Conformational Analysis of Azabicycloalkane Amino Acid Scaffolds as Reverse-Turn Inducer Dipeptide Mimics

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In an effort to design structural mimics of protein and peptide reverse-turns, the conformations of 5,5-, 6,5-, and 7,5-fused 1-aza-2-oxobicycloalkane amino acids have been evaluated. The conformational preferences of these proline-derived bicyclic lactams have been studied by Monte Carlo molecular mechanics searches, and the reverse-turn inducing properties of the calculated structures have been quantitatively assessed using various geometrical parameters. All of the four possible diastereoisomers arising from two of the three stereogenic centres [C3 and bridgehead carbon atom; Pro Cα is (*S*) in all compounds] have been considered for each bicyclic scaffold. These studies have revealed that the (3*S*)-Pro Cα(*S*) configuration is an effective turn-inducer, although the torsion angles of the backbone do not always mimic those of classical β-turns. A dependence of the turn-inducing ability on lactam ring size and bridgehead stereochemistry has also been found. Reverse-turn mimetic bicyclic lactams have been shown to exhibit a tendency to form an inverse γ-turn or a type II′ β-turn. Experimental ^{1}H-NMR and FT-IR spectroscopic data of model compounds in chloroform solutions have complemented our computer modelling studies and have confirmed our conclusions.

Introduction

In the field of peptidomimetics,[1] much effort has been focused on the design and synthesis of conformationally constrained compounds that mimic or induce certain secondary structural features of peptides and proteins that are thought to play important roles in recognition and biological activity.[2]

A common motif in protein structures is the reverse-turn, which is defined as a site where the peptide backbone reverses the direction of propagation by adopting a U-shaped conformation. The terms γ- and β-turn have more specific definitions and describe turns of three or four consecutive residues, respectively. These turns may (classical γ- or β-turn) or may not (open γ- or β-turn) 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 classification into specific β-turn or γ-turn structural types is based on the geometry of the peptide backbone, as described by the φ (C−N−Cα−C) and ψ (N−Cα−C−N) torsion angles in residues *i*+1 and *i*+2 (β-turn) or in residue *i*+1 (γ-turn).[3]

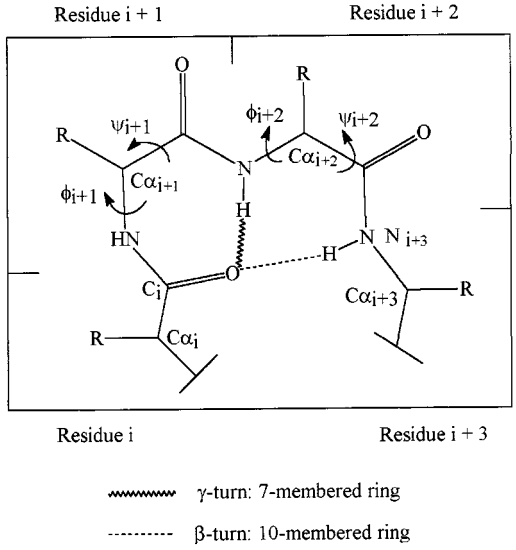

Figure 1. Structures of γ- and β-turns

As a consequence of the prevalence of this motif in recognition events, several non-peptide systems have been designed to mimic different types of reverse-turns.[4] The incorporation of some of these scaffolds into biologically active peptides has led to peptidomimetic drugs with enhanced activities and metabolic stabilities. Reverse-turn mimics are generally cyclic or bicyclic dipeptide analogues which, as a result of their constrained structure, force a peptide chain to fold back upon itself. Many of these molecules feature the 1-azaoxobicyclo[*X*.*Y*.0]alkane skeleton (Figure 2); such a basic ring system could also encompass heteroatom analogues, in which carbon is replaced by sulfur,

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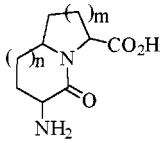


Figure 2. Azabicyclo[*X. Y.*0]alkane amino acids

oxygen, or nitrogen, and/or allow the attachment of specific substituents. A comprehensive review of the synthesis of these compounds has been provided by Hanessian et al.,[5] while conformational analyses of some azabicycloalkane amino acids have been reported by Marshall[6] and Olson[7] as part of extensive computational studies on representative collections of dipeptide mimetics and reverse-turn constraints.

In the course of our studies on peptide secondary structure mimics, we have synthesized several 5,5-, 6,5-, and 7,5-fused 1-aza-2-oxobicyclo[*X*.3.0]alkane amino acids.[8] These lactams can be viewed as conformationally restricted X−Pro dipeptide units, and, if their conformations meet certain criteria, they might be used as synthetic analogues of the $i+1$ and $i+2$ elements of the four consecutive residues of β-turn motifs.

In this paper, we report on the conformational analysis of a series of bicyclic lactams (Figure 3), which has been accomplished by a combination of computer modelling and ^{1}H-NMR and FT-IR spectroscopy.

In order to elucidate the consequences of incorporating these systems into peptides, we have performed Monte Carlo molecular mechanics calculations[9] on dipeptide analogues and have analyzed their reverse-turn mimetic properties by computing various geometrical parameters. The abilities of these structures to induce turn-like conformations are discussed as a function of ring size and stereochemistry. All of the four possible diastereoisomers arising from two of the three stereogenic centres [bridgehead carbon atom and C3; Pro Cα is (*S*) in all compounds] have been considered for each bicyclic scaffold (Figure 3). In selected cases, the effect of solvation ($CHCl_3$ and H_2O) has been included by using the implicit GB/SA model.[10]

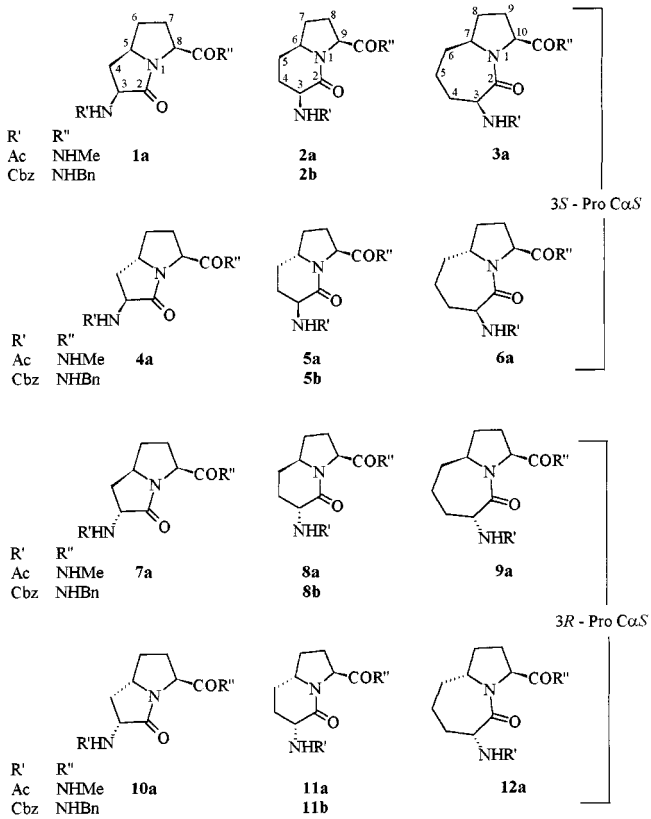


Figure 3. Bicyclic dipeptide mimics used in modelling (**a** series) or in spectroscopic experiments (**b** series)

In these constrained structures, intramolecular hydrogen bonding may provide the principal driving force for turn formation. For this reason, experimental investigations have been carried out in a relatively non-polar solvent (chloroform), which does not offer strong hydrogen-bonding competition.[11] The ^{1}H-NMR chemical shifts of the amide protons, the temperature dependence of the amide proton chemical shifts [$\Delta\delta(NH)/\Delta T$], and the positions of the IR bands have been used to assess which amide protons and which carbonyl groups are involved in intramolecular hydrogen bonds.

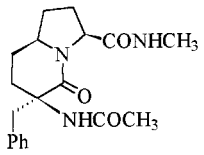
The bicyclic dipeptide derivatives **1**−**12** (Figure 3) used in this study were synthesized according to the protocols reported in the accompanying paper.[8d] They all incorporate a natural [Cα(*S*)] proline residue, but vary in the lactam ring size (5, 6, or 7), and in the stereochemistry at the bridgehead and at the N-bearing carbon C3. They are classified according to the C3 configuration as (3*S*) (**1**−**6**) or (3*R*) (**7**−**12**) bicycles. For convenience, to indicate the bridgehead configuration, the "*cis*" and "*trans*" descriptors are also used, depending on the relative configuration of the bridgehead and the Pro Cα carbon. Thus, compounds **1**−**3** and **7**−**9** are "*cis*", while **4**−**6** and **10**−**12** are "*trans*".

Results and Discussion

Molecular Modelling

Computational studies designed to investigate the abilities of the bicyclic scaffolds to adopt reverse-turn conformations were performed on the *N*-acetyl *N*′-methylamide dipeptide analogues **1a**−**12a** (Figure 3).

Each structure was subjected to an extensive, unconstrained Monte Carlo/energy minimization (MC/EM) conformational search[9] by molecular mechanics methods in vacuo (see Computational Methods in the Experimental Section). The turn propensity of the minimum energy conformations was assessed by computing and analyzing various geometrical parameters, specifically the $C\alpha_i-C\alpha_{i+3}$ distance ($d\alpha$) between capping groups on the N- and C-termini, the φ and ψ backbone torsion angles in residues $i+1$ and $i+2$, the virtual torsion angle β ($C_i-C\alpha_{i+1}-C\alpha_{i+2}-N_{i+3}$),[12] and parameters indicative of hydrogen bonding (i.e. the distance between the carbonyl oxygen and the amide hydrogen atom, and directional requirements). These data are available for each representative low-energy conformation as electronic supporting information.



13

Figure 4. Dipeptide mimic **13**

The criteria adopted in the present work to quantitatively characterize the turn-inducing potential of the various bicyclic lactams were the same as those described in a recent study of tetrapeptide mimics incorporating the 7,5-fused bicyclic unit **3** and the 6,5-fused lactam **13** (Figure 4), featuring a quaternary carbon bearing a benzyl substituent.[13]

Briefly, values of the $C\alpha_i-C\alpha_{i+3}$ distance ($d\alpha$) of less than 7 Å were taken to indicate the presence of a reverse-turn.[3] The virtual torsion angle β ($C_i-C\alpha_{i+1}-C\alpha_{i+2}-N_{i+3}$), suggested as an alternative method of characterizing a reverse-turn by Ball et al.[12] on the basis of a topographical analysis of β-turns, was also measured. The range $0 \pm 30°$ is usually taken to indicate a tight reverse-turn.[6]

The φ and ψ backbone torsion angles in residues $i+1$ and $i+2$ (β-turn) or in residue $i+1$ (γ-turn) were taken as a basis for the 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.[3]

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

A summary of the reverse-turn mimetic properties of the calculated structures of compounds **1a−12a** is presented in Table 1. The results relating to lactam **13** are included for comparison purposes. The turn propensity was quantitatively assessed by computing the percentage of conformations within 6 kcal/mol of the global minimum for which the aforementioned parameters assume typical turn values.

The percentage of all conformers with a virtual torsion angle β (absolute value) of less than 30° or 60° clearly shows that **1a−6a**, i.e. all the compounds having the carboxy and amino groups on the same face of the bicyclic system [(3*S*)-Pro Cα(*S*) configuration] are of potential use as reverse-turn mimetics, although there is a dependence on the lactam ring size and bridgehead stereochemistry. On the contrary, among the diastereoisomers **7a−12a** [(3*R*)-Pro Cα(*S*)], only the 7,5-fused lactam **12a** is able to enforce a turn that actually induces a reversal of direction in the peptide backbone. Analysis of the $C\alpha_i-C\alpha_{i+3}$ distances $d\alpha$ reveals that some of the reverse-turn mimetic scaffolds, in particular **3a** and **12a**, tend to induce rather "open" turns in "tweezer-like" structures.

The percentages of conformers forming intramolecular hydrogen bonds show that the reverse-turn mimetic units **1a**, **2a**, and **4a−6a** can promote either a γ-turn (7-membered ring H-bond) or a β-turn (10-membered ring H-bond). The 7,5-fused reverse-turn mimics **3a** and **12a** exclusively induce γ-turn conformations, resulting in a poor alignment of the entry and exit peptide bonds. In the remaining bicyclic lactams **7a−11a**, the formation of a γ-turn is not sufficient to reverse the peptide direction, as indicated by the values of the torsion angle β and the distance $d\alpha$.

All the parameters clearly indicate that the (3*S*) "*trans*" lactams **4a**, **5a**, and **6a** are better turn-inducers than the (3*S*) "*cis*" lactams **1a**, **2a**, and **3a**. This trend is particularly evident when considering the β-turn mimetic properties. Only the "*cis*" lactam **13**, featuring a quaternary carbon at $C\alpha_{i+1}$, exhibits mimetic properties similar to those of the "*trans*" series.

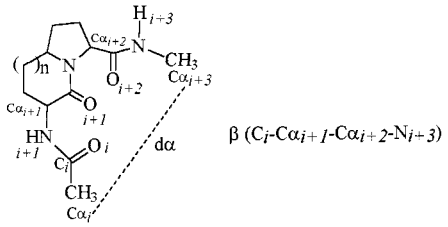
In general, the data reported in Table 1 suggest that the azabicycloalkane amino acid scaffolds are more effective as reverse-turn than as β-turn mimics, in agreement with the results obtained by Marshall.[6]

The principal types of backbone geometries calculated for compounds **1a−12a** and **13** and their relative stabilities are given in Table 2. In vacuo, the lowest energy conformer of all these systems features an inverse γ-turn centred at the Pro residue of the bicyclic lactam. The $NH_{i+3}O_{i+1}$ distances, as well as the directional parameters, are consistent with the intramolecular hydrogen-bond characteristics of a γ-turn, i.e. with a seven-membered ring. Low-energy conformations mimicking a type II′ β-turn or an inverse γ-turn/distorted type II′ β-turn are found at different energy values relative to the global minimum, depending on the lactam ring size and stereochemistry. An unusual situation is observed for compound **6a** in that type I β-turn and inverse γ-turn conformations are almost isoenergetic.

The dependence of the reverse-turn mimetic properties on the solvent has been investigated by performing MC/EM conformational searches[9] of selected dipeptide bicyclic mimics in chloroform and water, as implicitly represented by the GB/SA solvation model.[10] On comparing these results (Table 3) with those calculated in vacuo, it can be seen that there is an improvement of the reverse-turn (particularly the β-turn) mimetic properties of the bicyclic scaffolds in chloroform. Not surprisingly, a decrease in the hydrogen bonding percentages is observed in water. However, the computations generally show a stabilization of the type II′ β-turn conformation both in chloroform and water.

In conclusion, the computational studies show that the dipeptide mimics **1a−12a** may form an inverse γ-turn or a type II′ β-turn[14] through intramolecular hydrogen bonding, depending on the nature of the lactam system. It should be noted that calculations are known to overestimate the stability of the γ-turn conformation relative to other turn geometries. Experimental evidence for the relative importances of the γ- and β-turn conformations of the bicyclic scaffolds under study is provided by ^{1}H-NMR and FT-IR spectroscopic studies (see below).

Table 1. Quantitative characterization of the reverse-turn forming abilities of the conformers (MC/EM, AMBER*, in vacuo) calculated for dipeptide mimics **1a**−**12a** and **13**



1a-12a

Cmpd.	No. of conf. <6 kcal /mol	%$d\alpha$[a] < 7 Å	%|β|[b] < 30°	%|β|[b] < 60°	%H-bond[c] $NH_{i+3}O_{i+1}$ 7-membd. ring, γ-turn	%H-bond[c] $NH_{i+3}O_i$ 10-membd. ring, β-turn	%H-bond[c] $NH_{i+1}O_{i+2}$ 8-membd. ring
1a	19	42 (8)	21 (4)	74 (14)	21 (4)	11+5[d] (2+1)	16 (3)
2a	26	27 (7)	69 (18)	92 (24)	27 (7)	8+4[d] (2+1)	19 (5)
3a	12	0	0	50 (6)	67 (8)	0+0[d]	0
4a	9	22 (2)	44 (4)	78 (7)	44 (4)	22+0[d] (2+0)	0
5a	11	46 (5)	82 (9)	91 (10)	64 (7)	27+18[d] (3+2)	0
6a	33	48 (16)	27 (9)	67 (22)	48 (16)	21+15[d] (7+5)	9 (3)
7a	24	0	0	0	25 (6)	0+0[d]	0
8a	8	0	0	0	50 (4)	0+0[d]	0
9a	24	0	8 (2)	21 (5)	63 (15)	0+0[d]	0
10a	3	0	0	0	100 (3)	0+0[d]	0
11a	17	0	0	12 (2)	71 (12)	0+0[d]	0
12a	15	20 (3)	80 (12)	100 (15)	53 (8)	0+7[d] (0+1)	0
13	47	68 (32)	66 (31)	96 (45)	45 (21)	11+15[d] (5+7)	17 (8)

[a] % $d\alpha$ is the percentage of all conformers for which the distance between $C\alpha_i$ and $C\alpha_{i+3}$ is < 7 Å. The occurrence numbers are given in parentheses. − [b] % |β| is the percentage of all conformers in which the virtual torsion angle β (absolute value) is < 30° (or 60°). The occurrence numbers are given in parentheses. − [c] % H-bond is the percentage of all conformers in which HO distance < 2.5 Å, N−HO bond angle > 120°, and HO=C angle > 90°. The occurrence numbers are given in parentheses. − [d] Percentage of conformers in which 2.5 Å < HO distance < 4 Å.

Table 2. Characteristics of low-energy conformers (MC/EM, AMBER*, in vacuo) calculated for dipeptide mimics **1a**−**12a** and **13**

Cmpd.	Δ*E* (kcal/mol) $NH_{i+3}O_{i+1}$ H-bond 7-membd. ring inverse γ-turn	$NH_{i+3}O_i$ H-bond 10-membd. ring II′ β-turn/I β-turn	$NH_{i+1}O_{i+2}$ H-bond 8-membd. ring
1a	0.0	2.7/−	2.3
2a	0.0	1.2/−	2.0
3a	0.0	−/−	−
4a	0.0	2.1/−	−
5a	0.0	0.9/−	−
6a	0.0	1.2/0.0	2.7
7a	0.0	−/−	−
8a	0.0	−/−	−
9a	0.0	−/−	−
10a	0.0	−/−	−
11a	0.0	−/−	−
12a	0.0	4.7[a] /−	−
13	0.0	0.7/−	1.2

[a] Distorted type II′ β-turn.

Variable-Temperature ^{1}H-NMR Spectroscopy and Discussion of the IR Data

The 6,5-fused peptide mimics **2b**, **5b**, **8b**, and **11b** were selected for spectroscopic studies. Their conformational behaviour was elucidated on the basis of their ^{1}H-NMR spectral characteristics. NMR experiments were conducted with the aim of detecting intramolecular H-bonds by measuring the chemical shifts of the N−H protons H^1 and H^2 and their temperature coefficients ($\Delta\delta/\Delta T$) (Table 4).

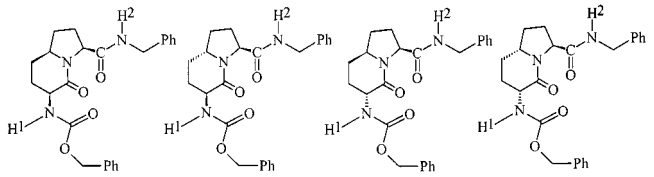
In chloroform, a relatively non-polar solvent, it was necessary to carry out such experiments at sufficient dilution to preclude intermolecular hydrogen bonding. For all compounds described in the following, preliminary variable-concentration experiments showed that no intermolecular aggregation occurred at concentrations < 5 mM at 300 K. However, the chemical shift of the H^1 proton of compound **5b** was found to be concentration-dependent at 240 K in the same concentration range. Thus, the variable-

Table 3. Quantitative characterization of the reverse-turn forming abilities of the conformers (MC/EM, AMBER*, $CHCl_3$, or H_2O GB/SA) calculated for dipeptide mimics **2a**, **3a**, **5a**, **6a**, and **13**

Cmpd.	%$d\alpha$[a] < 7 Å	%$\|\beta\|$[b] $< 30°$	%$\|\beta\|$[b] $< 60°$	%H-bond[c] 7-membd. ring, γ-turn	%H-bond[c] 10-membd. ring, β-turn	%H-bond[c] 8-membd. ring	ΔE inv γ/βII′ kcal/mol
2a							
$CHCl_3$	39	77	100	27	12+8[d]	12	0.0/0.4
H_2O	34	52	96	18	5+9[d]	9	1.3/1.3
3a							
$CHCl_3$	0	0	60	53	0+0[d]	0	0.0/>6
H_2O	5	3	36	26	0+5[d]	0	0.2/>6
5a							
$CHCl_3$	50	75	92	67	25+25[d]	0	0.3/0.0
H_2O	32	68	94	26	10+7[d]	0	0.7/0.3
6a							
$CHCl_3$	47	25	63	44	22+13[d]	13	0.5/0.6[e]
H_2O	44	34	71	23	11+11[d]	1	1.3/1.4[e]
13							
$CHCl_3$	84	60	95	32	19+19[d]	22	1.9/0.0
H_2O	70	61	97	20	9+18[d]	10	1.1/0.0

[a] % $d\alpha$ is the percentage of all conformers for which the distance between $C\alpha_i$ and $C\alpha_{i+3}$ is < 7 Å. – [b] % $|\beta|$ is the percentage of all conformers in which the virtual torsion angle β (absolute value) is $< 30°$ (or 60°). – [c] % H-bond is the percentage of all conformers in which HO distance < 2.5 Å, N–HO bond angle $> 120°$, and HO=C angle $> 90°$. – [d] Percentage of conformers in which 2.5 Å $<$ HO distance < 4 Å. – [e] The global minimum energy conformation features a type I β-turn.

Table 4. ^{1}H-NMR chemical shifts and temperature dependence of amide proton signals of dipeptide mimics **2b**, **5b**, **8b**, and **11b** in 2 mM $CDCl_3$ solutions



2b **5b** **8b** **11b**

Cmpd.	δH^1 (ppm)	δH^2 (ppm)	$\Delta\delta H^1/\Delta T$ (ppb/K)	$\Delta\delta H^2/\Delta T$ (ppb/K)
8b	5.48	6.70	−3.95	−0.7
11b	5.72	7.09	−1.66	−1.8
2b	5.71	7.43	−2.4	−1.0
5b	5.59	7.54	−8.0	−4.1

temperature NMR (VT NMR) experiments are invalidated for H^1 of **5b** by the formation of intermolecular aggregates.

Since interconversion between hydrogen-bonded and non-hydrogen-bonded forms is rapid on the NMR time scale, each δNH value represents a weighted average of contributions from the hydrogen-bonded and non-hydrogen-bonded states of the compound in question. Previous data[13] suggest that $\delta NH < 6.2$ corresponds to a completely non-hydrogen-bonded peptide amide or carbamate proton.

The temperature dependence of the ^{1}H-NMR chemical shifts of amide protons also reflects their hydrogen-bonding state. 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 are involved in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state exhibit a large temperature dependence.[13]

For all the compounds examined, the chemical shift values of the carbamate protons H^1 were found in the range $\delta = 5.48-5.72$, clearly showing that these protons are not involved in H-bonding.

The chemical shift of the amide proton H^2 of compound **8b** was at found at $\delta = 6.7$ and showed only a small temperature dependence (-0.7 ppb K^{-1}). This suggests that there is some, albeit very slight internal hydrogen bonding in this compound.

In compounds **11b** and **2b**, the signals of the amide protons H^2 were found at $\delta = 7.09$ and 7.43, respectively, and exhibited relatively small temperature dependences (-1.8 and -1.0 ppb K^{-1}). These observations are consistent with the involvement of these protons in hydrogen-bonding interactions.

The chemical shift ($\delta = 7.54$ ppm) of the amide proton H^2 of compound **5b** is typical of that of an amide proton in a hydrogen-bonded state. Its relatively large temperature coefficient (-4.1 ppb K^{-1}) reflects the increased population of the hydrogen-bonded state at lower temperatures. In the case of **5b**, the chemical shift of H^2 was found to be independent of concentration in the range 240–300 K, in marked contrast to that of H^1. A possible explanation for this behaviour is the involvement of H^2 in intramolecular ring formation driven by hydrogen bonding, which would leave H^1 available for a selective intermolecular association.

The chemical shifts of amide protons H^2 in the studied lactams **2b**, **5b**, **8b**, and **11b** proved to be very sensitive to the nature of the three stereogenic centres. Thus, in (3*S*) lactams **2b** and **5b** the H^2 resonances appeared at $\delta = 7.43$ and 7.54, respectively, downfield shifted by 0.73 and 0.45 ppm relative to the corresponding H^2 signals in (3*R*) lactams **8b** and **11b**.

CD_3OD addition experiments showed very fast H/D exchange for all the NH's of the bicyclic compounds, indicating that in these small peptide mimics even intramolecul-

Table 5. IR stretching of the amide proton bonds and the carbonyl groups of dipeptide mimics **2b**, **5b**, **8b**, and **11b** in 2 mM $CHCl_3$ solution; the corresponding band numbers from Figure 5 are given in parentheses

Cmpd.	νN−H (cm^{-1}) non-bonded	νN−H (cm^{-1}) bonded	νC=O (cm^{-1}) (CO−NH)	νC=O (cm^{-1}) (O−CO−NH)
8b	3438 (1)	3307 (2)	1678 1645	1718
11b	3431 (1) 3390 (2)	3340 (3)	1680 1651	1717
2b	3430 (1) 3410 (2)	3307 (3)	1676 1648	1718
5b	3454 (1)	3365 (2) 3285 (3)	1653	1708

arly hydrogen-bonded protons are rapidly accessible to the solvent.[13]

With a view to confirming the NMR data and to assess their tendencies to adopt γ- or β-turn arrangements, the conformations of compounds **2b**, **5b**, **8b**, and **11b** were also studied by IR spectroscopy using 2 mM solutions in chloroform (Table 5 and Figure 5). These peptidomimetics were found to display both non-hydrogen-bonded and intramolecularly hydrogen-bonded states, as evidenced by the presence of non-hydrogen-bonded (3454−3390 cm^{-1}) and hydrogen-bonded (3350−3285 cm^{-1}) absorptions in the NH stretch region for each molecule.[15] It is clear from the IR spectra that **5b** exists predominantly in the intramolecularly hydrogen-bonded state under these conditions, whereas compounds **2b**, **8b**, and **11b** exist largely in the non-hydrogen-bonded state.

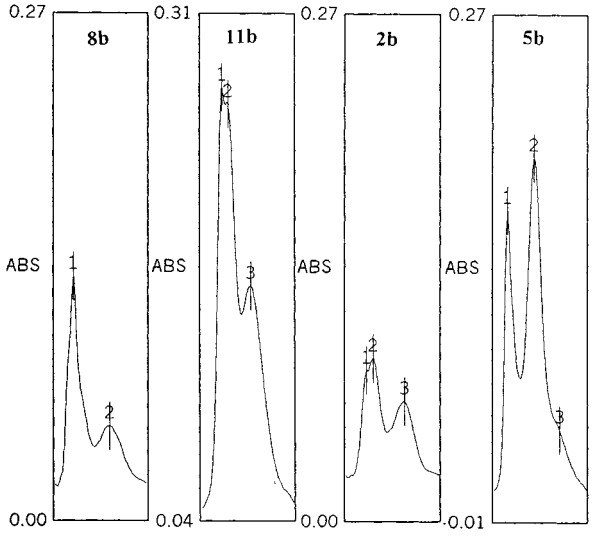


Figure 5. N−H stretch FT-IR data for 1 mM samples in $CHCl_3$ at room temperature after subtraction of the spectrum of pure $CHCl_3$; from left to right: **8b**, maxima at 3438(1) and 3307(2) cm^{-1}; **11b**, maxima at 3431(1), 3390(2), and 3340(3) cm^{-1}; **2b**, maxima at 3430(1), 3410(2), and 3307(3) cm^{-1}; **5b**, maxima at 3454(1), 3365(2), and 3285(3) cm^{-1}

For compounds **2b**, **5b**, **8b**, and **11b**, we assign the bands at 3454−3390 cm^{-1} to the carbamate proton H^1, which appears to be free of intramolecular hydrogen-bonding (cf. the chemical shifts), while the bands below 3350 cm^{-1} we attribute to the hydrogen-bonded fraction of H^2. This proton can form an intramolecular hydrogen bond either to the lactam carbonyl group with formation of a seven-membered ring (γ-turn) or to the carbamate carbonyl group with formation of a ten-membered ring (β-turn). To further characterize the hydrogen-bonding properties of these compounds, the C=O stretchings in the amide I region were used.

The amide I region of the infrared spectrum is predominately due to the C=O stretching vibration; **2b**, **5b**, **8b**, and **11b** possess three different types of carbonyls: a secondary amide, a tertiary amide, and a carbamate. On the basis of model compounds,[16] these are known to give rise to three distinct absorbances, at 1680−1675 cm^{-1} for the free secondary amide, at 1665 cm^{-1} for the free tertiary amide of the 6,5-fused bicyclic lactam, and at 1730 cm^{-1} for the free carbamate. Hydrogen bonding to the carbonyl shifts the band at lower frequency by 20−30 cm^{-1}.

In compounds **2b**, **5b**, **8b**, and **11b**, a H-bonded amide band is seen in the amide I region (1653−1645 cm^{-1}) (Table 5). Since the H^1 protons are not H-bonded, this suggests that the H^2 protons of these compounds are involved in a seven-membered ring H-bond with the lactam carbonyl (γ-turn).

Interestingly, the absorption of the carbamate carbonyl group of **5b** at 1708 cm^{-1} is shifted by ca. 22 cm^{-1} to lower energy compared to the standard compound. This shift is of the magnitude expected for the participation of this carbonyl in a hydrogen-bond. Thus, mimic **5b** can clearly access a β-turn-like conformation. This result seems to indicate that in **5b** an equilibrium between two hydrogen-bonded conformations (γ- and β-turn) across the proline residue is set up.

Conclusions

Computational and spectroscopic studies have been performed in order to assess the propensities of bicyclic lactams **1**−**12** to mimic the reverse-turn peptide conformation. Conformational searches have been run, and the turn-like character has been evaluated using the geometrical parameters β ($C_i-C\alpha_{i+1}-C\alpha_{i+2}-N_{i+3}$ virtual torsion angle) and $d\alpha$ ($C\alpha_i-C\alpha_{i+3}$ distance), as well as by studying the H-bond patterns. All the (3*S*) compounds **1**−**6** were found to be potential reverse-turn inducers, as judged on the basis of their β parameters. On the contrary, with the exception of the 7,5-fused lactam **12**, all the (3*R*) compounds **7**−**11** were found to have poor turn-inducing character. Evaluation of the $d\alpha$ distance has shown that, among the reverse-turn mimics, **3** and **12** tend to induce rather "open" turns. Computational studies of the H-bond patterns have indicated that the reverse-turn mimetic units **1**, **2**, and **4**−**6** can promote either an inverse γ-turn or a type II′ β-turn, the "*trans*" lactams **4**−**6** being better β-turn inducers than the "*cis*" lactams **1** and **2**. On the contrary, the 7,5-fused reverse-turn mimics **3** and **12** exclusively induce inverse γ-turn conformations.

Experimental ^{1}H-NMR and FT-IR spectroscopic data collected for 6,5-fused peptide mimics **2**, **5**, **8**, and **11** have

confirmed the dependence of the intramolecular hydrogen-bonding properties on the stereochemistry of the bicyclic scaffold and have provided evidence for the H-bond interaction involved in the γ-turn conformation. The contribution of the hydrogen-bonded β-turn geometry to the conformational equilibrium was evident only for the "*trans*" (3*S*)-lactam **5**.

Experimental Section

NMR and IR Methods: All NMR experiments were performed using 2 mM $CDCl_3$ solutions. Variable-temperature NMR (VT NMR) measurements on the amides were performed on a Bruker AC-300 spectrometer in the temperature range 240−300 K. − IR measurements were performed on a Jasco 400 FT-IR instrument. − All experiments were performed using 2 mM $CHCl_3$ solutions at room temperature. In each case, the spectrum of the pure solvent was subtracted from that of the solution under investigation and baseline corrections were applied.

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, where necessary, the implicit chloroform or water GB/SA solvation model of Still et al.[10] 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.[19] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search of Chang, Guida, and Still.[9] A ring-closure bond was defined in the six- and seven-membered rings of the 6,5- and 7,5-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[20] 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.

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[14] The percentages of conformers with the *i* to *i*+3 hydrogen bond (β-turn) reported in the paper for the 6,5-fused dipeptide mimics **2a** and **5a** are higher than the values obtained by Marshall and Takeuchi[6b] for the corresponding tetrapeptides Ac-Ala-indolizidinone-Ala-NHMe. No interaction was observed by Marshall between the carbonyl oxygen of residue *i* and the amide hydrogen atom of residue *i*+3 in the conformations sampled during a 1000 ps, 300 K Monte Carlo/stochastic dynamics (MC/SD) simulation[21] using the all-atom AMBER* parameters and the GB/SA solvation water model in MacroModel 5.5. The percentage of β-turn hydrogen bonds determined by MC/SD simulations in GB/SA chloroform or water of dipeptide and tetrapeptide analogues of the indolizidinone ring system is generally lower than the corresponding value calculated by the MC/EM protocol. A better agreement between the two computational approaches is observed for the benzyl-substituted dipeptide mimic **13** and its longer derivatives.[13] It should also be noted that MC/SD simulations of some bicyclic systems have shown convergence problems. NMR and IR spectroscopic studies of sequences of different lengths will play an important role in assessing the β-turn-inducing potentials of the bicyclic mimics.

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