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Protonation of the Side Group in β- and γ-Aminated Proline Analogues: Effects on the Conformational Preferences

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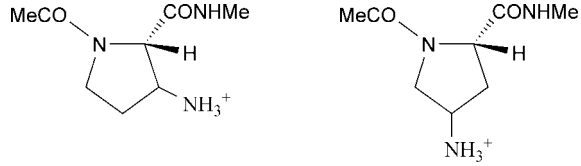
Received January 26, 2009



Ac-βAmH⁺p-NHMe Ac-γAmH⁺p-NHMe

This work shows the influence of the side-chain protonation on the conformational properties, relative stabilities, and peptide bond isomerization of four aminoproline isomers. Thus, this research has been useful to define the rules that allow control the conformation of aminoproline with the pH. Comparison of the results obtained using density functional theory calculations for the *N*-acetyl-*N*′-methylamide derivatives of the protonated isomers, which differ in the β- or γ-position of the substituent and its *cis* or *trans* relative disposition, with those reported for the corresponding neutral analogues (*J. Phys. Chem. B* **2008**, *112*, 14045) has allowed us to reach the following conclusions: (i) protonation of the amino group produces a reduction of the backbone conformational flexibility and a destabilization of the *cis* configuration of the amide bond involving the pyrrolidine nitrogen; (ii) the planarity of the peptide bond is broken in some cases to form strong side chain···backbone interactions, which induce a very significant pyramidilization at the amide nitrogen atom; (iii) as was also detected for the neutral analogues, the formation of side chain···backbone intraresidue interactions favor the *cis* disposition of the substituent; and (iv) protonation of the amino side group increases the energy gaps that separate the different investigated isomers resulting in an enhancement of the destabilization of the dipeptides with the substituent attached in a *trans* position.

Introduction

The design and application of synthetic amino acids with restricted conformational mobility in different fields of nanobiology, e.g., the re-engineering of physical protein modules and the generation of nanodevices,[1,2] is a topic of growing interest. Within this context, we recently observed that the insertion of chemically constrained residues with suitable backbone conformational tendencies enhance the thermodynamic stability of the nanotubular structures constructed by self-assembling protein fragments.[3]

Among the large variety of amino acids that can be designed, those achieved by introducing chemical modifications to proline

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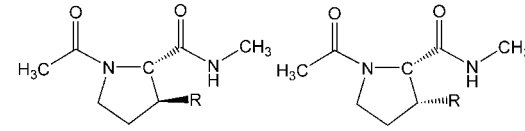
10.1021/jo900169s CCC: $40.75 © 2009 American Chemical Society
Published on Web 03/18/2009

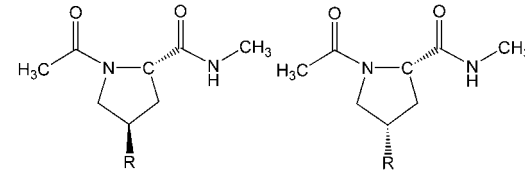
(Pro) are particularly attractive. This is because the side chain of Pro is bonded to both the α-carbon and its preceding amide nitrogen providing conformational properties that are unique among naturally occurring amino acids. As a consequence, rotation about the N−C^{α} bond is prohibited, and the φ torsion angle is confined to values of around −60°. Accordingly, Pro is overwhelmingly found in the α-helical ($\varphi,\psi \approx -60°,-30°$) and semiextended ($\varphi,\psi \approx -60°,140°$) regions of the conformational map.[4] In addition, Pro shows a higher propensity to promote γ-turn conformations ($\varphi,\psi \approx -70°,60°$) than other proteinogenic amino acids.[4d,5] Another effect derived from its cyclic structure is that the peptide bond preceding Pro (that involving the pyrrolidine nitrogen) has a relatively high probability of accommodating a *cis* arrangement[6] as compared to other peptide bonds, for which the *cis* form is almost inexistent.

The conformational properties of a relative wide number of synthetic Pro derivatives have been reported. These compounds were obtained by incorporating a substituent at the C^{α} atom (α-substituted Pro analogues)[7,8] or in the pyrrolidine ring (e.g., hydroxylated, fluorinated, and aminated Pro analogues),[9−11] or altering the chemical nature of the own pyrrolidine ring (*e.g.* diminishing or enlarging the ring size,[12] replacing a carbon atom by an heteroatom,[13] and incorporating a double bond through a deshydrogenation[14]). Within this context, we recently reported the intrinsic conformational properties of different aminoproline (Amp) derivatives,[11] which have been already used to construct β- and γ-peptides with helical secondary structures.[15] Specifically, we investigated the *N*-acetyl-*N*'-methylamide derivatives of both the *cis* and *trans* Amp isomers that incorporate an amino group to the C^{β}- or C^{γ}-positions of the pyrrolidine ring. Theoretical calculations based on density functional theory (DFT) methods on these four compounds, which were denoted Ac-βtAmp-NHMe, Ac-βcAmp-NHMe, Ac-γtAmp-NHMe, and Ac-γcAmp-NHMe (Scheme 1), evidenced that the incorporation of the amino group reduces the intrinsically low conformational flexibility of conventional Pro. Furthermore, the stability of the conformations with the peptide bond involving the pyrrolidine nitrogen arranged in *cis* was higher for Amp derivatives than for Pro. This was attributed to the formation of stable intramolecular hydrogen bonds with the nitrogen of the amino substituent acting as acceptor.

SCHEME 1



R= NH_2	Ac-β*t*Amp-NHMe	Ac-β*c*Amp-NHMe
R= NH_3^+	Ac-β*t*AmH^+p-NHMe	Ac-β*c*AmH^+p-NHMe



R= NH_2	Ac-γ*t*Amp-NHMe	Ac-γ*c*Amp-NHMe
R= NH_3^+	Ac-γ*t*AmH^+p-NHMe	Ac-γ*c*AmH^+p-NHMe

Although these results suggested that Amp derivatives have potential interest for many nanobiological applications, the conformational properties of these amino acids may be easily altered by transforming the amino group, a reasonably weak base, into the positively charged ammonium group, i.e., amines react readily with acids. This equilibrium is especially important in aqueous solution, in which the pH can be used to control the conformation of the Amp derivatives. However, the conformational preferences of the protonated Amp derivatives, hereafter denoted Ac-βtAmH^+p-NHMe, Ac-βcAmH^+p-NHMe, Ac-γtAmH^+p-NHMe, and Ac-γcAmH^+p-NHMe (see Scheme 1) remain totally unknown yet. In this work, we have used DFT calculations to explore the potential energy hypersurfaces of these four dipeptides, the influence of the solvent being evaluated through a self-consistent reaction field (SCRF) method. Furthermore, we have examined the influence of the protonation on both the *trans*/*cis* disposition of the peptide bond involving the pyrrolidine nitrogen and the relative stability of the four isomers generated by the substitution at different positions.

Methods

All calculations were carried out using the Gaussian 03 computer program.[16] DFT calculations were performed using the B3LYP

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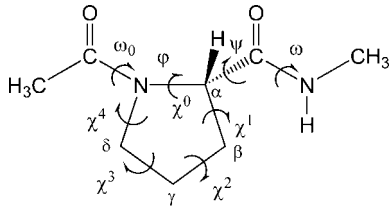

FIGURE 1. Dihedral angles used to identify the conformations of the *N*-acetyl-*N*′-methylamide derivatives of the AmH^+p analogues studied in this work. The dihedral angles ω_0, φ, ψ, and ω are defined using backbone atoms while the endocyclic dihedral angles χ^i are given by the atoms of the five-membered ring. In particular, the sequence of atoms used to define φ and χ^0 are C(=O)−N−C^α−C(=O) and C^δ−N−C^α−C^β, respectively.

method[17] combined with the 6-31+G(d,p) basis set,[18] which was previously employed to study the *N*-acetyl-*N*′-methylamide derivatives of conventional Pro[7] and Amp.[11]

Dihedral angles for the backbone and side chain of the AmH^+p derivatives studied in this work are defined in Figure 1. Since each flexible backbone dihedral angle is expected to have three minima, i.e., *gauche*$^+$ (60°), *trans* (180°), and *gauche*$^-$ (−60°), and φ is fixed by the geometry of the five-membered ring, the number of minima that may be anticipated for the potential energy surface $E = E(\psi)$ of each AmH^+p dipeptide is 3. Additionally, each amide bond (given by the dihedral angles ω_0 and ω) can be arranged in *trans* or *cis* positions, even though only the peptide bond involving the pyrrolidine nitrogen (ω_0) is likely to adopt a *cis* configuration. Therefore, both the *trans* and *cis* states were considered for ω_0, while the amide bond involving the *N*-methylamide blocking group (ω) was arranged in *trans* only. Furthermore, due to the cyclic nature of the side chain, two puckering states (denoted *down* and *up*) are expected for each backbone minimum energy conformation. The *down* and *up* arrangements refer to the displacement of the $C^\beta$and C^γ atoms form the main plain of the pyrrolidine ring. Specifically, they are defined as those in which one of such atoms and the carbonyl group of the AmH^+p residue lie on the same (*down*) and opposite sides (*up*) of the plane.

Accordingly, for each of the four dipeptides under study (Scheme 1), 3(Ψ backbone) × 2(ω_0 *trans*-or-*cis*) × 2(cyclic side chain) = 12 structures were considered as starting points for complete geometry optimizations at the B3LYP/6-31+G(d,p) level. Frequency analyses were carried out to verify the nature of the minimum state of all the stationary points obtained and to calculate the zero-point vibrational energies (ZPVE) as well as both thermal and entropic corrections. These statistical terms were used to evaluate the conformational Gibbs free energies in the gas phase (ΔG^{gp}) at 298 K.

To examine the solvation effects on the conformational stability, single-point calculations were conducted on the B3LYP/6-31+G(d,p) optimized structures using a self-consistent reaction-field (SCRF) model. SCRF methods treat the solute at the quantum mechanical level, and the solvent is represented as a dielectric continuum. Specifically, we chose the polarizable continuum model (PCM) developed by Tomasi and co-workers to describe the solvent.[19] The PCM represents the polarization of the liquid by a charge density appearing on the surface of the cavity created in the solvent, i.e., the solute/solvent interface. This cavity is built using a molecular shape algorithm. PCM calculations were performed in the framework of the B3LYP/6-31+G(d,p) level using the standard protocol, and considering the dielectric constant of water ($\varepsilon = 78.1$). The conformational free energies in solution (ΔG^{WAT}) were estimated by adding the free energies of solvation to the ΔG^{gp} values.

The minimum energy conformations of the four dipeptides studied in this work have been denoted using the same three-label code that was previously used for the Amp derivatives,[11] which specifies the arrangement of the ω_0 peptide bond, the φ,ψ-backbone conformation, and the puckering of the five-membered ring. The first letter refers to the *trans* (t) or *cis* (c) arrangement of the peptide bond involving the pyrrolidine nitrogen. The second label identifies the backbone conformation using the nomenclature introduced by Perczel et al.[20] more than 15 years ago. In the case of Pro and its derivatives, only the γ_L (γ-turn or C_7), α_L (α-helical), and ε_L (polyproline II-like) conformations are accessible to the backbone since φ is fixed in the neighborhood of −60°. Finally, the *up* or *down* puckering of the five-membered ring is indicated using the [u] and [d] labels, respectively. The puckering of the ring was described using the classical pseudorotational algorithm, which uses a very simple model based on the puckering amplitude and the state of the pucker in the pseudorotation pathway. This model was previously applied by Perczel et al.[21] to describe conventional Pro.

Results and Discussion

Calculations at the B3LYP/6-31+G(d,p) level led to 3, 5, 5, and 3 minimum energy conformations characterized for Ac-$\beta t$$AmH^+$p-NHMe, Ac-$\beta c$$AmH^+$p-NHMe, Ac-$\gamma t$$AmH^+$p-NHMe, and Ac-$\gamma c$$AmH^+$p-NHMe, respectively. Table 1 lists the more relevant structural parameters together with the relative energy (ΔE^{gp}) of all these structures, which are displayed in Figures 2−5. Table 1 also shows the relative stability of the four dipeptides ($\Delta E^{\#gp\#}$), which corresponds to the energy relative to the lowest energy conformation of the most stable isomer. Table 2 compares the relative free energies in the gas phase (ΔG^{gp}) and aqueous solution (ΔG^{H2O}) for the minima of the four dipeptides. Furthermore, the relative free energies in these two environments calculated in each case with respect to the conformation of lowest free energy of the most stable isomer, $\Delta G^{\#gp\#}$ and $\Delta G^{\#H2O\#}$, have been also included in Table 2.

Ac-βtAmH^+p-NHMe. The lowest energy conformation found for this dipeptide corresponds to the t-γ_L[u] (Figure 2a), which is the only minimum identified with ω_0 arranged in *trans*. This structure is stabilized by a backbone···backbone hydrogen bonding interaction that involves the NHMe and Ac blocking groups, and by an intraresidue interaction between the ammonium side group and the carbonyl oxygen atom of the $\beta t$$AmH^+$p. The latter side chain···backbone interaction is consequence of the change in the pyrrolidine puckering produced by the protonation of the lowest energy minimum of Ac-βtAmp-NHMe, which was the t-γ_L[d].[11] Thus, the C^β-exo ($_\beta$E) conformation found for the pyrrolidine ring in this minimum transforms into C^β-endo ($^\beta$E) when the neutral peptide transforms into Ac-$\beta t$$AmH^+$-NHMe.

The peptide bond ω_0 shows a *cis* arrangement in the other two minima characterized for Ac-$\beta t$$AmH^+$p-NHMe, c-$\alpha_L$[u] (Figure 2b) and c-$\alpha_L$[d] (Figure 2c), which only differ in the puckering of the five membered ring. Thus, the *up* puckering ($^\beta$E) favors the formation of a side chain···backbone interaction in the former structure, while this interaction is precluded by the down arrangement ($_\beta$E) showed in the latter conformation. The energy difference between such two minima, 5.9 kcal/mol, provides an estimation of the strength of the interaction between

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TABLE 1. Backbone Dihedral Angles (deg), Pseudorotational Parameters (*A* and *P*, deg), Relative Energy (ΔE^{gp}, kcal/mol), and Relative Energy with Respect to the Lowest Energy Conformation of the Most Stable Dipeptide ($\Delta E^{\#gp\#}$, kcal/mol) of the Minimum Energy Conformations Characterized for Ac-βtAmH$^+$p-NHMe, Ac-βcAmH$^+$p-NHMe, Ac-γtAmH$^+$p-NHMe, and Ac-γcAmH$^+$p-NHMe at the B3LYP/6-31+G(d,p) Level in the Gas Phase

conf	ω_0	φ	ψ	ω	(*A*, *P*)	ΔE^{gp}	$\Delta E^{\#gp\#}$
			Ac-βtAmH$^+$p-NHMe				
t-γ_L[u]	−170.5	−66.2	33.5	176.0	(42.3, 73.0)[a]	0.0[b]	6.2
c-α_L[u]	15.5	−88.7	4.1	−178.5	(42.3, 83.5)[c]	6.8	13.0
c-α_L[d]	7.2	−97.1	12.1	178.0	(35.6, −131.9)[d]	12.7	18.9
			Ac-βcAmH$^+$p-NHMe				
t-γ_L[d]	−171.5	−83.7	65.3	−179.9	(34.0, −114.0)[e]	0.0[f]	2.8
t-γ_L[u]	−170.9	−79.2	68.6	179.1	(38.2, 90.0)[g]	1.6	4.4
c-α_L[d]	14.3	−109.9	22.0	176.8	(42.6, −128.7)[h]	7.2	10.0
c-α_L[u]	21.2	−113.1	20.4	175.2	(39.1, 133.1)[i]	10.3	13.1
c-ε_L[u]	13.0	−45.9	117.2	178.6	(43.2, 41.7)[j]	11.0	13.8
			Ac-γtAmH$^+$p-NHMe				
g$^+$-δ_L[u]	63.0	−147.6	33.8	175.9	(43.8, 154.7)[k]	0.0[l]	13.1
g$^+$-δ_L[u]	60.0	−149.0	104.6	−177.6	(44.1, 154.6)[m]	0.2	13.3
t-γ_L[d]	−177.5	−83.6	85.8	−176.8	(40.8, −118.3)[n]	2.0	15.1
t-γ_L[u]	172.4	−75.2	87.0	−176.9	(42.8, 128.8)[o]	2.8	15.9
c-ε_L[d]	−2.3	−91.2	115.8	−179.2	(38.5, 145.0)[p]	4.7	17.8
			Ac-γcAmH$^+$p-NHMe				
t-γ_L[d]	−173.5	−83.5	95.9	176.8	(33.1, −113.9)[q]	0.0[r]	0.0
c-ε_L[d]	7.2	−81.5	121.0	178.9	(33.6, −104.6)[s]	4.3	4.3
c-α_L[d]	13.6	−98.4	−12.4	172.9	(36.1, −112.7)[t]	15.9	15.9

[a] $\chi^0 = 12.4°$, $\chi^1 = -34.4°$, $\chi^2 = 42.6°$, $\chi^3 = -33.9°$, and $\chi^4 = 13.7°$. [b] $E = -629.0390489$ au. [c] $\chi^0 = 4.8°$, $\chi^1 = -29.4°$, $\chi^2 = 41.2°$, $\chi^3 = -37.6°$, and $\chi^4 = 21.0°$. [d] $\chi^0 = -23.8°$, $\chi^1 = 34.1°$, $\chi^2 = -32.6°$, $\chi^3 = 18.3°$, and χ^4,= 3.6°. [e] χ^0= −16.0°, χ^1= 34.0°, χ^2= −39.7°, χ^3= 29.0° and χ^4= −8.1°. [f] $E = -629.044445$ au. [g] $\chi^0 = 0.0°$, $\chi^1 = -22.8°$, $\chi^2 = 36.4°$, $\chi^3 = -36.0°$, and $\chi^4 = 22.6°$. [h] $\chi^0 = -22.6°$, $\chi^1 = 40.8°$, $\chi^2 = -39.9°$, $\chi^3 = 23.8°$, and $\chi^4 = 2.2°$. [i] $\chi^0 = -26.8°$, $\chi^1 = 3.9°$, $\chi^2 = 18.6°$, $\chi^3 = -34.3°$, and $\chi^4 = 38.9°$. [j] $\chi^0 = 32.2°$, $\chi^1 = -42.3°$, $\chi^2 = 37.5°$, $\chi^3 = -18.1°$, and $\chi^4 = -9.4°$. [k] $\chi^0 = -39.6°$, $\chi^1 = 20.5°$, $\chi^2 = 5.1°$, $\chi^3 = -29.4°$, and $\chi^4 = 43.5°$. [l] $E = -629.028031$ au. [m] $\chi^0 = -39.8°$, $\chi^1 = 20.4°$, $\chi^2 = 5.2°$, $\chi^3 = -29.3°$, and $\chi^4 = 43.9°$. [n] $\chi^0 = -19.3°$, $\chi^1 = 35.4°$, $\chi^2 = 40.4°$, $\chi^3 = 28.8°$, and $\chi^4 = -5.6°$. [o] $\chi^0 = -26.8°$, $\chi^1 = 0.8°$, $\chi^2 = 23.4°$, $\chi^3 = -38.6°$, and $\chi^4 = 41.4°$. [p] $\chi^0 = -31.5°$, $\chi^1 = 36.9°$, $\chi^2 = -30.9°$, $\chi^3 = 12.6°$, and $\chi^4 = 12.3°$. [q] $\chi^0 = -13.4°$, $\chi^1 = 28.3°$, $\chi^2 = -33.1°$, $\chi^3 = 24.6°$, and $\chi^4 = -7.0°$. [r] $E = -629.049002$ au. [s] $\chi^0 = -8.5°$, $\chi^1 = 25.9°$, $\chi^2 = -33.8°$, $\chi^3 = 28.1°$, and $\chi^4 = -12.4°$. [t] $\chi^0 = -13.9°$, $\chi^1 = 30.5°$, $\chi^2 = -36.1°$, $\chi^3 = 27.5°$, and $\chi^4 = -8.4°$.

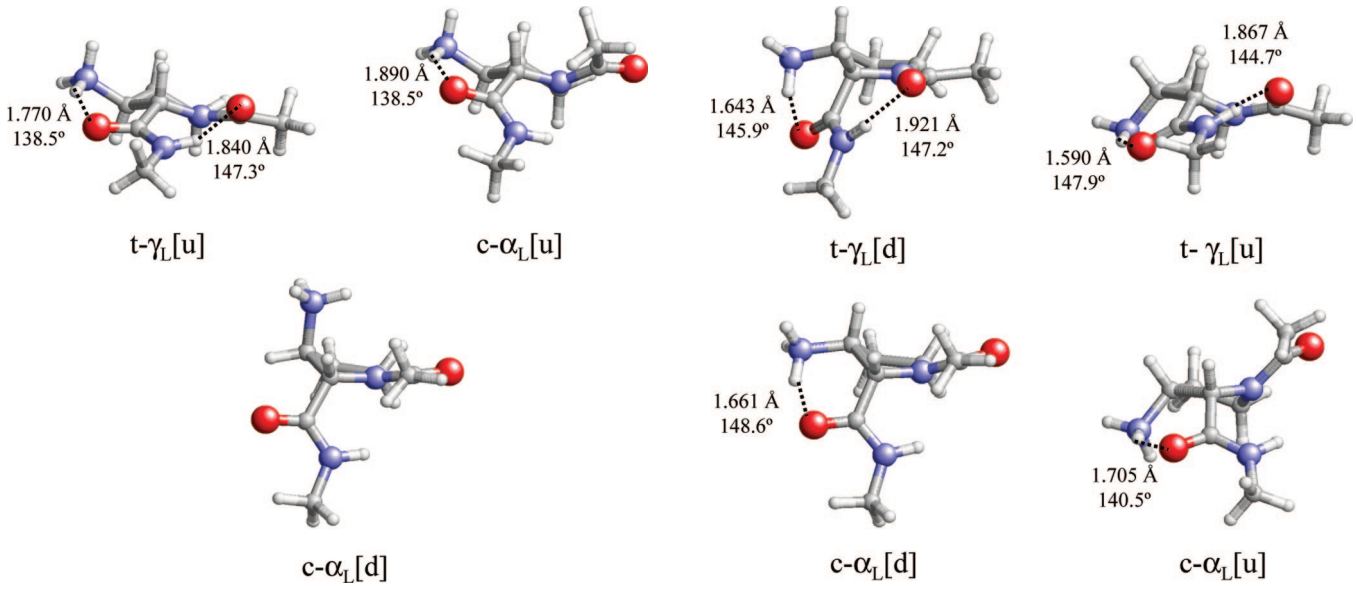

FIGURE 2. Representation of the minimum energy conformations characterized in the gas-phase for the Ac-βtAmH$^+$p-NHMe dipeptide.

the charged ammonium group and the carbonyl oxygen atom of the same residue. Comparison with the results obtained for Ac-βtAmp-NHMe evidence the annihilation upon protonation of the ε_L backbone conformation, which was identified in two minima of the neutral peptide. On the other hand, the c-α_L[u] minimum was detected for both Ac-βtAmp-NHMe and Ac-βtAmH$^+$p-NHMe, even although the two dipeptides differ significantly in the φ,ψ backbone dihedral angles, i.e., φ,ψ = −69.6°,−35.1° for the neutral compound. Indeed, the dihedral

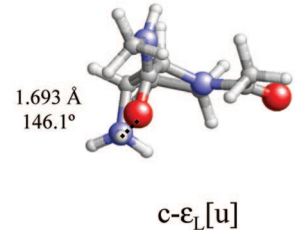

FIGURE 3. Representation of the minimum energy conformations characterized in the gas-phase for the Ac-βcAmH$^+$p-NHMe dipeptide.

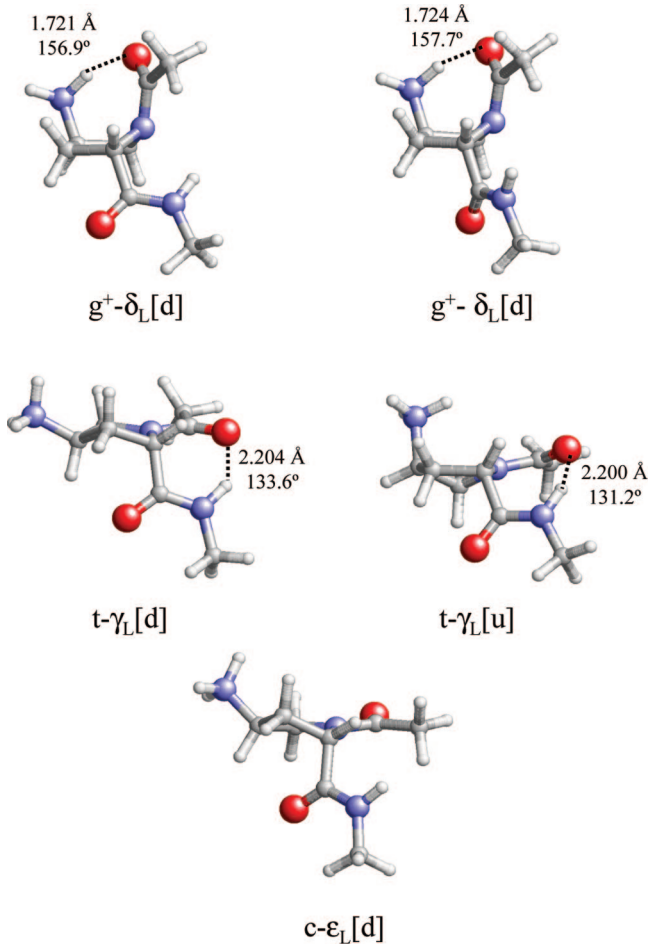

FIGURE 4. Representation of the minimum energy conformations characterized in the gas-phase for the Ac-γ*t*AmH$^+$p-NHMe dipeptide.

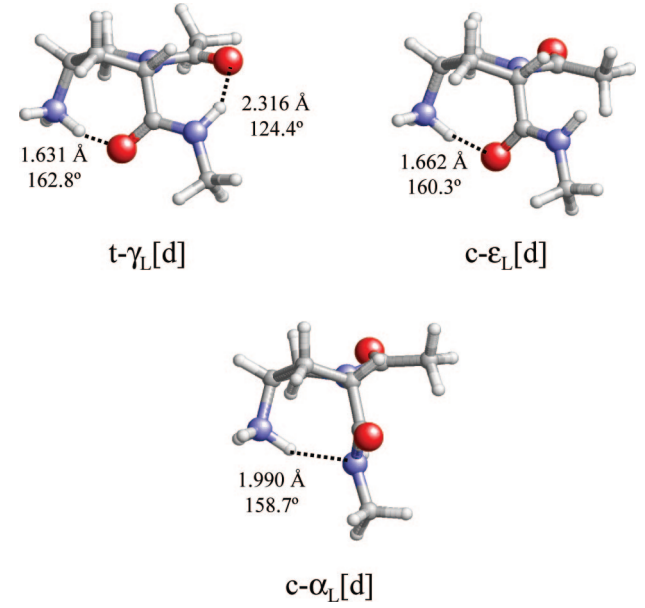

FIGURE 5. Representation of the minimum energy conformations characterized in the gas-phase for the Ac-γ*c*AmH$^+$p-NHMe dipeptide.

angles of the two c-α_L minima found for Ac-β*t*AmH$^+$p-NHMe are relative close to those usually observed in the so-called *bridge* region ($\varphi,\psi = -80°,0°$),[22] which are typically adopted by amino acids accommodated in the $i + 2$ position of types I and II β-turns.

TABLE 2. Relative Free Energy in the Gas Phase and in Aqueous Solutions (ΔG^{gp} and ΔG^{H2O}, Respectively, kcal/mol) for the Minimum Energy Conformations Characterized for Ac-β*t*AmH$^+$p-NHMe, Ac-β*c*AmH$^+$p-NHMe, Ac-γ*t*AmH$^+$p-NHMe, and Ac-γ*c*AmH$^+$p-NHMe at the B3LYP/6-31+G(d,p) level and the Relative Free Energy in the Gas Phase and in Aqueous Solutions Calculated with Respect to the Lowest Energy Minimum of the Most Stable Isomer ($\Delta G^{\#gp\#}$ and $\Delta G^{\#H2O\#}$, Respectively, kcal/mol)

conf	ΔG^{gp}	$\Delta G^{\#gp\#}$	ΔG^{H2O}	$\Delta G^{\#H2O\#}$
		Ac-β*t*AmH$^+$p-NHMe		
t-γ_L[u]	0.0[a]	7.6	0.5	8.1
c-α_L[u]	8.2	15.8	1.7	9.3
c-α_L[d]	14.8	22.4	0.0	7.6
		Ac-β*c*AmH$^+$p-NHMe		
t-γ_L[d]	0.0[b]	1.7	0.0	3.6
t-γ_L[u]	2.2	3.9	3.2	6.8
c-α_L[d]	9.3	11.0	3.9	7.5
c-α_L[u]	9.1	10.8	3.7	7.3
c-ε_L[u]	13.8	15.5	3.7	7.3
		Ac-γ*t*AmH$^+$p-NHMe		
g$^+$-δ_L[u]	0.0[c]	17.4	11.9	16.3
g$^+$-δ_L[u]	0.2	17.6	1.0	5.4
t-γ_L[d]	2.0	19.4	1.9	6.3
t-γ_L[u]	2.4	19.8	3.3	7.6
c-ε_L[d]	2.9	20.3	0.0	4.4
		Ac-γ*c*AmH$^+$p-NHMe		
t-γ_L[d]	0.0[d]	0.0	1.3	1.3
c-ε_L[d]	5.3	5.3	0.0	0.0
c-α_L[d]	20.9	20.9	10.5	10.5

[a] $G = -628.831142$ au. [b] $G = -628.840562$ au. [c] $G = -628.815530$ au. [d] $G = -628.843287$ au.

Inspection of the free energies listed in Table 2 shows the importance of the ZPVE, thermal, and entropic corrections to the relative stability of the minima characterized for Ac-β*t*AmH$^+$p-NHMe. Consideration of these statistical terms for the transformation of ΔE^{gp} into ΔG^{gp} produces a relative destabilization of 1.4 and 2.1 kcal/mol for the c-α_L[u] and c-α_L[d] conformations, respectively. According to a Bolzmann distribution of minima, the ΔG^{gp} values indicate that the only populated conformation in the gas phase at room temperature is the t-γ_L[u].

Table 2 includes the relative free energies in aqueous solution. As can be seen, this solvent introduces significant changes in the relative stability of the three minima. Thus, the c-α_L[d] conformation, which was the least stable in the gas-phase, becomes the most favored in water. However, it is worth noting that the ΔG^{H2O} values of the other two conformations are relatively close. Indeed, the population predicted at room temperature for the c-α_L[d], t-γ_L[u] and c-α_L[u] conformations in water is 67.3%, 28.9%, and 3.7%, respectively. Our previous calculations on Amp derivatives also evidenced a significant stabilization of the conformers with the peptide bond ω_0 arranged in *cis*,[11] i.e., the most stable conformation found for Ac-β*t*Amp-NHMe in aqueous solution was the c-ε_L[d], the other minima being destabilized by more than 3.5 kcal/mol in this environment.

(22) (a) Valle, G.; Crisma, M.; Toniolo, C.; Holt, E. M.; Tamura, M.; Bland, J.; Stammer, C. H. *Int. J. Pept. Protein Res* **1989**, *34*, 56. (b) Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Santini, A.; Crisma, M.; Valle, G.; Toniolo, C. *Biopolymers* **1989**, *28*, 175. (c) Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Santini, A.; Barone, V.; Fraternalli, F.; Lelj, F.; Bavoso, A.; Crisma, M.; Toniolo, C. *Int. J. Biol. Macromol.* **1989**, *11*, 353. (d) Fabiano, N.; Valle, G.; Crisma, M.; Toniolo, C.; Saviano, M.; Lombardi, A.; Isernia, C.; Pavone, V.; Di Blasio, B.; Pedone, C.; Benedetti, E. *Int. J. Pept. Protein Res.* **1993**, *42*, 459.

Previous studies[7,11] evidenced that, although the stability of the *cis* conformers in protic solvents able to form specific hydrogen bonds with the solute is overestimated by PCM calculations, the general tendencies provided by this theoretical method for Pro derivatives describe very satisfactorily the experimental observations from a qualitative point of view. Thus, PCM calculations recently predicted that ω_0 exhibits a considerably smaller probability of adopting a *cis* disposition in α-methylproline and α-phenylproline than in Pro,[7] which was in good agreement with experimental information. Comparison of the results provided in Table 2 for Ac-βtAmH$^+$p-NHMe with those reported for Ac-βtAmp-NHMe[11] and Ac-Pro-NHMe[7] at the same theoretical level suggests that, although the incorporation of the amino group to the pyrrolidine ring enhances the stability of the conformers with ω_0 arranged in *cis*, its protonation tends to compensate this effect. Thus, although the c-ε_L[u] conformation was predicted as the most favored for Ac-Pro-NHMe in water, the t-α_L and t-γ_L were unfavored by only 1.3 and 0.3 kcal/mol, respectively.[7] These differences are similar to that found for the t-γ_L[u] conformation of Ac-βtAmH$^+$p-NHMe (0.5 kcal/mol in Table 2) and significantly smaller than that reported for the t-γ_L[d] minimum of Ac-βtAmp-NHMe (6.4 kcal/mol in reference [11]). However, caution is required in the analysis of PCM results when protic solvents able to form specific solute−solvent interactions are considered.

Ac-βcAmH$^+$p-NHMe. Five minimum energy conformations, including the three with the peptide bond ω_0 arranged in *cis*, were obtained for Ac-βcAmH$^+$p-NHMe, while six were found for the nonprotonated Ac-βcAmp-NHMe. In both cases the lowest energy minimum corresponds to the t-γ_L[d] (Figure 3a) evidencing that the stability provided by the side chain···backbone interaction, which forms simultaneously to the backbone···backbone hydrogen bond typically associated to the γ_L conformation, is independent of the protonation state. In contrast, the t-γ_L[u] (Figure 3b) was only characterized as minimum in the potential energy surface of Ac-βcAmH$^+$p-NHMe dipeptide. This structure, which also presents an intramolecular hydrogen bond between the side ammonium group and the *O*═C of the own AmH$^+$p residue, only differ from the global minimum in the arrangement of the five membered ring, i.e., $_\beta$E and $^\beta$E for the t-γ_L[d] and t-γ_L[u], respectively. The t-α_L[d] and t-α_L[u] conformations of Ac-βcAmp-NHMe, which were found to be destabilized by 4.0 and 8.8 kcal/mol, respectively,[11] annihilate upon protonation of the side amino group.

The c-α_L[d] (Figure 3c) is the most stable minimum of Ac-βcAmH$^+$p-NHMe with the peptide bond ω_0 arranged in *cis*. Although this conformation presents a strong side chain···backbone intraresidue interaction, it is unfavored by 7.2 kcal/mol with respect to the lowest energy minimum. These results are fully consistent with those showed above for Ac-βtAmH$^+$p-NHMe, which indicated that the structures with ω_0 in *cis* become strongly destabilized upon protonation of the amino group attached to the C$^\beta$ atom. Thus, for Ac-βcAmp-NHMe the energy of the c-α_L[d] was higher than that of the global minimum by 4.3 kcal/mol only. On the other hand, the other two minima found for Ac-βcAmH$^+$p-NHMe correspond to the c-α_L[u] (Figure 3d) and c-ε_L[u] (Figure 3e) with ΔE^{gp} values of 10.3 and 11.0 kcal/mol, respectively. Interestingly, the φ,ψ values of the two minima that show a α_L conformation are significantly distorted with respect to those expected for an ideal conformation. These deformations, which were also detected in the two c-α_L minima of Ac-βtAmH$^+$p-NHMe, are consequence of the interaction between the ammonium group and the peptide bond ω.

Inspection of the ΔG^{gp} values indicates that the statistical corrections added to the electronic energies produce a relative destabilization of the t-γ_L[u], c-α_L[d], and c-ε_L[u] structures, which range from 0.6 to 2.8 kcal/mol. In opposition, the c-α_L[u] conformation becomes more stable by 1.2 kcal/mol, even although its population in the gas-phase is negligible. Thus, the only structure of Ac-βcAmH$^+$p-NHMe that presents a significant population (97.6%) in the gas-phase is the t-γ_L[d]. Similarly, this minimum is the only populated conformation in aqueous solution, the ΔG^{H2O} of the other four conformers ranging between 3.2 and 3.9 kcal/mol. The fact that the global minimum in the gas-phase is also the most favored conformation in water represents a remarkable difference with respect to the Ac-βcAmp-NHMe dipeptide. Thus, for the latter system, the t-γ_L[d] was destabilized by 3.3 kcal/mol in aqueous solution, the c-ε_L[d] becoming the most favored in this polar environment. These results are fully consistent with the destabilization of the structures with ω_0 arranged in *cis* discussed above for Ac-βtAmH$^+$p-NHMe.

Ac-γtAmH$^+$p-NHMe. This dipeptide shows a distinctive conformational behavior. As can be seen in Table 1, two almost isoenergetic minima with the peptide bond ω_0 arranged in a *gauche*$^+$ conformation (labeled as g$^+$) are the most favored for Ac-γtAmH$^+$p-NHMe. The distortion from the planarity of the peptide bond in these structures, g$^+$-δ_L[u] (Figure 4a,b), must be attributed to the strength of the side chain···backbone interaction, which shows a favorable geometry because of the N-exo ($_N$E) conformation of the pyrrolidine ring, i.e., this envelope conformation breaks the planar disposition of the peptide bond. Such distortion is evidenced by a notable pyramidalization of the amide nitrogen, the sum of the valence angles around this atom (θ) being 338.5 and 340° for the two g$^+$-δ_L[u] minima. This large deformation indicates that the pyramidalization of ω_0 in the g$^+$-δ_L[u] structures is similar, or even higher, than that observed for the bicyclic amide nitrogen of highly constrained Pro analogues.[23] Interestingly, the two g$^+$-δ_L[u] minima only differ in the dihedral angle ψ, which defines the orientation of peptide bond ω. The orientation of the polar −CONH− moiety in such minima does not affect to their intrinsic stability in the gas-phase, i.e., the ΔG^{gp} values differ by 0.2 kcal/mol only, even though their relative stabilities in aqueous solution are completely different. Thus, the strength of the solute···solvent attractive interactions increases with the accessibility of this peptide group to the solvent, i.e., the g$^+$-δ_L[u] conformation with $\psi = 104.6°$ is favored by 11.1 kcal/mol, which explains the difference found in their ΔG^{H2O} values (Table 2).

The next two minima, which correspond to the conventional t-γ_L[d] (Figure 4c) and t-γ_L[u] (Figure 4d) conformations, are destabilized by 2.0 and 2.8 kcal/mol, respectively. These structures, in which the two peptide bonds adopt a planar *trans* arrangement, are stabilized by the backbone···backbone intramolecular hydrogen bond only, no side chain···backbone interaction being detected. This feature explains the lower stability of the two t-γ_L conformations with respect to the two g$^+$-δ_L minima. Thus, the strong side chain···backbone interaction of the latter, which is more attractive than the seven-

(23) Alemán, C.; Jiménez, A. I.; Cativiela, C.; Pérez, J. J.; Casanovas, J. *J. Phys. Chem. B* **2005**, *109*, 11836.

membered intramolecular hydrogen bond, almost compensates the energy penalty associated to the geometric deformation of the peptide bond ω_0. Finally, the least stable conformation, c-ε_L[d] (Figure 4e), which is unfavored by 4.7 kcal/mol with respect to the global minimum, corresponds to the only conformation with ω_0 arranged in *cis*. As can be seen, this structure does not show any N−H···O intramolecular interaction.

Comparison of the ΔE^{gp} and ΔG^{gp} values reveals that, in this case, the influence of the statistical corrections is very small. Thus, with the exception of two minima of higher energy, which become stabilized by 0.4 (t-γ_L[u]) and 1.8 kcal/mol (c-ε_L[d]) by the addition of the thermal and entropic terms, the relative stability of the other three structures remained unaltered. On the other hand, the relative free energy order undergoes a drastic change in aqueous solution. Specifically, the most stable structure in water is the c-ε_L[d], which was the least favored in the gas-phase. This feature is consistent with the overestimation of the conformers with ω_0 arranged in *cis* previously attributed to the PCM solvation model. Furthermore, the lowest energy minimum in the gas phase is the least favored in aqueous solution, this feature being a consequence of the poor interaction between the C═O group of the peptide bond ω and the solvent (Figure 4a). In contrast, the second minimum with a distorted peptide bond results less favored in aqueous solution than the c-ε_L[d] by 1.0 kcal/mol only, which is due to the very favorable interactions of the peptide bond ω with the environment. Finally, the stability in water of the two structures with the t-γ_L backbone conformation is similar to that obtained in the gas-phase. The overall of these results indicate that the conformational populations predicted for Ac-γtAmH$^+$p-NHMe in aqueous solution, considering Boltzmann distribution of the identified minima, are 81.7% c-ε_L[d], 15.0% g$^+$-δ_L[d], and 3.3% t-γ_L[d].

Ac-γcAmH$^+$p-NHMe. Only three minima were detected for Ac-γcAmH$^+$p-NHMe, showing that the protonation of the amino side group drastically reduces the conformational flexibility of neutral Ac-γcAmp-NHMe.[11] Thus, seven minima with relative energies of up 7.9 kcal/mol were found for the latter dipeptide: three with ω_0 arranged in *trans* and four in *cis*. The lowest energy minimum found for Ac-γcAmH$^+$p-NHMe corresponds to the t-γ_L[d] (Figure 5a), which presents both backbone···backbone and side chain···backbone favorable interactions, the latter being facilitated by the $_\beta$E arrangement of the pyrrolidine ring. This conformation was also identified as the most stable conformation of Ac-γcAmp-NHMe, even although the t-γ_L[u] and t-α_L[d] minima annihilate when the amino group of this dipeptide transforms into ammonium.

The second minimum of Ac-γcAmH$^+$p-NHMe shows a c-ε_L[d] structure (Figure 5b) with the pyrrolidine ring arranged like in the global minimum, i.e., $_\beta$E conformation. This structure, which is unfavored by 4.3 kcal/mol, is similar to the least stable minimum of Ac-γtAmH$^+$p-NHMe, even although the *cis* disposition of the substituent allows the formation of an attractive side chain···backbone interaction that was not possible in the latter compound. Finally, the last minimum, c-α_L[d] (Figure 5c), is strongly destabilized, i.e., ΔE^{gp} = 15.9 kcal/mol. This should be attributed to the simultaneous combination of a number of factors: (i) the *cis* arrangement of ω_0; (ii) the lack of backbone···backbone intramolecular hydrogen bond; and, especially, (iii) the nature of side chain···backbone interaction, which is of the N−H···N type. Thus, the stabilizing effect provided by the latter interaction is lower than that achieved through the N−H···O(═C) interaction.[24] It is worth noting that the c-ε_L[d] and c-α_L[d] minima of Ac-γcAmp-NHMe were unfavored by 6.0 and 2.7 kcal/mol,[11] respectively, which reflects the large change that the ionization of the side group produces in the potential energy surface of this dipeptide.

The most significant change produced by the transformation of ΔE^{gp} into ΔG^{gp} is the destabilization of the c-α_L[d] conformation, which increases 5.0 kcal/mol. Accordingly, the t-γ_L[d] is predicted to be the only populated conformation in the gas phase. On the other hand, inspection to the ΔG^{H2O} values indicates again that the PCM model produces a considerable stabilization of the c-ε_L[d] structure. Thus, the latter conformation becomes the most favored in aqueous solution, whereas the t-γ_L[d] is higher in energy by 1.3 kcal/mol, i.e., the populations of the c-ε_L[d] and t-γ_L[d] conformations in aqueous solution are 90.1% and 9.9%, respectively. These preferences are significantly different from those reported for Ac-γcAmp-NHMe, in which the c-ε_L[d] was predicted to be only conformation with a significant population in aqueous solution;[11] i.e., all of the other minima were destabilized by more than 2 kcal/mol.

Relative Stability of the Four Isomers. The $\Delta E^{\#gp\#}$ and $\Delta G^{\#gp\#}$ values displayed in Tables 1 and 2, respectively, indicate that Ac-γcAmH$^+$p-NHMe is the most stable isomer, Ac-βcAmH$^+$p-NHMe being unfavored by only 1.7 kcal/mol (2.8 kcal/mol in terms of $\Delta E^{\#gp\#}$). The stability of these isomers, which is significantly higher than that of the analogues with a *trans* disposition of the charged side group, should be attributed to the formation of side chain···backbone interactions. Thus, intraresidue interactions are clearly stronger when the substitution is attached in *cis*. This feature is reflected by the H···O distances displayed in Figures 3 and 5, which are ∼1.65 Å for the minima of the γcAmH$^+$p- and βcAmH$^+$p-containing dipeptides. In contrast, these distances are 1.770 and 1.890 Å for the two minima of Ac-βtAmH$^+$p-NHMe that present a side chain···backbone interaction (Figure 2a,b), this isomer being unfavored by 7.6 kcal/mol (6.2 kcal/mol in terms of $\Delta E^{\#gp\#}$) with respect to the most stable. Similarly, the values of the ∠N−H···O angles are consistent with a more favorable interaction when the substituent is attached in *cis*. The Ac-γtAmH$^+$p-NHMe is not able to form side chain···backbone interactions without induce a remarkable distortion of the peptide bond, which produces a significant energy penalty. Consequently, this isomer is unfavored by 17.4 kcal/mol (13.1 kcal/mol in terms of $\Delta E^{\#gp\#}$) with respect to the Ac-γcAmH$^+$p-NHMe dipeptide.

The relative stability order obtained for the neutral Amp-containing dipeptides was identical, i.e., Ac-γcAmp-NHMe > Ac-βcAmp-NHMe > Ac-βtAmp-NHMe > Ac-γtAmp-NHMe.[11] However, in this case, the energy differences among the different isomers were significantly lower than those obtained for the AmH$^+$p derivatives. Thus, the lowest $\Delta G^{\#gp\#}$ value of the βcAmp-, βtAmp-. and γtAmp-containing dipeptides was 0.6, 1.0, and 1.4 kcal/mol, respectively. The remarkable energetic difference between Amp and AmH$^+$p dipeptides should be attributed to the strength of the intraresidue interaction, which is significantly higher when the side group is ionized.

On the other hand, $\Delta G^{\#H2O\#}$ values indicate that the solvent does not alter the preferences by the isomers with the ammonium

(24) (a) Alemán, C.; Zanuy, D.; Casanovas, J. *J. Phys. Chem. A* **2003**, *107*, 4151. (b) Barril, X.; Alemán, C.; Orozco, M.; Luque, F. J. *Proteins* **1998**, *32*, 67. (c) Alemán, C.; Navas, J. J.; Muñoz-Guerra, S. *J. Phys. Chem.* **1995**, *99*, 17653.

group attached in *cis* with respect to those in *trans*. Thus, Ac-γcAmH$^+$p-NHMe is the most stable in water followed by Ac-βcAmH$^+$p-NHMe, which is unfavored by 3.6 kcal/mol. Accordingly, the solvent destabilizes the latter isomer 1.9 kcal/mol with respect to the gas phase. Regarding Ac-βtAmp-NHMe and Ac-γtAmp-NHMe, their relative stabilities are exchanged with respect to the gas phase, the latter being favored with respect to the former by 3.2 kcal/mol. Thus, these isomers are 5.4 and 7.6 kcal/mol, respectively, less stable than the Ac-γcAmH$^+$p-NHMe one. Comparison with the results reported in aqueous solution for the neutral Amp-containing dipeptides reveals considerable differences. Specifically, the relative energy order reported for these dipeptides were[11] Ac-γcAmp-NHMe $\approx$ Ac-βtAmp-NHMe ($\Delta G^{\#H2O\#} = 0.2$ kcal/mol) > Ac-γtAmp-NHMe ($\Delta G^{\#H2O\#} = 1.7$ kcal/mol) > Ac-βcAmp-NHMe ($\Delta G^{\#H2O\#} = 5.9$ kcal/mol). Accordingly, the protonation of the amino substituent in water produces a pronounced stabilization of the isomers substituted in *cis* with respect to those with the substituent in *trans*.

Conclusions

DFT calculations at the B3LYP/6-31+G(d,p) level have been used to examine the conformational preferences of Ac-βtAmH$^+$p-NHMe, Ac-βcAmH$^+$p-NHMe, Ac-γtAmH$^+$p-NHMe, and Ac-γcAmH$^+$p-NHMe, both in the gas phase and aqueous solution, which have been compared with those reported for the neutral analogues Ac-βtAmp-NHMe, Ac-βcAmp-NHMe, Ac-γtAmp-NHMe, and Ac-γcAmp-NHMe. The results allow us to draw the following conclusions:

(i) Protonation of the amino group attached to the β- or γ-position of the pyrrolidine ring reduces the backbone conformational flexibility of the Amp derivatives, which was low compared to that conventional Pro. Specifically, the number of minima identified for the four AmH$^+$p-containing dipeptides was smaller than that found for their Amp analogues. Furthermore, the relative energies and free energies increase upon protonation of the side group.

(ii) The stability of conformations with ω_0 in *cis* is significantly lower for AmH$^+$p than for Amp. This is a very remarkable result because the incorporation of the nonprotonated amino group to the pyrrolidine ring of conventional Pro produced a stabilization of such conformations. Accordingly, the population of *cis* conformers in Amp/AmH$^+$p derivatives could be easily controlled with the pH.

(iii) The intrinsic conformational preferences of the Ac-γtAmH$^+$p-NHMe dipeptide show that the strength of the side chain$\cdots$backbone attractive interaction allows compensate the energy penalty associated to the deformation of the peptide bond. Thus, in order to reach such interactions, this compound tends to break the planarity of the peptide bond inducing a large pyramidalization of the amide nitrogen atom.

(iv) The Ac-γcAmH$^+$p-NHMe dipeptide, followed by the Ac-βcAmH$^+$p-NHMe peptide, are the more stable isomers in both the gas phase and aqueous solution. The *cis* disposition of the substituent is favored because of the attractive side chain$\cdots$backbone interactions. Comparison between Amp and AmH$^+$p derivatives reveals that the energy gap between the different isomers increases upon ionization of the side group. This should be attributed to the strength of the intraresidue interactions, which is higher when a positively charged ammonium group is involved.

Acknowledgment. Gratitude is expressed to the Centre de Supercomputació de Catalunya (CESCA). Financial support from the "Ministerio de Educación y Ciencia" (HF2007-0049 and CTQ2008-00423-E/BQU) is gratefully acknowledged. The French−Spanish collaborative action (Programme Hubert Curien, Picasso no. 17075XM) is thanked for covering the travel expenses of X.A. in Barcelona. A.F.-O. and X.A. acknowledge the financial support of "Région Lorraine" for the visit in Nancy of A.F.-O. during autumn 2008.

JO900169S