Crystal Structure and Conformation of the Cyclic Hexapeptide *cyclo*-(Gly-L-Pro-D-Ala)$_2$

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Abstract: The crystal structure and conformation of *cyclo*-(Gly-L-Pro-D-Ala)$_2$, $C_{20}H_{30}N_6O_6$, has been determined by single-crystal X-ray diffraction analysis. The crystals have the symmetry of the orthorhombic space group $P2_12_12_1$ with a = 9.389 (6), b = 10.379 (8), and c = 22.006 (16) Å. The structure was solved by direct methods and refined to a final R of 0.055 on 1575 unique reflections. The hexapeptide shows significant deviation from internal twofold symmetry. This asymmetric conformer apparently optimized one single 4 → 1 transannular hydrogen bond at the expense of other possible interactions. Conformation angles are compared with those obtained by CD and NMR techniques, and bond distances are discussed. This hexapeptide contains two type II β turns. All of the peptide bonds are in the trans conformation and the C_γ atom in one of the proline rings is disordered.

In recent years cyclic peptides have been used with increasing frequency for the study of peptide backbone conformations. Despite a large number of NMR studies of cyclohexapeptide conformations in solution, few X-ray crystal studies have been accomplished.[2-4] Unhindered cyclopeptides such as cyclohexaglycyl and *cyclo*-(Gly-Gly-Gly-Gly-D-Ala-D-Ala) may assume many low-energy conformations; therefore, conformational analysis is quite difficult. Crystal and solution analyses for this type of compound generally differ with respect to the description of the predominant conformers.

The addition of proline residues to the cyclopeptide reduces the number of possible conformers and both Blout[5] and Kopple[6] have synthesized and studied (by NMR) C_2-symmetric hexapeptides containing proline. We report here the crystal structure and conformation of *cyclo*-(Gly-L-Pro-D-Ala)$_2$, which, unlike *cyclo*-(D-Ala-L-Pro-D-Phe)$_2$,[3] does not retain the twofold symmetry in the solid state.

Experimental Section

Crystals of the title compound were grown by slow evaporation from D_2O solution and have the symmetry of the orthorhombic space group

Table I. Fractional Atomic Coordinates (×10^4)

atom	x	y	z
1N	530 (6)	7725 (5)	942 (2)
1C$_\alpha$	291 (8)	8409 (6)	1503 (3)
1C	1371 (7)	7952 (6)	1957 (3)
1O	2147 (5)	7012 (4)	1854 (2)
2N	1497 (5)	8596 (5)	2482 (2)
2C$_\alpha$	2622 (7)	8255 (6)	2921 (3)
2C$_\beta$	2290 (9)	9102 (6)	3472 (3)
2C$_\gamma$	1562 (9)	10276 (7)	3201 (3)
2C$_\delta$	713 (7)	9769 (7)	2672 (3)
2C	2557 (7)	6803 (6)	3077 (3)
2O	1496 (5)	6306 (4)	3291 (2)
3N	3813 (6)	6188 (5)	2981 (2)
3C	4007 (7)	4836 (6)	3163 (3)
3C$_\beta$	5530 (8)	4653 (6)	3408 (3)
3C	3691 (7)	3893 (6)	2645 (3)
3O	3926 (5)	2714 (4)	2738 (2)
4N	3181 (6)	4292 (5)	2114 (2)
4C$_\alpha$	2754 (8)	3365 (7)	1654 (3)
4C	2379 (8)	4015 (6)	1068 (3)
4O	2776 (5)	5132 (4)	968 (2)
5N	1636 (6)	3350 (5)	664 (2)
5C$_\alpha$	1369 (7)	3868 (6)	55 (3)
5C$_\beta$	672 (9)	2748 (7)	−284 (3)
5C$_\gamma$1[a]	967 (13)	1582 (10)	50 (5)
5C$_\gamma$2[a]	192 (14)	1874 (12)	194 (6)
5C$_\delta$	1178 (8)	1982 (6)	719 (3)
5C	327 (7)	5012 (6)	92 (3)
5O	−905 (5)	4914 (5)	259 (2)
6N	889 (5)	6164 (5)	−89 (2)
6C$_\alpha$	9 (7)	7324 (6)	−144 (3)
6C$_\beta$	724 (8)	8276 (6)	−579 (3)
6C	−297 (7)	7981 (6)	463 (3)
6O	−1265 (5)	8769 (4)	500 (2)

[a] Occupancy is 0.50.

Table II. Calculated Hydrogen Coordinates (×10^3)

H bonded to atom	x	y	z
1N	132	705	91
1C$_\alpha$	−78	821	167
1C$_\alpha$	41	944	143
2C$_\alpha$	370	842	275
2C$_\beta$	158	860	379
2C$_\beta$	327	938	371
2C$_\gamma$	86	1073	353
2C$_\gamma$	235	1098	305
2C$_\delta$	−37	953	281
2C$_\delta$	68	1047	230
3N	463	667	278
3C$_\alpha$	324	462	352
3C$_\beta$	565	360	340
3C$_\beta$	565	520	380
3C$_\beta$	580	500	298
4N	309	525	203
4C$_\alpha$	183	283	182
4C$_\alpha$	363	270	157
5C$_\alpha$	233	422	−17
5C$_\beta$[a]	−47	290	−31
5C$_\beta$[a]	111	268	−74
5C$_\beta$'[a]	144	227	−58
5C$_\beta$'[a]	−23	309	−55
5C$_\gamma$1[a]	8	91	1
5C$_\gamma$1[a]	193	113	−12
5C$_\gamma$2[a]	−88	214	34
5C$_\gamma$2[a]	19	89	2
5C$_\delta$[a]	19	190	97
5C$_\delta$[a]	200	140	94
5C$_\delta$'[a]	63	180	115
5C$_\delta$'[a]	208	132	68
6N	195	622	−19
6C$_\alpha$	−102	701	−32
6C$_\beta$	5	900	−70
6C$_\beta$	170	860	−40

[a] Occupancy is 0.50.

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Table III. Bond Lengths (Å) and Angles (deg)

bonds	$i = 1$	2	3	4	5	6	av for *cyclo*-(Gly-Pro-D-Ala)$_2$	av for polypeptides[b]
N_i-$C\alpha_i$	1.441	1.474	1.471	1.452	1.465	1.465	1.461	1.455
$C\alpha_i$-C_i	1.500	1.546	1.532	1.498	1.541	1.526	1.524	1.51
C_i-O_i	1.237	1.217	1.259	1.238	1.218	1.226	1.232	1.24
C_i-N_{i+1}	1.341	1.358	1.330	1.323	1.367	1.338	1.343	1.325
$C\alpha_i$-$C\beta_i$		1.531	1.540		1.529	1.531		
$C\beta_i$-$C\gamma_i$		1.519			1.443 (1.460)[a]			
$C\gamma_i$-$C\delta_i$		1.507			1.541 (1.484)[a]			
$C\delta_i$-N_i		1.482			1.489			
angles	Gly	L-Pro	D-Ala	Gly	L-Pro	D-Ala		
$C_{i-1}N_iC\alpha_i$	119.2	120.5	120.9	120.4	120.9	121.7	120.6	122
$N_iC\alpha_iC_i$	108.0	110.6	112.5	111.5	110.0	113.6	111.0	111
$C\alpha_iC_iN_{i+1}$	118.4	112.9	121.7	117.9	114.4	119.5	117.5	116
$C\alpha_iC_iO_i$	121.7	122.1	117.8	120.2	123.7	119.8	120.9	120.5
$N_{i+1}C_iO_i$	119.9	124.9	120.5	121.9	121.9	120.7	121.6	123.5
$C_iC\alpha_iC\beta_i$		112.1	111.2		109.9	110.0		
$N_iC\alpha_iC\beta_i$		103.6	109.2		104.0	109.6		
$C\alpha_iC\beta_iC\gamma_i$		104.4			107.8 (104.6)[a]			
$C\beta_iC\gamma_iC\delta_i$		105.2			106.6 (108.8)[a]			
$C\gamma_iC\delta_iN_i$		104.0			102.5 (100.9)[a]			
$C\alpha_iN_iC\delta_i$		111.7			111.9			
$C_{i-1}N_iC\delta_i$		127.5			126.6			

[a] Values for alternate disordered position. [b] See R. Marsh and J. Donohue, *Adv. Protein Chem.*, **22**, 235 (1967).

Table IV. Proline Dihedral Angles[a] (deg)

	χ_1	χ_2	χ_3	χ_4
Pro_2	−28.5	34.8	−27.0	9.1
Pro_5	−17.6	26.9	−25.1	14.3
Pro_5 disordered	17.7	−31.0	30.6	−18.9

[a] χ_1 = N-Cα-Cβ-Cδ, χ_2 = Cα-Cβ-Cγ-Cδ, χ_3 = Cβ-Cγ-Cδ-N, χ_4 = Cγ-Cδ-N-Cα.

$P2_12_12_1$. The unit cell has dimensions $a = 9.389$ (6), $b = 10.379$ (8), and $c = 22.006$ (16) Å ($\lambda = 1.541\ 78$ Å) and contains four molecules and no solvent. Calculated and observed[7] densities are 1.395 and 1.39 ± 0.01 g/cm^3, respectively. The crystal used for data collection had dimensions 0.07 × 0.23 × 0.40 mm. Integrated intensities of 1705 independent reflections ($2\theta_{max} = 115°$) were measured with Ni-filtered Cu Kα radiation on a Syntex $P2_1$ four-circle diffractometer. The data were collected using ω scans (1°/min) with 10-s background counts at each end of the scan. Three reference reflections were measured every 50 reflections and these remained constant throughout data collection. Reflection intensities were corrected for background, polarization, and Lorentz effects. Those 1575 reflections (92%) which had values of $F_o \geq 3\sigma(F_o)$ were included in the refinement.

Structure Determination and Refinement. The structure was solved using the weighted multiple solution tangent formula approach of direct methods.[8] A total of 64 phase sets each consisting of those 200 normalized structure factors, $|E|$'s, having $|E| \geq 1.50$ was generated. An E map calculated from the phase set with the highest absolute figure of merit revealed 30 of the 32 atoms in the structure. The remaining two atoms were located from an F_o map based on this model.

Block-diagonal matrix least-squares refinement utilizing individual isotropic temperature factors and a fractional weighting scheme[9] reduced the R value to 0.123, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. At this point we noticed an unusually large temperature factor associated with the $5C_\gamma$ atom of a proline ring. Also, the $5C_\beta$-$5C_\gamma$-$5C_\delta$ angle was abnormally large (115°). These conditions are indicative of a disordered C_γ carbon atom. Careful inspection of a difference Fourier map generated without the $5C_\beta$, $5C_\gamma$, and $5C_\delta$ carbons revealed two peaks of equal magnitude near the $5C_\gamma$ site (one above and one below the proline ring plane). The $5C_\beta$ and $5C_\delta$ atoms appeared as single, though elongated, peaks. The structural model was altered to include two positions of one-half occupancy for the $5C_\gamma$ carbon.

After several cycles of full-matrix least-squares refinement employing individual anisotropic temperature factors, a difference Fourier map was calculated. Because of the disorder some of the hydrogens could not be located. Consequently, the calculated positions of the hydrogen atoms (including alternate positions necessitated by the disordered C_γ carbon) were included as constants in the final least-squares cycles. An isotropic thermal parameter, B, equal to 5.0 Å^2 was assigned to each hydrogen. The approximate positions of the six methyl hydrogens used in the calculations were derived from the difference map. The refinement converged at a final residual, R, of 0.055.

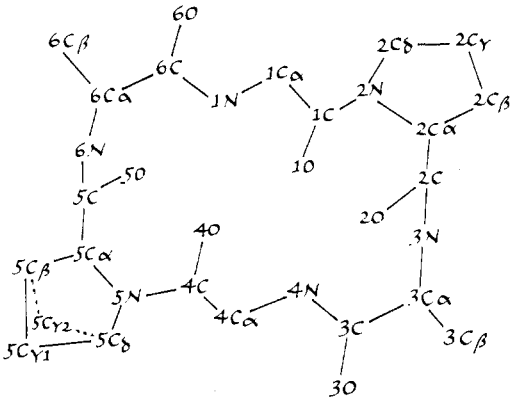

Figure 1. The chemical structure and numbering scheme of *cyclo*-(glycyl-L-prolyl-D-alanyl)$_2$.

Results

Figure 1 is a drawing of the molecule indicating the labeling scheme. The positional parameters for the nonhydrogen atoms, including estimated standard deviations, are listed in Table I. Table II contains the calculated coordinates of the hydrogen atoms and Table III lists the hexapeptide bond lengths and angles. The largest estimated standard deviations are less than 0.03 Å for the lengths and 1.7° for the angles. A comparison of the average backbone distances and angles for this structure with standard values shows that the lengths and angles are close to expected values. Figure 2 is a stereoscopic view[10] of *cyclo*-(Gly-L-Pro-D-Ala)$_2$ perpendicular to the peptide ring.

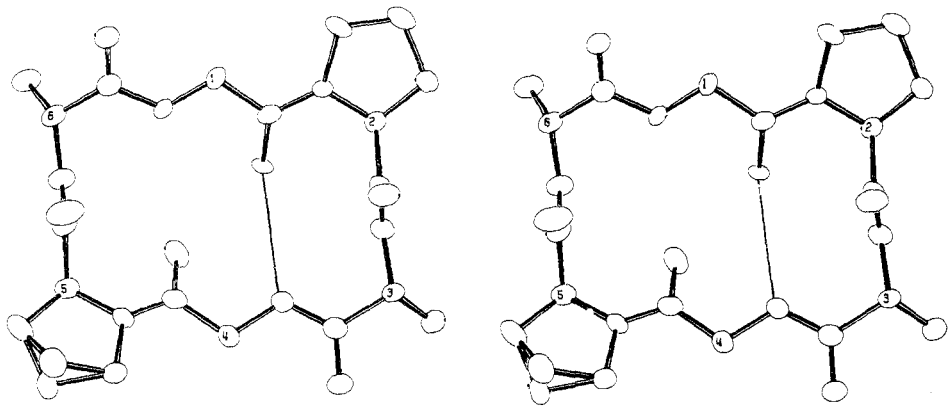

Figure 2. A stereoscopic view of *cyclo*-(Gly-L-Pro-D-Ala)$_2$ perpendicular to the peptide ring. The α carbons are numbered. Note the disordered atoms in Pro$_5$ at lower left. The 4→1 transannular H bond is indicated by the thin line.

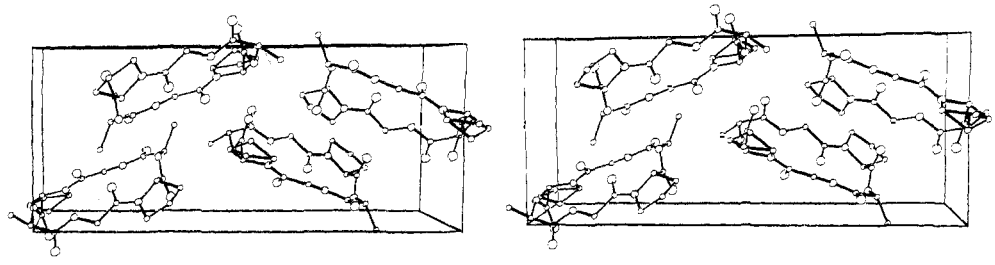

Figure 3. A stereoscopic view of the unit cell contents of *cyclo*-(Gly-L-Pro-D-Ala)$_2$ parallel to *b*.

The anomalously short 5C$_\beta$–5C$_\gamma$ bonds most probably result from the disordered 5C$_\gamma$ atom and the large thermal motions associated with the adjacent atoms. Similar behavior of a C$_\gamma$ pyrrolidine atom has been observed in the analysis of L-Leu-L-Pro-Gly.[11] The alternate 5C$_\gamma$ positions are separated by 0.85 Å. The γ carbon in pyrrolidine rings usually exhibits a large thermal motion. It is interesting that just one of the carbons in the structure displays the disorder. Table IV lists the proline dihedral angles for both ordered and disordered residues. Pro$_2$ assumes a C$_2$–C$_\gamma$-exo conformation,[12] while the Pro$_5$ disorder creates intermediate conformations. The peptide bonds are all close to the ideal trans conformation, which indicates a relatively unstrained molecule. The average deviation from trans is 5.7°, whereas the maximum is 7°. The structure contains two type II β turns[13] and a single 4→1 transannular hydrogen bond. This bond is characterized by a 4N to 1O distance of 3.041 Å and a 156° 4N–H···1O angle. By contrast, the 1N to 4O distance, at 3.418 Å, is too long to be considered a hydrogen bond. The 1O–4O carbonyl oxygens are separated by a 2.835-Å distance across the peptide ring.

Each hexapeptide molecule participates in two intermolecular hydrogen bonds. The first is a relatively strong bond of 2.805 Å between atom 6N and atom 6O′ of an adjacent molecule. The 6N–H···6O′ atoms form a 177° angle. The second hydrogen bond, which forms with a different adjacent molecule, is a weaker one with a 3N to 3O′ distance of 3.096 Å and a 172° 3N–H···3O′ angle. Thus, the carbonyls parallel to the peptide rings participate in a hydrogen-bonding network with the amide hydrogens perpendicular to the rings. Figure 3 shows a stereoscopic view of the unit cell contents.

Discussion

Crystal structure data are now available for two cyclic hexapeptides containing proline residues. The crystal conformation of *cyclo*-(Gly-L-Pro-D-Ala)$_2$ differs from that of *cyclo*-(L-Ala-L-Pro-D-Phe)$_2$ inasmuch as the latter structure preserves the C_2 symmetry observed in the solution studies,[6] whereas the former does not. Both *cyclo*-(Gly-L-Pro-D-Ala)$_2$ and its analogue, *cyclo*-(Gly-L-Pro-D-Phe)$_2$, have been examined in solution by ^{1}H and ^{13}C nuclear magnetic resonance and by circular dichroism (CD).[14] These data indicate that the preferred conformation of both molecules consists of all-trans peptide bonds and is stabilized by 4→1 intramolecular hydrogen bonds in type II β turns. These cyclopeptides are C_2 symmetric on the NMR time scale and coupling constants, together with model building and examination of theoretical CD spectra, support the following approximate Φ, Ψ angles: Gly (−160, 160°), Pro (−70, 90°), and D-Phe or D-Ala (90, 0°). It was concluded that averaging around this structure in solution was probable on the basis of the difficulty in fitting all of the data by one unique structure. Solution and X-ray data are compared in Table V.

Table V. Comparison of Crystal and Solution[13] (Approximate) Peptide Backbone Conformational Angles[a] (deg)

	i = 1,4-Gly		*i* = 2,5-L-Pro		*i* = 3,6-D-Ala	
	cryst	soln	cryst	soln	cryst	soln
ϕ_i	−179, −173	−160	−54, −70	−70	94, 79	90
ψ_i	170, −163	160	125, 116	90	−5, 19	0
ω_i	−175, −173	180[b]	174, 174	180[b]	−174, 176	180[b]

[a] Using the conventions set forth by IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970). [b] Ideal trans conformation assumed in NMR studies. Solution conformation is C_2 symmetric.

The crystal and NMR data for *cyclo*-(Gly-L-Pro-D-Ala)$_2$ agree qualitatively. The largest discrepancies occur in the ψ angles and the loss of twofold rotation symmetry. The observation of any asymmetry in solution is precluded by the dynamics of the experiment.[15]

The presence of C_2 symmetry in the *cyclo*-(L-Ala-L-Pro-D-Phe)$_2$ crystal structure allows no transannular hydrogen bond formation because of close contact distances. Apparently,

an asymmetric conformer can optimize one hydrogen bond in a β turn at the expense of the other and still maintain undistorted backbone angles. This is the case for *cyclo*-(Gly-L-Pro-D-Ala)$_2$. The intermolecular hydrogen bonds seem to support the effect. The stronger one reinforces the peptide twist away from a possibly favorable 1N-H $\cdots$ 4O interaction and the weaker one reinforces the twist toward the existing favorable 4N-H $\cdots$ 1O hydrogen bond.

We take this opportunity to correct ϕ_1 from 109° to −109° in the study[16] of the naturally occurring cyclic peptide, β-amanitin.

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Supplementary Material Available: Temperature factors (1 page). Ordering information is given on any current masthead page.

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(15) In one case, that for *cyclo*-(D-Phe-L-Pro-Gly)$_2$, the molecule appeared to be C_2 symmetric from NMR spectra, but displayed four amide I stretches in an infrared spectrum (in $CHCl_3$).[13] This result suggests that some conformational asymmetry may occur in this type of peptide, yet go unobserved by NMR. Unfortunately, the solubility of *cyclo*-(Gly-L-Pro-D-Ala)$_2$ in $CHCl_3$ was too low to examine it in the same manner.
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Kinetic Applications of Electron Paramagnetic Resonance Spectroscopy. 33. Diazirinyl Radicals[1]

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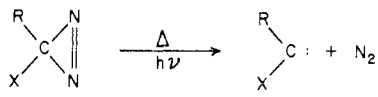
Abstract: Some 3-substituted diazirinyl radicals, RC$\overline{=NN}$·, have been generated by photolysis of the parent bromides in the presence of hexa-*n*-butylditin. The principal EPR parameters for 3-alkyldiazirinyl and 3-phenyldiazirinyl are similar: $a^N(2N)$ = 7.8 G, g = 2.0042. INDO calculations give ^{14}N and ^{13}C hyperfine splittings in good agreement with experiment. Diazirinyls are Π radicals, the two nitrogens' $2p_z$ atomic orbitals making the major contribution to the semioccupied orbital. Diazirinyls decay with second-order kinetics to yield the corresponding nitrile. Like other N-centered three-membered ring radicals, they do not form nitroxides. Studies on the products of reaction of aziridinyl, $\overline{CH_2CH_2N}$·, with *tert*-butylperoxy have revealed that a nitroxide is probably formed, but it decomposes (to ethylene and NO) too rapidly for it to be detected. It is suggested that analogous processes occur with diazirinyls and other N-centered three-membered ring radicals.

The thermal[3-7] and photolytic[8-13] decomposition of 3-alkyl-3-halodiazirines and 3-aryl-3-halodiazirines, **1**,[14] have been studied as sources of "free" halocarbenes, **2**. The possibility that free radicals are involved in some of the systems investigated does not appear to have been explored, nor even suggested. We have discovered that 3-organodiazirinyl radicals, **3**, can be derived from a variety of **1**. These species represent a hitherto unidentified class of nitrogen-containing radicals. In this paper we report on their generation, identification by EPR spectroscopy, decay kinetics, and decay products.



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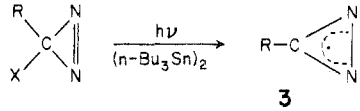

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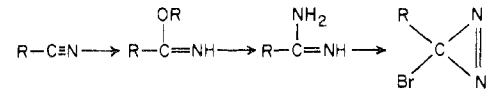
Experimental Section

Materials. Bromodiazirines. The 3-alkyl-3-bromodiazirines and 3-phenyl-3-bromodiazirine were prepared from the corresponding amidine hydrochlorides by oxidation with freshly prepared aqueous sodium hypobromite in Me_2SO according to the general method described by Graham.[14] The volatile alkylbromodiazirines (R = CH_3 and CH_3CH_2) were collected continuously by means of a vacuum



pump which pulled them through a train of four U-tubes held at −35, −80, and −80 °C with the gases bubbling through *n*-pentane, and −196 °C. These diazirines were retained in the pentane-filled U-tube. The less volatile organobromodiazirines (R = $(CH_3)_3C$, C_6H_5, and $C_6H_5CH_2$) were extracted continuously into *n*-pentane and were then purified by column chromatography through silica gel. The infrared

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