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Conformation of *cyclo*-Bis(-L-valyl-L-prolyl-D-alanyl-), a Synthetic Cyclic Hexapeptide

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Abstract. $C_{26}H_{42}N_6O_6$, $M_r = 534{\cdot}7$, monoclinic, $C2$, $a = 20{\cdot}526\ (2)$, $b = 4{\cdot}923\ (1)$, $c = 17{\cdot}092\ (2)$ Å, $\beta = 126{\cdot}37\ (1)°$, $V = 1390{\cdot}9$ Å^3, $Z = 2$, D_m not measured, $D_x = 1{\cdot}28$ g cm^{-3}, λ(Cu $K\alpha$) = 1·5418 Å, $\mu = 7{\cdot}1$ cm^{-1}, $F(000) = 576$, $R = 0{\cdot}050$, $wR = 0{\cdot}049$ for 1012 reflections [$I > 2\sigma(I)$], 1501 unique reflections measured at room temperature (296 K). The synthetic cyclic hexapeptide, *cyclo*-bis(-L-Val-L-Pro-D-Ala-), exhibits exact C_2 symmetry in the crystalline state with *cis* peptide links [$\omega = -13{\cdot}1\ (7)°$] between Val and Pro residues; there are no intramolecular hydrogen bonds. The cyclic ring consists of two type VI*b* *cis* proline turns fused at the D-Ala residue. The backbone dihedral angles are all in the extended range except for ψVal [72·9 (5)°] and φPro [−78·9 (5)°] on either side of the *cis* peptide link. The carbonyl O atoms and the amide N atoms in the extended portion of the cyclic peptide form intermolecular hydrogen bonds with another cyclic hexapeptide molecule translated by a cell edge along the crystallographic *b* axis, forming an infinite stretch of β-sheets. The parallel β-sheet structures are separated by about 3·15 Å.

Introduction. Cyclic hexapeptides are good model compounds to study β-turns since a cyclic hexapeptide can be constructed by fusing two β-turns. We have studied the conformation of a number of cyclic hexapeptides of the general type *cyclo*-bis(-L-*Xxx*-L-Pro-D-*Yyy*-) in solution and the crystalline state. In solution, these peptides exist in two conformations (Kopple, Schamper & Go, 1974; Kopple, Sarkar & Giacometti, 1981), one with all-*trans* and the other with two *cis* *Xxx*-L-Pro peptide links. In the all-*trans* form, these hexapeptides are found to contain two β-turns with the L-Pro and D-*Yyy* residues at the corners and 4→1 type intramolecular hydrogen bonds. In the two *cis* forms, however, there are no intramolecular hydrogen bonds. Examples of the *trans* forms can be found in the crystal structures of *cyclo*-bis(-Gly-Pro-D-Phe-) (Brown & Yang, 1979), *cyclo*-bis(-Ala-Pro-D-Phe-) (Brown & Teller, 1976), *cyclo*-bis(-Gly-Pro-D-Ala-) (Kostansek, Lipscomb & Thiessen, 1979) and *cyclo*-bis(-L-Phe-L-Pro-D-Gln-) (Bhandary & Kopple, 1985). Two *cis* forms are found in the crystal structures of two crystal forms of *cyclo*-bis(-L-Phe-L-Pro-D-Ala-) (Kartha, Bhandary, Kopple, Go & Zhu, 1984; Bhandary, Kartha & Kopple, 1986), a second form of *cyclo*-bis(-L-Phe-L-Pro-D-Gln-) (Bhandary & Kopple, 1987) and in an Mg^{2+} complex of *cyclo*-bis(-Pro-Pro-Gly-) (Karle & Karle, 1981). In the crystal structure of the uncomplexed *cyclo*-bis(-Pro-Pro-Gly-), however, there are one *trans* and one *cis* Pro-Pro peptide linkages (Czugler, Sasvari & Hollosi, 1982). In this paper we report another example of a cyclic hexapeptide containing two *cis* L-Xxx-L-Pro peptide links.

Experimental. Needle-shaped crystal, 0·12 × 0·12 × 0·4 mm in size, of the synthetic cyclic hexapeptide, *cyclo*-bis(-L-Val-L-Pro-D-Ala-), grown by slow

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evaporation of a solution of the compound in ethanol was used to determine cell dimensions and for intensity data collection on an Enraf–Nonius CAD-4 diffractometer equipped with a graphite monochromator. Data to $2\theta = 154°$ ($0 < h < 25$, $0 < k < 6$, $-21 < l < 21$) were collected by the ω–2θ scan technique using Ni-filtered Cu $K\alpha$ radiation. A total of 1501 unique reflections were measured, of which 1012 had intensities greater than $2\sigma(I)$. Lattice parameters determined using 25 reflections in the range $16 < \theta < 21°$. Three standard reflections measured every 2 h showed no intensity variation. Intensity data were corrected for Lorentz and polarization factors and an empirical absorption correction (North, Phillips & Mathews, 1968) based on a series of ψ scans was also applied. Relative transmission coefficients ranged from 0·957 to 0·996 with an average value of 0·979. The structure was solved by the application of direct phase-determining procedures using the multisolution program *MULTAN*80 (Main, Fiske, Hull, Lessinger, Germain, Declercq & Woolfson, 1980). The asymmetric unit contained a tripeptide, a total of 19 non-H atoms. Full-matrix least-squares techniques were used to refine the structure. The function minimized was of the form $\sum w(|F_o| - |F_c|)^2$, where $w = 4|F_o|^2/\sigma(|F_o|^2)$. H atoms, located in a difference Fourier map, were given isotropic temperature factors of the atoms to which they are attached and were included in the refinement. Non-H atoms were refined anisotropically. Final $R = 0{\cdot}050$ and $wR = 0{\cdot}049$. $S = 1{\cdot}31$. $\Delta/\sigma = 0{\cdot}01$. The final difference electron density map had a peak of maximum height $\Delta\rho_{max} = 0{\cdot}20$ e Å^{-3} and a minimum peak $\Delta\rho_{min}$ of $-0{\cdot}18$ e Å^{-3}. Atomic scattering factors were taken from Cromer & Waber (1974). A secondary-extinction correction (Zachariasen, 1963) was also applied, the final refined coefficient being $4{\cdot}6 \times 10^{-6}$. All calculations were performed on a MicroVAX II computer using *SDPVAX* software package (Frenz, 1978).

Discussion. Final atomic coordinates and equivalent isotropic thermal parameters for the non-H atoms are listed in Table 1.* Bond lengths, angles and torsion angles are given in Table 2. Main-chain bond distances averaged over three residues are 1·460 (5) Å for N_i—C_iA, 1·527 (6) Å for C_iA—C'_i, 1·217 (5) Å for C'_i—O_i and 1·337 (4) Å for C'_i—N_{i+1} and the characteristic bond angles are 122·9 (4)° for C'_{i-1}—N_i—C_iA, 110·9 (4)° for N_i—C_iA—C'_i, 121·4 (4)° for C_iA—C'_i—O_i, 115·8 (4)° for C_iA—C'_i—N_{i+1} and 122·7 (5)° for O_i—C'_i—N_{i+1}. These values agree well, within experimental error, with those found for other cyclic hexapeptides (Kartha, Bhandary, Kopple, Go & Zhu, 1984; Bhandary, Kartha & Kopple, 1986).

Table 1. *Positional parameters and their e.s.d.'s*

Anisotropically refined atoms are given in the form of the equivalent isotropic thermal parameter defined as: $4/3[a^2\beta(1,1) + b^2\beta(2,2) + c^2\beta(3,3) + (ac\cos\beta)\beta(1,3)]$, where a, b and c are reciprocal cell dimensions.

	x	y	z	B_{eq}(Å^2)
N1	0·1783 (1)	1·4099	0·6671 (2)	2·93 (7)
C1*A*	0·2369 (2)	1·303 (1)	0·7644 (2)	3·07 (9)
C1*B*	0·3247 (2)	1·328 (1)	0·7983 (2)	4·1 (1)
C1*G*1	0·3340 (2)	1·200 (2)	0·7243 (2)	6·0 (2)
C1*G*2	0·3822 (2)	1·189 (2)	0·8955 (3)	6·1 (2)
C1′	0·2251 (2)	1·445 (1)	0·8359 (2)	3·4 (1)
O1	0·2741 (2)	1·6132 (9)	0·8922 (2)	5·74 (9)
N2	0·1610 (1)	1·3830 (8)	0·8332 (2)	2·89 (7)
C2*A*	0·0894 (2)	1·2291 (9)	0·7561 (2)	2·79 (9)
C2*B*	0·0479 (2)	1·161 (1)	0·8055 (2)	3·6 (1)
C2*G*	0·0693 (2)	1·403 (1)	0·8726 (2)	4·2 (1)
C2*D*	0·1527 (2)	1·490 (1)	0·9065 (2)	3·5 (1)
C2′	0·0317 (2)	1·406 (1)	0·6679 (2)	2·82 (8)
O2	0·0328 (1)	1·6523 (7)	0·6724 (2)	3·65 (7)
N3	−0·0239 (2)	1·2686 (7)	0·5869 (2)	2·62 (7)
C3*A*	−0·0923 (2)	1·416 (1)	0·5065 (2)	2·92 (9)
C3*B*	−0·1525 (2)	1·493 (1)	0·5286 (2)	5·0 (1)
C3′	−0·1359 (2)	1·2555 (9)	0·4131 (2)	2·65 (8)
O3	−0·1355 (1)	1·0065 (6)	0·4117 (1)	3·63 (7)

Table 2. *Bond distances* (Å) *and angles and torsion angles* (°) *for cyclo-bis*(-L-*Val*-L-*Pro*-D-*Ala*-)

		Val	Pro	D-Ala
	i =	1	2	3
N_i—C_iA		1·456 (4)	1·475 (5)	1·448 (5)
C_iA—C'_i		1·548 (6)	1·523 (6)	1·510 (5)
C'_i—O_i		1·213 (5)	1·213 (6)	1·226 (5)
C'_i—N_{i+1}		1·324 (6)	1·344 (4)	1·342 (4)
C_iA—C_iB		1·538 (6)	1·550 (6)	1·538 (6)
C_iB—C_iG1		1·521 (7)		
C_iB—C_iG2		1·515 (6)		
C_iB—C_iG			1·526 (7)	
C_iG—C_iD			1·508 (6)	
C_iD—N_i			1·459 (5)	
C'_{i-1}—N_i—C_iA		123·9 (3)	126·4 (4)	118·5 (4)
N_i—C_iA—C'_i		109·1 (4)	111·6 (4)	111·9 (4)
C_iB—C_iA—C'_i		111·3 (4)	107·9 (4)	109·2 (4)
C_iB—C_iA—N_i		112·9 (4)	101·7 (4)	111·1 (4)
C_iA—C'_i—O_i		119·4 (4)	122·4 (4)	122·5 (4)
C_iA—C'_i—N_{i+1}		119·0 (4)	114·7 (4)	113·8 (4)
O_i—C'_i—N_{i+1}		121·6 (5)	122·8 (4)	123·7 (4)
C'_{i-1}—N_i—C_iD			120·4 (4)	
C_iA—N_i—C_iD			113·0 (4)	
N_i—C_iA—C_iB			101·7 (4)	
C_iA—C_iB—C_iG			103·6 (4)	
C_iB—C_iG—C_iD			105·4 (5)	
C_iG—C_iD—N_i			104·6 (4)	
C_iA—C_iB—C_iG1		110·5 (5)		
C_iA—C_iB—C_iG2		110·6 (5)		
C_iG1—C_iB—C_iG2		109·0 (5)		
C'_{i-1}—N_i—C_iA—C'_i	φ	−136·7 (6)	−78·9 (5)	164·1 (6)
N_i—C_iA—C'_i—N_{i+1}	ψ	72·9 (5)	164·5 (6)	−156·6 (6)
C_iA—C'_i—N_{i+1}—$C_{i+1}A$	ω	−13·1 (7)	165·8 (6)	−168·1 (6)
N_i—C_iA—C_iB—C_iG1	$\chi^{1,1}$	−53·7 (6)		
N_i—C_iA—C_iB—C_iG2	$\chi^{1,2}$	−174·6 (6)		
C_iD—N_i—C_iA—C_iB	χ^0		−19·3 (5)	
N_i—C_iA—C_iB—C_iG	χ^1		31·7 (5)	
C_iA—C_iB—C_iG—C_iD	χ^2		−33·7 (5)	
C_iB—C_iG—C_iD—N_i	χ^3		21·9 (6)	
C_iG—C_iD—N_i—C_iA	χ^4		−1·2 (5)	

* Lists of structure factors, H-atom parameters and anisotropic thermal parameters (U_{ij}) have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53659 (11 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

The molecular conformation of the cyclic hexapeptide is shown in Fig. 1. The hexapeptide exhibits exact C_2 symmetry and the backbone consists of two *cis* peptide links [$\omega = -13{\cdot}1\ (7)°$] between Val and Pro residues. The remaining four peptide links are *trans* with the non-planarity parameter, ω, varying by as much as 16°. There are none of the transannular 4→1 hydrogen bonds that are observed in the all-*trans* forms of the cyclic hexapeptides. Turns containing *cis* prolines are classified as type VI β-turns (Richardson, 1981). In the present structure there are two type VI*b* turns [$\varphi_2 = -136{\cdot}7\ (6)$, $\psi_2 = 72{\cdot}9\ (5)$, $\varphi_3 = -78{\cdot}9\ (5)$ and $\psi_3 = 164{\cdot}5\ (6)°$] fused together at the D-Ala residues. With two type VI*b* turns in the cyclic backbone, the carbonyl O atom of the first amino acid and the amide H atom of the fourth residue are directed perpendicular to the plane of the α-carbons of the cyclic ring and hence transannular hydrogen bonding does not occur. The valine side chain has a (g^-,t) conformation. The proline ring is in ${}^{\beta}E$ [$P = 34{\cdot}6\ (4)$, $\tau_m = 164{\cdot}5\ (6)°$] conformation (Hasnoot, DeLeeuw, DeLeeuw & Altona, 1981).

The backbone dihedral angles are all in the extended range except for ψVal [72·9 (5)°] and φPro [−78·9 (5)°] which are on either side of the *cis* peptide link. The carbonyl O atoms and the amide N atoms in the extended portion of the cyclic peptide form intermolecular hydrogen bonds (Table 3) with another cyclic hexapeptide molecule translated along the crystallographic *b* axis, which is only 4·923 Å long, thus forming infinite stretches of parallel β-sheet structures separated by about 3·15 Å (Fig. 2). This type of hydrogen bonding exhibiting a parallel β-sheet arrangement has been observed in small linear oligopeptides (Chatterjee & Parthasarathy, 1984; Marsh & Glusker, 1961). The present structure is, probably, the first case of a cyclic oligopeptide forming intermolecular hydrogen bonding perpendicular to the plane of the cyclic ring to give parallel β-sheet-type structure. Packing of the molecules in the crystal is shown in Fig. 3. The unit cell does not contain water or solvent of crystallization. The molecules pack in such a way as to form columns of hydrophobic (side chains) and hydrophilic (amide and carbonyl groups) regions along the crystallographic *b* axis.

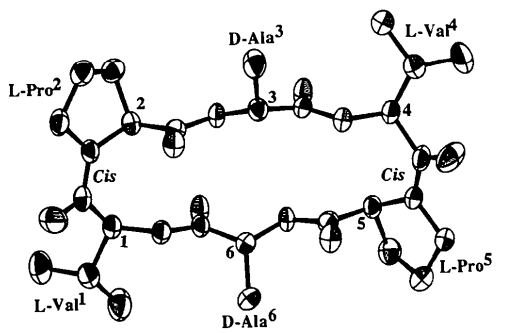

Fig. 1. Conformation of the cyclic hexapeptide *cyclo*-bis(-L-Val-L-Pro-D-Ala-). Amino-acid residues 4, 5 and 6 are related by symmetry $-x, y, 1-z$ to residues 1, 2 and 3, respectively. α-C atoms are numbered and the two *cis* Val-Pro peptide links are shown.

Table 3. *Hydrogen-bonding distances* (Å) *less than* 3·5 Å *and angles* (°) *in the crystal structure of cyclo-bis*(-L-*Val*-L-*Pro*-D-*Ala*-)

D	H	*A*	Symmetry*	Distance *D*—*A*	Distance H···*A*	Angle *D*—H···*A*
N1	HN1	O3	(ii) (011)	3·132 (3)	2·32 (5)	160 (4)
N3	HN3	O2	(i) (0$\bar{1}$0)	3·268 (5)	2·59 (5)	140 (5)

* Symmetry: (i) x, y, z; (ii) $-x, y, -z$.

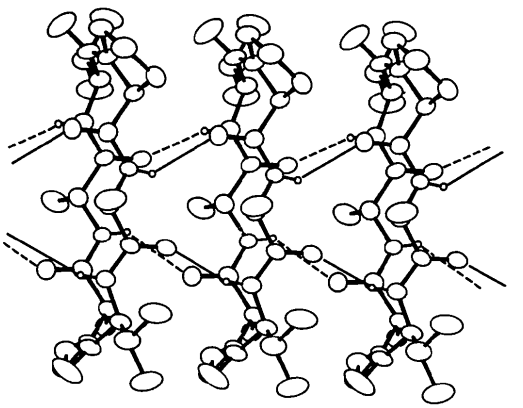

Fig. 2. Hydrogen-bonding pattern (parallel β-sheet) observed between molecules translated by a unit-cell edge along the crystallographic *b* axis. Solid lines represent the hydrogen bonding in the sheet above while the dashed lines represent hydrogen bonding in the sheet below. The two sheets are separated by about 3·15 Å.

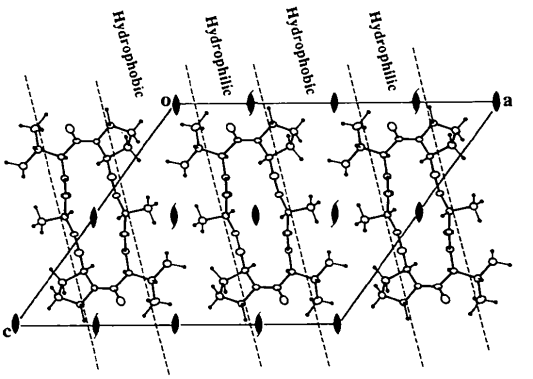

Fig. 3. Crystal packing as viewed down the crystallographic *b* axis. The molecules pack in such a way as to form alternating hydrophobic and hydrophilic columns.

There are three examples of cyclic hexapeptides having two *cis* peptide links in the crystalline state; this one, two crystalline forms of *cyclo*-bis(-L-Phe-L-Pro-D-Ala-) and *cyclo*-bis(-L-Phe-L-Pro-D-Gln-). The important feature of the two-*cis* forms is the absence of transannular 4→1 hydrogen bonding. The D-*Yyy* residue in the present structure is in a fully extended conformation whereas in the other two examples it is in a semi-extended conformation with average φ and ψ values of 80 and $-143°$ respectively. The hexapeptide molecule in the present case has an exact twofold symmetry whereas in the other two examples it is only approximate, with differences in values of ψ(Pro) and φ(D-*Yyy*) in the two halves of the molecule.

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Structure of 2,6-Dibromo-*N*-methyl-4-nitroaniline (DBNMNA); a New Electro-Optic Organic Crystal

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Abstract. $C_7H_6Br_2O_2N_2$, $M_r = 309{\cdot}94$, orthorhombic, *Fdd*2, $a = 11{\cdot}745\ (1)$, $b = 29{\cdot}640\ (2)$, $c = 10{\cdot}807\ (2)$ Å, $V = 3762{\cdot}1\ (6)$ Å^3, $Z = 16$, $D_x = 2{\cdot}189$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54178 Å, $\mu = 108{\cdot}31$ cm^{-1}, $F(000) = 2368$, $T = 296\ (1)$ K, $R = 0{\cdot}043$ based on 714 reflections [$I > 3{\cdot}00\sigma(I)$] of 799 unique reflections measured, and 117 variables. The DBNMNA molecule has a dihedral angle between the plane of the nitro group and the plane of the ring of 2 (2)° and a dihedral angle between the plane of the *N*-methylamino group and the plane of the ring of 28 (2)°. The 16 molecules of the asymmetric unit pack with all the nitro groups pointing in the same sense, resulting in a polar *c* axis. The net polar orientation was confirmed using second harmonic generation and electro-optic measurements.

Introduction. The high intrinsic optical nonlinearities and low dielectric constants measured for organic crystals (Zyss & Chemla, 1987) have generated sig-

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