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Influence of *N*-Terminal Residue Stereochemistry on the Prolyl Amide Geometry and the Conformation of 5-*tert*-Butylproline Type VI β-Turn Mimics

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Abstract: The effects of *N*-terminal amino acid stereochemistry on prolyl amide geometry and peptide turn conformation were investigated by coupling both L- and D-amino acids to (2*S*, 5*R*)-5-*tert*-butylproline and L-proline to generate, respectively, *N*-(acetyl)dipeptide *N*′-methylamides **1** and **2**. Prolyl amide *cis*- and *trans*-isomers were, respectively, favored for peptides **1** and **2** as observed by proton NMR spectroscopy in water, DMSO and chloroform. The influence of solvent composition on amide proton chemical shift indicated an intramolecular hydrogen bond between the *N*′-methylamide proton and the acetamide carbonyl for the major conformer of dipeptides (*S*)-**1**, that became less favorable in (*R*)-**1** and **2**. The coupling constant ($^3J_{NH,\alpha}$) values for the *cis*-isomer of (*R*)-**1** indicated a ϕ_2 dihedral angle value characteristic of a type VIb β-turn conformation in solution. X-ray crystallographic analysis of *N*-acetyl-D-leucyl-5-*tert*-butylproline *N*′-methylamide (*R*)-**1b** showed the prolyl residue in a type VIb β-turn geometry possessing an amide *cis*-isomer and ψ_3-dihedral angle having a value of 157°, which precluded an intramolecular hydrogen bond. Intermolecular hydrogen bonding between the leucyl residues of two turn structures within the unit cell positioned the *N*-terminal residue in a geometry where their ϕ_2 and ψ_2 dihedral angle values were not characteristic of an ideal type VIb turn. The circular dichroism spectra of *tert*-butylprolyl peptides (*S*)- and (*R*)-**1b** were found not to be influenced by changes in solvent composition from water to acetonitrile. The type B spectrum exhibited by (*S*)-**1b** has been previously assigned to a type VIa β-turn conformation [Halab L, Lubell WD. *J. Org. Chem.* 1999; **64**: 3312–3321]. The type C spectrum exhibited by the (*R*)-**1b** has previously been associated with type II′ β-turn and α-helical conformations in solution and appears now to be also characteristic for a type VIb geometry. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *cis*-amide bond; 5-*tert*-butylproline; stereochemistry; steric interactions; type VI β-turn mimics

INTRODUCTION

Turns play essential roles in protein folding and recognition [1,2]. Conformationally constrained turn mimics are thus valuable tools for replicating these secondary structures to study their effects in such phenomenon [3,4]. Modified prolines have been particularly useful in the preparation of turn mimics because of the high preference of proline at the central residues of β-turn structures [3–6]. Specific turn geometries have been achieved by using alkylprolines to affect the energy barrier for prolyl amide isomerization as well as the conformation about the proline residue [7–18].

Conformational preferences of peptide turns are contingent on the configuration of their amino acid components. For example, studies of the influence of stereochemistry on the conformation and amide equilibrium of peptides possessing sequences incorporating a D-amino acid at the *N*-terminal position of a proline residue have demonstrated preferences

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for the amide *trans*-isomer as well as type II and II′ β-turn geometry [2]. In the dipeptide H-D-Phe-Pro-OH, only the prolyl amide *trans*-isomer was observed by NMR spectroscopy, whereas the incorporation of L-Phe augmented the *cis*-isomer population in D_2O [19]. In the case of linear peptides, X-ray diffraction has shown that *N*-pivaloyl-D-alanyl-proline *N*′-isopropylamide adopted a type II′ β-turn conformation with an intramolecular hydrogen bond between the oxygen of the pivaloyl carbonyl and the hydrogen of the isopropylamide [20]. Spectroscopic and crystallographic data of depsipeptides Ac-X-Pro-Glyco-D-Leu-NMe_2 and Ac-X-Pro-D-Lac-D-Leu-NMe_2 (where X = L- or D-Val, Glyco = glycolic acid and Lac = lactic acid) have shown them to adopt type II β-turn conformations in CD_2Cl_2, CD_3CN and DMSO-d_6 when X = D-Val [21]. In these cases, greater amounts (15–25%) of prolyl amide *cis*-isomer were observed in DMSO relative to the other two solvents [21].

The influences of an *N*-terminal residue with D-stereochemistry on the conformations about proline residues have also been studied with cyclic peptides both in solution and in the crystalline state. For example, *cyclo*-(X-Pro-Y)$_2$ was shown by a combination of NMR, CD and X-ray experiments to adopt type II or II′ β-turn conformations when a D-residue preceded the proline (X = D-Ala, D-Phe) [22]. The cyclic pentapeptide, *cyclo*-(Gly^1-Pro-Gly^2-D-Ala-Pro) adopted a γ-turn conformation about the D-Ala-Pro-Gly residues with an intramolecular hydrogen bond between the carbonyl oxygen of D-Ala^4 and the amide hydrogen of Gly^1 as observed in the crystal state and in solution [23,24].

A rare turn conformation is the type VI β-turn secondary structure, which uniquely features an amide *cis*-isomer *N*-terminal to a proline residue situated at the $i+2$ position of the peptide bend (Figure 1) [25]. The type VI β-turn is classified into type VIa and type VIb conformations, which have been defined based on the dihedral angle values of the central $i+1$ and $i+2$ residues [25]. In the type VIa β-turn, an intramolecular hydrogen bond is found between the i residue carbonyl oxygen and the $i+3$ residue amide hydrogen. This intramolecular hydrogen bond cannot be formed in the type VIb β-turn because of the proline ψ dihedral angle value. Type VI β-turn conformations play important roles in the recognition and reactivity of bioactive peptides and proteins [26–30].

Previously, we demonstrated that the steric interactions of 5-*tert*-butylproline can influence the prolyl amide isomer geometry in peptides [7–12,14]. Incorporation of (2S, 5*R*)-5-*tert*-butylproline [31] at the *C*-terminal of a series of *N*-acetyl-dipeptide *N*′-methylamides (S)-**1** has led to a means for reproducing the structural elements of type VIa β-turns. For example, replacement of proline by (2S, 5*R*)-5-*tert*-butylproline in *N*-acetyl-L-Xaa-L-proline *N*′-methylamides (S)-**2** (Xaa = Gly, Ala, Met, Leu, Val, Phe) perturbed the naturally favored prolyl amide *trans*-isomer and promoted a dominant *cis*-isomer population as shown by NMR experiments in chloroform, DMSO and water [9,10]. Conformational analyses of the *N*-acetyl-L-Xaa-5-*tert*-butylproline *N*′-methylamides (Xaa = Ala, Leu) by circular dichroism spectroscopy exhibited characteristic type B spectrum for a β-turn conformation in both water and acetonitrile, and indicated that the *tert*-butylprolyl type VIa β-turn geometry was adopted independently of solvent composition [9]. Furthermore, crystallographic analysis of

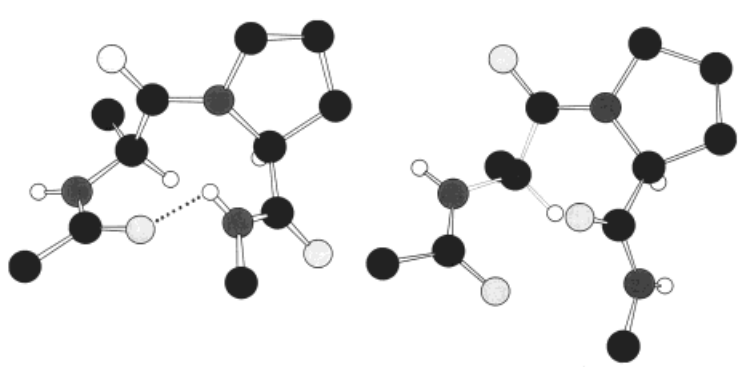

Figure 1 Type VIa and VIb turn conformation found respectively in the central $i+1$ and $i+2$ residues of Ribonuclease S and Bence–Jones protein (C, black; N, dark gray; O, light gray; H, white).

N-acetyl-L-leucyl-5-*tert*-butylproline *N*′-methylamide by X-ray diffraction demonstrated the presence of an amide *cis*-isomer in a geometry characteristic of the central $i+1$ and $i+2$ residues of an ideal type VIa β-turn [9]. An intramolecular hydrogen bond between the *N*′-methylamide and the acetamide carbonyl was inferred from their interatomic distance (2.13 Å) in the X-ray structure.

The influence of the *N*-terminal residue stereochemistry on the conformation of dipeptides possessing (2*S*, 5*R*)-5-*tert*-butylproline has now been investigated through a comparison of *N*-acetyl-D-Xaa-5-*tert*-butylproline *N*′-methylamides (*R*)-**1** and *N*-acetyl-D-Xaa-proline *N*′-methylamide (*R*)-**2** (Xaa = **a**, Ala; **b**, Leu and **c**, Phe). Employing 5-*tert*-butyproline to sterically disfavor the prolyl amide *trans*-isomer, we have explored its potential to disrupt the type II and II′ β-turn conformations, expected for the natural D-Xaa-Pro diastereomers, to induce a type VI geometry. Conformational analyses of dipeptides **1** and **2** by proton NMR and circular dichroism spectroscopy, as well as X-ray diffraction, all have shown that (*R*)-**1a**–**c** preferred the prolyl amide *cis*-isomer in a turn geometry that was less likely to adopt an intramolecular hydrogen bond relative to its diastereomeric counterpart (*S*)-**1a**–**c**. Changing the stereochemistry at the *N*-terminal residue of 5-*tert*-butylproline peptides seems thus to disrupt the hydrogen bond of the type VIa β-turn conformation adopted by *N*-acetyl-L-Xaa-5-*tert*-butylproline *N*′-methylamides (*S*)-**1a**–**c** and gives rise to an alternative type VI β-turn conformation.

RESULTS AND DISCUSSION

Synthesis of Dipeptides 1 and 2

Dipeptide analogs Ac-D-Xaa-5-*t*-BuPro-NHMe (*R*)-**1a**–**c** were synthesized using D-amino acids and protocols described previously for the respective L-Xaa series (*S*)-**1a**–**c** [9,10]. The D-*N*-BOC-amino acids were coupled to the *N*-terminal of 5-*tert*-butylproline *N*′-methylamide using BOP-Cl, DIEA in dichloromethane in lower yields (60–64%) than had been obtained with the L-*N*-BOC-amino acids (75–94%, Figure 2). The *N*-acetyl-D-Xaa-5-*tert*-butylproline *N*′-methylamides (*R*)-**1a**–**c** (Xaa = Ala, Leu, Phe) were finally produced by solvolysis of the BOC group with trifluoroacetic acid and *N*-acetylation with acetic anhydride and potassium carbonate in dichloromethane [11]. For comparison, dipeptides possessing natural L-proline (*N*-acetyl-D-Xaa-L-proline *N*′-methylamides (*R*)-**2a**–**c**) were synthesized by coupling D-*N*-(BOC)-amino acids to proline *N*′-methylamide using TBTU, and DIEA in acetonitrile, followed by BOC group removal and acetylation of the amine as described for the *tert*-butylproline analogs.

Conformational Analysis of Dipeptides 1 and 2 by NMR Spectroscopy

A series of solvents were used to study the influence of environment on the conformation of prolyl dipeptides **1** and **2**. The relative populations of the amide *cis*- and *trans*-isomers *N*-terminal to the prolyl residues of peptides **1** and **2** were measured by

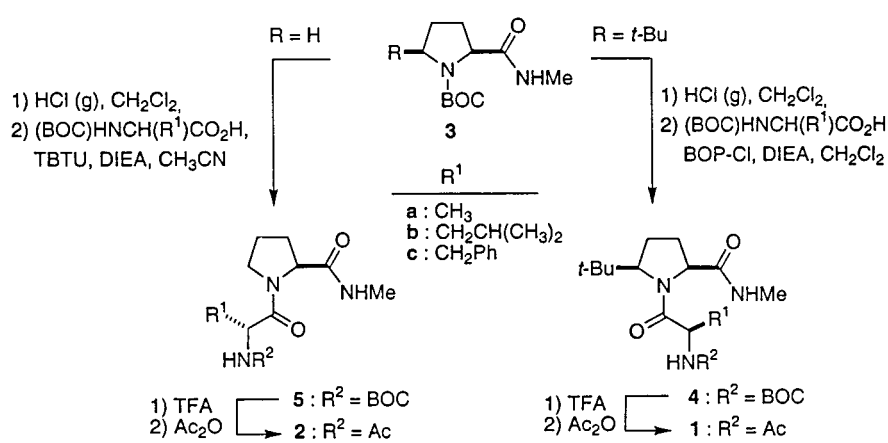

Figure 2 Synthesis of *N*-(acetyl)dipeptide *N*′-methylamides **1** and **2**.

integration of the isomeric *tert*-butyl singlets or *N*′-methyl doublets in their proton NMR spectra in $CDCl_3$, DMSO-d_6 and water. The *tert*-butyl singlet of the amide *cis*-isomer appeared always upfield from that of the *trans*-isomer in the *tert*-butylprolyl peptides. The cross-peak between the *N*-terminal amino acid and proline α-hydrogens arising from the nuclear Overhauser effect in the NOESY and ROESY spectra confirmed the assignment of the *cis*-isomer in dipeptides **1** and **2**.

In the proline dipeptides (*R*)-**2a**–**c**, as is typically observed for linear prolyl peptides [32–35], the *trans*-amide geometry *N*-terminal to the prolyl residue was the major conformer. On the other hand, the incorporation of 5-*tert*-butylproline into the dipeptides favored the *cis*-amide geometry *N*-terminal to the 5-*tert*-butylprolyl residue. In $CDCl_3$ and water, the D-Xaa-5-*t*-BuPro *cis*-isomer population was lower for (*R*)-**1a**–**c** than that observed in the L-series (*S*)-**1a**–**c**. Switching solvent from $CDCl_3$ to DMSO augmented more significantly the *cis*-isomer population in the D-series than had been previously observed in the dipeptide counterparts with L-amino acids (Table 1). In DMSO, the highest (> 90%) *cis*-isomer populations were observed when the D-amino acid side-chain was aliphatic.

The influence of solvent composition on the chemical shifts of the NH signals was used to identify amides engaged in intramolecular hydrogen bonds (Table 1). In the major *cis*-amide conformer of peptide **1** and the major *trans*-amide conformer of **2**, the signal for the *N*′-methylamide proton was always observed downfield relative to the signal for the acetamide proton in all three solvents. This downfield shift was most evident in chloroform in which the *N*′-methylamide proton signals were shifted furthest downfield for (*S*)-**1** (8.27–8.37 ppm) relative to (*R*)-**1** (7.29–7.37 ppm), (*R*)-**2** (6.90–6.95 ppm) and (*S*)-**2** (6.61–6.68 ppm). In contrast, the acetamide proton signal varied between 5.97–6.43 ppm for all four series (Table 1). The downfield shifted amide proton signal can be used to suggest an intramolecular hydrogen bond between the *N*′-methylamide proton and the acetamide carbonyl in a type VIa β-turn conformation for (*S*)-**1**. Taking into consideration that the chemical shift of the amide protons represents an average value from a conformational equilibrium, we may conclude that relative to (*S*)-**1**, the D-Xaa-5-*tert*-butylproline dipeptide (*R*)-**1** is less likely to adopt a type VIa β-turn possessing an intramolecular hydrogen bond. An opposite influence of stereochemistry on

Table 1 Influence of Solvent on the Chemical Shifts[a] and Amide Isomer Equilibrium of **1** and **2**

***Trans*-isomer** ⟷ ***Cis*-isomer**

Entry	*N*-Terminal residue	*R*	% *Cis*-isomer ± 3%			($CDCl_3$)		($CDCl_3 \rightarrow$ DMSO)		($CDCl_3 \rightarrow D_2O$)	
			D_2O	DMSO	$CDCl_3$	δ NH^{Xaa}	NH^{Me}	$\Delta\delta$ NH^{Xaa}	NH^{Me}	$\Delta\delta$ NH^{Xaa}	NH^{Me}
(S)-**1a**	L-Ala	*t*-Bu	79	79	83	6.07	8.30	2.39	0.28	2.22	0.28
(*R*)-**1a**	D-Ala	*t*-Bu	68	91	71	6.43	7.34	1.71	0.62	1.71	0.51
(S)-**1b**	L-Leu	*t*-Bu	81	67	85	5.97	8.27	2.43	0.22	2.27	0.12
(*R*)-**1b**	D-Leu	*t*-Bu	78	93	60	6.19	7.29	1.89	0.71	1.94	0.60
(S)-**1c**	L-Phe	*t*-Bu	90	79	89	6.09	8.37	2.53	0.35	–	0.26
(*R*)-**1c**	D-Phe	*t*-Bu	58	73	82	6.32	7.37	1.86	1.12	–	0.16
(S)-**2a**	L-Ala	H	14	30	19	6.33	6.61	1.79	1.06	1.82	1.20
(*R*)-**2a**	D-Ala	H	19	36	6	6.30	6.90	1.90	0.63	1.93	0.75
(S)-**2b**	L-Leu	H	19	17	20	6.03	6.68	2.00	1.65	2.13	1.10
(*R*)-**2b**	D-Leu	H	29	48	9	6.19	6.95	1.97	0.57	2.03	0.73
(*R*)-**2c**	D-Phe	H	14	39	4	6.31	6.91	2.07	0.59	2.03	0.65

[a] Values are for the major conformer at 5 mM concentration, 25°C, determined by 600 MHz NMR.

the preference for an intramolecular hydrogen bond may also be suggested from the results with the proline dipeptides **2**, where a type II′ β-turn is better accommodated in the D-series (*R*)-**2** relative to its diastereomer (*S*)-**2** [22,36].

In all cases, the signal for the *N*′-methylamide proton was less affected by changes in solvent relative to the signal for the acetamide proton in the NMR spectra for the major amide *cis*-isomer of **1** and major amide *trans*-isomer of **2**. The signal for the acetamide proton of dipeptides **1** and **2** was strongly shifted (1.71–2.53 ppm) with changes in solvent. On switching solvents from $CDCl_3$ to DMSO, the signal for the *N*′-methylamide proton was less shifted downfield (0.22–0.35 ppm) for (*S*)-**1** relative to (*R*)-**1** (0.62–1.12 ppm), (*R*)-**2** (0.57–0.63 ppm) and (*S*)-**2** (1.06–1.65 ppm). Similarly, on changing from $CDCl_3$ to water, the signal for the *N*′-methylamide proton was less shifted downfield (0.12–0.28 ppm) for (*S*)-**1** relative to (*R*)-**1** (0.16–0.60 ppm), (*R*)-**2** (0.65–0.75 ppm) and (*S*)-**2** (1.10–1.20 ppm, Table 1).

In the *tert*-butylproline dipeptides, the influences of solvent on the *N*′-methylamide NH chemical shift were more pronounced in the D-series (*R*)-**1** relative to (*S*)-**1**. Since independence from solvent composition is characteristic of an amide proton engaged in a shielded environment such as an intramolecular hydrogen bond, this result also indicated a greater preference for a type VIa β-turn conformation in the *cis*-amide conformer of the L-series (*S*)-**1** relative to the D-series (*R*)-**1**. The opposite trend was again observed in the proline dipeptides **2**, where the *N*′-methylamide proton was less shifted downfield in the D-series indicative of its greater preference for an intramolecular hydrogen bond in a β-turn.

The coupling constant ($^3J_{NH,\alpha}$) between the amide and α-protons of the *N*-terminal amino acid residue can be used to determine the ϕ dihedral angle value in solution [37]. The $^3J_{NH,\alpha}$ values for the major amide *cis*-isomer of the *tert*-butylprolyl dipeptides (*S*)-**1** were 1.0–2.5 Hz lower than for the major amide *cis*-isomer of the *tert*-butylprolyl dipeptides (*R*)-**1**. In DMSO, the $^3J_{NH,\alpha}$ values varied from 5.2 to 5.5 Hz for the amide *cis*-isomer of (*S*)-**1** and from 6.2 to 8.0 Hz for the major conformer of (*R*)-**1**. Since the ideal ϕ dihedral angle values, $-60°$ and $-120°$, for the $i+1$ residue in a type VIa and VIb β-turn correspond respectively to $^3J_{NH,\alpha}$ coupling constants of 4.2 and 6.9 Hz, the observed values support the hypothesis that the major *cis*-isomer of (*S*)-**1** and (*R*)-**1** adopt type VIa and VIb β-turn geometry, respectively, in solution. In the prolyl dipeptides in DMSO, the $^3J_{NH,\alpha}$ values (6.7–7.2 Hz) for the major amide *trans*-isomer of (*R*)-**2** corresponded with the ideal ϕ_2 dihedral angle value of 60° inside a type II′ β-turn conformation.

Conformational Analysis of Dipeptides 1 and 2 by Circular Dichroism Spectroscopy

To examine the influence of solvent composition on peptide conformation, circular dichroism (CD) spectra of dipeptides **1b** and **2b** were measured in water and acetonitrile (Figure 3). The CD spectra of the *N*-acetyl-L-leucyl-*tert*-butylproline *N*′-methylamide (*S*)-**1b** (Figure 3(A)) exhibited a strong negative band at 188 nm, a strong positive band at 209 nm and a weak negative band at 227 nm [9]. The shape of the CD curves for (*S*)-**1b** remained constant as the solvent was changed from acetonitrile to water [9]. Because this CD curve shape had been classified as a type B spectrum [38], previously assigned to β-turn conformations [39], we concluded that a type VIa β-turn conformation was adopted by the *tert*-butylprolyl peptide (*S*)-**1b**, which was shown to be independent of solvent composition [9]. Modifying the stereochemistry on the *N*-terminal amino acid of 5-*tert*-butylproline had an important effect on the CD spectra. The CD curves of *N*-acetyl-D-leucyl-*tert*-butylproline *N*′-methylamide (*R*)-**1b** (Figure 3(B)) were characterized by a minimum at 205 nm in both water and acetonitrile and a shape similar to a type C spectrum [38]. Type C curves were also observed for *N*-acetyl-D-leucyl-proline *N*′-methylamide (*R*)-**2b**, which exhibited minima at 200 and 206 nm in water that converged to a single band at 206 nm in acetonitrile (Figure 3(D)). Type C curves have been reported for cyclic peptides, such as gramicidin S, which possess D-Xaa-L-Pro sequences and adopt type II′ β-turn conformations [40,41]. On the other hand, the CD spectra of the prolyl peptide (*S*)-**2b** varied significantly with changes in solvent composition and exhibited a $n-\pi^*$ band near 225 nm in acetonitrile and a significant $\pi-\pi^*$ band near 195 nm in water (Figure 3(C)) [9], similar to CD spectra reported for *N*-(acetyl)proline *N*′-methylamide [42]. The similarity of the CD curves of (*R*)-**1b** and (*R*)-**2b** also indicated that the prolyl amide geometry has a limited influence on the molecular ellipticity exhibited by the D-Xaa-Pro residues within the peptide. A similar set of CD curves was obtained from examination of dipeptides **1a** and **2a** in which alanine preceded the prolyl residues (Figure 4).

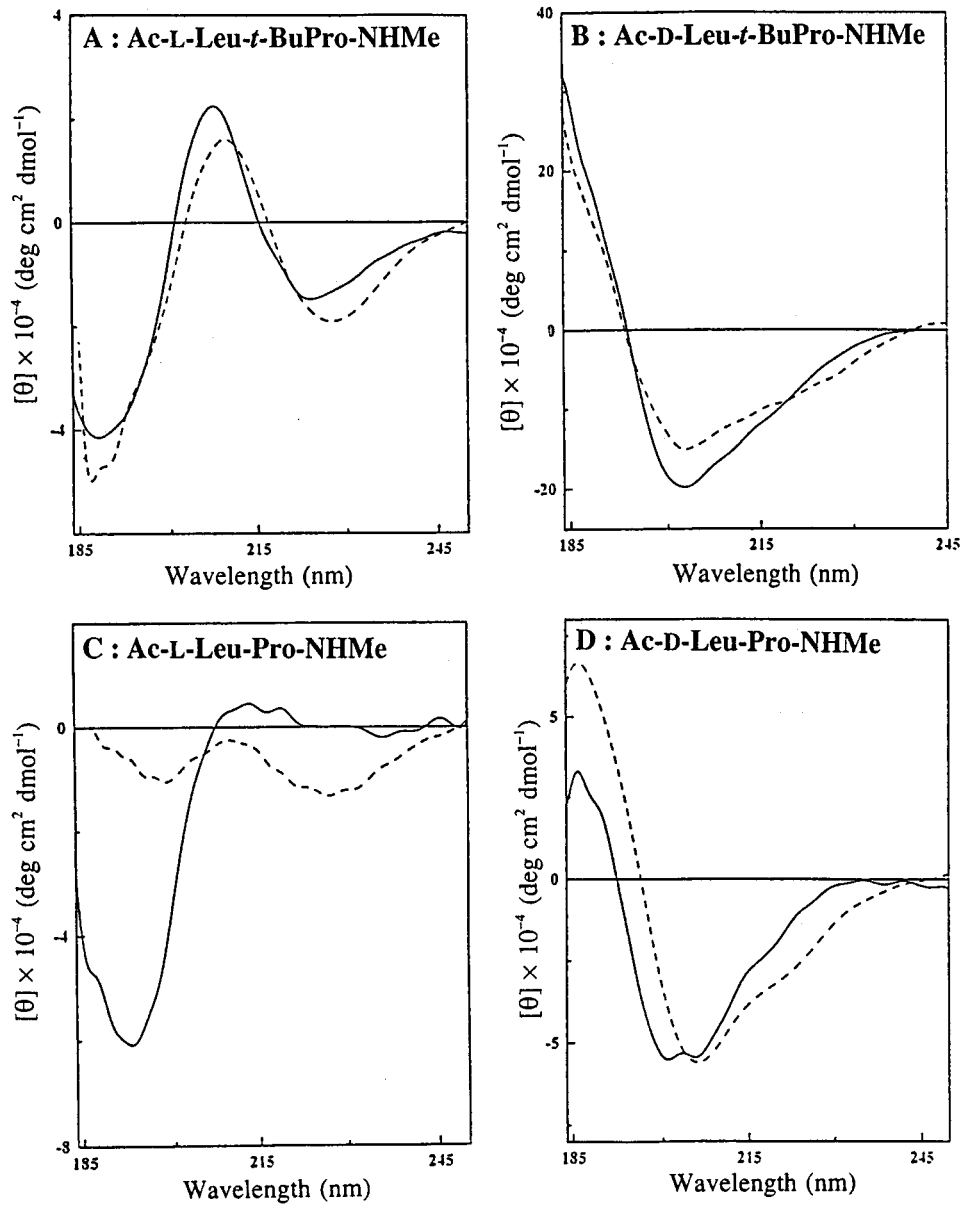

Figure 3 Circular dichroism spectra of *N*-(acetyl)dipeptide *N*′-methylamides (S)-**1b** (A), (*R*)-**1b** (B), (S)-**2b** (C) and (*R*)-**2b** (D) in water (—) and acetonitrile (---).

X-Ray Crystallographic Analysis of Dipeptide (*R*)-1b

N-Acetyl-D-leucyl-5-*tert*-butylproline *N*′-methylamide (*R*)-**1b** was crystallized from a mixture of ethyl acetate and hexane. The analysis of the crystal structure by X-ray diffraction revealed a unit cell with eight molecules, which adopted one of two similar turn conformations. Intermolecular hydrogen bonds were observed between the *N*-(acetyl)-leucyl residues with the leucine carbonyl oxygen and the acetamide nitrogen at an interatomic distance of 2.86–2.87 Å (Figure 5), as well as between the *N*′-methylamide residues with the carbonyl oxygen and the nitrogen at an equal interatomic distance (2.86–2.87 Å). Both turn structures possessed *cis*-amide bonds *N*-terminal to the 5-*tert*-butylproline residues with significantly twisted dihedral angle values of $\omega = 28.9°$ and 29.0°. In addition, no intramolecular hydrogen bond was observed between the methylamide nitrogen and the acetamide oxygen in the crystal structure of (*R*)-**1b**.

For comparison, the dihedral angles of peptides (*S*)-**1b** and (*R*)-**1b** are illustrated in Table 2 with the

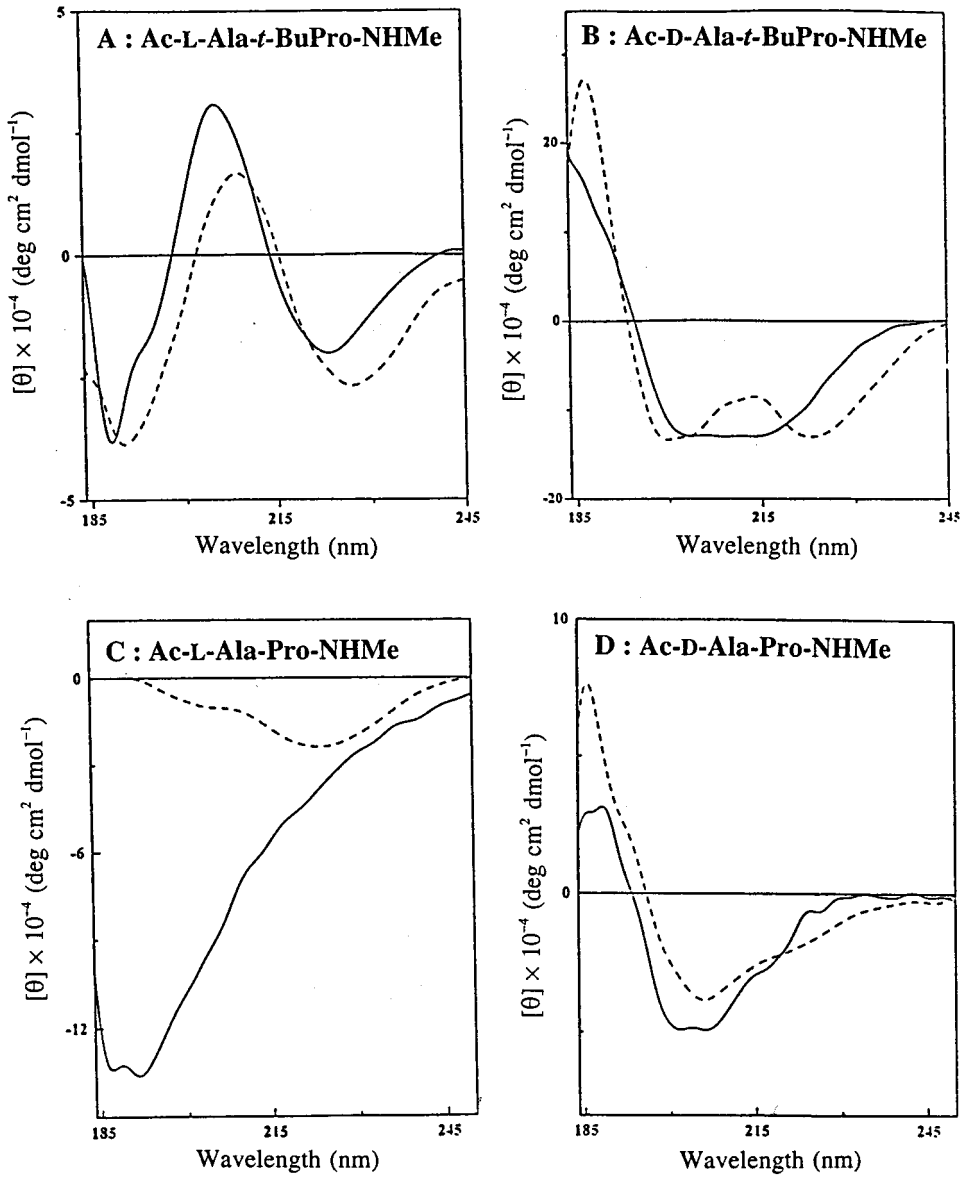

Figure 4 Circular dichroism spectra of *N*-(acetyl)dipeptide *N*′-methylamides (S)-**1a** (A), (*R*)-**1a** (B), (S)-**2a** (C) and (*R*)-**2a** (D) in water (—) and acetonitrile (---).

ideal values of type VIa and VIb β-turn conformations [9,25], as well as those for the L-Leu-Pro residues of the cyclic peptide evolidine [43] and the Bence–Jones immunoglobulin [44], which adopt type VIa and VIb β-turns, respectively, as observed in their X-ray structures. The ϕ_3 and ψ_3 dihedral angle values of peptide (*R*)-**1b** were similar to the values of a type VIb geometry. However, the ϕ_2 and ψ_2 dihedral angle of peptide (*R*)-**1b** possessed opposite values from those of the type VIb structure, an effect that could be attributed to the intermolecular hydrogen bonds in the unit cell. The distance between the carbons of the acetamide methyl group and the methylamide at the i and $i+3$ residues is 7.5 Å, indicative of a β-turn conformation.[1] The sterically bulky 5-*tert*-butyl substituent exhibited an influence on the amide bond *N*-terminal to the prolyl residue that distorted it from planarity. The X-ray structure of (S)-**1b** had shown the presence of a twisted amide geometry *N*-terminal to the 5-*tert*-butylprolyl residue with an ω dihedral angle value of 17° [9]. A more important effect was observed for (*R*)-**1b**, where the ω dihedral angle value was 29°.

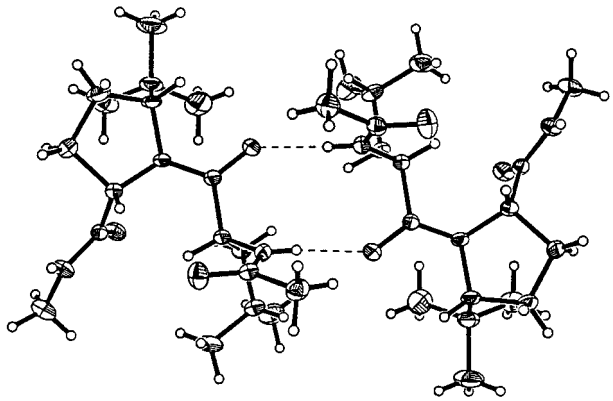

Figure 5 Two dipeptide turn structures (*R*)-**1b** and (*R*)-**1b**′ engaged in intermolecular hydrogen bonds between their leucyl residues in the crystal structure. Ellipsoids drawn at 40% probability level. Hydrogens represented by spheres of arbitrary size.[2]

CONCLUSIONS

Comparing the effects of 5-*tert*-butylproline and L-proline at the *C*-terminal of *N*-(acetyl)dipeptide *N*′-methylamides in which the *N*-terminal residue was varied using both L- and D-amino acids, we have itemized the influences of steric bulk and stereochemistry on prolyl amide geometry and peptide turn conformation. Proton NMR experiments indicated that prolyl dipeptides (*S*)-**2** and (*R*)-**2**, both adopted dominant amide *trans*-isomer geometry in chloroform, DMSO and water. Further, analysis by CD spectroscopy in water and acetonitrile revealed that prolyl peptides (*S*)-**2a** and (*S*)-**2b** possessed conformations that were dependent on solvent composition. On the other hand, type C curves were obtained for prolyl peptides (*R*)-**2a** and (*R*)-**2b**, which were unaffected by changes in solvent composition. The type C curve shape had previously been assigned to a type II′ β-turn conformation in cyclic peptides [41]. Prolyl peptide (*R*)-**2** was thus assumed to adopt a predominant type II′ β-turn conformation in solution, where its diastereomer (*S*)-**2** exhibited no preferred conformation.

The stereochemistry of the *N*-terminal residue in peptides possessing 5-*tert*-butylproline was shown to exhibit a significant effect on their conformation. Spectral analysis by NMR experiments in various solvents indicated that 5-*tert*-butylprolyl peptide (*S*)-**1** adopted predominantly the amide *cis*-isomer in a type VIa β-turn conformation whereas a type VIb β-turn conformation was exhibited by 5-*tert*-butylprolyl dipeptide (*R*)-**1**. This shift from type VIa to VIb β-turn conformation on switching the *N*-terminal amino acid stereochemistry from L- to D-configuration was also suggested by the significant differences in the CD curve shapes for (*S*)- and (*R*)-**1a** and **1b**: Ac-Xaa-5-*t*BuPro-NHMe (Xaa = Ala, Leu) exhibited, respectively, type B and type C spectra with L- and D-Xaa residues. The conformations adopted by (*S*)- and (*R*)-**1** were also shown by CD analysis to be independent of solvent composition. Because both B and C curve types have been associated with β-turn conformations possessing, respectively, L- and D-amino acids at the $i+1$ position, we may infer that a similar change in stereochemistry has given rise to this switch in type VI β-turn geometry for the *tert*-butylproline dipeptides. Accordingly, the different conformations adopted by the peptides (*S*)- and (*R*)-**1b** was supported by their X-ray structures. In the solid state, *N*-acetyl-L-leucyl-5-*tert*-butylproline *N*′-methylamide (S)-**1b** adopted a type VIa β-turn conformation with an intramolecular hydrogen bond between the acetamide oxygen and the methylamide hydrogen [9]. The *tert*-butylprolyl residue in the X-ray structure of *N*-acetyl-D-leucyl-5-*tert*-butylproline *N*′-methylamide (*R*)-**1b** adopted a type VIb geometry; however, the ϕ_2 and ψ_2 dihedral angle

Table 2 Comparison of the Dihedral Angles of Ideal Type VI β-Turn and X-Ray Structure of *N*-(Acetyl)-D-Leucyl-5-*tert*-butylproline *N*′-Methylamide (*R*)-**1b**

Entry	ϕ_2 (°)	ψ_2 (°)	ω (°)	ϕ_3 (°)	ψ_3 (°)
Ideal type VIa β-turn [25]	−60	120	0	−90	0
Ac-L-Leu-5-*t*-BuPro-NHMe (*S*)-**1b** [9]	−61	139	17	−95	19
L-Leu-Pro residues in X-ray of evolidine [43]	−65	151	2	−93	13
Ideal type VIb β-turn [25]	−120	120	0	−60	150
Ac-D-Leu-5-*t*-BuPro-NHMe (*R*)-**1b**	93	−141	29	−81	157
(*R*)-**1b**	95	−139	29	−82	164
L-Leu-Pro residues in X-ray of Bence–Jones immunoglobulin [44]	−88	154	−27	−56	149

values deviated from that of an ideal type VIb conformation, perhaps due to intermolecular hydrogen bonds within the unit cell. Thus, replacement of proline by 5-*tert*-butylproline in *N*-acetyl dipeptide *N*′-methylamides provided, respectively, type VIa and VIb β-turn conformations when the *N*-terminal amino acids possessed L-configuration and D-configuration. Steric interactions have thus been used to destabilize the natural predisposition for type II′ β-turn geometry and in turn augment the population of a rare type VI β-turn conformation. These results demonstrate further the importance of steric interactions and stereochemistry for dictating peptide secondary structure.

EXPERIMENTAL SECTION

General

Unless otherwise noted, all reactions were run under a nitrogen atmosphere and distilled solvents were transferred by syringe. CH_2Cl_2 was distilled over P_2O_5, CH_3CN was distilled over CaH_2, and DIEA was distilled over ninhydrin and CaH_2. Final reaction mixture solutions were dried over Na_2SO_4. Chromatography was on 230–400 mesh silica gel, and TLC was on aluminium-backed silica plates. Melting points are uncorrected. Mass spectral data, HRMS (EI and FAB) were obtained by the Université de Montréal Mass Spectroscopy facility.

NMR Measurements

^{1}H and ^{13}C NMR experiments were performed on Brucker DMX600 and ARX400 spectrometers. The chemical shifts are reported in ppm (δ units) downfield of the internal tetramethylsilane ($(CH_3)_4Si$). Coupling constants are in hertz. The chemical shifts for the carbons and the protons of the minor isomers are respectively reported in parentheses and in brackets. All 2D NMR experiments were carried out at a concentration of 5 mM. COSY, NOESY and ROESY spectra were obtained with 2048 × 512 data points. A mixing time of 500 ms was used for the NOESY and ROESY spectra.

Circular Dichroism Measurements

CD spectra of 0.1 mM solutions of peptides in H_2O and CH_3CN were measured on a Jasco J-710 spectropolarimeter using a circular quartz cell with a path length of 1 mm at 23°C. Band intensities are expressed as molar ellipticities [θ]. Spectra were run with a bandwidth of 1 nm, a response time of 0.25 s and a scan speed of 100 nm min^{-1}. Each measurement was the average result of ten repeated scans in steps of 0.2 nm. Baseline spectra of the solvents were subtracted.

General Procedure for Coupling to 5-*tert*-Butylproline *N*′-Methylamide

A solution of (2*S*, 5*R*)-5-*tert*-butylproline *N*′-methylamide hydrochloride (100 mg, 0.45 mmol) prepared according to [9], *N*-(BOC)-D-amino acid (0.54 mmol) and DIEA (0.3 ml, 1.8 mmol) in CH_2Cl_2 (4.5 ml) was cooled to 0°C, treated with BOP-Cl (138 mg, 0.54 mmol), stirred for 1 h and allowed to warm to room temperature with stirring for 18 h. Brine was added and the solution was extracted with EtOAc. The combined organic layers were washed with 0.1 M HCl (2 × 10 ml), 5% $NaHCO_3$ (2 × 10 ml) and brine (10 ml), dried and evaporated to a residue that was purified by chromatography on silica gel using 35% EtOAc in hexane as eluant. Evaporation of the collected fractions furnished *N*-(BOC)-dipeptide *N*′-methylamides.

N-(BOC)-(2*R*)-Alanyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-4a

This was obtained in 60% yield as a white solid: m.p. 165–166°C; $[\alpha]_D^{20}$ −122.9° (c 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.86 (s, 5 H) [0.89 (s, 4 H)], 1.18 (d, 1.7 H, $J = 6.6$) [1.23 (d, 1.3 H, $J = 6.5$)], [1.33 (s, 4 H)] 1.37 (s, 5 H), 1.80 (m, 1 H), 1.98 (m, 2 H), [2.22 (m, 0.4 H)] 2.56 (m, 0.6 H), 2.71 (d, 1.7 H, $J = 4.7$) [2.78 (d, 1.3 H, $J = 4.0$)], [3.52 (d, 0.4 H, $J = 4.0$)] 3.67 (d, 0.6 H, $J = 8.2$), 4.22 (m, 1 H), 4.52 (t, 1 H, $J = 8.5$), 4.50–1.72 (m, 1 H), [5.25 (m, 0.4 H)] 5.38 (d, 0.6 H, $J = 6.0$), [6.48 (br s, 0.4 H)] 7.33 (br s, 0.6 H); ^{13}C NMR ($CDCl_3$) δ (19.0) 19.2, 25.0 (25.7), 26.1 (30.7), 26.2, 27.3, 28.1, 35.3 (36.1), (47.6) 48.1, 61.7 (62.2), (65.7) 67.5, 79.4, 154.6 (155.3), 171.7 (172.6), 175.8 (176.4). HRMS calcd for $C_{18}H_{34}O_3N_3$ (MH^+) 340.2600, found 340.2618.

N-(BOC)-(2*R*)-Leucyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-4b

This was obtained in 62% yield as a white solid: m.p. 156–157°C; $[\alpha]_D^{20}$ −99.6° (c 0.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.81–0.99 (m, 15 H), 1.12 (m, 1 H), [1.36 (s, 3 H)] 1.40 (s, 6 H), 1.60–1.88 (m, 3 H), 2.01 (m, 1.4 H) [2.21 (m, 0.6 H)], 2.59 (m, 1 H), 2.75 (d, 2.1 H, $J = 4.7$) [2.80 (d, 0.9 H, $J = 4.5$), [3.70 (d, 0.3 H, $J = 7.9$)] 4.23 (m, 0.7 H), 4.54 (dd, 0.7 H, $J = 7.7$, 9.5) [4.60 (m, 0.3 H)], 4.78 (t, 1 H, $J = 8.4$),

[5.03 (d, 0.3 H, $J = 9.4$)] 5.23 (d, 0.7 H, $J = 8.3$), [6.46 (br s, 0.3 H)] 7.34 (br s, 0.7 H); ^{13}C NMR ($CDCl_3$) δ (20.8) 21.6, (23.1) 23.4, (24.2) 24.4, 25.0 (25.8), 26.0 (30.9), 26.2, 27.2, 28.1, 35.2 (36.1), (41.7) 43.5, (50.2) 50.7, 61.6 (62.2), (65.8) 67.4, 79.4, 155.0 (155.8), 171.8 (172.5), 176.0 (176.2). HRMS calcd for $C_{21}H_{40}O_4N_3$ (MH^+) 398.3019, found 398.3007.

N-(BOC)-(2*R*)-Phenylalanyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-4c

This was obtained in 64% yield as an oil: $[\alpha]_D^{20}$ $-124.4°$ (c 0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.71 (s, 7.2 H) [0.88 (s, 1.8 H)], [1.26 (s, 1.8 H)] 1.38 (s, 7.2 H), 1.47 (m, 2 H), 2.27 (m, 1 H), 2.69 (d, 3 H, $J = 4.3$), 2.79 (m, 1 H), 3.05 (dd, 1 H, $J = 4.8$, 12.4), 3.22 (d, 1 H, $J = 8.3$), 4.29 (m, 1.6 H) [4.36 (m, 0.4 H)], [4.66 (t, 0.2 H, $J = 8.5$)] 4.92 (m, 0.8 H), [5.22 (d, 0.2 H, $J = 8.7$)] 5.43 (d, 0.8 H, $J = 8.4$), 7.13–7.24 (m, 5 H), [6.31 (br s, 0.2 H)] 7.38 (br s, 0.8 H); ^{13}C NMR ($CDCl_3$) δ 24.6 (25.8), 24.8 (30.3), 26.2, 26.6 (27.4), 28.1, 35.0 (36.0), (39.2) 41.3, 53.6, 61.4 (62.3), (66.2) 67.9, 79.7, (126.4) 127.0, (128.1) 128.6, 129.0 (129.2), 135.8 (137.2), 154.4 (155.4), 171.7 (172.4), (175.1) 174.4. HRMS calcd for $C_{24}H_{38}O_4N_3$ (MH^+) 432.2862, found 432.2849.

General Procedure for Acetamide Synthesis

A solution of *N*-(BOC)dipeptide *N*′-methylamide (20.5 mg, 0.05 mmol) in 25% TFA in CH_2Cl_2 (1 ml) was stirred for 1 h and the solvant was evaporated. The residue was dissolved in CH_2Cl_2 (1 ml), treated with K_2CO_3 (65.6 mg, 0.5 mmol) and acetic anhydride (45 ml, 0.5 mmol) and stirred for 18 h. The solution was filtered, washed with CH_2Cl_2 (2×5 ml) and evaporated to give the *N*-acetyl dipeptide *N*′-methylamide.

N-Acetyl-(2*R*)-alanyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-1a

This was isolated in 98% yield as a white solid: m.p. 186–187°C; $[\alpha]_D^{20}$ $-125.7°$ (c 0.4, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.94 (s, 6.3 H) [1.0 (s, 2.7 H)], 1.29 (d, 2.1 H, $J = 6.7$) [1.35 (d, 0.9 H, $J = 6.4$)], 1.88 (m, 2 H), [1.99 (s, 0.9 H)] 2.05 (s, 2.1 H), 2.09 (m, 1 H), [2.29 (m, 0.3 H)] 2.69 (m, 0.7 H), 2.84 (d, 2.1 H, $J = 4.6$) [2.90 (d, 0.9 H, $J = 4.4$)], 3.79 (d, 0.7 H, $J = 8.6$) [4.30 (d, 0.3 H, $J = 8.7$)], [4.55 (t, 0.3 H, $J = 7.0$)] 4.60 (t, 0.7 H, $J = 8.5$), [4.73 (t, 0.3 H, $J = 9.2$)] 5.13 (m, 0.7 H), [6.20 (br s, 0.3 H)] 6.43 (d, 0.7 H, $J = 6.8$), [6.13 (br s, 0.3 H)] 7.34 (br s, 0.7 H); ^{13}C-NMR ($CDCl_3$) δ (18.4) 19.0, (22.6) 23.1, 25.2 (25.8), 26.1 (30.7), (26.2) 27.2, 27.3, 35.4 (36.2), (46.9) 47.1, 62.0 (62.2), (65.8) 67.6, 169.0 (170.2), 171.6 (172.7), 175.6 (175.9). HRMS calcd for $C_{15}H_{28}O_3N_3$ (MH^+) 298.2131, found 298.2120.

N-Acetyl-(2*R*)-leucyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-1b

This was isolated in 92% yield as a white solid that was recristallized from EtOAc and hexane: $[\alpha]_D^{20}$ $-73.8°$ (c 0.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ [0.87 (d, 1.2 H, $J = 6.3$)] 0.95 (d, 1.8 H, $J = 8.5$), [0.93 (d, 1.2 H, $J = 6.2$)] 1.05 (d, 1.8 H, $J = 6.5$), 0.96 (s, 5.4 H) [1.01 (s, 3.6 H)], [1.20 (m, 0.4 H)] 1.50 (m, 0.6 H), 1.79–1.96 (m, 2 H), [2.01 (s, 1.2 H)] 2.06 (s, 1.8 H), 2.08–2.16 (m, 2 H), 2.25 (m, 1 H), 2.65–2.75 (m, 1 H), 2.83 (d, 1.8 H, $J = 4.8$) [2.88 (d, 1.2 H, $J = 4.8$)], 3.80 (d, 0.6 H, $J = 8.0$) [4.27 (d, 0.4 H, $J = 8.5$)], 4.56–4.69 (m, 1.4 H), 5.21 (m, 0.6 H), [5.97 (d, 0.4 H, $J = 9.4$)] 6.19 (d, 0.6 H, $J = 8.5$), [6.31 (br s, 0.4 H)] 7.29 (br s, 0.6 H); ^{13}C-NMR ($CDCl_3$) δ (20.9) 21.6, (22.9) 23.1, (23.2) 23.4, (24.4) 24.6, 25.1 (30.8), (25.9) 26.0, 26.1 (26.3), 27.2 (27.3), 35.3 (36.2), (41.4) 43.5, (49.3) 49.4, 61.8 (62.4), (65.8) 67.5, 169.3, 171.7 (172.6), 175.7. HRMS calcd for $C_{18}H_{34}O_3N_3$ (MH^+) 340.2600, found 340.2594.

N-Acetyl-(2*R*)-phenylalanyl-(2*S*, 5*R*)-5-*tert*-butylproline *N*′-Methylamide (*R*)-1c

This was isolated in 94% yield as a white solid: m.p. 96–97°C; $[\alpha]_D^{20}$ $-108.0°$ (c 0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.67 (m, 1 H), 0.79 (s, 7.2 H) [0.98 (s, 1.8 H)], 0.90 (m, 1 H), 1.58 (m, 1 H), 2.07 (s, 1.6 H) [1.91 (s, 1.4 H)], 2.38 (m, 1 H), 2.83 (d, 1.6 H, $J = 4.8$), 2.85–2.89 (m, 2.4 H), 3.19 (dd, 0.8, $J =$ 4.9, 12.7) [3.27 (dd, 0.4 H, $J = 5.0$, 14.0)], 3.35 (dd, 0.8 H, $J = 1.4$, 8.7) [4.74 (m, 0.2 H)], [4.33 (d, 0.2 H, $J = 8.7$)] 4.40 (m, 0.8 H), [4.80 (t, 0.2 H, J = 8.4)] 5.36 (m, 1 H), 6.36 (d, 1 H, J = 8.1), 7.19–7.36 (m, 5 H), [6.06 (br s, 0.2 H)] 7.37 (br s, 0.8 H); ^{13}C-NMR ($CDCl_3$) δ 23.2, 24.7 (24.8), 26.3 (26.5), 26.7, 27.4, 35.2, 41.0, 52.5, 61.7, 68.0, 127.3, (128.4) 128.7, (128.5) 129.2, 135.7, 168.9, 171.7, 174.4. HRMS calcd for $C_{21}H_{32}O_3N_3$ (MH^+) 374.2444, found 374.2434.

General Protocol For The Synthesis of *N*-Acetyl Dipeptide *N*′-Methylamides Possessing Natural Proline

A solution of *N*-(BOC)-D-amino acid (2.1 mmol) in CH_3CN (10 ml) was treated with DIEA (0.7 ml, 4.4

mmol), proline *N*′-methylamide hydrochloride (150 mg, 1.1 mmol) and TBTU (0.68 g, 2.2 mmol), stirred at room temperature for 18 h, and partitioned between brine (10 ml) and EtOAc (10 ml). The organic phase was washed with 0.1 M HCl (2 × 8 ml), 5% $NaHCO_3$ (2 × 8 ml) and brine (10 ml), dried and evaporated to a residue that was purified by chromatography on silica gel (35% EtOAc in hexane). The *N*-(BOC)dipeptide *N*′-methylamide was treated with 25% TFA in CH_2Cl_2 (10 ml) for 1 h and evaporated. The resulting dipeptide *N*′-methylamide trifluoroacetate was dissolved in CH_2Cl_2 and treated in the same acetylation conditions as described above.

N-Acetyl-(2*R*)-alanylproline *N*′-Methylamide (*R*)-2a

This was isolated as a white precipitate in 56% overall yield for the three steps: $[\alpha]_D^{20}$ −61.6° (c 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ [1.31 (d, 0.18 H, $J = 6.8$)] 1.39 (d, 2.82 H, $J = 6.9$), 1.94–2.04 (m, 1 H), 2.05 (s, 3 H), 2.06–2.15 (m, 2 H), 2.42 (m, 1 H), 2.81 (d, 2.82 H, $J = 4.8$) [2.90 (d, 0.18 H, $J = 4.7$)], 3.51 (q, 0.94 H, $J = 6.9, 9.4$) [3.66 (m, 0.06 H), 3.92 (m, 1 H), 4.62 (m, 1.88 H) [4.71 (m, 0.12 H)], 6.30 (d, 1 H, $J = 5.1$), 6.90 (br s, 1 H); ^{13}C-NMR ($CDCl_3$) δ 16.1, 22.3, 24.0, 26.2, 29.0, 46.9, 47.6, 60.6, 171.3, 171.5, 172.5; HRMS calcd for $C_{11}H_{20}O_3N_3$ (MH^+) 242.1505, found 242.1498.

N-Acetyl-(2*R*)-leucylproline *N*′-Methylamide (*R*)-2b

This was isolated as an oil in 52% overall yield for the three steps: $[\alpha]_D^{20}$ −52.4° (c 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ [0.89 (d, 0.3 H, $J = 6.6$), 0.93 (d, 0.3 H, $J = 6.6$)] 1.01 (d, 5.4 H, $J = 6.6$), 1.52 (m, 1 H), 1.73 (m, 1 H), 2.03 (m, 1 H), 2.05 (s, 3 H), 2.41 (m, 1 H), 2.79 (d, 2.7 H, $J = 4.8$) [2.88 (d, 0.3 H, $J = 4.8$)], 3.50 (m, 0.9 H) [4.63 (m, 0.1 H), 4.00 (m, 1 H), 4.60 (m, 1.8 H) [4.65 (m, 0.1 H), 4.73 (m, 0.1 H)], 6.19 (d, 1 H, $J = 6.6$), 6.95 (br s, 1 H); ^{13}C-NMR ($CDCl_3$) δ 21.6, 22.2, 23.2, 23.9, 24.5, 26.1, 29.1, 39.6, 46.8, 50.6, 60.7, 171.5, 172.0, 172.6. HRMS calcd for $C_{14}H_{26}O_3N_3$ (MH^+) 284.1974, found 284.1983.

N-Acetyl-(2*R*)-phenylalanylproline *N*′-Methylamide (*R*)-2c

This was isolated as an oil in 75% overall yield for the three steps: $[\alpha]_D^{20}$ −107.2° (c 0.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.60 (m, 1 H), 1.65 (m, 1 H), 1.84 (m, 1 H), 2.04 (s, 3 H), 2.26 (m, 1 H), 2.63 (m, 1 H), [2.76 (d, 0.1 H, $J = 5.1$)] 2.78 (d, 2.9 H, $J = 4.8$), 3.05 (m, 2 H), 3.66 (m, 1 H), 4.49 (d, 1 H, $J = 8.1$), 4.71 (m, 1 H), 6.31 (d, 1 H, $J = 5.9$), 6.91 (br s, 1 H), 7.25–7.36 (m, 5 H); ^{13}C-NMR ($CDCl_3$) δ 22.1, 23.6, 26.1, 28.9, 37.3, 46.6, 54.1, 60.5, 127.2, 128.5, 129.1, 135.6, 171.6, 171.8. HRMS calcd for $C_{17}H_{24}O_3N_3$ (MH^+) 318.1818, found 318.1832.

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NOTES

1. The interatomic distance between the α carbons of the i and $i+3$ residues in a β-turn conformation has been defined to be between 4–7 Å [45].
2. The structure of (*R*)-**1b** was solved at l'Université de Montréal X-ray facility using direct methods (SHELXS96) and refined with NRCVAX and SHELXL96: $[C_{18}H_{33}N_3O_3]_2$ $[C_4H_8O_2]$; $M_r = 767.052$; orthorhombic, colorless crystal; space group $P2_12_12_1$; unit cell dimensions (Å) $a = 9.685(3)$, $b = 18.001(11)$, $c = 26.586(17)$; volume of unit cell (Å^3) 4635(4); $Z = 4$; $R_1 = 0.0619$ for $F^2 > 2\sigma(F^2)$, $wR_2 = 0.1534$ for all data; GoF = 1.056. The author has deposited the atomic coordinates for the structure of (*R*)-**1b** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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