Conformation of the Cyclic Pentapeptide Gly-L-Pro-L-Ser-D-Ala-L-Pro in the Crystalline State and an Example of Rotational "Isomerism" between Analogues

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Abstract: Cyclic Gly[1]-L-Pro[2]-L-Ser[3]-D-Ala[4]-L-Pro[5] ($C_{18}H_{27}N_5O_6$) contains all trans peptide units with significant deviations from planarity in three of them where $\omega_3 = -165°$, $\omega_4 = 160°$, and $\omega_5 = 169°$. If the side groups are disregarded, the conformation of the cyclic backbone is almost identical with that found in cyclic Gly[1]-L-Pro[2]-Gly[3]-D-Ala[4]-L-Pro[5], previously reported, with the exception that the backbones in the two molecules are related by a mirror. If the peptide backbones of both molecules are held in the same orientation, the side groups of the Ser-containing molecule have progressed by one peptide unit along the backbone as compared to the Gly[3] molecule. One transannular 4→1 hydrogen bond (type II′) is formed encompassing Gly[1]-L-Pro[2] with $\phi_1 = 58°$, $\psi_1 = -128°$, $\phi_2 = -75°$, and $\psi_2 = 20°$. This is a first observation, at least in crystals of relatively small peptides, for the second corner of a β turn to contain a Pro group. The space group is $P2_1$ with $a = 12.392$ (7) Å, $b = 9.149$ (8) Å, $c = 10.428$ (4) Å, $\beta = 97.3°$, and $Z = 2$. The peptide molecules are stacked in the crystal with NH···O=C and OH···O=C hydrogen bonds between molecules in a stack. The stacks of peptide molecules are separated by layers of solvent molecules, CH_2Cl_2.

Parallel studies on the solution and crystalline conformations of cyclic Gly[1]-L-Pro[2]-L-Ser[3]-D-Ala[4]-L-Pro[5] have been carried out by NMR and X-ray diffraction analysis, respectively. The results of the conformational analysis of this cyclic pentapeptide in solution are reported in an accompanying paper.[1] This paper concerns the conformation and structure in the crystalline state. There are both similarities and significant differences in conformation and intramolecular hydrogen bonding between the present cyclic pentapeptide and its analogue,[2,3] cyclic Gly[1]-L-Pro[2]-Gly[3]-D-Ala[4]-L-Pro,[5] where the only chemical difference is the substitution of L-Ser[3] for Gly[3].

Experimental Section

Clear, lath-shaped crystals of c-Gly-Pro-Ser-D-Ala-Pro grown from CH_2Cl_2/hexane solution were obtained from Pease and Niu.[1] X-ray intensity data were collected on a four-circle automatic diffractometer with the θ–2θ scan mode using a scan of $2.0° + 2\theta(\alpha_2) - 2\theta(\alpha_1)$ and a scan speed of 2°/min. Data were collected with Cu Kα radiation to a scattering angle of $2\theta = 120°$ for a total of 1864 reflections. Lorentz and polarization corrections were applied and normalized structure factors were derived with the aid of a K curve. The space group is $P2_1$ with cell parameters $a = 12.392$ (7) Å, $b = 9.149$ (8) Å, $c = 10.428$ (4) Å, $\beta = 97.31$ (3)°, $z = 2$, $v = 1172.7$ Å^3, and a calculated density of 1.400 g/cm^3 for two peptide molecules plus two CH_2Cl_2 molecules per unit cell.

The structure of the crystal was solved with some difficulty by the direct method[4] of phase determination using symbolic addition. The fragment of the molecule obtained from the determined phases was misplaced with respect to the twofold screw axis. The procedure for reducing the symmetry of the cell to space group $P1$, and consequently doubling the number of atoms to be placed in the cell, yielded the structure.[5] Subsequently, the structure was solved more directly by a new automated procedure.[6]

Full-matrix least-squares refinement[7] on the 32 nonhydrogen atoms, with weighting based on counting statistics,[8] led to an R factor of 17% for isotropic thermal parameters and 10.4% for anisotropic thermal parameters. Scattering factors used were those listed in Vol. III of the "International Tables for Crystallography" and the function minimized was $\Sigma w(|F_o| - |F_c|)^2$. Most of the hydrogen atoms were located in a difference map and coordinates for the remaining hydrogen atoms were calculated for idealized positions. In the final cycles of refinement the coordinates of the hydrogen atoms were not varied, except for the H on N_3. The R factor, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, for all the observed data was 8.5%, and 8.2% for the 1816 reflections greater than σ. The large decrease in the value of the R factor between the isotropic and anisotropic refinements, and the relatively large value for the final R factor, must be due in large part to the CH_2Cl_2 molecule that cocrystallizes with the peptide molecule. The thermal parameters for the Cl atoms are very large and anisotropic. In fact, in the E map the weights of the Cl atoms are no larger than the weights of the C and O atoms. Moreover, atoms in the side groups of the peptide, particularly C_5^γ and C_4^β, also have very large thermal parameters, indicating a considerable degree of disorder, and contributing to a large R factor.

The labeling of the atoms is shown in Figure 1, the coordinates for the atoms, other than hydrogen, are listed in Table I, the bond lengths and bond angles are shown in Table II, while the conformational angles are shown in Table III.

Results

The Molecule. The most striking feature of this molecule is the similarity of the conformation of the backbone with that in the Gly[3] analogue,[2] which can be seen by comparing Figures 1 and 2. The backbones in the two molecules are related by a mirror. The side groups, however, have been moved by one peptide unit along the backbone (Figure 3). A possible explanation for the rotation of the side groups may be that the conformation for the ring is quite stable for the chiral sequence DLDDL (or the mirror image LDLLD). From the values of the torsional angles ϕ and ψ plotted on the graph in Figure 4, it is seen that the Gly residues in the Gly[3] pentapeptide behave as if they had the D hand. A replacement of such a Gly residue with L-Ser results in the chiral sequence DLLDL. If the side groups are shifted around the ring to the next C^α, then the chiral sequence becomes LDLLD, the mirror image of the first pentapeptide. Perhaps, if D-Ser rather than L-Ser were substituted for Gly[3], the two pentapeptide analogues would remain isostructural, rather than exhibit rotational "isomerism", i.e., the same backbone shape but a shifting of the side groups to the next C^α atom.

A comparison of the ϕ and ψ values for the two analogues (Figure 4) shows that the difference in the values are very small around the two C^α atoms which form the corners of the 4→1 transannular hydrogen bonds and the differences become larger, 30° and up to 50°, for the remaining three C^α atoms. These differences in ϕ and ψ are accounted for by the occurrence of a 3→1 transannular hydrogen bond encompassing Pro[5] in the Gly[3] analogue and the impossibility for such hydrogen bonding in the Ser[3] analogue since a Pro residue has

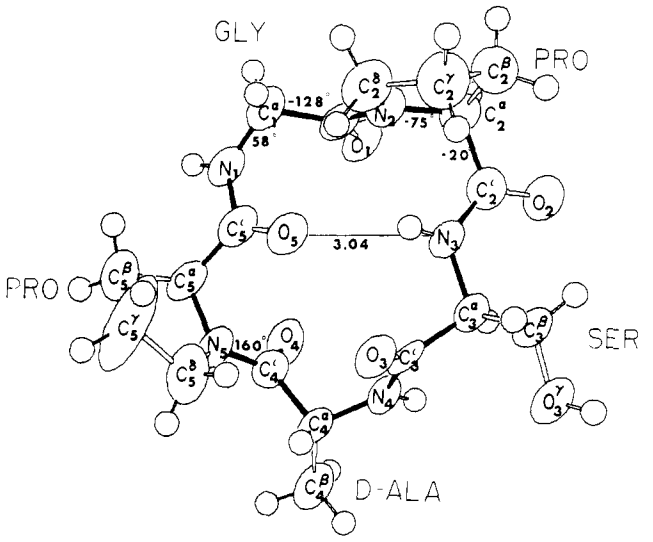

Figure 1. Conformation of cyclic (Gly-Pro-Ser-D-Ala-Pro) in the crystalline state. The thermal ellipsoids for the C, N, and O atoms are drawn at the 50% probability level. Hydrogen atoms are indicated by small spheres and the hydrogen bond (4→1, type II′) is indicated by the thin line.

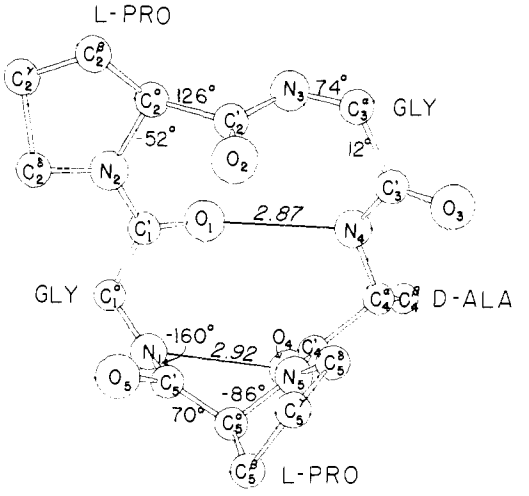

Figure 2. Conformation of cyclic (Gly-Pro-Gly-D-Ala-Pro) in the crystalline state,[2] an analogue and rotational "isomer" of molecule in Figure 1.

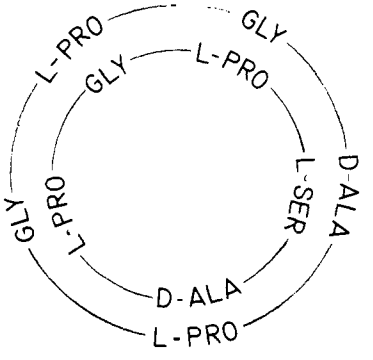

Figure 3. A schematic comparison of the placement of the side groups in two cyclic pentapeptide analogues that illustrate rotational "isomerism". The Ser[3] analogue is represented by the inner circle and the Gly[3] analogue is represented by the outer circle.

moved into the position occupied by a Gly residue in the earlier analogue and there no longer exists an available NH.

The values of ω, the torsional angle about the amide bond,

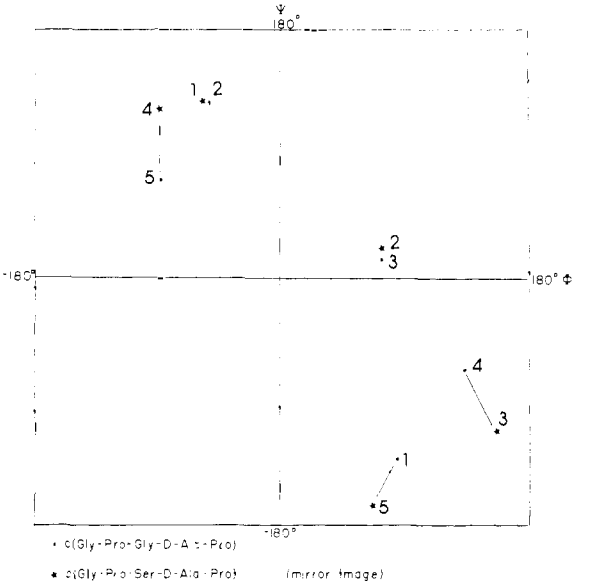

Figure 4. The conformational angles ϕ and ψ representing the torsions about the N_i–C_i^α bonds and the C_i^α–C_i' bonds, respectively. The signs for all the values for the Ser[3] analogue have been changed (the equivalent of a mirror image) in order to compare directly with the Gly[3] analogue.

Table I. Fractional Coordinates

atom	x	y	z
N_1	0.4394 (6)	−0.0741 (9)	0.3919 (6)
C^α_1	0.5540 (8)	−0.0890 (11)	0.3857 (7)
C'_1	0.6121 (7)	0.0543 (11)	0.4077 (7)
O_1	0.6085 (5)	0.1289 (8)	0.5087 (5)
N_2	0.6772 (6)	0.1013 (9)	0.3210 (6)
C^α_2	0.7429 (7)	0.2330 (11)	0.3404 (7)
C'_2	0.6783 (7)	0.3767 (12)	0.3188 (7)
O_2	0.7233 (5)	0.4910 (9)	0.3539 (6)
C^β_2	0.8241 (8)	0.2153 (13)	0.2450 (9)
C^γ_2	0.7597 (9)	0.1356 (14)	0.1341 (8)
C^δ_2	0.6838 (8)	0.0328 (12)	0.1929 (7)
N_3	0.5788 (5)	0.3693 (11)	0.2478 (6)
C^α_3	0.5152 (6)	0.5039 (10)	0.2176 (7)
C'_3	0.3999 (7)	0.4520 (9)	0.1658 (7)
O_3	0.3823 (4)	0.3801 (8)	0.0676 (4)
C^β_3	0.5617 (7)	0.5900 (10)	0.1100 (8)
O^γ_3	0.4958 (5)	0.7147 (9)	0.0864 (5)
N_4	0.3228 (5)	0.4880 (9)	0.2425 (6)
C^α_4	0.2161 (7)	0.4145 (11)	0.2209 (8)
C'_4	0.2315 (7)	0.2744 (11)	0.2989 (7)
O_4	0.2651 (5)	0.2808 (9)	0.4174 (5)
C^β_4	0.1278 (9)	0.5096 (13)	0.2618 (14)
N_5	0.2118 (6)	0.1439 (9)	0.2398 (6)
C^α_5	0.2599 (9)	0.0120 (12)	0.3042 (9)
C'_5	0.3819 (8)	0.0218 (11)	0.3094 (8)
O_5	0.4286 (5)	0.1065 (9)	0.2439 (6)
C^β_5	0.2138 (11)	−0.1113 (14)	0.2110 (16)
C^γ_5	0.1842 (21)	−0.0410 (18)	0.0855 (15)
C^δ_5	0.1755 (9)	0.1205 (13)	0.1025 (9)
SC[a]	−0.1053 (9)	0.7285 (16)	0.3775 (10)
SCl_1[a]	−0.0021 (3)	0.8327 (8)	0.4533 (3)
SCl_2[a]	−0.1081 (3)	0.7318[b]	0.2112 (3)

[a] Solvent molecule. [b] Held constant during refinement to fix origin along b axis in space group $P2_1$.

are a measure of the planarity of the peptide groups. In the Gly[3] analogue the ω values are 174, −179, 177, 178, and −160°, whereas in the Ser[3] analogue they are −175, −177, −165, 160, and 169°. Thus in each molecule, the amide bonds have twists up to 20° from the planar trans conformation. These observations indicate that the nature of the backbone conformation is more important than the location of side

Table II. Bond Lengths (Å) and Angles (deg)[a]

	Gly 1	Pro 2	Ser 3	D-Ala 4	Pro 5	av
			Bonds			
N_i-C_i^α	1.436	1.454	1.474	1.474	1.470	1.462
C_i^α-C_i'	1.499	1.541	1.537	1.517	1.508	1.520
C_i'-O_i	1.260	1.220	1.213	1.254	1.226	1.235
C_i'-N_{i+1}	1.357	1.356	1.363	1.351	1.365	1.358
C_i^α-C_i^β		1.512	1.542	1.502	1.550	1.526
C_i^β-C_i^γ		1.507			1.462[b]	
C_i^γ-C_i^δ		1.514			1.494[b]	
C_i^δ-N_i		1.487			1.460	
C_i^β-O_i^γ			1.407			
			Angles			
$C'_{i-1}N_iC_i^\alpha$	118.3	122.7	119.9	118.9	118.5	119.7
$N_iC_i^\alpha C_i'$	111.6	114.5	105.4	104.7	108.3	108.9
$C_i^\alpha C_i'N_{i+1}$	119.3	117.2	114.2	119.9	115.2	117.2
$C_i^\alpha C_i'O_i$	122.3	118.5	121.6	119.5	124.0	121.2
$N_{i+1}C_i'O_i$	118.2	123.8	124.2	120.6	120.7	121.5
$C_i'C_i^\alpha C_i^\beta$		111.9	108.6	112.6	110.7	109.5
$N_iC_i^\alpha C_i^\beta$		103.1	110.1	111.4	102.4	
$C_i^\alpha C_i^\beta C_i^\gamma$		103.0			105.7	
$C_i^\beta C_i^\gamma C_i^\delta$		106.6			110.0[b]	
$C_i^\gamma C_i^\delta N_i$		102.1			104.1	
$C_i^\delta N_iC_i^\alpha$		112.4			112.5	
$C_i^\delta N_iC_{i-1}'$		124.8			126.3	
$C_i^\alpha C_i^\beta O_i^\gamma$			106.2			

[a] Standard deviations are of the order of 0.011 Å for bond lengths and 1.0° for bond angles in the peptide ring and increase to 0.015 Å and 1.3° for the side groups. [b] The large value for the B_{11} thermal parameter for atom $C^\gamma{}_5$ indicates a disorder among several positions (probably two) for this atom. The coordinates listed for the average position give unrealistically small bond lengths for $C^\beta{}_5$-$C^\gamma{}_5$ and $C^\gamma{}_5$-$C^\delta{}_5$ and unrealistically large $C^\beta{}_5C^\gamma{}_5C^\delta{}_5$ angle.

Table III. Conformational Angles (deg)[a]

angle	Gly 1	Pro 2	Ser 3	D-Ala 4	Pro 5
$\phi_i(N_i$-$C_1^\alpha)$	58	−75	−167	86	−66
$\psi_i(C_i^\alpha$-$C_i')$	−128	−20	114	−123	165
$\omega_i(C_i'$-$N_{i+1})$	−175	−177	*−165*	*160*	169
χ_{i1}		33	179		22
χ_{i2}		−35			−18
χ_{i3}		22			6
χ_{i4}		0			10
$C_i^\delta N_iC_i^\alpha C_i^\beta$		−21			−20

[a] The convention followed for labeling the atoms and conformational angles is that proposed by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970). In the fully extended chain $\phi_i = \psi_i = \omega_i = 180°$.

groups, the number of transannular hydrogen bonds, or the planarity of the peptide units.

Hydrogen Bonds. The molecule has three NH groups and one OH group which act as donors in four hydrogen bonds, one intramolecular and three intermolecular (Table IV). The carbonyl oxygen atoms O_1, O_3, and O_5 act as acceptors, with O_1 participating in two intermolecular hydrogen bonds, while O_2 and O_4 are not involved in any hydrogen bonding.

A β turn of type II′ [9] encompasses a Gly, L-Pro sequence, as compared to an L-Pro, Gly sequence in the Gly[3] analogue. The ϕ and ψ values at the two corner C^α atoms are nearly identical in the two molecules, except for changes in sign, since one has an effective D,L sequence and the other an effective L,D sequence. The ϕ and ψ values for the 4→1 type II and II′ bonds in these cyclic pentapeptides are also very close to the ϕ and ψ values occurring for the L-Ser,Gly sequence in the cyclic hexapeptide ring of ferrichrome A, the first substance for which the conformation of a type II intramolecular bond was established.[10] This investigation represents a first observation in a crystal of a Pro residue in the second corner of an intramolecular 4→1 hydrogen bond, although Schwyzer et al.[11] in 1964 had already proposed a similar conformation for c(Gly-Gly-L-Pro)$_2$. The presence of the pyrrolidine ring and the accompanying inflexibility imparted to the peptide backbone do not affect the ϕ and ψ values in the β turn.

Packing. Molecules of the cyclic peptide and the solvent, CH_2Cl_2, crystallize in a 1:1 ratio. The packing in the crystal, illustrated in Figure 5, is a classic layered type. The lipophilic CH_2Cl_2 molecules lie in layers along the *bc* faces of the unit cells. The peptide rings are stretched between the lipophilic CH_2Cl_2 layers with the lipophilic side group of Pro[2] adjacent to one CH_2Cl_2 layer and the lipophilic side groups of Ala[4] and Pro[5] adjacent to the next CH_2Cl_2 layer. The closest contacts

Table IV. Hydrogen Bonds

donor	acceptor	symmetry equivalent of acceptor	distance D···A, Å	distance[c] H···A, Å	angle[c] DH···A, deg
N_1H	O_1	$1-x; -\frac{1}{2}+y; 1-z$	2.99[b]	2.2	132
N_3H	O_5	x, y, z	3.04[a]	2.3	146
N_4H	O_1	$1-x; \frac{1}{2}+y; 1-z$	2.93[b]	2.0	153
$O_3{}^\gamma H$	O_3	$1-x; \frac{1}{2}+y; -z$	2.79[b]	2.0	160
	O_2, O_4	no participation			

[a] Transannular. [b] Intermolecular. [c] Approximate values since the coordinates of the H atoms were not refined.

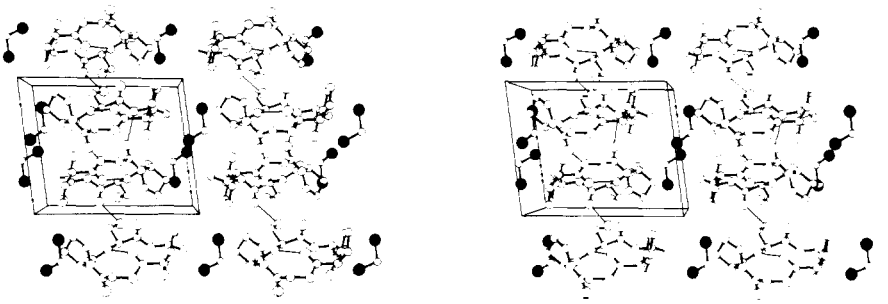

Figure 5. A stereodiagram of the packing. Layers of solvent molecules CH_2Cl_2, where the Cl atoms are represented by ●, intersperse layers of peptide molecules. Hydrogen bonds between peptide molecules are indicated by thin lines. The lipophilic side groups of the Pro and Ala residues are directed toward the lipophilic solvent layers.

between the peptide and the CH_2Cl_2 molecules are $O_2\cdots C_{CH_2Cl_2}$ and $O_4\cdots C_{CH_2Cl_2}$ at 3.03 and 3.13 Å respectively.

The central portion of the peptide stacks contains the polar regions of the peptide molecules, N_1H, N_3H, N_4H, and $O_3{}^{\gamma}H$, that participate in hydrogen bond formation with O_1, O_3, and O_5. The intermolecular hydrogen bonds are located around the screw axes at ($x = 1/2$, $z = 0$) and ($x = 1/2$, $z = 1/2$). The hydrogen bonds connect the stacked peptide molecules into infinite layers parallel to the solvent layers. Thus in proceeding parallel to the *a* axis, one encounters the lipophilic solvent layer, a lipophilic region of the peptide, the polar region of the peptide, a lipophilic region of the peptide, and the next lipophilic solvent layer.

A comparison of the conformation of cyclic Gly-Pro-Ser-D-Ala-Pro in the crystalline state with the conformation in solution deduced from NMR data will be presented in the accompanying paper.[1]

Supplementary Material Available: Listings of observed and calculated structure factors as well as tables of anisotropic thermal parameters for the nonhydrogen atoms and coordinates for hydrogen atoms (10 pages). Ordering information is given on any current masthead page.

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Solution Conformation of *cyclo*-(Gly-Pro-Ser-D-Ala-Pro). Hydrogen-Bonded Reverse Turns in Cyclic Pentapeptides

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Abstract: The synthesis and conformational analysis in solution of *cyclo*-(glycyl-L-prolyl-L-seryl-D-alanyl-L-prolyl) [*cyclo*-(Gly-Pro-Ser-D-Ala-Pro)] are reported. ^1H and ^{13}C NMR results indicate that one conformer (all-trans) predominates in CD_2Cl_2 and Me_2SO-d_6, and that intramolecular hydrogen bonding involving the Gly N–H is a feature of the preferred conformer. ^1H and ^{13}C NMR results suggest a likelihood of a 1←3 hydrogen bond, though not analogous to that observed in the related molecule *cyclo*-(Gly-Pro-Gly-D-Ala-Pro). A structure is proposed which contains a D-Ala-Pro type II′ β turn, with the Gly N–H involved in the 1←4 hydrogen bond. The Ser N–H is suggested to participate in the 1←3 interaction though to remain accessible to solvent or other peptide molecules. Comparisons are discussed between the conformer proposed and the crystal structure reported in an accompanying paper. In addition, these structures are compared with other proline-containing cyclic pentapeptides which have been studied.

Recently recognized to be important and widespread conformational features in proteins are so-called "turns", wherein the polypeptide chain reverses direction, often with concomitant formation of a hydrogen bond.[2-5] Indeed, it has been suggested that the formation of turns may nucleate polypeptide chain folding.[6] Cyclic peptides serve as ideal models for studies of the details of conformation in turns, and offer the attractive possibility of examining various amino acid sequences to establish conformational variables for different types of turns.

In previous studies,[7,8] a cyclic pentapeptide, *cyclo*-(Gly(1)-Pro-Gly(2)-D-Ala-Pro),[9] was observed to take up an all-trans β,γ-turn conformation both in the crystal and in solution in a variety of solvents. The observed conformation contained transannular hydrogen bonds from the N–H of

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