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Impact of Azaproline on Peptide Conformation

Ye Che† and Garland R. Marshall*

Center for Computational Biology and Department of Biochemistry and Molecular Biophysics, Washington University, St. Louis, Missouri 63110

garland@pcg.wustl.edu

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The amino acid analog azaproline (azPro) contains a nitrogen atom in place of the C_α of proline. Peptides containing azPro were shown to stabilize the *cis*-amide conformer for the acyl-azPro bond and prefer type VI β-turns both in crystals and in organic solvents by NMR. The increased stability for *cis*-amide conformers was relatively minor with respect to the *trans*-conformers. Further, their conformational preferences were depended on solvent. To elucidate the impact of azPro substitution on amide *cis*−*trans* isomerism and peptide conformation, this paper reports ab initio studies on azPro derivatives and a comparison with their cognate Pro derivatives: 1-acetyl-2methyl pyrrolidine (**1**), 1-acetyl-2-methyl pyrazolidine (**2**), Ac-Pro-NHMe (**3**), Ac-azPro-NHMe (**4**), Ac-azPro-NMe_2 (**5**), Ac-azAzc-NHMe (**6**), and Ac-azPip-NHMe (**7**). Conformational preferences were explored at the MP2/6-31+G** level of theory in vacuo. Solvation effects for **1** and **2** were studied implicitly using the polarizable continuum model and explicitly represented by interactions with a single water molecule. An increase in the conformational preference for the *cis*-amide conformer of azPro was clearly seen. An intramolecular hydrogen bond occurred solely in the *trans*-amide conformer that reduced the preference for the *cis*-conformer by 2.2 kcal/mol. The larger ring homolog aza-pipecolic acid (azPip), in which this internal hydrogen bond was diminished, significantly augmented stabilization of the *cis*-amide conformer. In aqueous solution, the preference for the *cis*-amide conformers was greatly reduced, mainly as a result of interaction between water and the lone pair of the α-nitrogen in the *trans*-amide conformer that was 3.8 kcal/mol greater than that in the *cis*-conformer. In the azPro analog, the energy barrier for *cis*−*trans* amide isomerization was 6 kcal/mol less than that in the cognate Pro derivative. Because the azPro derivatives can stabilize the *cis*-amide bond and mimic a type VI β-turn without incorporation of additional steric bulk, such a simple chemical modification of the peptide backbone provides a useful conformational constraint when incorporated into the structure of selected bioactive peptides. Such modifications can scan receptors for biological recognition of reverse turns containing *cis*-amide bonds by the incorporation of type VI β-turn scaffolds with oriented appended side chains.

Introduction

The β-turn is a well-studied motif in both proteins and peptides[1,2] and has often been implicated as a recognition site in protein interactions.[3−5] A β-turn consists of four sequential residues, which are designated as i, $i+1$, $i+2$, and $i+3$, making an almost complete 180° turn in the direction of peptide chain. Several different types of β-turns are possible depending upon the Φ and Ψ backbone torsional angles of the $i+1$ and $i+2$ residues.[6] In addition, these turns may (classic β-turn) or may not (open β-turn) be stabilized by an intramolecular hydrogen bond between the carbonyl oxygen of the first residue (i) and the amide hydrogen of the fourth residue ($i+3$).[1] One special type of β-turn is the type VI turn defined by an amino acid, usually proline, with a *cis*-amide bond located at the third residue ($i+2$). Richardson[2] divided this class into two different subclasses, type VIa and VIb. Type VIa usually has an internal hydrogen bond, whereas type VIb does not usually make a hydrogen bond. The reported values for (Φ_2, Ψ_2), (Φ_3, Ψ_3) torsions for the two types of VI turns are shown in Table 1.[1]

TABLE 1. Ideal ϕ, ψ, and ω Backbone Torsional Angles for Type VI β-Turns[1]

β-turns	ϕ_2	ψ_2	ω_2	ϕ_3	ψ_3
VIa	−60°	120°	0°	−90°	0°
VIb	−120°	120°	0°	−60°	0°

β-Turns are intrinsically polar structures with backbone groups that pack together closely and side chains that project outward. Such an array of atoms often constitute a site for molecular recognition, and indeed, an example of the type VI β-turn as a loci for molecular recognition can be found in the solution structure of an

† Current address: Laboratory of Biophysical Chemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

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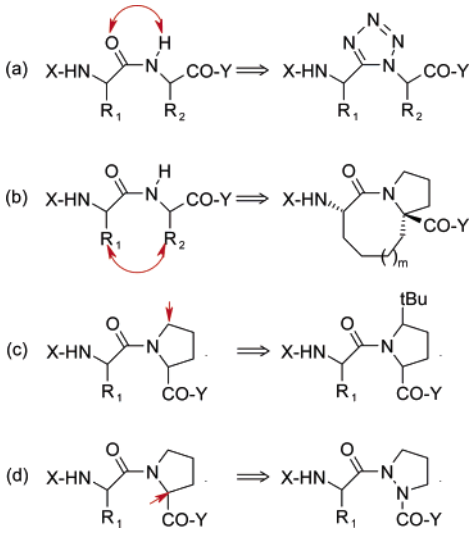
antibody-bound HIV-1_{IIIB} V3 peptide.[7] Further, such a type VI β-turn conformation in the HIV-1_{IIIB} V3 peptide was entirely consistent with the antibody recognition deduced from structure−activity studies.[8] The importance of type VI β-turn in recognition has led to efforts to stabilize the *cis*-amide bonds and mimic type VI β-turns in peptide chains.

In native proteins, the proline residue plays a special role in stabilizing the preceding *cis*-amide bond. As the steric properties of proline due to disubstitution on the α-nitrogen are quite similar in either *cis*- or *trans*-conformations, a high degree of the *cis*-amide conformer of proline has often been observed in peptides and proteins.[9] The intrinsic probability for a *cis*-amide bond compared with the *trans*-conformation of the amide bond preceding a proline is 0.1−0.3 as compared to less than 10^{-3} for the rest of naturally occurring amino acids in proteins.[10] The energy barrier for *cis*−*trans* isomerization is also less for proline as compared to other amino acids. The loss of the amide hydrogen results in lack of resonance stabilizing and a redistribution of charge with greater length of the Xxx-Pro bond, 1.36 Å for proline as compared to 1.33 Å for a usual amide bond. The impact of Pro on *cis*−*trans* distribution was studied by Zimmerman and Scheraga[11] using the ECEPP force field (limited to torsional variables) and attributed primarily to negative van der Waals interaction between adjacent C_α's connected to the *cis*-amide bond for the non-Pro case, which were essentially equivalent for *cis*−*trans* conformers in the dimethyl amide case such as proline. The conformational space for a set of tripeptides containing a *cis*-amide bond was studied by Nagarajaram et al.,[12] who reported the minimum-energy conformation for a set of Xxx-*cis*-Pro combinations including Ala-Pro and Pro-Pro. These studies ruled out the occurrence of a *cis*-amide bond for the first proline in Pro-*cis*-Pro because of its high energy. Two types of hydrogen bond were found, type 4 → 1 and type 1 → 2. The effect of the preceding side chain on *cis*-amide content in the pentapeptide series Ac-Ala-Xxx-Pro-Ala-Lys-NH_2 was investigated by Reimer et al.[13] with magnetization transfer NMR. Those data showed that Pro-Pro had the least *cis*-amide content. The results also indicated that aromatic stacking with preceding residues as Phe, Tyr, or Trp stabilized the *cis*-amide bonds. *Cis*−*trans* isomerization in biological and organic chemistry and its implications for therapeutics was recently reviewed by Dugave and Demange.[14]

Several peptidomimetics approaches to mimic type VI β-turns by stabilizing the *cis*-amide bond through using the 1,5-disubstitued tetrazole ring,[15−21] 1,2,5-triazole,[22] or 1,2,4-triazole[23] as a *cis*-amide bond surrogate (Scheme 1a); incorporating vicinal disulfide bonds,[24−28] bicyclic dipeptide analogs,[22,29−37] and certain sequences into cyclic peptides[38] (Scheme 1b); or substituting sterically bulky groups on the C_δ-atom of the proline residue[8,39−45] (Scheme

SCHEME 1. *cis*-Amide Bond Mimetics[a]



[a] The 1,5-disubstituted tetrazole as a surrogate for the *cis*-amide bond (a), the bicyclic dipeptide analog (b), the C_δ-substituted Pro analog (c), and the azPro peptide analog.

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1c). Unfortunately, many of methods listed above for stabilizing *cis*-amide bonds dramatically change the steric bulk of the turn structure and modify its hydrogen-bonding characteristics, which can severely affect the recognition specificity and limit possibilities of incorporation of side-chain functional groups as chimeric amino acids into turns. Another less investigated modification is the aza-amino acid, especially azaproline (azPro, Scheme 1d). In an aza-amino acid, the α-carbon is changed to nitrogen, and incorporation of such residues into biologically active peptides was pioneered by Dutta and Morley.[46] Several azPro-containing model peptides and azPro analogs of biologically active peptides have been prepared. For example, analogs of thyrotropin-releasing hormone (TRH) containing azPro, [azPro^3]-TRH, and [Phe^2, azPro^3]-TRH were recently prepared in our group to probe the receptor-bound conformation of TRH.[47] The Kessler group has developed solid-phase synthetic routes to azapeptides and azapeptoids and applied them to RGD analogs.[48] Numerous aza-analogs of biologically active peptides have been prepared; for example, angiotensin II,[49] oxytocin,[50] eledoisin,[51] enkephalin,[52] and luliberin (LHRH),[53,54] with one analog ([D-Ser(*t*-Bu),^{6} azGly^{10}]-LHRH, a commercial product, Zoladex, ICI 118630) used for the treatment of prostate carcinoma. More recently, azaglycine has been studied as a replacement for the central residue of the RGD recognition motif of integrins.[55,56] The azapeptide linkage also appears to confer resistance to degradation by many proteolytic enzymes as originally discovered by Oehme et al.[57] and by Dutta and Giles.[58] Azapeptide linkages have been incorporated into inhibitors of various enzymes, such as angiotensin converting enzyme,[59] cysteine protease,[60,61] renin,[62] human leukocyte elastase,[63] and human rhinovirus 3C protease.[64] HIV protease inhibitor Atazanavir (BMS-232632) contains a *para*-substituted azaphenylalanine and is active against multiple resistant strains.[65] A special example of azapeptides is the azatide that is defined as a "pure azapeptide" where the α-carbon for each amino acid is changed to nitrogen.[66,67] An inhibitor of renin prepared by Gante et al.[62] was the first example of a biologically active azatide.

TABLE 2. Crystal and Solution Structures of Various AzPro Peptides

azPro peptides[a]	exptl methods	ω
Z-azPro-NH^{i}Pr[b]	X-ray	−18.8°
Ac-azPro-NH^{i}Pr[c]	X-ray	15°
Z-azPro-Ala-NH^{i}Pr[b]	X-ray	−22.7°
Boc-Ala-azPro-NH^{i}Pr[b]	X-ray	14.2°
Z-azPro-azPro-Obzl[d]	X-ray	177.2°, 188.2°, −173.0°, 179.7
Boc-Ala-azPro-Ala-NH^{i}Pr[c]	X-ray	21°
Boc-Ala-azPro-D-Ala-NH^{i}Pr[c]	X-ray	18°
pGlu-His-azPro-NH$_2$[e]	NMR (MeOH, −10 °C)	40% *cis*-amide
pGlu-Tyr-azPro-NH$_2$[e]	NMR (MeOH, −10 °C)	20% *cis*-amide

[a] Z = carbobenzoxy, Ac = acetyl, Boc = tert-butyloxycarbonyl, iPr = isopropyl, bzl = benzyl, pGlu = pyroglutamic acid. [b] Reference 67. [c] Reference 69. [d] Reference 68. [e] Reference 46.

AzPro-containing peptides have been characterized in crystals,[68−70] organic solvent,[47,71,72] and aqueous solutions.[47] These azPro studies are summarized in Table 2. Most of the crystal structures obtained for the azPro-containing peptides have the amide bond preceding the azPro residue with the *cis*-amide conformation. The two adjacent nitrogens in the pyrazolidine ring showed a clear pyramidal character in the reported crystals. The amide bond was also longer, giving a lower barrier for *cis*−*trans* isomerization. However, in aqueous solutions and organic solvents, the increased stability for the *cis*-amide conformer with respect to the *trans*-conformer as determined by NMR was not as significant as in the X-ray studies. Thus, the effects of azPro on *cis*−*trans* isomerism and its impact on peptide conformations needed further investigations both theoretically and experimentally.

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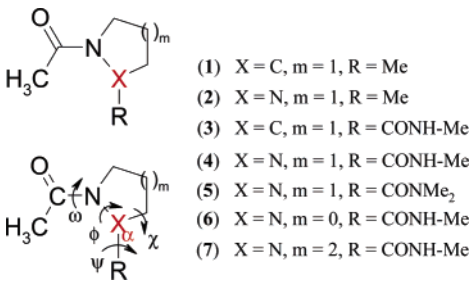

FIGURE 1. Chemical structures and torsion angles (ω, ϕ, ψ, and χ) for 1-acetyl-2-methyl pyrrolidine (**1**), 1-acetyl-2-methyl pyrazolidine (**2**), Ac-Pro-NHMe (**3**), Ac-azPro-NHMe (**4**), Ac-azPro-NMe_2 (**5**), Ac-azAzc-NHMe (**6**), and Ac-azPip-NHMe (**7**).

Several ab initio and density functional calculations were performed in vacuo on model compounds to examine the general structural properties of azapeptides. Reynolds at al.[73] explored the structure and rotational flexibility of diformylhydrazine using ab initio calculations (MP2/6-31+G**). The calculations were focused on the (*Z*,*Z*), (*Z*,*E*), and (*E*,*E*) conformations of diformylhydrazine as well as the rotational barrier for the CO−N−N−CO torsional angles. In these calculations, the two adjacent nitrogens had a pyramidal conformation for all conformations. The fact that diformylhydrazine has a flat conformation in crystal structure is probably due to crystal packing forces in that a planar conformation allows stacked sheets of hydrogen-bonded networks. Lee et al.[74] studied the structural perturbation introduced into formyl-amino acid-amides by changing the α-carbon to nitrogen with ab initio calculations (HF/6-31G*). The global minimum energy conformation for these compounds (azGly, azAla, azLeu) suggested a β-turn motif with the aza residue at the $i + 2$ position. Lee et al.[75] further examined the conformational properties of *N*-methyl azapeptides derivatives by ab initio (HF/6-31G*) and density functional theory (B3LYP/6-31G*) calculations. *N*-Methyl azapeptides were found to preferentially adopt various types of β-turn, polyproline II, α-helical, or even extended conformations depending on the orientation of the acetyl group and the positions of the *N*-methyl groups.

The focus of this paper is quantitative determination of the conformational influence of azPro in stabilizing the *cis*-amide bond and mimicking the type VI β-turn structure. To elucidate these fundamental questions, we have undertaken ab initio (MP2/6-31+G**) and DFT (density functional theory, B3LYP/6-31+G**) studies on azPro derivatives in comparison with their cognate Pro derivatives: 1-acetyl-2-methyl pyrrolidine (**1**) vs 1-acetyl-2-methyl pyrazolidine (**2**), Ac-Pro-NHMe (**3**) vs Ac-azPro-NHMe (**4**), Ac-azPro-NMe_2 (**5**), Ac-azAzc-NHMe (**6**), and Ac-azPip-NHMe (**7**), as shown in Figure 1. The solvation effects for **1** and **2** were studied implicitly using the polarizable continuum model (PCM)[76] or when explicitly represented by interactions with a single water molecule.

These calculations of azPro derivatives provide insight into the structural preferences and dynamics of azapeptides, which should aid in the development of novel inhibitors based on aza-amino acids. The detailed results (conformers, energies, and coordinates) for compounds **1**−**7** (Figure 1) are found in Supporting Information.

Computational Methods

The Gaussian03 program[77] was used to perform ab initio and DFT calculations. For the ab initio studies, all structures were first optimized at the HF/6-31+G* level and then completely reoptimized at the MP2/6-31+G** level to account for correlation effects. DFT calculations were done at the level of B3LYP/6-31+G**. Though there were some structural and energetic differences between the ab initio and DFT results, conclusions on conformational preferences for the azPro derivatives were identical. Only the MP2/6-31+G** results will be discussed in the following, and the DFT studies will be mentioned only as needed for comparison purposes.

The large basis set 6-31+G**, with polarization functions on all atoms and diffuse functions on the heavy atoms, was employed because polarization functions are often required to reproduce correct pyramidalization of nitrogen lone pairs.[73] In addition, any energetic comparisons are likely to be sensitive to interaction between the diffuse adjacent nitrogen lone pairs. The presence of diffuse and polarization functions should represent these interaction energies more accurately. The 6-31+G** basis set is large enough that it should give excellent results for relative conformational energies of aza-amino acids. The calculations were supplemented by computation of the correlation energy via second-order Møller−Plesset perturbation theory. The correlation effect is important to accurately describe the intramolecular hydrogen bond and several strong polar groups in close proximity. All stationary points located at the MP2/6-31+G** level were characterized as minima or transition states by harmonic vibrational frequency calculations. The zero-point vibrational energies and the thermal corrections were not included in the conformational analysis because of small energetic differences found between different conformers in this study.

1-Acetyl-2-methyl pyrrolidine (**1**) and 1-acetyl-2-methyl pyrazolidine (**2**) are the smallest compounds modeled in this study. The conformational preferences for the *cis*- and *trans*-amide conformers of **1** and **2** are affected only by the chemical modifications just adjacent to the amide bond. Under physiological conditions, the C_α-atom is a nonconvertible chiral center with absolute configurations of either *R* or *S*. It is well-known that the N_α-atom, as a result of its sp^3 character, is able to invert at room temperature from one trigonal pyramidal conformation to the other through a planar, sp^2-hybridized intermediate, namely, amine inversion, with a relatively small energy barrier of about 6 kcal/mol.[78] As reported by Boussard et al.,[68,70] the out-of-plane distance for the α-nitrogen is

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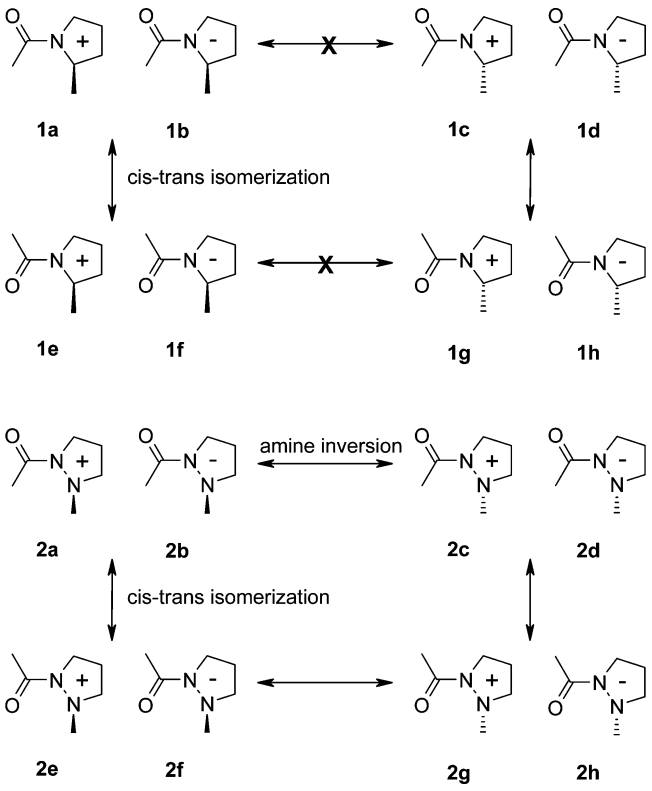
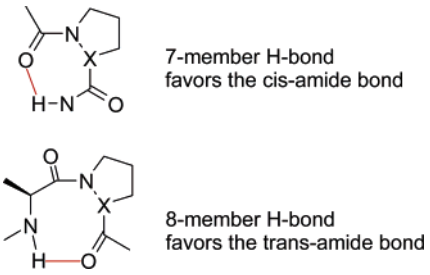


FIGURE 2. All possible conformations for 1-acetyl-2-methyl pyrrolidine (a) and 1-acetyl-2-methyl pyrazolidine (b). "+" and "−" stand for the up- and down-puckering of the five-member pyrazolidine or pyrrolidine ring.

between 0.18 and 0.40 Å. Pyramidalization of the two adjacent nitrogens in azPro derivatives was described by the sum of the bond angles around the nitrogen atom with a pyramidal sp^3-structure close to 328° and a planar sp^2-structure close to 360°. The conformations of **1** and **2** were characterized by the *cis*- and *trans*-amide bond, the chiral center at the α-position, and the up- and down-puckering of the pyrrolidine and pyrazolidine rings. Four conformers exist for 1-acetyl-2-(*R*)-methyl pyrrolidine (**1a**, **1b**, **1e**, and **1f**), and four exist for 1-acetyl-2-(*S*)-methyl pyrrolidine (**1c**, **1d**, **1g**, and **1h**). On the other hand, eight conformers (**2a**−**2h**) are available to 1-acetyl-2-methyl pyrazolidine as a result of amine inversion (Figure 2).

Usually, for short linear peptides, we would not expect much internal hydrogen bonding in aqueous solutions; however, significant intramolecular hydrogen bonding might be expected in organic solvents, and many experimental data are available in this regard. Even in aqueous solutions, if azPro or Pro were incorporated in a cyclic peptide template, it would be more likely to have intramolecular hydrogen bonding. Such internal hydrogen bonds can significantly affect the relative populations of *cis*/*trans* amide isomers. Depending on the sequence and geometry, the internal hydrogen bond can favor *cis*- or *trans*-amide as shown in Scheme 2. To elucidate to what extent the seven-membered hydrogen bond favors the *trans*-amide conformer in both Pro and azPro-containing peptides, Ac-Pro-NHMe (**3**) and Ac-azPro-NHMe (**4**) were studied as simple model peptides. In this study, only the most stable *cis*- and *trans*-amide conformers, in vacuo or nonpolar solvent, of **3** and **4** were considered. Experimental and high-level ab initio results[79,80] are well documented for Ac-Pro-NHMe. The predominant *trans*-amide conformer is the C_7-conformer with

SCHEME 2. Effects of Intramolecular Hydrogen Bonds on *cis*/*trans*-Amide Isomers[a]



[a] The seven-membered hydrogen bond ($i + 2 \rightarrow i$) stabilizes the *trans*-amide conformer, and the eight-membered hydrogen bond ($i \rightarrow i + 1$) stabilizes the *cis*-amide conformer.

backbone torsion $\Psi = 74.3°$ and a seven-membered intramolecular hydrogen bond. The *cis*-amide conformer has the backbone torsion $\Psi = -9.1°$. The complete rotational energy profiles of the Ψ-angle for both the *cis*- and *trans*-amide conformer of **3** and **4** were calculated at the MP2/6-31+G**//HF/6-31+G** level of theory to define the major features of the potential energy surface, which is important to assess the structure and flexibility of azPro peptides as to whether they can stabilize a type VI β-turn structure.

The seven-membered hydrogen bond seen in Scheme 2 can be eliminated by further *N*-methylation at the *N*-methylcarboxamide terminus. Ac-azPro-NMe_2 (**5**) was studied in a similar fashion to examine the impact of changing the amide proton for a methyl group. Internal hydrogen bonding can also be impacted by changing the ring size to the smaller four-membered ring homolog aza-azetidine acid (azAzc), Ac-azAzc-NHMe (**6**), or the larger six-membered ring homolog aza-pipecolic acid (azPip), Ac-azPip-NHMe (**7**).

The α-nitrogen has more sp^3 character, which allows it to serve as a hydrogen-bonding acceptor due to the existence of a lone pair. Solvation effects on the conformational preferences of **1** and **2** were estimated implicitly utilizing the polarizable continuum model (PCM)[76] with the integral-equation formalism.[81−83] Calculations were performed in several solvents with the following dielectric constants (ϵ): carbon tetrachloride (CCl_4, $\epsilon = 2.228$), chloroform ($CHCl_3$, $\epsilon = 4.9$), methanol (MeOH, $\epsilon = 32.63$), dimethyl sulfoxide (DMSO, $\epsilon = 46.7$), and water (H_2O, $\epsilon = 78.39$). Interactions with water were also explicitly studied by complete optimizations of water-bound complexes with the complexation energy corrected for the basis set superposition error (BSSE) by the counterpoise method.[84]

Results and Discussion

Optimized geometries, coordinates, and energies of compounds **1**−**7** are listed comprehensively in Supporting Information. Puckering effects of pyrrolidine and pyrazolidine were only minor relative to the conformational preferences observed. In general, the down-puckering conformations ($\chi_1 > 0$) were more stable by about 0.5−1.0 kcal/mol in vacuo relative to the up-puckering conformation ($\chi_1 < 0$). In the following sections, only the down-puckering conformations are discussed as representative of both ring conformers.

1-Acetyl-2-methyl pyrrolidine (1) and 1-Acetyl-2-methyl-pyrazolidine (2). Profound differences between

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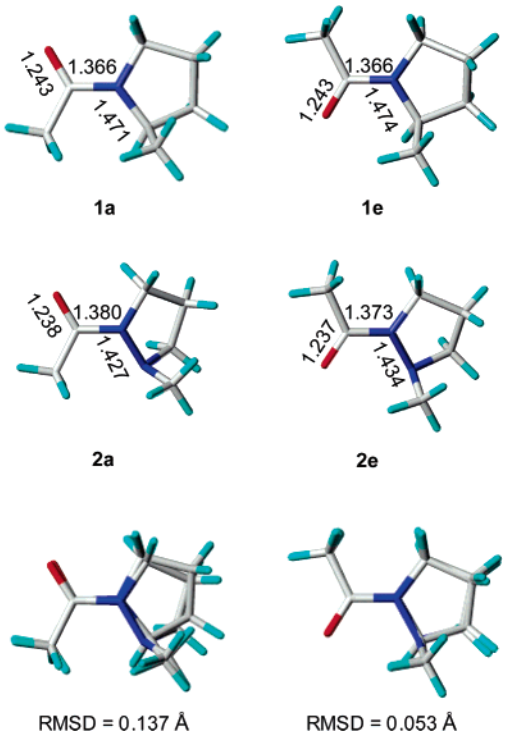

FIGURE 3. Optimized structures (MP2/6-31+G**) of the down-puckering *cis*- and *trans*-amide conformers of **1** and **2**. The root-mean-square displacement of backbone atoms between **1** and **2** was 0.137 and 0.053 Å for the *cis*- and *trans*-amide conformers, respectively.

the *cis*- and *trans*-amide preferences of **1** and **2** were seen. The energy difference between the *cis*- and *trans*-amide conformers for compound **1** was small. In this case, the *trans*-amide conformer had been found to be favored over the *cis*-amide one by only 0.7−1.0 kcal/mol. Its conformational preference was controlled mainly by substituents on the C_α- and C_δ-atoms. In contrast, the *cis*-amide conformer of compound **2** was 5.2 kcal/mol (MP2/6-31+G**) or 4.4 kcal/mol (B3LYP/6-31+G**) more stable than the *trans*-amide conformer. In other words, in vacuo, the probability of occurrence of a *cis*-amide bond in 1-acetyl-2-methyl-pyrazolidine (**2**) was almost 100%. The conformational preference in **2** was mainly due to an unfavorable lone-pair/lone-pair repulsion between the carbonyl oxygen and the α-nitrogen in the *trans*-amide conformer.

The optimized structures of compounds **1** and **2** are shown in Figure 3, along with their superimposed structures for the *cis*- and *trans*-amide conformers. Compound **2** had greater amide-bond (C−N) lengths, 0.007−0.014 Å, compared to those of **1** for both the *cis*- and *trans*-amide conformers, indicating reduced resonance of the amide bond and decreased energy barrier for *cis*−*trans* isomerization. The ω-angle of **2** was calculated to be nearly 18° from planarity for the *cis*-amide conformer and 8° for the *trans*-amide one. Both deviations were doubled compared to those seen for compound **1**. The ϕ-angle, rigid for both Pro and azPro derivatives, was ±94° in the *cis*-amide conformer of **1** and ±121° in **2**. Both α-atoms, carbon in **1** and nitrogen in **2**, are pyramidal. The root-mean-square displacement of backbone atoms between **1** and **2** was 0.137 and 0.053 Å for *cis*- and *trans*-amide conformers, respectively.

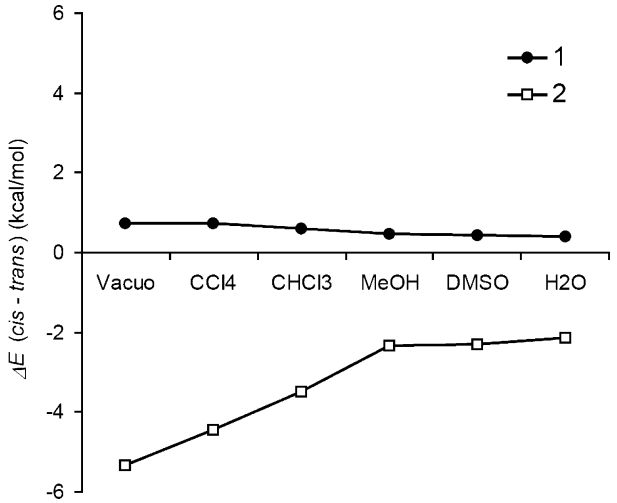

FIGURE 4. The *cis*-*trans*-amide relative energies for **1** and **2** in different environments: in vacuo (ϵ = 1.0), carbon tetrachloride (ϵ = 2.2), chloroform (ϵ = 4.9), methanol (ϵ = 32.6), dimethyl sulfoxide (ϵ = 46.7), and water (ϵ = 78.4), calculated at the MP2/6-31+G** level using the PCM solvation model.

The solvation effect was estimated empirically with PCM calculations (Figure 4). Consistent with experimental observations[85] and computer simulations,[86] the *cis*-*trans* relative population of **1** was less sensitive to different solvent environments. The relative conformational energy, ΔE (*cis* − *trans*), changed only 0.35 kcal/mol from that in vacuo to that in water. In contrast, the conformational preference of **2** was highly sensitive to solvent. In water, the *cis*-amide conformer was only favored by 2.15 kcal/mol determined by MP2/6-31+G**, and by DFT calculations, the *cis*-amide conformer was even 0.24 kcal/mol less stable than the *trans*-amide conformer. The calculations were consistent with our previous NMR study on azPro analogs of TRH, [azPro^3]-TRH and [Phe^2, azPro^3]-TRH, in methanol, which suggested only 20−40% of the populations were *cis*-amide conformers. The unfavorable lone-pair/lone-pair repulsion between the carbonyl oxygen and the α-nitrogen in the *trans*-conformer was mainly electrostatic in nature and should be dampened by an increase in the dielectric of the medium.

Interactions with water molecules were further investigated by calculations on water-bound complexes with **1** and **2**. The structures and BSSE-corrected complexation energies (MP2/6-31+G**) are illustrated in Figure 5. Water formed hydrogen bonds only with the carbonyl oxygens in both the *cis*- and *trans*-amide conformer of compound **1**. Hydrogen-bond lengths and complexation energies were almost identical for both of the *cis*- and *trans*-amide isomers. In contrast, the water molecule can donate its hydrogen to either the carbonyl oxygen or the α-nitrogen of compound **2** to form intermolecular hydrogen bonds. The strength of hydrogen bonding to the carbonyl oxygens of either the *cis*-amide conformer or the *trans*-conformer was still similar, although both strengths

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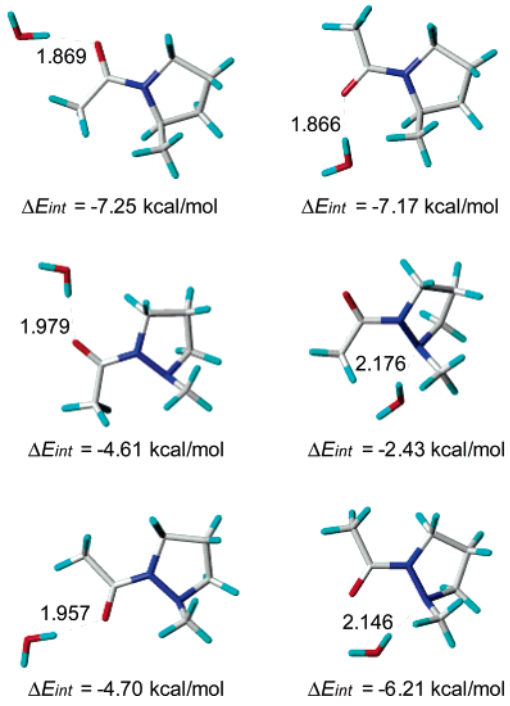

FIGURE 5. Low-energy structures and the BSSE-corrected complexation energies for the water-bound complexes with **1** and **2** from optimizations at the MP2/6-31+G** level of theory.

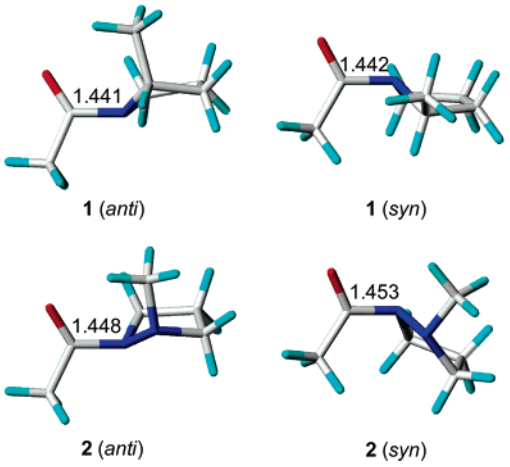

FIGURE 6. Structure of the *anti*- and *syn*-transition states optimized with the MP2/6-31+G** calculations for compounds **1** and **2**. The amide bond lengths (Å) are given.

were reduced by about 2.6 kcal/mol and both bond lengths increased by 0.1 Å compared to those of **1**. Profound differences were seen for hydrogen bonding with the pyramidal α-nitrogens. The interaction was 3.8 kcal/mol stronger in the *trans*-conformer, though the hydrogen-bond length was only 0.03 Å shorter. It suggested that significant differences between the two hydrogen bonds might be due to less repulsion between the two lone pairs of the carbonyl oxygen and α-nitrogen.

Structures for transition states with the amide-nitrogen lone pair *anti* and *syn* to the oxygen were optimized with the MP2/6-31+G** calculations (Figure 6). The energy barrier for *cis*−*trans* isomerization was nearly 6 kcal/mol less for **2** compared to that of compound **1**. Upon rotation to the transition states, the most

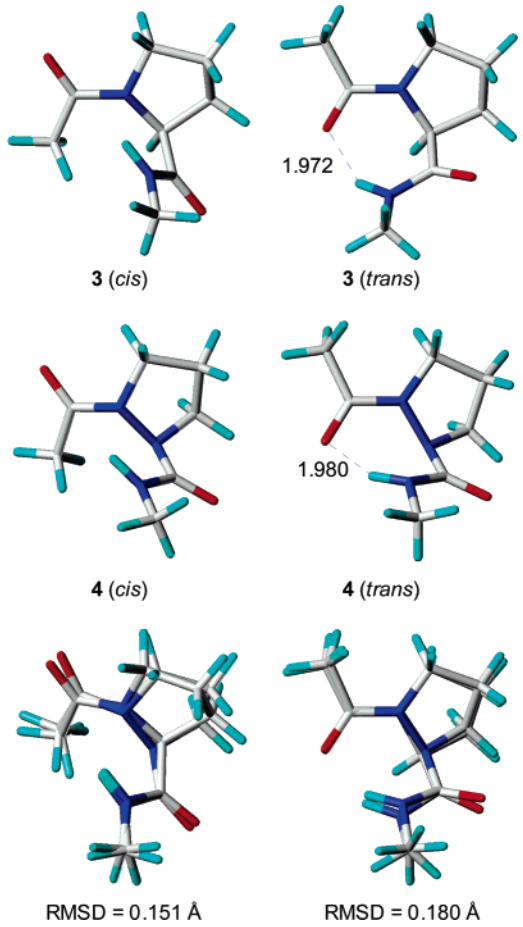

FIGURE 7. Optimized structures (MP2/6-31+G**) of the *cis*- and *trans*-amide conformers of **3** and **4**. The root-mean-square displacement of backbone atoms between **3** and **4** was 0.151 and 0.180 Å for the *cis*- and *trans*-amide conformers, respectively.

significant structural changes were that the amide nitrogen was pyramidalized and the C−N amide bond lengthened from 1.37 to 1.44 Å for compound **1** and 1.38 to 1.45 Å for compound **2**. A modest contraction of the C=O bond by 0.01−0.02 Å was also observed.

Ac-Pro-NHMe (3) and Ac-azPro-NHMe (4). In non-polar solvents or within cyclic peptides, an internal hydrogen bond can greatly favor one conformer relative to another. The seven-membered hydrogen bond ($i + 2 \rightarrow i$), seen in the γ-turn, has been suggested to exert such an effect. Ac-Pro-NHMe favors the *trans*-amide conformer by 2.25 kcal/mol (MP2/6-31+G**) or 3.34 kcal/mol (B3LYP/6-31+G**). In contrast, Ac-azPro-NHMe favors the *cis*-amide conformer by 3.15 kcal/mol (MP2/6-31+G**) or 1.83 kcal/mol (B3LYP/6-31+G**). Comparing the results to those of **1** and **2**, if conformational preferences for *cis*- or *trans*-amide isomers were only attributed to an intrinsic effect around the amide bond and the internal hydrogen bond, then such an intramolecular hydrogen bond contributed 1.5−2.0 kcal/mol (MP2/6-31+G**) or 2.5 kcal/mol (B3LYP/6-31+G**) to the *trans*-amide conformations of both the Pro and azPro analogs.

Optimized structures of **3** and **4** are shown in Figure 7, along with their superimposed structures for the *cis*- and *trans*-amide conformers. Both *cis*-amide bonds of **3** and **4** were greater than their *trans* ones: 0.014 Å greater

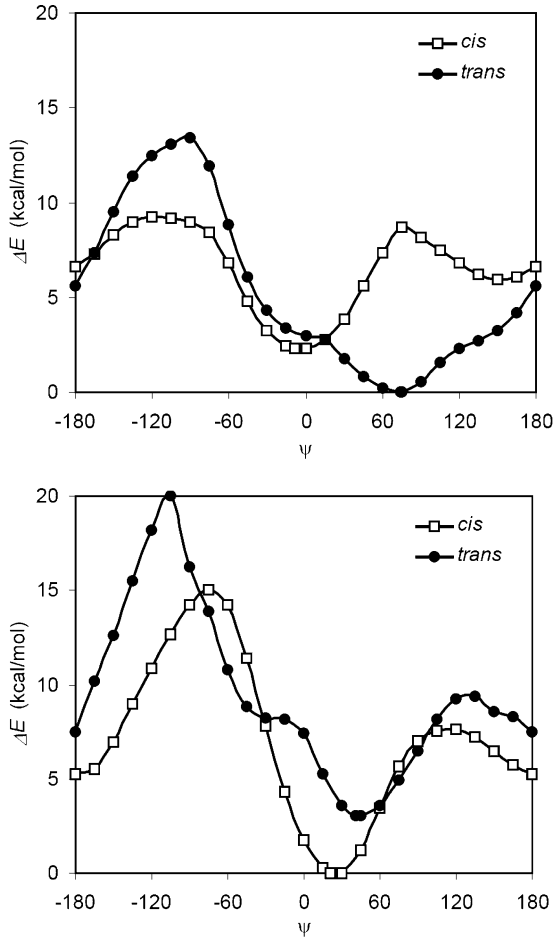

FIGURE 8. The relative energy as a function of Ψ angle for both *cis*- and *trans*-amide conformations of Ac-Pro-NHMe (a) and Ac-azPro-NHMe (b) at the level of MP2/6-31+G**//HF/6-31+G**.

in **3** and 0.030 Å greater in **4**. Such large differences in amide bond length between *cis*- and *trans*-amide isomers were due to the urea-like chemical structure and the internal hydrogen bond in the *trans*-conformer. Both *trans*-amide conformers had an internal hydrogen bond with length of about 1.97−1.98 Å. The rigid ϕ-angle torsions were ±94° in the *cis*-conformer of **3** and ±121° in **4**, identical to those in compounds **1** and **2**. The root-mean-square displacement of backbone atoms between **3** and **4** was 0.151 and 0.180 Å for *cis*- and *trans*-amide conformers, respectively.

The complete rotational-energy profiles of the Ψ-angle for both the *cis*- and *trans*-amide conformer of **3** and **4** were calculated at the MP2/6-31+G**//HF/6-31+G** level of theory to define the major features of the potential-energy surface (Figure 8). Because two amides exist in the azPro peptides, there was a second minimum for both the *cis*- and *trans*-amide conformer. The second minimum were near Ψ = 180°, corresponding to the (*E*,*Z*)-conformer for the *cis*-amide bond and (*Z*,*Z*)-conformer for the *trans*-amide bond. Both were 5 kcal/mol less stable than the corresponding minima at Ψ = 0°.

Ac-azPro-NMe$_2$ (5). Bis-*N*-methylation at the C-terminal carboxamide of azPro should disrupt any internal hydrogen bond, but such a modification could also severely perturb the typical β-turn conformation of

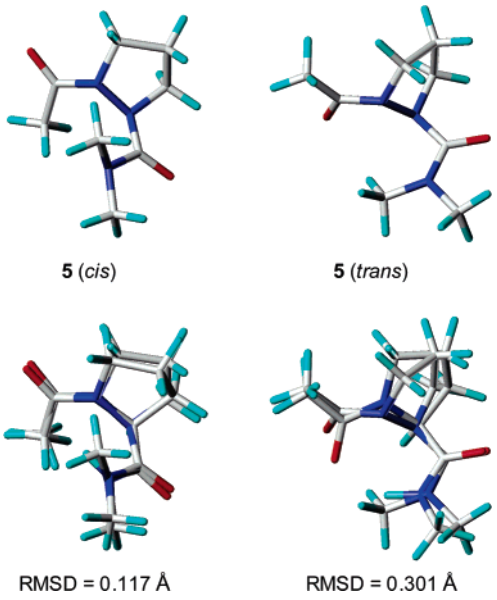

FIGURE 9. Optimized structures (MP2/6-31+G**) of the *cis*- (upper left) and *trans*-amide (upper right) conformers of **5**. The root-mean-square displacement of backbone atoms between **3** and **5** was 0.117 Å (lower left) and 0.301 Å (lower right) for the *cis*- and *trans*-amide conformers, respectively.

azapeptides. The calculated energy difference between the *cis*-amide conformer and the *trans*-conformer was small. The *cis*-amide conformer was favored by 0.27 kcal/mol at the HF/6-31+G** level, but the *trans*-conformer was 0.55 kcal/mol more stable at the MP2/6-31+G** level. The backbone (ϕ, ψ) angles for both *cis*- and *trans*-amide conformers of Ac-azPro-NMe$_2$ (**5**) were greatly altered with respect to those for Ac-azPro-NHMe (**4**). The *cis*-amide conformer had a backbone torsion angle ϕ = −101.2° and ψ = 14.7°, still consistent with a type VI β-turn structure (Figure 9). The *trans*-conformer has a backbone torsion angle ϕ = −138.9° and ψ = 54.2°, values usually associated with residues preceding Pro[87]. Additional *N*-methylation also altered the nitrogen pyramidalization. In Ac-azPro-NMe$_2$ (**5**), the sum of bond angles around N and N$_\alpha$ were closer to each other in both the *cis*- and *trans*-amide conformers compared to those of Ac-azPro-NHMe (**4**). These results indicated that conjugation at the C-terminal of azPro was partially reduced as a result of the additional N-methylation. Furthermore, the *cis*-amide conformer of **5** has a shorter C−N amide bond compared to that of the *trans*-amide conformer, and the opposite trend was found in **4**. The N−N$_\alpha$ bond also decreased 0.013 Å compared to that of the *trans*-amide conformer. These calculations were consistent with those done by Lee et al.[75] Their calculations at the B3LYP/6-31G* level showed that, in Ac-azAla-NHMe, the *cis*-amide conformer was 1.36 kcal/mol more stable than the *trans*-amide conformer and, in Ac-azAla-NMe$_2$, the *trans*-amide conformer was favored by 1.24 kcal/mol.

Ac-azAzc-NHMe (6) and Ac-azPip-NHMe (7). To see if changing the ring size of cyclic aza-amino acid would affect *cis*−*trans*-amide isomerism and allow conformations more compatible with type VI β-turns by allowing a narrower or wider variation in (ϕ, ψ) backbone torsion angles, the effect of replacing azaproline by aza-

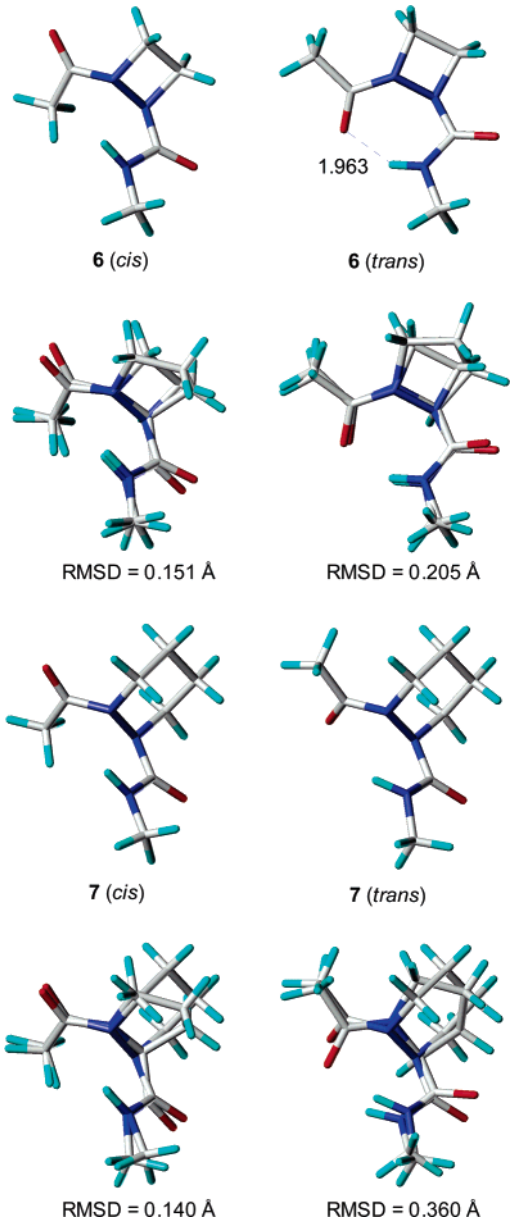

FIGURE 10. Optimized structures (MP2/6-31+G**) of the *cis*- and *trans*-amide conformers of **6** and **7**. The root-mean-square displacement of backbone atoms between **3** and **6** was 0.151 and 0.205 Å for the *cis*- and *trans*-amide conformers. The root-mean-square displacement of backbone atoms between **3** and **7** was 0.140 and 0.360 Å for the *cis*- and *trans*-amide conformers.

azetidine acid (azAzc), which contains a four-membered ring, or by aza-pipecolic acid (azPip), which contains a six-membered ring, were calculated (Figure 10). Ac-azAzc-NHMe (**6**) showed better stabilization of the *trans*-amide conformer due to a strong internal hydrogen bond, which was 0.017 Å shorter than that of Ac-azPro-NHMe (**4**). The *trans*-amide conformer was stabilized by 1.65 kcal/mol relative to the *cis*-amide conformer. Both nitrogen atoms in **6** were more pyramidalized than those in **4** regardless of the *cis*- or *trans*-amide isomers. Lengths of both the C−N amide bond and the N−N_α bond increased by about 0.01 Å compared to those in the azPro counterpart. Although the backbone torsion angles (ϕ, ψ) in **6** were still close to those values of the azPro peptide, both of the *cis*- and *trans*-amide bond torsions ω were significantly perturbed from ideal values with ω (cis) = 34.7° and ω (trans) = −155.6°.

In contrast to the four-membered ring analog **6**, an increased stability for the *cis*-amide conformer of Ac-azPip-NHMe (**7**) was clearly shown in the calculations. The intramolecular hydrogen bond diminished in the *trans*-amide conformer as a result of the increase of ring size. The *cis*-amide conformer was almost 5 kcal/mol more stable than the *trans*-amide conformer in vacuo. Both nitrogen atoms in the *cis*-amide conformer showed more planar sp^2 characters, and the ω-torsion angles were closer to the ideal amide-bond value. The length of the N−N_α bond decreased about 0.02 Å, because strain was partially relieved by the increased ring size. Compared to those of azPro or azAzc, the backbone torsion angles (ω, ϕ, ψ) of the azPip peptide were closer to those in the *cis*-amide conformer of the cognate Pro peptide. It was also demonstrated by the root-mean-square displacement of backbone atoms. The RMSD was 0.140 Å between azPip and Pro peptides and 0.151 Å for both azPro and azAzc. So, incorporation of the larger ring homolog azPip in the third position may strongly stabilize a *cis*-amide, which in turn stabilizes a type VI β-turn. If there was no ring constraint at all for the aza-amino acid, as shown by Lee at el.[75] in Ac-azGly-NHMe, Ac-azAla-NHMe, Ac-NMe-azGly-NHMe, and Ac-NMe-azAla-NHMe investigated with B3LYP/6-31G*, the *cis*-amide conformer was stabilized by 1−3 kcal/mol in vacuo, depending on the sequences and N-methylation. These aza-peptides unconstrained by the ring would be more flexible as both backbone torsion angles (ϕ, ψ) can vary. The conformational preferences for *cis*- or *trans*-amide bonds were still highly dependent on the environments as demonstrated by experiments. Didierjean et al.[70] reported the crystal structure of Boc-Ala-NMe-azAla-Ala-NHiPr where the aza-amino acid residue adopted a type VIa β-turn conformation, but this model compound in solution was found to coexist in a ratio of 80(*cis*):20(*trans*) in $CDCl_3$ and 25(*cis*):75(*trans*) in DMSO-d_6. The fact that azPro or azPip with ring constraints stabilizes the *cis*-amide bond and restrains conformational spaces suggests they are more suitable for mimicking type VI β-turns and their properties should be studied more thoroughly, especially in aqueous solutions. In fact, Takeuchi and Marshall[88] showed that inclusion of D-Pro-Pip caused significant nucleation of reverse-turn structure, and it was superior to simple N-methylation (Pro-D-NMeAA and D-Pro-NMeAA) or di-proline sequences (Pro-D-Pro, D-Pro-Pro). As a result of their similar structures and energetic differences, the larger ring homolog aza-pipecolic acid should receive more attention in mimicking type VI β-turn conformations for probing peptide-receptor interactions.

Cyclo-(azPro)$_4$. Another way to augment the effect of azPro on stabilizing *cis*-amide conformers is to incorporate the dipeptide azPro-azPro into head-to-tail cyclic arrangements. A prototypical compound, cyclo(D-Pro-L-Pro-D-Pro-L-Pro), has been well characterized in water

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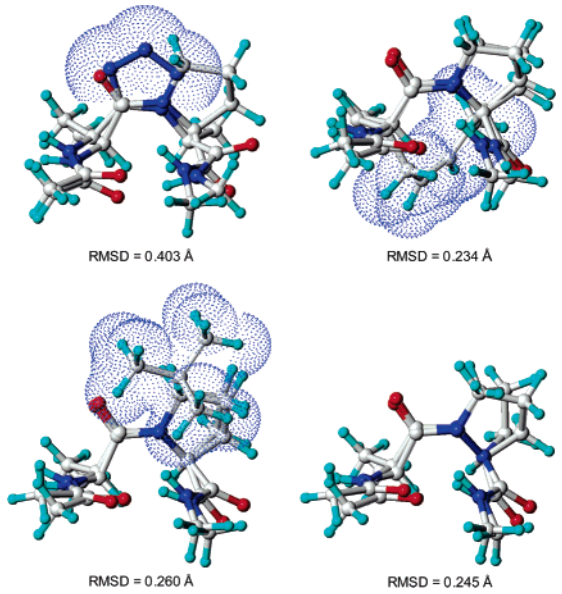

FIGURE 11. The superimposed structures of the AMBER*-minimized type VI β-turn mimetics within an ideal type VIa β-turn dipeptide: tetrazole (a), bicyclic analog (b), 5-*t*Bu-Pro analog (c), and azPro (d). The extra steric bulk required by atoms introduced in each mimetics are highlighted with van der Waals dot surfaces.

and in the solid state[89] exclusively in conformations having alternative *cis*- and *trans*-amide bonds: either the *cis-trans-cis-trans* (*ctct*) or the *trans-cis-trans-cis* (*tctc*) conformation. We found such arrangement was mainly due to the fact that the large dipole moment of the *ctct*- or *tctc*-conformers was favored by the aqueous solution by DFT at the level of B3YLP/6-31G* theory. In vacuo, the all-*trans*-amide (*tttt*) conformer was 7.7 kcal/mol more stable than the *ctct*- or *tctc*-conformer. When all four prolines were replaced by azPro, the new tetrapeptide, cyclo-(azPro)$_4$, could not form any internal hydrogen bonds. The cumulative effects of four azPro residues in this environment are dramatic. The most stable conformer of cyclo-azPro$_4$ in vacuo is all-*cis*-amide (*cccc*), 14.8 kcal/mol more stable than any other conformations found by MC/MD search. In aqueous solution, the all-*cis*-amide conformer was still the global minimum, 6.9 kcal/mol more stable than any other. Experimental validation of this prediction is underway, and a detailed conformational analysis of azPro in cyclic peptides will be published elsewhere.

Comparison with Other Type VI β-turn Mimetics. So far all efforts have focused on stabilizing type VI β-turns by stabilizing a *cis*-amide bond conformer. To compare different modifications, we built a capped dipeptide, Ac-Ala-Pro-NHMe, with a *cis*-amide bond preceding the Pro residue, and set the classical torsional angles for the type VIa β-turn in the dipeptide. The structure was generated by constraining the backbone angles to be close to the ideal values and minimizing with MacroModel[90] version 8.0 using the AMBER* all-atom force field, followed by relaxation of the constraints and further minimization, and was used to locate a local minimum near ideal values for the classical type VIa β-turn. Structural modifications corresponding to incorporation of tetrazole, bicyclic, *t*Bu and azPro were done manually through MacroModel[90] followed by minimization. The resulting conformations were compared to that of the capped dipeptide for qualitative evaluation of the structural perturbations. The minimized structures were superimposed on the ideal type VIa-turn dipeptide as shown in Figure 11.

Tetrazole. The 1,5-tetrazole ring is a surrogate for the *cis*-amide and fixes the amide bond in a *cis*-geometry in excellent agreement for bond lengths and angles, as shown by the X-ray crystal structure of Z-Proψ[CN$_4$]-Ala-OBzl.[16−18] Takeuchi and Marshall[88] investigated a tetrapeptide, Ac-Ala-Proψ[CN$_4$]-Ala-Ala-NHMe, with the Monte Carlo/Stochastic Dynamic (MC/SD) hybrid simulation[91] using the GB/SA continuum solvation model[92] for water. The data showed that $|\beta|$ (β, the virtual torsion angle, is defined by the atoms C$_{\alpha1}$, C$_{\alpha2}$, C$_{\alpha3}$, and N$_4$) is less than 30° in 26% of the equilibrium structures and 40% have a distance of C$_{\alpha1}$-C$_{\alpha4}$ less than 7 Å. The amide hydrogen and carbonyl oxygen are less than 4 and 2.5 Å apart in 15% and 5% of the equilibrium structures, respectively. The root-mean-square displacement of backbone atoms between an ideal type VIa-turn and the AMBER*-minimized tetrazole mimetics was 0.403 Å. These values suggested that the tetrazole is a good *cis*-amide surrogate, and the increased percentage for type VI-turn was comparable to the Pro-Pro sequence for residue i +1 and i + 2 where the *cis*-amide conformer for ω_{23} is stable. However, the tetrazole surrogate changes the steric bulk of the amide and modifies its hydrogen-bonding characteristics (Figure 11a). Lack of biological activity of an analog with a tetrazole modification could be due to one of these perturbations rather than the *cis*-conformational constraint.

Bicyclic Analogs. The bicyclic analog is a surrogate for *cis*-peptidyl proline and forms a lactam-bridged type VI β-turn mimetic. In the tetrazole case, the constraint incorporates the nitrogen of the peptide bond, an alteration that forces a replacement of the proline amino acid by ring opening, and elimination of the Φ-constraint might also result in lose of activity. The bicyclic approach, instead, incorporates a second ring in the opposite direction of the pyrrolidine ring to further constraint the *cis*-amide bond. Most bicyclic analogs incorporated a six-membered ring constraint. On the other hand, Hoffman et al.[37] studied different bicyclic analogs using MD simulations to determine which ring size and configuration was most promising in terms of possible intramolecular hydrogen-bond interactions between the carbonyl oxygen in the first residue and the amide proton in the fourth residue. The calculations were performed in the gas phase using the AM1 Hamiltonian with no continuum model, or explicit solvent molecules, present. Among the bicyclic analogs studied, the fused eight-membered ring exhibited the most promising behavior to form the hydrogen-bonding pattern, which could lead

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to a successful type VIa β-turn mimetic. During the 120 ps semiempirical MD simulation at 400 K, no conformational interchange could be observed, which suggested the conformation of the eight-membered ring bicyclic analog was rather rigid and stable. The root-mean-square displacement of backbone atoms between the ideal type VIa-turn and the AMBER*-minimized eight-membered bicyclic analog was 0.234 Å, which is the smallest among all four mimetics. However, similar to the tetrazole mimetic, the bicyclic analog introduces even a larger steric perturbation, though on the other side of the β-turn plane compared to the tetrazole or the pyrrolidine ring (Figure 11b). This could also severely impact peptide-receptor recognition.

Bulky Substitution on C_δ-Pro. Introducing a sterically bulky group, such as *tert*-butyl (*t*Bu), on the C_δ-atom of the proline residue changes the interactions of atoms adjacent to the amide bond. Because the substitution group on the C_δ-atom is larger than that of the C_α-atom, it favors the *cis*-amide bond preceding the proline residue. The *cis*-*trans*-amide preference in a *t*Bu-substitution analog, 1-acetyl-2-tertbutyl-5-methyl pyrrolidine, was calculated by DFT (B3LYP/6-31+G**). The data showed that in vacuo the *cis*-amide conformer was stabilized by 2.51 kcal/mol compared to the *trans*-amide conformer. This stabilization was reduced to 1.17 kcal/mol in aqueous solution evaluated with the PCM solvation model. At the same level of theory, in 1-acetyl-2-methyl pyrazolidine (**2**), the *cis*-amide conformer was stabilized by 4.43 kcal/mol in vacuo and 1.95 kcal/mol in aqueous solution. Even when introducing such a large functional group as *t*Bu, the effect on stabilizing the *cis*-amide bond was still minor compared to the simple azPro modification. In addition, Halab and Lubell[40−42] have synthesized and used (2*S*,5*R*)-5-*tert*-butylproline to explore the effect of *t*Bu substitutions on stabilizing type VI β-turns. The results showed that in a dipeptide, Ac-Xxx-*t*BuPro-NHMe, the *t*Bu substitution stabilized type VIa and VIb β-turn conformations contingent upon the stereochemistry of the N-terminal residue. Dipeptides possessing Xxx = Ala and Leu residues adopted type VIa and VIb β-turn conformations when the N-terminal amino acid possessed, respectively, L- and D-configuration, as shown by NMR and CD spectroscopy, as well as X-ray analysis. Furthermore, the presence of Phe at the N-terminal of *t*BuPro caused a remarkable increase in the *cis*-amide conformation (>90% in water). Further in tetrapeptide Ac-Xaa-Yaa-*t*BuPro-Zaa-XMe high (> 80%) *cis*-amide populations were obtained with alkyl groups at the Xaa position, an aromatic residue at the Yaa position, and either an alanine or a lysine residue at the Zaa position of the *t*BuPro-tetrapeptide methyl esters in water. Such tetrapeptides with high *cis*-amide content adopted type VIa β-turn conformations, though the results did not indicate strong hydrogen bonding in water. As well as its power to augment the population of *cis*-amide conformers, the *tert*-butyl substituent reduced the barrier for *cis*−*trans*-amide isomerization by 3.7 kcal/mol relative to its proline counterpart. The root-mean-square displacement of backbone atoms between the ideal type VIa-turn and the AMBER*-minimized *t*BuPro analog was 0.260 Å (Figure 11c), but among all of the AMBER*-minimized mimetic structures, the *t*BuPro analog has an atom closest to the amide hydrogen of the fourth residue (1.709 Å), which is involved in forming a type VIa β-turn structure. This might partially explain the weak hydrogen bonding seen in *t*BuPro-containing tetrapeptides. Except for the *t*Bu modifications, the 2,2-dimethylated oxazolidine and thiazolidine derivatives were also studied in model peptides, in which the percentage of *cis*-amide conformations were observed between 5% and 100% determined spectrophotometrically with a chymotrypsin-couple assay and the transition barrier for *cis*−*trans*-amide isomerization was reduced by about 2−4 kcal/mol in comparison to that for Pro.[44,45] As in the tetrazole and bicyclic analogs, the sterically bulky substituents pose important limitations to peptide-receptor recognition.

AzPro. The azaproline residue has unique conformational properties due to its diacylhydrazide backbone. It favors the *cis*-amide bond preceding the azPro residue as a result of the unfavorable lone-pair/lone-pair repulsion in the *trans*-amide conformation. As compared to most types of type VI β-turn mimetics mentioned, azPro strongly stabilizes the *cis*-amide conformation, makes only a small change to the overall steric/geometric structure of Pro, and does not introduce additional steric bulk that could compromise receptor interactions. The root-mean-square displacement of backbone atoms between the ideal type VIa-turn and the azPro analog is about 0.245 Å (Figure 11d). The backbone torsion Φ was constrained to a small range, $\Phi = \pm 120°$, for the *cis*-conformation as a result of the pyrazolidine ring. The conformational preferences of azPro-containing peptides for the *cis*-amide bond conformer were sensitive to different solvents, and those atoms attached to the nitrogens in the azPro ring can invert from *R* to *S* and vice versa. Furthermore, the nitrogen-substitution of the α-carbon also modified the hydrogen-bonding characteristics of the parent peptide backbone.

Comparison of Different Force Fields for azPro. Compounds containing two or more strongly polar functional groups in close proximity, such as in azPro peptides, have proven to be the most problematic in force field development. We examined three different all-atom force fields, AMBER*,[93,94] OPLS-AA,[95] and MMFF94s,[96−102] which are implemented in the MacroModel 8.0 program, on their performances on Ac-azPro-NHMe. The minimized geometries and *cis*-*trans*-amide energy differences are summarized in Table 3. Only the AMBER* force field has explicitly included parameters for aza-amino acids. It was not surprising, therefore, that only the AMBER* force field provided a close estimation for the energy difference between the *cis*- and *trans*-amide conformations. The results showed that the *cis*-conformation was 2.50 kcal/mol more stable than the *trans*-amide one using AMBER*, compared to 3.15 kcal/mol with MP2/6-31+G**

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TABLE 3. Geometries and *cis-trans*-Amide Energy Differences (kcal/mol) of 3−7 Optimized with MP2/6-31+G**

		d(C−N)	d(C=O)	d(N−X_α)	ω	ϕ	ψ	ΣN	ΣX_α	d(C=O···H−N)	ΔE (*cis*−*trans*)
3	*cis*	1.374	1.240	1.466	12.4	−94.2	−4.2	357.0	327.1		2.25
	trans	1.360	1.250	1.477	−172.6	−85.8	71.8	358.6	325.3	1.972	
4	*cis*	1.397	1.233	1.421	21.7	−121.3	25.5	345.6	334.6		−3.15
	trans	1.367	1.242	1.423	−167.2	−92.2	44.5	357.0	335.6	1.980	
5	*cis*	1.388	1.236	1.408	18.7	−101.2	14.7	349.0	344.9		0.55
	trans	1.395	1.232	1.421	−168.6	−138.9	54.2	346.5	330.3		
6	*cis*	1.405	1.231	1.467	34.7	−119.5	28.4	327.8	322.1		1.65
	trans	1.376	1.238	1.463	−155.6	−91.5	46.8	338.4	324.5	1.963	
7	*cis*	1.393	1.234	1.402	18.0	−121.9	23.4	353.1	347.9		−4.92
	trans	1.398	1.231	1.400	−170.4	−124.2	19.0	351.1	350.8	3.364	

and 1.83 kcal/mol with B3LYP/6-31+G**. In contrast, both the OPLS-AA and the MMFF94s force fields underestimated the propensity for the *cis*-amide conformation.

There were several noticeable differences in the geometries from optimizations with MP2/6-31+G** and those from AMBER*. First, the internal γ-turn hydrogen bond was 0.2 Å longer than that from the ab initio calculations. On the other hand, it was 0.2 Å shorter for the OPLS-AA and MMFF94s results. Second, in the AMBER*-minimized structures, the two nitrogen atoms were less pyramidal and the sum of angles around the two nitrogens was 10−20° greater than those found in the MP2/6-31+G**-optimized structures. Third, the *cis*-amide conformer from minimization with AMBER* was closer to the *cis*-Pro structure than the *cis*-azPro structure found in the MP2/6-31+G** studies. Last, all three force fields underestimated the C−N length by 0.01−0.04 Å and the N−N_α length by 0.02−0.05 Å. AzPro-containing peptides were previously studied using MC/SD simulations with the AMBER* force field mainly due to the energetic considerations,[47] but some conclusions need reconsideration because of the structural discrepancies between the empirical and ab initio studies.

AMBER*, OPLS-AA, and MMFF94s all use the static atomic-charge model, which lacks conformational dependence for atomic charges. For example, the atomic charges fitted according to the CHelpG scheme[103] was up to 0.1 e different between the *cis*- and *trans*-amide conformation for azPro derivatives. The static partial-charge model neglects both higher order multipoles and molecular polarizability. Because of such simplicity, the force field employs enhanced charge distributions that emulate the effect of polarizability by amplifying electrostatic interactions for favorable contacts in high-dielectric medium. Unfortunately, these enhanced charge distributions also amplify electrostatically unfavorable interactions, whereas the proper account of polarizability would diminish them. They also improperly enhance electrostatic interactions in gas-phase or low-dielectric environments. They may not be optimal for describing intramolecular interactions and may thereby limit the ability of the force fields to account for differences in conformational energies.[104] Our ab initio results provide valuable data for the improvements of current force-field parameters and further development of a polarizable force field with explicit atomic multipoles and electronic polarizability.

Conclusions

In the search for a new type *cis*-amide inducer and type VI β-turn mimetic, azPro was of significant interest on the basis of initial crystal structures and NMR studies in organic solvent. As compared to most other types of type VI β-turn mimetics, azPro strongly stabilizes the *cis*-amide conformation, makes only a small change to the overall geometrical structure of Pro, and does not introduce additional steric bulk that could compromise receptor interactions.

As compared to its cognate residue Pro and other type VI β-turn mimetics, azPro has very unique conformational properties as a result of its diacylhydrazide backbone. The ab initio and DFT calculations indicated that, in vacuo or nonpolar solvent, an azPro stabilized the *cis*-amide bond preceding the azPro residue and, by formation of that *cis*-amide bond, the likelihood for a type VI β-turn. In aqueous solution, the propensity for the *cis*-conformation was significantly reduced, because water molecules partially shielded the unfavorable lone-pair/lone-pair repulsion in the *trans*-amide conformer. The internal γ-turn-like hydrogen bond can also contribute to favoring the *cis*-amide conformer relative to the *trans*-amide one. The introduction of the larger six-membered ring homolog azPip diminished the internal hydrogen bond in the *trans*-amide conformer, and that in turn increased the propensity for the *cis*-amide conformation.

Similarly to proline, the backbone torsion Φ of azPro was constrained to a smaller range. It was around Φ = ±120° for the *cis*-conformation of azPro as compared to the Φ = ±90° of Pro. The azPro residue has one more degree of freedom than that of Pro: the nitrogens in azPro, especially the α-nitrogen, are not planar. The two nitrogens can readily invert at room temperature between the two diastereomers: *R*,*R* or *S*,*S*.

Developing a general strategy for the incorporation of aza-amino acids at any position in a peptide chain with the Boc-protecting group tactics is of great interest and under active development.[105] This will allow aza-amino acids to be accessible by the solid-phase peptide synthesis in a combinatorial way. The availability of chimeric proline analogs,[106−111] where side-chain functional groups have been attached to the proline ring, suggests the

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preparation of similar chimeric azPro or azPip analogs with diverse side-chain functional groups as receptor probes for type VI β-turns. Besides approaches based on solid-phase peptide synthesis to incorporate chimeric amino acids, selective electrochemistry of nitrogen-containing compounds[112] to generate an *N*-acyliminium ion with subsequent addition may provide an alternative route for synthesis of chimeric analogs.

The azPro-containing peptides with predetermined conformational effects and accessible synthetic routes for fixed side-chain placement provide ideal structural probes for applications in combinatorial libraries. Analysis of activity from screening of such libraries can be used to test for a consistent hypothesis concerning the receptor-bound conformation of the parent peptide. This hypothetical receptor-bound conformation can then be used to select additional classes of modifications or transcend from the peptide into true peptidomimetics with improved pharmacological/pharmacodynamic properties.

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Supporting Information Available: Geometries, coordinates, and relative energies (kcal/mol) of different conformers for compounds **1** and **2** and their water complexes optimized with MP2/6-31+G**, as well as data for *cis*- and *trans*-amide conformers of compounds **3**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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