Crystal Structure of *cyclo*(Gly-L-Pro-D-Phe-Gly-L-Val): An Example of a New Type of Three-Residue Turn

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Abstract: *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Val_5) was designed and synthesized to explore the influence of a β-branched residue on the reverse-turn preferences within a cyclic pentapeptide. Its conformation in the solid state as determined by X-ray crystallography contains the now-familiar hydrogen-bonded type II β-turn in the Gly_1-L-Pro_2-D-Phe_3-Gly_4 portion of the molecule. By contrast with several related peptides (e.g.: Karle, I. L. *J. Am. Chem. Soc.* **1978**, *100*, 1286; **1979**, *101*, 181), there is not a γ-turn in the Gly_4-L-Val_5-Gly_1 region; instead, the conformational angles of the valine are approximately those of a right-handed α-helix and no hydrogen bond is present in this turn. We suggest that this type of chain reversal be termed a *helical turn* (H_R-turn). A recent study of a cyclic pentapeptide closely related to the title peptide found a similar combination of a β-turn and a helical turn, but in that case a left-handed helical turn (H_L-turn) (Karle, I. L. *Int. J. Pept. Protein Res.* **1986**, *28*, 420). Distortion of peptide bonds from planarity in the title peptide was observed for the residues flanking Val_5 ($\omega_4 = 167°$ and $\omega_5 = 164°$). Positional disorder of the proline C^γ resulted in refinement of two conformations for the pyrrolidine ring. The orthorhombic $P2_12_12_1$ unit cell contains four peptide molecules and eight water molecules with $a = 5.914$ (2) Å, $b = 17.148$ (7) Å, and $c = 25.820$ (7) Å.

Cyclic pentapeptides are convenient models for studying reverse turns,[1] which are frequent structural features in proteins and naturally occurring peptides.[2] Reverse turns are nonperiodic regions of structure in polypeptides where the backbone folds back on itself. The size and rigidity of cyclic pentapeptides allow detailed analysis of their conformations by various techniques such as nuclear magnetic resonance, circular dichroism, and X-ray crystallography.[1,3-5] A series of cyclic pentapeptides having two L-proline residues and a DLDDL chiral sequence (considering glycines and D residues as formally of the D configuration) displayed one strongly favored backbone conformation, both in solution and in crystals. This structure consisted of a type II β-turn (which has a hydrogen bond from the carbonyl oxygen of the residue in the *i* position to the amide hydrogen of the residue in the $i + 3$ position) with proline in the $i + 1$ position and an inverse γ-turn (which has a hydrogen bond from the carbonyl oxygen of the residue in the *i* position to the amide hydrogen of the residue in the $i + 2$ position) with proline in the $i + 1$ position.[6-8] An example of this conformation is shown in Figure 1 for *cyclo*(D-Phe_1-L-Pro_2-Gly_3-D-Ala_4-L-Pro_5).[7] In order to explore the participation of residues other than proline in the $i + 1$ position of a γ-turn, we have synthesized a new series of cyclic pentapeptides having only one proline (Pro_2) and retaining a DLDDL chiral sequence. Removal of one of the prolines may lead to greater flexibility in the cyclic pentapeptide backbone; nonetheless, retention of L-Pro_2 followed by a D residue or glycine still favors a type II β-turn in the new series of cyclic pentapeptides.[9]

The crystal structure of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Ala_5), the first peptide in this new series, has recently been reported.[10] A type II β-turn, analogous to that present in the cyclic pentapeptides with two prolines, was found within the sequence Gly_1-L-Pro_2-D-Phe_3-Gly_4. In contrast to the cyclic pentapeptide shown in Figure 1, the residue in position 5 (L-Ala) was not found in a γ-turn. Instead a conformation typical of a left-handed α-helix was observed for the L-Ala_5 residue: $\phi = 58°$, $\psi = 65°$. Interestingly, a similar conformation has been observed in the crystal structure of a related cyclic pentapeptide that contains an α,β-unsaturated amino acid in position 5: *cyclo*(D-Ala_1-L-Pro_2-Gly_3-Gly_4-$\Delta^z Phe_5$).[8] A D-Ala_1-L-Pro_2-Gly_3-Gly_4 type II β-turn was present while the achiral dehydrophenylalanine residue also had dihedral angles of a left-handed α-helix: $\phi = 53°$, $\psi = 35°$. Crystal packing of these two cyclic pentapeptides involves the residue-5 carbonyl oxygen and amide proton in intermolecular hydrogen bonding.

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Table I. Crystal Data, Data Collection, and Refinement of $C_{23}H_{34}N_5O_5 \cdot 2H_2O$

	(a) Crystal Data		
formula	$C_{23}H_{31}N_5O_5 \cdot 2H_2O$	V, Å^3	2618 (1)
crystal system	orthorhombic	Z	4
space group	$P2_12_12_1$	crystal color	colorless
a, Å	5.914 (2)[a]	crystal dim, mm	0.19 × 0.27 × 0.31
b, Å	17.148 (7)	μ(Mo Kα), cm^{-1}	0.78
c, Å	25.820 (7)	D(calcd), g cm^{-3}	1.168
	(b) Data Collection		
diffractometer	Nicolet R3m/μ	rflns collected	2021
radiation	Mo Kα (λ = 0.71073 Å)	unique rflns	2021
temp, °C	23	unique reflns [$F_o \geq 3\sigma(F_o)$]	1733
scan method	ω	std rflns	3 std/97 rflns
scan speed, deg min^{-1}	var 4-20	decay	7% (cor)
2θ limits, deg	$4 \leq 2\theta \leq 45$		
	(c) Refinement		
R_F, R_{wF}, %	4.7, 5.8	highest peak, e Å^{-3}	0.27
GOF	1.32	slope, norm prob plot	1.021
Δ/σ	0.11		
data/parameter	4.1		

[a] Twenty-five reflections, $21° \leq 2\theta \leq 28°$, with Friedel-related reflections to check optical and diffractometer alignment.

In the present paper we report the crystal structure of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Val_5), which has a β-branched, valine

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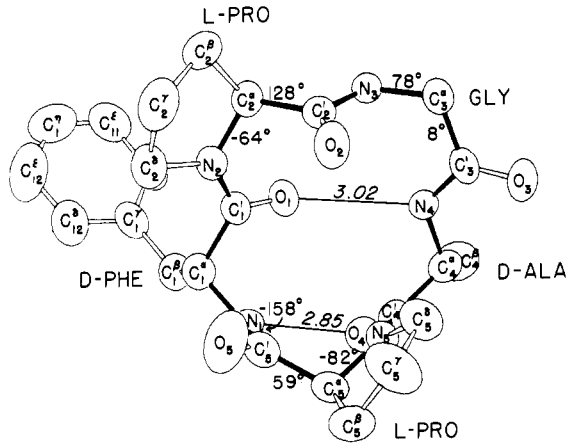

Figure 1. Conformation of *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$)[7] typical of the cyclic pentapeptides with two prolines and the chiral sequence DLDDL. Other peptides of this family are *cyclo*(Gly$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$)[6] and *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-D-Ala$_4$-L-Pro$_5$).[8]

Table II. Fractional Coordinates[a] and Thermal Parameters[b] for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$)

atom	x	y	z	B_{eq}, Å^2
N_1	0.0935	0.4447	0.6644	4.0
C^{α}_1	0.2392	0.4788	0.7040	4.3
C'_1	0.2353	0.4270	0.7520	3.2
O_1	0.2103	0.3559	0.7475	3.9
N_2	0.2602	0.4601	0.7986	3.3
C^{α}_2	0.2594	0.4113	0.8458	3.3
C'_2	0.0389	0.3646	0.8485	3.5
O_2	−0.1486	0.3974	0.8485	4.1
C^{β}_2	0.2788	0.4712	0.8896	5.2
C^{γ}_2	0.4053	0.5405	0.8634	6.0
(C^{γ}_2)[c]	0.2246	0.5430	0.8696	8.1
C^{δ}_2	0.2885	0.5446	0.8108	4.5
N_3	0.0588	0.2864	0.8429	3.4
C^{α}_3	−0.1467	0.2375	0.8566	3.2
C'_3	−0.2892	0.2360	0.8073	3.5
O_3	−0.4871	0.2101	0.8111	4.7
C^{β}_3	−0.0783	0.1526	0.8701	3.7
C^{γ}_3	0.0529	0.1434	0.9202	4.0
C^{δ}_{31}	−0.0413	0.1692	0.9670	4.7
C^{δ}_{32}	0.2603	0.1064	0.9228	5.9
C^{ϵ}_{31}	0.0667	0.1588	1.0131	6.5
C^{ϵ}_{32}	0.3659	0.0946	0.9707	7.4
C^{η}_3	0.2663	0.1221	1.0148	8.6
N_4	−0.1977	0.2588	0.7637	3.4
C^{α}_4	−0.3169	0.2579	0.7133	3.8
C'_4	−0.1359	0.2670	0.6710	3.5
O_4	0.0360	0.2264	0.6718	4.6
N_5	−0.1775	0.3203	0.6345	3.5
C^{α}_5	0.0031	0.3457	0.5990	3.9
C'_5	0.1767	0.3946	0.6286	4.0
O_5	0.3829	0.3893	0.6182	6.0
C^{β}_5	−0.0999	0.3883	0.5521	4.1
C^{γ}_{51}	0.0855	0.4260	0.5196	7.0
C^{γ}_{52}	−0.2446	0.3326	0.5202	6.2
W_1	−0.2498	0.4675	0.7441	4.8
W_2	−0.2184	0.6290	0.7526	4.9

[a] Esd's for x, y, and z are near 0.0013, 0.0003, and 0.0002, respectively, for the backbone atoms and increase up to 0.0022, 0.0008, and 0.0003 for some of the atoms in the side groups. [b] $B_{eq} = (4/3)\sum_i\sum_j a_i a_j$. [c] The proline C^{γ} was located in two positions, one at a frequency of occupation of 64%, C^{γ}_2, and the other at 36%, (C^{γ}_2).

residue in the site occupied by Pro$_5$ in the cyclic pentapeptides with two prolines. We find that *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) also has a type II β-turn within the sequence Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$ in the crystal state. However, the L-Val$_5$ is found in neither a γ-turn nor a left-handed helical conformation as described above. Instead, the conformational angles for the valine approximate those of a right-handed α-helix: $\phi = -70°$, $\psi = -40°$. It is striking that a constrained three-residue fragment can adopt several very different conformations depending on sequence and packing influences.

Table III. Bond Lengths (Å) and Angles (Deg)[a]

	Gly$_1$	L-Pro$_2$	D-Phe$_3$	Gly$_4$	L-Val$_5$	av
			Bonds			
N_i–C^{α}_i	1.460	1.480	1.480	1.481	1.473	1.475
C^{α}_i–C'_i	1.525	1.531	1.526	1.536	1.530	1.530
C'_i–O_i	1.233	1.243	1.256	1.231	1.252	1.243
C'_i–N_{i+1}	1.338	1.351	1.309	1.337	1.354	1.338
C^{α}_i–C^{β}_i		1.533	1.549		1.540	1.541
C^{β}_i–C^{γ}_i		1.559	1.517		1.524	
		1.374[b]			1.524	
C^{γ}_i–C^{δ}_i		1.526	1.404			
		1.564[b]	1.382			
C^{δ}_i–C^{ϵ}_i			1.361			
			1.400			
C^{ϵ}_i–C^{η}_i			1.338			
			1.365			
N_i–C^{δ}_i		1.491				
			Angles			
$C'_{i-1}N_iC^{\alpha}_i$	121.1	120.0	119.8	123.8	120.5	121.0
$N_iC^{\alpha}_iC'_i$	109.0	109.7	114.1	106.9	109.8	109.9
$C^{\alpha}_iC'_iO_i$	120.2	121.5	117.1	120.4	120.4	119.9
$N_{i+1}C'_iO_i$	121.1	121.8	123.8	123.4	123.2	122.7
$C_iC'_iN_{i+1}$	118.7	116.7	119.0	116.2	116.4	117.4
$C'_iC^{\alpha}_iC^{\beta}_i$		112.4	108.4		113.5	
$N_iC^{\alpha}_iC^{\beta}_i$		103.2	109.4		110.0	
$C^{\alpha}_iC^{\beta}_iC^{\gamma}_i$		103.1	115.0		110.4	
		107.8[b]			110.5	
$C^{\beta}_iC^{\gamma}_iC^{\delta}_i$		101.8	119.9			
		108.9[b]	123.0			
$C^{\gamma}_iC^{\delta}_iC^{\epsilon}_i$			121.6			
			120.4			
$C^{\delta}_iC^{\epsilon}_iC^{\eta}_i$			120.3			
			119.5			
$C^{\gamma}_iC^{\beta}_iC^{\gamma}_i$					111.9	
$C^{\delta}_iC^{\gamma}_iC^{\delta}_i$			117.0			
$C^{\epsilon}_iC^{\eta}_iC^{\epsilon}_i$			121.1			
$C^{\gamma}_iC^{\delta}_iN_i$		101.2				
		99.3[b]				
$C^{\delta}_iN_iC^{\alpha}_i$		112.1				
$C^{\delta}_iN_iC'_{i-1}$		128.0				

[a] Esd's are of the order of 0.006 Å for bond lengths and 0.4° for bond angles in the peptide ring and increase up to 0.018 Å for bond lengths and 1.3° for bond angles in some of the side groups. [b] Two positions were found for the proline C^{γ}.

Experimental Section

Synthesis of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) is described elsewhere.[11]

X-ray Crystallographic Structure Determination. The best of seven screened samples of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) obtained by recrystallization from 1:1 acetonitrile/water at 4 °C was affixed to a fine glass fiber with varnish and found by preliminary photographic characterization to belong to the orthorhombic crystal system. Systematic absences in the diffraction data uniquely determined the space group. Details of the data collection and refinement are provided in Table I. Correction for *Lp* effects, secondary extinction, and decay, but none for absorption, were applied to the intensity data. A profile analysis was used to improve the precision in the measurement of weak reflections. The last 41 reflections in the data set could not be collected due to an abrupt, destructive phase change (the crystal became opaque); only a linear 7% decay was observed up to the change. No attempt was made to re-collect these reflections using another specimen as all mounted samples had experienced the same disintegration and the lost data (all $h = 6$) were at $2\theta > 42°$ where fewer than 10% of the data were observed. The phase change was not radiation induced.

All hydrogen atoms except for those attached to the two water molecules were located, and with the exception of one of the proline protons, all hydrogen atoms were refined. Positional disorder was found at the proline γ; two sites, C^{γ}_2 and (C^{γ}_2), were refined with unit-constrained

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Table IV. Conformational Angles (Deg) for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$)[a]

angle	Gly$_1$	L-Pro$_2$	D-Phe$_3$	Gly$_4$	L-Val$_5$
$\phi_i(N_i\text{-}C^\alpha_i)$	−92	−57	67	−165	−70
$\psi_i(C^\alpha_i\text{-}C'_i)$	−149	125	17	−132	−40
$\omega_i(C'_i\text{-}N_{i+1})$	−179	179	178	167	164
χ_{i1}		−27 (17)	58		171
χ_{i2}		42 (−29)	58		−65
χ_{i3}		−39 (29)			
χ_{i4}		24 (−18)			
$C^\delta_i N_i C^\alpha_i C^\beta_i$		2			
$\tau_i(N_i C_i C'_i)$	108	109	114	106	110

[a] χ's for proline C$^\gamma$ with the 36% occupancy are given in parentheses. χ_5^{12} and χ_5^{22} are listed for valine.

Table V. Hydrogen Bonds of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$)

donor	acceptor	NH···O, Å	symmetry of acceptor
HN_1	OW_1	2.015	x, y, z
HN_3	O_3	2.275	$1 + x, y, z$
HN_4	O_1	2.112	x, y, z
HN_5	O_5	1.985	$-1 + x, y, z$
donor	acceptor	OW···O Å	symmetry of acceptor
OW_1	OW_2	2.785	x, y, z
OW_1	O_2	3.011	x, y, z
OW_2	O_4	2.786	$-x, 0.5 + y, 1.5 - z$
OW_2	O_3	2.770	$-1 - x, 0.5 + y, 1.5 - z$

occupancy and found to be, respectively, 64 (1) and 36 (1)% occupied. The final model used anisotropic temperature factors for all non-hydrogen atoms. No attempt was made to assign hydrogen atom positions for the water molecules. The correct enantiomorph could not be crystallographically determined but was assigned from chemical evidence.

Table II provides the atomic coordinates, Table III bond distances and angles, Table IV conformational angles, and Table V hydrogen bonds for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$). Additional crystallographic data are available as Supplementary Material (see note at end). SHELXTL software (version 5.1) was used for all computations (Nicolet Corp., Madison, WI).

Results and Discussion

Overall Description. The conformation of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) in the crystal (see Figure 2) contains all trans peptide bonds and is stabilized by one intramolecular hydrogen bond. As observed in other cyclic pentapeptides, including cyclic pentapeptides with two prolines, a distorted peptide bond occurs between the residue not involved in a β-turn (here L-Val$_5$) and the first position of the β-turn (here Gly$_1$). In the present case, this distortion is 16° from planarity, ω_5 = 164° (see Table IV). Unlike other cyclic pentapeptides, a second distorted peptide bond is observed between the Gly$_4$ and Val$_5$ residues, ω_4 = 167°. A type II β-turn is found for the residues Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$, but the valine residue is not in a γ-turn. In contrast with *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$) where the L-Ala$_5$ had conformational angles typical of a left-handed α-helix,[10] the L-Val$_5$ residue has conformational angles typical of a right-handed α-helix.

Three-Residue Turns Observed in Cyclic Pentapeptides. The three experimentally observed backbone conformations for cyclic pentapeptides with the DLDDL chiral sequence and all trans peptide bonds show remarkable similarity when the residues in the corner positions of the β-turn are compared. This is shown in a map of ϕ vs ψ in Figure 3 and in comparative ball-and-stick structures in Figure 4. Residues 2 and 3, which occupy the corner positions of the β-turn, are closely grouped. The residues occupying the fifth position, as depicted, differ widely in their preferred conformational space. The Pro$_5$ in *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$) has a typical inverse γ-turn conformation. The L-Ala$_5$ of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$) is in a special region of conformational space for L-amino acids, having dihedral angles typical of a left-handed α-helix. The L-Val$_5$ of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) has approximately the mirror image dihedral angles, placing this residue in conformational space typical of a right-handed α-helix.

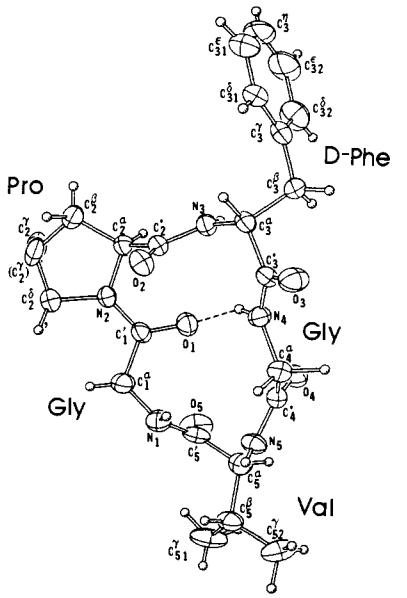

Figure 2. Conformation of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) determined by X-ray crystallography. The dashed line depicts the intramolecular hydrogen bond of the type II β-turn. The proline γ-carbon was located in two positions, C$^\gamma$ with a frequency of occupation of 64% and (C$^\gamma$) with a frequency of occupation of 34%. Ellipsoids represent thermal parameters at the 50% probability level.

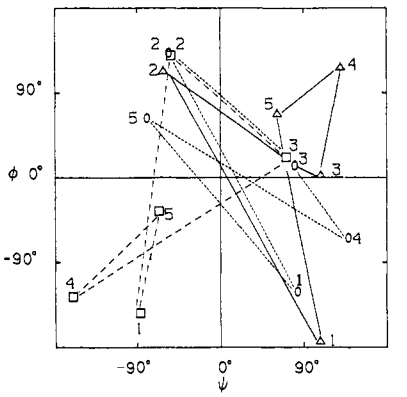

Figure 3. ϕ vs ψ map of the three experimentally observed conformations in the crystalline state for cyclic pentapeptides with a DLDDL chiral sequence: (Δ) *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$);[10] (□) *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$); (○) *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$).[7] Dihedral angles for the residues in the corner positions of the type II β-turn (residues 2 and 3) are virtually identical. Residues in the fifth position are found in all regions of ϕ,ψ allowed for an L residue.

The observed conformations of the title peptide and its analogue with L-Ala in place of the L-Val illustrate that the peptide chain may reverse its direction by the presence of an isolated residue in a helical conformation. We suggest that the resulting non-hydrogen-bonded three-residue turn be termed a helical turn and that it is an alternative chain reversal to β- or γ-turns.

Comparison with Theoretical Conformations. Manjula et al.[12] have calculated three stereochemically favored structures using contact criteria and potential energy calculations for a cyclic pentapeptide containing a β-turn. The dihedral angles of these structures, named A$_1$, A$_2$, and B, together with the dihedral angles for the crystal conformation of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$), *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$), and *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$) are listed in Table VI. As can be seen in the table, the calculated conformation termed A$_1$ by Manjula et al. roughly matches the experimental angles for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$).[10] The conformation termed

(12) Manjula, G.; Ramakrishnan, C. *Int. J. Pept. Protein Res.* **1979**, *13*, 353–362.

Table VI. Dihedral Angles for Calculated[12] and Experimental Conformations of Cyclic Pentapeptides[a]

	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_4	ϕ_5	ψ_5
					Calculated					
A_1	130	−140	−60	90	90	40	110	140	40	30
A_2	−130	−140	−60	90	90	40	150	−130	−40	−50
B	−120	−130	−50	−40	−110	40	130	−110	−40	−30
					Experimental					
1	81	−126	−64	128	78	8	135	−69	−82	59
2	104	−176	−65	112	105	0	126	115	58	65
3	−91	−149	−57	125	67	17	−165	−132	−70	−40

[a] **1** is *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$),[7] **2** is *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$),[10] and **3** is *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) (this paper). Note the similarity between A_1 and **2** and A_2 and **3**. The γ-turn conformation of **1** was not one of the calculated minimum-energy conformations. The calculations of Manjula et al. did not take into account deformations of peptide bonds, which may account for the absence of the γ-turn conformation and the differences in dihedral angles for the other conformations.

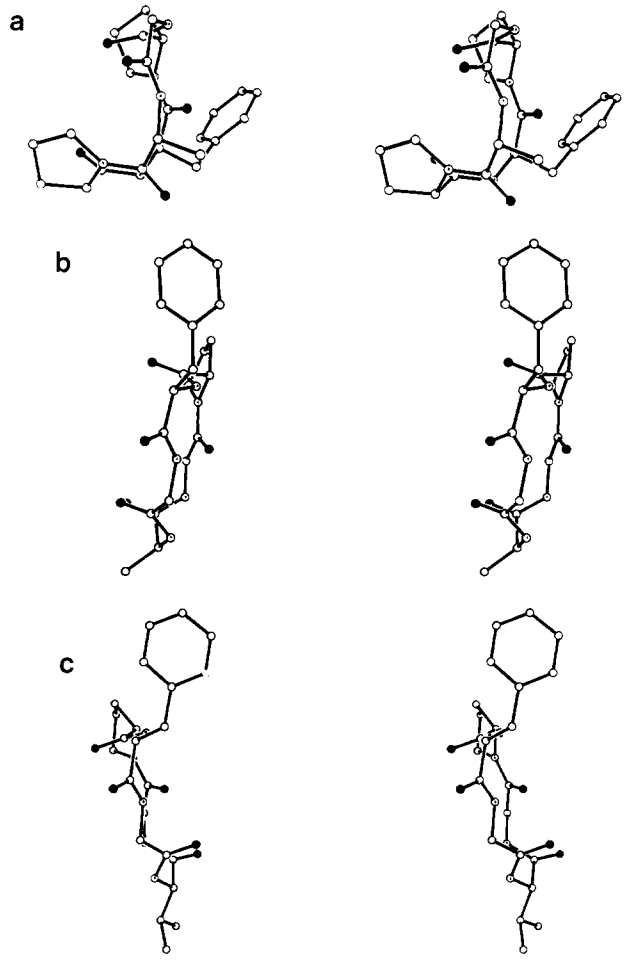

Figure 4. Stereoviews of the backbone conformations of *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$) (a), *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Ala$_5$) (b), and *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) (c) comparing three types of three-residue turns; (a) γ-turn; (b) H_L-turn; (c) H_R-turn. Oxygens are shown as solid spheres and nitrogens have dotted centers. The type II β-turns are oriented toward the top, viewing down the peptide bond between the $i + 1$ and $i + 2$ residues. The three-residue turns are on the bottom portion of the molecule. Differences between the three types of turn are clearly seen by the positions of the carbonyl oxygens.

A_2 by Manjula et al. is approximately that observed for the crystal conformation of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$). The cyclic pentapeptide backbone conformation with a β-turn and a γ-turn was not one of the minimum-energy conformations calculated by Manjula et al. Nonetheless, this is the conformation observed for the series of cyclic pentapeptides with prolines in the second and fifth positions and exemplified by both solution and crystal structures of *cyclo*(Gly$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$).[1,6] It appears that proline in the 5-position biases the cyclic pentapeptide to adopt a backbone conformation with a γ-turn. When other amino acids are present, the conformations observed reflect alternative low-energy regions of ϕ,ψ space. Clearly, packing interactions may strongly influence the conformation adopted in the crystal lattice.

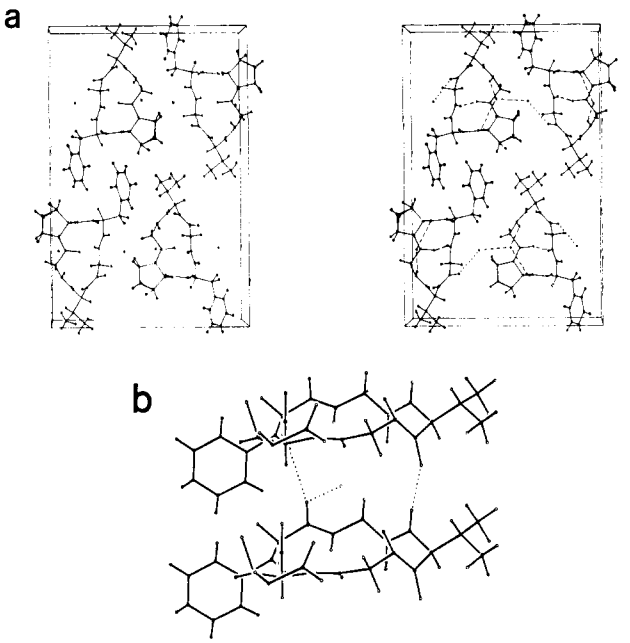

Figure 5. Packing of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$) in the crystal lattice. (a) Interactions between molecules in a sheet where hydrogen bonding within the asymmetric unit is shown with dashed lines and hydrogen bonding between asymmetric units is shown with dotted lines. Only the oxygens of water molecules have been located. Axial directions are $b\uparrow$, $c\rightarrow$, and a directed out from the page. (b) View of the columns of peptide molecules. Only hydrogen bonds between molecules in a column are shown. Axial direction are $a\uparrow$, $c\rightarrow$, and b directed out from the page.

While the cyclic pentapeptide backbone seems to have limited conformational choice, previous studies argue that there are in fact several available conformations. Additional conformations, including ones with cis peptide bonds[3,5] have been observed for these peptides and for their metal ion complexes. The complexes can adopt an all-trans conformation or a one-cis conformation.[1,4a,13] In solution in a poor hydrogen-bonding solvent such as chloroform, the conformation consisting of a β-turn and a γ-turn appears to be preferred even for the cyclic pentapeptides with only one proline (unpublished results). This result suggests that the alternative conformations are favored by intermolecular interactions (solvation or packing) and/or by ligand binding. We are currently examining various cyclic pentapeptides by molecular dynamics simulation in order to assess the relative energies of the accessible conformational states.

Crystal Packing. All amide hydrogens and carbonyl oxygens of the cyclic peptides are involved in hydrogen-bonding interactions, either with other peptide groups or with the eight water molecules that are present in the unit cell (Table V). The two

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Table VII. Three-Residue Turns in Cyclic Pentapeptides and an Example from Lysozyme

peptide[a]	ω_i, deg	ϕ_{i+1}, deg	ψ_{i+1}, deg	ω_{i+2}, deg	C^{α}_i–C^{α}_{i+2}, Å	type	ref
cyclo(Gly-Pro-D-Phe-Gly-*Val*)	167	−70	−40	164	5.03	H_R-turn	
cyclo(Gly-Pro-D-Phe-Gly-*Ala*)	−173	58	65	−161	5.21	H_L-turn	10
cyclo(D-Ala-Pro-Gly-Gly-Δ^z*Phe*)	−168	53	35	−166	4.90	H_L-turn	8
cyclo(Gly-Pro-D-Phe-D-Ala-*Pro*)	−176	−84	78	−158	5.21	γ-turn	8
cyclo(D-Phe-Pro-Gly-D-Ala-*Pro*)	179	−82	59	−158	5.24	γ-turn	7
cyclo(Gly-Pro-Gly-D-Ala-*Pro*)	178	−86	70	−160	5.18	γ-turn	6
Ser_{85}-*Ser_{86}*-Asp_{87} HEWL	177	−67	−16	171	5.36	H_R-turn	
		−60	−60			α-helix	17
		−60	−30			3_{10}-helix	17

[a] Italicized residues are in the $i + 1$ position of the three-residue turn. H-turn designates a helical turn while the subscripts R and L describe the conformation as either a right-handed helical residue or a left-handed helical residue. HEWL is hen egg white lysozyme. Coordinates for HEWL were from the Brookhaven Protein Data Bank.

waters in each asymmetric unit link together a column of peptide molecules in the *a* axial direction and a sheet of peptide molecules oriented along the *b* axis. Figure 5a shows the sheet of peptide where two waters are extensively hydrogen bonded within the asymmetric unit (Table V). The waters tie the sheet of peptide molecules via a hydrogen-bonded chain from peptide to waters to the next peptide in the sheet. Figure 5b is a view of the hydrogen bonding of the columns in the *a* direction. A peptide in the column participates in four hydrogen bonds with other peptide molecules and two hydrogen bonds between waters. W_2 links both a column and a sheet by hydrogen bonding from W_2 to O_4 of a sheet and to O_3 of a column. This arrangement differs from the crystal structures of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Ala_5) and *cyclo*(D-Phe_1-L-Pro_2-Gly_3-D-Ala_4-L-Pro_5) which are hydrogen bonded only between molecules in a column.

Proline Ring Puckering. Puckering of the proline ring often leads to large thermal ellipsoids for proline side chains. In this case, the proline C^{γ} has been crystallographically located in two sites; one refined with a frequency of occupation of 64% and the other 36%. The major form of the proline ring has the γ ring carbon exo with respect to the proline carbonyl group, and in the minor form it is endo. Similar findings have been reported for other proline-containing peptides.[14] Analysis of the coupling constants for the proline in a β-turn (Pro_2) of *cyclo*(Gly_1-L-Pro_2-Gly_3-D-Ala_4-L-Pro_5) and *cyclo*(D-Phe_1-L-Pro_2-Gly_3-D-Ala_4-L-Pro_5) indicated the exo and endo forms were nearly equally populated in solution.[15]

Presence of Helical Turns in Proteins. A limited search was performed to locate turns similar to the helical turn observed in the crystals of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Val_5) within a representative globular protein, hen egg white lysozyme. Criteria used to locate helical turns were a short C^{α}_i–C^{α}_{i+2} distance (less than 5.6 Å) and helical dihedral angles for the $i + 1$ residue. Turns for lysozyme have been previously located by using a method which searches for minima in the radius of curvature for the polypeptide backbone.[16] A strikingly similar structure to that seen in the cyclic pentapeptide with Val_5 (this paper) was observed for residues 85–87 of lysozyme (Ser_{85}-Ser_{86}-Asp_{87}; see Table VII). These residues are flanked by two helices, although they are not a part of either, and they change the direction of the backbone by ~90°. According to a recently proposed classification of turns, the helical turns within a cyclic pentapeptide resemble a class 1, three-residue β-hairpin, for which a single example has been tabulated.[18] A more general search of the Brookhaven database is planned for three-residue, non-hydrogen-bonded turns with either the left- or right-handed helical conformation.

Conclusion

The conformation of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Val_5) in the crystal contains a new type of three-residue turn or reversal of polypeptide chain direction. The dihedral angles observed for the central residue (Val) are those of a right-handed α-helix. There is no hydrogen bond stabilizing this turn. We suggest the term "helical turn" for the observed chain reversal. The cyclic pentapeptide backbone in this peptide and those studied previously has served to illustrate the conformational options available to a three-residue segment constrained to share α-carbon positions with residues i and $i + 3$ of a type II β-turn: viz., a distance of 5.2 Å and acceptable geometry. These results provide a detailed picture of a local peptide chain folding that may be of importance in proteins and naturally occurring peptides.

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Registry No. *cyclo*(Gly-L-Pro-D-Phe-Gly-L-Val), 110590-39-1; *cyclo*(Gly-L-Pro-D-Phe-Gly-L-Val)·$2H_2O$, 110658-89-4.

Supplementary Material Available: Hydrogen atom coordinates (Table SI) and anisotropic temperature factors (Table SII) (4 pages); observed and calculated structure factors for *cyclo*(Gly_1-L-Pro_2-D-Phe_3-Gly_4-L-Val_5) (10 pages). Ordering information is given on any current masthead page.

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