the reaction rate, compared with Cu^{2+}, it is suggested that this is enough, but also that both of these binding sites are used for coordination to the triphosphate chain. As the reaction most probably proceeds also in this case via an intramolecular nucleophilic OH^- attack from a M—OH unit (section 3), one may tentatively suggest the following structure and mechanistic path for the reactive $[(tn)_2Co(OH)(ATP)Cu(bpy)]^0$ species: Cu-(bpy)$^{2+}$, coordinating to the α,β phosphate group of ATP^{4-}, leads to ring-opening of the cobalt chelate and to a monodentate coordination of $(tn)_2Co(OH)^{2+}$ at the γ group, thus allowing for an intramolecular attack at the γ phosphorus by the cobalt-bound OH^-.

In conclusion, it is evident that systems containing two different metal ions can also be rather reactive toward the dephosphorylation

of nucleoside 5′-triphosphates. This observation corresponds to many biological phosphoryl and nucleotidyl transfers, where two different metal ions are also often involved.[9,10]

Crystal and Solution Structures of *cyclo*(Ala-Pro-Gly-D-Phe-Pro): A New Type of Cyclic Pentapeptide Which Undergoes Cis-Trans Isomerization of the Ala-Pro Bond

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Abstract: *cyclo*(L-Ala-L-Pro-Gly-D-Phe-L-Pro) has been synthesized and its conformation determined in the crystalline state and in solution in chloroform and dimethyl sulfoxide. This cyclic pentapeptide was designed to explore the conformational response of the restricted pentapeptide ring to the presence of an L residue preceding proline; it is the first cyclic pentapeptide with an LLDDL chiral sequence to be studied. In crystals, a cis Ala–Pro bond exists, and there are no intramolecular hydrogen bonds. The Ala NH is buried in the interior of the backbone ring and does not participate in any hydrogen bonding. In both prolines, the C^γ atom is disordered between at least two positions corresponding to two different envelope conformations for the pyrrolidine ring. The peptide crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters $a = 9.142$ (2) Å, $b = 11.000$ (4) Å, and $c = 23.885$ (5) Å. In solution in dimethyl sulfoxide, the same one-cis form of the peptide is observed, but a conformational change occurs to an all-trans form in chloroform. There appear to be hydrogen-bonding interactions within the ring in the all-trans form, but they are not well-defined. The most likely conformation based on proton and carbon NMR data for the all-trans form contains a type II Pro-Gly β turn within which is a weak γ turn with a hydrogen bond between the Gly NH and the Ala C=O.

Cyclic pentapeptides have been extensively studied as conformational models, particularly for reverse turns.[1-8] In cases where both solution and crystal structure analyses have been carried out (Table I), an all-trans conformation containing an internal 4 → 1 hydrogen bond in a type I or II β turn and often an internal 3 → 1 hydrogen bond in a γ turn is observed for this family of peptides. Yet all these examples fall into a single class of backbone sequence chirality: DLLDL or its mirror image LDLLD (with Gly residues occupying either L or D sites).

The peptide *cyclo*(Ala-Pro-Gly-D-Phe-Pro) (all residues are of the L configuration unless otherwise noted) was originally designed to explore a question raised in the solution conformational analysis of *cyclo*(Gly^1-Pro^2-Gly^3-D-Ala^4-Pro^5).[2] While a predominant all-trans conformer with both β and γ turn intramolecular hydrogen bonds exists for this latter peptide in several solvents, a small proportion (<20%) of a conformer containing one-cis peptide bond is observed in water. Complexation with cations perturbs the conformational distribution, leading to higher proportion of the one-cis form. Model building led to the proposal that it is the Gly^1-Pro^2 bond that undergoes isomerization in *cyclo*(Gly-

Pro-Gly-D-Ala-Pro). Furthermore, introduction of an L residue in place of Gly^1 was predicted to favor the one-cis form; replacement of Gly^1 with a D residue was predicted to preclude formation of the one-cis form. These predictions have been substantiated by synthesis of the title peptide and by synthesis and conformational analysis of *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro). No cis form has been seen for this latter peptide, even upon complexation by cations.[11] A complete conformational analysis

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Table I. Conformations of Cyclic Pentapeptides Analyzed Both in Solution and in Crystals[a]

peptide	hydrogen bonding	ref
cyclo(Gly[1]-Pro[2]-Gly[3]-D-Ala[4]-Pro[5])		
soln[b-d]	Pro-Gly type II β turn; γ turn around Pro[5]	2
crystal	same	3a
cyclo(D-Phe[1]-Pro[2]-Gly[3]-D-Ala[4]-Pro[5])		
soln[b,c]	Pro-Gly type II β turn; γ turn around Pro[5]	6, 12
crystal	same	5
cyclo(Gly[1]-Pro[2]-Ser[3]-D-Ala[4]-Pro[5])		
soln[b,c]	Pro-Ser type I β turn; γ turn around Pro[5]	4
crystal	Gly-Pro type II' β turn	3b
cyclo(Gly[1]-Val[2]-Gly[3]-Val[4]-Pro[5])		
soln[b,c]	Val-Gly type II β turn; cis Val[4]-Pro[5]	10a
crystal	same	10b
cyclo(Thr-D-Val-Pro-Sar-MeAla)		
soln[b,c,e]	Sar-MeAla type I β turn	9
crystal[f]	same	9

[a] All residues are of the L configuration unless noted otherwise. [b] In chloroform. [c] In dimethyl sulfoxide. [d] In acetonitrile. [e] In acetone. [f] The crystal structure was solved for the Thr(OBzl) derivative.

of *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro) by quantitative nuclear Overhauser effect spectroscopy has been reported.[12] Preliminary studies of cation complexation by *cyclo*(Ala-Pro-Gly-D-Phe-Pro) showed the predicted strong preference for a one-cis conformation.[11]

The preferred conformations of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) are described in the present paper. Indeed, in solution this peptide readily undergoes a conformational change from an all-trans form preferred in chloroform (70:30) to a one-cis Ala-Pro conformer preferred in dimethyl sulfoxide (~100%). In the crystalline state, this cyclic pentapeptide takes up the one-cis form. Presence of an L substituent preceding Pro and existence of a different chiral sequence have major effects on the preferred conformation of cyclic pentapeptides.

Experimental Section

Synthesis. Procedures used in the synthesis of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) were as previously described.[2,4] Peptide bond couplings were accomplished by the mixed anhydride method, using *N*-methylmorpholine and isobutyl chloroformate. N-termini were protected by the *tert*-butyloxycarbonyl (*t*-Boc) group and C-termini by formation of a benzyl ester. Deprotection of the former was by treatment with HCl in anhydrous ether and of the latter by hydrogenation in the presence of a Pd/carbon catalyst. Precursors were crystallized whenever possible, and their identity and purity checked by thin-layer chromatography, ^{1}H NMR, infrared spectroscopy, and melting point determination. The pentapeptide was cyclized as the *p*-nitrophenyl ester, which was formed by coupling the pentapeptide acid and *p*-nitrophenol by using dicyclohexylcarbodiimide. Removal of the *t*-Boc group yielded an amorphous solid (2.72 g from 3.08 g, 97%) which was dissolved in acidified dimethylformamide (63 mL) and added (dropwise) over 3 h to spectral grade pyridine (800 mL, dried over alumina) and stirred at 50 °C for 3 days. Removal of the pyridine yielded a yellowish oil that was dissolved in 50:50 ethanol/water (100 mL) and treated with Rexyn I-300 mixed-bed ion-exchange resin (10 g) for several hours. Evaporation of the solvent and trituration with ether yielded a crude product (1.36 g, 70% yield, ~90% pure); a portion of this material was subsequently crystallized from methylene chloride/ether: mp 144–149 °C, R_f (93:7, CHCl$_3$/CH$_3$OH) = 0.50. Appropriate ^{1}H, ^{13}C NMR spectra were observed; the identity and the monomeric nature of the peptide were con-

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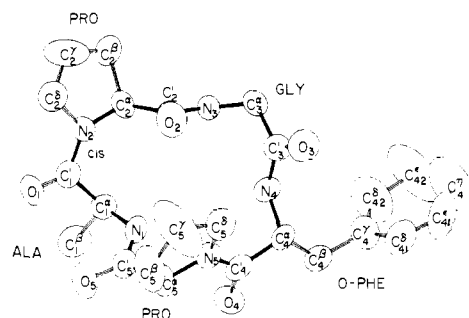

Figure 1. Diagram drawn by computer (ORTEP 11, ORNL-5138: Johnson, C. K. Oak Ridge National Laboratory, Oak Ridge, TN, 1976) using experimentally determined coordinates of *cyclo*(Ala-Pro-Gly-D-Phe-Pro). Ellipsoids represent the "thermal" parameters at the 50% probability level. The thermal parameters for the average structure presented here include positional disorder from molecule to molecule. Note the cis peptide bond between Ala[1] and Pro[2].

Table II. Fractional Coordinates[a] and Thermal Parameters[b] for *cyclo*(L-Ala-L-Pro-Gly-D-Phe-L-Pro)

atom	x	y	z	B_{eq}, Å^2
N1	0.1087	0.6802	0.7878	5.1
C1α	0.0489	0.7070	0.7325	4.9
C1'	-0.0623	0.6039	0.7156	5.6
O1	-0.0219	0.5112	0.6932	8.0
C1β	0.1638	0.7201	0.6865	6.9
N2	-0.2010	0.6195	0.7337	4.0
C2α	-0.2657	0.7285	0.7564	4.5
C2'	-0.2473	0.7395	0.8196	4.3
O2	-0.2412	0.6531	0.8501	5.7
C2β	-0.4317	0.7141	0.7416	5.9
C2γ	-0.4384	0.5903	0.7210	17.3
C2δ	-0.3123	0.5278	0.7203	6.3
N3	-0.2493	0.8582	0.8374	3.7
C3α	-0.2678	0.8820	0.8964	4.9
C3'	-0.1402	0.8574	0.9342	4.9
O3	-0.1570	0.8222	0.9832	6.2
N4	-0.0061	0.8792	0.9117	4.8
C4α	0.1347	0.8555	0.9416	4.3
C4'	0.2227	0.7711	0.9051	5.0
O4	0.3307	0.8075	0.8789	5.6
C4β	0.2136	0.9744	0.9529	5.8
C4γ	0.1393	1.0571	0.9942	5.8
C4δ1	0.1459	1.0412	1.0504	7.0
C4δ2	0.0522	1.1499	0.9744	11.0
C4ϵ1	0.0830	1.1174	1.0854	10.6
C4ϵ2	-0.0234	1.2282	1.0114	15.3
C4η	-0.0003	1.2096	1.0690	13.4
N5	0.1706	0.6557	0.8992	4.5
C5α	0.2419	0.5706	0.8609	5.1
C5'	0.2008	0.5874	0.8001	5.0
O5	0.2480	0.5157	0.7655	6.5
C5β	0.1866	0.4459	0.8817	8.6
C5γ	0.0616	0.4737	0.9141	15.2
C5δ	0.0616	0.5991	0.9353	6.1

[a] The esd's for x, y, and z are near 0.0009, 0.0008, and 0.0003, respectively, for the backbone atoms and increase up to 0.0020, 0.0018, and 0.0009 for some of the atoms in the side groups. [b] B_{eq} = $^4/_3 \Sigma_i \Sigma_j \beta_{ij} a_i \cdot a_j$.

firmed by mass spectrometry (*m/e* obsd for C$_{24}$H$_{31}$N$_5$O$_5$ 469.232, predicted 469.232).

X-ray Crystallography. Clear, colorless crystals were grown from CH$_3$CN. X-ray intensity data were collected from a platelet of dimensions 0.28 × 0.35 × 0.08 mm by using an automatic four-circle diffractometer in the $\theta/2\theta$ scan mode and a variable scan speed dependent upon the intensity. Data were collected with Cu Kα radiation and a graphite monochromator. During the data collection, reflections 032, 1 1 13 and 506, serving as monitors and remeasured at intervals of 60 measurements, did not indicate any decay. The data were corrected for Lorentz and polarization effects, and normalized $|E|$ values were obtained by means of a K curve. The space group is $P2_12_12_1$ with cell parameters a = 9.142 (2) Å, b = 11.000 (4) Å, c = 23.885 (5) Å, Z = 4, V = 2401.9 Å^3, and a calculated density of 1.298 g cm^{-3} for the molecular formula C$_{24}$H$_{31}$-N$_5$O$_5$ and a molecular weight of 469.54.

Table III. Bond Lengths (Å) and Angles (deg) for Crystal Structure^a

	L-Ala (1)	L-Pro (2)	Gly (3)	D-Phe (4)	L-Pro (5)	av
Bonds						
$N_i-C_i^\alpha$	1.460	1.443	1.442	1.495	1.463	1.460
$C_i^\alpha-C_i'$	1.576	1.523	1.501	1.508	1.511	1.524
$C_i'-O_i$	1.209	1.198	1.243	1.235	1.220	1.221
$C_i'-N_{i+1}$	1.351	1.373	1.359	1.363	1.356	1.360
$C_i^\alpha-C_i^\beta$	1.527	1.567		1.518	1.545	1.539
$C_i^\beta-C_i^\gamma$		1.449		1.505	1.415^b	
$C_i^\gamma-C_i^\delta$		1.343^b		1.355^b / 1.378	1.469	
$C_i^\delta-C_i^\epsilon$				1.317^b / 1.414		
$C_i^\epsilon-C_i^\eta$				1.328^b / 1.405		
$N_i-C_i^\delta$		1.468			1.457	
Angles						
$C_{i-1}'N_iC_i^\alpha$	125.4	127.6	118.5	123.8	120.2	123.1
$N_iC_i^\alpha C_i'$	109.1	113.2	117.6	106.9	114.4	112.2
$C_i^\alpha C_i'N_{i+1}$	115.6	112.4	115.5	116.5	117.0	115.4
$C_i^\alpha C_i'O_i$	121.5	122.9	121.8	121.3	118.9	121.3
$N_{i+1}C_i'O_i$	122.4	124.5	122.6	122.0	124.1	123.1
$C_i'C_i^\alpha C_i^\beta$	109.1	109.8		112.3	109.7	
$N_iC_i^\alpha C_i^\beta$	114.4	103.2		110.1	102.8	
$C_i^\alpha C_i^\beta C_i^\gamma$		102.2		115.0	104.4	
$C_i^\beta C_i^\gamma C_i^\delta$		116.7^b		123.5 / 118.9	113.0^b	
$C_i^\gamma C_i^\delta C_i^\epsilon$				121.8 / 121.2		
$C_i^\delta C_i^\epsilon C_i^\eta$				123.2^b / 116.7^b		
$C_i^\delta C_i^\gamma C_i^\delta$				117.5^b		
$C_i^\epsilon C_i^\eta C_i^\epsilon$				119.1		
$C_i^\gamma C_i^\delta N_i$		103.9			101.5	
$C_i^\delta N_i C_i^\alpha$		111.7			113.7	
$C_i^\delta N_i C_{i-1}'$		119.6			125.2	

^{a} Estimated standard deviations are of the order of 0.010 Å for bond lengths and 0.7° for bond angles in the peptide ring and increase up to 0.03 Å for bond lengths and 1.7° for bond angles in some of the side groups. ^{b} Elongated ellipsoids for the thermal parameters of some of the atoms in the side groups of Pro^2, D-Phe^4, and Pro^5 indicate positional disorder for these atoms. The bond lengths and angles listed represent a nonphysical model that is an average of several conformations.

The structure of the crystal was solved by determining phases directly from the intensities. After initial refinement with full-matrix least squares of the 34 C, N, and O atoms and anisotropic thermal parameters, hydrogen atoms were placed in idealized positions. Further least-squares refinement, holding the coordinates for the hydrogen atoms constant, resulted in an agreement factor of $R = 8.4\%$ for 1544 reflections with $|F|_{obsd} > 2$ and $R_w = 7.0\%$ for all 1817 data. The somewhat high R values can be correlated with local disorder and high thermal values for the side groups in the Pro and D-Phe residues. The structure of the molecule is depicted in the computer drawing in Figure 1. The coordinates for the C, N, and O atoms are listed in Table II, bond lengths and angles are shown in Table III, and conformational angles are shown in Table IV.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were obtained in the Fourier transform mode on Bruker WM250 or AM250 spectrometers operating at 250.13 MHz for ^{1}H and 62.90 MHz for ^{13}C. Samples were referenced to internal tetramethylsilane. Deuterated solvents were purchased from Cambridge Isotopes. Two-dimensional spectra (COSY and NOESY) were obtained by using standard Bruker software.

Results and Discussion

Conformation in the Crystal State. The conformation of cyclo(Ala^1-Pro^2-Gly^3-D-Phe^4-Pro^5) in crystals is shown in Figure 1. For purposes of comparison, the crystal conformation of a similar peptide with a D residue in place of the L-Ala of the title peptide is shown in Figure 2. An immediately obvious difference is the occurrence of a cis peptide bond between Ala^1 and Pro^2 in Figure 1. As a result of the cis bond, O$_1$ is directed to the exterior of the backbone ring and cannot participate in an intramolecular hydrogen bond with N$_4$H. A difference of 57° in ψ_5 has changed the direction of O$_4$ away from N$_1$H so that the resulting geometry

Table IV. Conformational Angles for Crystal Conformation (deg)^{a,b}

angle	L-Ala (1)	L-Pro (2)	Gly (3)	D-Phe (4)	L-Pro (5)
$\phi_i(N_i-C_i^\alpha)$	69	−89	74	124	−80
$\psi_i(C_i^\alpha-C_i')$	86	+154	34	−68	2
$\omega_i(C_i'-N_{i+1})$	14	166	−177	175	176
χ_{i1}		9^c		67	18^c
χ_{i2}		0		79	−23
χ_{i3}		−9			17
χ_{i4}		15			−4
$C_i^\delta N_i C_i^\alpha C_i^\beta$		−15			−9

^{a} The convention followed for labeling atoms and conformational angles is that proposed by the IUPAC-IUB Comission on Biochemical Nomenclature (*Biochemistry* **1970**, *9*, 3741-3479). In the fully extended $\phi_i = \psi_i = \omega_i = 180°$. ^{b} Esd's for the torsional angles are near 1.4°. ^{c} The C$^\gamma$ atoms in pyrrolidine rings in Pro^2 and Pro^5 are highly disordered (see text) representing two possible envelope conformations. The angles listed are for a nonphysical model of the average of the different conformations.
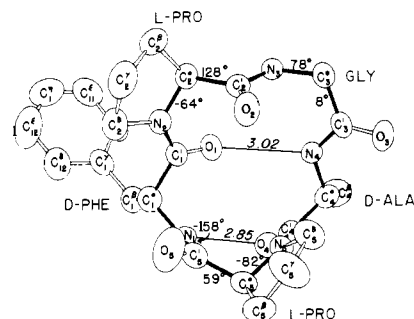
Figure 2. Diagram of *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro), having a typical backbone conformation for a DLDDL sequence.^{5} Note that the Gly residue has assumed ϕ,ψ angles characteristic for a D residue.

in the molecule in Figure 1 is inappropriate for forming a 3 → 1 hydrogen bond (γ turn) as exists in the molecule in Figure 2. Furthermore, the N$_1$···O$_4$ distance in Figure 1 is 3.29 Å. In fact, N$_1$ is buried in the interior of the present molecule and does not participate in any hydrogen bond formation, intra- or intermolecular.

The substitution of an L residue for a D residue in position 1 makes the conformation shown in Figure 2 untenable for steric reasons. Space-filling models show a collision between the methyl side chain in Ala^1 and O$_5$ as well as C$_2^\delta$ of the Pro^2 residue.

Bond angles differ for the cis-Pro^2 and trans-Pro^5 residues. For example, C$_1$N$_2$C$_2$ is 127.6° while C$_4$N$_5$C$_5$ is only 120.2° (Table IV). This difference is consistent with the difference in values of bond angles observed for cis and trans residues in other peptides.[13,14]

An examination of the "thermal"parameters, represented in Figure 1 by ellipsoids at the 50% probability level and listed in the B_{eq} column in Table II, shows that very large ellipsoids are associated with C$^\gamma$ atoms in the two Pro residues and in the outermost atoms of the D-Phe residue. The thermal ellipsoids represent not only the natural vibrations of the atoms but also positional disorder of the atoms from molecule to molecule in the crystal. The elongated ellipsoids for the C$_2^\gamma$ and C$_5^\gamma$ atoms in the two pyrrolidine rings indicate puckering disorder. Within each ellipsoid there are two different positions for each of these atoms which correspond to the two envelope conformations that are endo and exo with respect to the interior of the backbone ring of the peptide. The thermal ellipsoids for the phenyl ring in D-Phe^4, seen from different vantage points in Figures 1 and 3, increase in elongation from C$_4^\gamma$ to C$_4^\eta$. The relative size and orientation of the ellipsoids suggest the presence of different rotamers for the phenyl ring in the different unit cells. The different orientations may be produced by small rotations about the C$_4^\alpha$–C$_4^\beta$ bond and

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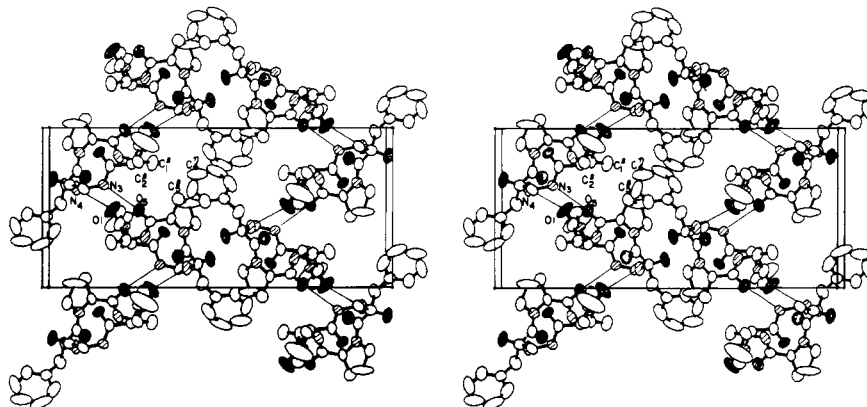
Figure 3. Stereo diagram of the packing in the crystal of *cyclo*(Ala-Pro-Gly-D-Phe-Pro). The axial directions for the unit cell are $b \uparrow$, $c \rightarrow$, and a directed into the page. Pairs of hydrogen bonds between NH and O groups, indicated by thin lines, link molecules into infinite zigzag ribbons parallel to the b axis. Oxygen atoms are shaded and nitrogen atoms are striped.

Table V. NMR Parameters for One-Cis Solution Conformation of *cyclo*(Ala-Pro-Gly-D-Phe-Pro)[a]

parameter	solvent	Ala	Pro	Gly	D-Phe	Pro
δ_{NH}	$Me_2SO\text{-}d_6$	6.95		8.43	8.73	
	$CDCl_3$	6.24		~7.3	7.58	
$\Delta\delta/\Delta T$-(NH)[b]	$Me_2SO\text{-}d_6$	<-1		-6.1	-5.7	
	$CDCl_3$	-2.0		<|4|[c]	-9.0	
J_{NH-H^α}, Hz	$Me_2SO\text{-}d_6$	4.5		4.6, 6.3	7.8	
	$CDCl_3$	<2		8, <2	4	
δ_{H^α}[d]	$Me_2SO\text{-}d_6$	3.70	4.58	3.6, 3.15	4.25	4.20
	$CDCl_3$	3.9		4.3, 3.4	4.5	
δ_{H^β}[d]	$Me_2SO\text{-}d_6$	1.3	2.1, 1.9		2.9, 2.75	1.9, 1.1
	$CDCl_3$	1.5			3.1, 2.9	
$J_{H^\alpha-H^\beta}$	$Me_2SO\text{-}d_6$				6.3, 8.8	
	$CDCl_3$				11, 4	
δ_{C^β}[e]	$Me_2SO\text{-}d_6$		31.64			28.41
	$CDCl_3$		31.77			28.64
δ_{C^γ}[e]	$Me_2SO\text{-}d_6$		21.78			23.94
	$CDCl_3$		22.19			25.01

[a] Unless noted otherwise, 25 °C, 5 mg/0.5 mL in $Me_2SO\text{-}d_6$, 20 mg/0.5 mL in $CDCl_3$, 250 MHz (^1H), 62.9 MHz (^{13}C). Chemical shifts in ppm downfield from internal tetramethylsilane. Where measurable, parameters are given in both $Me_2SO\text{-}d_6$ and $CDCl_3$. Upfield region of spectrum in $CDCl_3$ was highly overlapped due to 30:70 mixture of one-*cis* and all-*trans* conformers. [b] Parts per billion/deg temperature range 22–110 °C (Me_2SO), 22–52 °C ($CDCl_3$). [c] Under the aromatics, estimated maximum slope. [d] Centers of multiplets; rough positions in many cases from two-dimensional correlated spectra. [e] Proline assignments are not definitive; those presented are based on comparisons with other pentapeptides.

small rotations about the C_4^β–C_4^γ bond. The multiple conformations for the side groups in this structure are possible owing to the relatively large space in the unit cell surrounding each of these groups, thus giving them some freedom of motion. The packing diagram of the molecules in the crystal lattice, illustrated in Figure 3, shows that the pyrrolidine and phenyl rings are adjacent to voids where the nearest approaches across the voids are greater than 4.0 Å.

The stability of the lattice is maintained by pairs of hydrogen bonds that link adjacent molecules firmly into zigzag ribbons. The hydrogen bonds are between $N_3\cdots O_5$ (3.007 Å) and $N_4\cdots O_1$ (2.907 Å) in molecules related by the twofold screw operations parallel to the b axis. Relatively weaker van der Waals' attractions between hydrophobic groups hold the ribbons of molecules together. For example, in the a direction, that is, perpendicular to the view in Figure 3, there are attractions between the methyl group of Ala[1] and the pyrrolidine ring of Pro[2] in the molecule directly underneath where $C_1\cdots C_2$ is 3.93 Å, and between the pyrrolidine ring of Pro[5] and the phenyl ring of D-Phe[4] from a molecule in an adjacent ribbon (see the middle of Figure 3) where $C_4\cdots C_5$ is 3.54 Å.

Conformations in Solution. The preferred conformation of this cyclic pentapeptide in dimethyl sulfoxide ($Me_2SO\text{-}d_6$) retains the
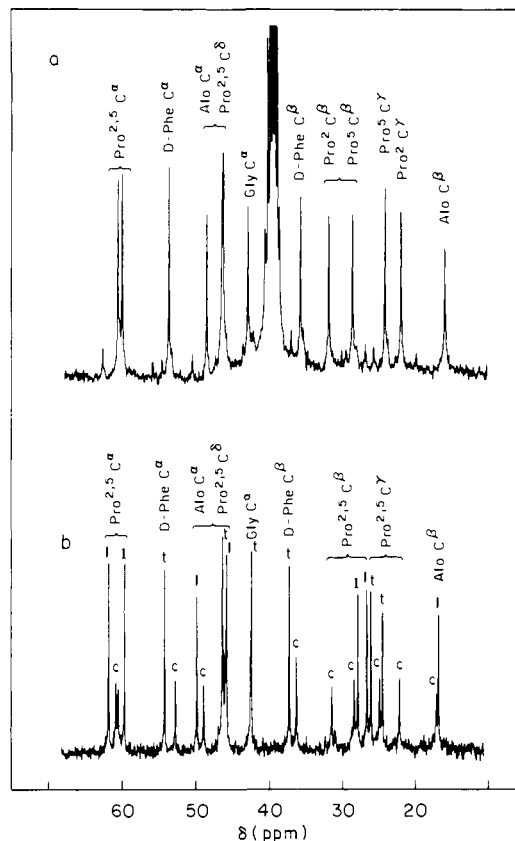

Figure 4. Upfield regions of 62.9-MHz ^{13}C NMR spectra of *cyclo*-(Ala-Pro-Gly-D-Phe-Pro) (a) $Me_2SO\text{-}d_6$, 40 mg/mL, and (b) in $CDCl_3$, 40 mg/mL. Temperature 25 °C. In (b), resonances for the one-cis conformer are labeled "c" and those of the all-trans conformer "t".

principal features of its conformation in the crystal state: a cis peptide bond between L-Ala[1] and L-Pro[2] and an unusual *buried* Ala NH that is not intramolecularly hydrogen bonded. Evidence supporting this conformation includes the following: (1) A ^{13}C NMR spectrum of the peptide reveals the characteristic pattern of proline C^β and C^γ resonances for one cis and one trans X-Pro bond (Figure 4a). (2) ^1H NMR data for this peptide in $Me_2SO\text{-}d_6$ (Table V) indicate that the Ala NH is inaccessible to intermolecular interactions (small $\Delta\delta/\Delta T$) but resonates at a high chemical shift, consistent with little or no hydrogen bonding. (3) A sizable nuclear Overhauser effect was observed between Ala[1] H^α and the downfield Pro H^α resonances, as expected for a cis Ala-Pro bond. (4) The D-Phe and Gly NH's behave as though they are solvated by Me_2SO molecules (high $\Delta\delta/\Delta T$, low δ).

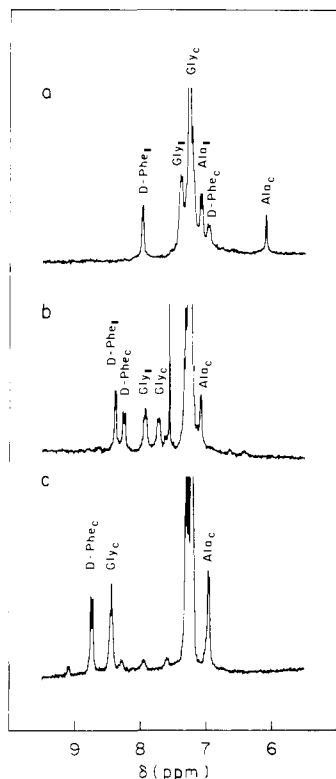A comparison in greater detail between the crystal conformation of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) and its conformation in

Figure 5. Downfield (amide) regions of 250-MHz ^{1}H NMR spectra of *cyclo*(Ala-Pro-Gly-D-Phe-Pro). Representative points during a CDCl$_3$/Me$_2$SO-d_6 titration (percentage given as v/v) of the pentapeptide are shown. Concentration 5 mg/0.5 mL. Temperature 25 °C. (a) 100% CDCl$_3$; (b) 85% CDCl$_3$, 15% Me$_2$SO-d_6; (c) 100% Me$_2$SO-d_6. Resonances from the one-cis conformer are labeled "c" and those from the all-trans conformer "t".

Me$_2$SO-d_6 suggests that there may be some minor rearrangements or averaging in solution. Observed vicinal coupling constants ($J_{NH-H^{\alpha}}$) differ from those expected if the crystal conformation were strictly retained in Me$_2$SO-d_6. For example, $J_{NH-H^{\alpha}}$ for Ala (4.5 Hz) can be attributed to a ϕ dihedral angle of ca. 100°, while the X-ray derived ϕ angle (69°) would be expected to show a $J_{NH-H^{\alpha}}$ of 6-8 Hz.[15] Conformational averaging around ϕ would be expected to reduce the observed coupling constant, since the X-ray derived ϕ is near a maximum in the J vs. ϕ function.

Model building together with fitting of observed NMR parameters yields a possible conformation for the one-cis form of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in Me$_2$SO-d_6:

	Ala	Pro	Gly	D-Phe	Pro
ϕ	100	−80	~90	120	−80
ψ	120	160	80	−100	0

The glycine $J_{NH-H^{\alpha}}$ values (4.6 and 6.3 Hz) suggest some degree of averaging around this residue. Note from Table V that the NMR parameters for the one-cis form present as 30% of the conformational distribution in CDCl$_3$ are somewhat different from those in Me$_2$SO-d_6. Hence, while the same peptide bond states of isomerization obtain, perturbations around the backbone have occurred in this weakly hydrogen-bond-donating solvent. For example, the Ala $J_{NH-H^{\alpha}}$ coupling constant is less than 2 Hz, suggesting an Ala ϕ angle close to 120°.[15] The region of the structure around this Ala residue is extremely strained and crowded; there is no arrangement, given the constraints of cyclization, that seems devoid of unfavorable contacts. Not only is the Ala methyl sterically hindered by the carbonyl oxygens of Pro^5 and Ala^1, but also these two oxygens are subject to close
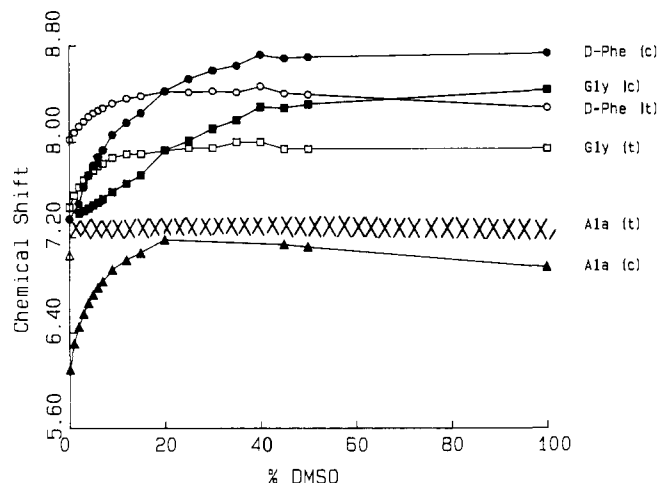
(15) (a) Bystrov, V. F.; Portnova, S. L.; Balashova, T. A.; Koz'min, S. A.; Gavrilov, Yu. D.; Afanas'ev, V. A. *Pure Appl. Chem.* **1973**, *36*, 19–34. (b) Ramachandran, G. N.; Chandrasekaran, R.; Kopple, K. D. *Biopolymers* **1971**, *10*, 2113.


Figure 6. Plot of CDCl$_3$/Me$_2$SO-d_6 titration data: NH resonances as a function of solvent composition. The cross-hatched band indicates the position of the aromatic resonances. Open symbols: all-trans conformer; filled symbols: one-cis conformer.

approach given certain combinations of dihedral angles (as in the crystal).

The conformational lability of this constrained peptide is illustrated further by its response to a change of solvent. As shown in Figure 4b, the ^{13}C NMR spectrum of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in CDCl$_3$ displays Pro C$^{\beta}$ and C$^{\gamma}$ resonances indicative of two populations of slowly interconverting conformers: one, all-trans, present in 70% proportion and the other, one-cis, making up the remaining 30%. Titration of the peptide in CDCl$_3$ with Me$_2$SO-d_6 confirms that the minor form present in CDCl$_3$ has the same arrangement of peptide bond configurations as the major form in Me$_2$SO-d_6 (Figures 5 and 6). Spectral parameters for this one-cis form are presented in Table V, and its conformation is discussed above.

The other form present in CDCl$_3$ has all-trans peptide bonds, as judged by its ^{13}C NMR spectrum (Figure 4b). The complexity of ^{1}H NMR spectra for the pentapeptide in CDCl$_3$ presents problems for analysis of the all-trans form. Two-dimensional NMR spectroscopy was used to separate and assign the resonances of the two interconverting conformers. J-coupled spin systems were analyzed by using two-dimensional correlated (COSY) spectra, in which the normal one-dimensional spectrum appears along the diagonal (usually presented as a contour plot) and cross-peaks occur at the intersections of chemical shifts for two J-coupled resonances. The COSY spectrum for *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in CDCl$_3$ is shown in Figure 7. The strength of the two-dimensional method of spectral analysis is clearly demonstrated in this example of a highly overlapped mixture of conformers. Another two-dimensional NMR experiment, the NOE-correlated or NOESY spectrum (not shown), was very informative in this study. Resonances that exchange spin polarization by dipole–dipole interactions, usually through space, give rise to cross-peaks in NOESY spectra. Alternatively, spin polarization can be transferred by chemical exchange, provided that chemical exchange occurs slowly relative to the rate of polarization transfer. Here, resonances arising from one of the two slowly interconverting conformers give rise to cross-peaks with corresponding resonances in the other conformer. By analyzing these results, the assignments to the one-cis or all-trans form were confirmed. When these approaches were used, the key spectral data (given in Table VI) were obtained, and taken as a whole, they lead to a consistent overall model. There is a suggestion of some conformational dependence on concentration, temperature, or solvent. Spectral parameters for the NH resonances indicate that the D-Phe and Ala NH's are less accessible to intermolecular interactions than is the Gly NH, but the Ala NH appears not to be intramolecularly hydrogen-bonded. Evidence for this interpretation includes the following: (1) the higher chemical shift of the Ala NH than those of the D-Phe and the Gly in pure CDCl$_3$
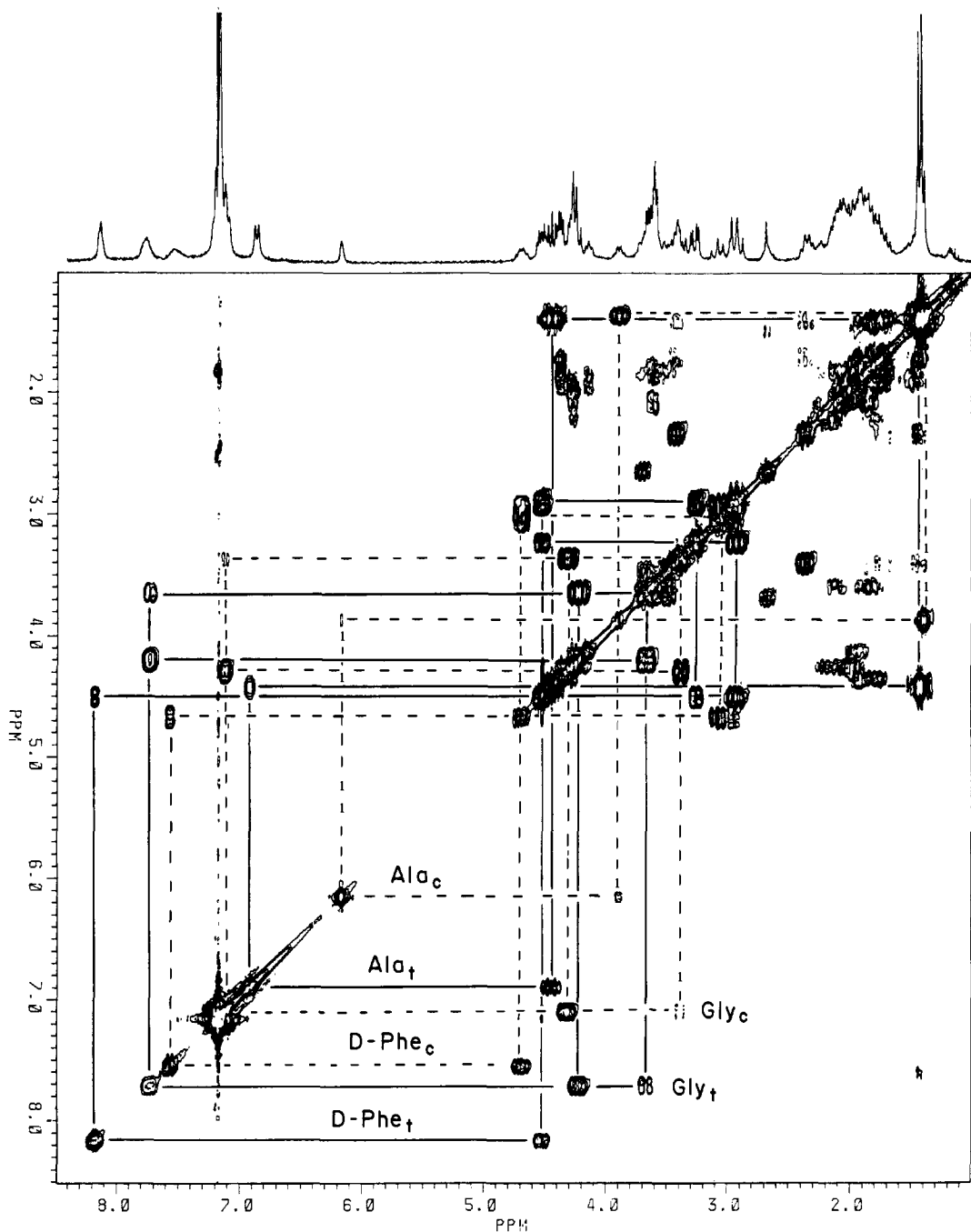
Figure 7. Two-dimensional correlated (COSY) spectrum of cyclo(Ala-Pro-Gly-D-Phe-Pro) in $CDCl_3$. For reference the one-dimensional spectrum is shown across the top. The spin systems for Ala, D-Phe, and Gly residues in each conformer are connected by dashed (one-cis conformer) or solid (all-trans conformer) lines. These data were obtained by using standard Bruker software, with a $90-t_1-90$ acquire sequence, 2048 points zero-filled to 4096 in F2, number of t_1's 256, were zero-filled to 512 points in F1, number of scans 96, sine-bell apodization in both dimensions. Peptide concentration 20 mg/0.5 mL. Note that in two dimensions, adequate resolution is achieved to identify all the Ala, D-Phe, and Gly resonances. Examination of rows in the two-dimensional matrix enabled rough measurement (resolution 2.4 Hz/point) of J_{NH-H^α} for the Gly residues and $J_{H^\alpha-H^\beta}$ for the D-Phe residues (Tables V and VI).

(at concentrations dilute enough to prevent intermolecular effects); (2) the small, positive $\Delta\delta/\Delta T$ of the Ala NH which contrasts with the negative $\Delta\delta/\Delta T$ of the D-Phe, which in turn has a smaller magnitude than that of the Gly (also negative); and (3) the shifts caused by addition of Me_2SO to a $CDCl_3$ solution of the peptide—greater for the Gly than the D-Phe NH resonances, which were both apparently larger than the shifts of the Ala NH resonance (Figure 6). The unusual positive $\Delta\delta/\Delta T$ of the Ala NH and small changes in coupling constants (upon solvent titration) indicate some degree of conformational change.

The ^{13}C NMR spectrum of the cyclic pentapeptide in $CDCl_3$ (Figure 4b) has an unusual pattern of Pro C^β and C^γ resonances for the all-trans conformer. One C^γ signal is at lower field, and one C^β signal is at higher field than typical "unconstrained" values.

Although unique assignments of carbon signals to particular prolines in the sequence are not possible, these positions are reminiscent of proline signals in the all-trans conformers of the cyclo(L-X-Pro-D-Y[Gly])₂ hexapeptides, which, like this cyclic pentapeptide, undergo a cis–trans isomerization.[16] In the case of the cyclic hexapeptides, consideration of a variety of spectral data led to a proposal of a bifurcated hydrogen bond: a γ turn within a β turn, where two NH's, the NH's of the $(i + 2)$th and the $(i + 3)$th residues in the β turn, both interact with the C=O of the ith residue.[16] This arrangement, coupled with the presence of an L-residue preceding Pro, may be correlated with the unusual

(16) Gierasch, L. M.; Deber, C. M.; Madison, V.; Niu, C. H.; Blout, E. R. *Biochemistry* **1981**, *20*, 4730–4738.

Table VI. NMR Parameters for All-Trans Solution Conformation of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in $CDCl_3$[a]

parameter	Ala	Pro	Gly	D-Phe	Pro
δ_{NH}	6.95		7.84	8.21	
$\Delta\delta/\Delta T(NH)$[b]	+3.0		-7.7	-4.7	
$\Delta\delta/\Delta$solvent[c]	<0.25[d]		0.50	0.25	
J_{NH-H^α}, Hz	6.9		8, ~2	4.5	
δ_{H^α}[e]	4.5		4.2, 3.7	4.7	
δ_{H^β}	1.5			3.3, 2.9	
$J_{H^\alpha-H^\beta}$				2, 11	
δ_{C^β}[f]		28.08			26.79
δ_{C^γ}[f]		24.27			26.20

[a] Unless otherwise noted, 25 °C and 20 mg/0.5 mL, 250 MHz for ^1H, 62.9 MHz for ^{13}C, chemical shifts in ppm from internal tetramethylsilane. [b] Parts per billion/deg, temperature range 22–52 °C. [c] $\delta(Me_2SO-d_6)-\delta(CDCl_3)$; carried out at 5 mg/0.5 mL concentration (Figures 5 and 6). [d] Under the aromatics, estimated maximum chemical shift. [e] Upfield resonance positions are estimated from two-dimensional NMR spectra. *J*-coupled systems were observed via COSY spectra and conformational isomers via two-dimensional NOE spectra. Prolines could not be uniquely assigned. [f] Proline assignments are not definitive; those presented are based on arguments from previous cyclic pentapeptides.

trans-Pro ring carbon resonance positions. The other proline in the cyclic pentapeptide has ^{13}C resonance positions with typical values for prolines not participating in γ-turn conformations and experiencing no unusual steric effects.

Titration of a $CDCl_3$ solution of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) with Me_2SO-d_6 causes decreased intensity and pronounced shifts of resonances of the all-trans conformer, increased intensity and shifting of resonances from the one-cis form, and some alterations of coupling constants for both species (Figures 5 and 6). It is evident that modest conformational rearrangement within the all-trans form occurs concurrent with the shift in the all-trans/one-cis equilibrium. The Gly NH of the all-trans form undergoes the largest shift in resonance position upon addition of small amounts of Me_2SO-d_6, followed by the NH of the D-Phe, then that of the Ala (Figure 6). At 100% Me_2SO-d_6, such a small amount of the all-trans form is present that analysis of its spectral parameters is not possible. Interestingly, another slowly interconverting conformer appears in small concentration in the high [Me_2SO] ranges of the titration. This minor conformer must arise from a two-cis or the alternate one-cis isomer of the pentapeptide ring.

Model building together with fitting of observed NMR parameters leads to the suggestion that the following all-trans conformation is a major contributor to the conformational distribution of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in $CDCl_3$:

	Ala	Pro	Gly	D-Phe	Pro
ϕ	-160	-60	90	150	-75
ψ	-170	90	40	-130	50

Note that this conformation (illustrated in Figure 8) is a somewhat modified version of the preferred all-trans conformation of *cyclo*(Gly-Pro-Gly-D-Ala-Pro)[2] and *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro)[12] (Figure 2). There is a weak γ turn within the Pro-Gly type II β turn, and the D-Phe-Pro-Gly sequence does not take up a γ
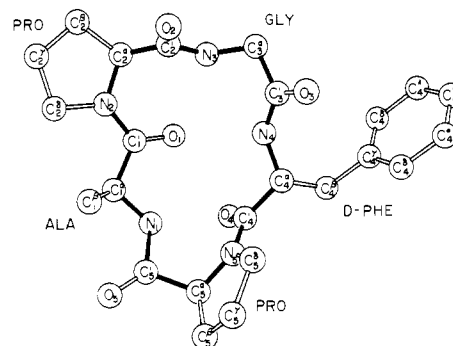

Figure 8. Diagram of *cyclo*(Ala-Pro-Gly-D-Phe-Pro) in the all-trans conformation proposed to exist in $CDCl_3$ solution on the basis of model building from NMR data. This conformation probably represents an average structure; other ϕ,ψ values for Gly[3] seem particularly accessible. In building this structure, cyclization was achieved most readily with the Pro-Ala bond adopting an ω value of -160°, as has been seen in other of the cyclic pentapeptides.[3,5]

turn. The altered backbone geometry appears to accommodate the Ala CH_3 with reduced crowding.

The introduction of an L residue preceding a proline in *cyclo*-(Ala-Pro-Gly-D-Phe-Pro) has markedly changed the conformational preferences of the pentapeptide backbone compared to analogous peptides with a D residue or Gly preceding Pro. Empirical energy calculations have shown that the L-X-L-Pro sequence places significant constraints on the conformational space available to a peptide.[17] Previous work with the *cyclo*(L-X-Pro-D-Y[Gly])$_2$ hexapeptides found a similar conformational interconversion to that seen here, which also was strongly correlated with the size of the side chain of L-X and with the polarity of the solvent.[16,18,19] As observed for the cyclic pentapeptide in the present study, Me_2SO favors the cis form of the cyclic hexapeptides and $CDCl_3$ the trans. Interestingly, in neither the cyclic hexa- nor pentapeptide cis conformers are there any intramolecular hydrogen bonds.[19] Fundamental characteristics of this local dipeptide sequence appear to influence strongly the relative energies of peptide conformational states; the preferred conformation is not only a function of constraints specific to the cyclic hexa- or pentapeptide ring.

Supplementary Material Available: Table of anisotropic thermal parameters, fractional coordinates of hydrogen atoms, and observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

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(18) Kopple, K. D.; Schamper, T. J.; Go, A. *J. Am. Chem. Soc.* **1974**, *96*, 2597–2605.
(19) Kartha, G.; Bhandary, K. K.; Kopple, K. D.; Go, A.; Zhu, P. P. *J. Am. Chem. Soc.* **1984**, *106*, 3844–3850.