

racemization in the presence of these bases.<sup>6</sup>

Another widely used procedure for synthesizing phosphoanhydrides is that introduced by Khorana and associates in which a nucleoside phosphoramidate reacts with a phosphate in pyridine to form the phosphoanhydride and an amine.<sup>7</sup> This reaction is reported to proceed best in tertiary amines, and pyridine is generally used as the solvent. It is conceivable that pyridine may serve as a nucleophilic catalyst in supporting the coupling of phosphoramidates to phosphates by a mechanism similar to that outlined in Scheme IV.

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**Registry No.**  $R_p$ -[ $\alpha$ -<sup>18</sup>O]AMPS trioctylammonium salt, 87226-42-4; [ $\beta$ -<sup>18</sup>O]ADPBS, 87302-51-0;  $S_p$ -[ $\alpha$ -<sup>18</sup>O]AMPS trioctylammonium salt, 87226-43-5; AMP trioctylammonium salt, 69098-20-0;  $S_p$ -[ $\beta$ -<sup>18</sup>O]-ATPBS, 87226-46-8;  $R_p$ -[ $\beta$ -<sup>18</sup>O]ATPBS, 87226-47-9;  $P^1$ -5'-adenosyl- $P^2$ -2',3'-(methoxymethylene)-5'-adenosyl 1-thio[1-<sup>18</sup>O]pyrophosphate, 87302-50-9; diphenylphosphorochloridate, 2524-64-3; 2',3'-(methoxymethylene)-AMP trioctylammonium salt, 81671-39-8; ( $R_p$ )- $P^1$ -5'-adenosyl- $P^2$ -bis(phNyl) 1-thio[1-<sup>18</sup>O]pyrophosphate, 87226-44-6; ( $S_p$ )- $P^1$ -5'-adenosyl- $P^2$ -bis(phenyl) 1-thio[1-<sup>18</sup>O]pyrophosphate, 87226-45-7; ( $R_p$ )- $P^1$ , $P^2$ -bis(5'-adenosyl) 1-thio[1-<sup>18</sup>O]pyrophosphate, 69010-06-6; ( $S_p$ )- $P^1$ , $P^2$ -bis(5'-adenosyl) 1-thio[1-<sup>18</sup>O]pyrophosphate, 68973-42-2.

## Crystal Structures, Molecular Conformations, Infrared Spectra, and <sup>13</sup>C NMR Spectra of Methylproline Peptides in the Solid State

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**Abstract:** Sterically hindered amino acids can form peptides of defined conformation. We have investigated the conformational influences of the methyl groups in *N*-acetyl, *N'*-methylamide derivatives of the seven isomeric monomethylprolines. X-ray crystallography demonstrated two molecular conformations ( $\alpha_R$  or  $P_{II}$ ) for these peptides. For each of the conformations, there are two types of intermolecular hydrogen bonding: either the proline CO or the acetyl CO accepts a hydrogen bond from the one NH. The previously determined crystal structure of the AcProNHMe fits one of the classes observed for the methylproline peptides. Infrared spectra of the crystalline peptides can be correlated with their molecular conformations and types of hydrogen bonding. The amide II frequencies differentiate the  $\alpha_R$  and  $P_{II}$  conformers. The amide I frequencies reveal the hydrogen-bond acceptor. The amide A frequencies seem to indicate the hydrogen-bond strengths. High-resolution <sup>13</sup>C NMR of the crystalline peptides also can be correlated with their conformations. The chemical shift differences between C $\beta$  and C $\gamma$  reveal the peptide  $\psi$  angles.

### Introduction

Peptide chains have a variety of conformations which are energetically competitive. In proteins and polypeptides, specific conformers are stabilized by long-range cooperative effects. For oligopeptides which lack these long-range effects, covalent restrictions have been introduced to reduce their flexibility. Methyl derivatives of proline provide one class of amino acids which will give conformationally restricted peptides. We previously have reported spectroscopic and thermodynamic studies in solution for *N*-acetyl, *N'*-methylamide derivatives of the seven isomeric monomethylprolines (AcMeProNHMe).<sup>2</sup>

The structure and conformational nomenclature of the AcMeProNHMe are illustrated in Figure 1. There are five accessible conformational regions for these peptides.<sup>3</sup> The three regions for the trans peptide bond isomers will be referred to as  $\alpha_R$ , C $\gamma$ , and  $P_{II}$  which have peptide  $\psi$  dihedral angles of ca. -60, 80, and 150°, respectively. The two regions for the cis peptide bond isomers have  $\psi$  angles of ca. -60 and 150°, respectively. In solution most of the peptides have about 75% trans isomer. In nonpolar solutions the intramolecularly hydrogen-bonded C $\gamma$  conformer dominates, while the  $P_{II}$  conformer is most prevalent in aqueous solution. Steric effects alter both the distribution of  $\psi$  values and cis-trans isomerism.<sup>2</sup>

In the solid state, intermolecular hydrogen bonding and crystal packing produce long-range cooperative effects which stabilize particular conformations among those sterically allowed. Methyl group placement influences both molecular conformation and crystal packing. Atomic coordinates have been determined by X-ray diffraction for the seven isomeric AcMeProNHMe. The

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(2) (a) Delaney, N. G.; Madison, V. *Int. J. Peptide Protein Res.* **1982**, *19*, 543-548. (b) Delaney, N. G.; Madison, V. *J. Am. Chem. Soc.* **1982**, *104*, 6635-6641. (c) Madison, V.; Delaney, N. G. *Biopolymers* **1983**, *22*, 869-877.

(3) Madison, V. *Biopolymers* **1977**, *16*, 2671-2692.

Table I. Unit Cell Dimensions and Other Experimental Parameters for Ac-X-NHMe, C<sub>9</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>

peptide (X)	Pro <sup>a</sup>	2-MePro	<i>anti</i> -3-MePro	<i>anti</i> -4-MePro	<i>syn</i> -4-MePro	<i>syn</i> -5-MePro	<i>syn</i> -3-MePro	<i>anti</i> -5-MePro
space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 1	<i>P</i> 2 <sub>1</sub> / <i>c</i>	<i>P</i> 2 <sub>1</sub> / <i>a</i>	<i>Pbca</i>
<i>Z</i>	4	4	4	4	4	4	4	4
<i>a</i> , Å	9.74	7.17 (2)	7.74 (3)	7.28 (3)	10.02 (2)	6.84 (4)	9.56 (3)	7.22 (2)
<i>b</i> , Å	13.20	8.79 (2)	9.32 (4)	9.77 (4)	10.30 (3)	18.08 (7)	7.36 (2)	9.08 (2)
<i>c</i> , Å	7.17	16.05 (4)	13.90 (6)	14.20 (6)	10.53 (3)	8.17 (4)	14.69 (3)	31.28 (6)
$\alpha$ (deg)					105.6 (3)			
$\beta$ (deg)					95.2 (3)	97.0 (4)	92.6 (3)	
$\gamma$ (deg)					91.7 (3)			
<i>d</i> <sub>c</sub> (g/cm <sup>3</sup> )		1.21	1.18	1.22	1.17	1.21	1.22	1.19
radiation		Cu K $\alpha$	Mo K $\alpha$	Mo K $\alpha$	Mo K $\alpha$	Mo K $\alpha$	Cu K $\alpha$	Cu K $\alpha$
no. of independent reflections		796	791	792	1953	1141	1345	1334
final <i>R</i>		5.6	4.7	3.9	7.0	5.0	7.5	4.8
final <i>R</i> <sub>w</sub>		5.9	4.2	4.3	5.2	5.0	8.1	5.9
av   <i>F</i> <sub>o</sub> - <i>F</i> <sub>c</sub>		1.1	0.8	0.7	0.9	0.7	1.1	0.6

<sup>a</sup> From ref 4.

conformations and hydrogen-bonding patterns of these peptides are compared with each other and to *N*-acetyl-L-proline-*N*'-methylamide (AcProNHMe) whose crystal structure was determined by Matsuzaki and Iitaka.<sup>4</sup> Infrared and high-resolution <sup>13</sup>C NMR spectra also were determined for the crystalline peptides. Knowledge of the crystal structures enables us to correlate the observed spectroscopic parameters with molecular conformations and types of hydrogen bonding. Hydrogen bond strengths, indicated by infrared spectra, are correlated with observed geometries.

### Experimental Methods

**Synthesis and Crystallization.** The synthesis of the seven monomethylprolines and their peptide derivatives has been described elsewhere.<sup>2</sup> Single crystals for X-ray diffraction studies were grown from mixtures of ethyl acetate and cyclohexane using racemic peptides, except for Ac-L-*anti*-3-MeProNHMe. The approximate v/v ratios of ethyl acetate to cyclohexane for crystallization of each of the Ac-X-NHMe were: 2-MePro 1:3 or 3:1, *syn*-3-MePro 1:3, *anti*-4-MePro 3:1, *syn*-4-MePro 3:1, *anti*-5-MePro 1:3, *syn*-5-MePro 1:2. For Ac-L-*anti*-3-MeProNHMe, 2:1:6 ethyl ether-ethyl acetate-cyclohexane was used.

**X-ray Diffraction.** X-ray data on all seven molecules were collected at room temperature on a Nicolet P3F automatic diffractometer with a graphite monochromator on the incident beam. Cell dimensions were determined from a least-squares refinement on several independently measured reflections. The  $\theta$ - $2\theta$  scan technique, with a variable scan rate, was used to measure intensities out to a  $2\theta_{\max} = 112^\circ$ . Cell dimensions and other experimental parameters are given in Table I.

The structures were solved by direct methods, using the sigma-one, sigma-two, and tangent formulas<sup>5</sup> to generate and refine phases for the structure factors. The MULTAN 80 system of computer programs was used to implement the phase determination.<sup>6</sup> The structures were refined using the restrained least-squares program RESLSQ.<sup>7</sup> Atomic scattering factors used were those listed in the "International Tables for X-ray Crystallography".<sup>8</sup> Hydrogen atoms were located in difference maps calculated at various points during the refinement and their positional parameters were refined. The hydrogen atom thermal parameters were not refined but were allowed to "ride" on the atoms to which they were bonded; i.e., after each cycle of refinement their thermal parameters were automatically reset to be equal to those of the atom to which they were bonded. The function minimized by the least squares was  $\sum w(|F_o| - |F_c|)^2$ , where the weights, *w*, were derived from esds of the observed intensities.<sup>9</sup> All reflections were included in the refinements. The final *R* factors, where  $R = \sum ||F_o| - |F_c|| / \sum |F_o|$  and  $R_w = [\sum w(|F_o| - |F_c|)^2 / \sum w F_o^2]^{1/2}$ , are given in Table I. Coordinates, *B*<sub>eq</sub> (equivalent isotropic thermal parameter) values, and anisotropic thermal parameters for the nonhydrogen atoms, and positional coordinates for the hydrogen

Table II. Average Bond Lengths for AcMeProNHMe<sup>a</sup>

bond	length (Å)	bond	length (Å)
C <sup>3</sup> C <sup>1</sup>	1.496 (4)	C <sup>α</sup> C <sup>4</sup>	1.519 (7)
C <sup>1</sup> O <sup>2</sup>	1.232 (8)	C <sup>4</sup> O <sup>6</sup>	1.232 (5)
C <sup>1</sup> N	1.351 (10)	C <sup>4</sup> N <sup>5</sup>	1.329 (11)
NC <sup>α</sup>	1.463 (9)	N <sup>5</sup> C <sup>7</sup>	1.451 (6)
NC <sup>δ</sup>	1.476 (8)		
C <sup>α</sup> C <sup>β</sup>	1.539 (9)		
C <sup>β</sup> C <sup>γ</sup>	1.523 (9)		
C <sup>γ</sup> C <sup>δ</sup>	1.520 (8)		
C <sup>X</sup> CM	1.525 (11)		

<sup>a</sup> Average bond lengths and standard deviations, in parentheses, from crystal structures of the seven peptides. The standard deviations are calculated from the actual distribution of values observed for the seven compounds. C<sup>X</sup> denotes the carbon in the proline ring to which CM is bonded.

Table III. Average Bond Angles for AcMeProNHMe<sup>a</sup>

atoms	bond angle (deg)	atoms	bond angle (deg)
C <sup>3</sup> C <sup>1</sup> O <sup>2</sup>	122.3 (1.0)	C <sup>α</sup> C <sup>4</sup> O <sup>6</sup>	120.3 (1.5)
C <sup>3</sup> C <sup>1</sup> N	117.3 (9)	C <sup>α</sup> C <sup>4</sup> N <sup>5</sup>	116.6 (1.7)
O <sup>2</sup> C <sup>1</sup> N	120.4 (6)	O <sup>6</sup> C <sup>4</sup> N <sup>5</sup>	123.0 (8)
C <sup>1</sup> NC <sup>α</sup>	120.9 (1.1)	C <sup>4</sup> N <sup>5</sup> C <sup>7</sup>	121.8 (9)
C <sup>1</sup> NC <sup>δ</sup>	126.0 (1.0)		
C <sup>α</sup> NC <sup>δ</sup>	112.4 (6)		
NC <sup>α</sup> C <sup>β</sup>	103.2 (1.1)	NC <sup>α</sup> C <sup>4</sup>	113.3 (1.9)
C <sup>α</sup> C <sup>β</sup> C <sup>γ</sup>	104.2 (1.3)	C <sup>4</sup> C <sup>α</sup> C <sup>β</sup>	110.8 (1.4)
C <sup>β</sup> C <sup>γ</sup> C <sup>δ</sup>	103.6 (1.0)	CMC <sup>X</sup> X	113.0 (1.4)
C <sup>γ</sup> C <sup>δ</sup> N	102.8 (9)		

<sup>a</sup> Average values and standard deviations, in parentheses, from the crystal structures of the seven peptides. The standard deviations are calculated from the actual distribution of values for the seven compounds. C<sup>X</sup> is the carbon in the proline ring to which CM is bonded. X is the two heavy atom neighbors of C<sup>X</sup>.

atoms, have been deposited as supplementary material.

**Infrared Spectroscopy.** For infrared spectra, ca. 1 mg of crystalline peptide was added to ca. 150 mg of dry potassium bromide. The solids were ground together in an agate mortar. Pellets were prepared in vacuo by applying about 10<sup>5</sup> psi pressure in a hydraulic press. Spectra were obtained on a Nicolet Model 7199 FTIR. Samples were ratioed against a blank KBr pellet prepared on the same day as the sample pellets. Crystals were grown from the same solvents used for the X-ray studies. For a number of the peptides, spectra were obtained from material crystallized using two different solvent ratios. No differences were observed in the infrared spectra due to varying the crystallization conditions.

**NMR Spectroscopy.** Crystalline peptides for the NMR studies were prepared employing conditions similar to those used in growing single crystals. The <sup>13</sup>C NMR spectra of polycrystalline peptides were obtained on a homebuilt double resonance spectrometer with a 3.5-T magnetic field. The <sup>13</sup>C magnetization was developed using a spin-lock cross polarization with a mix time of 1 ms. The proton decoupling field was 2.5 mT during the 100-ms data acquisition period. The spectra were signal averaged for 10<sup>3</sup> transients using spin temperature alternate and phase cycling to eliminate experimental artifacts. Magic angle sample spinning was carried out using Andrew-Beams rotors made from Delrin,

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(8) "International Tables for X-ray Crystallography"; Kynoch Press: Birmingham, 1974; Vol. IV.

(9) Gilardi, R. D. *Acta Crystallogr., Sect. B* 1973, 29, 2089-2095.

Table IV. Molecular Conformations for Ac-X-NHMe

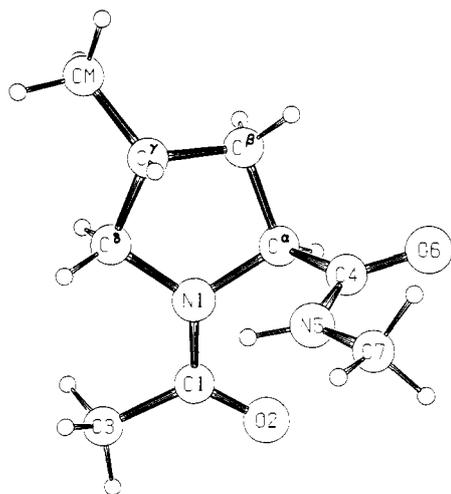
peptide (X)	class IA				class IB		class IIA	class IIB	
	Pro <sup>a</sup>	2-MePro	anti-3-MePro	anti-4-MePro	syn-4-MePro		syn-5-MePro	syn-3-MePro	anti-5-MePro
chirality <sup>b</sup>	L	L	L	L	(mol A)		L	L	L
$\omega_0^c$	-177.0	-179.8	-177.0	-171.6	-174.3	-174.1	-172.3	-179.2	172.8
$\Phi_1$	-76.3	-60.5	-63.7	-78.2	-74.3	-71.4	-65.0	-79.2	-66.1
$\psi_1$	-15.9	-24.9	-36.1	-20.5	-14.1	-18.5	149.9	146.0	152.3
$\omega_1$	-177.3	-176.2	-179.1	-175.6	-176.4	-177.4	174.7	174.5	178.0
$\chi_1$	27.0	-25.2	-29.3	26.0	-18.4	-19.9	-25.6	34.6	29.5
$\chi_2$	-36.2	36.2	40.6	-36.4	33.7	34.5	36.7	-35.5	-38.9
$\chi_3$	30.7	-32.0	-35.5	32.5	-35.9	-35.1	-32.5	22.2	32.3
$\chi_4$	-14.2	17.3	17.8	-17.4	25.5	23.9	17.3	0.4	-14.3
$\theta$	-7.7	4.6	7.4	-5.2	-4.5	-2.5	5.2	-22.2	-9.4

<sup>a</sup> From ref 4. <sup>b</sup> Chirality of atomic coordinates. Except for Ac-L-ProNHMe and Ac-L-anti-3-MeProNHMe, only the relative chirality has physical meaning. <sup>c</sup> Angles in degrees.

Table V. Hydrogen-Bond Parameters and Infrared Frequencies for Ac-X-NHMe in the Solid State

peptide (X)	class IA				class IB		class IIA	class IIB	
	Pro <sup>a</sup>	2-MePro	anti-3-MePro	anti-4-MePro	syn-4-MePro		syn-5-MePro	syn-3-MePro	anti-5-MePro
conformation	$\alpha_R$	$\alpha_R$	$\alpha_R$	$\alpha_R$	$\alpha_R$	$\alpha_R$	P <sub>II</sub>	P <sub>II</sub>	P <sub>II</sub>
hydrogen bond acceptor (CO)	Ac	Ac	Ac	Ac	Pro	(mol A) Pro	Ac	Pro	Pro
H-O <sup>b</sup>	2.05	2.05	2.00	2.08	1.93	1.94	2.06	1.93	1.98
N-O <sup>b</sup>	2.88	2.92	2.89	2.95	2.83	2.84	2.94	2.83	2.88
CN-O <sup>c</sup>	135	135	130	134	137	136	109	120	112
CO-N <sup>c</sup>	116	116	120	119	148	148	154	170	153
NH-O <sup>c</sup>	158	155	163	158	161	163	159	170	167
out-of-plane H(N) <sup>b</sup>	0.01	0.37	1.12	0.23	0.06	0.25	1.05	0.26	0.52
distance <sup>d</sup> O(C) <sup>b</sup>	0.11	0.01	0.58	0.02	0.12	0.07	0.40	0.36	0.36
infrared frequencies <sup>e</sup>									
amide A	3238	3286	3287	3270	3295		3332	3302	3307
amide I (Pro)	1669	1670	1668	1676	1652		1680	1656	1658
amide I (Ac)	1629	1627	1626	1632	1644		1628	1637	1640
amide II	1543	1540	1552	1546	1550		1564	1570	1572

<sup>a</sup> Structural data from ref 4. <sup>b</sup> Distance in Å. <sup>c</sup> Angles in deg. <sup>d</sup> Distance of hydrogen-bond donor or acceptor from plane of amide to which it is bonded. <sup>e</sup> Frequencies in cm<sup>-1</sup>.



**Figure 1.** ORTEP drawing of Ac-anti-4-MeProNHMe showing atom names and illustrating conformational nomenclature. Anti indicates that the methyl and carboxamide groups are on opposite sides of the proline ring (syn will indicate that they are on the same side). The trans peptide bond isomer (C3 trans to C<sup>α</sup>) is shown. The peptide is in the  $\alpha_R$  conformation.

with typical rotational rates of 3.5 kHz. Chemical shifts are reported relative to external tetramethylsilane.

## Results and Discussion

The atomic coordinates determined for the seven isomeric *N*-acetylmethylproline-*N'*-methylamides by X-ray diffraction form the basis for discussion of molecular conformation and hydrogen

bonding in the crystals. The structure and nomenclature of these peptides are defined in Figure 1. Mean values and standard deviations of the bond lengths and bond angles for the heavy atoms of the seven peptides are given in Tables II and III. Throughout the series the bond lengths are constant to within 0.01 Å and the bond angles to within 2°. Bond lengths and angles observed for the methylproline peptides are comparable with values determined by Matsuzaki and Iitaka for AcProNHMe<sup>4</sup> and to average values compiled for a variety of peptides.<sup>10</sup>

In the methylproline peptide crystals, only trans peptide bond isomers are observed. The conformations fall within narrow regions near either  $\alpha_R$  or P<sub>II</sub> (Table IV). In all of the structures, the pyrrolidine rings have C<sup>β</sup> and C<sup>γ</sup> displaced about 0.2 Å in opposite directions from the mean plane of the five ring atoms. The hydrogen-bond distances and angles fall within ranges generally considered to be characteristic of strong hydrogen bonds (Table V).

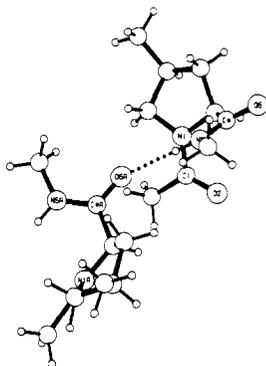
On the basis of their molecular conformations in crystals, the methylproline peptides will be divided into two classes. Each class will be subdivided based on hydrogen-bonding patterns. The classes are defined: class I,  $\alpha_R$  conformation; class II, P<sub>II</sub> conformation; subclass A, acetyl CO as hydrogen-bond acceptor; subclass B, proline CO as hydrogen-bond acceptor.

Class IA contains four peptides: AcProNHMe, Ac-2-MeProNHMe, Ac-anti-3-MeProNHMe, and Ac-anti-4-MeProNHMe. These four peptides crystallize in the orthorhombic space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with one molecule in the asymmetric unit

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**Figure 2.** Stereoview of two molecules of Ac-*anti*-3-MeProNHMe in the crystal structure. This peptide is in class IA ( $\alpha_R$  conformation, acetyl CO accepts the hydrogen bond). In Figures 2-6 the orientations are similar for the molecule containing the hydrogen-bond donor. These molecules have the *L* configuration.



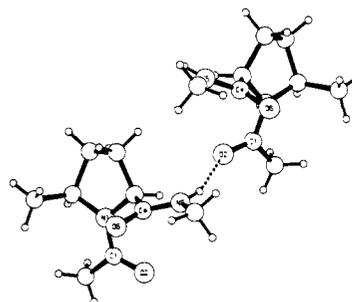
**Figure 3.** Ac-*syn*-4-MeProNHMe molecules in the crystal structure. This peptide is in class IB ( $\alpha_R$  conformation, proline CO accepts the hydrogen bond).

and four molecules in the unit cell. They have similar conformations near that of the right-handed  $\alpha$  helix ( $\alpha_R$ ,  $\Phi$ ,  $\psi = -57$ ,  $-48^\circ$ ). For the four peptides (Table IV),  $\Phi$  values range from  $-78$  to  $-60^\circ$ .  $\psi$  values range from  $-36$  to  $-16^\circ$ . Computed energy minima for the individual peptides have dihedral angles nearly coincident with those of the idealized  $\alpha$  helix. Intermolecular forces in the crystals shift  $\psi$  to larger values. Each of the peptide molecules in this class forms hydrogen bonds from its NH across the pyrrolidine ring to the acetyl CO of an adjacent molecule (Figure 2) and the symmetric hydrogen bond from its acetyl CO to the NH of another neighboring molecule. Linear chains of hydrogen bonds connect the molecules along one of the crystal axes. The hydrogen-bond lengths (N-O distances) are 2.88 to 2.95 Å. The hydrogen-bond angles CN-O and CO-N are near  $120^\circ$  as would be expected for  $sp^2$  hybridization of the atoms. The NH-O angles are nearly linear (ca.  $160^\circ$ ). The distance of each hydrogen-bonded atom to the plane of the peptide group to which it is bonded shows more variation than the other hydrogen-bond parameters. The atoms are most nearly in-plane for AcProNHMe (Table V).

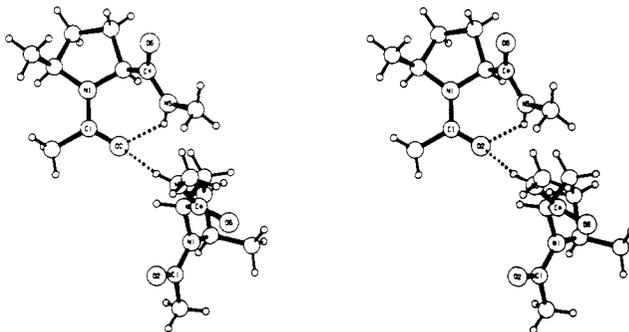
Ac-*syn*-4-MeProNHMe is the only member of class IB. This peptide has an  $\alpha_R$  conformation, but forms hydrogen bonds between the NH and the proline CO of adjacent molecules (Figure 3). There are two slightly different molecules in the asymmetric unit and a second pair of molecules in the unit cell which is related to the first pair through an inversion center. D-L pairs of molecules are hydrogen bonded. The hydrogen-bond parameters for Ac-*syn*-4-MeProNHMe are similar to those of class IA. Relative to the first class, the CO-N angle is increased to about  $150^\circ$  for Ac-*syn*-4-MeProNHMe.

The molecular conformations of the three class II peptides are similar to that of the polyproline II helix ( $P_{II}$ ,  $\Phi$ ,  $\psi = 77$ ,  $146^\circ$ ). For the three peptides,  $\Phi$  ranges from  $-79$  to  $-65^\circ$  and  $\psi$  from  $146$  to  $152^\circ$ . There is one molecule in each asymmetric unit, but the unit cells contain both D and L residues which are related by inversion centers. The D-L pairs are hydrogen bonded.

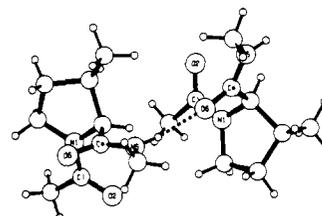
In class II, the hydrogen-bond lengths are similar to those of class I. The CN-O angles are near  $120^\circ$ , but the CO-N angles



**Figure 4.** Ac-*syn*-5-MeProNHMe molecules in the crystal structure. This peptide is in class IIA ( $P_{II}$  conformation, acetyl CO accepts the hydrogen bond). Note the large distance of the NH from the plane of the acetyl group to which it is hydrogen bonded.



**Figure 5.** Stereoview comparing the intramolecular  $C_7$  hydrogen bond with intermolecular one for Ac-*syn*-5-MeProNHMe. Note the similar out-of-plane distances of the two hydrogen bond donors. In this figure  $\psi$  of the upper proline residue was set at  $80^\circ$  to form the intramolecular hydrogen bond. The intermolecular hydrogen bond in the crystal is the lower one in the figure. Another view of this latter hydrogen bond is shown in Figure 4.



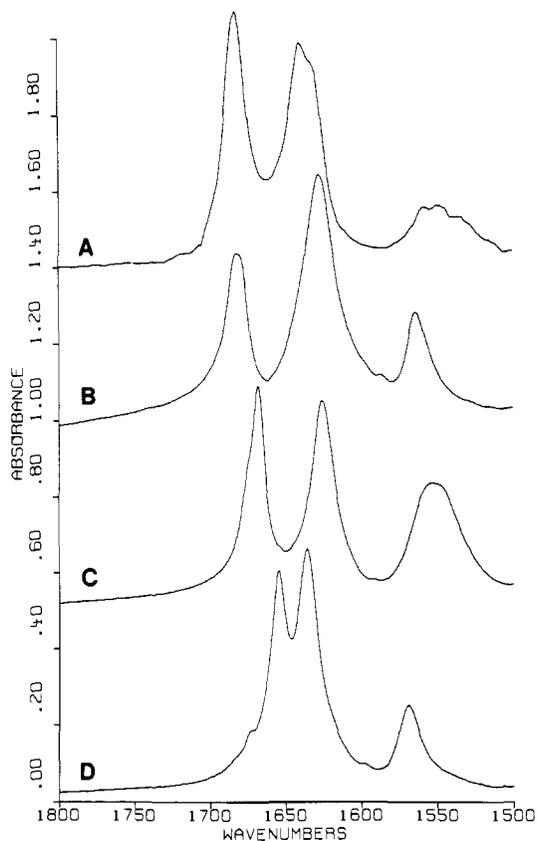
**Figure 6.** Ac-*syn*-3-MeProNHMe molecules in the crystal structure. This peptide is in class IIB ( $P_{II}$  conformation, proline CO accepts the hydrogen bond).

are closer to linear ( $150$  to  $170^\circ$ ) than for class IA. The NH-O angles range from  $160$  to  $170^\circ$ .

Ac-*syn*-5-MeProNHMe is the only member of class IIA. Its NH is distal to the pyrrolidine ring and hydrogen bonds to the acetyl CO of an adjacent molecule. Hydrogen-bond distances and angles observed for Ac-*syn*-5-MeProNHMe are typical of class II as a whole, but for this peptide the amide hydrogen is more than 1 Å from the plane of the peptide group to which it is hydrogen bonded (Figure 4, Table V). The out-of-plane disposition of the NH is, in fact, rather similar to that in the intramolecularly hydrogen-bonded  $C_7$  conformation. The two hydrogen bonds are compared in Figure 5.

For Ac-*syn*-3-MeProNHMe and Ac-*anti*-5-MeProNHMe, the two members of class IIB, the NH is distal to the pyrrolidine ring and it hydrogen bonds to a proline CO (Figure 6). The hydrogen-bond parameters are similar to those of classes IB and IIA, but the amide hydrogens are closer to the plane of the peptide group to which they hydrogen bond than in the case of class IIA (Table V).

Considering the entire series, most of the hydrogen-bond parameters fall within narrow ranges. Overall, the H-O distances range from 1.89 to 2.08 Å, the N-O distances from 2.83 to 2.95 Å, the CN-O angles from  $109$  to  $137^\circ$ , and the NH-O angles



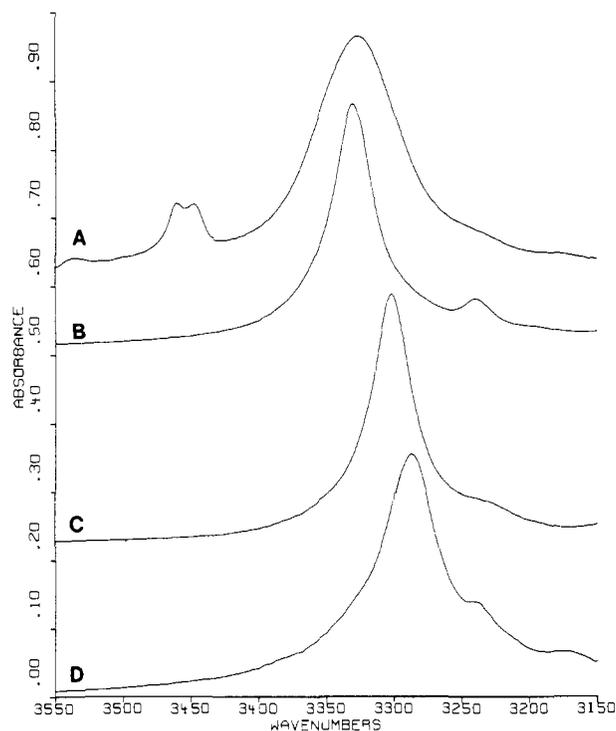
**Figure 7.** Infrared spectra covering the amide I and amide II regions. The spectra have been stacked, each has near zero absorbance at 1800  $\text{cm}^{-1}$ . (A) *Ac-anti-3-MeProNHMe* in carbon tetrachloride ( $C_7$  conformation, acetyl CO accepts intramolecular hydrogen bond). (B) *Ac-syn-5-MeProNHMe* crystals in KBr pellet (class IIA,  $P_{II}$  conformation, acetyl CO accepts intermolecular hydrogen bond). (C) *Ac-syn-3-MeProNHMe* crystals in KBr pellet (class IIB,  $P_{II}$  conformation, proline CO accepts intermolecular hydrogen bond). (D) *Ac-anti-3-MeProNHMe* crystals in KBr pellet (class IA,  $\alpha_R$  conformation, acetyl CO accepts intermolecular hydrogen bond).

from 155 to 170°. The CO-N angles can be divided into two groups with values: 116 to 120° for class IA, but 148 to 170° for classes IB, IIA, and IIB. Distances between hydrogen-bond donors or acceptors and the plane to which they hydrogen bond vary considerably. Distances range from 0.01 to 1.12 Å for amide hydrogens and 0.01 to 0.58 Å for carbonyl oxygens.

Infrared spectra of the methylproline peptides in the solid state are indicative of hydrogen-bonding patterns, molecular conformation, and hydrogen-bond strengths. The amide I frequencies (primarily CO stretching) reveal the hydrogen-bond acceptor, that is, differentiate subclasses A and B. Compare the 1650- $\text{cm}^{-1}$  region of Figure 7B and 7C (subclass A) with Figure 7D (subclass B). For the  $C_7$  conformation in solution, the acetyl carbonyl is the hydrogen-bond acceptor (subclass A). The amide I region in solution (Figure 7A) is similar to that of the subclass A peptides in the solid state (Figures 7B and 7C). For the A subclasses (NH hydrogen bonded to an acetyl CO), the amide I frequencies for the proline carbonyl range from 1668 to 1680  $\text{cm}^{-1}$  and those for the acetyl carbonyl from 1626 to 1632  $\text{cm}^{-1}$  (Table V). For the B subclasses (NH hydrogen bonded to a proline CO), the proline amide I frequencies range from 1652 to 1658  $\text{cm}^{-1}$  and the acetyl amide I frequencies from 1637 to 1644  $\text{cm}^{-1}$ . Hydrogen bonding lowers the frequency.

Within this series in which all of the NHs are hydrogen-bonded, the amide II frequencies (CN stretching coupled with NH bending) seem to be correlated with molecular conformation. For class I ( $\alpha_R$  conformation), the amide II frequencies range from 1540 to 1552  $\text{cm}^{-1}$ . For class II ( $P_{II}$  conformation), they range from 1564 to 1572  $\text{cm}^{-1}$  (Table V).

The strength of the peptide hydrogen bond may be affected by its chemical constituents, length, angles, and the degree of



**Figure 8.** Infrared spectra covering the amide A region. The spectra have been stacked, each has near zero absorbance at 3550  $\text{cm}^{-1}$ . The spectra are given in order of decreasing amide A frequency. (A) *Ac-anti-3-MeProNHMe* in carbon tetrachloride ( $C_7$  conformation, acetyl CO accepts intramolecular hydrogen bond). (B) *Ac-syn-5-MeProNHMe* crystals in KBr pellet (class IIA,  $P_{II}$  conformation, acetyl CO accepts intermolecular hydrogen bond). (C) *Ac-syn-3-MeProNHMe* crystals in KBr pellet (class IIB,  $P_{II}$  conformation, proline CO accepts intermolecular hydrogen bond). (D) *Ac-anti-3-MeProNHMe* crystals in KBr pellet (class IA,  $\alpha_R$  conformation, acetyl CO accepts intermolecular hydrogen bond).

coplanarity of the bonded peptide groups. Increasing hydrogen-bond strength is believed to correlate with lowering of the amide A frequency (NH stretch).<sup>11</sup> For the eight peptides considered herein, the amide A frequencies cover a range of nearly 100  $\text{cm}^{-1}$ . The progressive lowering of this frequency from peptides in class IIA, to those in class IIB, to class IA is shown in Figure 8B-D.

The amide A frequencies show considerable variation within class IA, even though there is little variation in hydrogen-bond lengths and angles. The N-O lengths fall within a range of 0.07 Å and no angle varies more than 7°. The degree of co-planarity shows more variation. The amide A frequencies correlate with the distance of the amide hydrogen to the plane of the peptide group to which it is hydrogen bonded. *AcProNHMe* has the lowest frequency by more than 30  $\text{cm}^{-1}$  and the most planar hydrogen bond (and also the shortest N-O distance). The other frequencies in this class increase with increasing distance of the hydrogen from the plane, but the correlation is far from linear.

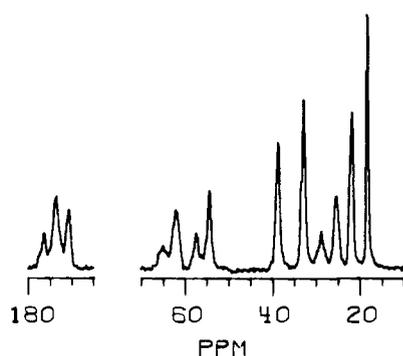
The amide A frequencies for peptides in classes IB and IIB are higher than those of class IA. The most likely reason for this increase is the increase in the CO-N angle. However, change of the hydrogen-bond acceptor from the carbonyl group of the tertiary amide (acetyl CO) to that of the secondary amide (proline CO) may also play a role. The amide A frequency for *Ac-syn-5-MeProNHMe*, class IIA, is the highest observed for any of the peptides in the solid state (Table V, Figure 8). In this case many of the hydrogen-bond parameters deviate from optimum values. The bond lengths are long, and the angles, especially CO-N, deviate from  $sp^2$  values. In addition, the amide hydrogen is 1.05 Å from the plane of the acetyl group to which it is hydrogen bonded. The geometry of this hydrogen bond is similar to that

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Table VI.  $^{13}\text{C}$  NMR Parameters for Ac-X-NHMe

peptide (X)	class IA				class IB		class IIA	class IIB	
	Pro	2-MePro	<i>anti</i> -3-MePro	<i>anti</i> -4-MePro	<i>syn</i> -4-MePro		<i>syn</i> -5-MePro	<i>syn</i> -3-MePro	<i>anti</i> -5-MePro
parameter					(mol A)				
H-bond acceptor <sup>a</sup> (CO)	Ac	Ac	Ac	Ac	Pro	Pro	Ac	Pro	Pro
$\psi$ (deg in solid) <sup>a</sup>	-16	-25	-36	-20	-14	-18	150	146	152
$\theta$ <sup>b</sup>	76	85	96	80	74	78	90	86	92
$\Delta\delta_{\beta\gamma}$ (ppm in solid) <sup>c</sup>	7.47	16.91	5.31	5.95	8.00		-4.28	4.81	-3.37
$\Delta\delta_{\beta\gamma}$ (ppm in solution) <sup>d</sup>									
isomer (solvent)									
trans (CHCl <sub>3</sub> )	2.01	15.37	2.42	2.50	2.55		-5.80	3.88	-6.51
trans (H <sub>2</sub> O)	5.81	16.61	6.38	5.37	5.10		-3.94	3.75	-3.58
cis (H <sub>2</sub> O)	9.05		9.98	8.64	8.22		-1.11	7.08	$\pm 0.24$

<sup>a</sup> From X-ray crystallographic data. <sup>b</sup>  $\theta = |\psi - 60^\circ|$ , i.e., the torsional angle from  $\text{C}^\beta$ . <sup>c</sup>  $\Delta\delta_{\beta\gamma} = \delta_{\text{C}^\beta} - \delta_{\text{C}^\gamma}$ . <sup>d</sup> From ref 2. The peptide bond isomers of the acetyl group are indicated.



**Figure 9.**  $^{13}\text{C}$  NMR spectrum of polycrystalline Ac-*anti*-4-MeProNHMe obtained with magic angle spinning and proton decoupling as described in the experimental section. Assignments were based on spectra in solution (ref 2). Chemical shifts in ppm from external  $(\text{CH}_3)_4\text{Si}$  are:  $\text{C}^\text{M}$  18.18,  $\text{C}^\beta$  21.77,  $\text{C}^\gamma$  26.42,  $\text{C}^\delta$  32.65,  $\text{C}^\epsilon$  38.60,  $\text{C}^\zeta$  55.32,  $\text{C}^\alpha$  62.99,  $\text{C}^1$  and  $\text{C}^4$  171.44 and 174.28. Asymmetrical doublets are observed for  $\text{C}^\gamma$ ,  $\text{C}^\beta$ ,  $\text{C}^\alpha$ ,  $\text{C}^1$ , and  $\text{C}^4$  since they are adjacent to  $^{14}\text{N}$  atoms.

of the intramolecular hydrogen bond in the  $\text{C}_7$  conformer (Figure 5), and the infrared spectra of peptides containing the two hydrogen bonds are also similar (compare Figures 7A and 7B and 8A and 8B).

The  $^{13}\text{C}$  NMR spectrum of polycrystalline Ac-*anti*-4-MeProNHMe is shown in Figure 9. This high-resolution spectrum has well-resolved resonances for each of the carbons in the molecule. The carbons bonded to nitrogens have resonances that are broadened asymmetrical doublets due to the influence of the  $^{14}\text{N}$  with its substantial quadrupole interaction<sup>12</sup> on the nearby  $^{13}\text{C}$ . In the following discussion, we will focus on the region which contains the proline  $\text{C}^\beta$  and  $\text{C}^\gamma$  resonances (20–40 ppm).

Siemion et al.<sup>13</sup> have proposed a correlation between the chemical shift difference  $\delta_{\text{C}^\beta} - \delta_{\text{C}^\gamma}$  ( $\Delta\delta_{\beta\gamma}$ ) and the  $\text{C}^\beta\text{C}^\alpha\text{C}^\gamma\text{O}$  dihedral angle ( $\theta = |\psi - 60^\circ|$ ). We have confirmed the correlation between  $\Delta\delta_{\beta\gamma}$  and conformation for the methylproline peptides in solution.<sup>2</sup> Since the methyl substituent has different effects on the chemical shifts of  $\text{C}^\beta$  and  $\text{C}^\gamma$ ,  $\Delta\delta_{\beta\gamma}$  depends on the position of substitution as well as conformation for the methylproline peptides. In the solid state  $\theta$  is in a narrow range ( $85 \pm 11^\circ$ ), but  $\Delta\delta_{\beta\gamma}$  ranges from -4 to 17 ppm owing to effects of the methyl substituent (Table VI).

For each of the peptides,  $\Delta\delta_{\beta\gamma}$  values have been related to conformations observed in solution.<sup>2</sup> In chloroform solution, each of the peptides, except Ac-*syn*-3-MeProNHMe, assumes the  $\text{C}_7$  conformation ( $\psi \approx 80^\circ$ ,  $\theta \approx 20^\circ$ ), and  $\Delta\delta_{\beta\gamma}$  values are small relative to those observed for the same peptide under different conditions (Table VI). In aqueous solution, the  $\text{P}_{\text{II}}$  conformation ( $\psi = 150 \pm 30^\circ$ ,  $\theta = 90 \pm 30^\circ$ ) has been firmly established for AcProNHMe<sup>14</sup> and there is strong evidence for this conformation

for all of the other peptides.<sup>2</sup> For each of the peptides  $\Delta\delta_{\beta\gamma}$  is considerably larger in water than in chloroform. Even larger values are observed for the cis peptide bond isomers in solution (Table VI). The exceptional case, Ac-*syn*-3-MeProNHMe, has the  $\text{P}_{\text{II}}$  conformation under almost all circumstances and little variation in  $\Delta\delta_{\beta\gamma}$  is observed.

On the average, the  $\Delta\delta_{\beta\gamma}$  values observed for the peptide crystals are within 1.0 ppm of those for their aqueous solutions. The  $\text{C}^\beta\text{C}^\alpha\text{C}^\gamma\text{O}$  dihedral angles are similar in the two cases ( $\theta = 85 \pm 11^\circ$  in the crystals,  $\theta = 90 \pm 30^\circ$  in aqueous solution). Only for Ac-*syn*-4-MeProNHMe is the difference in  $\Delta\delta_{\beta\gamma}$  for the crystal vs. aqueous solution substantially larger than the average. Nevertheless, we observe no unusual intermolecular contacts in this case.

Results for the methylproline peptides indicate that  $\Delta\delta_{\beta\gamma}$  values will be indicative of conformations in the solid state and that they will be useful in correlating solution and solid-state conformations.

## Conclusions

Crystallography revealed that the methylproline peptides assume either the  $\alpha_{\text{R}}$  or  $\text{P}_{\text{II}}$  conformations. Peptides with each of these conformations form intermolecular hydrogen bonds accepted by the acetyl or proline carbonyl groups. The hydrogen-bond acceptor can be deduced from the amide I region of infrared spectra. The two molecular conformations can be differentiated from the amide II frequencies. Similar absolute values of the  $\text{C}^\beta\text{C}^\alpha\text{C}^\gamma\text{O}$  torsional angle in the crystals and in aqueous solution are indicated by the comparable  $\Delta\delta_{\beta\gamma}$  values observed for each peptide in the solid state and in solution. Hydrogen-bond strengths inferred from the amide A frequencies in conjunction with the structural data indicate that stronger hydrogen bonds occur by placement of the amide hydrogen in the plane of the accepting peptide group aligned with the  $\text{sp}^2$  orbitals on the accepting carbonyl oxygen.

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**Registry No.** Ac-(2-MePro)-NHMe, 87173-11-3; Ac-(*anti*-3-MePro)-NHMe, 83434-50-8; Ac-(*anti*-4-MePro)-NHMe, 87136-68-3; Ac-(*syn*-4-MePro)-NHMe, 87136-69-4; Ac-(*syn*-5-MePro)-NHMe, 87136-70-7; Ac-(*syn*-3-MePro)-NHMe, 87173-12-4; Ac-(*anti*-5-MePro)-NHMe, 87136-71-8; Ac-(Pro)-NH-Me, 19701-85-0.

**Supplementary Material Available:** Positional coordinates and  $B_{\text{eq}}$  for the non-hydrogen atoms (Tables S-I through S-VII), positional coordinates for the hydrogen atoms (Tables S-VIII through S-XIV), and anisotropic thermal parameters for the non-hydrogen atoms (Tables S-XV through S-XXI) (21 pages). Ordering information is given on any current masthead page.

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