Steric Effects on the Amide Isomer Equilibrium of Prolyl Peptides. Synthesis and Conformational Analysis of *N*-Acetyl-5-*tert*-butylproline *N*′-Methylamides

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Abstract: The influence of a bulky 5-position substituent on the amide isomer equilibrium *N*-terminal to proline has been explored via the synthesis and analysis of *N*-(acetyl)proline *N*′-methylamide (**1**) and its respective *cis*- and *trans*-5-*tert*-butylproline amide diastereomers **2** and **3**. The relative populations of the amide *cis*- and *trans*-isomers as well as the energy barriers for amide isomerization of **1**−**3** in D_2O were ascertained using NMR with coalescence and magnetization transfer experiments. The relative populations of free *C*-terminal amide and hydrogen-bonded amide in the γ-turn conformation were also estimated by integrating the N−H stretch absorbances in the FT-IR spectra of **1**−**3** in $CHCl_3$ and CCl_4. In the prolyl peptides, the 5-*tert*-butyl substituent was found to exhibit profound effects on the amide isomer equilibrium, on the energy barrier for amide isomerization, and on the stability of the γ-turn conformation. Steric interactions between the 5-position substituent and the *N*-acetyl group disfavor the amide *trans*- and augment the *cis*-isomer population: 25% in **1**, 48% in **2**, and 66% in **3**. In the case of *cis*-5-*tert*-butylproline **2**, the energy barrier for amide isomerization is observed to be 3.9 kcal/mol lower than that of **1**. On the other hand, the amide isomerization barrier for *trans*-5-*tert*-butylproline **3** is similar to that for **1**. Only a single amide N−H stretch band is observed at 3454 cm^{-1} in the FT-IR spectrum of **3** in $CHCl_3$ and indicates that the NH group is free of intramolecular hydrogen bonding. Hence, *trans*-5-*tert*-butylproline amide **3** does not adopt a seven-membered γ-turn conformation, which is a favored conformer for **1** and **2** in $CHCl_3$. Maps, in which the ψ- and ω-dihedral angles are plotted at 30° intervals against the calculated energy of the local minimum conformation, predict qualitatively and display clearly all of the observed effects of the 5-*tert*-butyl substituent on the amide isomer in the *N*-(acetyl)-proline *N*′-methylamides. The results of this study suggest the use of 5-*tert*-butylprolines to prepare both X-Pro *cis*-amide isomers and twisted amide surrogates for examining prolyl residue conformations in bioactive peptides.

Introduction

The rational design of therapeutics based on peptide lead structures requires detailed knowledge of their conformational requirements for biological activity. Since energetically similar amide *cis*- and *trans*-isomers *N*-terminal to proline may create multiple low energy conformers (Figure 1), prolyl residues can complicate the characterization of peptide structures using techniques such as X-ray diffraction and NMR spectroscopy.[1] Conformationally rigid surrogates of the *cis*- and the *trans*-isomers of X-Pro amide bonds have thus emerged as important tools for studying the relationship between isomer geometry and peptide bioactivity.[2−10] Since conformational changes about proline can influence the stability, folding, denaturation, and recognition of prolyl peptides,[11] analogues that restrict prolyl geometry can also serve as sensitive probes for examining protein biology at the molecular level.

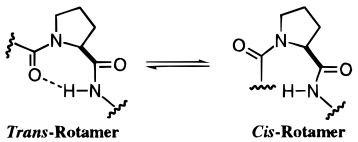

Figure 1. Amide isomer equilibrium *N*-terminal to proline derivatives.

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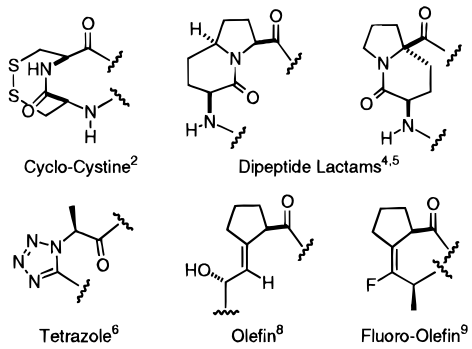

Figure 2. Representative examples of conformationally rigid amide surrogates.[2−9]

Attempts to understand the relationship between X-Pro amide isomer geometry and protein bioactivity have led to many strategies for preparing conformationally rigid isosteres of X-Pro amide bonds (Figure 2).[2−10] Efforts to mimic X-Pro dipeptide residues in peptides have focused on the geometry of the backbone, the hydrogen acceptor properties of the amide carbonyl, as well as the shape, function and geometry of the amino acid side-chains. For example, *cyclo*-cystine[2] and *cyclo*-lanthione[3] derivatives have been examined as amide *cis*-isomer mimics that replicate the backbone geometry of X-Pro residues.[2a] Dipeptide lactams in which either the 2- or the 5-position carbons of the pyrrolidine ring is tethered to the α-carbon of the *N*-terminal amino acid residue have been used to mimic the backbone geometry of type VI and II′ β-turn conformations.[4,5] In addition, rigid sp^2 hybridized amide isomer surrogates, that forfeit the potential hydrogen acceptor properties of the carbonyl group, have been generated using heterocycles such as tetrazoles[6] and pyrroles[7] as well as olefin[8] and fluoro-olefin[9] amide bond replacements.

Rigid amide isomer analogues with biological activities similar to the parent peptide can support the importance of a particular conformation for bioactivity. For example, the replacement of the Phe-Pro residues in a somatostatin analogue[12] with *cyclo*-cystine,[2a] dipeptide lactams[4d] and tetrazole derivatives[6c] all have given potent peptide mimetics that implicate the significance of a type VI β-turn conformation for bioactivity. Inhibitors of proline specific enzymes, such as peptidyl prolyl isomerase (PPIase)[8] and proline-specific protease,[9b] have also been designed using olefins and fluoro-olefins as non-isomerizable, non-hydrolizable amide isosteres. Since analogues without biological activity may arise both from mimicking an inactive conformation and from using a surrogate having structural features that interfere with receptor recognition, various innovative approaches to generate constrained X-Pro amide isomers are needed in order to precisely probe prolyl peptide geometry.

The employment of alkylprolines and their steric interactions to control amide isomer geometry is a particularly attractive method for preparing conformationally rigid amide isomers because libraries of X-Pro derivatives may be prepared by coupling different amino acid residues to the *N*-terminal of the alkylproline. Pioneering studies on the influence of methyl substituents on the amide isomer equilibrium of *N*-(acetyl)-proline *N*′-methylamides have provided fundamental understanding of the steric effects of alkylprolines as well as an effective means for preparing rigid X-Pro amide *trans*-isomers.[13−17] For example, only the *trans*-isomer (>98%) was observed in the ^{13}C NMR spectrum of *N*-acetyl-2-methylproline *N*′-methylamide.[13] This result motivated synthesis of 2,4-methanoproline,[14] a natural achiral proline analogue,[15] that was also shown to furnish X-Pro amide *trans*-isomer (>95% by ^{13}C NMR) in model peptides.

The steric interactions of methylprolines have also been employed to augment the population of the X-Pro amide *cis*-isomer.[16,17] A single methyl substituent at the proline 5-position was shown to have a subtle influence on the X-Pro amide isomer equilibrium of *N*-(acetyl)proline *N*′-methylamide.[16] This effect was contingent on the relative stereochemistry of the 5-methylproline. In the case of the *trans*-diastereomer, the steric interactions of the 5-methyl group destabilized the amide *trans*-isomer in *N*-acetyl-5-methylproline *N*′-methylamide without affecting the isomerization energy barrier. A 5% increase in the amide *cis*-isomer population was observed in water for the 5-methyl *trans*-diastereomer. In the case of the *cis*-diastereomer, the presence of the 5-methyl group had no effect on the ratio of amide isomers. However, the amide isomerization energy barrier was observed to be 1.2 kcal/mol lower for *N*-acetyl-5-methylproline *N*′-methylamide *cis*-diastereomer in water.[16] The combined effect of two methyl substituents at the proline 5-position was recently studied in *N*-BOC-phenylalanyl-5,5-dimethylproline methyl ester which was reported to exist as a 9:1 mix of amide *cis*:*trans* isomers.[17] The results so far obtained with methyl substituents[16,17] all point to the use of alkylprolines with bulkier 5-position substituents in order to prepare X-Pro amide analogues with greater *cis*-isomer populations. In addition, since the lower energy barrier for amide isomerization of the 5-methylproline *cis*-diastereomer arises via destabilization of both the *cis*- and *trans*-isomer conformations, larger 5-position substituents may give "twisted" amide mimetics by perturbing the planar amide sp^2 hybridized geometry.[18]

We have recently developed efficient methodology for synthesizing all four stereoisomers of 5-*tert*-butylprolines from glutamic acid as an inexpensive chiral educt.[19] We are presently using these 5-alkylprolines to alter the *cis*−*trans* isomer equilibrium of the amide bond *N*-terminal to prolyl residues in order to study the importance of X-Pro *cis*-isomer populations in the recognition and reactivity of bioactive peptides.[20] In this manuscript, we describe the synthesis and conformational analysis of *N*-acetyl-5-*tert*-butylproline *N*′-methylamides. These simple prolyl peptides are ideal model systems for illustrating the influence of the bulky 5-*tert*-butyl substituent on the amide isomer equilibrium *N*-terminal to proline. In the model peptides, the 5-*tert*-butyl substituent exhibits profound effects on both the amide isomer equilibrium and the energy barrier for amide isomerization. Moreover, we observe that the 5-*tert*-butyl group can alter the stability of the γ-turn conformation. In addition, the effects of the 5-*tert*-butyl substituent on the amide isomer in *N*-(acetyl)proline *N*′-methylamides were predicted and dis-

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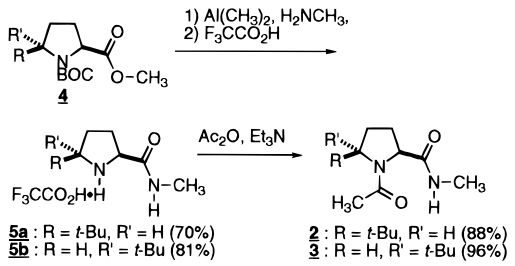
Scheme 1. Synthesis of *N*-(Acetyl)proline *N*′-Methylamides **2** and **3**



played qualitatively by maps portraying energy vs conformation as the ψ- and ω-dihedral angles are rotated about 30° intervals. In sum, our results indicate that 5-*tert*-butylprolines may be used to generate prolyl peptide libraries possessing X-Pro amide *cis*-isomers that mimic type VI β-turn as well as twisted amide conformations.

Results and Discussion

Synthesis of *N*-(Acetyl)proline *N*′-Methylamides. 5-*tert*-Butylprolines were synthesized from glutamic acid as described.[19] Acylation of the dianion of *N*-(9-(9-phenylfluorenyl))-glutamate γ-methyl ester with pivaloyl chloride, hydrolysis of the methyl ester and decarboxylation gave 6,6-dimethyl-5-oxo-2-[*N*-(9-(9-phenylfluorenyl)amino]heptanoates that were diastereoselectivity reduced to the *cis*- and *trans*-5-*tert*-butylprolines. The *cis*- and *trans*-diastereomers were purified as methyl *N*-BOC-5-*tert*-butylprolinates **4**.

N-Acetyl-*cis*-5-*tert*-butylproline *N*′-methylamide (**2**) and *N*-acetyl-*trans*-5-*tert*-butylproline *N*′-methylamide (**3**) both were synthesized from their respective *N*-BOC-5-*tert*-butylproline methyl esters **4** (Scheme 1). 5-*tert*-Butylproline *N*′-methylamides **5** were initially synthesized in 70% and 81% respective yield by treatment of methyl ester **4** in CH_2Cl_2 with a solution of methylamine hydrochloride and trimethyl aluminum in benzene,[21] followed by solvolysis of the BOC group with trifluoroacetic acid. Subsequent *N*-acetylation of *N*′-methylamides **5** with acetic anhydride and triethyl amine provided the respective *N*-(acetyl)proline *N*′-methylamides **2** and **3** in 88% and 96% yield after purification by chromatography. Amide **1** was obtained from proline methyl ester hydrochloride via treatment with a premixed solution of $Al(CH_3)_3$ and H_2NCH_3·HCl in benzene as described for **4** followed by *N*-acetylation as described for **5** in the Experimental Section.[22]

NMR Analysis of *N*-(Acetyl)proline *N*′-Methylamides 1−3. Water (H_2O and D_2O) was chosen as the solvent for measuring the populations of the amide *cis*- and *trans*-isomers as well as the energy barrier for amide isomerization. The rate of amide isomerization *N*-terminal to proline is known to be slower in water than in polar non-protic and in nonpolar solvents.[23] Since

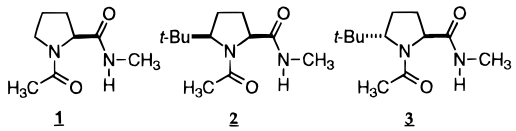


Figure 3. *N*-(acetyl)proline *N*′-methylamides **1**−**3** (amide *trans*-isomers shown).

Table 1. Selected Carbon Chemical Shift Values of Prolyl Amides **1**−**3**

	C^α		C^β		C^γ		C^δ	
prolyl amide	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
1	62.3	60.8	31.9	30.2	22.8	24.5	47.5	49.1
2	69.9	67.6	36	35.9	28.3	30.7	62.6	63.6
3	69.4	66.7	31.6	29.5	25.4	26.6	62.4	63.1

the conformational distribution of *N*-(acetyl)proline *N*′-methylamides has been shown to be solvent-dependent,[24] the effects of solvent on the isomer population in **1**−**3** are presently being examined and will be reported in due time.

The assignments of the isomer geometry were made based on the chemical shift values for the signals of the α- and δ-carbons in D_2O (Table 1).[25] In the ^{13}C NMR spectra, the α-carbon signal of the *trans*-isomer appears upfield to that of the *cis*-isomer. The δ-carbon signal of the *trans*-isomer appears downfield from that of the *cis*-isomer. Furthermore, the γ-carbon of the *cis*-isomer appears upfield to that of the *trans*-isomer and the β-carbon of the *cis*-isomer appears downfield from that of the *trans*-isomer. The populations of the amide isomers were measured in the ^1H NMR spectra by integration of the isomeric α- and *N*-acetyl proton signals in **1** as well as the δ- and *tert*-butyl proton signals in **2** and **3**. The ratios of amide isomers in **1**−**3** are listed as the percent of *cis*-isomer in Table 2.

The energy barriers ($\Delta G^\ddagger$) for amide isomerization in **1**−**3** were determined by two different NMR techniques. In the case of *cis*-diastereomer **2**, a series of ^1H NMR spectra were recorded at increasing temperatures until the resonances for the two isomer populations were observed to coalesce at 45 °C. The energy barrier for amide isomerization in **2** was calculated to be 16.5 kcal/mol in D_2O.[26] Insufficient exchange broadening below the boiling point of water prevented us from obtaining the coalescence temperatures for *N*-(acetyl)proline *N*′-methyl amide (**1**) and 5-*tert*-butylproline *trans*-diastereomer **3**. Instead, a series of magnetization transfer experiments were performed in order to ascertain the energy barriers for amide isomerization in **1** and **3**. In these experiments, the signal of the α-carbon of the major amide isomer was irradiated in H_2O and the rate for magnetization transfer to the minor isomer α-carbon signal was measured over a temperature range of 60−85 °C. The barriers for amide isomerization in **1** and **3** were respectively calculated to be 20.4 and 20.2 ± 0.2 kcal/mol.[27] The results of these studies are presented in Table 2 in which data for *N*-acetyl-5-methylproline *N*′-methyl amides in water has also been listed for comparison.[16]

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(22) ^1H NMR δ (D_2O) 1.95 (m, 3 H), [2.03 (s, 0.75 H)] 2.13 (s, 2.25 H), 2.2−2.4 (m, 1 H), 2.74 (s, 2.25 H) [2.8 (s, 0.75 H)], 3.5−3.8 (m, 2 H), 4.35 (m, 0.75 H) [4.52 (m, 0.25 H)]. ^{13}C NMR δ (D_2O) (21.6) 21.9, (22.8) 24.5, 26.3 (26.4), 30.2 (31.9), (47.4) 49, 60.8 (62.3), 173.5 (173.7), 175.1) 175.2.

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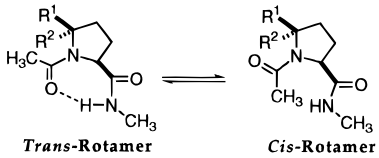
Table 2. Amide Isomer Equilibrium of Prolyl *N*-Acetyl *N*′-Methyl Amides in D_2O[d]



R^1	R^2	ΔE (calcd) (kcal/mol)[a,e]	*cis*-rotamer % calcd[a]	*cis*-rotamer % ±3%[b]	T_c (°C)[c]	$\Delta G^{\ddagger}$ (kcal/mol)[c]	ΔG (*cis*−*trans*) (kcal/mol)[b,e]
H	H	1.02	15	27	>85	20.4	0.57
Me	H			25	82	19.0	0.65
t-Bu	H	0.23	46	49	45	16.5	0.03
H	Me			30	>85		0.50
H	*t*-Bu	−0.72	77	66	>85	20.2	−0.38

[a] Calculated with Macromodel 3.5× and the AMBER force field. [b] Determined by 300 MHz NMR in D_2O at 25 °C. [c] Determined by 300 MHz NMR in D_2O. [d] Values for 5-Me examples are from ref 16. [e] Negative values indicate a predominant population of *cis*-isomer.

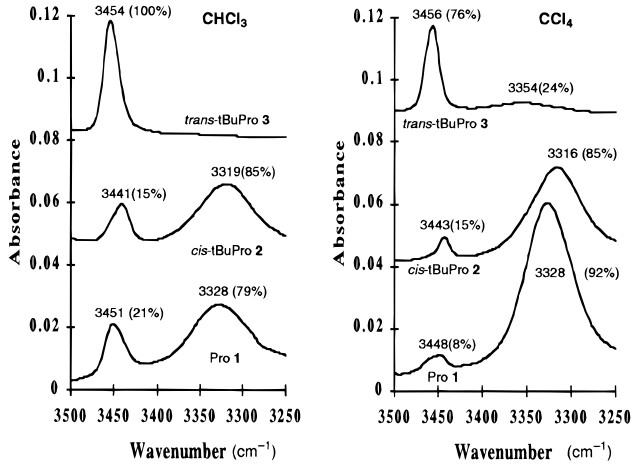


Figure 4. FT-IR spectra of the N−H stretch region of prolyl *N*-acetyl *N*′-Methylamides **1**−**3** in $CHCl_3$ and CCl_4.

FT-IR Analysis of *N*-(Acetyl)proline *N*′-Methylamides 1−3. The FT-IR spectra of prolyl amides **1**−**3** were measured at 25 °C at 1×10^{-4} M concentrations in $CDCl_3$ and in CCl_4 in order to examine the presence of a γ-turn conformation in which a seven-membered-ring intramolecular hydrogen bond exists between the *C*-terminal amide NH and the carbonyl of the *N*-terminal amide *trans*-isomer.[28,29] The solvents $CDCl_3$ and CCl_4 were chosen in order to directly compare spectra for **2** and **3** with the reported spectra of *N*-(acetyl)proline *N*′-methylamide (**1**).[13] The presence of the free N−H stretch band was observed at 3441−3454 cm^{-1} for the non-hydrogen bonded amide in all cases (Figure 4). In prolyl amide **1** and *cis*-diastereomer **2** in $CHCl_3$, an additional N−H stretch band was respectively observed at 3328 and 3319 cm^{-1} as the result of hydrogen bonding in the γ-turn conformation.[28] A predominance of hydrogen-bonded amide in **1** and **2** is indicated by the ratios of the relative areas of the lower vs higher energy absorbance for the N−H stretches which are 79:21 in **1** and 85:15 in **2**. On the other hand, no second lower energy N−H stretch band was observed for *trans*-diastereomer **3**. This result indicates the absence of a γ-turn conformation for **3** in $CHCl_3$.

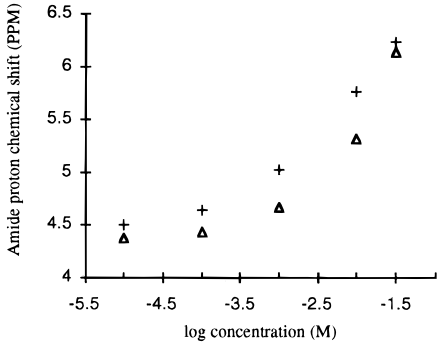


Figure 5. Amide proton NMR chemical shifts in CCl_4 at room temperature, as a function of the logarithm of the concentration of **3**: (Δ), NH amide *cis*-isomer; (+), NH amide *trans*-isomer.

In CCl_4, the area of the lower energy N−H stretch band increased in **1** and remained the same in **2**. When the FT-IR spectrum of prolyl amide **3** was measured at 1×10^{-4} M concentration in CCl_4 a second broader N−H stretch band was observed at 3354 cm^{-1}. The relative areas of the lower energy absorbance and that of higher energy for **3** in CCl_4 was 1:3. Since the N−H stretch for **3** at 3354 cm^{-1} in CCl_4 could be due to intermolecular instead of intramolecular hydrogen bonding, the effect of concentration on the chemical shift of the NH signal of **3** was measured by ^{1}H NMR (Figure 5). The $\Delta\delta/\Delta$concentration for the NH signal was still noticeable at 1×10^{-4} M and indicated intermolecular hydrogen bonding. Attempts to record the FT-IR spectrum of **3** at lower concentration were, however, unsuccessful at 1×10^{-5} M. The predominance of the N−H stretch at 3456 cm^{-1} demonstrates clearly that the γ-turn conformation is not energetically favored for *trans*-diastereomer **3**.

Molecular Mechanics Calculations and Maps of Energy vs Conformation. In recent years, considerable effort has been made to model the conformation preferences of the *cis*- and *trans*-isomers of *N*-acetylproline *N*′-methylamide (**1**) and the transition states for X-Pro amide isomerization.[30,31] These investigations have focused on calculating the energy difference between the *cis*- and *trans*-isomers and the energy barrier for amide isomerization.[30,31] We initially used the MacroModel 3.5× program and the AMBER force field with the GB/SA solvent model for water in order to calculate the energy differences between the amide *cis*- and *trans*-isomers in **1**−**3** by comparing the minima for each isomer (Table 2).[32]

(28) (a) Tsuboi, M.; Shimanouchi, T.; Mizushima, S. *J. Am. Chem. Soc.* **1959**, *81*, 1406. The intensity of this stretch band results mostly from intramolecular C_7 hydrogen bonding, yet may be due in part to intermolecular hydrogen bonding as discussed: (b) Rao, C. P.; Balaram, P.; Rao, C. N. R. *Biopolymers* **1983**, *22*, 2091 as well as in the references in 29.

(29) The use of IR spectroscopy to study intramolecular hydrogen bonds in model peptides is presented: (a) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105. (b) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. P.; Scheraga, H. A. *Biopolymers* **1979**, *18*, 2507. (c) Avignon, M.; Huong, P. V. *Biopolymers* **1970**, *9*, 427.

(30) Fischer, S.; Dunbrack, Jr. R. L.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931.

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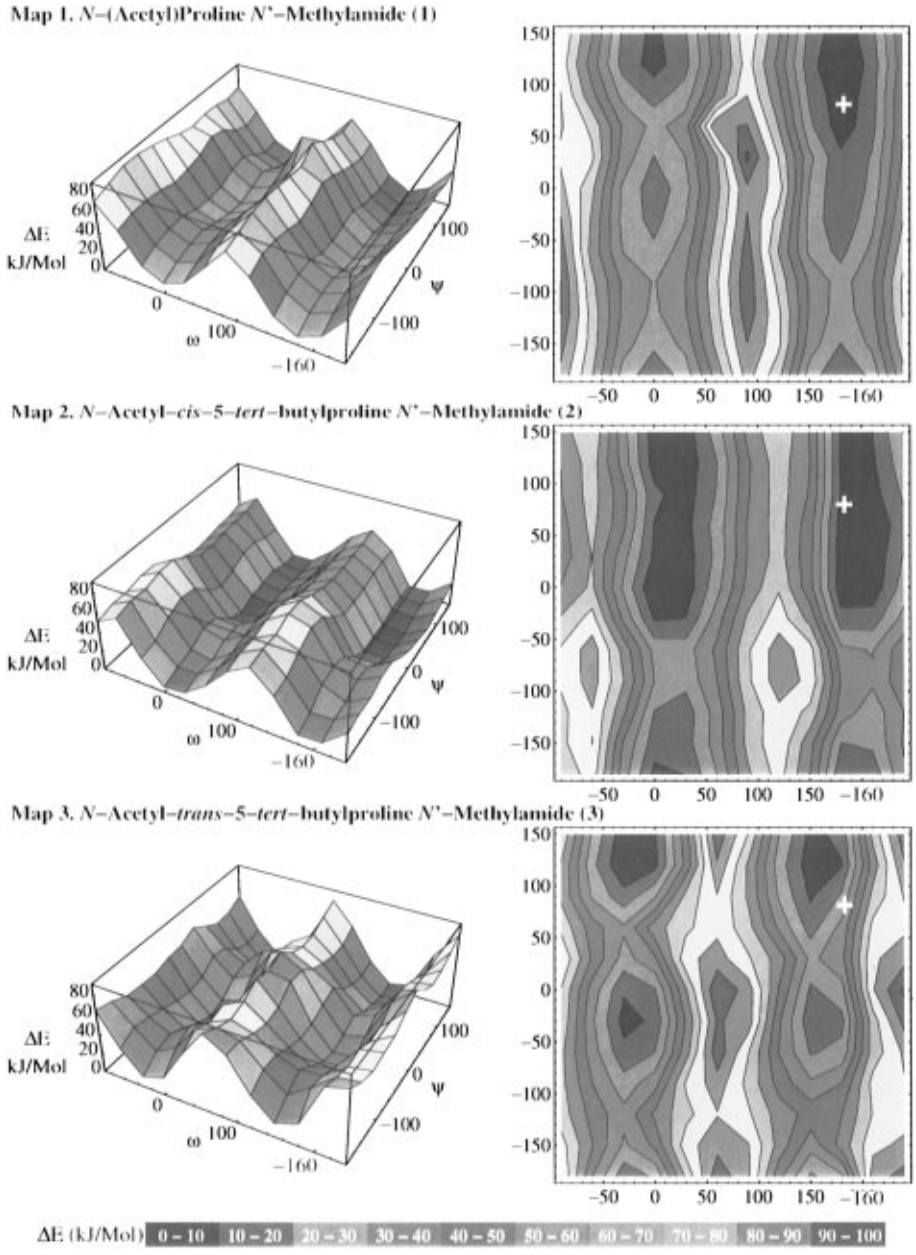
Chart 1. Maps 1−3: Conformation vs Energy of Prolyl Amides **1**−**3**



We sought next to develop a visual model by which the effects of the 5-alkyl substituent could be compared with proline **1**. A systematic analysis was performed in which the ψ- and ω-dihedral angles were rotated at 30° intervals and the energy of the local minimum was calculated for each conformation. Maps 1−3 (Chart 1) were then constructed for *N*-acetylproline *N*′-methylamides **1**−**3** by plotting the minimum energy value at each interval against the values for the ψ- and ω-dihedral angles.

Maps 1−3 provide a useful graphical representation of the effects of the 5-*tert*-butyl substituent on the amide isomer in *N*-(acetyl)proline *N*′-methylamides **1**−**3**. For example, the relative sizes of the regions of minimum energy (blue lakes) at the *cis*- and *trans*-isomers ($\omega \approx 0°$ and 180°) compare well with the populations observed by proton NMR. The magnitude of the saddles between the minima reflect the energy barriers ascertained by coalescence and magnetization transfer experiments. Furthermore, the white cross at $\omega = 180°$ and $\psi = 80°$ marks an ideal γ-turn conformation,[24] which is located at energy minima in the maps for **1** and **2**, yet appears at higher energy in the map for **3**. Maps 1−3 provide a visual aid that shows the conformational liberty about the ψ dihedral angle. Relative to proline **1**, the conformational freedom about ψ appears to be greater in *cis*-5-*tert*-butylproline **2** and more restricted in *trans*-5-*tert*-butylproline **3**.

Conclusion

Amide *cis*-isomers *N*-terminal to prolyl residues in peptides are presently being identified as important recognition elements in protein biology. For example, the type VI β-turn conformation, which features a *cis*-isomer, has been suggested to be a key intermediate in the mechanism for PPIase catalyzed

(32) The ψ, ϕ, and ω values for the calculated energy minima of the *trans*- and *cis*-isomers in **1**−**3** are as follows: **1** *trans*-isomer; 128°. −57°, −179°; **1** *cis*-isomer; 131°, −59°, 0°; **2** *trans*-isomer; 5°, −77°, −169°; **2** *cis*-isomer; 1°, −82°, 9°; a second minima was also obtained with slightly higher energy ($\Delta = 0.6$ kj/mol) for **2** *cis*-isomer: 133°, −70°, 17°; **3** *trans*-isomer; 122°, −25°, 152°; **3** *cis*-isomer; 126°, −26°, −32°. The ring puckering for all minima in **1**−**3** was consistent with an *S* conformation.

isomerization of X-Pro amide from *cis*- to *trans*-isomer.[33] In addition, a type VI conformation has been suggested as a requirement for thrombin catalyzed cleavage of the V_3 loop of HIV gp120, a prerequisite to viral infection.[34] Further examination of X-Pro amides in type VI β-turns as well as in alternative structural motifs, such as type I poly-proline helices,[35] is likely to provide additional examples of *cis*-isomer significance in other recognition events. In order to facilitate their study, methodology is thus desired for generating libraries of different peptide structures possessing conformationally restrained X-Pro amide *cis*-isomers.

Steric effects have been used to control the X-Pro amide conformation and favor the *cis*-isomer population. 5-*tert*-Butylprolines were synthesized with stereocontrol and examined in model peptides **2** and **3**. In *N*-(acetyl)proline *N*′-methylamides **1**−**3**, the interactions of the *tert*-butyl group on the amide geometry augment significantly the *cis*-isomer population in water (Table 2). The barrier for amide isomerization is 3.9 kcal/mol lower in *cis*-diastereomer **2** relative to proline **1**. Maps 2 and 3 illustrate that the bulky 5-position substituent skews the amide bond away from planarity such that twisted amide conformations appear with energy minima at $\omega > 0°$ and $> 180°$ for *cis*-diastereomer **2** and $\omega < 0°$ and $< 180°$ for *trans*-diastereomer **3**. Moreover, the *tert*-butyl group influences the ψ dihedral angle such that minima are found at $\psi \approx 125°$ for **1** and **3**, and at $\psi \approx 0°$ for **2**.[32] The lower barrier for isomerization in *cis*-diastereomer **2** may be due in part to this effect on the ψ dihedral angle, because the *C*-terminal NH group at $\psi \approx 0°$ may stabilize the pyramidalized amide transition states by interacting with either the nitrogen lone pair or the carbonyl oxygen.[30] In *trans*-diastereomer **3**, a combination of effects on the ω and ψ dihedral angles leads to a destabilization of the γ-turn conformation and no N−H stretching band corresponding to a hydrogen-bonded amide is observed by FT-IR spectroscopy in $CHCl_3$ (Figure 4).

In conclusion, by studying model peptides **1**−**3**, we have been able to itemize both the effects of steric bulk and stereochemistry of 5-position substituents on prolyl conformation. Since the bulky 5-position substituent influences amide geometry by disfavoring the *trans*-isomer, the population of *cis*-isomer will augment as the size of the *N*-terminal group increases. Furthermore, because the *tert*-butyl group distorts the amide away from planarity, potential exists to use 5-*tert*-butylprolines to generate twisted amide mimetics. Our preliminary results demonstrate already that 5-*tert*-butylproline can be introduced into different amide and peptide structures.[19,20] In future work, incorporation of 5-*tert*-butyl proline into a library of X-Pro analogues will be explored as a promising new means for studying the importance of *cis*-isomer geometry in protein chemistry and biology.

Experimental Section

Experiments involving chemical reactions were performed under the general guidelines described in reference 19. The chemical shifts for the carbons and protons of minor isomers are respectively reported in parentheses and in brackets.

General Procedure for the Synthesis of *N*-Acetyl-5-*tert*-butylproline *N*′-Methylamides 2 and 3. Methylamine hydrochloride (1.35 g, 20 mmol) in benzene (20 mL) at 5 °C was treated with a solution of trimethylaluminum in hexane (2 M, 10 mL, 20 mmol), brought to room temperature, and stirred until the loss of CH_4 gas was no longer detectable.[21] From the resulting solution (0.067 M), 2.7 mL (1.74 mmol, 300 mol %) was added dropwise to a room temperature solution of *cis*-*N*-BOC-5-*tert*-butylproline methyl ester (*cis*-**4**, 166 mg, 0.58 mmol, 100 mol %) in CH_2Cl_2 (6 mL). The mixture was stirred for 72 h, treated with another 1.73 mL of the benzene solution, and stirred 48 h when TLC showed complete disappearance of starting ester. The reaction mixture was treated with 6 mL of saturated K_2CO_3 and extracted with CH_2Cl_2 (3 × 15 mL). The organic layers were combined, washed with brine, dried with Na_2SO_4, filtered, and evaporated to 122 mg of an oil (74%) that was used in the next step without further purification. *cis*-*N*-BOC-5-*tert*-Butylproline *N*′-methylamide: ^{1}H NMR δ ($CDCl_3$) 0.88 (s, 9 H), 1.47 (s, 9 H), 1.9 (m, 2 H), 2.15 (m, 1H), 2.25 (m, 1 H), 2.83 (d, 3 H, $J = 4.9$), 3.87 (dd, 1 H, $J = 3.1, 7.8$), 4.32 (t, 1 H, $J = 8.7$), 6.8 (s, 1 H); ^{13}C NMR δ ($CDCl_3$) 26.15, 26.18, 26.5, 27.4, 28.3, 35.6, 62.9, 67.6, 80.8, 157.8, 173.2.

cis-*N*-BOC-5-*tert*-Butylproline *N*′-methylamide (100 mg, 0.35 mmol) was dissolved in 4 mL of dichloromethane, treated with 260 μL of trifluoroacetic acid (3.5 mmol), and stirred at room temperature for 5 h. Evaporation of the volatiles on a rotary evaporator gave 99 mg (95%) of *cis*-5-*tert*-butylproline *N*′-methylamide trifluoroacetate (**5a**): ^{1}H NMR δ ($CDCl_3$) 1.0 (s, 9 H), 1.6 (m, 1 H), 1.9 (m, 1H), 2.1 (m, 1 H), 2.3 (m, 1 H), 2.76 (d, 3 H, $J = 4.7$), 3.36 (m, 1 H), 4.49 (m, 1 H), 6.86 (br s, 1 H), 7.57 (br s, 1 H), 10.43 (br s, 1 H).

trans-5-*tert*-Butylproline *N*′-methylamide trifluoroacetate (**5b**) was generated in a similar way from *trans*-**4** in 81% overall yield: ^{1}H NMR δ ($CDCl_3$) 0.9 (s, 9 H), 1.38 (m, 1 H), 1.77 (m, 2 H), 2.25 (m, 1 H), 2.8 (d, 3 H, $J = 5$), 2.81 (m, 1 H), 3.73 (t, 1 H, $J = 7.5$), 7.5 (br s, 1 H).

cis-5-*tert*-Butylproline *N*′-methylamide trifluoroacetate (**5a**, 50 mg, 0.31 mmol) and Et_3N (60 μL, 0.5 mmol) in CH_2Cl_2 (3 mL) were treated with acetic anhydride (44 μL, 0.47 mmol), stirred for 48 h at room temperature, and evaporated to a residue that was purified by chromatography using an eluant of 5% methanol in $CHCl_3$. Evaporation of the collected fractions gave 61 mg (88%) of *N*-acetyl-*cis*-5-*tert*-butylproline *N*′-methylamide (**2**): ^{1}H NMR δ (D_2O) 0.92 (s, 4.5 H) [0.97 (s, 4.5 H)], 1.8−2.5 (m, 4 H), 2.02 (s, 1.5 H) [2.24 (s, 1.5 H)], 2.75 (s, 1.5 H) [2.8 (s, 1.5 H)], 3.97 (d, 0.5 H, $J = 7.6$) [4.1 (d, 0.5 H, $J = 8.2$)], 4.55 (m, 1 H); ^{13}C NMR δ (D_2O) 22.3 (23), 26 (26.2), 26.9, 27.4, (28.3) 30.7, 35.9 (36), (62.6) 63.6, 67.6 (69.9), 175.3, 176.6; FAB MS *m*/*e* 227.2 $[MH]^+$ (100%), 185.2, 168.2, 126.2. HRMS calcd for $C_{12}H_{23}N_2O_2$ (MH^+) 227.1760, found 227.1768.

N-Acetyl-*trans*-5-*tert*-butylproline *N*′-methylamide (**3**) was prepared from **5b** in a similar way in 96% yield; ^{1}H NMR δ (D_2O) 0.85 (s, 3 H) [0.9 (s, 6 H)], 1.7−2.2 (m, 3 H), 1.92 (s, 1 H) [2.16 (s, 2 H)], 2.4 (m, 1 H), 2.69 (s, 2 H) [2.73 (s, 1 H)], 4.02 (m, 0.66 H) [4.19 (d, 0.33 H, $J = 6.2$)], 4.32 (d, 0.66 H, $J = 8.5$) [4.57 (d, 0.33 H, $J = 10$)]; ^{13}C NMR δ (D_2O) (22.9) 23.6, (25.4) 26.1, (26.3) 26.6, 27.3 (27.6), 29.5 (31.6), (37.3) 37.9, 62.4 (63.1), (66.7) 69.4, 175.3 (175.5), 176 (176.1); FAB MS *m*/*e* 227.2 $[MH]^+$ (100%), 196.2, 168.2, 126.2. HRMS calcd for $C_{12}H_{23}N_2O_2$ (MH^+) 227.1760, found 227.1766.

Magnetization transfer NMR experiments were performed on Bruker AMX600 and DMX600 MHz spectrometers, both equipped with selective excitation units, and ^{1}H and broadband heteronuclear probes. Experiments were performed over several temperatures between 313 and 348 K for each compound. Selective inversion of C^α carbon *trans*- and *cis*-isomer signals were done with gaussian pluses centered on resonance ±25 Hz. Relaxation delays of 20 seconds and inversion-recovery delays of between 1 ms and 20 s were used. Data for each inversion recovery point were averaged over 32 to 128 points, depending on the concentration of the compound used. Amides **1** and **3** were dissolved in distilled and deionized H_2O or D_2O at concentrations between 1×10^{-2} M and 2×10^{-2} M and extensively exchanged with nitrogen gas in NMR tubes. For each compound at an individual temperature, inversion-recovery results were collected with selective inversion of the C^α *trans*-isomer peak. Data were fitted according to equations 1 and 2.[27]

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$$M_Z(A)/M_\infty(A) = 1 - (2k_A/\beta)I_A\{\exp[-1/2(\alpha - \beta)\tau] - \exp[-1/2(\alpha + \beta)\tau]\} \quad (1)$$

$$M_Z(X)/M_\infty(X) = 1 - (1/\beta)I_X\{(\beta + R_X - R_A)\exp[-1/2(\alpha + \beta)\tau] + (\beta + R_A - R_X)\exp[-1/2(\alpha - \beta)\tau]\} \quad (2)$$

Where $\alpha = R_A + R_X$, $\beta = [(R_A - R_X)^2 + 4k_Ak_X]^{1/2}$ and $R_A = k_A + (1/T_{1A})$, $R_X = k_X + (1/T_{1X})$. Where X is the inverted C^α signal; A is the passive signal; T_{1A} and T_{1X} are longitudinal relaxation times; k_A and k_X are rate constants for isomerization; $M_Z(A)$, $M_\infty(A)$, $M_Z(X)$, and $M_\infty(X)$ are longitudinal magnetizations; and I_A and I_X are proportionality constants used to correct the incomplete return of magnetization to equilibrium. We used conjugate-gradient optimization (Marquart−Levenberg method) to simultaneously fit all eight parameters in 1 and 2 starting with estimates of values for the individual parameters.[36] Analytical derivatives of 1 and 2 were calculated with the program Mathematica (version 2.0).

^{1}H NMR experiments to determine the effect of concentration on the chemical shift of **3** were performed in CCl_4 (freshly distilled from P_2O_5). The solutions were transferred to an NMR tube, and into the sample a second tube was inserted containing acetone-d_6 that was used for the Lock signal and as the internal reference.

FT-IR spectra were measured at 25 °C on a Perkin-Elmer 1600 FT-IR spectrometer using a DTGS detector at 4 cm^{-1} resolution. Chloroform and CCl_4 were distilled from P_2O_5. Peptide solutions of 1×10^{-4} M were examined in 1 cm quartz glass Infrasil cell and the spectra were recorded with 1024 scans.

Molecular mechanics calculations were performed on a Silicon Graphics Personal Iris Workstation using the AMBER force field and the GB/SA solvent model for water within the Macromodel program 3.5×.[37] The modeling protocol on amides **1**−**3** was conducted as follows. For each *N*-(acetyl)proline *N*′-methylamide, a *trans*-isomer amide bond was drawn and minimized in water using the Steepest Descent (SD) procedure followed by a Full Matrix Newton Raphson (FMNR) method until a 0.001 kJ/Å·mol gradient was reached. The resulting structure was then used as the starting point for the generation of the conformational energy maps. The ω and ψ dihedral angles were rotated about 30° increments in order to generate 144 conformers. For each conformer, two pyrrolidine ring puckerings were applied in order to generate 288 starting conformers that were then minimized in water using a Polack-Ribiere Conjugate Gradient (PRCG) and a force of 1000 kJ/mol in order to restrain the ω and ψ dihedral angles. The resulting minima were then plotted against the ω and ψ dihedral angles using the Mathematica program in order to furnish maps 1−3. The global minima for the *cis*- and *trans*-isomers of **1**−**3** were obtained using a similar protocol in which no constraints were placed on the ω and ψ dihedral angle geometries.[32]

Acknowledgment. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada, and the Ministère de l'Éducation du Québec. W.D.L. thanks Bio-Méga/Boehringer Ingelheim Recherche Inc. for a Young Investigator Award. We thank gratefully Professeur Stephen Michnick for his assistance in the execution and in the analysis of the magnetization transfer experiments. Similarly, we thank Ms. Sylvie Bilodeau and Dr. M. T. Phan Viet of the Regional High-Field NMR Laboratory for their assistance.

Supporting Information Available: The ^{1}H and ^{13}C NMR spectra of **1**−**3** and ^{1}H NMR of **5** and plots of intensity vs mixing time for the magnetization transfer experiments on **1** and **3** (8 pages). See any current masthead page for ordering and Internet access instructions.

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