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Conformational Studies of Oligopeptides Containing Proline and Glycine[1]

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ABSTRACT: Nuclear magnetic resonance, infrared, and circular dichroism methods were used to study the conformational properties of *N*-acetyl-*N'*-ethylprolineamide (*blocked* Pro single residue), *N*-acetyl-*N'*-methylglycineamide (*blocked* Gly single residue), *N*-acetyl-*N'*-methylglycylprolineamide (*blocked* Gly-Pro), and *N*-acetyl-*N'*-methylprolylglycineamide (*blocked* Pro-Gly) in various solvents. It was found that all four peptide molecules exist in solution at 29 °C as an ensemble of several conformations. In the blocked Pro single residue, the acetyl-Pro peptide bond was found to exist in both the cis and the trans forms, with $\Delta G^\circ_{trans \rightarrow cis} \approx 0.7$ kcal/mol in CD_2Cl_2 and ≈0.4 kcal/mol in DMSO at 29 °C. In the trans form, the hydrogen-bonded conformation (C_7^{eq}, with $\psi_{Pro} \approx 80°$) and nonhydrogen-bonded conformations (with ψ_{Pro} being unidentified) coexist. The blocked Gly single residue was found to exist as a flexible molecule, with the C_7 and other conformations present; no cis–trans isomerism was observed. Blocked Gly-Pro showed cis–trans isomerism about the Gly-Pro peptide bond with $\Delta G^\circ_{trans \rightarrow cis} \approx 0.7$ kcal/mol in DMSO and ≈1.5 kcal/mol in CD_2Cl_2 at 29 °C. The Gly residue in blocked Gly-Pro was found to exist, to a large extent, in the fully extended (C_5) structure, with the Pro residue in Gly-Pro assuming the C_7^{eq} conformation as well as other (nonhydrogen-bonded) conformations. No evidence for large amounts of β bend was detected in Gly-Pro. The temperature dependence of the NMR and CD data of blocked Gly-Pro in water was found to be similar to that of poly(Gly-Pro), indicating that the conformational properties of the polymer can be attributed to local interactions (i.e., those within the dipeptide unit). The predominant conformations of blocked Pro-Gly were found to contain a 4→1 hydrogen bond; the molecules also showed restricted segmental motion of the Gly residue. These observations indicate the presence of β bends (along with other conformations). The experimentally determined properties of the four peptide molecules were compared with those determined from previous theoretical studies using empirical conformational energy calculations and, with some exceptions, were found to be in good agreement.

The hypothesis has been presented[3,4] that the conformation of a given sequence of amino acids in a protein is determined largely, but not exclusively, by intra-residue interactions. If this hypothesis is correct, a short sequence of amino acid residues in a globular protein should exist in a conformation similar to one or more of the stable structures of the same amino acid sequence in an oligomer in solution. This is the basis for using random copolymers for determining the tendency of single amino acid residues to adopt the α-helical conformation[5] and for studying short peptides to determine their tendency toward formation of chain reversal (bend) conformations in proteins.[6–13] In this paper, we examine oligopeptides (specifically, *blocked* single residues and *blocked* dipeptides) containing proline and glycine. The α-amino and α-carboxyl groups are blocked to eliminate charge effects and to simulate the environment of the *poly*peptide chain. Earlier studies[14–16] have treated *unblocked* oligopeptides of proline and glycine, where the peptide molecules are ionizable. The proline and glycine residues are of interest in protein structure because they are found frequently in β-bend conformations, Pro-Gly having a high propensity for forming a bend but Gly-Pro having a very low one.[13,17,18] Proline is of special interest because cis conformations have been observed in peptide bonds preceding Pro in small molecules[14–16,19–25] and in proteins.[26]

The purposes of this paper are to employ proton nuclear magnetic resonance (NMR), infrared (IR), and circular dichroism (CD) methods to: (a) determine the preferred conformations (including possible cis and trans peptide forms) of the *N*-acetyl-*N'*-ethylamide of Pro and the *N*-acetyl-*N'*-methylamides of Gly, Gly-Pro, and Pro-Gly; (b) compare the calculated structures of the *N*-acetyl-*N'*-methylamides of Gly,[27] Pro,[27] Gly-Pro,[28,29] and Pro-Gly[28,29] with those observed experimentally; (c) determine the influence of solvent

Table I
NMR Data for Blocked Oligopeptides

Peptide	Solvent	Temp, °C	Chemcial shift, δ ppm					
			NH_{Gly}	$C^{\alpha}H_{Pro}$	$C^{\alpha}H_{Gly}$	CH_3CO	$NHCH_3$[a]	$NHCH_3$[b]
Pro	DMSO-d_6	29		4.15 (d)		1.79 (s)	7.64 (t)	0.97 (t)
				4.24 (d)		1.94 (s)	7.94 (t)	0.99 (t)
		64		4.17 (d)		1.94 (s)	7.78 (t)	0.98
							7.50 (t)	
	CD_2Cl_2	−21		4.39 (d)		2.07 (s)	7.19 (t)	1.07 (t)
				4.23 (q)		1.97 (s)		1.10 (t)
		29		4.41 (d)		2.04 (s)	6.93 (t)	1.07 (t)
				4.19 (q)	1.95 (s)	6.57 (t)	1.10 (t)	
Gly	DMSO-d_6	29	7.98 (t)		3.56 (d)	1.84 (s)	7.65 (q)	2.56 (d)
		64	7.76 (t)		3.56 (d)	1.84 (s)	7.43 (q)	2.56 (d)
	CD_2Cl_2		Too insoluble for NMR experiments					
Gly-Pro	DMSO-d_6	29	7.96 (t)	4.37 (q)	3.88 (d)	1.86 (s)	7.74 (q)	2.54 (d)
				4.22 (d)	3.75 (d)			2.60 (d)
		67	7.67 (t)	4.26 (t)[c]	3.84 (d)	1.85 (s)	7.50 (q)	2.55 (d)[c]
				~4.33 (q)	~3.76 (d)			~2.59 (d)
	CD_2Cl_2	−23	6.7[c]	4.22 (d)	3.78 (q)	1.80 (s)	6.7[c]	2.50 (d)
				4.02 (q)	3.55 (q)			2.58 (d)
		29	6.4[c]	4.41 (d)	3.95 (d)	1.97 (s)	6.4[c]	2.76 (d)
				4.22 (q)	3.78 (q)	1.69 (s)		2.70 (d)
Pro-Gly	DMSO-d_6	29	8.20 (t)	4.28 (q)	3.57 (d)	2.00 (s)[d]	7.78 (q)	2.55 (d)
				4.13 (t)			7.52 (q)	2.48 (d)
		64	8.02 (t)	4.20 (t)	3.59 (d)	1.99 (s)	7.69 (q)	2.58 (d)
							7.43 (q)	
	CD_2Cl_2	−51	7.93 (t)	4.18 (t)	3.72 (q)	2.09 (s)	7.59 (q)	2.67 (t)
		29	7.02[c]	4.24 (t)	3.78 (p)	2.07 (s)	7.02[c]	2.69 (d)
				3.95 (q)		1.89 (s)		2.73 (d)

[a] In the proline single residue, this column refers to the $NHCH_2CH_3$ proton. [b] In the proline single residue, this column refers to the $NHCH_2CH_3$ protons. [c] This resonance partially overlaps with the other resonance indicated. [d] This broad acetyl resonance (90 MHz) was shown to contain an upfield component (separated by 0.04 ppm) in the resolution enhanced correlation spectra obtained with a 250-MHz NMR spectrometer.

on the conformational properties of oligopeptides; and (d) determine the influence of local and long-range interactions on proline-containing oligo- and polypeptides.

Methods

A description of the syntheses and experimental methods is given in the Appendix.

Conformational Energy Calculations. The nomenclature, conventions, definitions, and methods of conformational energy calculations are those used earlier.[27-30] Rotations about the peptide bond between the N-terminal acetyl group ("residue" 1) and the first full residue (residue 2) are described by ω_1; rotations about the peptide bond following the first *full* residue are described by ω_2; those following the second residue by ω_3; and so on. Values of ω near 0° and 180° are referred to as cis and trans, respectively.

An energy contour diagram was drawn for ψ_{Gly} vs. ψ_{Pro} in blocked Pro-Gly by calculating the total conformational energy at 5° intervals over the range $0° \leq \psi_{Pro} \leq 180°$ and $-180° \leq \psi_{Gly} \leq 180°$ and then, by interpolation, determining lines of constant energy at 0.5-kcal/mol increments above the global minimum.

Results and Discussion

NMR spectra were determined for *N*-acetyl-*N'*-ethylprolineamide (blocked Pro), *N*-acetyl-*N'*-methylglycineamide (blocked Gly), and the *N*-acetyl-*N'*-methylamides of Gly-Pro and Pro-Gly, primarily in the deuterated solvents dimethyl sulfoxide (DMSO-d_6) and methylene chloride (CD_2Cl_2), at various temperatures. Some representative NMR results are shown in Table I.

The assignments of the resonances were made by comparing the spectra obtained here to published spectra[19-21,24,25] for the same amino acid residues in similar solvents and by use of coupling patterns, area ratios, and decoupling experiments.

Cis–trans isomerism about the X-Pro peptide bond in blocked Pro, blocked Gly-Pro, and blocked Pro-Gly was detected and measured quantitatively by the presence of, and the relative areas under, the doubled proton resonances,[19-21,24,25] particularly the Pro $C^{\alpha}H$ and the Gly $C^{\alpha}H$ or the end group CH_3CO resonances. Other doubled proton resonances also were used. For example, in Figure 1, the cis–trans ratio at 29 °C was determined by measuring the relative areas under the doubled NH resonance, where the upfield component was assigned to the trans form in DMSO-d_6 and the downfield component was assigned to the trans form in CD_2Cl_2. No doubling of resonances, and hence no cis–trans isomerism, was detected in blocked Gly. Table II gives the percent cis and the free energy difference between cis and trans, $\Delta G°_{trans \rightarrow cis}$, for the peptide molecules in various solvents at 29 °C. Table II also contains the values of $\Delta G°_{trans \rightarrow cis}$ as determined by conformational energy calculations.[27-29]

It is assumed, based on prior experimental[19] and theoretical[28] results, that the proportion of cis isomer of the peptide group *following* the proline ring is vanishingly small.

***N*-Acetyl-*N'*-ethylprolineamide. Cis–Trans Isomerism.** The coalescence of the CH_3CO resonance and of the $C^{\alpha}H$ resonance as the temperature is raised from 29 to ≈64 °C in DMSO-d_6 (see Table I) indicates, according to the method of Shanan-Atidi and Bar-Eli,[31] that the energy barrier between the cis and trans forms in the Pro single residue is approximately 18 kcal/mol, in agreement with cis–trans barriers in small peptides estimated previously.[19,21,25] Although the CH_3CO and $C^{\alpha}H$ resonances coalesce as the temperature is raised from 29 to 64 °C, the $NHCH_2CH_3$ resonance is still doubled at 64 °C because the *initial* difference (at 29 °C) in chemical shift between the two NH peaks is larger than that

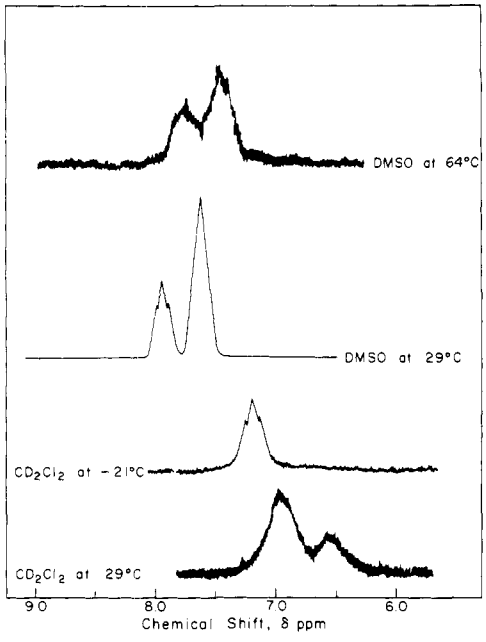

Figure 1. The NH region of the 90-MHz ^1H NMR spectrum of *N*-acetyl-*N'*-ethylprolineamide in dimethyl sulfoxide-d_6 (DMSO) and methylene chloride at the temperatures indicated. The chemical shifts are given in ppm with respect to the internal standard TMS. Spectra at different temperatures were obtained at different amplifications.

between the two CH_3CO or $C^{\alpha}H$ peaks. An estimate of the cis–trans barrier cannot be obtained in CD_2Cl_2 because of its low boiling point (40 °C), well below the expected coalescence temperature.

Upon lowering the temperature to −21 °C in CD_2Cl_2, the resonances of the CH_3CO and $C^{\alpha}H$ protons remain doubled, suggesting that both cis and trans are still present. The amount of cis (25%) is approximately the same at −21 °C as it was at 29 °C. Because the energy barrier between the cis and trans forms is high (18–20 kcal/mol), it is unlikely that the two forms are in equilibrium at the lower temperature, as was assumed at 29 °C, but rather that the relative amounts of the cis and trans forms are "frozen" into the approximate amounts found at 29 °C. Therefore, the value of $\Delta G^{\circ}_{\text{trans}\rightarrow\text{cis}}$ at −21 °C cannot be estimated.

At −21 °C in CD_2Cl_2, the $NHCH_2CH_3$ resonance is no longer doubled (see Figure 1). The NH group is shielded from the solvent, presumably because of hydrogen bonding, when the acetyl-Pro peptide bond is trans but not when the peptide bond is cis, giving rise to a reduced temperature coefficient of -5.1×10^{-3} ppm/deg for the trans form, compared with -8.9×10^{-3} ppm/deg for the cis form. Therefore, as the temperature is lowered, the peak corresponding to the cis form moves downfield at a greater rate than that of the trans form, until the two coalesce. It should be noted that the temperature coefficients evidently are not constant over the entire temperature range; otherwise, complete coalescence would not be expected to occur until about −70 °C.

The CD spectra of *N*-acetyl-*N'*-ethylprolineamide in water and dioxane (Figure 2) are similar to published spectra for *N*-acetyl-*N'*-methylprolineamide[19,22,23] in the same or related solvents. (Unfortunately, CD data cannot be obtained in DMSO nor in CD_2Cl_2 because these solvents absorb in the important wavelength region below 250 nm.) The fact that the temperature dependence is small supports the conclusion that there exists a statistical ensemble of conformations in water, and the crossover point near 213 nm (i.e., a point at which the ellipticities are equal for different temperatures) indicates the presence of cis–trans isomerism; see the discussion on Gly-Pro below. Although there is no crossover point for the Pro single residue in dioxane (Figure 2), it is still likely (based on NMR data) that cis–trans isomerism also occurs in this solvent.

Table II
Cis–Trans Isomerism in Peptide Bonds *Preceding* Proline in Blocked Oligopeptides

Peptide[a]	Solvent	% cis (exptl)[b]	$\Delta G^{\circ}_{\text{trans}\rightarrow\text{cis}}$, kcal/mol Exptl[b]	Calcd[c]
Pro	DMSO	33	0.4	
	Dioxane	29	0.5	
	CD_2Cl_2	25	0.7	
	D_2O	24	0.7	
				1.87
Gly-Pro	DMSO	25	0.7	
	D_2O	19	0.9	
	MeOD	17	1.0	
	CD_2Cl_2	9	1.5	
				1.87
Pro-Cly[d]	DMSO	22	0.8	
	CD_2Cl_2	<10	>1.5	

[a] The peptides are *N*-acetyl-*N'*-ethylprolineamide (listed as Pro), *N*-acetyl-*N'*-methylglycylprolineamide (Gly-Pro), and *N*-acetyl-*N'*-methylprolylglycineamide (Pro-Gly). The calculation on blocked Pro was performed on the *N*-acetyl-*N'*-*methyl*amide. [b] Experiments were carried out at 29 °C. [c] Calculations were carried out using T = 300 K. See ref 28 and 29. [d] The relative amount of cis in blocked Pro-Gly was not calculated.[29]

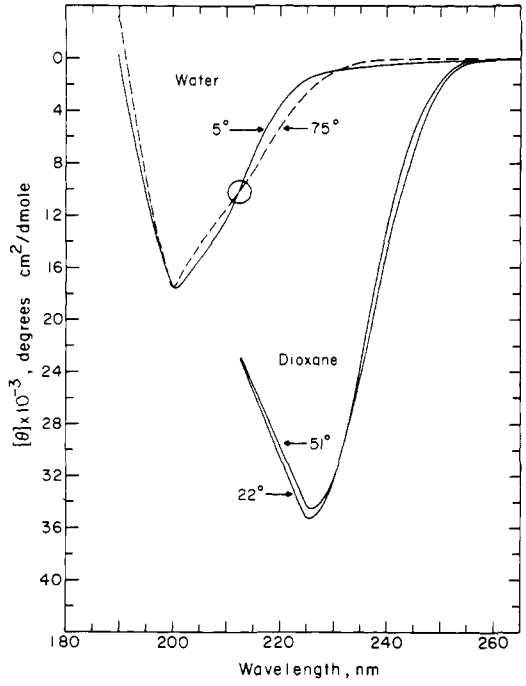

Figure 2. CD spectra of *N*-acetyl-*N'*-ethylprolineamide in dioxane at 22 and 51 °C (2.2×10^{-3} M) and in water at 5 and 75 °C (6.3×10^{-4} M). These curves were independent of concentration over a tenfold range in these solvents; the values of concentration, quoted here, are the upper limit of this concentration range. The circle on the spectra in water shows the location of a crossover point. The curves in dioxane do not cross.

The C_7^{eq} Conformation. Theoretical work[27–29,32] suggests several possible values of ψ_{Pro} for trans peptide groups. One of these, $\psi_{Pro} \approx 80°$, yields a C_7^{eq} conformation (Figure 3A) in which the N*H* proton of the peptide group *following* the proline ring, i.e., the $NHCH_2CH_3$ proton in *N*-acetyl-*N'*-ethylprolineamide, is hydrogen bonded to the carbonyl oxygen of the peptide group preceding the proline ring, i.e., the CH_3CO oxygen of the *N*-acetyl blocking group. Other possible

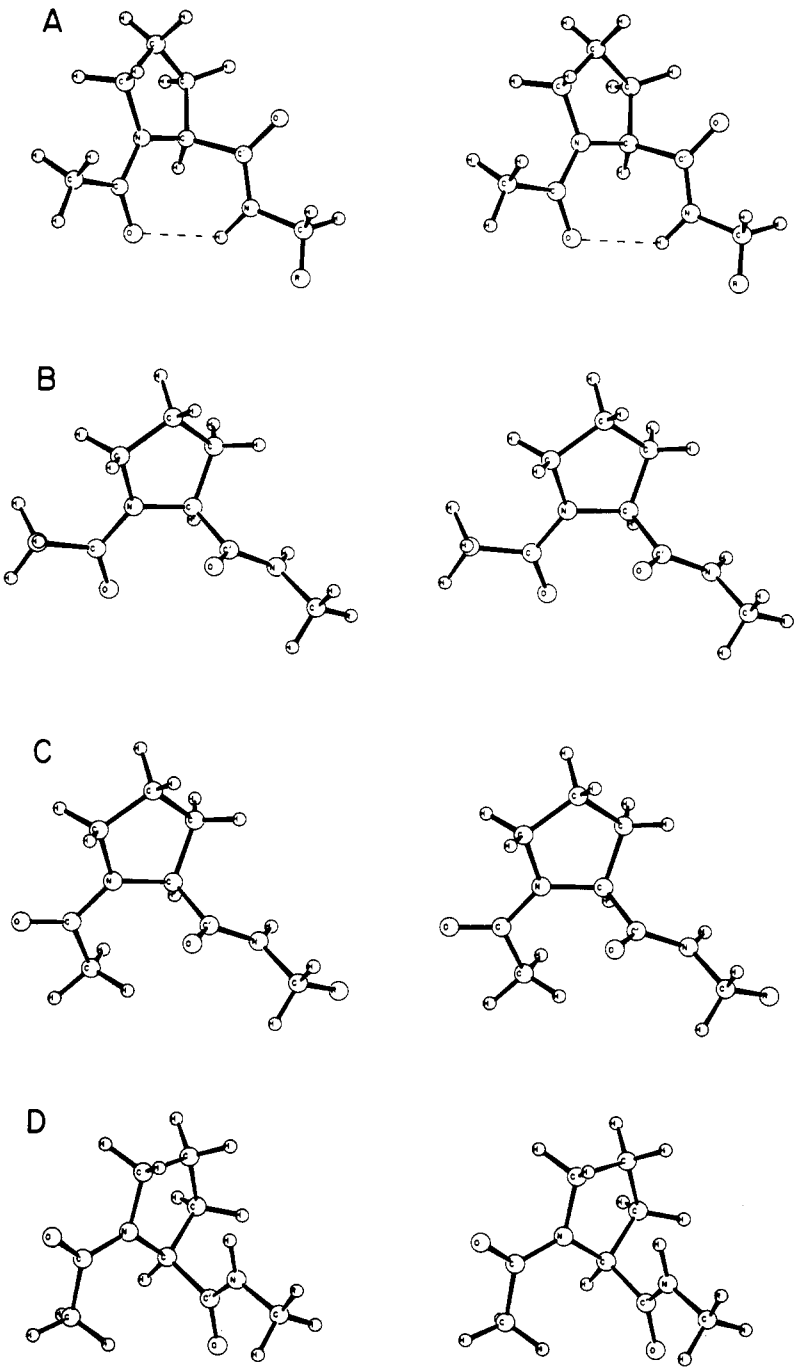

Figure 3. Stereo diagrams of several minimum-energy conformations of *N*-acetyl-*N'*-ethylprolineamide. Cis and trans refer to conformations involving ω_1 (which describes rotations about the acetyl-proline peptide bond); $\omega_1 \approx 180°$ is trans and $\omega_1 \approx 0°$ is cis. (A) A trans C_7^{eq} conformation with $(\omega_1, \phi_2, \psi_2, \omega_2) = 178°, -75°, 79°, 180°$); (B) a trans conformation with dihedral angles $(\omega_1, \phi_2, \psi_2, \omega_2) = 180°, -75°, 159°, 180°$); (C) a cis conformation for $(\omega_1, \phi_2, \psi_2, \omega_2) = (-5°, -75°, -48°, 180°)$; and (D) a cis conformation with $(\omega_1, \phi_2, \psi_2, \omega_2) = (-4°, -75°, 162°, 180°)$.

conformations arising from rotation about the C^{α}–C′ bond are[25-27,32] $\psi_{Pro} \approx -50°$ and $\approx 160°$ (Figure 3B). These cannot involve an intramolecular hydrogen bond and, therefore, are distinguishable from $\psi_{Pro} \approx 80°$ by NMR and IR spectroscopy, which can detect hydrogen bonding. In fact, the NMR and IR data do suggest conformational isomerism involving ψ of the Pro single residue. In DMSO at 29 °C, for example, the NH proton gives rise to a doubled resonance (Figure 1), presumably from the cis–trans isomerism involving ω_1. The two N*H* peaks behave differently as the temperature is varied, the one from the cis conformation at δ 7.94 at 29 °C (see Figure 1) having a temperature coefficient of -5.3×10^{-3} ppm/deg and the one from the trans conformation at δ 7.64 at 29 °C a temperature coefficient of -4.6×10^{-3} ppm/deg. We now make the admittedly simplistic assumptions that hydrogen-bonded and nonhydrogen-bonded NH groups have temperature coefficients[33] of -2×10^{-3} and -6×10^{-3} ppm/deg, respectively, and that the amount of hydrogen-bond character can be determined from the observed temperature coefficient by a linear interpolation between these values. Of course, a reduced temperature coefficient also can arise because of shielding from the solvent *without* the presence of a hydrogen bond, but, with peptides of these small sizes in which shielding of protons most likely would result from hydrogen bonding, we will assume that a reduced temperature coefficient implies the presence of hydrogen-bonded conformations. The observed -4.6×10^{-3} ppm/deg, therefore, suggests roughly 35% hydrogen-bond character for the NH group following Pro when $\omega_1 \approx 180°$ (trans), presumably arising from the C_7^{eq} conformation (Figure 3A), corresponding to $\psi_{Pro} \approx 80°$. Since

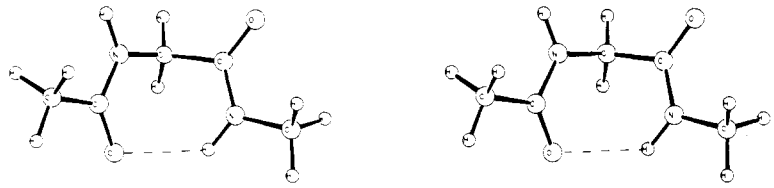

Figure 4. Stereo diagram of *N*-acetyl-*N'*-methylglycineamide showing a C_7^{eq} conformation and hydrogen bond (dashed line). The dihedral angles of Gly in this conformation are $(\phi, \psi) = (-83°, 76°)$.

the C_7^{eq} hydrogen bond leaves the NH proton only *partially* shielded from the solvent, the actual occurrence of the C_7^{eq} conformation may be somewhat higher than 35%. Although the temperature coefficient is -5.3×10^{-3} ppm/deg for the NH group following Pro when $\omega_1 \approx 0°$ (cis), the cis conformation cannot have a hydrogen-bonded NH group for steric reasons. The *partial* hydrogen-bond character of the $NHCH_2CH_3$ proton in the trans isomer implies the presence also of nonhydrogen-bonded structures, in which ψ_{Pro} must differ from 80° (presumably,[27-29,32] with $\psi_{Pro} = -50$ and 160°). This indicates that isomerism involving ψ_{Pro} takes place.

The existence of a hydrogen bond in the blocked Pro single residue is indicated further by its IR spectrum in DMSO (not shown here). A weak NH band at 3280 cm^{-1} and a band at 1650 cm^{-1} in the amide I region, at ~25 °C, suggest the presence of a hydrogen bond, with bands at 3450 and 1685 cm^{-1} arising from nonhydrogen-bonded conformations. Other possible interpretations of the existence of two bands in the amide I region are that the amide and imide groups give rise to different bands or that the cis and trans forms cause separate IR bands. However, because of the evidence for hydrogen bonding from other regions of the IR spectrum and from NMR temperature coefficients, we favor the assignment of the two bands in the amide I region to hydrogen-bonded and nonhydrogen-bonded conformations, not only for blocked Pro in DMSO but also for blocked Pro in CD_2Cl_2 and for other peptides in these two solvents (see below).

The C_7^{eq} conformation of the Pro single residue also appears to be present in CD_2Cl_2. The NMR spectrum in Figure 1 displays an $NHCH_2CH_3$ doubled resonance arising from the cis–trans isomerism, with a temperature coefficient of -5.1×10^{-3} and of -8.9×10^{-3} ppm/deg for the peaks which, at 29 °C, appear at δ 6.93 and 6.57, respectively. Unfortunately, the percent hydrogen-bond character of the NH protons cannot be estimated from these data since temperature coefficients in CD_2Cl_2 have not been related quantitatively to hydrogen bonding, as is the case in DMSO, but it is clear that the peak at δ 6.93 at 29 °C, which must correspond to the trans isomer (because the cis form cannot form a hydrogen bond), has a significantly reduced temperature coefficient indicative of partial hydrogen-bond character.

The IR spectrum at ~25 °C in CD_2Cl_2 (not shown here) shows the major NH band at 3435 cm^{-1}, characteristic of a nonhydrogen-bonded NH, and the minor (broader) band at 3313 cm^{-1} characteristic of a hydrogen-bonded group. The hydrogen bond also gives rise to a band at 1635 cm^{-1} near the (nonhydrogen-bonded) amide I band at 1672 cm^{-1}. These IR results are very similar to those for *N*-acetyl-*N'*-methylprolineamide[34] in CD_2Cl_2 and to those for *N*-acetyl-*N'*-ethylprolineamide,[35,36] with the exception that the 3446-cm^{-1} NH absorbance reported by Avignon[36] could be removed here by column chromatography (on Fluorosil) as *N*-methylacetamide, an impurity which arises during synthesis. The data, which imply the presence of a hydrogen bond, therefore suggest that the C_7^{eq} conformation is present in CD_2Cl_2.

Comparison with Calculations. The following comparisons can be made between these experimental results and previously published conformational energy calculations.[27] Although the experiments were carried out on *N*-acetyl-*N'-ethyl*prolineamide while the calculations were performed on *N*-acetyl-*N'-methyl*prolineamide, we assume that the differences between the ethyl and methyl end groups are negligible with respect to the conformational properties of the proline residue.

(1) The free energy difference, $\Delta G°_{trans \rightarrow cis}$, for isomerism about a peptide bond can be determined using data on minimum energy conformations (i.e., those in ref 27) and employing the method described in an earlier paper.[28] The peptide group preceding the pyrrolidine ring in *N*-acetyl-*N'*-methylprolineamide[27] is calculated to be 4.2% cis, which corresponds to $\Delta G°_{trans \rightarrow cis} = 1.87$ kcal/mol at $T = 300$ K (Table II). The experimental values of $\Delta G°_{trans \rightarrow cis}$ given in Table II for *N*-acetyl-*N'*-ethylprolineamide are ≈0.7 kcal/mol in CD_2Cl_2 and ≈0.4 kcal/mol in DMSO. It was concluded from the calculations[28] that $\Delta G°_{trans \rightarrow cis}$ of *N*-substituted peptides should decrease as the dielectric constant increases, since electrostatic repulsions destabilize the cis form. Hence, it is not unexpected that the calculated value of $\Delta G°_{trans \rightarrow cis}$, determined with an effective dielectric constant of 4,[30] is higher than the experimentally determined value in CD_2Cl_2 (dielectric constant of 8.93 at 25 °C), which is, in turn, higher than that in DMSO (dielectric constant of 46.68 at 25 °C). Hydrogen-bonded and nonhydrogen-bonded trans conformations are depicted in Figures 3A and 3B, respectively, and two different (nonhydrogen-bonded) cis conformations are shown in Figures 3C and 3D.

(2) An important similarity between the experimental and theoretical results is the presence of *several* stable conformations. Theory and experiment, therefore, show that the solution properties of Pro are a result of an ensemble of low-energy structures, not just one stable structure. Several conformations which may exist in solution are shown in Figure 3.

(3) The calculations and the experiments qualitatively agree that the C_7^{eq} conformation is one of the major low-energy structures (Figure 3A) of the Pro single residue.

***N*-Acetyl-*N'*-methylglycineamide. The C_7 conformation.** In DMSO, the temperature coefficients for the N*H* and $NHCH_3$ protons in blocked Gly (Table I) are -6.1×10^{-3} and -4.9×10^{-3} ppm/deg, respectively, suggesting that the $NHCH_3$ (but not the Gly N*H*) proton has partial (≈ 28%) hydrogen-bond character. This implies the presence of the C_7 conformation. [The C_7^{eq} conformation (shown in Figure 4) and its mirror image (in blocked Gly), the C_7^{ax} conformation, are equally probable. Unless otherwise specified, the term C_7 refers both to C_7^{eq} and to C_7^{ax} in blocked Gly but only to C_7^{eq} in blocked Pro since C_7^{ax} ($\phi, \psi \approx 80, -80°$) cannot exist because the proline ring fixes ϕ at ca. −75°.] Even though the Gly N*H* resonance does not show a lowered temperature coefficient, it is still possible that a significant amount of C_5 conformation is present, since the C_5 hydrogen bond is very nonlinear and its donor (NH) proton is quite exposed to the solvent.

The IR spectrum of blocked Gly in DMSO at 25 °C also indicates the presence of a hydrogen bond and hence the presence of the C_7 and/or C_5 conformation. A small shoulder at 3250 cm^{-1} on a strong, broad band centered at about 3440 cm^{-1} is interpreted as arising from a hydrogen-bonded NH

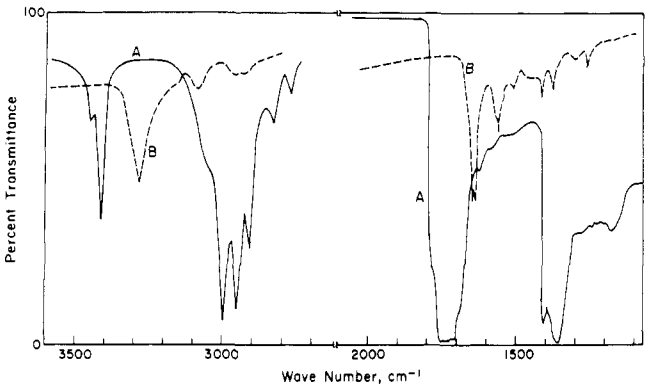

Figure 5. Infrared spectra of *N*-acetyl-*N'*-methylglycineamide, A (in CCl_4, at ~25 °C), and B (sublimed onto a KBr plate, and measured at ~25 °C).

group. The hydrogen bonding is evidently weak, however, since no shoulder is observed on the amide I band in the 1650–1685-cm^{-1} region nor on the amide II band in the 1500–1550-cm^{-1} region.

Although Gly is not sufficiently soluble in most nonpolar solvents to determine its NMR spectrum, its IR spectrum in CCl_4 had been determined by Avignon and Huong.[37] In an attempt to reproduce their work, we found their bands at 3461 and 3412 cm^{-1}; however, we could not detect the reported[37] band at 3360 cm^{-1} (Figure 5A). A study of the origin of this discrepancy has revealed[38] that the 3360-cm^{-1} band is due to impurities resulting from decomposition of the peptide in CCl_4. The IR spectrum of blocked Gly in the solid state is shown in Figure 5B and has bands at 3293 and 1642 cm^{-1} characteristic of hydrogen-bonded peptide groups. These hydrogen bonds, however, may be *inter-* rather than *intra*molecular and therefore tell us nothing about the conformation of the Gly residue. We have made no further effort to verify the existence of the C_7 conformation in *nonpolar* solvents.

Other Conformations. There are two pieces of evidence which indicate that other conformations, besides the C_7, are present in solutions of the blocked Gly single residue. First, the coupling constant $J_{C^\alpha H-NH}$ for blocked Gly in DMSO is 4.2 Hz, which corresponds (according to Bystrov et al.[39]) to $\langle\phi^2\rangle^{1/2} \approx 180°$, where the brackets indicate an average. If the C_7 structure (with $\phi, \psi \approx \mp 80, \pm 80°$) were the *only* conformation present in solution, the measured coupling constant would be consistent with $\phi \approx \mp 80°$, i.e., $\langle\phi^2\rangle^{1/2} \approx 80°$. Other conformations, therefore, must be present to yield the observed $\langle\phi^2\rangle^{1/2} \approx 180°$. The second piece of evidence for the presence of conformations other than the C_7 is that the N*H*CH_3 proton is only partially hydrogen bonded (as mentioned above), and therefore, some conformations not containing a (C_7) hydrogen bond also must be present. It is not possible to determine directly from the experimental data the values of ϕ and ψ in the nonhydrogen-bonded structures, but, as will be seen in the following paragraph, the experimental data are consistent with the calculated low-energy minima.[27]

Comparison with Calculations. Calculations on the Gly single residue[27] suggest that (a) no appreciable cis–trans isomerism can take place,[28] (b) Gly is very flexible, as indicated by the relatively flat conformational energy map,[27] (c) the C_7 conformation contributes significantly to the ensemble of conformations,[27] and (d) from the energies and dihedral angles at the minima we obtain a Boltzmann average value of $\langle\phi^2\rangle^{1/2} \simeq 160°$. All of these theoretical results are consistent with the experimental data presented in this paper. (The calculated and experimental values of $\langle\phi^2\rangle^{1/2}$ agree within experimental error.) Although the C_5 conformation (fully extended) is calculated to be the conformation of lowest free energy,[27] we do not expect to be able to detect the C_5 hydrogen bond from a lowering of the NMR temperature coefficient of the Gly NH proton since the proton would still be somewhat exposed to the solvent, and we obtain a calculated fraction of the C_5 conformation of only 21%. On the other hand, the combined fraction for the two C_7 conformations (C_7^{eq} and C_7^{ax}, which are mirror images in blocked Gly) is slightly higher, 27%, and the NH proton is shielded more from the solvent. The experimental results, therefore, are consistent with the interpretation that both the C_7 and the C_5 (as well as other conformations) are present in solution.

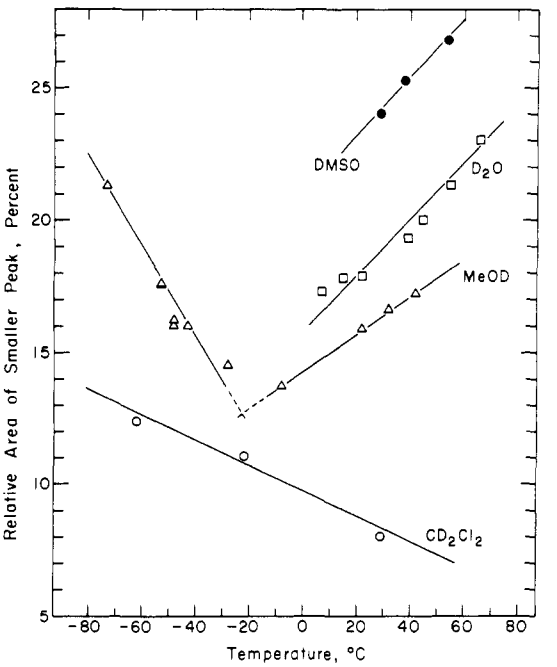

Figure 6. Temperature dependence of the ratio of peak areas for the resonances from the N*H*CH_3 protons of *N*-acetyl-*N'*-methylglycylprolineamide in four solvents.

***N*-Acetyl-*N'*-methylglycylprolineamide. Cis–Trans Isomerism.** Figure 6 shows a plot of the relative area of the smaller peak with respect to the total area of the two peaks of the doubled N*H*CH_3 resonance in blocked Gly-Pro as a function of temperature. Over most of the temperature range (see below), the doubling of the N*H*CH_3 resonance can be attributed to cis–trans isomerism, since the relative areas under the Pro $C^\alpha H$ or Gly $C^\alpha H$ peaks, which we use in Table II to measure the percent cis form, are approximately the same as the relative areas under the N*H*CH_3 peaks, in all these solvents and at temperatures greater than −15 °C.

In DMSO and in D_2O, the upfield peak is the trans isomer,[21] and we assume that the upfield peak (which is the larger of the two) is the trans form in MeOD at temperatures above −15 °C. In these polar solvents, the proportion of cis isomer increases with increasing temperature in the range above −15 °C. Evidently, the higher temperatures allow increased population of the higher energy cis form. Below −15 °C in MeOD, and over the entire temperature range in CD_2Cl_2, the relative area of the smaller peak increases with *decreasing* temperature. (DMSO and D_2O, of course, freeze at these low temperatures.) We do not understand the origin of this inverted temperature dependence at low temperatures. One possible interpretation is that, in the *higher* temperature range, the cis and trans forms are in equilibrium, since the barrier to rotation about the peptide bond can be surmounted. Cis–trans isomerism dominates the resonance of the N*H*CH_3 protons of the molecule at these *high* temperatures, although rotations about other bonds take place, e.g., variation of $\phi_{Gly}, \psi_{Gly}, \psi_{Pro}$.

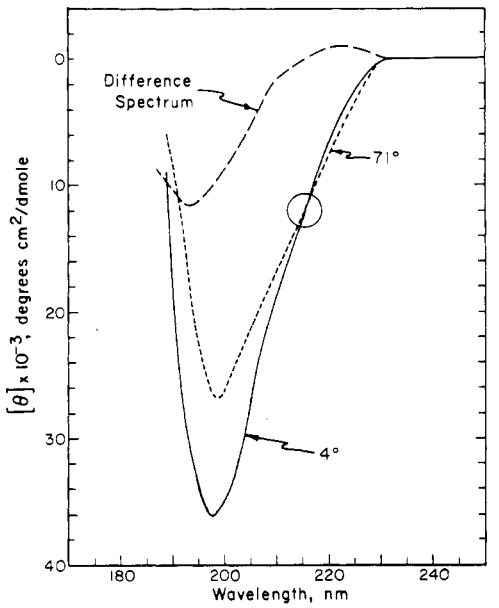

Figure 7. CD spectra of *N*-acetyl-*N'*-methylglycylprolineamide (3.6×10^{-4} M) in H_2O at 4 and 71 °C. The dashed curve is the difference spectrum between the 4 and 71 °C spectra obtained by subtracting one spectrum from the other. No concentration dependence was observed for $[\theta]$. The circle shows the location of a crossover point.

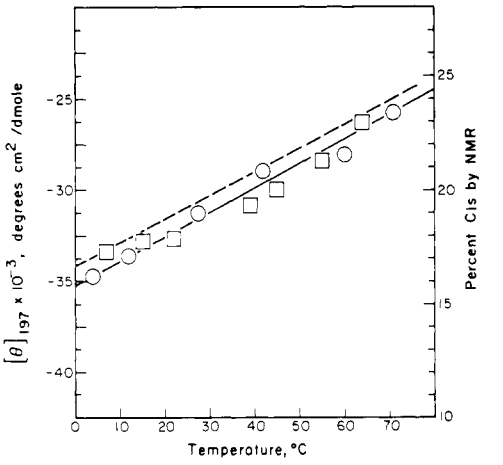

Figure 8. Plot of the ellipticity (○) at the minimum, $[\theta]_{197}$, and the percent of cis isomer, as determined from NMR (□), as a function of temperature in water for *N*-acetyl-*N'*-methylglycylprolineamide. The solid line is the best fit to the circles and squares. The dashed line represents the NMR data of Torchia[21] for poly(Gly-Pro) of molecular weight 13 200 in H_2O–CH_3COOH (99.5:0.5).

At *low* temperatures, on the other hand, variations of ω_2 cease, because the 18–20-kcal/mol barrier is too high, and the conformational isomers are no longer in equilibrium. Rotations about the other backbone bonds, however, have much smaller barriers and dominate the resonance of the NHC*H*$_3$ protons at the low temperatures, giving rise to the observed inverse temperature dependence. It is not clear, however, what particular conformations other than the cis and trans isomer would give rise to the doubled NHC*H*$_3$ resonances at low temperatures.

Another possible explanation may be that, since high dielectric constants favor the cis form, and since the dielectric constants of MeOD and CD_2Cl_2 increase with decreasing temperature, the amount of cis also increases with decreasing temperature in the range below −15 °C. This interpretation suffers from the fact that rotation about the Gly-Pro peptide bond is unlikely at very low temperatures because of the high cis–trans energy barrier. It also should be noted that we did not check for aggregation in these experiments. Further study may be required to explain the results of Figure 6 more fully.

The presence of both cis and trans forms of Gly-Pro in D_2O is indicated not only by the doubling of NMR peaks but also indirectly by the CD spectra shown in Figure 7. The observed temperature dependence[19] and the crossover point[21,40,41] near 216 nm (indicated in Figure 7 by the circle) are characteristic of an ensemble of structures which undergo isomerism between two states, the cis and trans forms, in water.

Other Conformations. Both the Gly N*H* and N*H*CH$_3$ resonances exhibit reduced temperature coefficients of their chemical shifts in DMSO (-4.3×10^{-3} and -4.1×10^{-3} ppm/deg, respectively), which suggests that both N*H* protons have partial hydrogen-bond character. The only possible intramolecular hydrogen bonds are those of the C_5 (fully extended) conformation in Gly, of the C_7^{eq} conformation in Pro, or of a bend conformation with a 4→1 hydrogen bond. Since the best interpretation of Figure 6 indicates that several conformations are present in solution (see discussion in preceding section), at this point it appears that all three of these structures contribute to the average conformational properties (however, see below). Of course, the fact that the N*H* protons show only moderately reduced temperature coefficients likely means that other nonhydrogen-bonded conformations also exist in DMSO.

The IR spectra of blocked Gly-Pro at 25 °C in DMSO and in CD_2Cl_2 verify the existence of hydrogen-bonded NH protons. In DMSO, there is a strong broad NH band centered at 3300 cm^{-1}, indicating a hydrogen bond, with another broad NH band (nonhydrogen-bonded) near 3450 cm^{-1}. [The other IR bands in DMSO are not as definitive, the amide I band occurring (unresolved) between 1610 and 1650 cm^{-1} and the amide II band appearing at 1540 cm^{-1}.] In CD_2Cl_2, blocked Gly-Pro gives rise to a spectrum which also shows NH hydrogen bonding. Two strong NH bands occur at 3440 and 3410 cm^{-1}, with a weak broad shoulder at ~3340 cm^{-1} arising from a hydrogen-bonded NH group. The shoulder at 1645 cm^{-1} on the strong amide I band at 1670 cm^{-1} also suggests the presence of some hydrogen bonding. These IR results are consistent with the interpretation derived from the NMR temperature coefficients that the Gly N*H* and the N*H*CH$_3$ protons are involved in some hydrogen bonding, which arises from the C_5 conformations in the Gly residue and from the C_7^{eq} conformation in the Pro residue or a 4→1 hydrogen bond in Gly-Pro, and that other (nonhydrogen-bonded) structures also exist.

Comparison with Poly(Gly-Pro). In Figure 8, the ellipticity at the 197-nm minimum, $[\theta]_{197}$, in the CD spectrum of the blocked dipeptide Gly-Pro in water is plotted against temperature. The proportion of cis isomer present under these conditions (determined by NMR) is also plotted and, by suitable alignment of scales, may be seen to fall on the same straight (solid) line. The dashed line in Figure 8 shows a plot of the proportion of cis isomer for poly(Gly-Pro) in water–acetic acid (99.5:0.5), reported by Torchia.[21] Considering the slight difference in solvents, the agreement between the solid and dashed lines is excellent and strongly suggests that cis–trans isomerism in the polypeptide is determined mainly by local interactions, i.e., within a dipeptide moiety, and not by long-range interactions.

The NMR and CD data of poly(Gly-Pro) correlate with those of blocked Gly-Pro but not, as we shall see later, with those of blocked Pro-Gly. This is because, as has been found in many studies,[28,29,32,42,43] the conformational space of Gly is severely restricted by a proline residue which follows it, as in blocked Gly-Pro and in poly(Gly-Pro), but not by the proline residue which precedes it, as in blocked Pro-Gly.

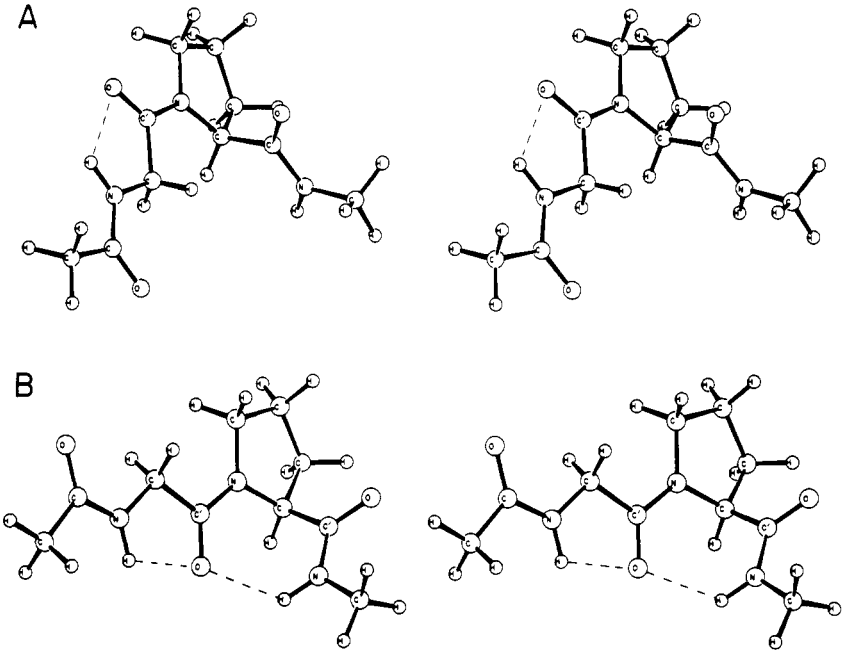

Figure 9. Stereo diagrams of *N*-acetyl-*N'*-methylglycylprolineamide. Cis and trans refer to conformations with $\omega_1 \approx 0°$ and $\approx 180°$, respectively. (A) A cis conformation with dihedral angles (ω_1, ϕ_2, ψ_2, ω_2, ϕ_3, ψ_3, ω_3) = (180°, 180°, 178°, −2°, −75°, 163°, 180°). (B) A trans conformation with dihedral angles (ω_1, ϕ_2, ψ_2, ω_2, ϕ_3, ψ_3, ω_3) = (180°, 178°, 175°, 177°, −75°, 79°, 180°).

Therefore, the conformation of the Gly residue in blocked Pro-Gly does not correspond to that in blocked Gly-Pro or poly(Gly-Pro). This means that, while the similarity in the CD and NMR behavior of blocked Gly-Pro and poly(Gly-Pro) is evidence for the dominance of *local* interactions over *long-range* interactions, the lack of similarity between the CD and NMR spectra of blocked Pro-Gly and poly(Gly-Pro) means that the local interactions also dominate over *short-range* interactions (the latter being those within the individual amino acid residues), since there are inter-residue interactions between Pro and Gly in the Gly-Pro sequence. Thus, even though poly(Gly-Pro) and poly(Pro-Gly) are different designations of the same polymer, the conformational behavior of Gly in this polymer is dominated by the proline residue which *follows* it.

It should be noted that there is no quantitative agreement between the cis–trans isomerism of H-Gly-Pro-OH and that of poly(Gly-Pro) since the charged end groups in the unblocked dipeptide play an important role in determining the cis–trans ratio.[14-16]

Comparison with Calculations. The following comparisons can be made between the conclusions derived from the experimental results given above and the theoretical results reported earlier.[28]

(1) The quantitative agreement in the experimental and the calculated values of the proportion of cis isomer is good. In our least polar solvent CD_2Cl_2, $\Delta G°_{trans \rightarrow cis} \approx 1.5$ kcal/mol, compared with the calculated value of $\Delta G°_{trans \rightarrow cis} \approx 1.87$ kcal/mol (for an effective dielectric constant of 4[30]). As discussed above in the section on the Pro single residue, the theoretical work[28] suggests that the proportion of cis isomer should increase with increasing dielectric constant. This *general* trend (except for the anomalous behavior in D_2O) is observed experimentally in Figure 6, where the relative area under the small peak of the $NHCH_3$ doubled resonance at temperatures above −15 °C is ascribed to the cis isomer. Of course, other solvent effects are also present, since the cis content in water is lower than that in DMSO, even though the dielectric constant of water is higher. An example of a Gly-Pro conformation with a cis peptide bond is shown in Figure 9A.

(2) As with the single residues, the dipeptide Gly-Pro exists in several conformations in solution, in agreement with the conformational energy calculations.[28,29] Two of the possible conformations, consistent with the experimental and theoretical results, are shown in Figure 9.

(3) The experimental results suggest that the Gly residue in Gly-Pro occurs to some degree in the C_5 conformation. This is in agreement with the predicted[29] 57% occurrence of C_5 in Gly of Gly-Pro. (As discussed above, the amount of C_5 conformation in the blocked Gly *single* residue is below the level of experimental detection, consistent with the predicted[29] percent occurrence of only 27%). It should be noted that both low-energy conformations in Figure 9 show Gly in a C_5 conformation. Recent reports[44,45] on the X-ray structure of *t*-BOC-Gly-L-Pro-OH also indicate that the Gly residue preceding Pro exists in the extended (C_5) conformation.

(4) The calculations[29] show that Pro in blocked Gly-Pro should exist in a C_7^{eq} conformation with a mole fraction of about 0.55. This is compatible with the experimental data which indicate the existence of a hydrogen-bonded $NHCH_3$ group. The calculated global minimum-energy conformation of blocked Gly-Pro is the trans form depicted in Figure 9B and shows that both NH groups are hydrogen bonded.

(5) The experimental data are not compatible with the existence of large amounts of the 4→1 hydrogen bond nor of significant amounts of β-bend structure in blocked Gly-Pro, because (a) the formation of a 4→1 hydrogen bond is incompatible with the existence of a C_5 conformation that is observed in the Gly residue (and the amount of nonhydrogen-bonded Gly NH is less than 50%), and (b) the CD spectrum for blocked Gly-Pro in water (Figure 7) is not that expected for a dipeptide with significant amount of bend structure (cf. Pro-Gly results below). We can conclude, therefore, that blocked Gly-Pro has a low propensity for formation of β bends, in agreement with the observed low fraction of occurrence of bends in Gly-Pro sequences in globular proteins[13] and with the calculations.[13,28,29]

(6) A comparison of the experimental results on blocked Gly-Pro with those on poly(Gly-Pro)[21] indicates that cis–trans isomerism is determined primarily by local rather than long-range interactions. This conclusion is in agreement with

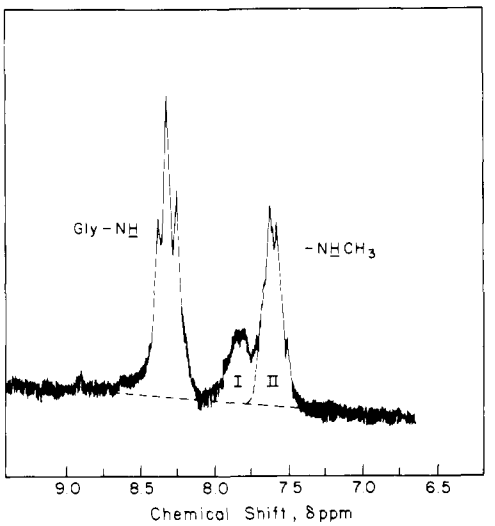

Figure 10. The N*H* region of the 90-MHz ^{1}H NMR spectrum of *N*-acetyl-*N'*-methylprolylglycineamide in DMSO-d_6 at 29 °C. The chemical shifts are given in ppm, with respect to the internal standard TMS.

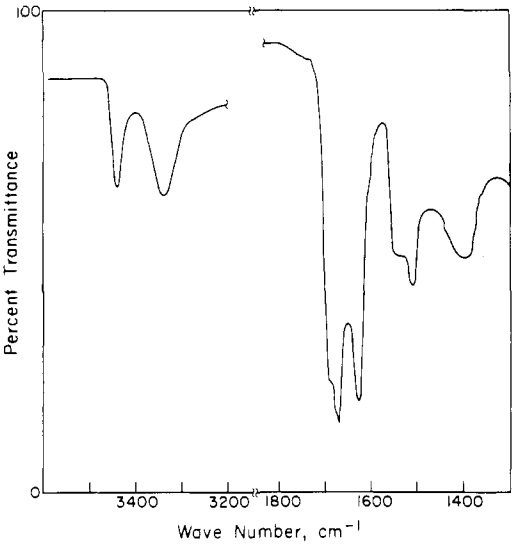

Figure 11. Infrared spectra of *N*-acetyl-*N'*-methylprolylglycineamide in CD_2Cl_2 at ~25 °C and 1.3×10^{-2} M. These curves were independent of concentration in the range of 4×10^{-3} to 4×10^{-2} M.

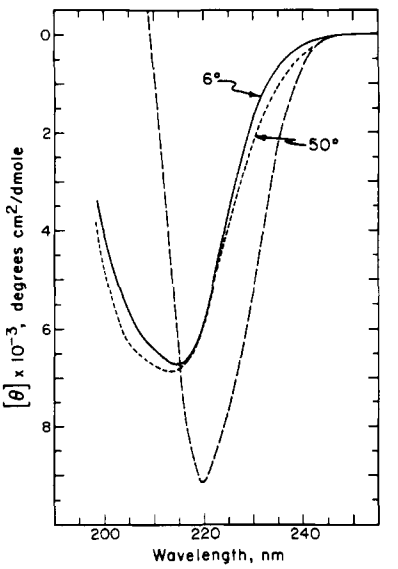

Figure 12. CD spectra of *N*-acetyl-*N'*-methylprolylglycineamide (8.8×10^{-4} M) in H_2O at 6 and 50 °C. The dashed curve represents the CD spectrum (1.1×10^{-3} M) in trifluoroethanol at 5 °C.

earlier calculations from this laboratory[28] but in disagreement with those of Tonelli.[46]

***N*-Acetyl-*N'*-methylprolylglycineamide. The 4→1 Hydrogen Bond.** The temperature coefficients for the doubled resonance of the N*H*CH_3 (amide) proton in DMSO (see Table I and peaks I and II in Figure 10) on the upfield and downfield side, respectively, are -3.0×10^{-3} and -3.3×10^{-3} ppm/deg. If we assume again that a nonhydrogen-bonded proton gives a temperature coefficient of -6×10^{-3} ppm/deg, and a completely hydrogen-bonded proton (well shielded from the solvent) gives a temperature coefficient of -2×10^{-3} ppm/deg, then both resonances from the N*H*CH_3 proton show partial (75 and 68%, respectively) hydrogen-bond character. This can be interpreted to indicate that each of the two resonances is a result of several conformations, some hydrogen bonded and some not.

The IR spectrum of Pro-Gly in DMSO verifies the presence of both hydrogen-bonded and nonhydrogen-bonded NH groups. A band at 3250 cm^{-1} arises from a hydrogen-bonded NH. This hydrogen-bonded NH group must be the N*H*CH_3 group and not the Gly NH group of Pro-Gly since the latter amide hydrogen shows an NMR temperature coefficient in DMSO of -5.6×10^{-3} ppm/deg, indicating little or no hydrogen-bond character.

The $NHCH_3$ group in CH_3CO-Pro-Gly-$NHCH_3$ can hydrogen bond either with the carbonyl oxygen of the CH_3CO end group (a 4→1 hydrogen bond) or with the carbonyl oxygen of the Pro C'=O group (in which the Gly residue is in a C_7 conformation). Since both peaks of the doubled N*H*CH_3 resonance have reduced temperature coefficients, suggesting two types of hydrogen bonds, we interpret the NMR data as indicating the presence of several conformations, some having a C_7 hydrogen bond (the Gly residue being in the C_7 conformation) and some having a 4→1 hydrogen bond. The interpretation that a 4→1 hydrogen bond exists in blocked Pro-Gly is consistent with those of Urry and co-workers,[6,7,12] Kopple et al.,[8] and Wüthrich et al.[47] in other peptides having a -Pro-Gly- sequence.

A 4→1 hydrogen bond can occur only in a bend conformation (although many bends can and do occur[13,29] with no 4→1 hydrogen bond). Therefore, bends play an important role among the stable conformations of blocked Pro-Gly. The data obtained in this study do not allow us to determine the particular types of bends present in solution, but the data do eliminate certain bend types. The type V bend[29] (involving a C_7^{eq} conformation for Pro), and others in which the Pro residue is in a C_7^{eq} conformation, do not seem to occur appreciably in DMSO, since the NMR temperature coefficient of the Gly N*H* proton is -5.6×10^{-3} ppm/deg, indicative of a proton with little or no hydrogen-bond character. Of course, bend types I', II', III', and V' (see Table I of ref 29 for definitions) cannot occur in Pro-Gly due to the restricted rotation about the N-C$^\alpha$ bond of Pro because of the pyrrolidine ring. The experimental data do not distinguish between bend types I, II, III, IV, and VII, and it is likely that several, if not all, of these bend types occur.

The bend probability P_b, i.e., the probability or mole fraction of Pro-Gly occurring in a bend conformation, cannot be determined quantitatively from the present work, but the NMR experiments suggest that Pro-Gly favors bend conformations over nonbends, and hence $P_b > 0.50$. This is indicated by the following observation. The T_1 value of the Gly C$^\alpha$*H* protons is 0.7 ± 0.1 s in Pro-Gly compared with 0.9 ± 0.1 s in Gly-Pro and 0.91 s in triglycine.[48] The reduced value of T_1 indicates that the segmental motion of the Gly residue in

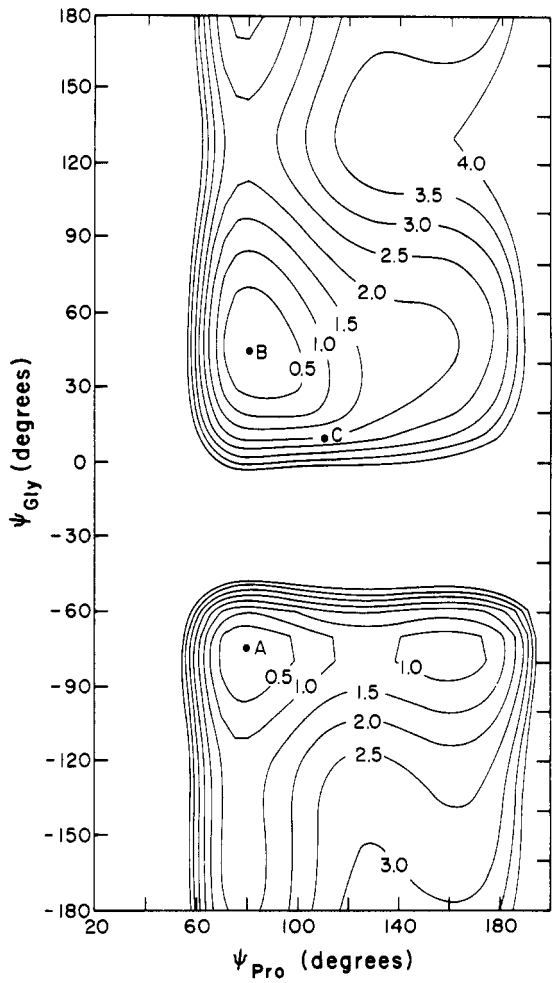

Figure 13. Conformational energy contour map for ψ_{Gly} vs. ψ_{Pro} in *N*-acetyl-*N'*-methylprolylglycineamide. Other dihedral angles were fixed at $(\omega_1, \phi_2, \omega_2, \phi_3, \omega_3) = (180°, -75°, 180°, 77°, 180°)$. The labeled points represent (A) a type V bend (ΔE = 0.0 kcal/mol); (B) the minimum-energy position of the conformation of lowest *free* energy (ΔE = 0.10 kcal/mol), a type II bend; and (C) the position of a type II bend having an $i + 3$ to i hydrogen bond (see Figure 14). The labels on the contour lines refer to total conformational energy in kcal/mol above the global minimum at point A.

blocked Pro-Gly is more restricted compared to that of the Gly residue in other oligopeptides. This restricted motion may be due to a high bend probability since the position of the $C^{\alpha}H$ relative to the rest of the molecule is approximately the same in all the bend conformations. It should be noted that the semilog plots of eq 1 (given in the Appendix) are not linear for large τ ($\tau > 3T_1$), possibly implying that more than one conformation is present, each having a different T_1, consistent with the interpretation that several bend types are stable in DMSO.

The experimental data for blocked Pro-Gly in solvents other than DMSO, such as CD_2Cl_2 and water, are more difficult to interpret, since the temperature coefficients in these other solvents have not been characterized and documented as extensively. Nevertheless, we can see some similarities between the conformational properties of blocked Pro-Gly in DMSO and those in other solvents. For example, the IR spectrum of blocked Pro-Gly in CD_2Cl_2, as shown in Figure 11, indicates a high degree of hydrogen bonding. The NH bands occur at 3440 and 3340 cm^{-1}, the amide I bands at 1670 and 1625 cm^{-1}, and the amide II bands at 1505 and 1540 cm^{-1}; in each pair, the latter band arises from hydrogen-bonded protons. Taken together with the NMR and IR data in DMSO, these data suggest that C_7 and 4→1 hydrogen bonds are present also in CD_2Cl_2. The CD spectrum of blocked Pro-Gly in H_2O, given in Figure 12, has a minimum at 214–215 nm and a low ellipticity ($\sim 7 \times 10^3$ deg cm^2/dmol), very different from the CD spectrum of Gly-Pro in H_2O (see Figure 7). Although these CD spectra cannot identify the presence or absence of bend conformations unequivocally, the spectrum of blocked Pro-Gly is similar to the calculated[49] CD spectra of types I and II bends of dipeptides, suggesting that Pro-Gly favors bends. The insensitivity of the CD spectrum of Pro-Gly to temperature, in contrast to that of Gly-Pro (which showed stronger temperature dependence because of the dominance of cis–trans isomerism), can be explained best by the presence of an ensemble of conformations in solution, without the cis–trans isomerism dominating the spectrum, as was also indicated by the NMR results in DMSO (see above).

Comparison with Calculations. The experimental results and the earlier calculations[29] on blocked Pro-Gly are in good agreement in the following ways. (a) Both show a distribution of stable conformations, rather than just one or two. (b) Both indicate that Pro-Gly has a high bend probability (as opposed to Gly-Pro, which does not). And (c) both theory and experiment suggest that the Gly residue in Pro-Gly can exist in a stable C_7 conformation. The calculations and experiments disagree, however, on the presence of the Pro C_7 conformation, the calculations[29] indicating that it is a stable structure (at least in solvents of low dielectric constant) and experiments indicating that it does not appear significantly in DMSO. The experiments and calculations further disagree on the presence of a 4→1 hydrogen bond, the IR and NMR experiments indicating that this hydrogen bond occurs to a significant extent while the calculations[29] show no strong 4→1 hydrogen bond. These two discrepancies likely arise from the same source, viz., the weak attraction between an amide hydrogen and a carbonyl oxygen in the hydrogen-bond potential of ECEPP.[30] Figure 13 shows the problem graphically: the minimum-energy conformation[29] of Pro-Gly is a type V bend at $(\phi_{Pro}, \psi_{Pro}, \phi_{Gly}, \psi_{Gly}) = (-75°, 79°, 84°, -74°)$, close to point A in Figure

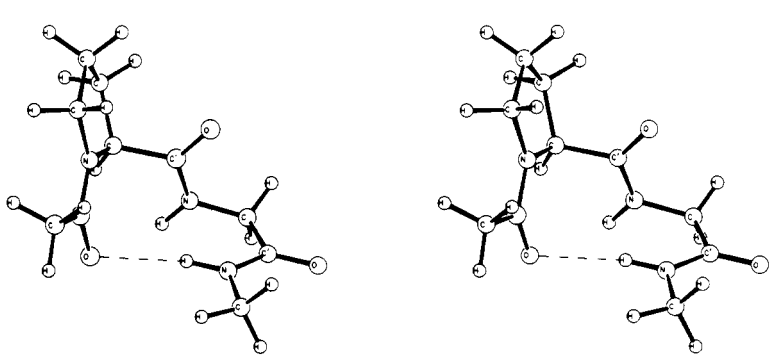

Figure 14. Stereo diagram of *N*-acetyl-*N'*-methylprolylglycineamide showing a type II β bend with $i + 3$ to i hydrogen bond (dashed line). The dihedral angles $(\omega_1, \phi_2, \psi_2, \omega_2, \phi_3, \psi_3, \omega_3) = (180°, -75°, 110°, 180°, 80°, 10°, 180°)$.

Appendix

Experimental Procedures

Materials

All solvents used in syntheses were spectral grade. Isobutylchloroformate was purchased from Eastman. Tertiary-butoxycarbonyl azide (t-Boc-azide), N-hydroxy succinimide (HOSu), N,N'-dicyclohexyl carbodiimide (DCCI), 1-hydroxy benzotriazole (HOBT), triethylamine (TEA), L-proline and glycine were purchased from Aldrich Chemical Co. Dimethylformamide (DMF), tetrahydrofuran (THF), ethyl acetate, hexane, dioxane, acetic anhydride, chloroform, methylene chloride and butanol were purchased from Fisher Scientific Co. Acetic acid was purchased from J. T. Baker Chem. Co.; anhydrous ethyl ether from Mallinckrodt, Inc.; and absolute ethanol from Commercial Solvents Corp. Monomethyl amine and monoethyl amine were purchased from Matheson Co. Deuterated solvents (≥ 99.8% isotopic purity) were from Merck & Co. Florisil®, an activated magnesium silicate for gel chromatography, was obtained from Fisher Scientific Co.

Unless mentioned below, all solvents and reagents were used without further purification. DCCI and HOBT were recrystallized. TEA was refluxed and distilled with acetic anhydride, and then dried and distilled over KOH. Dioxane was purified shortly before use by refluxing and distilling over sodium. Hexane was dried over calcium sulfate and filtered prior to use. Ethyl acetate was stored over molecular sieves and decanted just before use. DMF was vacuum distilled from ninhydrin. Chloroform, methylene chloride and carbon tetrachloride for IR measurement were dried with $CaCl_2$ and then treated to remove carbonyl containing impurities by percolation through a Celite column impregnated with 2,4-dinitrophenyl-hydrazine, followed by careful fractional distillation. Spectra of the solvents were taken prior to use to insure that all IR active impurities had been removed.

Synthesis of N-Acetyl-N'-methylglycylprolineamide

Tertiary butyloxycarbonyl glycine (Boc-Gly-OH) was prepared as described by Schnabel.[51]

Boc-Gly-Pro-OH was synthesized as follows. Boc-Gly-OH (5.25 g; 0.03 mole) was dissolved in THF (100 ml), and distilled, triethylamine (4.2 ml; 0.03 mole) was added. The solution was cooled to -10°C and stirred vigorously, while isobutylchloroformate (3.9 ml; 0.03 mole) was added. After five minutes, L-proline (5.2 g; 0.045 mole) in DMF (100 ml) and triethylamine (6.3 ml; 0.045 mole) were added. The reaction mixture was stirred for 5 hours while slowly warming it to room temperature, and the solvent was then removed under vacuum. A 10% citric acid solution (20 ml) was added to the residue, and the aqueous layer was extracted with ethyl acetate (5 x 50 ml). The combined ethyl acetate extract was washed with saturated sodium chloride solution (3 x 40 ml), and the ethyl acetate solution was dried over anhydrous magnesium sulfate. The solution was then filtered, and the solvent was removed (under vacuum). The residue was crystallized twice from ethyl acetate-hexane. Yield 6.0 g (73%); m.p. 142-144°; R_f: 0.61 [Butanol : acetic acid : water (60:20:20)]; Lit.[52] m.p. 142-144°.

Boc-Gly-Pro-OH was then converted to Boc-Gly-Pro-$NHCH_3$ by the following procedure. Boc-Gly-Pro-OH (2.72 g; 0.01 mole) was dissolved in amine-free DMF (30 ml) at 0°C, and one equivalent each of recrystallized 1-hydroxybenzotriazole (HOBT) and purified N,N'-dicyclohexylcarbodiimide (DCCI) were added. The reaction proceeded in an ice bath for one hour, and the mixture was then warmed slowly to room temperature for another two hours. The dicyclohexylurea (DCU) was removed by filtration, and an equivalent of liquified methylamine was added and allowed to react for 4 hours until the Kaiser test[53] for residual amine was negative. The solvent was removed under reduced pressure, and the residual oil was taken up into ethyl acetate (100 ml) from which the product solidified as a white crystalline material. The product was recrystallized twice from ethyl acetate. Yield: 1.8 g (61%); m.p. 178-180°; R_f: 0.64 [Butanol : acetic acid : water (60:20:20)]; NMR (CD_3OD, TMS): δ 1.4 s (9H) Boc, 2.0 m (4H) βγ Pro, 2.8 s (3H) NCH_3, 3.7 m (2H) δ Pro, 3.9 s (2H) Gly α, 4.5 m (1H) Pro α.

The tertiary butyloxy carbonyl group was then replaced by an acetyl group. Boc-Gly-Pro-$NHCH_3$ (1.8 g; 0.006 mole) was dissolved in 3N HCl-dioxane solution (20 ml), and allowed to react for ten minutes. The HCl-Gly-Pro-$NHCH_3$ was then precipitated with ether, filtered, collected and dried. This salt was then dissolved in water, and the pH was adjusted to ~8.7 with 1 N NaOH. An equivalent of acetic anhydride was added, and the reaction proceeded at room temperature until the starting material was converted to the acetyl form, as determined by thin layer chromatography (TLC). The aqueous solution was evaporated to dryness (in vacuum), then the residue repeatedly was dissolved in absolute ethanol, and the ethanol was evaporated. When all the water was thus removed by azeotropic distillation, the NaCl precipitate was filtered from the ethanolic solution, and the filtrate was evaporated (in vacuum) to give a white amorphous powder. Yield: 0.83 g (58%); m.p. 151°. A trace impurity was removed by passing the material through a Florisil® column (90 g), using ethanol-methylene chloride (1:9) as eluent. M.p : 151°; R_f: 0.36 [butanol:acetic acid:water (60:20:20)]; Anal. for C, H, N: Exp. 53.07, 7.42, 18.27, Theor. 52.85, 7.54, 18.49.

Synthesis of N-Acetyl-N'-methylprolylglycineamide

Boc-Pro-OH was prepared as described by Schnabel,[51] and converted to the N-hydroxysuccinimide ester (Boc-Pro-OSU) by the method of Anderson et al.[54] The latter was used to prepare Boc-Pro-Gly-OH as follows. Glycine (3.4 g; 0.045 mole) was dissolved in water (100 ml), and sodium bicarbonate (7.56 g; 0.090 mole) was added with stirring. Boc-Pro-OSu (8.56 g; 0.040 mole) was dissolved in dioxane (100 ml), added to the aqueous solution, and allowed to react for 24 hours at room temperature. The dioxane was removed under reduced pressure, and the aqueous phase diluted to 400 ml. The solution was chilled in ice, and the pH was slowly adjusted to 1.5 with 1N HCl. The solution was extracted with ethyl acetate (5 x 100 ml), and the extracts were washed with cold water (3 x 5 ml) and dried over anhydrous magnesium sulfate. The ethyl acetate solution was filtered, and the filtrate evaporated to dryness under reduced pressure yielding a white powder which was recrystallized twice from ethyl acetate. Yield: 4.3 g (32%); m.p. 173-174°; R_f: 0.30 [butanol:acetic acid:water (40:30:30)]; R_f 0.67 [butanol:acetic acid:water (60:20:20)]; NMR (DMSO-d_6, TMS): δ 1.4 s (9H) Boc, 1.9 m (4H) βγ Pro, 3.3 m (2H) δ Pro, 3.8 d (2H) Gly α, 4.2 m (1H) Pro α, 8.0 m (1H) Gly NH.

Boc-Pro-Gly-OH was then converted to Boc-Pro-Gly-$NHCH_3$ by the following procedure. Boc-Pro-Gly-OH (1.36 g, 0.005 mole) was dissolved in amine-free DMF (25 ml) and cooled to 0°C. An equivalent each of DCCI and HOBT were then added, and the solution mixed for an hour at 0°C before slowly warming the mixture to room temperature for an additional 2 hours. The DCU was removed by filtration, and an equivalent of liquified methylamine added and allowed to react until the Kaiser test[53] proved negative. The solvent was removed under reduced pressure, and the product (dissolved in methylene chloride) was separated on a Florisil® column with ethanol:methylene chloride (1:9) as eluent. Yield: 1.02 g (71%); m.p. 117.5-118°; R_f: 0.47 [ethanol:methylene chloride (1:9)]; NMR: (CD_3OH, TMS): δ 1.5 s 9H) Boc, 2.0 m (4H) βγ Pro, 2.9 d (3H) $NHCH_3$, 3.5 m (2H) δ Pro, 3.9 s (2H) Gly α, 4.2 m (1H) Pro α, broad NH.

The tertiary butyloxycarbonyl group was then replaced by an acetyl group. Boc-Pro-Gly-$NHCH_3$ (1.02 g; 3.6 mmole) was dissolved in 3N HCl-dioxane solution (20 ml), and allowed to react for ten minutes. The HCl-Pro-Gly-$NHCH_3$ was then precipitated with ether, filtered, collected and dried, it was then dissolved in water, and the pH was adjusted with 1N NaOH to ~8.7 before an equivalent of acetic anhydride was added Although the mixture was allowed to react for 2 days at room temperature, the reaction did not go to completion, when followed by TLC. The aqueous solution was evaporated to dryness (in vacuum), and the residue was dissolved in absolute ethanol and filtered; the filtrate was reduced in volume, and then applied to a Florisil® column. Fractions containing only the desired product were pooled and dried to give a chromatographically pure (by TLC) hygroscopic glass. Yield 166 mg (20%), R_f: 0.38 [butanol:acetic acid:water (60:20:20)], Anal. for C/N ratio: Exp. 2.900, Theor. 2.858. (Ratio given in order to avoid adjusting analysis for small fractional number of water molecules.)

Synthesis of N-Acetyl-N'-methylglycineamide

N-acetyl glycine N'-methyl amide was synthesized by the procedure of Applewhite and Niemann,[55] and purified by sublimation. M.p. 158°, Lit.[56] m.p. 157.5-158°. R_f: 0.48 [butanol:acetic acid:water (40:30:30)]; Anal. for C, H, N: Theor., 46.14, 7.75, 21.52; Expt., 46.28, 7.83, 21.51.

Synthesis of N-Acetyl-N'-ethylprolineamide

Boc-Pro-OH (2.1 g; 0.01 mole) was converted to N-acetyl proline N'-ethyl amide by means of the same procedures as described above for the conversion of Boc-Gly-Pro-OH to N-acetyl glycine proline N'-methyl amide with the exception that ethylamine was used instead of methylamine. The chromatographically pure (by TLC) oily product solidified in the cold without crystallizing. Yield: 956 mg (52%); R_f: 0.53 [butanol: acetic acid:water (40:30:30)], R_f: 0.22 [ethanol:methylene chloride (5:95)], mass spectroscopy: CI(M+1)-parent peak at 185, EC(M-1)-parent peak at 183. theor., M = 184.

Methods

Check for racemization. Checks for possible racemization during synthesis were made by the L-leucyl-dipeptide method of Manning and Moore.[57] For convenience (i.e., to avoid having to remove the L-leucyl-glycine later), the proline was isolated (before preparing the leucyl-proline derivatives) from the HCl-propionic (1:1) hydrolysis mixture[58] from the peptide under test by separating it on, and extracting it from, an analytical TLC plate (with Soxhlet extractor); R_f (Pro): 0.60 [phenol:water (75:25)]; R_f (Gly): 0.15 [phenol:water (75:25)]. The leucyl-proline dipeptides were analyzed on a Technicon NC-1 amino acid analyzer with Technicon pH 3.8 buffer. By this technique, the dipeptides were found to contain less than 1% D-proline, even without correction for possible racemization during hydrolysis.

Circular dichroism. CD spectra were obtained (in water, dioxane, and trifluoroethanol) with a Cary Model 60 spectropolarimeter modified with a Model 6001 attachment and equipped with a thermostat. The sample temperature was controlled to within ±0.5° in a water-jacketed 3 mm-pathlength quartz cell. The data are expressed in terms of [θ], molar ellipticity (in units of deg. cm^2/dmole).

Infrared spectra. IR spectra were recorded on Perkin-Elmer spectrophotometers Models 337 and 521. IR spectra of dilute amide solutions in halogenated hydrocarbons were measured with 5 cm length cells with KBr windows. The solvent was placed in the reference cell and the variable scale expansion (10 to 20 X) feature of the Perkin-Elmer Model 521 was used to enlarge the amide spectra. Sample temperature was ~25°C.

Proton magnetic resonance. NMR spectra were obtained on a Brüker HX-90 spectrometer equipped with an NMR-3 Digilab data system. Trimethylsilane (TMS) was used as an internal standard (<1%) in nonaqueous solutions and as an external standard (coaxial insert) in aqueous solutions. Spin-lattice relaxation time measurements, T_1, were made using a (180°, τ, 90°, T)$_n$ pulse sequence, for several values of τ, by Fourier transformation of the resulting free induction decay (FID). A delay at least equal to $5T_1$ was allowed between successive pulse sequences. Values of T_1 were obtained by a least squares fit of the data to the equation[48]

$$\ln(A_\infty - A_t) = \ln(2A_\infty) - t/T_1 \quad (1)$$

where A_t and A_∞ are the area or height of the peak at time t and at infinite time, respectively. The samples were deoxygenated with pure nitrogen gas for at least 15 min by the method of Coates et al.[48] This method of deoxygenating was used in order to compare our relaxation times with those of Coates et al.[48] and also to be able to use a column of liquid (with a vortex plug) as small as possible in order to minimize diffusion.

13 (ϕ_{Gly} was fixed at 77° in computing point A). Point B is the calculated conformation of next lowest energy and is a type II bend with Pro in the C_7 conformation. Neither of these bends has a 4→1 hydrogen bend (as defined in ref 29), although the $NHCH_3$ proton in the structure at point B would be shielded from the solvent and appear hydrogen bonded in a measurement of the NMR temperature dependence (but probably not appear hydrogen bonded in an IR spectrum). The structure having a 4→1 hydrogen bond, proposed from the experimental data to be present in DMSO, is shown in Figure 14 and corresponds to point C in Figure 13, only a short distance from B and only 2 kcal/mol higher in energy. It is probable that if the NH···OC hydrogen bond potential is changed to make the interaction more attractive, the minimum could shift from point B to point C in Figure 13. This is because an increase in the stability of the NH···OC interaction would greatly stabilize the linear 4→1 hydrogen bond (see Figure 14) but not stabilize the nonlinear C_7 hydrogen bond appreciably. The correct value of the NH···OC hydrogen-bond interaction parameter is being redetermined[50] presently from crystal data on model compounds by the procedure given in ref 30. Initial studies[50] indicate that this interaction should be changed from −1.1 kcal/mol[30] at the optimal interatomic distance to about −3.5 kcal/mol,[50] a sufficient change to stabilize the 4→1 hydrogen bond.

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Note Added in Proof. M. Marraud recently has brought to our attention his work [e.g., G. Boussard, M. Marraud, and J. Néel, *J. Chim. Phys. Phys.-Chim. Biol.*, **71**, 1081 (1974)] on some of the same blocked dipeptides treated in our present and earlier work (ref 29). Boussard et al. showed the presence of the 4 → 1 hydrogen bond, the C_5 and C_7^{eq} structures, and other conformations of *N*-acetyl-*N'*-methylprolylglycineamide in nonpolar solvents, which our paper confirms. After our paper had been accepted for publication, a paper by T. Higashijima, M. Tasumi, and T. Miyazawa was published in *Biopolymers*, **16**, 1269 (1977), on ^{1}H-NMR studies of *N*-acetyl-*N'*-methylprolineamide, a molecule closely related to *N*-acetyl-*N'*-ethylprolineamide, one of the molecules examined in the present work. Higashijima et al. determined the relative amounts of cis and trans isomers and investigated the presence of the C_7^{eq} structure (which they referred to as the γ-turn structure), in a variety of solvents, temperatures, and concentrations. They examined a greater variety of experimental conditions for *N*-acetyl-*N'*-methylprolineamide, and our work essentially confirms their results under comparable conditions. Higashijima et al. did not compare quantitatively their results with those of conformational energy calculation nor with work on related oligo- and polypeptides.

Miniprint Material Available: Full-sized photocopies of the Appendix (9 pages). Ordering information is given on any current masthead page.

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