3a**, H^5 is involved in a 7-membered ring H-bond with the lactam carbonyl oxygen atom within an inverse γ-turn (75% of the conformers, including the lowest energy conformer). In the (5,6)-fused compound **7b**, H^5 can form an H bond with either the lactam carbonyl oxygen atom (7-membered ring, 13% of the conformers) in an inverse γ-turn, or with the carbonyl oxygen atom of residue 2 (10-membered ring, 53% of the conformers, including the lowest energy conformer) within a type-II′ β-turn.

The percentage of conformers of the (5,6)-fused derivative **7b** that satisfy the distance and directional requirements to form a hydrogen bond between the H^2 proton and the carbonyl oxygen atom of residue 5 (14-membered ring, 63% of the conformers, including the lowest energy conformer)

is significantly higher than the corresponding value calculated for the (5,7)-fused compound **3a** (8% of the conformers). This trend qualitatively agrees with the experimental behaviour exhibited by the H^2 proton in the **a**- and **b**-series tetrapeptide methyl esters.

Computational results suggest that the H^3 proton could form H bonds with either the carbonyl oxygen atom of residue 5 (11-membered ring) or with the carbonyl oxygen atom of the acetyl group (7-membered ring) within an inverse γ-turn. The corresponding percentage values in the **b**-series derivative are higher than those calculated for the **a**-series compound. Moreover, the interaction between H^3 and O^5 in some conformers of **3a** (14% of the conformers, including the lowest energy conformation) is characterized by directional parameters optimal for H-bond formation, but with a donor−acceptor distance larger than 2.5 Å. This is the only exception to the general rule observed in the computed structures, where N−H···O and H···O=C bond angles larger than 120° and 90°, respectively, invariably imply an H···O distance smaller than 2.5 Å. The close connection between H-bond angles and distances is also valid in the opposite sense.

The data reported in Table 7 give an insight into the individual propensities of the various amide hydrogen atoms to be intramolecularly hydrogen-bonded and show a qualitative agreement with the conclusions derived from the spectroscopic studies. An indication of the preferred intramolecular hydrogen-bonding patterns and of their relative stabilities is given in Table 8, as derived from molecular mechanics conformational minima.

Table 8. Preferred intramolecular hydrogen-bonding patterns in the calculated conformers (MC/EM, AMBER*, $CHCl_3$ GB/SA) of the *N*-acetylated tetrapeptide mimics

Compd.	H-bond patterns		Relative energy [kcal/mol]
3a	(7 + 11)	(NH5···O3 + NH3···O5)	0.0
	(7)	(NH5···O3)	0.6
7b	(10 + 14 + 7)	(NH5···O2 + NH2···O5 + NH3···O1)	0.0
	(10 + 14)	(NH5···O2 + NH2···O5)	0.5
	(7 + 14 + 7)	(NH5···O3 + NH2···O5 + NH3···O1)	1.0
	(11)	(NH3···O5)	1.5
8a	non-bonded		0.0
	(10)	(NH6···O3)	0.2
	(7 + 7)	(NH5···O3 + NH6···O4)	1.1
	(7 + 11)	(NH5···O3 + NH3···O5)	1.8
9b	(10 + 14 + 10)	(NH5···O2 + NH2···O5 + NH6···O3)	0.0

Two forms are predominant among the conformers of the (5,7)-fused mimic **3a**: The first pattern is characterized by the presence of the 7- and 11-membered-ring hydrogen bonds (NH5···O3 and NH3···O5), while the second features only the 7-membered-ring H-bond within an inverse γ-turn centered at the Pro residue.

A more complex conformational behaviour is exhibited by the (5,6)-fused mimic **7b**: Four significant H-bond patterns were identified among its conformers (Table 8). Three of them involve β-hairpin formation: H^5 forms either a 10-membered-ring hydrogen bond with $C=O^2$ of alanine or a 7-membered-ring H bond with the lactam $C=O^3$, while H^2 is involved in a 14-membered-ring hydrogen bond with $C=O^5$ of valine. The β-hairpin structure can be combined with the 7-membered-ring NH3···O1 hydrogen bond within an inverse γ-turn centered at the alanine residue, giving rise to (10 + 14 + 7) or (7 + 14 + 7)-membered-ring H-bond patterns. A non-hydrogen-bonded state as well as an 11-membered-ring hydrogen bond with $C=O^5$ of valine can also be observed for H^3, giving rise to (10 + 14) and 11-membered-ring H-bond patterns, respectively.

The intramolecular hydrogen-bonding patterns suggested by molecular mechanics conformers of *N*-acetylated tetrapeptide mimics (methyl esters) nicely agree with those identified on the basis of the experimental data, lending support to the conformational equilibria depicted in Figure 7.

N-Acetylated Tetrapeptide Mimics (Benzyl Amides)

A summary of the results from Monte Carlo conformational searches of compounds **8a** and **9b** is given in Table 9.

Table 9. Analysis of intramolecular hydrogen-bond interactions in the calculated structures (MC/EM, AMBER*, $CHCl_3$ GB/SA) for the *N*-acetylated tetrapeptide mimics **8a** and **9b**[a]

	8a	**9b**
no. of conformers < 3 kcal /mol	105	9
% β ($C^1-C\alpha^2-C\alpha^3-N^4$) < 30 deg	3	0
% β ($C^2-C\alpha^3-C\alpha^4-N^5$) < 30 deg	0	100
% β ($C^3-C\alpha^4-C\alpha^5-N^6$) < 30 deg	62	56
% H-bond NH5···O3 (7-membd. ring)	25% (1.1)[b]	0% (> 3.0)
% H-bond NH5···O2 (10-membd. ring)	0% (> 3.0)	100% (0.0)
% H-bond NH3···O5 (11-membd. ring)	1 + 4[c]% (1.8)	0% (> 3.0)
% H-bond NH3···O1 (7-membd. ring)	0% (> 3.0)	0% (> 3.0)
% H-bond NH2···O5 (14-membd. ring)	1% (3.0)	100% (0.0)
% H-bond NH6···O4 (7-membd. ring)	14% (1.1)	0% (> 3.0)
% H-bond NH6···O3 (10-membd. ring)	23% (0.2)	100% (0.0)

[a] % β is the percentage of all conformers in which the virtual torsion angle β (absolute value) is < 30°;% H bond is the percentage of all conformers in which H···O distance < 2.5 Å, N−H···O bond angle > 120° and H···O=C angle > 90°. − [b] The number in parentheses indicates the relative energy [kcal/mol] of the lowest energy conformer exhibiting this H bond. − [c] Percentage of conformers in which 2.5 Å < H···O distance < 4 Å, N−H···O bond angle > 120°, and H···O=C angle > 90°.

The number of conformers within 3 kcal/mol of the global minimum and the H-bond occurrence percentages suggest that the conformational behaviour of the **a**-series tetrapeptide benzyl amide is significantly different from that of the **b**-series derivative.

High conformational flexibility and an absence of strongly preferred intramolecular hydrogen-bond interac-

tions emerge for the (5,7)-fused compound **8a**, in accordance with the ^{1}H-NMR temperature coefficients observed for all the NH protons of this compound (Table 6). The agreement between calculated and experimental (NH proton NMR chemical shifts) data of **8a** is less good when the protons mostly involved in equilibria between non-hydrogen-bonded and hydrogen-bonded states are considered.

Computational results suggest that the amide NH (H^6) can form either a 10-membered-ring H bond with the lactam C=O^3 within a type-I β-turn (23% of the conformers) or a 7-membered-ring H bond with C=O^4 of proline within an inverse γ-turn (14% of the conformers). The H^5 proton is involved in a 7-membered-ring H bond with the lactam carbonyl oxygen atom within an inverse γ-turn (25% of the conformers), in accordance with the observations made for the **a**-series dipeptide and tetrapeptide (methyl esters) mimics. The data reported in Table 9 show that the H^2 and H^3 protons are only weakly hydrogen-bonded in the (5,7)-fused compound **8a**, in contrast with the experimentally observed behaviour of H^3.

The preferred intramolecular hydrogen-bonding patterns observed in the molecular mechanics derived conformers of **8a** are given in Table 8. A non-hydrogen-bonded conformation, a β-turn conformation across the Pro−Val residues with the 10-membered-ring NH6···O3 H bond, a (7 + 7)-membered-ring H-bond pattern, corresponding to two consecutive inverse γ-turns at proline and valine, and, to a lesser extent, a (7 + 11)-membered-ring H-bond pattern, appear to contribute to the conformational equilibrium of the (5,7)-fused tetrapeptide mimic **8a**.

The behaviour of the (5,6)-fused benzyl amide **9b** is completely different (Tables 8 and 9). Very few conformers and a strongly preferred intramolecular hydrogen-bonding pattern were identified by molecular mechanics calculations. β-Hairpin formation with an additional β-turn is observed in all the minima: The H^5 proton forms a 10-membered-ring H bond with C=O^2 of alanine within a type-II′ β-turn,[16] H^2 forms a 14-membered-ring H bond with C=O^5 of valine, while H^6 forms a 10-membered-ring H bond with C=O^3 of the bicyclic lactam within a type-I β-turn. The resulting folding pattern is identical to that proposed for **9b** on the basis of the spectroscopic data (Figure 9).

Conclusions

In *N*-acetylated tetrapeptide mimics (methyl esters) incorporating a bicyclic lactam (**a** series and **b** series), H^5 is available for either a γ-turn (7-membered ring with the carbonyl group of the bicyclic lactam) or a β-turn (10-membered ring with the carbonyl group of residue 2), as shown in Figure 7. All the above data indicate that the **a** series incorporating the (5,7)-bicyclic lactam predominantly induces the γ-turn conformation, while the **b** series incorporating the (5,6)-bicyclic lactam can promote either a γ-turn or a β-turn conformation, with varying degrees of β-hairpin formation (14-membered ring involving N−H^2 and C=O^5).

The above trend is more pronounced in *N*-acetylated tetrapeptide mimics (benzyl amides), which possess an additional NH (H^6) (Figure 9). The (5,7)-bicyclic lactam induces an equilibrium between a γ-turn conformation, with intramolecular hydrogen bonds involving both H^5 and H^3, and a non-hydrogen-bonded conformation. These structures (**a** series) resemble tweezer-like molecules, and may be potentially useful as receptors for binding substrates through hydrogen bonding. On the contrary, the (5,6)-bicyclic lactam promotes considerable β-hairpin formation (H^5 forms a 10-membered-ring hydrogen bond with C=O^2, H^2 a 14-membered-ring hydrogen bond with C=O^5) with an additional β-turn involving H^6 and C=O^3 of the bicyclic lactam (10-membered-ring hydrogen bond). This type of compact folding is typical of longer sequences [*N*-acetylated hexapeptide mimics containing the (5,6)-bicyclic lactam, **b** series], which are presently under investigation, and the conformational properties of which will be reported in due course.

Experimental Section

NMR and IR Methods: NMR spectra were acquired in $CDCl_3$ with a Bruker AC-300 spectrometer operating at 300 MHz for ^{1}H. Chemical shifts are reported relative to the residual proton peak in $CDCl_3$ (δ = 7.23). Solutions of the compounds at concentrations of 0.5−20 mM were used for assessing 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. Sample temperatures were controlled with the variable-temperature unit of the instrument. Complete proton resonance assignments were made with the aid of COSY and NOESY experiments. NOESY experiments were performed in phase-sensitive mode. FT-IR spectra were recorded at room temperature with a Jasco FT-400 spectrophotometer.

Computational Methods: Molecular mechanics calculations were performed within the framework of MacroModel[17] version 5.5 using the MacroModel implementation of the Amber all-atom force field[18] (denoted AMBER*) and the implicit chloroform GB/SA solvation model of Still et al.[19] The AMBER* force field in MacroModel 5.5 contains a new set of parameters for proline-containing peptides, recently developed on the basis of high-level ab initio calculations.[20] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search of Chang−Guida−Still.[21] A ring-closure bond was defined in the six- and seven-membered rings of the (5,6)- and (5,7)-fused bicyclic systems, respectively. Amide bonds were included among the rotatable bonds. − For each search, at least 2000 starting structures for each variable torsion angle were generated and minimized until the gradient was less than 0.05 kJ/Å·mol using the truncated Newton−Raphson method[22] implemented in MacroModel. Duplicate conformations and those with an energy in excess of 6 kcal/mol above the global minimum were discarded. The nature of the stationary points individuated was tested by computing the eigenvalues of the second-derivative matrix.

Acknowledgments

We thank the Commission of the European Union (Fixed Contribution Contract for Training through Research ERB FMB ICT 960586) for financial support and for a postdoctoral fellowship to

Dr. A. Mielgo, N.A.T.O. (Collaborative Research Grant CRG-951194 to C. G. and H. P. N., Cold Spring Harbor Laboratory, N.Y., U.S.A.), Merck (Merck's Academic Development Program Award to C. G., 1996−97), and MURST COFIN 1998−99 (Rome) for financial support.

[1] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Prot. Chem.* **1985**, *37*, 1−109.

[2] [2a] T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 6975−6985. − [2b] B. L. Sibanda, J. M. Thornton, *J. Mol. Biol.* **1988**, *203*, 221.

[3] D. S. Kemp, T. P. Curran, J. G. Boyd, T. J. Allen, *J. Org. Chem.* **1991**, *56*, 6683−6697.

[4] [4a] A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303−1326; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244−1267. − [4b] 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. − [4c] R. M. J. Liskamp, *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1−19. − [4d] 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. − [4e] S. Hanessian, G. McNaughton-Smith, H.-G. Lombart, W. D. Lubell, *Tetrahedron* **1997**, *38*, 12789−12854. − [4f] K. Burgess, K.-K. Ho, *J. Am. Chem. Soc.* **1994**, *116*, 799−800. − [4g] R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461−7472.

[5] [5a] D. S. Kemp, P. McNamara, *Tetrahedron Lett.* **1982**, *23*, 3761−3764. − [5b] M. Feigel, *J. Am. Chem. Soc.* **1986**, *108*, 181−182. − [5c] D. S. Kemp, W. E. Sites, *Tetrahedron Lett.* **1988**, *29*, 5057−5060. − [5d] M. Feigel, *Liebigs Ann. Chem.* **1989**, 459−468. − [5e] V. Brandmeier, M. Feigel, *Tetrahedron* **1989**, *45*, 1365−1376. − [5f] V. Brandmeier, M. Feigel, *Angew. Chem.* **1989**, *102*, 466−468; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 486−488. − [5g] U. Nagai, K. Sato, R. Nakamura, R. Kato, *Tetrahedron* **1993**, *49*, 3577−3592. − [5h] H. Díaz, J. R. Espina, J. W. Kelly, *J. Am. Chem. Soc.* **1992**, *114*, 8316−8318. − [5i] B. Gardner, H. Nanashi, M. Khan, *Tetrahedron* **1993**, *49*, 3433−3448. − [5j] H. Díaz, K. Y. Tsang, D. Choo, J. W. Kelly, *Tetrahedron* **1993**, *49*, 3533−3545. − [5k] W. C. Ripka, G. V. De Lucca, A. C. Bach II, R. S. Pottorf, J. M. Blaney, *Tetrahedron* **1993**, *49*, 3609−3628. − [5l] H. Díaz, K. Y. Tsang, D. Choo, J. R. Espina, J. W. Kelly, *J. Am. Chem. Soc.* **1993**, *115*, 3790−3793. − [5m] J.-P. Dumas, J. P. Germanas, *Tetrahedron Lett.* **1994**, *35*, 1493−1496. − [5n] K. Y. Tsang, H. Diaz, N. Graciani, J. W. Kelly, *J. Am. Chem. Soc.* **1994**, *116*, 3988−4005. − [5o] V. Brandmeier, W. H. B. Sauer, M. Feigel, *Helv. Chim. Acta* **1994**, *77*, 70−85. − [5p] D. S. Kemp, Z. Q. Li, *Tetrahedron Lett.* **1995**, *36*, 4175−4178. − [5q] D. S. Kemp, Z. Q. Li, *Tetrahedron Lett.* **1995**, *36*, 4179−4180. − [5r] S. R. Labrenz, J. W. Kelly, *J. Am. Chem. Soc.* **1995**, *117*, 1655−1656. − [5s] J. P. Schneider, J. W. Kelly, *J. Am. Chem. Soc.* **1995**, *117*, 2533−2546. − [5t] R. R. Gardner, G.-B. Liang, S. H. Gellman, *J. Am. Chem. Soc.* **1995**, *117*, 3280−3281.

[6] [6a] W. F. Michne, J. D. Schoroeder, *Int. J. Protein Res.* **1996**, *47*, 2. − [6b] K. C. L. Nesloney, J. W. Kelly, *J. Org. Chem.* **1996**, *61*, 3127−3137. − [6c] K. McWilliams, J. W. Kelly, *J. Org Chem.* **1996**, *61*, 7408−7414. − [6d] J. S. Nowick, D. L. Holmes, G. Mackin, G. Noronha, A. J. Shaka, E. M. Smith, *J. Am. Chem. Soc.* **1996**, *118*, 2764−2765. − [6e] C. L. Nesloney, J. W. Kelly, *J. Am. Chem. Soc.* **1996**, *118*, 5836−5845. − [6f] E. M. Smith, D. L. Holmes, A. J. Shaka, J. S. Nowick, *J. Org. Chem.* **1997**, *62*, 7906−7907. − [6g] J. S. Nowick, M. Pairish, I. Q. Lee, D. L. Holmes, J. W. Ziller, *J. Am. Chem. Soc.* **1997**, *119*, 5413−5420. − [6h] D. L. Holmes, E. M. Smith, J. S. Nowick, *J. Am. Chem. Soc.* **1997**, *119*, 7655−7669. − [6i] K. Kyonghee, J. P. Germanas, *J. Org. Chem.* **1997**, *62*, 2847−2852. − [6j] K. Kyonghee, J. P. Germanas, *J. Org. Chem.* **1997**, *62*, 2853−2860. − [6k] C. Strässler, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 1528−1551. − [6l] T. S. Haque, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 2303−2304. − [6m] S. Krauthäuser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719−11720. − [6n] T. Kayashi, T. Asai, H. Ogoshi, *Tetrahedron Lett.* **1997**, *38*, 3039−3042. − [6o] S. Hanessian, H. Yang, *Tetrahedron Lett.* **1997**, *38*, 3155−3158. − [6p] B. E. Fink, P. R. Kym, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1998**, *120*, 4334−4344.

[7] C. Gennari, A. Mielgo, D. Potenza, C. Scolastico, U. Piarulli, L. Manzoni, *Eur. J. Org. Chem.* **1999**, 379−388.

[8] E. B. Garcia-Moreno, J. J. Dwyer, A. G. Gittis, E. E. Lattman, D. S. Spencer, W. E. Sites, *Biophys. Chem.* **1997**, *64*, 211.

[9] [9a] J. B. Ball, R. A. Hughes, P. F. Alewood, P. R. Andrews, *Tetrahedron* **1993**, *49*, 3467−3478. − [9b] Y. Takeuchi, G. R. Marshall, *J. Am. Chem. Soc.* **1998**, *120*, 5363−5372.

[10] G. Boussard, M. Marraud, *J. Am. Chem. Soc.* **1985**, *107*, 1825−1828.

[11] G. B. Liang, C. J. Rito, S. H. Gellman, *J. Am. Chem. Soc.* **1992**, *114*, 4440−4442.

[12] G. C. Pimentel, A. L. McClellan, *The Hydrogen Bond*, Freeman, San Francisco, **1960**.

[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. − [13d] J. Yang, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 9090−9091.

[14] In competitive solvents, such as water and DMSO, the chemical shifts of peptide amide protons exhibit differential temperature dependences. Solvent-exposed protons exhibit a large temperature dependence, while intramolecular hydrogen-bonded (solvent-shielded) protons exhibit only a small temperature dependence.

[15] I. G. Jones, W. Jones, M. North, *J. Org. Chem.* **1998**, *63*, 1505−1513.

[16] This result is in striking contrast to the results obtained by Marshall and Takeuchi[9] for the tetrapeptide Ac−Ala−(*S*)-indolizidinone−Ala−NHMe. The (5,6)-fused bicyclic turn mimetic employed in our study features a benzyl substituent on the (*S*)-indolizidinone ring system. No interaction was observed by Marshall between the carbonyl oxygen group of residue *i* ($C^{=}O^{2}$ of **9b**) and the amide hydrogen atom of residue *i* + 3 (H^{5} of **9b**) in the conformers sampled during a 1000 ps, 300 K Monte Carlo/Stochastic Dynamics (MC/SD) simulation[23] using the all-atom AMBER* parameters and the GB/SA solvation water model in Macromodel 5.5. Improved conformational analysis using the MC/SD protocol in GB/SA water was performed on compound **9b**. A 10-membered-ring NH5···O2 H-bond interaction was observed in most of the conformers sampled during the simulation, thus suggesting that the benzyl substituent plays a critical role in determining the turn mimetic properties of the indolizidinone ring system.

[17] 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.

[18] S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comput. Chem.* **1986**, *7*, 230−252.

[19] W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.

[20] D. Q. McDonald, W. C. Still, *J. Org. Chem.* **1996**, *61*, 1385−1391.

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

[22] J. W. Ponder, F. M. Richards, *J. Comput. Chem.* **1987**, *8*, 1016−1024.

[23] F. Guarnieri, W. C. Still, *J. Comput. Chem.* **1994**, *15*, 1302−1310.

Received September 2, 1998
[O98400]