

Does C^α-methyl proline prefer the helical or the semi-extended conformation? A Janus-headed, C^α-tetrasubstituted α-amino acid

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Introduction

Long time ago, in the course of their theoretical investigations on the effects of N^α- or C^α- methylation on the energetically preferred peptide conformations, S.J. Leach and his students A.W. Burgess and Y. Paterson (University of Melbourne, Australia) predicted *inter alia* that methylation at the C^α-atom of an α-amino acid [e.g., L-Ala, which they showed it was able to induce an obligatory right- or left-handed helical character in the resulting achiral Aib (α-aminoisobutyric acid) residue], if combined with the L-Pro to generate C^α-methyl-L-proline [L-(αMe)Pro], would produce a φ,ψ energy surface uniquely restricted to a *single* region, namely the *right-handed* helical conformation [1].

Taking this study further, Madison and his colleagues solved the X-ray diffraction structure of Ac-D,L-(αMe)Pro-NHMe (Ac, acetyl; NHMe, methylamino), showing that in the crystalline state this derivative is indeed helical, with the L-enantiomer adopting the right-handed screw sense [2]. Nevertheless, in their conformational energy calculations Delaney and Madison reported that the total energy for Ac-L-(αMe)Pro-NHMe has a deep well at the right-handed C_{7'} (inverse γ-turn) conformation [3]. For this derivative both the *semi-extended* [also called poly-(L-Pro)_n II] and right-handed helical regions are destabilized. However, there is little barrier separating the C_{7'} and helical regions. In any case, it should be pointed out that the population of the C_{7'} conformation tends to be overestimated in calculations focusing on compounds as short as amino acid derivatives.

To clarify this issue, we are investigating the crystal-state preferred conformations of (αMe)Pro, in particular of terminally-blocked, homo- or hetero-chiral, dipeptide alkylamide systems of the type RCO-(αMe)Pro-Xxx-NHR or RCO-Xxx-(αMe)Pro-NHR, where Xxx is Ala, Aib, Gly, or (αMe)Pro, long enough to fold into C=O ... H-N intramolecularly H-bonded γ- or β-turns. In this paper we describe the results of our initial X-ray diffraction analyses, namely those of Ac-L-Ala-L-(αMe)Pro-NHiPr (NHiPr, *isopropylamino*), Ac-D-(αMe)Pro-D-Ala-NHiPr, Ac-D-(αMe)Pro-L-Ala-NHiPr, *i*Bu-L-Ala-D-(αMe)Pro-NHiPr, (*i*Bu, *isobutyryl*), and Ac-D-(αMe)Pro-Aib-NHiPr.

Results and Discussion

The X-ray diffraction structures of three relevant, N^α-acylated (αMe)Pro dipeptide alkylamides are illustrated in Figures 1 and 2. Table 1 lists the backbone (φ,ψ) torsion angles and the type of turn, if any, for the five peptide structures solved, compared with those of the corresponding peptide sequences based on the C^α-unmethylated α-amino acid (Pro) counterpart.

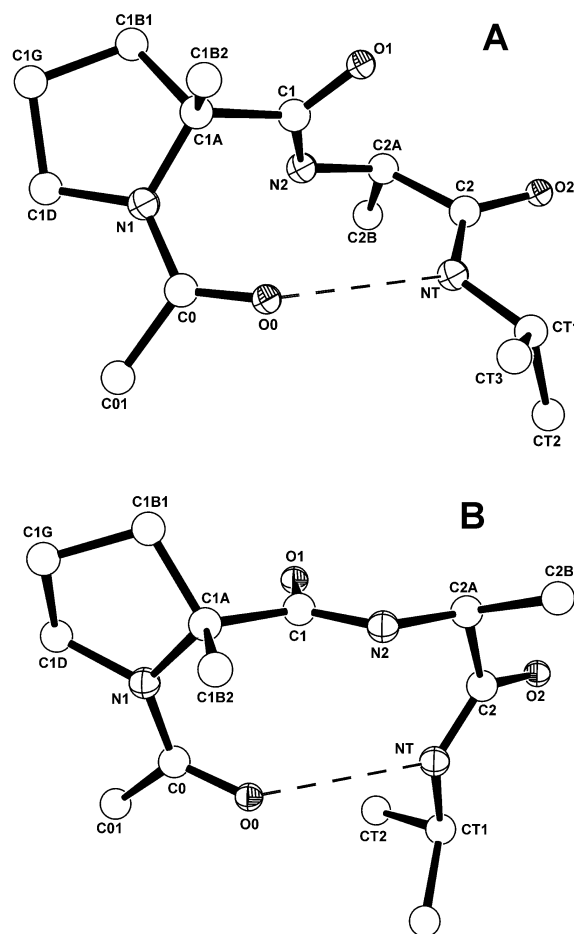


Fig. 1. X-Ray diffraction structures with atom numbering of the homo-chiral dipeptide amide Ac-D-(αMe)Pro-D-Ala-NHiPr (A) and the hetero-chiral dipeptide amide Ac-D-(αMe)Pro-L-Ala-NHiPr (B). In each structure the C=O ... H-N intramolecular H-bond stabilizing the β-turn conformation is indicated by a dashed line.

The main conclusions derived from our crystal-state experimental data may be summarized as follows:

(i) Although the region of the (φ,ψ) conformational map overwhelmingly preferred by L-(αMe)Pro would indeed be that typical of *right-handed* 3₁₀/α-helices, (with ψ ≈ -30° or *cis'*) as suggested in 1974 by Leach and coworkers [1], the *semi-extended*, type-II poly(L-Pro)_n region (with ψ ≈ 150° or *trans'*) can also be explored by this extremely sterically hindered C^α-tetrasubstituted α-amino acid, thus

highlighting its unique Janus-headed character. Conversely, the ϕ, ψ region ($\psi \approx 60^\circ$), exactly half-way between the two regions discussed above and corresponding to the C_7' (inverse γ -turn) conformation, does not seem to be accessible to L-(α Me)Pro.

(ii) In addition to the dramatic restriction of the ϕ torsion angle (to $\approx -60^\circ$) by its five-membered pyrrolidine ring structure, L-(α Me)Pro undergoes rigidification of the preceding tertiary peptide bond (*trans*, or 180° , ω torsion angle) as well, as shown by all C^α -methylated L- α -amino acids investigated to date [12].

(iii) The known high propensity of the L-Pro residue for β -turn formation is even enhanced in peptides based on its C^α -methylated derivative when it is located at the $i+1$ corner position. Despite this characteristics, L-(α Me)Pro seems to be unable to nucleate a β -turn when it is located at the $i+2$ corner position of a homo-chiral dipeptide sequence.

(iv) When incorporated at position 1 of the dipeptide sequence ($i+1$ corner position), L-(α Me)Pro tends to bias the β -turn to its helical type (III), as opposed to the non-helical type (II) typically induced by L-Pro.

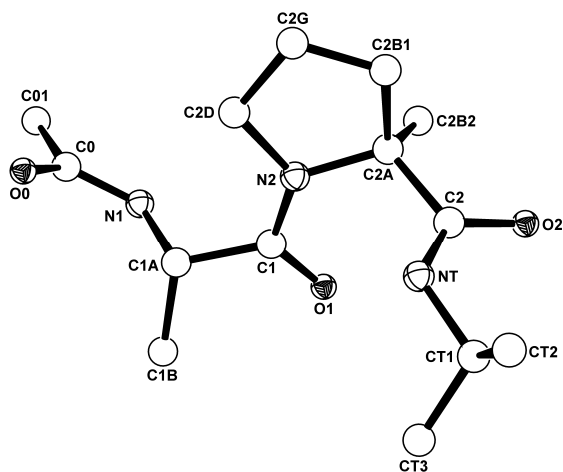


Fig. 2. X-Ray diffraction structure of Ac-L-Ala-L-(α Me)Pro-NHPr ("open" conformation) with atom numbering.

To complete the picture of the crystal-state conformational propensities of the C^α -tetrasubstituted α -amino acid (α Me)Pro in N^α -acylated dipeptide alkylamides, we are currently actively working on the synthesis, chemical characterization, and X-ray diffraction investigation of the homo- and hetero-chiral sequences RCO-(α Me)Pro-Xxx-NHR [Xxx = Gly, (α Me)Pro] and RCO-Xxx-(α Me)Pro-NHR [Xxx = Aib, Gly, (α Me)Pro]. In parallel, we are performing a detailed FT-IR absorption and NMR study in $CDCl_3$ solution of the (α Me)Pro and related Pro peptides to delineate the relative extent of *cis* / *trans* isomers at the Xxx-(α Me)Pro and Xxx-Pro tertiary amide bonds and to highlight their tendency to adopt C=O ... H-N intramolecularly H-bonded turn structures under those favorable environmental conditions.

Table 1. 3D-Structural parameters in the crystal state for the known Ala/Pro, Ala/(α Me)Pro, Aib/Pro, and Aib/(α Me)Pro dipeptide sequences

Peptide sequence	Backbone torsion angles				Type of turn	Ref.
	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}		
Ac-L-Ala-L-(α Me)Pro-NHPr	-135	77	-58	-37	—	^b
<i>i</i> Bu-L-Ala-L-Pro-NHPr	-129	76	-67	-22	—	4
Piv-D-Ala-D-Pro-NHPr ^a	74	-150	57	-142	—	5
	64	-152	83	-156	—	
Ac-D-(α Me)Pro-D-Ala-NHPr	53	32	66	25	β III'	^b
Ac-L-Pro-L-Ala-NHtBu ^a	-66	166	-71	154	—	6
<i>i</i> Bu-L-Pro-L-Ala-NHPr	-59	136	66	14	β II	7,8
Ac-D-(α Me)Pro-L-Ala-NHPr	53	-129	-77	-12	β II'	^b
Z-D-Pro-L-Ala-NHtBu	58	-137	-76	-14	β II'	6
<i>i</i> Bu-L-Pro-D-Ala-NHPr	-62	137	96	3	β II	7,8
<i>i</i> Bu-L-Pro-D-Ala-NHtBu	-60	133	82	15	β -II	9
<i>i</i> Bu-L-Ala-D-(α Me)Pro-NHPr	-55	133	78	0	β II	^b
Piv-D-Ala-L-Pro-NHPr	60	-140	-89	9	β II'	10
Ac-D-(α Me)Pro-Aib-NHPr	53	37	61	28	β III'	^b
Piv-L-Pro-Aib-NHMe	-58	139	61	25	β II	11

^aPiv, pivaloyl; NHtBu, *tert*-butylamino. ^bThis work.

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