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X-ray and NMR Studies of L-4-Hydroxyproline Conformation in Oligopeptides Related to Collagen

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Abstract: Correlative solid/solvated state analyses of some L-4-hydroxyproline containing oligopeptides, mainly Gly-L-4Hyp, L-Pro-L-4Hyp, *cyclo*-(L-Pro-L-4Hyp), and *N*-Ac-L-Pro-L-4Hyp, have been undertaken by use of X-ray (single crystal) and NMR techniques (solution). The main features deduced from these comparative analyses are as follows: (1) the rapid conformational flexibility of C^γ of L-Pro residues is largely inhibited in L-4Hyp, leading to a much more rigid structure in solution and in the crystal; (2) L-4Hyp rings are much more puckered than L-Pro; (3) the same dynamic deformations of the rings are derived from X-ray $\langle B \rangle$ thermal parameters compared to the carbon-13 NT_1 factors, despite different cis/trans isomerism around amide bonds. Very similar conformations are observed by both methods. This gives additional evidence for the key role played by the L-4Hyp residues in the stability of triple helical peptides related to collagen.

Introduction

Many studies have been performed to understand the structural role of the pyrrolidine residues L-Pro and L-4Hyp in collagen.[2,3] This polypeptide contains a great number of repeated Gly-L-Pro-L-4Hyp tripeptide sequences and experimental evidence has shown that Hyp residues contribute to the stability of the triple helical structure.[2b,3] On the other hand, recent studies[4] suggest that the cis ⇆ trans isomerism around the X-L-Pro peptide bond could be involved in the folding of proteins.

Both synthetic peptides $(\text{L-Pro-L-Pro-Gly})_n$ and $(\text{L-Pro-L-4Hyp-Gly})_n$, $n < 10$, form triple-helical structures[5-7] and the role of L-4Hyp in stabilizing the triple helix was demonstrated by the higher thermal transition temperature of the polymer containing L-4Hyp.[5,6,8,9] For Traub[10] and Ramachandran[2a,3] such an effect of L-4Hyp is due to participation of the γ OH group in additional hydrogen bonds between the chains via a water molecule. Evidence has been presented for the existence of intermolecular water bridges in poly(L-4Hyp)[2,3,9,11] and in the crystal of *N*-Ac-L-4Hyp.[44]

However, this proposed key role of L-4Hyp by specific interaction with a water molecule[8] in collagen and/or related peptides $(\text{L-Pro-L-Pro-Gly})_n$ and $(\text{L-Pro-L-4Hyp-Gly})_n$ may not fully account for a leading part in the triple-helix stabilization. As previously suggested,[12,13] when compared to L-Pro, the L-4Hyp residue seems to stabilize the helix by more subtle intrinsic effects (i.e., ring flexibility and restrictions of local backbone motion).

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Table I

A. Crystallographic Details

	Gly-L-4Hyp (1)	L-Pro-L-4Hyp[a] (2)	*N*-Ac-L-Pro-L-4Hyp (3)	*cyclo*-(L-Pro-L-4Hyp)[a] (4)
formula	$C_7H_{12}N_2O_4$	$C_{10}H_{16}N_2O_4$	$C_{12}H_{18}N_2O_5$	$C_{10}H_{14}N_2O_3$
system	orthorhombic	monoclinic	orthorhombic	orthorhombic
space group	$P2_12_12_1$ ($Z = 4$)	$P2_1$ ($Z = 2$)	$P2_12_12_1$ ($Z = 4$)	$P2_12_12_1$ ($Z = 8$)
a, Å	5.894	6.264	7.204	10.377
b, Å	7.894	8.940	8.322	11.777
c, Å	17.713	10.336	21.240	17.123
β, deg		101.5		
no. of measured reflections ($>2\sigma$)	932	2161	1038	1202
final *R*, %	8.6	5.9	6.7	4.8

B. ^{13}C NMR Chemical Shifts (ppm) in Proline and Hydroxyproline Rings, Referred to Me_4Si as External Reference in D_2O

		Gly-L-4Hyp		L-Pro-L-4Hyp		*N*-Ac-L-Pro-L-4Hyp		*cyclo*-(L-Pro-L-4Hyp)	*N*-Ac-L-Pro[c]	
		c (40%)	t	c (30%)	t	ct (20%)	tt (70%)		c (20%)	t
L-Pro	C^α			60.0	60.1	61.0	59.2	60.0	61.4	59.7
	C^β			29.6	29.1	30.9	29.4	28.4	31.6	30.1
	C^γ			25.2	25.2	23.5	25.2	23.9	23.2	25.0
	C^δ			47.7	47.6	48.0	49.6	46.4	47.5	49.2
	C^0			168.7	167.9	173.0	173.0	168.7[b]	173.0	173.0
L-4Hyp	C^α	62.5	63.0	61.4	61.8	59.3	59.2	61.7		
	C^β	41.8	39.9	40.6	38.4	37.5	37.6	36.9		
	C^γ	70.5	72.1	68.9	70.7	70.9	70.9	69.4		
	C^δ	56.8	56.5	55.7	55.7	55.5	55.5	54.5		
	C^0	179.7	180.5	177.6	178.0	176.0	176.0	169.1[b]		

[a] Crystallizes as monohydrate. [b] These attributions may be permuted. [c] See also ref 77.

These features can be studied by the use of NMR measurements,[14] CD[15] and ORD,[16] IR,[17] theoretical calculations,[18-23] and in idealized cases by X-ray diffraction methods (powder[24] or single-crystal techniques), on either synthetic polymers[25] or oligopeptides[26-36] as models.

In the literature, some reports have recently been made about the L-Pro ring conformations,[20,21,37] but results are not fully self-consistent as regards the conformation influences of the L-Pro environment.

Discussions are still open about relationships between averaged conformational parameters and dynamic features of these compounds in solvated and crystalline states. It must be emphasized that accurate descriptions may reasonably be achieved by assuming the existence of at least two major puckered conformations contributing through particular weights, which is a useful approach in NMR. The same problems have been encountered in the crystalline forms of Pro-containing peptides, as pointed out by Mitsui et al.[38] in the case of DL-Pro: are the observed conformations the average of two—or several—conformations? Clearly, a small local disorder is possible in the crystalline structure, as found in other Pro-containing structures, e.g., *cyclo*-(L-Pro-L-Pro-L-4Hyp).[39] However, from a solid state NMR study, Andrew et al.[40] concluded that the Pro ring is rigid. Although it is difficult to draw any conclusion in the case of the Pro ring, the L-4Hyp moiety is expected to exhibit more rigid structures owing to its stabilizing role in polymers.[2b,3,11-13]

In order to test the role played by the L-4Hyp residue in the stability of peptide chains, the systematic influence of the 4-OH function borne by the pyrrolidine ring will be herein analyzed in terms of static (averaged) and dynamic deformations of the rings. The results will be compared to the known data concerning the L-Pro residue.

Such an analysis requires (1) an investigation of the conformational behavior of L-Pro and L-4Hyp when preceded or followed by one or two residues, (2) elucidation of possible relationships between the preferential pyrrolidine ring conformations measured by NMR in solution and X-ray in the crystal, (3) a comparison of the segmental motions determined by ^{13}C T_1 measurements in solution and by thermal displacements in the solid.

The selected compounds we have investigated are Gly-L-4Hyp (**1**) and L-Pro-L-4Hyp (**2**). Unfortunately the triplet unit Gly-L-Pro-L-4Hyp failed to crystallize in suitable form for three-dimensional X-ray studies and the closest model we have included in the list is *N*-Ac-L-Pro-L-4Hyp (**3**). In the same way, the small cyclopeptide (diketopiperazine) *cyclo*-(L-Pro-L-4Hyp) (**4**) was taken as a model for a pure cis peptide junction.[41]

Experimental Section

X-ray. The most critical problem is the preparation of suitable crystals for the X-ray determinations. The four compounds **1–4** were conveniently crystallized from aqueous diglyme solutions (except mixtures of MeOH/H_2O for **2**) over a period of several weeks. Water is part of the crystal structure of some L-4Hyp derivatives and to avoid a slow dehydrative process they were included with mother liquor in Lindman capillaries and mounted on a four-circle diffractometer using the Cu Kα radiation monochromatized by graphite. All the structures were solved by direct methods.[42] Refinements were carried out with isotropic, then anisotropic thermal factors by block-diagonal least-squares procedures. Most of the hydrogen atoms were located during the refinements on Fourier-difference syntheses and introduced with an isotropic thermal factor equal to that of the bonded carbon. The rest of the hydrogen atoms were positioned at their theoretical places with $\angle CCH = 108°$ and C–H = 1.09 Å. In all these structures, hydrogen atom positions were not refined. The crystal data, as well as the final *R* indices,[43] are given in Table IA.

The bond angles and distances have been calculated. They are in agreement with those found in other structural reports on oligopeptides[20,21,44] and will not be discussed here. The estimated standard deviations of the bond lengths and angles are 10^{-2} Å and 0.3°, respectively. The positional parameters, bond distances, angles, and structural factors for all the structures have been prepared as supplementary material.

^1H NMR. ^1H NMR spectra were recorded at 250 or 300 MHz on a Cameca 250 or a Varian HR 300 spectrometer, both operating in CW mode at room temperature. D_2O was used as solvent and DSS (sodium 3-(trimethylsilyl)-1-propanesulfonate) as internal reference. NMR simulations for **1** and **2** were run with the DECHAMIT program[14a] as (2 × 6 + 2 × 2) and (2 × 6 + 2 × 7) spin systems, re-

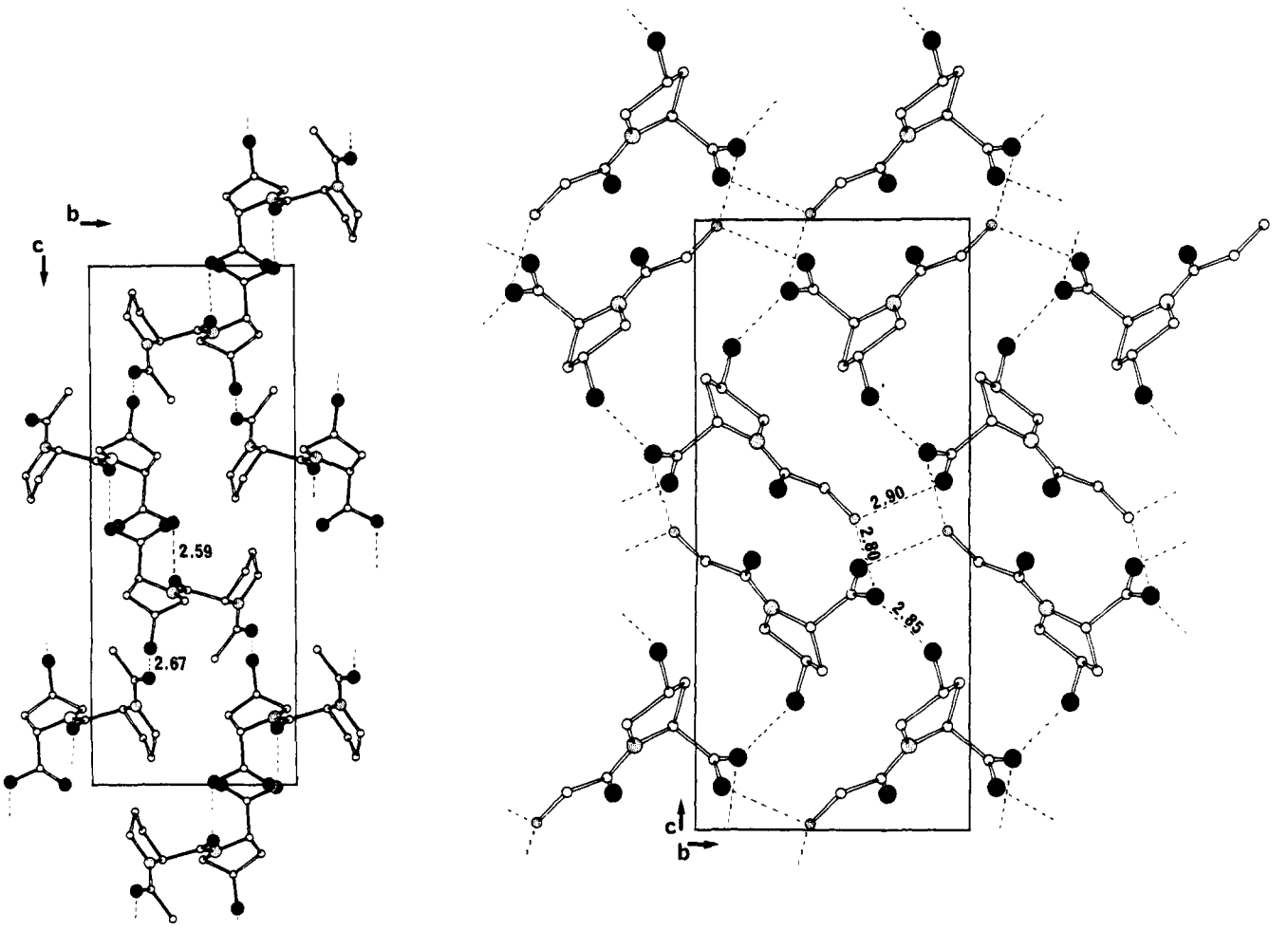


Figure 1. Packings of type 1: *N*-Ac-L-Pro-L-4Hyp (**3**) (left) with H bonds along the *c* axis and Gly-L-4Hyp (**1**) (right). **1** also displays a helical bond network along the *a* axis.

spectively. Figures were drawn with a Benson plotter using a Lorentzian profile (Figures 3 and 4) and the ORTEP program.[45]

^{13}C NMR. ^{13}C NMR spectra were recorded on a Varian XL 100 and/or a WH 270 Brucker spectrometer operating at 25.2 and 67.89 MHz, respectively, in Fourier transform mode. ^{13}C T_1 values were measured in aqueous solutions (5×10^{-2} M) using the inversion-recovery technique[46] and spectral data analyzed using a nonlinear least-squares regression technique. Complete nuclear Overhauser enhancements (NOE) were obtained for all proton-bearing carbons.[47]

Line Attributions. They are based upon previous works[14] and chemical shifts are reported in Table IB (Me_4Si as external reference). In the cases of Gly-L-4Hyp (**1**) and L-Pro-L-4Hyp (**2**) the cis/trans rotamers around the peptide bond are easily attributed through temperature equilibrations.[14b] After dissolution at low temperature of crystals exhibiting a unique and well-defined conformation (determined by X-ray), the apparition of a new form was detected and monitored by NMR until equilibrium was established.

Results

X-ray. 1. General Features. Figures 1 and 2 show the projections of the packing along the *a* axes. The analyses of the structures in the crystal show that complex hydrogen-bonding networks are present in all the structures.

These packings can be divided into the two classes I and II (Figures 1 and 2, respectively) according to the characteristics of the H bonds.

Type I (Gly-L-4Hyp (**1**) and *N*-Ac-L-Pro-L-4Hyp (**3**); Figure 1) is characterized by an extended H-bond network along the *c* axis, involving the L-4Hyp OH function.

Type II (L-Pro-L-4Hyp (**2**) and *cyclo*-(L-Pro-L-4Hyp) (**4**); Figure 2) are hydrated structures. They exhibit a more complicated hydrogen-bond network through molecules of water.

In the different types, the two dipeptides Gly-L-4Hyp (**1**) and L-Pro-L-4Hyp (**2**) (zwitterionic forms) exhibit important head-to-tail interactions between the different molecules of the packing. Typical N^+/COO^- distances are in the range 2.8–2.9 Å. Furthermore, the hydroxyl function at C-4 of the L-4Hyp ring is tightly involved in such an H bond, usually directed toward the carboxylic COO^- group of another molecule (Gly-L-4Hyp (**1**) and L-Pro-L-4Hyp (**2**)) or toward the CO of the acetyl group in *N*-Ac-L-Pro-L-4Hyp (**3**).

In the latter compound, it is interesting to observe that a similar but intramolecular interaction between the OH group of the carboxylic function and the CO of the preceding amino acid (trans peptide bond) is observed, and was postulated to stabilize the trans rotamer in solution.[14d] In *cyclo*-(L-Pro-L-4Hyp) (**4**), two independent molecules A and B are packed together in the asymmetric unit. No free carboxylic function is present, but the hydroxyl group of the L-4Hyp moiety is involved in two different H-bond paths: toward a water molecule and toward a carboxamide carbonyl function in molecules A and B, respectively (Figure 2).

None of the highly coordinating diglyme molecule, the solvent used in the crystallizations, is observed in the crystal structures.

2. Peptidic Bond Arrangement. Table II displays the different values for the dihedral angles ω, ψ, and ϕ describing the peptide backbone.[48]

(a) ω Angle. The ω angle depicts the cis or trans conformation around the peptidic bond. Gly-L-4Hyp (**1**) is trans as ob-

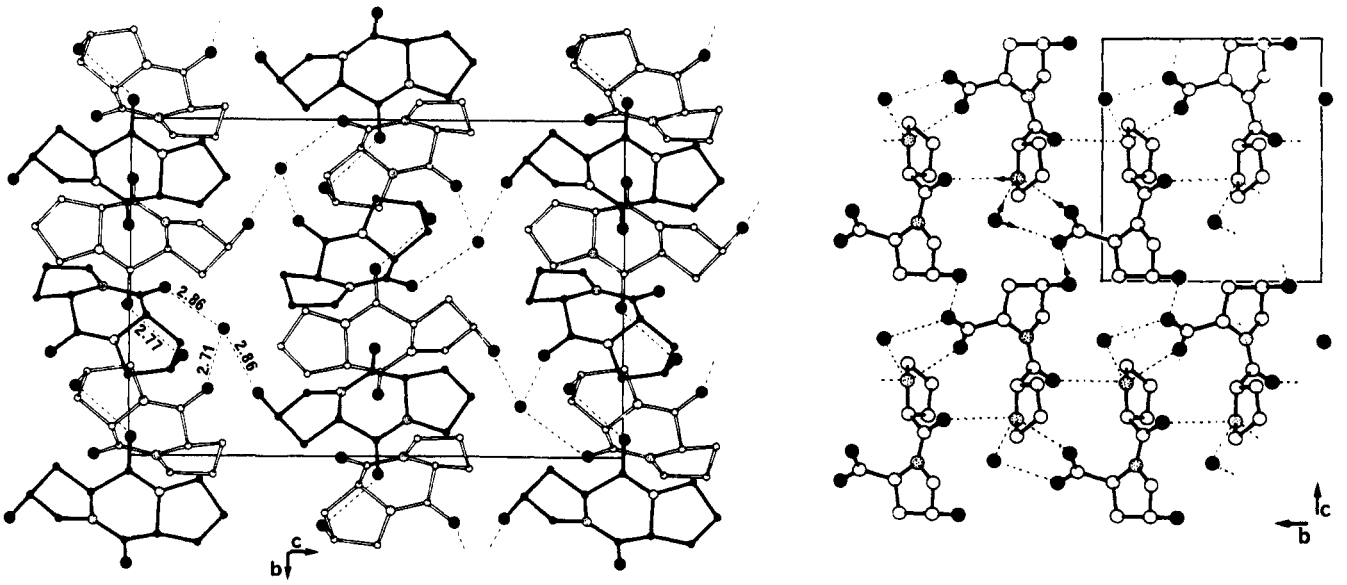


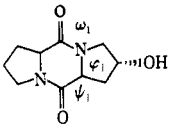
Figure 2. Packings of type 11: *cyclo*-(L-Pro-L-4Hyp) (**4**) (left) and L-Pro-L-4Hyp (**2**) (right). The water molecules are strongly coordinated in the cell. As in Figure 1, the same plane of projection was selected.

Table II

A. IUPAC Torsional Angles of the Amide Bonds in the X-ray Structures of Compounds **1**–**3**

	Gly-L-4Hyp **1**	Ac-L-4Hyp (ref 44) **5**	L-Pro-L-4Hyp **2**	Ac-L-Pro-L-4Hyp **3**	
angle	Gly/L-4Hyp	Ac/L-4Hyp	L-Pro/L-4Hyp	Ac/L-Pro	L-Pro/L-4Hyp
ψ	+173°	−30°	+158°		+170°
ϕ	−75.4°	−61°	−65.4°	−86°	−78°
ω	−177°	−174°	−3°	+10°	+171°
junction	trans	trans	cis	cis	trans

B. IUPAC Torsional Angles (X-ray) of the Diketopiperazines



4

	cyclo-(L-Pro-L-4Hyp) (**4**)		*cyclo*-(L-Pro)$_2$
	molecule A	molecule B	(from ref 32)
ψ_1	40°	28°	37°
ψ_2	36°	35.3°	36°
ω_1	1°	7°	0.7°
ω_2	5.5°	−1.5°	−0.7°
ϕ_1	−4.5°	−32°	−38°
ϕ_2	−40.5°	−40°	−37°

served in *N*-Ac-L-4Hyp.[44] L-Pro-L-4Hyp (**2**) and obviously *cyclo*-(L-Pro-L-4Hyp) (**4**) are cis with small values of ω (from −3 to +7°) as observed in the corresponding protected *N*-Ts-L-Pro-L-4Hyp.[36] On the contrary, in the tripeptide *N*-Ac-L-Pro-L-4Hyp (**3**), the closest model of collagen triplet Gly-L-Pro-L-4Hyp, the peptidic bond L-Pro-L-4Hyp is trans, whereas the acetyl-L-Pro bond (ω = +10°) is cis. Compared with compounds **1** and **2**, this result is somewhat surprising and the existence of a cis linkage may be correlated with the steric hindrance brought by the two bulky L-Pro and L-4Hyp residues following in the sequence.

In the collagen-like polypeptide (Gly-L-Pro-L-4Hyp)$_n$ such a cis bond between Gly and L-Pro residues followed by a trans bond between L-Pro and L-4Hyp was proposed by Berg et al.[9] in order to explain a possible hydrogen bond between the γ OH of one L-4Hyp residue belonging to one chain and the cis carbonyl group of the glycine of one adjacent chain.[6] However, in aqueous solution (see below), the preferential conformation for *N*-Ac-L-Pro-L-4Hyp and Gly-L-Pro-L-4Hyp corresponds to trans-trans arrangement.

(b) ψ Angle. The L-Pro- or L-4Hyp-containing peptides are classified into two groups according to their ψ values: the collagen type, with large positive ψ values, and the α-helix type, with small negative ones.[49] Accordingly, the compounds Gly-L-4Hyp (**1**, ψ = 173°), L-Pro-L-4Hyp (**2**, ψ = 158°), and *N*-Ac-L-Pro-L-4Hyp (**3**, ψ_2 = 170°) can be classified in the collagen type together with the protected dipeptide *t*-Boc-Gly-L-Pro[29] and several oligopeptides containing the repeated L-Pro unit such as amyloxycarbonyl-L-Pro-L-Pro-L-Pro-OH[26] or *t*-Boc-(L-Pro)$_4$-OBz.[27] On the other hand, the compound *N*-Ac-L-Pro-NH_2[50] and oligopeptides containing isolated L-Pro residues such as *p*-Br-Z-Gly-L-Pro-L-Leu-Gly[30] belong to the α-helix type.

In *N*-Ac-L-Pro-L-4Hyp (**3**), the backbone dihedral angles including the carboxylic group (ψ_2 = 170°, ϕ_2 = −86°, ψ_3 = 177°, ϕ_3 = −78°) agree with those (ψ_2 = 152°, ϕ_2 = −71°,

$\psi_3 = 173°, \phi_3 = -80°$) calculated by Ramachandran from the model proposed by Berg[6] (vide supra).

In *cyclo*-(L-Pro-L-4Hyp) (**4**), the different molecular shapes of the molecules A and B of the asymmetric unit are indicated by the values of the ψ angles, which are 40 and 28°, respectively.

(c) **ϕ Angle.** The ϕ angles in linear proline-containing peptides lie in a small range as depicted by the values listed in Table II ($-61° > \phi > -86°$) and average to $-37°$ in the cyclic dipeptide *cyclo*-(L-Pro-L-4Hyp) except ϕ_1 for molecule A. In the crystal structure, the ratio ϕ/ψ for molecule A is $-4.5/40°$ and for molecule B $-32/28°$. These differences between the two molecules of the asymmetric unit clearly arise from the hydrogen-bonded 4-OH function linked on the L-4Hyp moiety. This results in a less twisted conformation for molecule A since the 4-OH group is differently involved in the packing hydrogen bond network.

^{1}H NMR. The analyses of the crystalline states are dependent on the intermolecular H bonds of a molecule with its nearest neighbors. Whatever may be the results of such an investigation, they need to be compared with the solvated state of these oligopeptides. In that way, NMR spectroscopy of peptides gives access to their mean conformation and in the case of proline-containing peptides to the cis $\rightleftarrows$ trans isomerism around the peptide bond.[14,51-53] Nevertheless dynamic deformations may not be observed owing to the inherent time scale limitation of the method and models with two differently weighted forms led to consistent results.[23,54]

Gly-L-4Hyp (1). Part of the observed and calculated spectrum for the zwitterionic form is given in Figure 3. The theoretical part of the spectrum is obtained by matching 160 measured ν_{obsd} frequencies. The ^3J couplings of both cis and trans rotamers obtained after refinement are reported in Table IV; those of the trans form can be compared to the theoretical ^3J values deduced from the former X-ray study by the use of a Karplus relation.[55-57]

$$^3J_{H-H} = a\cos^2\theta_i + c \qquad 0° < \theta_i < 90°$$
$$= b\cos^2\theta_i + c \qquad 90° < \theta_i < 180°$$

θ_i = dihedral angle between the vicinal proline ring hydrogens; $a = 8.5$, $b = 10.5$, and $c = 1.4$.

L-Pro-L-4Hyp (2). As the ^{1}H NMR spectrum of **2** in D_2O was rather complicated, even at 300 MHz, chemical-shift assignments were achieved after successive additions of praseodymium perchlorate as LIS reagent in water.[58] It strongly complexes at the carboxyl function of L-4Hyp leading to important deshieldings in the L-4Hyp moiety. By this procedure J coupling constants could be obtained in the predominant (80%) trans rotamer. They were not extracted from the spectra of the lanthanide complex because of a possible conformational influence of the LIS reagent.[59-61]

Owing to the presence of a mixture of 80% of trans rotamer and 20% of cis, strong overlaps of the resonances occurred in the ^{1}H NMR spectrum in D_2O. It was possible to extract the chemical shifts of the preferential trans rotamer by extrapolation to zero concentration of LIS reagent as shown in Figure 4. The coupling constants in the trans form were then obtained from computed spectra. In order to obtain the chemical shifts in the cis form, an equilibration experiment[62] was performed by dissolving the crystalline form in D_2O and recording the spectra as soon as possible. However, at this stage the complexity of the spectra did not allow accurate measurements of all the cis lines.

***cyclo*-(L-Pro-L-4Hyp) (4).** In water solution, the complete assignment of the ^{1}H NMR resonances could be achieved with difficulty only by comparison with the spectrum of **4** in nonaqueous media.[52] It follows that the more sensitive parameters to solvent-induced conformational changes are the J couplings

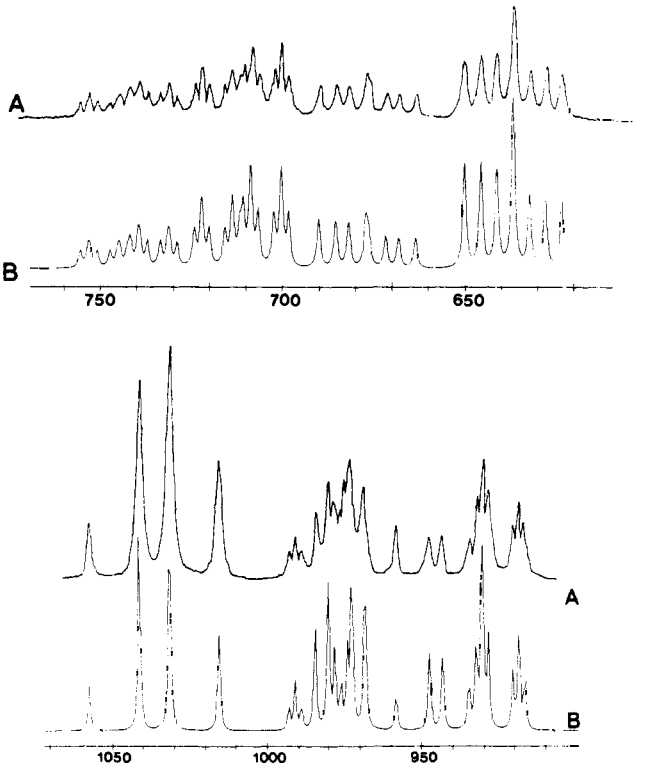


Figure 3. Observed (A) and calculated (B) 250-MHz ^{1}H NMR spectrum of Gly-L-4Hyp. Downfield region (lower part) shows the Gly and C^δ (Hyp ring) hydrogens. Upfield region (upper part) displays the C^β hydrogens of both cis and trans rotamers.

around the C^γ. These couplings can be used in the same way as are geminal couplings in the case of glycine-containing diketopiperazines.[63]

Discussion

1. Puckering of the Pyrrolidine Rings. X-ray. The χ_i values and puckering of the pyrrolidine rings are gathered in Table III. Balasubramanian et al. have proposed[49] that, in collagen-type peptide structure ($\psi > 0$), the prolyl residue would be C^γ endo or C^δ exo with respect to the C^0 atom and termed it conformation B with positive χ_1 value. The same authors proposed that in the α-helix type structure the prolyl residues would be C^γ exo corresponding to conformation A with negative χ_1 value. From the results of Table III, it appears that it is not easy to correlate the collagen or α-helix type structures with the pyrrolidine puckering for the three models Gly-L-4Hyp (**1**, $\psi = +173°$), L-Pro-L-4Hyp (**2**, $\psi = +158°$), and *N*-Ac-L-Pro-L-4Hyp (**3**, $\psi = +170$ and $+173°$). Only Gly-L-4Hyp (**1**) exhibits the proposed features for puckering of the pyrrolidine ring corresponding to conformation B. Moreover, in *N*-Ac-L-Pro-L-4Hyp (**3**), the closest model of collagen triplet Gly-L-Pro-L-4Hyp, the proline ring adopts a C^β exo/C^γ endo form characteristic of the collagen type, whereas the 4Hyp ring is C^δ endo/C^γ exo as would correspond to the α-helix type.

In the cyclic diketopiperazine *cyclo*-(L-Pro-L-4Hyp) (**4**), the conformational angles in the pyrrolidine rings (Table II) are in agreement with those reported in *cyclo*-(L-Pro-L-Leu)[31] and *cyclo*-$(L\text{-Pro})_2$[32] from X-ray analyses, and with those reported in *cyclo*-(L-Pro-D-Pro) on the basis of NMR measurements and minimum energy calculations.[34] The pyrrolidine ring symmetry is intermediate between C_s and C_2 (rather than pure C_s for the prolyl ring) with C^β endo/C^γ exo with respect to the C^0. The β hydrogen atoms are in quasi-equatorial positions and the two rings are puckered at the β carbon atoms which deviate from the best planes defined by the four re-

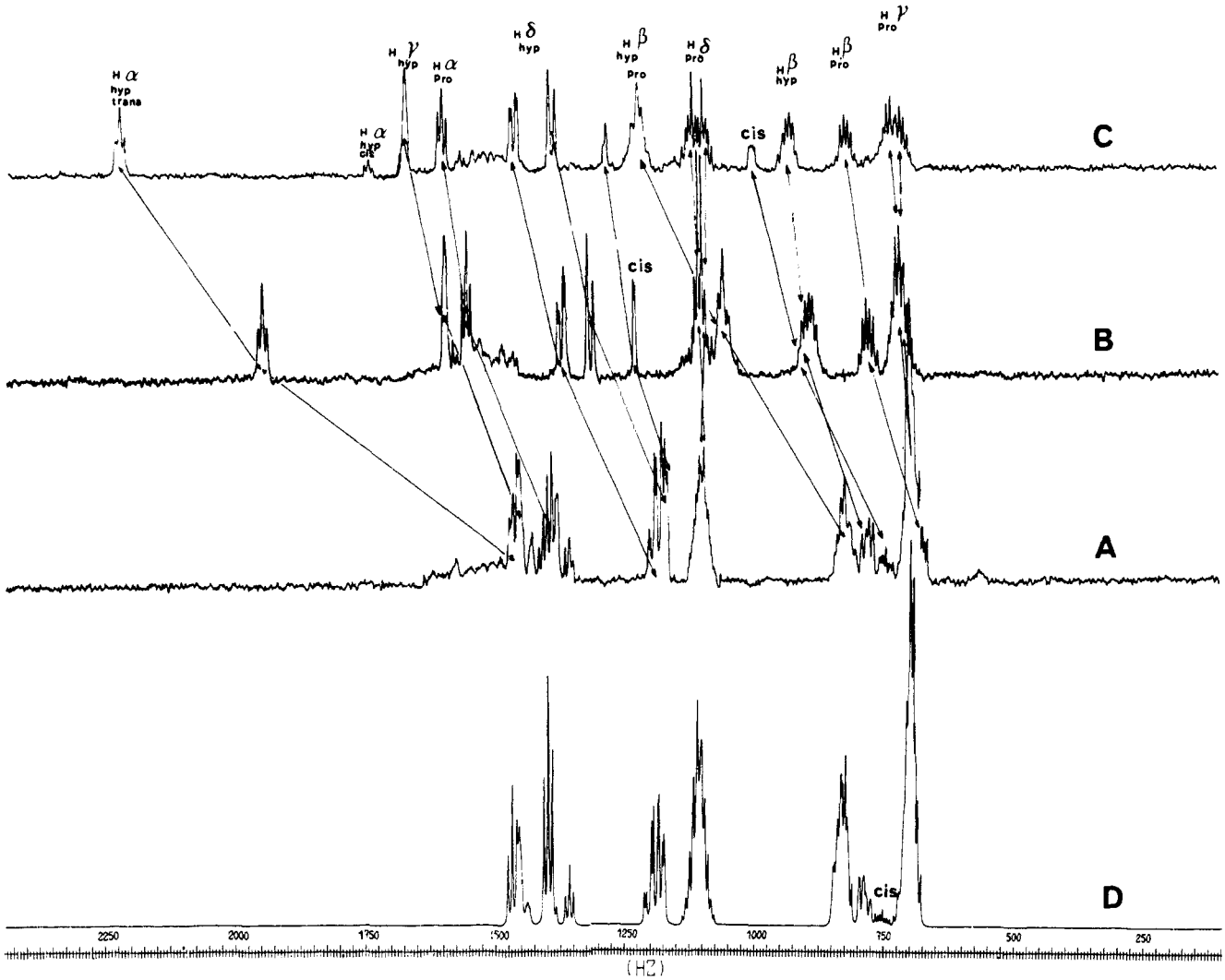


Figure 4. Modifications of (A) the zwitterionic L-Pro-L-4Hyp ^{1}H NMR spectrum (300 MHz) upon increasing quantities of LIS reagent;[52] (B) and (C) praseodymium perchlorate in D_2O; (D) simulation with the chemical shifts deduced from (B) and (C) and the J couplings calculated from X-ray data. The good agreement is indicative of similar conformation shapes in both solvated and crystalline states.

maining atoms (0.51 and 0.57 Å for the prolyl ring, 0.56 and 0.63 Å for the hydroxyprolyl ring in molecules A and B, respectively).

A simpler approach to the conformational problem of the L-4Hyp or L-Pro pyrrolidine ring is to consider one of the formalisms outlined by Altona et al.[64] These authors indicated that the knowledge of two conformational parameters, a phase angle Δ and the maximum puckering angle ϕ_m, permits a simple description of the overall conformation for a five-membered ring. These parameters are defined as follows:

$$\tan(\Delta/2) = \frac{(\phi_2 + \phi_4) - (\phi_1 + \phi_3)}{3.0777\phi_0}$$

$$\phi_m = \phi_0/\cos(\Delta/2)$$

ϕ_i angles are related to the IUPAC torsional angles by fixing ϕ_0 the greatest angle and ϕ_3 the smallest one, in absolute scale. Using this definition, $\Delta = 0$ (modulo $4\pi/10$) indicates a C_2 symmetry (half-chair conformation) while $\Delta = 36°$ (modulo $4\pi/10$) corresponds to a C_s (envelope) symmetry.

The ϕ_m and Δ angles, calculated for all the five-membered rings of the compounds selected, are given in Table III, in addition to the corresponding torsional angles χ_i.

From these values, no straightforward correlation is found between the nature of the L-Pro or L-4Hyp ring and its particular conformation, but the following observations can be made.

(1) A cis peptide junction leads to a preferential envelope conformation ($\Delta \sim 36°$) while a trans disposition shifts the conformation toward the half-chair ($\Delta \sim 0°$).

(2) The general conformations are closely related to the deformations induced in the packing of the molecules by the hydrogen-bond network.

(3) It is noteworthy that the cis disposition appears less hindered than the trans one. This observation may partly explain the easy trans $\rightleftarrows$ cis interconversion in the higher peptidic or collagen structures.

Solvated State. The situation is complicated by superposition of two (or several) spectra arising from the cis $\rightleftarrows$ trans isomerism around one (or more) peptide linkage ($\Delta G^{\ddagger} \sim 22$ kcal mol^{-1}). At the dissolving pD (zwitterionic conditions for **1** and **2**), ratios follow:[61] Gly-L-4Hyp, 56% trans; L-Pro-L-4Hyp, 80% trans.

Gly-L-4Hyp (1). The $^3J_{exp}$ vicinal H–H couplings were directly extracted from the ^{1}H spectra, whereas the $^3J_{calcd}$ were deduced from the X-ray data, using the above-mentioned Karplus rule. They are reported together in Table IVA. A good correlation between the $^3J_{exp}$ and $^3J_{calcd}$ in the trans rotamer is observed, except for two couplings involving H^{α} and $H^{\delta 1}$. Such a discrepancy suggests modification of the Karplus parameters for hydrogens in the vicinity of the nitrogen ring atom.[56] From NMR/X-ray comparison a more bent C_s (envelope), C^{γ} endo conformation with dihedral angles $\alpha\beta_1$ and $\alpha\beta_2$ close to 20 and 150° may be proposed for Gly-L-4Hyp (**1**)

Table III

compd	χ_1	χ_2	χ_3	χ_4	χ_5	ϕ_m	Δ,[a] deg
A. Conformational χ Angles (deg) of the L-4Hyp Pyrrolidine Rings (from X-ray Data), Compared to Known Data							
1	+27	−32	+24	−7.5	−12	−32 C^γ endo / C^β exo	9.5 ½ ch
2	−21	+37	−38	+25	−3	−40 C^γ exo	29 E
3	−27.4	+5	+17	−34	+39	+40 C^δ endo / C^γ exo	17.7 tw
4A	−31	+39	−31	+11.5	+13	+39 C^β endo / C^γ exo	1.4 ½ ch
4B	−39	+41	−26	+2	+24	+42.5 C^β endo / C^γ exo	31 E
5[b]	−28	+40	−36	+19	+6	+40	19.4 tw/½ ch
6[b]	−13	+31	−35	+20	−10	+35.5	15 ~½ ch
7[b]	−18	+32	−33	+23	−3		
B. Conformational χ Angles (deg) of the L-Pro Rings							
2	−10	−14.5	+33	−40	+31.6	−41 C^δ exo	5.5 ½ ch
3	−30	−35	+27	−7	−14.5	−35.5 C^β exo / C^γ endo	11 ½ ch
4A	−32	+31	−16	−5	+23.7	34.2 $C^{\beta'}$ endo / C^γ exo	20.4 tw
4B	−35	+35	−21	−2	+24	+37.4 C^β endo	30.8 ~E
8[b]	+33	−40	+31	−10	−14	+40	5.6 ½ ch

[a] ½ ch = half-chair; tw = twisted; E = envelope. [b] Compound **5**; *N*-Ac-L-4Hyp (trans) from ref 44. Compound **6**: tosyl-L-Pro-L-4Hyp (cis) from ref 36. Compound **7**: L-4Hyp (neutron study) from ref 35. Compound **8**: *N*-Ac-L-Pro-NH_2 (trans) from ref 50.

Table IV

A. ^{1}H NMR Data for Gly-L-4Hyp in Both Rotamers[a]

	X-ray dihedral angles, deg	trans J_{exp}	trans J_{calcd}	cis J_{exp}
$H_2C^\alpha C^\beta H_{3_A}$	100.5	$J^{\alpha\beta_1}$ = 8.4	2	$J^{\alpha\beta_1}$ = 8.2
$H_2C^\alpha C^\beta H_{3_B}$	−22.5	$J^{\alpha\beta_2}$ = 8.86	8	$J^{\alpha\beta_2}$ = 8.2
		$J^{\beta_1\beta_2}$ = −13.4[c]		$J^{\beta_1\beta_2}$ = −13.7
$H_{3_A}C^\beta C^\gamma H_4$	52.2	$J^{\beta_1\gamma}$ = 2.15	4.5	$J^{\beta_1\gamma}$ = 2.58
		$J^{\beta_1\delta_1}$ = 1.94[b]		$J^{\beta_1\delta_1}$ = 2.11
$H_{3_B}C^\beta C^\gamma H_4$	167.2	$J^{\beta_2\gamma}$ = 4.54	4	$J^{\beta_2\gamma}$ = 4.66
$H_4C^\gamma C^\delta H_{5_A}$	13.2	$J^{\gamma\delta_1}$ = 1.90	8.9	$J^{\gamma\delta_1}$ = 1.98
$H_4C^\gamma C^\delta{}_{5_B}$	−131	$J^{\gamma\delta_2}$ = 4.28	4.5	$J^{\gamma\delta_2}$ = 4.26
		$J^{\delta_1\delta_2}$ = −11.85[c]		$J^{\delta_1\delta_2}$ = −12.76

B. L-Pro-L-4Hyp: Conformational Parameters[d]

L-Pro				L-4Hyp			
	θ angles, deg (X-ray)	J_{exp} trans	J_{calcd} cis	θ angles, deg		J_{exp} trans	J_{calcd} cis
$H_2C_1{}^\alpha C_1{}^\beta H_{3_A}$	+120.1	7.5	4.0	$H_2C_2{}^\alpha C_2{}^\beta H_{3_B}$	+38.5	8.2	6.6
$H_2C_1{}^\alpha C_1{}^\beta H_{3_B}$	+22.0	8.5	8.5	$H_2C_2{}^\alpha C_2{}^\beta H_{3_B}$	+151.8	8.8	9.4
$H_{3_A}C_1{}^\beta C_1{}^\gamma H_{4_A}$	+32.5	7.5	7.2	$H_{3_A}C_2{}^\beta C_2{}^\gamma H_4$	+68.4	2.5	2
$H_{3_A}C_1{}^\beta C_1{}^\gamma H_{4_B}$	−87.5	2.5	1.4	$H_{3_B}C_2{}^\beta C_2{}^\gamma H_4$	−38.3	4	6.6
$H_{3_B}C_1{}^\beta C_1{}^\gamma H_{4_A}$	+133.2	5.5	6.5	$H_4C_2{}^\gamma C_2{}^\delta H_5$	−80.6	1.8	1.7
$H_{3_B}C_1{}^\beta C_1{}^\gamma H_{4_B}$	+13.2	7.5	9.5	$H_4C_2{}^\gamma C_2{}^\delta H_5$	+49.1	4.4	4.5
$H_{4_B}C_1{}^\gamma C_1{}^\delta H_{5_A}$	−36.0	5.5	7.0				
$H_{4_A}C_1{}^\gamma C_1{}^\delta H_{5_B}$	−152.8	7.5	9.5				
$H_{4_B}C_1{}^\gamma C_1{}^\delta H_{5_A}$	+85.7	5	1.4				
$H_{4_B}C_1{}^\gamma C_1{}^\delta H_{5_B}$	−31.2	8	7.2				

[a] J_{calcd} are the conformational parameters deduced from X-ray (Hz). [b] Long-range coupling relevant to HH coplanarity (see ref 66). [c] Geminal couplings. [d] J_{exp}: from ^{1}H NMR, in solution in the trans form. J_{calcd}: from X-ray dihedral angles in the cis form.

in D_2O solution. This is consistent with recent NMR studies in nonaqueous media and with results reported in the compound *p*-Br-Z-Gly-L-Pro-L-Leu-Gly-L-Pro.[65]

The comparison between the ^3J coupling of cis and trans conformers of **1** in solution does not provide measurable evidence of any fundamental difference in their ring puckering (Table IVA) in contrast to the compound *N*-Ac-L-4Hyp.[44]

In the two rotamers of Gly-L-4Hyp the carbon C^γ is a center of symmetry as shown by the similar values of $J^{\beta\gamma}$ and $J^{\gamma\delta}$ [$J^{\beta_1\gamma}$ = 2.1, $J^{\beta_2\gamma}$ = 4.5, and $J^{\gamma\delta_1}$ = 1.9, $J^{\gamma\delta_2}$ = 4.3 Hz in the trans rotamer (Table IV)].

L-Pro-L-4Hyp (2). In order to compare cis and trans proline puckering, as well as conformations between solvated and crystalline forms, the subsequent procedure was developed: (1) The $^3J_{calcd}$ couplings were computed from X-ray dihedral angles in the cis form and compared with the $^3J_{exp}$ derived from NMR trans data (Table IVB)—this led us to propose equivalent conformations in the cis and trans forms. (The same difference remains in the *J* data: H^α and $H^{\delta 1}$ show the same slight deviations pointed out in Gly-L-4Hyp). (2) An overall "hybrid" spectrum was then calculated using the experimental chemical shifts (δ) and theoretical couplings (from X-ray),

Table V

cyclo-(L-Pro-L-4Hyp)[a] 4	L-Pro ring			L-4Hyp ring	
	NT_1, s/⟨B⟩	τ_{eff}, 10^{-11} s	τ^l_{ring}, 10^{-11} s	NT_1, s/⟨B⟩	τ_{eff}, 10^{-11} s
C_α	0.68/2.8	6.9	47	0.59/3.4	8.1
C_β	0.78/3.8	6.0	47	0.74/3.2	6.4
C_γ	0.84/4.5	5.6	29	0.60/3.3	7.8
C_δ	0.76/3.7	6.2	59	0.74/2.6	6.4

L-Pro-L-4Hyp 2	L-Pro ring						L-4Hyp ring			
	NT_1, s/⟨B⟩		τ_{eff}, 10^{-11} s		τ^l_{ring}, 10^{-11} s		NT_1, s/⟨B⟩		τ_{eff}, 10^{-11} s	
	trans	cis-cis	trans	cis	trans	cis	trans	cis-cis	trans	cis
C_α	0.49	0.55/2.4	9.6	8.6			0.57	0.57/2.4	8.3	8.3
C_β	0.94	0.92/5.0	5.0	5.1	10.4	12.7	0.59	0.79/3.	8.0	8.3
C_γ	1.57	1.64/6.1	3.0	2.9	4.4	4.3	0.57	0.58/3.1	8.3	6.0
C_δ	1.12	0.92/3.2	4.2	5.1	7.5	12.7	0.56	/3.2	8.4	8.1
acetyl-L-Pro-L-4Hyp[b] 3										
C_α	0.48	0.45/3.8	9.8	10.5			0.45	0.44/5.5	9.8	10.7
C_β	0.76	0.62/5.8	6.2	7.6	16.9	27.5	0.60	0.58/5.9	7.9	8.1
C_γ	1.02	0.82/7.7	4.6	5.7	8.6	12.4	0.57	/5.7	8.3	
C_δ	0.60	0.54/7.0	7.9	8.7	40.7	50.7	0.52	0.48/6.9	9.1	9.8

[a] At 22.17 MHz. [b] At 67.89 MHz.

Figure 4A, curve D. This result, compared to the experimental spectrum—curve A—emphasizes that the ring conformations in solution remain roughly the same in both cis and trans forms.

***cyclo*-(L-Pro-L-4Hyp) (4).** The C^γ proton of L-4Hyp was used as a conformational probe. In D_2O, the more significant results are $J_{\gamma-\delta_A} = 0.6$ and $J_{\gamma-\delta_B} = 4.7$ Hz. The couplings lie in the range of those calculated fom X-ray data for the solid ($J_{calcd} = 1.3$ and 7.7 Hz, respectively). These couplings also support similar conformations for **4** in the solid and solvated states. The 4-OH group is thus nearly perpendicular to the mean plane of the pyrrolidine ring, similarly to the cases of *N*-Ac-L-4Hyp[14a] ($J_{\gamma-\delta_A} = 1.8$ Hz) and L-4Hyp[37] ($J_{\gamma-\delta_A} = 1.4$ Hz).

In conclusion there is a close similarity between the conformations of the H-bonded crystalline forms obtained by X-ray and the solvated mean conformations deduced from the vicinal and long-range coupling constants measured by ^1H NMR (Table IV). These similarities in both solid and solvated states imply that the L-4Hyp ring has a more bent conformation than L-Pro with a quasi-axial H-bonded γ-OH function. The ring conformation appears to be dependent on (1) the bonding of the OH group and to a lesser extent (2) the cis ⇄ trans isomerism around the peptide bond preceding the residue.

2. Dynamic Deformations of the Pyrrolidine Rings. The analyses of the conformational states are closely related to the thermodynamic parameters of the different main conformations. The presence of a pyrrolidine ring leads to an exo-endo interconversion of the ring through a planar transition state. NMR studies and calculations show that in the case of the L-Pro ring the barrier of energy is very small (<5 kcal mol^{-1}). A convenient approach to estimate the internal motion is to consider the vibrational parameters of the ring atoms by the use of the thermal parameters deduced from the X-ray studies (in the crystal) in correlation with the ^{13}C relaxation times deduced from the NMR measurements (in solution).[67,68] As far as the crystalline form may be considered as a solvated blocked form by an intermolecular hydrogen bonding network replacing the surrounding solvent molecules, these comparisons will provide valuable information about the behavior of Hyp vs. Pro. In each case, the same method was developed: a search for an overall thermal motion was initially performed and this motion subtracted, when found significant, from the individual thermal factors in order to obtain more accurate atomic displacements in the rings.

X-ray Thermal Factors. All the L-4Hyp-containing compounds **1–4** have been analyzed in term of the rigid-body approximation. A modified version of JMTFAC[69] was used to calculate the tensor components of the thermal ellipsoids (translation, rotation, and screw motions), using the method of Shomaker and Trueblood.[70] The motion of the atoms was deduced from the quadratic mean deviations of the U_{ij} parameters and from the criteria used by Burns et al.[69] (size, shape, and orientation of the ellipsoids). The inclusion of the overall linear peptides in the rigid-body model did not lead to conclusive results. Better fits were obtained when analyzing peptides residue by residue; this yields valuable information about segmental motion in the chains. Using this approach we find:

1. Solely the L-Pro ring atoms of compounds **2** and **3** appear to have a significant overall motion nearly perpendicular to the ring.

2. The best results were obtained when omitting C^γ, a sharp indication of its random thermal agitation.

3. *cyclo*-(L-Pro-L-4Hyp) (**4**) appears to have overall motion (in the rigid-body sense), especially a vibrating ("flipping") displacement (±0.2 Å) of the pyrrolidine rings, relative to the diketopiperazine mean plane.

More details about the numeric results—tensor components, moments of inertia, corrected anisotropic factors, etc.—are compiled in the supplementary material.

In order to have comparable data with the NMR results, the thermal ellipsoid parameters U_{ij} were normalized in the sense of the mean isotropic motion by calculation[71] of the ⟨B⟩ factors:

$$\langle B \rangle = 8\pi^2 \langle \Delta U_{ij} \rangle$$

and reported, together with the relaxation times T_1 deduced from ^{13}C NMR, in Table V. An alternative analysis would be to select $(\Delta U_{ij})_z$ components of the vibrational displacements, assuming the z axis perpendicular to the mean plane of the ring. However, the anisotropic parameters thereby obtained would not be directly comparable with the isotropic parameters given by ^{13}C T_1 measurements.

^{13}C NMR Analyses of the Flexibilities. ^{13}C NMR spectra, obtained in the complete proton decoupling mode, give well-separated individual lines. Both cis and trans rotamers are clearly observed and the evolution of the cis ⇄ trans equilibrium can easily be monitored as soon as the crystalline product is dissolved in D_2O.[14b] In the case of *N*-Ac-L-Pro-L-4Hyp (**3**), both cis ⇄ trans interconversions around the two amide bonds

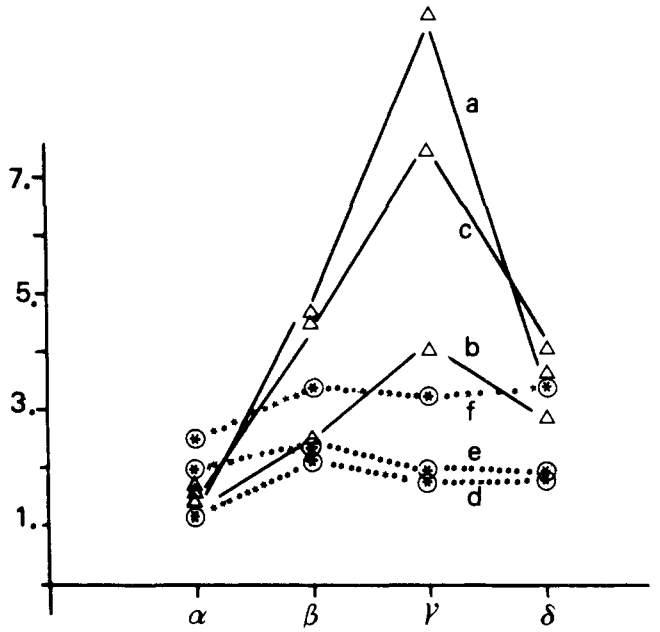


Figure 5. Plot of the $(NT_1 \times \langle B \rangle)$ product vs. the carbon sequence of the rings. Heavy lines: Pro in (a) L-Pro-L-4Hyp; (b) *cyclo*-(L-Pro-L-4Hyp); (c) *N*-Ac-L-Pro-L-4Hyp. Dashed lines: Hyp in the same peptides, (d), (e), and (f), respectively.

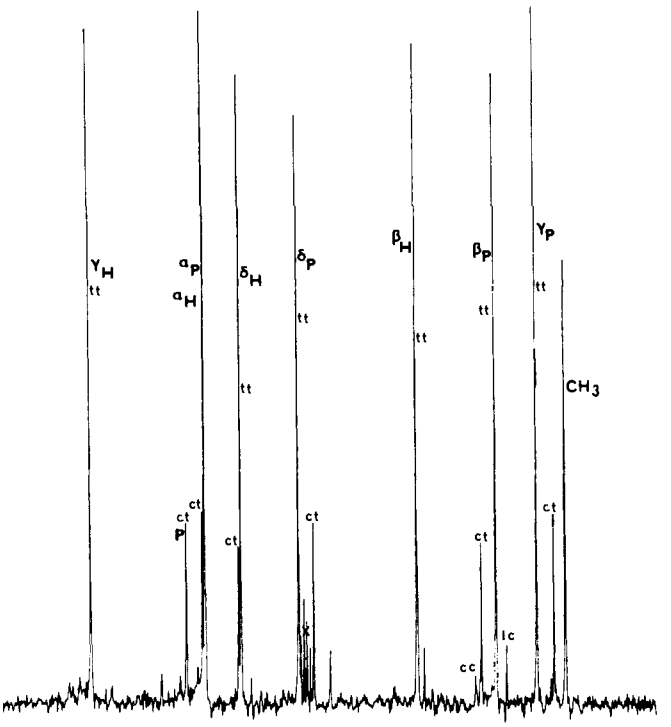


Figure 6. ^{13}C NMR spectrum of *N*-Ac-L-Pro-L-4Hyp (67.89 MHz). H and P refer to L-4Hyp and L-Pro rings, respectively. The spectrum shows for each carbon two major resonances (ct and tt) with additional small peaks (cc and tc), especially for the carbons of the proline residue. The assignment of the two major forms was based on the comparison of the L-4Hyp chemical shifts in *N*-Ac-L-Pro-L-4Hyp (**3**) with those of *N*-Ac-L-Pro (**9**) and L-Pro-L-4Hyp (**2**) (Table IB). The resonances of the two major forms correspond closely with those of the trans form of **2**. Thus, the two major rotamers of **3** (tt and ct) correspond to the trans form around the L-Pro-L-4Hyp peptide bond. The final assignment of these two forms to trans and cis rotamers around the *N*-Ac-L-Pro bond was made by comparison of the L-Pro resonances with those of the dipeptide *N*-Ac-L-Pro (**9**). The intensity ratios and the chemical shifts of **3** (see Table IB) correspond to 20% cis form, named ct, and 70% trans form, named tt, around the *N*-Ac-L-Pro amide bond. The minor forms cc and tc, corresponding to the last 10%, were attributed in a similar way. Thus, it can be observed that in *N*-Ac-L-Pro-L-4Hyp (**3**) and in *N*-Ac-L-Pro (**9**) the cis/trans ratios (20/80) around the Ac-L-Pro amide bond are identical. On the opposite, the cis/trans ratio around the L-Pro-L-4Hyp bond, which is 30/70 for the dipeptide L-Pro-L-4Hyp (**2**), reaches the value 20/80 in the tripeptide *N*-Ac-L-Pro-L-4Hyp (**3**).

are observed and the ^{13}C NMR spectrum is the superposition of four different species (cis-trans, cis-cis, trans-cis, and trans-trans), Figure 6. All the NT_1 values are gathered in Table V. In the case of **3**, only the T_1 values of the two major rotamers (tt and ct) were analyzed and reported in Table V.

In general the correlation time τ^i of protonated carbon atoms is related in a direct way[72] to the relaxation time T_1 provided that overall molecular reorientation is nearly isotropic, as is the case for small molecules. The overall correlation times τ^i_{eff} of C_i ($i = \alpha, \beta, \gamma, \delta, \ldots$) are then derived directly from the ^{13}C T_1's of the different carbons through the expression $\tau^i_{eff} = c/NT_1$ where $c = 4.72 \times 10^{-11}$ s^2 and N is the number of directly bonded hydrogen atoms, τ falling in the extreme narrowing limit ($(\omega_H + \omega_C)^2\tau^2 \ll 1$)).[73] This condition is satisfied since the measured Overhauser enhancements are maximal for the protonated carbons indicating that their relaxation is dominated by dipolar ^{13}C-^1H interaction. The effective correlation time τ^i_{eff} contains contributions from the internal motion of atoms as well as contributions from the overall molecular reorientation. Making the assumption that minimum motion occurs at C^α, τ^α_{eff} is assumed to be equal to τ_{eff}, the molecular tumbling correlation time.

At the same concentration (5×10^{-2} M) in the two conformers of the linear peptides L-Pro-L-4Hyp (**2**) and in the trans-trans (tt) and cis-trans (ct) conformers of *N*-Ac-L-Pro-L-4Hyp (**3**), L-Pro and L-4Hyp ^{13}C T_1's are lying in the same range. These results permit an evaluation of their molecular tumbling correlation time: $8.3 \times 10^{-11} < \tau_{eff} < 1.07 \times 10^{-10}$ s.

In *cyclo*-(L-Pro-L-4Hyp) (**4**), the molecular tumbling correlation time τ_{eff} ($\sim 7 \times 10^{-11}$ s) is close to that of the linear free dipeptide L-Pro-L-4Hyp (**2**) ($\sim 8.7 \times 10^{-11}$ s), consistent with their similar bulkiness. It differs more from the value reported for *cyclo*-(L-Pro)$_2$ and *cyclo*-(L-Pro-D-Pro)[34] ($\tau_{eff} \sim 3 \times 10^{-11}$ s). This is not surprising as the segmental motions of the L-Pro and L-4Hyp residues are very different.

In all the examined peptides including *cyclo*-(L-Pro-L-4Hyp) the τ^i_{eff} of all ring carbons are shorter than the τ^α_{eff}, indicating that changes in internal motion are smaller than changes in overall molecular tumbling. The decreasing difference between τ^α_{eff} and τ^i_{eff} on the L-Pro ring carbons in the series L-Pro-L-4Hyp (**2**), *N*-Ac-L-Pro-L-4Hyp (**3**), *cyclo*-(L-Pro-L-4Hyp) (**4**) is due to the increasing rigidity of the models.

From the effective correlation times τ^i_{eff}, Torchia and Lyerla[74] in the case of prolines derived the expression

$$1/\tau^i_{ring} = 1/\tau^i_{eff} - 1/\tau^\alpha_{eff} \qquad (I)$$

where $\tau^\alpha_{eff} = \tau_{eff}$ is assumed to contain the contribution of backbone motion and τ^i_{ring} the contribution of ring carbon motion, respectively. In proline rings, the calculated τ^i_{ring} values provide evidence for the greatest mobility of C^γ. Owing to the small differences between the NT_1 values of all the ring carbons, including C^α, calculation of the τ^i_{ring} in L-4Hyp residues was considered meaningless.

Using relaxation data analyzed on the basis of several models of pyrrolidine puckering, London[23] recently proposed correlating the NT_1^γ/NT_1^α ratio with the conformational behavior of the proline ring: a ratio $NT_1^\gamma/NT_1^\alpha \sim 1$ is consistent with a single conformation. If the NT_1^γ/NT_1^α ratio exceeds about 2, several puckered forms of the proline ring can be significantly populated.

In the hydroxyproline-containing peptides the ratio $NT_1^\gamma/NT_1^\alpha \sim 1$ is reliable with the existence of a single highly

stabilized conformation. On the contrary, the endo ⇄ exo interconversion of the proline ring in the L-Pro residue of the compounds **2** and **3** is clearly demonstrated by the greater mobility of the C^{γ} and in a lesser extent the C^{δ} carbons.[75] The $NT_1^{\gamma}/NT_1^{\alpha}$ ratios vary from 3.2 and 3.0 in trans and cis L-Pro-L-4Hyp forms, respectively, to 2.1 and 1.8 in trans-trans and cis-trans *N*-Ac-L-Pro-L-4Hyp rotamers. Such a variation is interpreted in two ways. First, a large restriction of internal motion, particularly evidenced for C^{δ} and C^{γ}, is brought about by the acetylation of the dipeptide. Then, the difference observed in $NT_1^{\gamma}/NT_1^{\alpha}$ ratios between the cis-trans (ct) and trans-trans (tt) rotamers in *N*-Ac-L-Pro-L-4Hyp indicates a smaller mobility of the L-Pro ring in the cis form. This effect may be due to a slight increase in the steric interactions which hinder the backbone segmental motion in the latter compound. These results are in accordance with calculations that propose a slightly constrained structure of the triple helix of (Gly-L-Pro-L-4Hyp)$_n$[19] when involving a cis Gly-L-Pro peptide bond.

The analyses of the $\langle B \rangle$ thermal parameters in crystal structures quite agree with the results obtained through ^{13}C data in solution. They clearly show in the proline rings that the C^{γ} atom and C^{β} to a lesser extent have the most important motion in the crystal, when compared to the other atoms of the ring. On the other hand, both $\langle B \rangle$ and τ_{eff} are nearly constant and largely smoothed in the case of the L-4Hyp ring.[76] These data are summarized in Figure 5 for the peptides having Pro and Hyp rings together in the same structure.

The present results may be considered as a general feature for oligopeptides and polypeptides both containing L-4Hyp, as supported by the restrained motion observed in poly-L-4Hyp compared to poly-L-Pro.[74]

In the melting of collagen or of models of this polymer, the mechanism of internal energy dissipation involves activated segmental mobility of the macromolecule. In the polymer containing L-4Hyp, the rigidity brought by this residue could alter the segmental molecular motion by itself and/or by the interaction of the $^{\gamma}$OH group with water, and may explain the increased stability of (Gly-L-4Hyp-L-Pro)$_n$ compared to that of (Gly-L-Pro-L-Pro)$_n$.[8] The same phenomenon, though on a smaller molecular scale, may be generalized to most of the proline-containing oligopeptides.

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Supplementary Material Available: Positional parameters, bond distances and angles, structure factors, and more details about the numeric results (tensor components, moments of inertia, and corrected anisotropic factors) (64 pages). Ordering information is given on any current masthead page.

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BC(DEF) Parameters. 1. The Intrinsic Dimensionality of Intermolecular Interactions in the Liquid State†

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Abstract: An average 95.7% of the variances in six physical properties (aqueous solvation energy, partition coefficient, boiling point, and molar refractivity, volume, and vaporization enthalpy) of 114 diverse pure liquid compounds is a linear function of two "**BC**" parameters characteristic of the compound, and derived by factor (principal components) analysis. The "**BC**" parameters are independent of the data set used in their derivation and can be identified with the "bulk" and "cohesiveness" of an individual molecule. Other physical properties which are well correlated ($r^2 > 0.9$) by the **BC** parameters include magnetic susceptibility, van der Waals' A and B, and critical temperature. Minor "**DEF**" parameters, also derived from the factor analysis, correlate further, to effect slight but significant reductions in the "unexplained" variance of critical pressure, surface tension, log (viscosity), solubility parameter, compressibility, and solvatochromic effects, as well as of the above properties. The "**BC(DEF)**" parameters are well correlated ($r^2 > 0.8$) with all the above 16 properties, except critical pressure, but only moderately correlated ($0.5 < r^2 < 0.8$) with thermal conductivity, dielectric constant, critical pressure, and the unrelated properties dipole moment, melting point, and molecular weight.

A fundamental objective in scientific research is the discovery of unifying relationships among a body of data. Ideally such relationships in chemistry are developed from a theoretical model of molecular behavior, such as the ideal gas law or the Schrodinger equation. However, some useful concepts, for example, the Hammett equation, have a primarily empirical rationale.

Noncovalent inter- and intramolecular forces, while of general chemical interest, are particularly important in the highly organized quasi-liquid structures and phenomena which are so characteristic of living organisms. Thus biochemical researchers have been prominent in demonstrating the unmistakable regularities in the liquid-state properties of molecules, particularly organic ones. Empirical additive-constitutive schemes give a completely adequate accounting of properties as diverse as partition coefficient,[1] boiling point,[2] molar volume,[3] and magnetic susceptibility.[4] Such regularities argue that, despite the potentially complex nature of intermolecular interactions within liquids, it may be that only a few relatively simple types of interaction are actually responsible for the major differences among the properties of compounds. An improved understanding of these regularities should help in the solution of a variety of biochemical problems, including the very practical challenges of drug design.[5]

Of the many mathematical methods which have been proposed for seeking regularities in chemical data,[6] the approach which best reveals the *intrinsic* linear structure of a data set is factor analysis,[7] particularly its subset methods such as principal components analysis or the Karhunen–Loeve transform. These techniques, first developed and long used in psychometrics, have recently been applied in chemical contexts to the derivation of substituent constants,[8] NMR shifts,[9] odor perception,[10] and structure/biological potency correlation.[11] Weiner has considered liquid-state properties as possible fundamental descriptors for sets of factorizable chromatographic data,[12a] and the partition coefficients of various solutes in various solvents have been factored by several groups.[12b] However, the factorization of an extremely varied set of liquid-state properties does not seem to have been attempted previously.

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