Conformational Preferences of α-Substituted Proline Analogues

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Abstract

DFT calculations at the B3LYP/6-31+G(d,p) level have been used to investigate how the replacement of the α hydrogen by a more sterically demanding group affects the conformational preferences of proline. Specifically, the *N*-acetyl-*N*'-methylamide derivatives of L-proline, L-α-methylproline and L-α-phenylproline have been calculated, with both the *cis*/*trans* isomerism of the peptide bonds and the puckering of the pyrrolidine ring being considered. The effects of solvation have been evaluated using a Self Consistent Reaction Field model. As expected, tetrasubstitution at the α carbon destabilizes the conformers with one or more peptide bonds arranged in *cis*. The lowest energy minimum has been found to be identical for the three compounds investigated, but important differences are observed regarding other energetically accessible backbone conformations. The results obtained provide evidence that the distinct steric requirements of the substituent at C^α may play a significant role in modulating the conformational preferences of proline.

Introduction

The incorporation of conformationally constrained amino acids into a peptide chain is a powerful tool to reduce its intrinsic flexibility. Among the residues whose structural rigidity can be exploited in the design of peptides with well-defined backbone conformations are α-tetrasubstituted α-amino acids.[1]

The simplest α-tetrasubstituted analogue of a proteinogenic amino acid that can be considered is that resulting from the replacement of the α hydrogen by a methyl group. In the last two decades, extensive efforts have been directed at the development of efficient methodologies for the synthesis of the α-methyl derivatives of all genetically coded amino acids[2] (glycine excluded, since it leads to alanine). The simplest one is α-methylalanine (α-aminoisobutyric acid, Aib), whose conformational properties have been deeply investigated and are well established.[1,3,4] In comparison, the α-methylated analogues of all other proteinogenic amino acids have been much less studied, mainly due to synthetic difficulties: α-methylation of Ala

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gives rise to a symmetric achiral residue, whereas two enantiomeric forms are possible for all other residues. Although not as extensively as for Aib, the study of the conformational properties of the α-methyl derivatives of other proteinogenic amino acids (mainly valine, leucine and phenylalanine) has been addressed.[1c,e,5] In general, these α-methylated residues behave as the prototype Aib, although they present particular conformational features derived from their chiral nature.

The unique properties of proline make the study of its α-methylated derivative (in general, α-substituted analogues) particularly intriguing. The singularity of proline lies in its cyclic structure, which includes the amino function. As a consequence, rotation about the N—C^α bond is prohibited and the φ torsion angle is confined to values around −60°. Accordingly, proline is overwhelmingly found in the α-helical [$(\varphi,\psi) \approx (-60°,-30°)$] and semi-extended [$(\varphi,\psi) \approx (-60°,140°)$] regions of the conformational map.[6] In addition, proline shows a higher propensity to promote γ-turn conformations [$(\varphi,\psi) \approx (-70°,60°)$] than other proteinogenic amino acids.[6d,7] Another effect derived from its cyclic structure is that the peptide bond preceding proline (that involving the pyrrolidine nitrogen) has a relatively high probability of accommodating a *cis* arrangement[8] as compared to other peptide bonds, for which the *cis* form is almost inexistent. Recent studies in proline dipeptides evidenced that the *cis/trans* isomerization is a enthalpy driven process that depends on the polarity of the environment.[9] Thus, although the electronic effects that stabilize the *cis* form become enhanced in polar environments, the *cis/trans* rotational barriers increases with the polarity of the enviroment.

Due to its particular structural properties, proline plays a key role in the structure and biology of peptides and proteins, and, hence, α-substituted derivatives are of great interest. The conformational preferences of the α-methylated analogue (αMePro) remain little explored.[10,11] Studies on the *N*-acetyl-*N'*-methylamide derivative indicated a preference for the γ-turn conformation in solution, [10c,d] whereas an α-helical structure was found in the solid state.[10b] Spectroscopic and computational studies on other peptides containing αMePro suggested a stabilization of the βI-turn in comparison with proline.[11] In contrast to the scarce structural studies, the large number of papers[11,12] and patents[13] dealing with the incorporation of αMePro into bioactive peptides and other biologically relevant systems provide evidence for the enormous potential of this amino acid. However, the exploitation of αMePro and other α-tetrasubstituted proline analogues in the design of peptides with controlled fold in the backbone relies on the previous knowledge of their conformational propensities.

In this work, we have investigated the intrinsic conformational preferences of α-methylproline (αMePro) and α-phenylproline (αPhPro) using Density Functional Theory (DFT) methods. Calculations were performed on the *N*-acetyl-*N'*-methylamide derivatives of the L-amino acids, hereafter denoted as Ac-L-αMePro-NHMe and Ac-L-αPhPro-NHMe, respectively. The influence of the methyl and phenyl groups has been determined by comparison with the proline derivative Ac-L-Pro-NHMe, which has been investigated for comparative purposes using the same quantum mechanical method. Specifically, we have examined how the substituent incorporated at the α position affects the preferred backbone conformation, the puckering of the pyrrolidine ring and the *cis/trans* disposition of the amide bonds. On the other hand, as was mentioned above, the role of the environment, in particular of the solvent, in the *cis/trans* rotational isomerism of proline was reported to be crucial.[9] In spite of this, no information about the solvent effects on the isomerization of the α-substituted proline analogs has been provided yet. Accordingly, we decided to evaluate the influence of the solvent polarity on the conformational preferences of the compounds under study using a Self Consistent Reaction Field method.

Methods

Computational Details

DFT calculations were carried out using the Gaussian 03 computer program,[14] combining the Becke′s three-parameter hybrid functional (B3)[15] with the Lee, Yang and Parr (LYP)[16] expression for the nonlocal correlation (B3LYP). This method provides a very satisfactory description of the conformational properties of cyclic constrained amino acids, including Pro and pseudoprolines.[17,18] Accordingly, all the calculations presented in this work were performed using the B3LYP method combined with the 6–31+G(d,p) basis set,[19] even although some additional single point calculations on selected conformations were performed using the aug-cc-pVTZ[20] basis set.

The backbone ($\omega_0, \phi, \psi, \omega$) and side chain ($\chi^i$; endocyclic) dihedral angles of the N-acetyl-N'-methylamide derivatives of Pro, αMePro and αPhPro are defined in Figure 1. Since φ is fixed by the geometry of the five-membered ring, only three minima may be anticipated for the potential energy surfaces $E=E(\psi)$ of the dipeptides for a given arrangement of the peptide bonds. The flexible angle ψ is expected to have three minima, *i.e. gauche*$^+$ (60°), *trans* (180°) and *gauche*$^-$ (−60°), while each amide bond (ω_0, ω) can be arranged in *cis* or *trans*. It should be noted that only the peptide bond preceding proline (that involving the pyrrolidine nitrogen, corresponding to the ω_0 torsion angle) is likely to adopt a *cis* configuration. However, we considered also the *cis* and *trans* states of the amide bond formed by the proline carbonyl (the methylcarboxamide group, –CONHMe, given by ω) with the aim of exploring how α-methylation affects the isomerism of this amide linkage. For the αPhPro derivative, only the *cis/trans* arrangement of ω_0 was considered.

The cyclic side chains of the compounds under study may adopt two main different conformational states, corresponding to the *down* and *up* puckering of the five-membered ring. They are defined as those in which the C^γ atom and the carbonyl group of the Pro residue (or analogue) lie on the same and opposite sides, respectively, of the plane defined by the C^δ, N and C^α atoms.

Accordingly, for Ac-ʟ-Pro-NHMe and Ac-ʟ-αMePro-NHMe, 3(ψ backbone) × 2(ω_0 *cis-or-trans*) × 2(ω *cis-or-trans*) × 2(cyclic side chain) = 24 structures were considered as starting points for complete geometry optimizations at the B3LYP/6–31+G(d,p) level. Regarding Ac-ʟ-αPhPro-NHMe, ω was kept in the *trans* configuration, while for the arrangement of the phenyl substituent three different orientations were considered. Therefore, the number of starting structures for geometry optimizations were 3(ψ backbone) × 2(ω_0 *cis-or-trans*) × 2(cyclic side chain) × 3(Ph substituent) = 36. 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) with both thermal and entropic corrections, the latter statistical terms being used to compute the conformational Gibbs free energies in the gas phase (ΔG^{gp}) at the B3LYP/6–31+G(d,p) level.

To obtain an estimation of the solvation effects on the relative stability of the different minima, single point calculations were also 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, while the solvent is represented as a dielectric continuum. Specifically, the Polarizable Continuum Model (PCM) developed by Tomasi and co-workers was used to describe the bulk solvent.[21] This method involves the generation of a solvent cavity from spheres centered at each atom in the molecule and the calculation of virtual point charges on the cavity surface representing the polarization of the solvent. The magnitude of these charges is proportional to the derivative of the solute electrostatic potential at each point calculated from the molecular wave function. The point charges may, then, be included in the

oneelectron Hamiltonian, thus inducing polarization of the solute. An iterative calculation is carried out until the wave function and the surface charges are self-consistent. PCM calculations were performed using the standard protocol and considering the dielectric constants of carbon tetrachloride ($\varepsilon = 2.228$), chloroform ($\varepsilon = 4.9$), methanol ($\varepsilon = 32.6$) and water ($\varepsilon = 78.4$). The conformational free energies in solution ($\Delta G^{\#sol\#}$, where #sol# refers to the solvent) were computed using the classical thermodynamics scheme, that is, the free energies of solvation provided by the PCM model were added to the ΔG^{gp} values.

Nomenclature and Pseudorotational Parameters

The minimum energy conformations of the three dipeptides studied in this work have been denoted using a four-label code that specifies the arrangement of the two peptide bonds, 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 preceding proline (ω_0). The second label identifies the backbone conformation using the nomenclature introduced by Perczel *et al.*[22] more than fifteen years ago. Accordingly, nine different backbone conformations can be distinguished in the potential energy surface $E=E(\varphi,\psi)$ of amino acids: γ_D, δ_D, α_D, ε_D, β_L, ε_L, α_L, δ_L and γ_L. In the case of proline, only the γ_L (γ-turn or C_7), α_L (α-helical), and ε_L (polyproline II-like) conformations are accessible due to φ being fixed in the neighborhood of $-60°$. Next, the *up* or *down* puckering of the five-membered ring is indicated using the [u] and [d] labels, respectively. In particular, the *down* ring puckering was identified when χ^1 and χ^3 were positive while χ^2 and χ^4 were negative. Conversely, the *up* ring puckering is characterized by negative values of χ^1 and χ^3 and positive values of χ^2 and χ^4. Finally, the last letter indicates the *trans* (t) or *cis* (c) arrangement of the amide bond involving the proline carbonyl group (ω).

The puckering of the five-membered ring was described using the classical pseudorotational algorithm, which uses a very simple model based on only two parameters, as previously applied to proline by Perczel *et al.*[23] The pseudorotational parameters A and P, which describe the puckering amplitude and the state of the pucker in the pseudorotation pathway, respectively, are derived from the endocyclic dihedral angles as follows:

$$A=\sqrt{(A \sin P)^2+(\chi^0)^2}, \text{where A} \sin \text{P}=\frac{\chi^1 - \chi^2+\chi^3 - \chi^4}{-2(\sin 144°+\sin 72°)},$$

and

$$P=\begin{cases} \arccos \frac{\chi^0}{A}, & \text{if A} \sin \text{P} \geq 0 \\ -\arccos \frac{\chi^0}{A}, & \text{if A} \sin P<0 \end{cases}$$

Accordingly, parameter A is defined to be positive while P falls between $-180°$ and $180°$.

Results and Discussion

Ac-L-Pro-NHMe

Table 1 lists the most relevant structural parameters together with the relative energy (ΔE^{gp}) and free energy (ΔG^{gp}) in the gas phase for the 14 minimum energy conformations characterized for Ac-L-Pro-NHMe (Figure 2). These minima are distributed according to the disposition of the peptide bonds (defined by the ω_0 and ω angles, Figure 1) as follows: both amide moieties adopt a *trans* arrangement in 3 minima (*trans-trans* conformers), one peptide bond is *cis* in 7 minima (4 *cis-trans* and 3 *trans-cis* conformers) and, finally, both peptide bonds

exhibit a *cis* configuration in 4 minima (*cis-cis* conformers). It is worth noting that the structural data and ΔE^{gp} values displayed in Table 1 for the 14 minima characterized for Ac-L-Pro-NHMe are in excellent agreement with the results recently reported by Csizmadia[24] and Kang[8b] at the B3LYP/6–31G(d) and B3LYP/6–311++G(d,p) levels, respectively.

As expected, the lowest energy minimum, denoted as t-γ_L[d]-t, was found to be *trans-trans* with the backbone defining a γ_L conformation, *i.e.* a seven-membered intramolecularly hydrogen-bonded ring [d(H···O) = 1.971 Å, ∠N-H···O = 146.4°] and the pyrrolidine moiety exhibiting a *down* puckering. The next minimum, t-γ_L[u]-t, only differs in the ring puckering and this change produces a destabilization of 1.0 and 1.3 kcal/mol in terms of ΔE^{gp} and ΔG^{gp}, respectively. The last *trans-trans* conformer combines the α_L backbone conformation with an *up*-puckered pyrrolidine and is disfavored by more than 4 kcal/mol.

The *cis-trans* conformer of lowest energy corresponds to c-α_L[d]-t, which is destabilized with respect to the global minimum by 2.3 kcal/mol in terms of ΔG^{gp}. A *down-to-up* transition of the pyrrolidine puckering leads to a further destabilization of 1.3 kcal/mol so that the ΔG^{gp} of the c-α_L[u]-t minimum is 3.6 kcal/mol. The remaining *cis-trans* minima as well as all the *trans-cis* and *cis-cis* conformers are highly destabilized with respect to the global minimum, with their ΔG^{gp} values ranging from 4.8 to 11.3 kcal/mol. Surprisingly enough, the least stable minimum, t-α_L[u]-c, corresponds to a *trans-cis* rather than to a *cis-cis* conformer. In order to check that the 6–31+G(d,p) basis set describes satisfactorily the stability of the different conformers, single point calculations were performed at the B3LYP/aug-cc-pVTZ level on all the *trans-trans* and *cis-trans* conformers of Ac-L-Pro-NHMe. As expected, differences between the relative energies provided by these two basis sets are very small (see Supporting Information) evidencing the suitability of the 6–31+G(d,p) one.

A detailed inspection of Table 1 allows the establishment of a clear relationship between the characterization of certain backbone conformations as energy minima and the *cis/trans* state of the amide bonds. Specifically, the γ_L structure appears as an energy minimum only when the two peptide bonds adopt a *trans* disposition (necessary for the formation of the intramolecular hydrogen bond), while minima in the ε_L region are located provided that at least one of the amide linkages is *cis*. Moreover, the latter becomes the preferred backbone conformation when the *cis* peptide bond is that involving the proline carbonyl group (– CONHMe, $\omega \approx 0°$). In contrast, if the acetamido group adopts a *cis* arrangement ($\omega_0 \approx 0°$) while ω remains close to 180°, the ε_L backbone conformation is an energy minimum but becomes less stable than the α_L arrangement. It is noteworthy that, even if the ε_L conformation was not detected as an energy minimum for all *trans* peptide bonds, proline is experimentally found to accommodate this disposition with high frequency.[6] Indeed, (ϕ,ψ) values in the ε_L region correspond to the $i+1$ position of a βII-turn,[6d] which is known to be among those preferred by proline.[6] Interestingly, calculations on Ac-L-Pro-NHMe at levels of theory lower than that used in this study, locate the *trans-trans* ε_L conformation as an energy minimum,[24] but it disappears when going to larger basis sets, as seen in the present and previous[8b,24,25] works.

Table 2 lists the relative free energies in carbon tetrachloride, chloroform, methanol and water solutions for the 14 minima mentioned above. PCM calculations were performed using the geometries optimized in the gas phase. It should be noted that previous studies indicated that solute geometry relaxations in solution and single point calculations on the optimized geometries in the gas phase give almost identical free energies of solvation.[26]

The solvent introduces significant changes in the relative stability of the different minima (Table 2). Carbon tetrachloride was found to considerably stabilize conformers with at least one *cis* amide bond. Thus, in this solvent, the c-α_L[d]-t conformer becomes almost isoenergetic

with the global minimum, t-γ_L[d]-t. Furthermore, the c-ε_L[d]-t and c-ε_L[u]-t minima are 3.2 and 2.5 kcal/mol, respectively, more stable than in the gas phase. In spite of the stabilization produced by this solvent in conformers with *cis* amide bonds, the *trans-cis* and *cis-cis* minima are not within the set of conformations energetically accessible at room temperature.

The higher polarity of chloroform results in a further stabilization of conformers with *cis* peptide bonds. In fact, the lowest energy minimum in this solvent is c-ε_L[u]-t, with the c-ε_L[d]-t and t-γ_L[d]-t conformations being disfavored by only 0.2 and 0.3 kcal/mol, respectively. Moreover, the ΔG^{CHCl3} value of the least stable *cis-trans* conformer is 2.1 kcal/mol, which provides evidence for the strong stabilizing effect of this solvent on *cis* peptide bonds. Although *trans-cis* and *cis-cis* conformers are not energetically accessible in chloroform solution, their ΔG^{CHCl3} are about half the values in the gas phase.

The c-ε_L[u]-t is again the most stable conformation in both methanol and aqueous solutions, even although in the latter environment the c-α_L[d]-t and t-α_L[u]-t are unfavored by only 0.2 and 0.3 kcal/mol, respectively. However, the most remarkable result in polar environments is the stabilization of the *cis-trans* conformers, which is consistent with the theoretical estimation previously reported for the Pro dipeptide.[8] Thus, in such studies it was found that polar environments favor the *cis* conformation in Pro peptidic bonds.

The overall of the results obtained in solution suggests that the conformational flexibility induced by the environment is considerable in terms of both the ψ dihedral angle and *cis/trans* isomerism. However, the latter effect seems to be overestimated by the PCM solvation model, this effect being very large when polar solvents like methanol and water are considered. According to the results in Table 2, conformers exhibiting a *cis* configuration for the peptide bond involving the Pro nitrogen ($\omega_0 \approx 0°$) should predominate over *trans* conformers, which contradicts experimental data.[6–9] Thus, although in condensed phases, *i.e.* solid state or solution, the peptide bond preceding Pro has a relatively high probability of adopting a *cis* arrangement, the *trans* is by far preferred. Accordingly, solvent-induced stabilizations seem to be considerably overestimated by the PCM method. Caution is therefore required when interpreting the results provided by PCM calculations, which should be considered only qualitatively.

Ac-L-αMePro-NHMe

The 17 minimum energy conformations characterized for Ac-L-αMePro-NHMe in the gas phase are displayed in Figure 3. Their structural and energy data are given in Table 3. According to the *cis/trans* state of the peptide bonds, these minima are distributed as 4 *trans-trans*, 4 *cis-trans*, 5 *trans-cis* and 4 *cis-cis*. Interestingly, α-methylation of Ac-L-Pro-NHMe produces an enlargement of the number of minimum energy conformations, which suggests a higher flexibility for the non-proteinogenic residue. However, a detailed inspection of the energy data in Table 3 reveals that the replacement of the α hydrogen in proline by a methyl group results in a general destabilization of the minimum energy conformations, particularly, of the *trans-cis* and *cis-cis* subgroups.

The lowest energy conformation characterized for Ac-L-αMePro-NHMe in the gas phase corresponds to a t-γ_L[d]-t conformer, which was also identified as the global minimum for Ac-L-Pro-NHMe. The geometric parameters of the hydrogen bond associated with this conformation [d(H···O) = 1.874 Å, ∠N–H···O = 151.4°] indicate that this intramolecular interaction is stronger in the α-methyl derivative. The other two *trans-trans* conformers found for Ac-L-Pro-NHMe, t-γ_L[u]-t and t-α_L[u]-t (Table 1), were also located as energy minima for Ac-L-αMePro-NHMe (Table 3), with similar geometries and energies. Thus, the main difference between Pro and αMePro when both peptide bonds exhibit a *trans* arrangement is the characterization of a minimum in the ε_L region for the α-methylated compound. No such

semi-extended backbone conformation was detected as an energy minimum for Ac-L-Pro-NHMe. This could be indicative of this backbone conformation being more favorable for αMePro than for the parent amino acid, which is contrary to the general observation that semi-extended and fully extended conformations are more stable for proteinogenic amino acids than for their α-methylated counterparts.[1c,e,3–5]

This singularity is specifically evidenced in Figure 4, where the potential energy curves $E = E(\psi)$ of Ac-L-Pro-NHMe and Ac-L-αMePro-NHMe for *trans* peptide bonds and an *up*-puckered ring are compared. As can be seen, the two profiles differ almost uniquely in the flat region that appears for the latter compound at ψ values ranging from 120° to 150°, that is, where the ε_L semi-extended conformation is located. However, as already mentioned, conformations in the ε_L region are very often observed experimentally[6] for Pro-containing peptides longer than that considered in the present work.

Also *cis-trans* α_L and ε_L conformers similar to those observed for proline were characterized for αMePro. They are disfavored with respect to the global minimum by about 3 and 7 kcal/mol, respectively, the influence of the pyrrolidine ring puckering being negligible (Table 3). Comparison between the ΔG^{gp} values obtained for the *cis-trans* conformers of Ac-L-Pro-NHMe and Ac-L-αMePro-NHMe indicates that, in general, α-methylation produces a destabilization of 1–2 kcal/mol. This result is not unexpected since the α-methyl group increases the steric hindrance around C^α, thus disfavoring the *cis* disposition between the acetyl methyl group and the α carbon ($\omega_0 \approx 0°$). The effect of α-methylation in the destabilization of *cis* amide bonds becomes more evident for the –CONHMe moiety (corresponding to ω). In fact, all *trans-cis* and *cis-cis* conformers exhibit ΔG^{gp} values above 10.8 kcal/mol (Table 3) and significantly higher than those obtained for the equivalent conformers of Ac-L-Pro-NHMe (Table 1).

Table 4 shows the effects of solvation on the 17 minima of Ac-L-aMePro-NHMe. As can be seen, the t-γ_L[d]-t is the most stable conformation not only in the gas phase but also in carbon tetrachloride and chloroform solutions. Significant differences are observed between the results obtained for Ac-L-Pro-NHMe (Table 2) and Ac-L-αMePro-NHMe (Table 4) in chloroform. Specifically, for the latter peptide, the ΔG^{CHCl3} values of all four *trans-trans* conformers lie below 1.6 kcal/mol, whereas all the *cis-trans* conformers show ΔG^{CHCl3} values above this limit, indicating that only *trans-trans* arrangements are energetically accessible in chloroform. This is in sharp contrast with the results obtained for Ac-L-Pro-NHMe, for which certain *cis-trans* conformers were found to exhibit a high stability.

Although the stability of the *cis-trans* conformers in Table 4 is probably overestimated in carbon tetrachloride, the general tendencies derived from PCM calculations in non-polar environments are fully consistent with data from NMR experiments, which showed no *cis* conformers for Ac-L-αMePro-NHMe in chloroform solution.[10d] In good agreement, our calculations predict the peptide involving the pyrrolidine nitrogen (ω_0) to exhibit a considerably smaller probability of adopting a *cis* disposition in αMePro than in Pro.

Finally, analysis of the results obtained for Ac-L-αMePro-NHMe in methanol and aqueous solution indicates that the *cis-trans* conformers are the most favored in these polar environments. Thus, the c-ε_L[u]-t and c-α_L[u]-t are the lowest energy minimum in methanol and water, respectively, and, in addition, the ΔG^{CH3OH} and ΔG^{H2O} of the remaining three *cis-trans* conformers are lower than 1.5 kcal/mol. These results clearly evidence that the stability of the *cis* configuration for the peptide bond involving the aMePro nitrogen ($\omega_0 \approx 0°$) is significantly overestimated by the PCM method when polar solvents are considered.

Ac-L-αPhPro-NHMe

Table 5 shows the structural parameters together with the ΔE^{gp} and ΔG^{gp} values for the 8 minimum energy conformations found for the αPhPro-containing peptide (Figure 5). Specifically, 4 minima with two *trans* amide bonds were characterized, while the other 4 correspond to *cis-trans* conformers. It should be noted that the *cis* arrangement of the –CONHMe peptide bond (corresponding to the ω angle) was not considered for this compound.

As observed before for Pro and αMePro, the γ_L backbone conformation with all *trans* peptide bonds is the most stable arrangement for αPhPro, with the *down* puckering of the pyrrolidine ring being preferred. Thus, t-γ_L[d]-t appears as the global minimum while t-γ_L[u]-t is destabilized by 1.3 kcal/mol. In spite of this parallelism, the conformational profile of the α-phenyl derivative shows important differences with respect to those described above for Pro and αMePro. The semi-extended structure t-ε_L[u]-t characterized as an energy minimum for the α-methylated compound, but not for the parent amino acid, was also located for αPhPro, 2.6 kcal/mol above the global minimum (Table 5). Moreover, an additional ε_L minimum with a *down* puckering was found for the latter compound, although this arrangement of the five-membered ring proved very unfavorable energetically. The overall of these results suggests that conformations in the ε_L region could be more favored for α-substituted proline derivatives than for proline itself, contrary to the general behavior expected for α-tetrasubstituted amino acids in comparison with their proteinogenic counterparts.[1c,e,3–5]

Another distinct feature in the conformational map of Ac-L-αPhPro-NHMe is the disappearance of *trans-trans* minima of the α-helical type. Thus, the t-α_L[u]-t, which was characterized for both Ac-L-Pro-NHMe and Ac-L-αMePro-NHMe, was not a minimum in the potential energy hypersurface of Ac-L-αPhPro-NHMe. Although calculations on small peptide systems like these in the present study are known to underestimate the stability of α-helical conformations (in general, of those lacking an intramolecular hydrogen bond) in favor of γ-turns, this finding is highly remarkable.

The effect of α-substitution on the *cis/trans* isomerism described above for Ac-L-αMePro-NHMe is also observed for αPhPro. The *cis-trans* conformers in Table 5 exhibit ΔG^{gp} values ranging from 3.8 to 7.6 kcal/mol, evidencing a destabilization of the *cis* disposition of the ω_0 amide bond with reference to that observed for Pro.

Table 6 compares the solvation effects estimated for the 8 minima characterized for Ac-L-αPhPro-NHMe. As can be seen, the conformational properties predicted in carbon tetrachloride and chloroform solutions are very similar to those obtained in the gas phase. The *trans-trans* conformers are scarcely affected by solvation, while the relative free energy of the *cis-trans* conformers decreases on going from the gas phase to solution, and with the solvent polarity. In spite of such stabilization, minima with a *cis* peptide bond remain inaccessible at room temperature. In fact, only the t-γ_L[d]-t and t-γ_L[u]-t conformers present energies below 2.0 kcal/mol in both solvents and are therefore predicted to be populated. However, results in methanol and aqueous solutions reflect again the limitations of the PCM model to describe the *cis/trans* isomerism of ω_0 in polar environments.

Conclusions

Quantum mechanical calculations at the B3LYP/6–31+G(d,p) level have been used to explore the conformational preferences of Ac-L-αMePhe-NHMe and Ac-L-αPhPro-NHMe. Comparison of the results with those obtained for Ac-L-Pro-NHMe at the same theoretical level allows us to draw the following conclusions:

i. Replacement of the α hydrogen in proline by a more bulky group destabilizes the *cis* configuration of the amide bond involving the pyrrolidine nitrogen. The percentage of *cis* conformers usually observed for the peptide bond preceding proline, if any, is thus predicted to be much inferior for α-tetrasubstituted proline derivatives.

ii. Another general structural trend associated with C^α-tetrasubstitution seems to be the stabilization of the semi-extended polyproline II conformation (ε_L), which was identified as an energy minimum for both αMePro and αPhPro but not for the proteinogenic amino acid.

iii. Although α-tetrasubstitution results in general conformational changes like those outlined above, more subtle but equally important differences seem to be associated with the particular nature of the substituent incorporated at C^α. Thus, even if the γ-turn (γ_L) is the lowest energy minimum for both Ac-L-αMePhe-NHMe and Ac-L-αPhPro-NHMe in all the environmental conditions examined, the α-helical conformation (α_L) with *trans* amide bonds was also found to be accessible for the α-methyl derivative but was not located as an energy minimum for the αPhPro-containing peptide.

iv. PCM calculations in solution indicate that the stability of the conformers with a *cis* configuration for the peptide bond involving Pro nitrogen increases with the polarity of the environment. However, in this case results in solution must be analyzed with caution since SCRF calculations overestimate this effect significantly, especially when polar solvents (as water or methanol) are considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.
Dihedral angles used to identify the conformations of the *N*-acetyl-*N'*-methylamide derivatives of proline and its α-substituted 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$^\alpha$–C(=O) and C$^\delta$–N–C$^\alpha$–C$^\beta$, respectively.
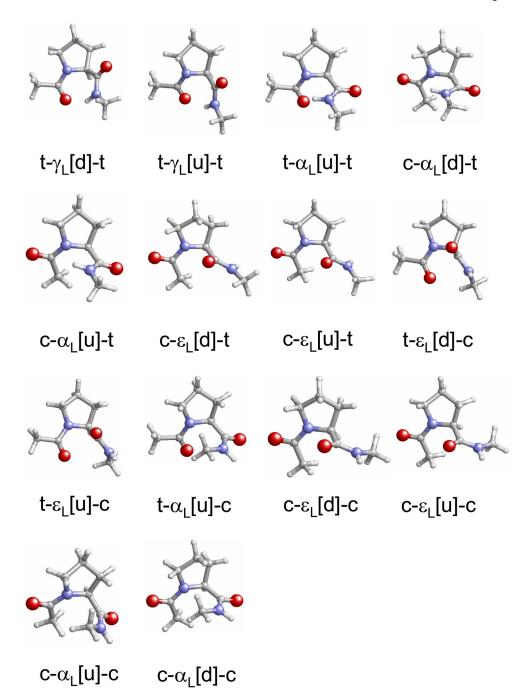
Figure 2.
Representation of the minimum energy conformations characterized for Ac-L-Pro-NHMe at the B3LYP/6–31+G(d,p) level.

t-γ_L[d]-t   t-γ_L[u]-t   t-ε_L[u]-t   t-α_L[u]-t

c-α_L[d]-t   c-α_L[u]-t   c-ε_L[u]-t   c-ε_L[d]-t

t-ε_L[u]-c   t-ε_L[d]-c   t-α_L[u]-c   t-ε_L[d]-c

t-α_L[d]-c   c-α_L[u]-c   c-α_L[d]-c   c-ε_L[d]-c

c-ε_L[u]-c
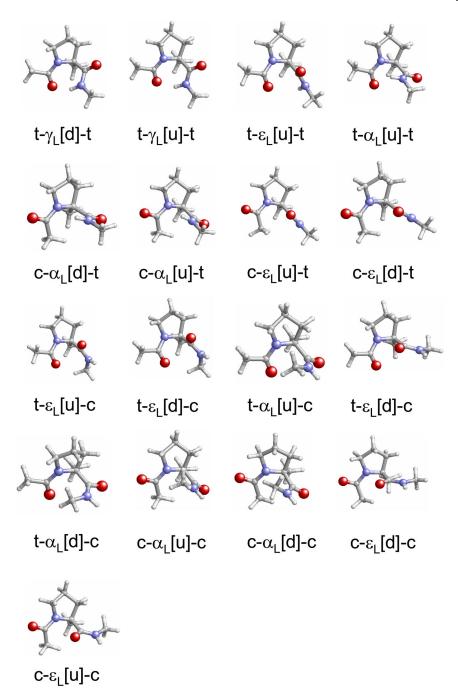
Figure 3.
Representation of the minimum energy conformations characterized for Ac-L-αMePro-NHMe
at the B3LYP/6–31+G(d,p) level.
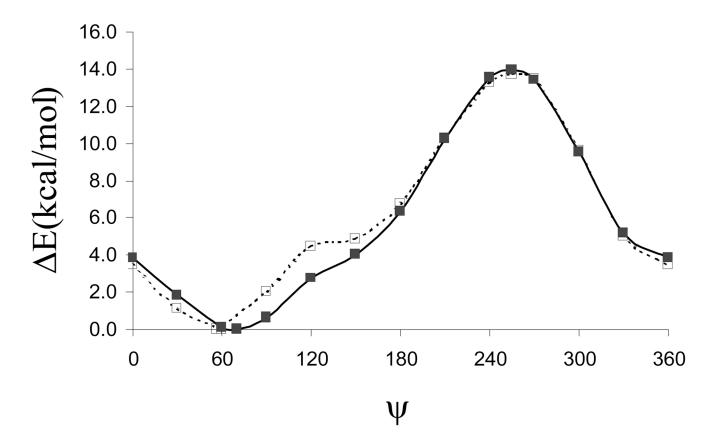
Figure 4.
Potential energy curves *E=E(ψ)* cross sections of the conformational potential energy surfaces of Ac-L-Pro-NHMe (filled squares and solid lines) and Ac-L-αMePro-NHMe (empty squares and dashed lines). In both compounds, the pyrrolidine ring is *up*-puckered and the peptide bonds are arranged in *trans*.

t-γ$_L$[d]-t   t-γ$_L$[u]-t   t-ε$_L$[u]-t   t-ε$_L$[d]-t
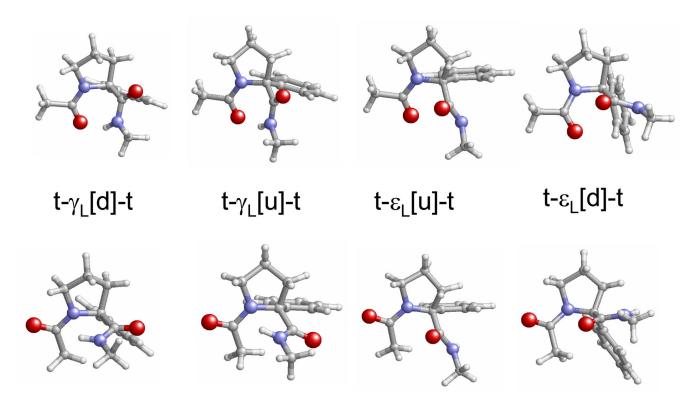
Figure 5.
Representation of the minimum energy conformations characterized for Ac-L-αPhPro-NHMe at the B3LYP/6–31+G(d,p) level.

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Table 1

Backbone dihedral angles (in degrees), pseudorotational parameters (A and P; in degrees) and relative energy (ΔE^{gp}; in kcal/mol) and free energy (ΔG^{gp}; in kcal/mol) of the minimum energy conformations characterized for Ac-L-Pro-NHMe at the B3LYP/6–31+G(d,p) level in the gas phase.

# Conf.	ω_0	φ	ψ	ω	(A, P)	ΔE^{gp}	ΔG^{gp}
t-γL[d]-t	−172.6	−83.4	70.3	−177.7	(37.4, −111.9)[a]	0.0[b]	0.0[c]
t-γL[u]-t	−173.9	−81.6	77.3	−175.9	(37.5, 75.8)[d]	1.0	1.3
t-αL[u]-t	−171.0	−77.5	−11.5	175.9	(37.8, 89.2)[e]	4.9	4.0
c-αL[d]-t	10.2	−90.9	−5.1	−179.6	(37.5, −111.5)[f]	3.3	2.3
c-αL[u]-t	8.0	−79.2	−18.4	−177.1	(37.5, 90.1)[g]	4.2	3.6
c-εL[d]-t	1.2	−75.2	147.9	174.6	(36.8, −114.4)[h]	6.3	4.8
c-εL[u]-t	−0.1	−61.2	145.3	177.5	(37.4, 88.0)[i]	6.6	5.0
t-εL[d]-c	−179.9	−76.7	125.9	−14.6	(35.2, −114.4)[j]	6.1	6.2
t-εL[u]-c	177.9	−63.3	128.1	−18.6	(37.6, −48.1)[k]	6.5	6.7
t-αL[u]-c	−173.4	−61.6	−35.4	12.0	(37.8, 77.9)[l]	11.3	11.3
c-εL[d]-c	0.6	−75.6	159.3	−6.0	(37.2, −116.4)[m]	9.3	8.5
c-εL[u]-c	−1.6	−61.9	155.1	−5.8	(37.4, 91.5)[n]	9.8	9.2
c-αL[u]-c	4.3	−63.5	−38.7	0.3	(37.2, 81.4)[o]	10.1	9.7
c-αL[d]-c	6.9	−82.5	−16.2	−0.8	(36.1, −111.2)[p]	10.8	10.7

[a] $\chi^0 = -13.9°$, $\chi^1 = 31.4°$, $\chi^2 = -37.6°$, $\chi^3 = 28.7°$ and $\chi^4 = -9.3°$.

[b] E=−573.315217 a.u.

[c] G=−573.132049 a.u.

[d] $\chi^0 = -10.3°$, $\chi^1 = -13.4°$, $\chi^2 = 31.0°$, $\chi^3 = -36.6°$ and $\chi^4 = 29.8°$.

[e] $\chi^0 = 0.5°$, $\chi^1 = -22.9°$, $\chi^2 = 36.1°$, $\chi^3 = -35.4°$ and $\chi^4 = 22.0°$.

[f] $\chi^0 = -13.8°$, $\chi^1 = 31.4°$, $\chi^2 = -37.6°$, $\chi^3 = 29.0°$ and $\chi^4 = -9.5°$.

[g] $\chi^0 = -0.1°$, $\chi^1 = -22.1°$, $\chi^2 = 35.7°$, $\chi^3 = -35.2°$ and $\chi^4 = 22.3°$.

[h] $\chi_0 = -15.2°$, $\chi^1 = 31.6°$, $\chi^2 = -36.7°$, $\chi^3 = 27.4°$ and $\chi^4 = -7.5°$.

[i] $\chi_0 = 1.3°$, $\chi^1 = -23.3°$, $\chi^2 = 36.0°$, $\chi^3 = -34.6°$ and $\chi^4 = 21.2°$.

[j] $\chi_0 = -14.5°$, $\chi^1 = 30.3°$, $\chi^2 = -35.1°$, $\chi^3 = 26.0°$ and $\chi^4 = -7.2°$.

[k] $\chi_0 = 25.1°$, $\chi^1 = -3.6°$, $\chi^2 = -19.5°$, $\chi^3 = 34.4°$ and $\chi^4 = -35.8°$.

[l] $\chi_0 = 7.9°$, $\chi^1 = -28.1°$, $\chi^2 = 37.7°$, $\chi^3 = -32.5°$ and $\chi^4 = 15.5°$.

[m] $\chi_0 = -16.5°$, $\chi^1 = 32.5°$, $\chi^2 = -36.9°$, $\chi^3 = 26.8°$ and $\chi^4 = -6.3°$.

[n] $\chi_0 = -1.0°$, $\chi^1 = -21.5°$, $\chi^2 = 35.3°$, $\chi^3 = -35.3°$ and $\chi^4 = 23.1°$.

[o] $\chi_0 = 5.5°$, $\chi^1 = -26.1°$, $\chi^2 = 36.8°$, $\chi^3 = -33.1°$ and $\chi^4 = 17.3°$.

[p] $\chi_0 = -13.1°$, $\chi^1 = 30.1°$, $\chi^2 = -36.2°$, $\chi^3 = 28.0°$ and $\chi^4 = -9.3°$.

Table 2

Relative free energy in the gas-phase (ΔG^{gp}; in kcal/mol) and in carbon tetrachloride, chloroform, methanol and aqueous solutions (ΔG^{CCl4}, ΔG^{CHCl3}, ΔG^{CH3OH} and ΔG^{H2O}, respectively; in kcal/mol) for the minimum energy conformations of Ac-L-Pro-NHMe at the B3LYP/6–31+G(d,p) level.

# Conf.	ΔG^{gp}	ΔG^{CCl4}	ΔG^{CHCl3}	ΔG^{CH3OH}	ΔG^{H2O}
t-γ$_L$[d]-t	0.0	0.0	0.3	4.5	1.3
t-γ$_L$[u]-t	1.3	1.4	1.7	5.9	2.8
t-α$_L$[u]-t	4.0	3.0	1.9	4.0	0.3
c-α$_L$[d]-t	2.3	0.1	1.0	3.8	0.2
c-α$_L$[u]-t	3.6	2.7	2.1	4.9	0.8
c-ε$_L$[d]-t	4.8	1.6	0.2	0.3	0.7
c-ε$_L$[u]-t	5.0	2.5	0.0	0.0	0.0
t-ε$_L$[d]-c	6.2	4.5	3.8	6.8	3.2
t-ε$_L$[u]-c	6.7	4.9	3.9	6.6	3.1
t-α$_L$[u]-c	11.3	7.3	4.8	5.7	1.7
c-ε$_L$[d]-c	8.5	5.3	3.2	3.3	0.4
c-ε$_L$[u]-c	9.2	6.5	3.7	4.8	0.9
c-α$_L$[u]-c	9.7	6.4	4.9	7.0	3.3
c-α$_L$[d]-c	10.7	7.4	5.9	8.2	4.6

Table 3

Backbone dihedral angles (in degrees), pseudorotational parameters (A and P; in degrees) and relative energy (ΔE^{gp}; in kcal/mol) and free energy (ΔG^{gp}; in kcal/mol) of the minimum energy conformations characterized for Ac-L-αMePro-NHMe at the B3LYP/6–31+G (d,p) level in the gas phase.

# Conf.	ω_0	φ	ψ	ω	(A, P)	ΔE^{gp}	ΔG^{gp}
t-γL[d]-t	−172.2	−77.2	57.1	178.7	(35.0, −108.7)[a]	0.0[b]	0.0[c]
tγL[u]-t	−176.2	−69.8	60.7	178.8	(31.2, 86.9)[d]	1.6	1.7
t-εL[u]-t	175.4	−55.1	123.4	−174.0	(31.3, 91.7)[e]	3.3	2.8
t-αL[u]-t	−172.6	−64.2	−20.1	177.6	(33.6, 72.8)[f]	4.2	3.2
c-αL[d]-t	10.8	−77.7	−17.1	−179.1	(37.3, −94.3)[g]	4.5	3.3
c-αL[u]-t	3.0	−63.5	−28.1	−177.9	(37.6, 74.9)[h]	4.6	3.1
c-εL[u]-t	−3.0	−48.5	141.5	176.4	(37.8, 74.9)[i]	7.6	6.9
c-εL[d]-t	0.0	−66.3	149.5	178.3	(36.8, −100.0)[j]	7.7	6.6
t-εL[u]-c	175.3	−55.5	125.9	−32.4	(38.3, 88.5)[k]	10.3	11.2
t-εL[d]-c	177.8	−61.1	127.7	−32.4	(36.1, −94.3)[l]	10.6	11.5
t-αL[u]-c	−174.4	−54.6	−41.1	15.8	(36.8, 68.3)[m]	10.6	11.1
t-εL[d]-c	173.9	−67.2	164.4	−2.0	(36.1, −116.5)[n]	11.6	11.7
t-αL[d]-c	−170.2	−73.7	−18.9	18.6	(37.7, −95.5)[o]	11.8	11.7
c-αL[u]-c	−0.2	−56.5	−40.0	3.5	(36.4, 71.2)[p]	10.6	10.8
c-αL[d]-c	12.9	−64.9	−38.6	−8.4	(32.2, −68.8)[q]	12.8	12.7
c-εL[d]-c	−2.5	−70.0	177.0	−4.1	(37.7, −111.0)[r]	13.9	14.1
c-εL[u]-c	−11.7	−57.5	177.3	3.7	(37.9, 94.5)[s]	14.6	14.4

[a] $\chi^0 = -11.2°$, $\chi^1 = 30.9°$, $\chi^2 = -39.4°$, $\chi^3 = 31.6°$ and $\chi^4 = -12.8°$.

[b] E=−612.629968 a.u.

[c] G=−612.419998 a.u.

[d] $\chi^0 = -0.9°$, $\chi^1 = -22.5°$, $\chi^2 = 37.1°$, $\chi^3 = -36.8°$ and $\chi^4 = 23.8°$.

[e] $\chi^0 = 1.7°$, $\chi^1 = -24.0°$, $\chi^2 = 37.0°$, $\chi^3 = -35.1°$ and $\chi^4 = 21.2°$.

[f] $\chi^0 = 9.9°$, $\chi^1 = -29.4°$, $\chi^2 = 38.1°$, $\chi^3 = -31.4°$ and $\chi^4 = 13.4°$.

[g] $\chi^0 = -2.8°$, $\chi^1 = 24.4°$, $\chi^2 = -36.5°$, $\chi^3 = 33.9°$ and $\chi^4 = -19.7°$.

[h] $\chi^0 = 9.8°$, $\chi^1 = -29.1°$, $\chi^2 = 37.9°$, $\chi^3 = -31.2°$ and $\chi^4 = 13.4°$.

[i] $\chi^0 = 9.8°$, $\chi^1 = -29.3°$, $\chi^2 = 38.0°$, $\chi^3 = -31.4°$ and $\chi^4 = 13.6°$.

[j] $\chi^0 = -6.4°$, $\chi^1 = 26.6°$, $\chi^2 = -36.7°$, $\chi^3 = 32.1°$ and $\chi^4 = -16.2°$.

[k] $\chi^0 = 1.0°$, $\chi^1 = -23.5°$, $\chi^2 = 36.9°$, $\chi^3 = -35.4°$ and $\chi^4 = 21.8°$.

[l] $\chi^0 = -2.3°$, $\chi^1 = 22.5°$, $\chi^2 = -33.7°$, $\chi^3 = 31.3°$ and $\chi^4 = -18.2°$.

[m] $\chi^0 = 13.6°$, $\chi^1 = -30.9°$, $\chi^2 = 317.1°$, $\chi^3 = -28.3°$ and $\chi^4 = 9.1°$.

[n] $\chi^0 = -16.1°$, $\chi^1 = 31.7°$, $\chi^2 = -36.0°$, $\chi^3 = 25.7°$ and $\chi^4 = -5.9°$.

[o] $\chi^0 = -3.6°$, $\chi^1 = 25.2°$, $\chi^2 = -37.1°$, $\chi^3 = 33.9°$ and $\chi^4 = -19.2°$.

[p] $\chi^0 = 11.3°$, $\chi^1 = -29.3°$, $\chi^2 = 36.7°$, $\chi^3 = -29.3°$ and $\chi^4 = -11.2°$.

[q] $\chi^0 = 11.6°$, $\chi^1 = 8.8°$, $\chi^2 = -24.9°$, $\chi^3 = 31.3°$ and $\chi^4 = -27.5°$.

[r] $\chi^0 = -13.5°$, $\chi^1 = 31.5°$, $\chi^2 = -38.0°$, $\chi^3 = 29.2°$ and $\chi^4 = -9.7°$.

[s] $\chi^0 = -3.0°$, $\chi^1 = -20.1°$, $\chi^2 = 35.1°$, $\chi^3 = -36.1°$ and $\chi^4 = 25.0°$.

Table 4

Relative free energy in the gas-phase (ΔG^{gp}; in kcal/mol) and in carbon tetrachloride, chloroform. methanol and aqueous solutions (ΔG^{CCl4}, ΔG^{CHCl3}, ΔG^{CH3OH} and ΔG^{H2O}, respectively; in kcal/mol) for the minimum energy conformations of Ac-L-αMePro-NHMe at the B3LYP/6–31+G(d,p) level.

# Conf.	ΔG^{gp}	ΔG^{CCl4}	ΔG^{CHCl3}	ΔG^{CH3OH}	ΔG^{H2O}
t-γ_L[d]-t	0.0	0.0	0.0	0.9	1.4
t-γ_L[u]-t	1.7	1.5	1.4	2.4	2.8
t-ε_L[u]-t	2.8	2.1	1.6	1.7	2.0
t-α_L[u]-t	3.3	2.4	1.2	0.2	0.3
c-α_L[d]-t	3.3	1.1	1.9	1.1	0.5
c-α_L[u]-t	3.1	0.8	1.7	0.4	0.0
c-ε_L[u]-t	6.9	2.7	4.5	0.0	0.6
c-ε_L[d]-t	6.6	2.3	4.3	0.5	0.6
t-ε_L[u]-c	11.2	8.9	8.0	7.7	7.5
t-ε_L[d]-c	11.5	9.4	8.6	8.5	8.4
t-α_L[u]-c	11.1	7.2	4.7	2.6	2.2
t-ε_L[d]-c	11.7	8.5	6.7	5.1	5.1
t-α_L[d]-c	11.7	8.2	6.2	4.8	4.5
c-α_L[u]-c	10.8	7.1	5.4	4.8	4.5
c-α_L[d]-c	12.7	9.0	7.2	6.7	6.2
c-ε_L[d]-c	14.1	9.8	7.2	5.2	4.8
c-ε_L[u]-c	14.4	10.0	7.4	5.5	5.0

Table 5

Backbone dihedral angles (in degrees), pseudorotational parameters (A and P; in degrees) and relative energy (ΔE^{gp}; in kcal/mol) and free energy (ΔG^{gp}; in kcal/mol) of the minimum energy conformations characterized for Ac-L-αPhPro-NHMe at the B3LYP/6–31+G (d,p) level in the gas phase.

# Conf.	ω_0	φ	ψ	ω	(A, P)	ΔE^{gp}	ΔG^{gp}
t-γ_L[d]-t	−174.9	−75.4	59.1	178.9	(39.1, −107.0)[a]	0.0[b]	0.0[c]
t-γ_L[u]-t	−179.6	−67.9	66.2	−179.9	(39.2, 89.8)[d]	1.7	1.3
t-ε_L[u]-t	172.0	−46.5	120.3	−172.0	(39.9, 76.6)[e]	2.6	2.6
t-ε_L[d]-t	176.7	−61.2	161.7	176.6	(37.8, −98.4)[f]	7.8	7.5
c-γ_L[d]-t	10.8	−85.5	5.5	−176.4	(38.7, −103.8)[g]	4.5	4.0
c-α_L[u]-t	3.2	−59.9	−28.1	−175.9	(38.6, 68.2)[h]	5.1	3.8
c-ε_L[u]-t	−5.0	−43.3	129.6	−179.5	(39.9, 66.0)[i]	5.6	5.5
c-ε_L[d]-t	−4.7	−73.3	−162.6	−177.4	(39.7, −118.2)[j]	8.2	7.6

[a] $\chi^0 = -11.4°$, $\chi^1 = 31.2°$, $\chi^2 = -39.5°$, $\chi^3 = 31.7°$ and $\chi^4 = -12.7°$.

[b] $E = -804.371070$ a.u.

[c] $G = -804.113301$ a.u.

[d] $\chi^0 = 0.1°$, $\chi^1 = -23.4°$, $\chi^2 = 37.5°$, $\chi^3 = -36.6°$ and $\chi^4 = 23.1°$.

[e] $\chi^0 = 9.3°$, $\chi^1 = -30.3°$, $\chi^2 = 40.2°$, $\chi^3 = -33.7°$ and $\chi^4 = 15.3°$.

[f] $\chi^0 = -5.5°$, $\chi^1 = -26.6°$, $\chi^2 = -37.4°$, $\chi^3 = 34.0°$ and $\chi^4 = -17.6°$.

[g] $\chi^0 = -92°$, $\chi^1 = 29.5°$, $\chi^2 = -38.9°$, $\chi^3 = 32.6°$ and $\chi^4 = -14.6°$.

[h] $\chi^0 = 14.3°$, $\chi^1 = -32.4°$, $\chi^2 = 38.9°$, $\chi^3 = -29.7°$ and $\chi^4 = 9.4°$.

[i] $\chi^0 = 16.2°$, $\chi^1 = -34.3°$, $\chi^2 = 40.1°$, $\chi^3 = -29.7°$ and $\chi^4 = 8.2°$.

[j] $\chi^0 = -18.8°$, $\chi^1 = 35.4°$, $\chi^2 = -39.5°$, $\chi^3 = 27.6°$ and $\chi^4 = -5.2°$.

Table 6

Relative free energy in the gas-phase (ΔG^{gp}; in kcal/mol) and in carbon tetrachloride, chloroform, methanol and aqueous solutions (ΔG^{CCl4}, ΔG^{CHCl3}, ΔG^{CH3OH} and ΔG^{H2O}, respectively; in kcal/mol) for the minimum energy conformations of Ac-L-αPhPro-NHMe at the B3LYP/6–31+G(d,p) level.

# Conf.	ΔG^{gp}	ΔG^{CCl4}	ΔG^{CHCl3}	ΔG^{CH3OH}	ΔG^{H2O}
t-γ_L[d]-t	0.0	0.0	0.0	0.0	0.3
t-γ_L[u]-t	1.3	1.2	1.3	1.4	1.6
t-ε_L[u]-t	2.6	2.4	2.2	1.6	1.3
t-ε_L[d]-t	7.5	6.9	6.1	4.5	4.4
c-γ_L[d]-t	4.0	2.8	2.5	2.3	2.1
c-α_L[u]-t	3.8	2.4	2.0	1.4	0.9
c-ε_L[u]-t	5.5	3.5	2.5	1.3	2.8
c-ε_L[d]-t	7.6	4.8	2.8	0.5	0.0