Use of Steric Interactions To Control Peptide Turn Geometry. Synthesis of Type VI *β*-Turn Mimics with 5-*tert*-Butylproline

Liliane Halab and William D. Lubell*

Département de chimie, Université de Montréal, C. P. 6128, Succursale Centre Ville, Montréal, Québec, Canada H3C 3J7

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The influences of steric interactions on peptide geometry were studied to develop a novel means for generating type VIa *β*-turn mimics. (2*S*,5*R*)-5-*tert*-Butylproline and L-proline were respectively introduced at the *C*-terminal residue of *N*-(acetyl)dipeptide *N*′-methylamides **1** and **2**. The relative populations of prolyl *cis*- and *trans*-amide isomers in dipeptides **1** and **2** were measured in chloroform, DMSO, and water by proton NMR spectroscopy. Although the *trans*-amide isomer was favored in prolyl peptide **2**, the Xaa-Pro peptide bond adopted preferably the *cis*-amide isomer in the case of 5-*tert*-butylprolyl peptide **1**. Measurements of the influence of solvent and temperature on the chemical shift values for the amide proton signals of **1** in the *cis*-amide conformer indicated that the *N*′-methylamide was engaged in a hydrogen bond with the acetamide carbonyl in a type VIa *β*-turn conformation. Analysis of *N*-(acetyl)leucyl-5-*tert*-butylproline *N*′-methylamide (**1d**) in the solid state by X-ray diffraction showed the *cis*-amide conformer which adopted a geometry characteristic of the central, $i + 1$ and $i + 2$ residues of an ideal type VIa *β*-turn. In contrast to prolyl peptides **2b** and **2d**, *N*-(acetyl)alanyl- and *N*-(acetyl)leucyl-5-*tert*-butylproline *N*′-methylamides (**1b** and **1d**) maintained ordered *β*-turn conformations in solution that were shown to be independent of solvent composition by a comparison of their circular dichroism spectra obtained in water and acetonitrile. The NMR, X-ray, and CD data all confirm that the steric interactions of the 5-*tert*-butylprolyl residue induced dipeptide **1** to adopt a type VIa *β*-turn conformation.

Introduction

The spatial requirements for peptide biology may be elucidated by employing constrained analogues of native secondary structures to probe relationships between conformation and activity. Because of the importance of *β*-turns in protein folding and recognition,[1] considerable effort has focused on developing conformationally restricted mimics of the backbone geometry, intramolecular hydrogen bonding, and side-chain orientations exhibited by these secondary structures.[2] Among such designs, the use of modified prolines has often led to successful surrogates because of the high frequency of this amino acid at the central residues of the *β*-turn conformation.[2,3] We report now the use of the steric interactions of 5-*tert*-butylproline to generate conformationally rigid mimics of the type VIa *β*-turn.

The type VI *β*-turn is a unique secondary structure that features an amide *cis*-isomer *N*-terminal to a prolyl residue situated at the $i + 2$ position of the peptide bend.[4]

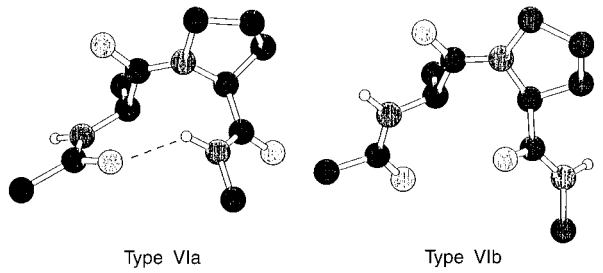

Figure 1. Central, $i + 1$ and $i + 2$ residues of type VIa and VIb turn conformations found respectively in ribonuclease S[5b] and Bence-Jones protein.[5c] Only amide protons shown (C, black; N, dark gray; O, light gray; H, white).

Two classes of type VI *β*-turns have been defined based on the backbone dihedral angle values of their central, $i + 1$ and $i + 2$ residues (Figure 1).[1,4] In the type VIa *β*-turn, the proline *ψ*-dihedral angle is near 0° and an intramolecular hydrogen bond exists between the carbonyl oxygen of the i and amide hydrogen of the $i + 3$

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residues. The proline ψ-dihedral angle is situated around 150° in the type VIb geometry which cannot form an intramolecular hydrogen bond. Identified on the surfaces of globular proteins,[5] type VI β-turns are commonly located in cyclic peptides possessing prolyl residues.[6] In addition, the minor *cis*-amide conformers of certain linear peptides, particularly those possessing aromatic amino acids *N*-terminal to proline and pipecolate residues, have been observed to adopt type VI β-turn geometry.[7−10]

Type VI β-turn conformations play important roles in the recognition and reactivity of bioactive peptides and proteins. For example, a type VI β-turn conformation has been suggested as a requirement for thrombin-catalyzed cleavage of the V_3 loop of HIV gp120, a prerequisite to viral infection.[11] Furthermore, peptidyl prolyl isomerases (PPIases) which catalyze the isomerization of Xaa-Pro amide bonds and thereby accelerate the folding of particular proteins have been proposed to bind preferably to peptides possessing type VI β-turn conformations.[12,13] Computational analysis revealed that the PPIase FKBP bound *N*-acetyl-Leu-Pro-Phe-methylamide in a type VIa β-turn.[12] In the solid state, the tetrapeptide substrate, *N*-acetyl-Ala-Ala-Pro-Ala-amidomethylcoumarin was shown by X-ray diffraction to adopt a type VIb β-turn geometry when bound to the PPIase cyclophilin.[13] In addition, the nature of the amino acid (Xaa) *N*-terminal to the prolyl residue was found to influence PPIase activity.[14]

Among the *cis*-amide prolyl peptide surrogates, competent replacements for the backbone geometry of the central residues of the type VIa β-turn have been synthesized by tethering the α-carbon of the *N*-terminal amino acid residue to the proline 2-position in a dipeptide lactam.[15,16] These azabicycloalkane amino acids have been effectively used in constrained analogues of peptides that require a type VI β-turn for bioactivity.[15b] In model peptides, these dipeptide lactams have oriented the *N*- and *C*-terminal amides to form intramolecular hydrogen bonds in 10-member β-turn and 14-member β-hairpin secondary structures.[16c,d] Although they may replicate the backbone and hydrogen-bonding elements of type VI β-turns, because of difficulties in appending substituents onto the *N*-terminal amino acid residue of dipeptide lactams, these azabicycloalkane amino acids do not effectively mimic side-chain pharmacophores that may influence peptide turn recognition.[14] A second approach for replicating the type VIa β-turn, which may allow diversification at the $i + 1$ position, has employed azaproline analogues in which the prolyl α-carbon is replaced by nitrogen.[17] In the solid state, azaproline analogues adopted a type VIa conformation as demonstrated by X-ray diffraction.[17a] Furthermore, spectroscopic studies of *N*-(BOC)alanyl-azaprolinyl-alanine *N*′-isopropylamides by NMR in solution indicated that intramolecular hydrogen bonding was maintained in a type VIa β-turn conformation as solvent composition was changed from chloroform to DMSO.[17b] Although azaproline is not chiral and the configurations of the neighboring residues may influence the ring puckering and ψ-dihedral angle of this prolyl residue in peptides, this approach may provide a variety of type VI β-turn mimics should the pyrazolidine moiety be efficiently introduced into peptide structures. Type VI β-turn surrogates may also be procured from alternative strategies for replicating *cis*-amide geometry, such as cyclo-cystine[18] and cyclolanthione[19] derivatives, diazabicycloalkane amino acids,[20] and heterocycle[21] olefin[22] and fluoro-olefin[23] amide bond replacements; however, less is known about the influence of these constraints on the overall peptide conformation. Moreover, approaches involving amide isosteres may not be readily amenable to the construction Xaa-Pro dipeptide surrogate libraries by diversification of the *N*-terminal amino acid.

Toward an approach for generating libraries of conformationally constrained type VI β-turn mimics, we have employed 5-alkylprolines to control the prolyl amide isomer geometry. The steric interactions between a 5-*tert*-butyl substituent and the *N*-terminal residue disfavor the Xaa-Pro peptide bond *trans*-isomer and increase the *cis*-isomer population.[24] By studying analogues of *N*-(acetyl)-proline *N*′-methylamide, we demonstrated that incorporation of 5-*tert*-butylproline into this model peptide increased the *cis*-isomer population, influenced the energy barrier for prolyl amide isomerization, and restricted the proline ψ-dihedral angle.[24] We have now incorporated (2*S*,5*R*)-5-*tert*-butylproline at the *C*-terminal of a series of *N*-(acetyl)dipeptide *N*′-methylamides **1** in order to

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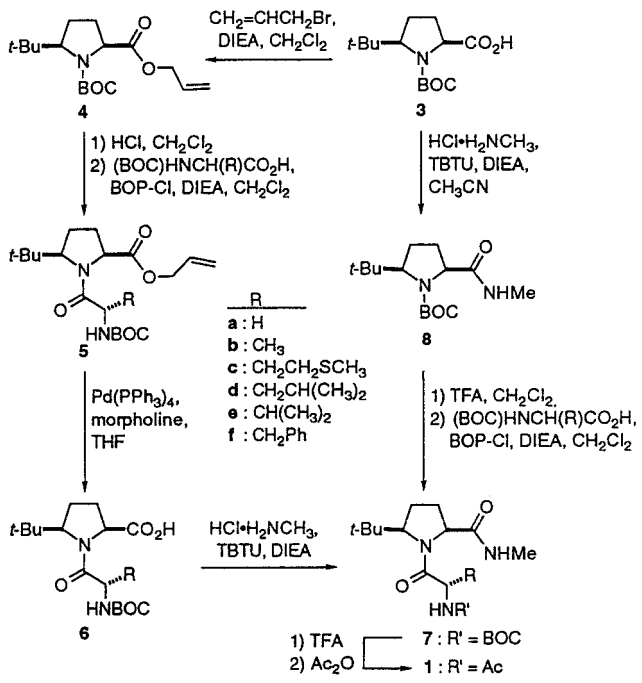
Scheme 1. Synthesis of *N*-(Acetyl)dipeptide *N′*-Methylamides 1



examine if the constraints on the prolyl ω- and ψ-dihedral angles would stabilize the type VI β-turn geometry. Conformational analyses of dipeptides **1** by NMR experiments, X-ray diffraction and circular dichroism spectroscopy, and comparison of **1** with dipeptide counterparts **2** possessing natural proline all have shown that (2*S*,5*R*)-5-*tert*-butylproline stabilized the type VIa β-turn geometry possessing an intramolecular hydrogen bond between the *N′*-methylamide proton and the acetamide carbonyl. By simply coupling a variety of different amino acid residues to the *N*-terminal of (2*S*,5*R*)-5-*tert*-butylproline, we have demonstrated the means for generating a library of dipeptide surrogates that mimic the backbone geometry, hydrogen bonding, and side-chain elements of type VIa β-turns. These 5-*tert*-butylproline surrogates may thus reproduce both the structural and recognition elements of type VI β-turns.

Results

Synthesis of Ac-Xaa-Pro-NHMe Dipeptides 1 and 2. (2*S*,5*R*)-*N*-(BOC)-5-*tert*-butylproline (**3**) was synthesized in seven steps from glutamic acid as an inexpensive chiral educt using our acylation/diastereoselective reductive amination sequence.[25] Two routes were investigated to introduce **3** into *N*-acetyl-Xaa-5-*tert*-butylproline *N′*-methylamides **1** (Scheme 1). In the first route, *N*-(BOC)-5-*tert*-butylproline allyl ester (**4**) was synthesized by alkylation of acid **3** with allyl bromide and DIEA in dichloromethane. Solvolysis of the BOC group with HCl gas in dichloromethane and coupling to *N*-(BOC)amino acids provided the *N*-(BOC)-dipeptide allyl esters **5** that were converted to *N*-(BOC)-dipeptides **6** by palladium-catalyzed ester cleavage. We have introduced *N*-BOC-Xaa-5-*tert*-butylprolines **6** into peptide structures by conventional coupling techniques using both solution- and solid-phase strategies.[26] In the context of the present project, dipeptides **6** were coupled to methylamine using benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[27] in acetonitrile to furnish *N*-BOC-Xaa-5-*tert*-butylproline *N′*-methylamides **7**. The respective *N*-acetyl-Xaa-5-*tert*-butylproline *N′*-methylamides **1** were synthesized from **7** by solvolysis of the BOC group with trifluoroacetic acid and *N*-acetylation with acetic anhydride and potassium carbonate in dichloromethane.

In the second route, *N*-(BOC)-5-*tert*-butylproline (**3**) was coupled to methylamine using TBTU in acetonitrile to provide *N*-(BOC)-5-*tert*-butylproline *N′*-methylamide (**8**).[24] Solvolysis of the BOC group with TFA in dichloromethane and coupling to *N*-(BOC)-amino acids provided *N*-BOC-Xaa-5-*tert*-butylproline *N′*-methylamides **7** that were acetylated as described above. Among the reagents explored for coupling to the *N*-terminal of 5-*tert*-butylproline allyl ester and 5-*tert*-butylproline *N′*-methylamide, *N*,*N′*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOPCl)[28] in CH_2Cl_2 at 5 °C gave the best yields of protected dipeptides **5** and **7**.[29] For comparison with dipeptides possessing natural L-proline, we synthesized *N*-(acetyl)alanylproline *N′*-methylamide (**2b**) and *N*-(acetyl)leucylproline *N′*-methylamide (**2d**) by respectively coupling *N*-(BOC)alanine and *N*-(BOC)leucine to proline *N′*-methylamide, followed by BOC group solvolysis and acetylation as described in the Experimental Section.

Conformational Analysis of Prolyl Dipeptides 1 and 2 by NMR Spectroscopy. The relative populations of the amide *cis*- and *trans*-isomers *N*-terminal to the prolyl residues of peptides **1** and **2** were ascertained by NMR spectroscopy in chloroform, dimethyl sulfoxide, and water. The amide populations of **1** and **2** could be determined for all peptides in water; however, coalescence of signals prevented their measurement for *N*-acetylglycyl-5-*tert*-butylproline *N′*-methylamide (**1a**) in chloroform and DMSO at room temperature. The *cis*-isomer was assigned on the basis of observation of the cross-peak arising from the nuclear Overhauser effect between the *N*-terminal amino acid and proline α-hydrogens in the NOESY and ROESY spectra in DMSO. The populations of the amide isomers were measured by integration of the isomeric *tert*-butyl singlets and *N′*-methyl doublets in the ^{1}H NMR spectra of **1** and **2**. The *tert*-butyl singlet of the amide *trans*-isomer appeared always downfield from that of the *cis*-isomer. The ratios of amide isomers in **1b**−**f** and **2** for each solvent are listed as the percent of *cis*-isomer in Table 1.

As is typically observed for linear prolyl peptides,[7−11] the major conformer of peptides **2** possessed the *trans*-amide geometry *N*-terminal to the prolyl residue. On the other hand, the major conformer of peptides **1** adopted the *cis*-amide geometry *N*-terminal to the 5-*tert*-butylprolyl residue. *N*-Acetyl-glycyl-5-*tert*-butylproline *N′*-meth-

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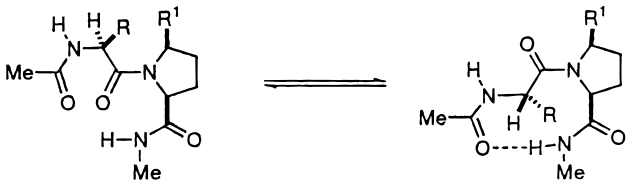
Table 1. Influence of Solvent on the Chemical Shifts and Amide Isomer Equilibrium of 1 and 2[a]



Trans-Isomer *Cis*-Isomer

			% *cis*-isomer ± 3%			($CDCl_3$)		($CDCl_3$ → DMSO)		($CDCl_3$ → D_2O)	
entry	R	R^1	D_2O	DMSO	$CDCl_3$	δ NH^{Xaa}	NH^{Me}	$\Delta\delta$ NH^{Xaa}	NH^{Me}	$\Delta\delta$ NH^{Xaa}	NH^{Me}
1b	CH_3	*t*-Bu	79	79	83	6.07	8.30	2.39	0.28	2.22	0.28
1c	$CH_2CH_2SCH_3$	*t*-Bu	74	72	73	6.41	8.27	2.08	0.32	1.95	0.16
1d	$CH_2CH(CH_3)_2$	*t*-Bu	81	67	85	5.97	8.27	2.43	0.22	2.27	0.12
1e	$CH(CH_3)_2$	*t*-Bu	81	73	89	6.18	8.48	2.13	0.32	1.86	0.13
1f	CH_2Ph	*t*-Bu	90	79	89	6.09	8.37	2.53	0.35		0.26
2b	CH_3	H	14	30	19	6.33	6.61	1.79	1.06	1.82	1.20
2d	$CH_2CH(CH_3)_2$	H	19	17	20	6.03	6.68	2.00	1.65	2.13	1.10

[a] Values are for the major conformer at 5 mM concentration.

ylamide (**1a**) exhibited only 55% *cis*-isomer population in water, which was similar to the amount of *cis*-isomer (48%) previously observed with (2*S*,5*R*)-*N*-acetyl-5-*tert*-butylproline *N*′-methylamide and indicated that the additional acetamide group had a limited influence on the prolyl amide equilibrium.[24] The presence of an alkyl substituent at the α-position of the *N*-terminal amino acid residue augmented significantly the *cis*-isomer population in peptides **1b**−**f** (Table 1). Additional alkyl branching at the β- and γ-positions gave a relatively minor increase to the *cis*-isomer population. As previously noted in prolyl peptides,[8−10] the presence of an aromatic amino acid *N*-terminal to proline caused a notable increase in the population of the *cis*-isomer in water and *N*-acetylphenylalanyl-5-*tert*-butylproline *N*′-methylamide (**1f**) exhibited the largest amounts of *cis*-amide among the examples studied.

In the major *cis*-amide conformer of peptides **1**, the signal for the *N*′-methylamide proton was 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 signal appeared between 8.27 and 8.48 ppm at the same time the acetamide proton signal came between 5.97 and 6.41 ppm (Table 1). The downfield shifted amide proton signal was indicative of an intramolecular hydrogen bond between the *N*′-methylamide proton and the acetamide carbonyl in a type VI β-turn conformation.[30]

The signal for the *N*′-methylamide proton was much less affected by changes in solvent relative to the signal for the acetamide proton in the NMR spectra for the *cis*-amide conformer of dipeptides **1**. The *N*′-methylamide proton signal was shifted 0.22−0.35 ppm downfield on switching solvent from chloroform to DMSO and 0.12−0.28 ppm downfield on changing solvent from chloroform to water (Table 1). On the contrary, the signal for the acetamide proton was shifted 2.08−2.53 ppm downfield on switching solvent from chloroform to DMSO and 1.86−2.27 ppm downfield on changing solvent from chloroform to water. The influence of solvent on the chemical shifts of the amide proton signals supported a type VI β-turn conformation for the *cis*-amide conformer of **1** by indicating that the *N*′-methylamide proton was engaged in an intramolecular hydrogen bond.[30]

Table 2. Influence of Temperature on the N−H Chemical Shifts of *N*-(Acetyl)dipeptide *N*′-Methylamides 1 and 2 in DMSO

				$\Delta\delta/\Delta T$ (−ppb/K)	
entry	R	R^1	isomer	NH^{Xaa}	NH^{Me}
1b	CH_3	*t*-Bu	*cis*	5.6	3.7
			trans	4.9	4.4
2b	CH_3	H	*cis*	6.3	4.3
			trans	5.7	4.4
1d	$CH_2CH(CH_3)_2$	*t*-Bu	*cis*	5.3	3.0
			trans	4.3	4.4
2d	$CH_2CH(CH_3)_2$	H	*cis*	6.1	4.1
			trans	5.6	5.3

A comparison of the measured temperature coefficients for the amide protons in peptides **1b**, **1d**, **2b**, and **2d** in DMSO provided additional evidence for a type VI β-turn conformation in **1** (Table 2). In the case of the *cis*- and *trans*-conformers of Ac-Ala-Pro-NHMe (**2b**) and Ac-Leu-Pro-NHMe (**2d**), all of the amide proton signals exhibited chemical shift temperature coefficients that were less than −4 ppb/K, within the range for unstructured peptides.[31] Similarly, in the case of the corresponding *tert*-butylprolyl peptides **1b** and **1d**, the amide signals for the minor *trans*-amide conformer and the acetamide proton signal of the *cis*-amide conformer all possessed chemical shift temperature coefficients less than −4 ppb/K in DMSO. Only the *N*′-methylamide proton signal for the major *cis*-amide conformer of *tert*-butylprolyl peptides **1b** and **1d** exhibited a temperature coefficient that was greater than −4 ppb/K in DMSO. Although values greater than −3 ppb/K have been suggested to indicate a solvent-shielded amide proton engaged in an intramolecular hydrogen bond in DMSO,[31] such temperature coefficients are usually measured on cyclic peptides and peptide structures larger than those studied in this report. The respective values of −3.7 and −3.0 ppb/K for the *N*′-methylamide proton signal of the major *cis*-amide conformer of dipeptides **1b** and **1d** fall into the region between temperature coefficients associated with hydrogen-bonded and solvent-exposed amide; however, their size is most probably due to the inability of the acetamide and *N*′-methyl groups in model peptides **1** to provide adequate solvent shielding of the hydrogen-bound *N*′-methylamide proton.

(30) Reviewed in: Dyson, H. J.; Wright, P. E. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 519.

(31) Reviewed in: Kessler, H. *Angew Chem., Int. Ed. Engl.* **1982**, *21*, 512.

Smaller values were consistently measured for the vicinal coupling constant ($^3J_{NH,\alpha}$) between the amide and α-protons of the *N*-terminal amino acid residue in the major *cis*-amide conformer of *tert*-butylprolyl peptides **1b**−**f** than were observed for the minor *trans*-amide conformer of **1** and the *cis*- and *trans*-amide conformers of **2**. For example, $^3J_{NH,\alpha}$ values were 0.8−3.5 Hz lower for the *cis*-amide than for the *trans*-amide conformer of **1b**−**f** in DMSO. The $^3J_{NH,\alpha}$ values varied from 5.2 to 6.4 Hz for the *cis*-amide conformers of **1b**−**f** in DMSO. Since the ideal ϕ-dihedral angle of −60° for the $i + 1$ residue in a type VIa β-turn corresponds to a $^3J_{NH,\alpha}$ of 4.2 Hz, the observed reduced coupling constant values support the hypothesis that the major *cis*-amide conformer of peptides **1b**−**f** adopts a significant population of type VI β-turn in solution.[32]

Evidence for the presence of a twisted amide geometry in solution was obtained by examining the chemical shift value for the carbonyl carbon of the *N*-terminal amino acid residue in peptide **1b**.[33] After assigning the amide carbonyl resonances by using two-dimensional HMBC NMR experiments,[34] we observed that 5-*tert*-butylprolyl peptide **1b** exhibited carbonyl chemical shift values for the *N*-terminal amino acid residue that were 3.1 ppm downfield relative to those of prolyl peptide **2b** in water. Because inhibition of amide resonance by factors that distort the N−C(O) bond deshields the carbonyl carbon,[33a] the downfield-shifted ^{13}C NMR chemical shift value indicated that the prolyl amide bond of **1b** was twisted from planarity by steric interactions between the *N*-terminal residue and 5-*tert*-butyl substituent.

X-ray Crystallographic Analysis of *N*-Acetyl-L-leucyl-5-*tert*-butylproline *N*′-Methylamide (1d). Crystals of *N*-acetyl-L-leucyl-5-*tert*-butylproline *N*′-methylamide (**1d**) were grown from a mixture of ether and hexane. Crystallographic analysis of **1d** by X-ray diffraction demonstrated the presence of the amide *cis*-isomer *N*-terminal to the 5-*tert*-butylprolyl residue (Figure 2).[35] Furthermore, the dihedral angles of peptide **1d** resembled those of the central, $i + 1$ and $i + 2$ residues of a type VIa β-turn. For comparison, the dihedral values for the crystal structure of **1d** are listed in Table 3 with those of an ideal type VIa geometry[4] and the values for the Leu-Pro residues found in the central positions of the type VIa β-turn in the X-ray structure of the cyclic peptide evolidine.[6d] An intramolecular hydrogen bond between the *N*′-methylamide proton and the acetamide carbonyl oxygen was clearly inferred from the interatomic distance of 2.13 Å in the X-ray structure of **1d**.

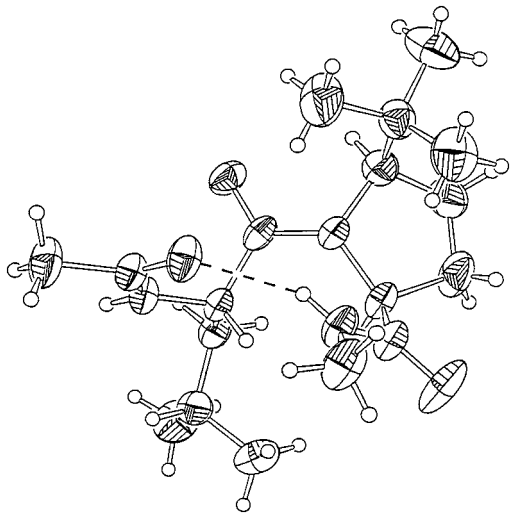


Figure 2. ORTEP view of Ac-Leu-5-*t*-BuPro-NHMe **1d**. Ellipsoids drawn at 40% probability level. Hydrogens represented by spheres of arbitrary size.[35]

Table 3. Comparison of the Dihedral Angles of Ideal Type VIa β-Turn and X-ray Structure of *N*-(Acetyl)leucyl-5-*tert*-butylproline *N*′-Methylamide 1d

entry	ϕ_2	ψ_2	ω	ϕ_3	ψ_3
ideal type VIa β-turn[4]	−60°	120°	0°	−90°	0°
Ac-Leu-5-*t*-BuPro-NHMe **1d**[35]	−61°	139°	17°	−95°	19°
Leu-Pro residues in X-ray of evolidine[6d]	−65°	151°	2°	−93°	13°

The ω, ψ, and ϕ values for the dihedral angles of the 5-*tert*-butylprolyl residue in the X-ray structure of peptide **1d** were similar to those calculated for the energy minimum of the *cis*-isomer of *N*-acetyl-5-*tert*-butylproline *N*′-methylamide.[24] A twisted amide conformation *N*-terminal to the 5-*tert*-butylprolyl residue was observed on measuring the ω-dihedral angle value of 17.3° for **1d** in the X-ray structure and indicated that the bulky 5-position substituent skewed the amide bond away from planarity.[33] The twisted amide geometry was substantiated by the 1.36 Å carbonyl carbon to nitrogen bond distance for the amide *N*-terminal to the 5-*tert*-butylprolyl residue, which was longer than the 1.33 Å bond lengths for the other amides.[33b] The measured ψ-dihedral angle value of 18.5° placed the *N*′-methylamide hydrogen at a 2.45 Å interatomic distance from the prolyl nitrogen. These constraints account for the observed acceleration of amide isomerization *N*-terminal to (2*S*,5*R*)-5-*tert*-butylproline. Ground-state destabilization results from the bulky 5-*tert*-butyl substituent distorting the amide bond away from planarity. Stabilization of the pyramidalized amide transition state arises from the *N*′-methylamide hydrogen interacting with the nitrogen lone pair of the rotating prolyl amide.[12,36] In the case of *N*-(acetyl)-glycyl-5-*tert*-butylproline *N*′-methylamide (**1a**), such an acceleration of the rate of prolyl amide isomerization was illustrated by the coalescence of the isomeric *cis*- and *trans*-amide signals in DMSO and chloroform at room

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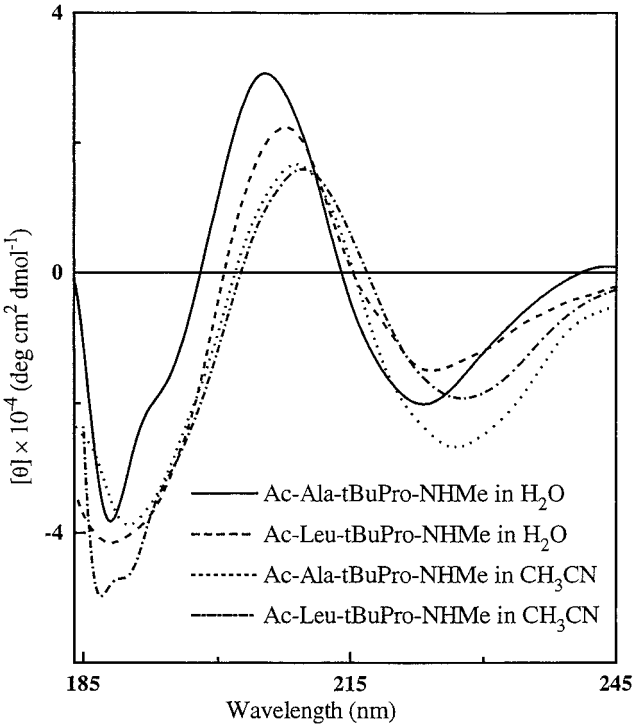

Figure 3. Circular dichroism spectra of *N*-(acetyl)alanyl- and *N*-(acetyl)leucyl-5-*tert*-butylproline *N*′-methylamides (**1b** and **1d**) in water and acetonitrile.

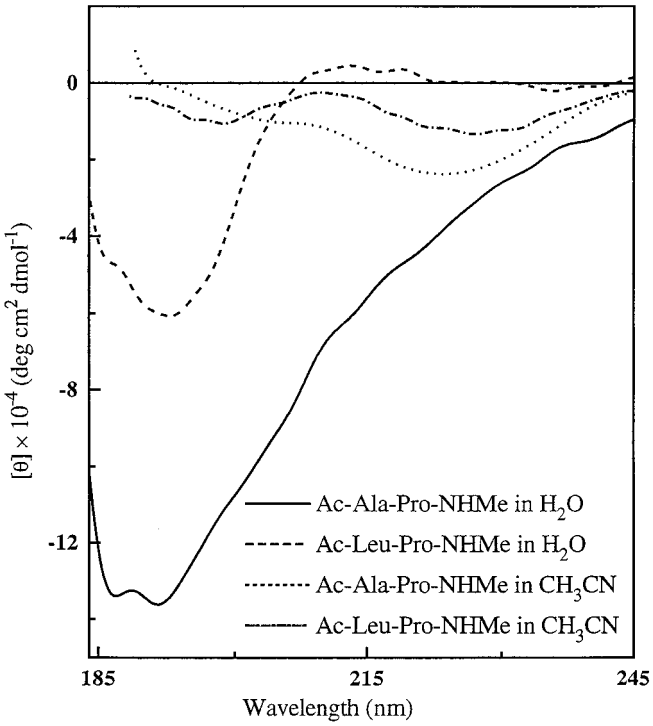

Figure 4. Circular dichroism spectra of *N*-(acetyl)alanyl- and *N*-(acetyl)leucyl-proline *N*′-methylamides (**2b** and **2d**) in water and acetonitrile.

temperature, because amide isomerization *N*-terminal to proline proceeded faster in the nonprotic and comparatively nonpolar solvent than in water which stabilized the polar amide ground states relative to the less polar transition state.[37]

Conformational Analysis of Dipeptides 1 and 2 by Circular Dichroism Spectroscopy. Circular dichroism (CD) spectra of **1b**, **1d**, **2b**, and **2d** were measured in both water and acetonitrile to examine the influence of solvent composition on peptide conformation. The CD spectra of **1b** and **1d** in acetonitrile exhibited a strong negative band at 188 nm, a strong positive band at 209 nm, and a weak negative band at 227 nm (Figure 3). This type of CD curve shape has previously been assigned to β-turn conformations in studies of model peptides in water.[38] Aside from a slight blue shift,[39] the shape of the CD curves for **1b** and **1d** remained constant as solvent was changed from acetonitrile to water. The type VIa β-turn conformation adopted by 5-*tert*-butylprolyl peptide **1** was thus shown to be independent of solvent composition. On the other hand, the CD spectral characteristics of prolyl peptides **2** were similar to those reported for *N*-(acetyl)proline *N*′-methylamide and varied significantly with changes in solvent composition exhibiting a n$-\pi^*$ band near 225 nm in acetonitrile that shifted to a significant $\pi-\pi^*$ band near 195 nm in water (Figure 4).[40]

Discussion

Steric interactions have been employed to restrain peptide geometry and favor particular secondary structures. For example, amino isobutyric acid (Aib) residues can create steric interactions with neighboring residues that favor 3_{10}- and α-helical geometries such as those found in antibiotic peptides such as althemethicin.[41] By similar interactions, the related α,α-dialkyl glycines induce 3_{10}-helices and type III β-turn geometries in linear peptides.[42] In pioneering studies of substituted polyproline oligomers, the steric effects of 2-methylproline were found to stabilize polyproline type II helical conformation.[43] Recently, the combination of $A^{1,3}$- and $A^{1,2}$-strain of alkyl-substituted olefin amide bond isosteres has been used to induce the formation of β-turn conformations exhibiting 10-member intramolecular hydrogen bonds[44] and a β-hairpin mimic possessing a 14-member intramolecular hydrogen bond.[45] On the other hand, disruption of the γ-turn conformation has been caused by the placement of alkyl substituents at the 3-position of a central prolyl residue.[46]

In this report, we have demonstrated that the steric interactions of 5-*tert*-butylproline can induce the forma-

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tion of type VI β-turn conformations. Although 5-alkylprolines and their related 4-thia- and 4-oxaproline analogues have previously been employed for augmenting the prolyl amide *cis*-isomer population in peptide structures,[24,47,48] prior to our examination, the influence of the 5-alkyl substituent on the prolyl peptide turn conformation had not been reported.

We synthesized *N*-(acetyl)dipeptide *N*′-methylamides **1** and **2** possessing respectively (2*S*,5*R*)-5-*tert*-butylproline and L-proline at the *C*-terminal residue. Conformational analysis of dipeptides **1** and **2** by NMR spectroscopy showed that the Xaa-Pro peptide bond adopted preferably the *cis*-amide isomer in 5-*tert*-butylprolyl peptides **1** in contrast to prolyl dipeptides **2** which preferred the *trans*-amide isomer in solution. The limited influence of solvent composition and temperature on the chemical shift value of the *N*′-methylamide in the *cis*-amide conformer of dipeptide **1** indicated that it was engaged in an intramolecular hydrogen bond with the acetamide carbonyl in a type VIa β-turn conformation. The presence of significant type VI β-turn populations for *tert*-butylprolyl peptides **1b**−**f** in solution was also supported by the reduced value for the vicinal coupling constant between the α and amide protons of the *N*-terminal amino acid residue in the major *cis*-amide conformer. Furthermore, in the solid state, *N*-acetyl-L-leucyl-5-*tert*-butylproline *N*′-methylamide (**1d**) existed in a type VIa β-turn geometry as shown by X-ray diffraction. Finally, because they exhibited circular dichroism spectra characteristic of β-turn conformation in both water and acetonitrile, *tert*-butylprolyl peptides **1b** and **1d** were shown to adopt type VIa β-turn geometry independent of solvent composition.

Steric interactions between the bulky 5-position substituent and the *N*-terminal residue contorted the (2*S*,5*R*)-5-*tert*-butylprolyl amide away from the planar sp^2 hybridized geometry as illustrated by spectroscopic and crystallographic data. In the ^{13}C NMR spectrum of *N*-acetyl-L-alanyl-5-*tert*-butylproline *N*′-methylamide **1b**, the carbonyl carbon signal of the *N*-terminal residue was downfield shifted 3.1 ppm relative to the amide carbon resonance of its prolyl dipeptide counterpart **2b** in water. The *tert*-butylprolyl peptide bond exhibited a 17° ω-dihedral angle and an extended N−C(O) bond length in the X-ray structure of dipeptide **1d**. Contortion from planarity is the primary force diminishing the barrier for 5-*tert*-butylprolyl amide isomerization. For example, amide isomerization *N*-terminal to (2*S*,5*R*)-*N*-acetyl-5-*tert*-butylproline *N*′-methylamide was reduced by 3.7 kcal/mol compared to the barrier for *N*-(acetyl)proline *N*′-methylamide in water.[24] However, the 5-*tert*-butyl substituent influences the prolyl carboxylate to adopt a ψ-dihedral angle around $\psi \approx 0°$, which may also lead to a lower barrier for amide isomerization by enabling stabilization of the pyramidalized transition state via interactions between the *C*-terminal amide NH with the nitrogen lone pair of the rotating *N*-terminal amide.[12,36]

In conclusion, we have developed a novel approach for mimicking type VIa β-turns that features the employment of (2*S*,5*R*)-5-*tert*-butylproline to constrain the conformational liberty of *N*-(acetyl)dipeptide *N*′-methylamides **1**. An ensemble of spectroscopic and crystallographic data verified the presence of the type VIa β-turn geometry in both solution and the solid state. Because (2*S*,5*R*)-5-*tert*-butylproline can now be conveniently introduced into peptide structures, we are presently generating type VIa β-turn libraries in order to explore the importance of this structure in peptide chemistry and biology.[26]

Experimental Section

General. Unless otherwise noted, all reactions were run under a nitrogen atmosphere and distilled solvents were transferred by syringe. THF was distilled from sodium/benzophenone, 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 aluminum-backed silica plates. Melting points are uncorrected. Mass spectral data, HRMS (EI and FAB), were obtained at the Université de Montréal Mass Spectroscopy facility.

NMR Measurements. ^1H and ^{13}C NMR experiments were performed on Bruker 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. COSY, NOESY, and ROESY spectra were obtained with 2048 by 512 data points. A mixing time of 500 ms was used for the NOESY and ROESY spectra. The temperature coefficients of the amide proton chemical shifts in DMSO-d_6 were measured for at least five different temperatures in 5 deg steps by varying the temperature between 298 and 328 K. The value of the temperature coefficient was obtained by a linear least-squares fit of the data.

Circular Dichroism Measurements. CD spectra of 0.1 mM solutions 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. 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 10 repeated scans in steps of 0.2 nm. Baseline spectra of the solvents were subtracted.

(2*S*,5*R*)-*N*-(BOC)-5-*tert*-butylproline Allyl Ester (4). A solution of (2*S*,5*R*)-*N*-(BOC)-5-*tert*-butylproline (**3**, 0.71 g, 2.62 mmol, prepared according to ref 25) in CH_2Cl_2 (26 mL) was treated with DIEA (1.0 mL, 5.76 mmol) and allyl bromide (2.3 mL, 26.2 mmol), heated to a reflux, stirred for 18 h, cooled to room temperature, and evaporated. The residue was dissolved in EtOAc (50 mL), and the solution was washed with cold 0.1 M HCl (2 × 10 mL) and a phosphate buffer solution at pH 9.5 (15 mL), dried, and evaporated to give **4** (0.28 g, 98%) as an oil: $[\alpha]^{20}_D$ −29.7° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (s, 9

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H), 1.35 (s, 9 H), 1.85 (m, 3 H), 2.17 (m, 1 H), 3.76 (m, 1 H), 4.24 (m, 1 H), 4.55 (m, 2 H), 5.30 (m, 2 H), 5.86 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 26.4, 27.2, 28.0, 29.5, 36.2, 61.4, 65.1, 66.5, 79.7, 118.2, 131.8, 155.8, 172.8; HRMS calcd for $C_{17}H_{30}O_4N$ (MH^+) 312.2175, found 312.2193.

(2*S*,5*R*)-5-*tert*-Butylproline Allyl Ester Hydrochloride. A solution of (2*S*,5*R*)-*N*-(BOC)-5-*tert*-butylproline allyl ester (**4**, 1.91 g, 6.14 mmol) in CH_2Cl_2 (60 mL) was saturated with HCl (g) bubbles at 0 °C, stirred for 2 h at room temperature, and evaporated to provide (2*S*,5*R*)-5-*tert*-butylproline allyl ester hydrochloride in 99% (1.51 g) yield as a white precipitate: $[\alpha]^{20}_D$ −24.6° (*c* 0.7, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.12 (s, 9 H), 1.79 (m, 1 H), 2.13 (m, 1 H), 2.3−2.4 (m, 2 H), 3.55 (dd, 1 H, *J* = 6.3, 12.0), 4.58 (dd, 1 H, *J* = 3.8, 9.2), 4.77 (m, 2 H), 5.33 (m, 2 H), 6.00 (m, 1 H); ^{13}C NMR (CD_3OD) δ 25.7, 26.7, 29.5, 33.1, 60.6, 68.4, 72.9, 120.0, 132.5, 170.1; HRMS calcd for $C_{12}H_{22}O_2N$ (MH^+) 212.1651, found 212.1656. Anal. Calcd for $C_{12}H_{22}O_2NCl$: C, 58.17; H, 8.95; N, 5.65. Found: C, 58.20, H, 9.34, N, 5.65. (2*S*,5*R*)-5-*tert*-Butylproline allyl ester trifluoroacetate was prepared by stirring a solution of allyl ester **4** (0.84 g, 2.70 mmol) in 1:3 TFA:CH_2Cl_2 at room temperature for 2 h to give an oil (0.86 g, 98%) after evaporation of the volatiles: ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 9 H), 1.65 (m, 1 H), 2.04 (m, 1 H), 2.33 (m, 1 H), 2.50 (m, 1 H), 3.65 (m, 1 H), 4.59 (dd, 1 H, *J* = 2.1, 9.9), 4.74 (m, 2 H), 5.36 (m, 2 H), 5.91 (m, 1 H).

General Procedure for Peptide Coupling to 5-*tert*-Butylproline Residues. A solution of (2*S*,5*R*)-5-*tert*-butylproline allyl ester hydrochloride (355 mg, 1.43 mmol), *N*-(BOC)-amino acid (1.72 mmol), and DIEA (1.0 mL, 5.72 mmol) in CH_2Cl_2 (14 mL) was cooled to 0 °C, treated with BOP-Cl (430 mg, 1.72 mmol), stirred for 1 h ,and allowed to warm to room temperature with stirring for 18 h. Brine (5 mL) was added to the reaction solution which was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with 0.1 M HCl (2 × 5 mL), 5% $NaHCO_3$ (2 × 5 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 afforded *N*-(BOC)dipeptide allyl ester **5**. The same protocol was used to couple *N*-(BOC)amino acids and (2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide trifluoroacetate to provide *N*-(BOC)dipeptide *N*′-methylamides **7**.

***N*-(BOC)-(2*S*)-alanyl-(2*S*,5*R*)-5-*tert*-butylproline allyl ester (5b)** was obtained in 68% yield as an oil: $[\alpha]^{20}_D$ −92.4° (*c* 0.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ [0.83 (s, 3.7 H)] 0.93 (s, 5.3 H), [1.17 (d, 1.2 H, *J* = 6.4)] 1.26 (d, 1.8 H, *J* = 6.5), 1.33 (s, 5.3 H) [1.35 (s, 3.7 H)], 1.75−1.98 (m, 3 H), 2.25 (m, 1 H), 4.12 (d, 1 H, *J* = 7.9), 4.31 (m, 1 H), 4.55−4.65 (m, 3 H), 4.95 (d, 1 H, *J* = 8.8), 5.27 (m, 2 H), 5.83 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.1 (19.0), 25.6, (27.3) 27.5, 28.0 (28.1), 28.8, 35.7 (36.0), 46.5 (47.9), (59.8) 60.4, 65.1 (66.3), 66.6, (79.0) 79.4, 117.9 (118.9), (131.3) 131.8, (154.3) 154.9, (171.2) 171.5, (173.7) 174.8; HRMS calcd for $C_{20}H_{35}O_5N_2$ (MH^+) 383.2546, found 383.2560.

***N*-(BOC)(2*S*)-leucyl-(2*S*,5*R*)-5-*tert*-butylproline allyl ester (5d)** was obtained in 82% yield as an oil: $[\alpha]^{20}_D$ −91.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89−1.01 (s, 15 H), 1.41 (m, 10 H), 1.70−1.93 (m, 3 H), 2.04 (m, 1 H), 2.30−2.35 (m, 2 H), 4.19 (d, 1 H, *J* = 8.6), 4.38 (m, 1 H), 4.63−4.74 (m, 4 H), 5.21−5.35 (m, 2 H), 5.92 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.5 (21.7), (25.8) 27.3, 27.4 (29.1), (23.4) 27.6, (24.2) 28.0, (24.3) 28.1, 35.6 (36.1), 40.5 (43.3), 49.4 (50.4), (59.9) 60.4, (66.4) 65.2, 66.5 (66.6), (78.8) 79.3, 118.0 (118.9), (131.5) 131.8, (154.7) 155.2, (171.3) 171.5, (173.9) 174.6; HRMS calcd for $C_{23}H_{41}O_5N_2$ (MH^+) 425.3015, found 425.3023.

***N*-(BOC)-(2*S*)-valyl-(2*S*,5*R*)-5-*tert*-butylproline allyl ester (5e)** was obtained in 43% yield as an oil: $[\alpha]^{20}_D$ −72.5° (*c* 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89−1.01 (m, 15 H), 1.41 (s, 9 H), 1.75−2.32 (m, 5 H), 4.20 (m, 1 H), 4.42 (m, 1 H), 4.55−4.75 (m, 3 H), 5.33 (m, 2 H), 5.89 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.5 (17.9), 19.4 (19.8), 26.0 (28.9), 27.4 (27.5), (27.7) 28.1, 28.2, (31.1) 32.5, (35.6) 36.2, 56.5 (56.6), 60.0 (60.1), (65.4) 66.4, (66.2) 66.5, 78.9 (79.6), 118.3 (118.9), (131.6) 131.8, 155.0 (155.3), 171.4 (171.7), 173.2 (173.7); HRMS calcd for $C_{22}H_{39}O_5N_2$ (MH^+) 411.2859, found 411.2852.

***N*-(BOC)-(2*S*)-phenylalanyl-(2*S*,5*R*)-5-*tert*-butylproline allyl ester (5f)** was obtained in 94% yield as a white solid: mp 118−119 °C; $[\alpha]^{20}_D$ −67.4° (*c* 0.84, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 7.4 H) [0.96 (s, 1.6 H)], [1.28 (s, 1.6 H)] 1.42 (s, 7.4 H), 1.51−1.65 (m, 2 H), 1.83 (m, 1 H), 2.05 (m, 1 H), 2.83 (m, 1 H), 3.02 (m, 1 H), 3.48 (t, 1 H, *J* = 8.4), 4.07 (d, 0.8 H, *J* = 8.5) [4.29 (d, 0.2 H, *J* = 7.9)], 4.62 (m, 3 H), 4.91 (br s, 1 H), 5.29 (m, 2 H), 5.84 (m, 1 H), 7.19−7.27 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 25.4, 27.3 (27.5), (27.4) 28.0, (28.1) 28.2, (35.6) 36.0, (38.3) 41.0, (52.0) 53.7, 59.5 (60.5), (65.3) 66.2, 66.6, 79.2 (79.5), (118.1) 118.8, (126.4) 126.9, (128.2) 128.6, 129.3, 131.4 (131.8), 136.4 (136.9), 154.4 (154.9), 171.4 (171.5), 172.8 (173.7); HRMS calcd for $C_{26}H_{39}O_5N_2$ (MH^+) 459.2859, found 459.2872.

***N*-(BOC)-glycyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7a)** was obtained in 87% yield as a white solid: mp 63−66 °C; $[\alpha]^{20}_D$ −113.7° (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 9 H), 1.45 (s, 9 H), 1.85 (m, 2 H), 2.04 (m, 0.7 H) [2.20 (m, 0.3)], [2.35 (m, 0.3 H)] 2.63 (m, 0.7 H), 2.80 (m, 3 H), 3.71 (d, 0.7 H, *J* = 8.1) [3.85 (m, 0.3 H)], 4.11 (m, 1 H), [4.30 (m, 0.3 H)] 4.68 (t, 0.7 H, *J* = 8.5), 5.40 (br s, 1 H), [6.81 (br s, 0.3 H)] 7.24 (br s, 0.7 H); ^{13}C NMR ($CDCl_3$) δ 24.7 (25.6), 26.1, 26.5, 27.4, 28.1, 35.6 (35.9), (42.9) 43.3, 61.7, (67.2) 67.6, 79.6, 155.6, (171.4) 171.7, 172.2; HRMS calcd for $C_{17}H_{32}O_4N_3$ (MH^+) 342.2393, found 342.2399.

***N*-(BOC)-(2*S*)-alanyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7b)** was obtained in 75% yield as a white solid: mp 100−101 °C; $[\alpha]^{20}_D$ −81.5° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 7.6 H) [0.84 (s, 1.4 H)], 1.16 (d, 2.6 H, *J* = 6.8) [1.27 (d, 0.4 H, *J* = 6.6)], 1.34 (s, 9 H), 1.75 (m, 2 H), 2.15 (m, 1 H), 2.33 (m, 1 H), [2.72 (d, 0.4 H, *J* = 6.5)] 2.75 (d, 2.6 H, *J* = 4.6), 4.07 (m, 1 H), 4.22 (m, 2 H), 5.29 (d, 1 H, *J* = 3.8), 8.32 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.2 (18.2), (24.9) 25.2, 26.2, 27.4, 28.0, (26.3) 29.1, (35.1) 35.6, (46.6) 48.9, (61.5) 61.9, 67.0 (67.5), (79.7) 80.3, 156.4, 171.7, 175.6; HRMS calcd for $C_{18}H_{34}O_4N_3$ (MH^+) 356.2549, found 356.2556.

***N*-(BOC)-(2*S*)-methionyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7c)** was obtained in 64% yield as a white solid: mp 102−103 °C; $[\alpha]^{20}_D$ −88.4° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 6.4 H) [0.93 (s, 2.6 H)], 1.42 (s, 9 H), 1.83 (m, 3 H). 2.01−2.09 (m, 5 H), 2.56 (m, 3 H), [2.78 (d, 0.9 H, *J* = 4.7)] 2.83 (d, 2.1 H, *J* = 4.4), 4.28 (m, 2 H), 4.44 (m, 1 H), 5.12 (d, 1 H, *J* = 7.8), 8.29 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ (14.0) 15.6, (25.1) 25.2, 26.1 (26.2), (26.3) 27.1, (27.4) 28.0, 28.7, 30.3, 30.7, (35.1) 35.6, (50.5) 52.5, (61.5) 61.8, 67.0 (67.5), (80.0) 80.6, (155.1) 157.0, 171.3 (171.9), 174.6 (175.2); HRMS calcd for $C_{20}H_{38}O_4N_3S$ (MH^+) 416.2583, found 416.2593.

***N*-(BOC)-(2*S*)-valyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7e)** was obtained in 35% yield as a white solid: mp 56−58 °C; $[\alpha]^{20}_D$ −80.8° (*c* 0.57, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (m 9 H), 0.96 (d, 3 H, *J* = 6.9), 1.03 (d, 3 H, *J* = 6.7), 1.43 (s, 9 H), 1.61 (m, 1 H), 1.84 (m, 3 H), 2.57 (m, 1 H), 2.81 (d, 3 H, *J* = 4.6), 3.95 (t, 1 H, *J* = 8.0), 4.32 (t, 1 H, *J* = 7.3), 4.46 (dd, 1 H, *J* = 3.5, 8.1), 4.99 (d, 1 H, *J* = 8.5), 8.48 (br s, 1 H);^{13}C NMR ($CDCl_3$) δ (17.9) 18.3, 19.5 (20.0), (24.9) 25.2, 26.0, (26.2) 27.1, (27.6) 28.0, 28.1, 30.5 (31.1), (35.0) 35.6, (56.9) 58.6, (61.3) 61.7, 66.7 (66.9), (79.8) 80.4, 156.9, 171.1 (172.0), 175.0; HRMS calcd for $C_{20}H_{38}O_4N_3$ (MH^+) 384.2862, found 384.2872.

General Procedure for Allyl Ester Removal. A solution of allyl ester **5** (1.0 g, 2.18 mmol) in THF (22 mL) was treated with morpholine (1.9 mL, 21.8 mmol) and $Pd(PPh_3)_4$ (25 mg, 0.2 mmol), stirred for 3 h at room temperature, and evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), washed with 0.1 M HCl (2 × 15 mL) and brine (2 × 15 mL), dried, and evaporated to a residue that was purified by chromatography on silica gel using 5% MeOH in $CHCl_3$ as eluant. Evaporation of the collected fractions afforded *N*-(BOC)-dipeptide **6**.

***N*-(BOC)-(2*S*)-leucyl-(2*S*,5*R*)-5-*tert*-butylproline (6d)** was isolated in 79% yield as a white solid: mp 152−153 °C; $[\alpha]^{20}_D$ −170.4° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86−0.91 (m, 15 H), 1.36 (s, 9 H), 1.42 (m, 1 H), 1.66−1.86 (m, 3 H), 2.01 (m, 1 H), 2.34 (m, 2 H), [4.19 (m, 0.3 H)] 4.44 (m, 1.2 H), 4.62 (m, 1 H), 5.21 (d, 0.6 H, *J* = 9.5) [5.68 (d, 0.4 H, *J* = 8.9)]; ^{13}C NMR

($CDCl_3$) δ 21.4 (21.6), 23.4, 24.1 (24.3), (25.6) 26.0, 26.5 (29.0), (27.3) 27.5, 28.1, 35.3 (35.9), 40.1 (41.9), 49.5 (51.1), (60.2) 61.4, (66.9) 67.4, 79.6 (80.0), 155.3 (156.0), 172.8 (173.0), (174.5) 176.8; HRMS calcd for $C_{20}H_{37}O_5N_2$ (MH^+) 385.2702, found 385.2710.

***N*-(BOC)-(2*S*)-phenylalanyl-(2*S*,5*R*)-5-*tert*-butylproline (6f)** was isolated in 64% yield as a white solid: mp 149−151 °C; $[\alpha]^{20}_D$ −66.7° (*c* 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 6.3 H) [1.03 (s, 2.7 H)], [1.29 (s, 2.7 H)] 1.39 (s, 6.3 H), 1.48 (m, 1 H), 1.76−1.96 (m, 3 H), 2.87 (m, 2 H), 3.75 (t, 1 H, J = 8.7), 4.06 (d, 1 H, J = 8.5), 4.35 (d, 1 H, J = 7.5), 5.60 (m, 1 H), 7.24 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 26.7 (28.0), 28.3, (28.6) 28.8, (28.5) 30.2, (36.7) 37.2, (38.7) 41.0, (54.2) 55.3, 62.8 (63.8), 68.0 (68.3), (80.4) 80.9, (127.5) 128.0, (129.3) 129.7, (130.5) 130.6, 138.0 (138.9), 157.1 (157.4), 174.5 (176.0), 179.5.

General Procedure for Amidation of the Dipeptides. A solution of *N*-(BOC)dipeptide **6** (104 mg, 0.26 mmol) in CH_3-CN (2.6 mL) was treated with DIEA (181 μL, 1.0 mmol), methylamine hydrochloride (21 mg, 0.31 mmol), and TBTU (100 mg, 0.31 mmol), stirred at room temperature for 18 h, and partitioned between brine (2 mL) and EtOAc (10 mL). The organic phase was washed with 0.1 M HCl (2 × 3 mL), 5% $NaHCO_3$ (2 × 3 mL), and brine (4 mL), dried, and evaporated to yield the *N*-(BOC)dipeptide methylamide **7**.

***N*-(BOC)-(2*S*)-leucyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7d)** was isolated in 97% yield as a white solid: mp 152−153 °C; $[\alpha]^{20}_D$ −95.9° (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87−0.94 (m, 15 H), 1.25 (m, 1 H), 1.42 (s, 9 H), 1.72−1.84 (m, 4 H), 2.17 (m, 1 H), 2.44 (m, 1 H), [2.78 (d, 0.5 H, J = 4.7) 2.83 (d, 2.5 H, J = 4.6), 4.12 (m, 1 H), 4.28 (m, 2 H), 4.92 (d, 1 H, J = 7.7), 8.32 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.3 (21.5), 23.4 (23.5), (24.3) 24.5, (24.8) 25.2, 26.1 (26.2), 27.2 (27.5), 28.0, 29.0 (29.6), (35.0) 35.7, 40.0 (41.0), (49.5) 51.9, (61.5) 61.7, 67.0 (67.5), (79.8) 80.4, (155.1) 157.1, 171.5 (172.0), 175.2 (176.2); HRMS calcd for $C_{21}H_{40}O_4N_3$ (MH^+) 398.3019, found 398.3031.

***N*-(BOC)-(2*S*)-phenylalanyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (7f)** was isolated in 49% yield as a white solid: mp 51−52 °C; $[\alpha]^{20}_D$ −28.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 9 H), 1.15 (m, 1 H), [1.36 (s, 1.4 H)] 1.43 (s, 7.6 H), 1.59 (m, 2 H), 2.08 (m, 1 H), 2.76 (d, 3 H, J = 4.6), 2.92 (m, 2 H), 3.57 (dd, 1 H, J = 4.4, 8.9), 4.25 (dd, 1 H, J = 5.2, 8.6), 4.37 (m, 1 H), 5.12 (d, 1 H, J = 7.0), 7.17−7.33 (m, 5 H), 8.38 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.0, 25.9, 27.1, 27.9, 28.0, 35.6, 38.4, 54.5, 61.6, 66.9, 80.7, 127.5, 129.0, 129.3, 135.2, 156.2, 171.3, 174.6; HRMS calcd for $C_{24}H_{38}O_4N_3$ (MH^+) 432.2862, found 432.2871.

General Procedure for Acetamide Synthesis. A solution of *N*-(BOC)dipeptide *N*′-methylamide **7** (62 mg, 0.16 mmol) in 1:3 TFA:CH_2Cl_2 (1.6 mL) was stirred at room temperature for 2 h and evaporated on a rotary evaporator. The resulting dipeptide *N*′-methylamide trifluoroacetate was dissolved in CH_2Cl_2 (1.6 mL), treated with K_2CO_3 (0.22 mg, 1.6 mmol) and Ac_2O (148 μL, 1.6 mmol), stirred for 18 h, filtered, washed with CH_2Cl_2 (3 × 3 mL), and evaporated to give the *N*-acetyldipeptide *N*′-methylamide **1**.

***N*-Acetyl-glycyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1a)** was isolated in 93% yield as a white solid: mp 71−73 °C; $[\alpha]^{20}_D$ −99.5° (*c* 0.46, $CHCl_3$); mixture of 1:1 rotamers ^{1}H NMR ($CDCl_3$) δ 0.93 (s, 9 H), 1.27 (m, 1 H), 1.64 (m, 2 H), 1.85 (m, 1 H), 2.05 (s, 3 H), 2.82 (m, 3 H), 3.74 (d, 1 H, J = 9.5), 4.20 (m, 2 H), 4.66 (m, 1 H), 6.43 (br s, 1 H), 7.14 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 22.7, 25.0, 25.7, 26.2, 26.5, 27.4, 29.5, 30.2, 31.4, 35.6, 36.0, 42.2, 42.4, 61.7, 61.9, 67.4, 67.7, 170.2, 170.9, 171.1, 171.7, 171.9; HRMS calcd for $C_{14}H_{26}O_3N_3$ (MH^+) 284.1974, found 284.1968.

***N*-Acetyl-(2*S*)-alanyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1b)** was isolated in 97% yield as a white solid: mp 119−121 °C; $[\alpha]^{20}_D$ −60.3° (*c* 0.36, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (s, 7.5 H) [0.93 (s, 1.5 H)], 1.31 (d, 2.5 H, J = 6.9) [1.39 (d, 0.5 H, J = 6.6)], 1.85 (m, 2 H), [1.97 (s, 0.5 H)] 2.01 (s, 2.5 H), 2.22 (m, 1 H), 2.45 (m, 1 H), [2.80 (d, 0.5 H, J = 4.7)] 2.86 (d, 2.5 H, J = 4.6), 4.32 (m, 3 H), 6.54 (d, 1 H, J = 6.1), 8.35 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ (15.8) 16.0, 22.1 (22.6), (24.5) 25.2, 26.2 (26.4), 27.3 (27.5), 28.9, 35.6, 48.5, (61.6) 62.0, 67.4 (68.0), (169.8) 171.5, 171.7 (171.9), 175.4 (176.3); HRMS calcd for $C_{15}H_{28}O_3N_3$ (MH^+) 298.2131, found 298.2120.

***N*-Acetyl-(2*S*)-methionyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1c)** was isolated in 99% yield as a white solid: mp 53−56 °C; $[\alpha]^{20}_D$ −71.7° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (s, 6.6 H) [0.93 (s, 2.4 H)], 1.82 (m, 2 H), 1.96 (m, 4 H), 2.07 (s, 5 H), 2.53 (m, 3 H), [2.76 (d, 0.8 H, J = 4.6)] 2.83 (d, 2.2 H, J = 4.6), 4.23 (dd, 1 H, J = 5.3, 8.2), 4.48 (m, 2 H), 7.72 (d, 1 H, J = 6.2), 8.41 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ (15.5) 15.6, 22.2 (22.5), 25.1 (25.5), (26.2) 26.3, 27.2, (27.4) 28.4, 30.3, 30.5, (35.1) 35.5, (49.7) 52.1, (61.6) 61.7, 67.4 (67.8), (170.1) 171.0, (171.8) 172.2, 174.5 (175.2); HRMS calcd for $C_{17}H_{32}O_3N_3$ (MH^+) 358.2131, found 358.2152.

***N*-Acetyl-(2*S*)-leucyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1d)** was isolated in 98% yield as a white solid: mp 172−173 °C; $[\alpha]^{20}_D$ −88.2° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 7.7 H) [0.91 (s, 1.3 H)], 0.92 (d, 3 H, J = 4.0), 0.97 (d, 3 H, J = 6.6), 1.29 (m, 1 H), 1.58−1.72 (m, 3 H), 1.85 (m, 1 H), [1.98 (s, 0.4 H)] 2.04 (s, 2.6 H), 2.17 (m, 1 H), 2.48 (m, 1 H), [2.79 (d, 0.4 H, J = 4.6)] 2.87 (d, 2.6 H, J = 4.6), 4.29 (m, 3 H), 5.96 (d, 1 H, J = 6.9), 8.26 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.2 (21.4), 22.4 (22.7), 23.3 (23.5), (24.4) 24.8, 25.2, 26.3 (26.4), 27.3 (27.6), 28.7, (35.0) 35.6, 39.5 (40.7), (48.6) 51.5, (61.5) 61.8, 67.3 (67.9), 171.2, (171.8) 172.0, 174.8; HRMS calcd for $C_{18}H_{34}O_3N_3$ (MH^+) 340.2600, found 340.2611.

***N*-Acetyl-(2*S*)-valyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1e)** was isolated in 97% yield as a white solid: mp 166−167 °C; $[\alpha]^{20}_D$ −93.8° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 8 H), 0.97 (m, 4 H), 1.05 (d, 3 H, J = 6.7), 1.81−2.06 (m, 7 H), 2.59 (m, 1 H), [2.80 (d, 0.3 H, J = 4.6)] 2.84 (d, 2.7 H, J = 4.6), 4.18 (t, 1 H, J = 7.5), 4.30 (t, 1 H, J = 7.3), 4.50 (m, 1 H), 6.18 (d, 1 H, J = 6.2), 8.48 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ (18.0) 18.7, 19.3 (19.9), 22.6, (25.0) 25.2, 26.2 (26.5), 27.2, 27.9, 30.5, (35.0) 35.5, (55.6) 58.1, (61.3) 61.8, 67.0 (67.2), (169.2) 170.9, 171.5 (171.8), 174.3 (175.1); HRMS calcd for $C_{17}H_{32}O_3N_3$ (MH^+) 326.2444, found 326.2455.

***N*-Acetyl-(2*S*)-phenylalanyl-(2*S*,5*R*)-5-*tert*-butylproline *N*′-methylamide (1f)** was isolated in 94% yield as a white solid: mp 81−82 °C; $[\alpha]^{20}_D$ −32.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 9 H), 1.16 (m, 1 H), 1.51−1.67 (m, 2 H), [1.90 (s, 0.3 H)] 2.00 (s, 2.7 H), 2.13 (m, 1 H), 2.79 (d, 3 H, J = 4.6), 2.97−3.09 (m, 2 H), 3.71 (dd, 1 H, J = 4.5, 8.9), 4.25 (dd, 1 H, J = 4.9, 8.7), 4.57 (m, 1 H), 6.72 (d, 1 H, J = 5.6), 7.20−7.35 (m, 5 H), 8.37 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.6 (22.7), 24.9 (25.1), 26.2 (26.4), 27.2 (27.5), 27.8, 35.5, 38.1 (38.4), 54.0, (61.5) 61.7, 67.3 (67.9), (127.0) 127.6, (128.4) 129.1, 129.2 (129.3), 134.5, 171.2, 173.9; HRMS calcd for $C_{21}H_{32}O_3N_3$ (MH^+) 374.2444, found 374.2449.

***N*-Acetyl-(2*S*)-alanylproline *N*′-methylamide and *N*-acetyl-(2*S*)-leucylproline *N*′-methylamide** were synthesized in solution phase from *N*-(BOC)-L-proline *N*′-methylamide using the TBTU coupling and acetylation conditions as described above.

***N*-Acetyl-(2*S*)-alanylproline *N*′-methylamide (2a)**: mp 176−177 °C; $[\alpha]^{20}_D$ −145.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ [1.31 (d, 0.6 H, J = 7.0)], 1.36 (d, 2.4 H, J = 6.9), [1.78 (m, 0.2 H)] 1.92 (m, 0.8 H), 2.00 (s, 2.4 H) [2.01 (s, 0.6 H)], 2.03 (m, 1 H), [2.09 (m, 0.2 H)] 2.14 (m, 0.8 H), 2.34 (m, 0.8 H) [2.55 (m, 0.2 H)], 2.79 (d, 2.4 H, J = 3.9) [2.85 (d, 0.7 H, J = 3.8)], 3.57 (m, 1 H), 3.68 (m, 1 H), [4.31 (m, 0.2 H, J = 8.2)] 4.53 (dd, 0.8 H, J = 2.9, 8.1), [4.25 (m, 0.2 H)] 4.76 (m, 0.8 H), 6.53 (d, 0.8 H, J = 6.3) [6.65 (br s, 0.2 H)], 6.72 (br s, 0.8 H) [7.58 (br s, 0.2 H)]; ^{13}C NMR ($CDCl_3$) δ (16.3) 17.8, (22.3) 22.8, (21.8) 24.8, 26.0 (26.4), 27.7 (31.3), 46.5 (48.0), (46.7) 47.2, 59.8 (60.7), 169.5 (170.9), (171.1) 171.5, (172.0) 172.4; HRMS calcd for $C_{11}H_{20}O_3N_3$ (MH^+) 242.1505, found 242.1498.

***N*-Acetyl-(2*S*)-leucylproline *N*′-methylamide (2b)**: mp 90−91 °C; $[\alpha]^{20}_D$ −130.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94 (m, 6 H), 1.53 (m, 1 H), 1.67 (m, 1 H), 1.88 (m, 3 H), 2.00 (s, 2.4 H) [2.02 (s, 0.6 H)], 2.15 (m, 1 H), 2.35 (m, 1 H), 2.77 (d, 2.4 H, J = 4.8) [2.85 (d, 0.6 H, J = 4.7)], 3.56 (m, 1 H), 3.76 (m, 1 H), 4.50 (dd, 1 H, J = 2.7, 8.1), 4.81 (m, 1 H), 6.18 (d, 0.8 H, J = 8.4) [6.29 (d, 0.2 H, J = 6.1)], 6.72 (br s, 0.8 H) [7.52 (br s, 0.2 H)]; ^{13}C NMR ($CDCl_3$) δ (21.1) 21.6, (22.3) 22.8, 23.2 (23.3), 24.6 (24.7), (21.8) 24.8, 26.0 (26.5), 27.4 (31.2), 39.8

(41.4), (46.7) 47.2, 49.0 (50.9), 59.6 (60.8), 170.0 (170.6), 171.5 (171.6), (172.0) 172.8; HRMS calcd for $C_{14}H_{26}O_3N_3$ (MH^+) 284.1974, found 284.1966.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1a**−**f**, **2b**, and **2d**; COSY and NOESY spectra and plots of temperature versus amide N−H chemical shift for **1b** and **2b**, and crystallographic data for **1d**. This material is availabl free of charge via the Internet at http://pubs.acs.org.

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