Conformation and Crystal Structures of Two Cycloisomeric Hexapeptides: *cyclo*-(L-Alanyl-L-alanylglycylglycyl-L-alanylglycyl) Monohydrate (I) and *cyclo*-(L-Alanyl-L-alanylglycyl-L-alanylglycylglycyl) Dihydrate (II)

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Abstract: The crystal and molecular structures of two cycloisomeric triglycyltrialanyls, *cyclo*-(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly-)·H_2O (I) and (L-Ala-L-Ala-Gly-L-Ala-Gly-Gly-)·$2H_2O$ (II) have been determined by single-crystal x-ray diffraction. The two molecules are found to be dimensionally similar but they differ significantly in their conformation, planarity, and crystal structures. All peptide units in the two molecules are in the trans conformation. Both molecules contain two intramolecular NH···OC hydrogen bonds of the type 4→1 and possess similar backbone structures with two β turns. Peptide I has the unique feature of having both β(I) and β(II) turns. One of the β turns in peptide II has unexpected conformational angles. The hydrogen bonding schemes in both structures are quite extensive and involve all the NH and CO groups. Peptide I crystallizes in the monoclinic space group $P2_1$ with $a = 12.312$ Å, $b = 4.959$ Å, $c = 15.876$ Å, and $\beta = 99.07°$, while peptide II crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 9.274$, $b = 24.357$, and $c = 9.168$ Å. Intensity data in each case were measured using an automatic diffractometer and Cu K$\bar{\alpha}$ radiation at -135 ± 2 °C. The structures were solved by direct methods and were refined by a least-squares technique. The final R factor for peptide I (2225 reflections) is 0.0445 and that of peptide II (2451 reflections) is 0.0373.

The conformations of cyclic polypeptides have attracted increasing attention in efforts to understand structure–function relations in biologically active compounds.[1] The available experimental results show that, in spite of being more rigid than their linear counterparts, cyclopeptides possess flexibility to the extent that they can exist in several different conformers as found in the cases of cyclohexaglycyl[2] and oxytocin.[3] The answers to questions such as how the conformational features of a molecule are dependent on environment must await further structure investigations. An important feature of cyclic peptide conformers is the requirement of a folded conformation in which the polypeptide chain reverses its direction over a few residues, two of which (usually the first and the fourth) are connected by intramolecular NH···O hydrogen bonds (type 4→1) resulting in an antiparallel β structure. Such chain reversal has variously been called "hair-pin bend", "U-turn", "β-bend", and "β-turn".[4-8] The fact that similar reversal of polypeptide chain direction has either been recognized[6,9] or proposed[10] to be a frequent component of protein structures has led to added interest in the conformational properties of cyclic peptides. It has also been suggested[11] that biological significance may be attached to particular types of β turns. Crystal-structure determinations have provided some important conformational data for β turns in cyclic peptides.[2,12-15] However, considering the flexibility of the cyclopeptide conformers, crystal-structure data in this field are still insufficient. Among cyclic peptides, hexapeptides with their 18-membered ring systems are of special interest, as such peptide rings occur quite frequently in many biologically important molecules like ferrichrysin,[11] ferrichrome A,[13] and ferrichrome.[16] The present investigation was undertaken to provide some more data on cyclohexapeptide conformations in the solid state. Added interest in these compounds was the fact that these structures would provide an opportunity to compare the molecular structures of two cycloisomeric compounds.

The compounds were originally prepared by Gerlach, Haas, and Prelog[17] for their initial studies on cyclic isomerism. The crystals of the compounds were made available to us by Dr. T. Emery of Utah State University who obtained the original materials from Dr. Gerlach. The originally synthesized peptide I contained no water, but the structure determination and subsequent refinements showed that a water molecule was included in the structure during recrystallization.

Experimental Section

The crystals of both compounds were obtained from water by slow evaporation. The crystals of peptide I were narrow, elongated plates with *b* as the long axis, while those of peptide II were large rectangular blocks. For both compounds the unit cell dimensions and intensity data were obtained at -135 ± 2 °C with a CAD-4 counter diffractometer (Enraf-Nonius) controlled by a PDP8/e computer and fitted with a low-temperature apparatus. Cell parameters, using Cu Kα_1 ($\lambda = 1.54051$ Å) radiation, were obtained by least-squares fit to $+2\theta$ and -2θ values of 25 reflections for peptide I and 36 reflections for peptide II. Crystal data for the two peptides are given in Table I.

In each case intensities of all independent reflections with $2\theta \leq 150°$ were measured using a θ–2θ scan technique with variable scan rates and Ni-filtered Cu K$\bar{\alpha}$ radiation ($\lambda = 1.5418$ Å). The scan width was also variable and was taken to be $(1.0 + 0.1 \tan \theta)°$ for each reflection. A receiving aperture with a variable width of $(3.8 + 0.86 \tan \theta)$ mm and a constant height of 6 mm was located at a constant distance of 173 mm from the crystal. The maximum scan time for a reflection was 50 s. During the intensity measurements, the intensity of a standard reflection was monitored after every 25 measurements. In all, 2225 independent reflections were measured for peptide I, out of which 242 reflections were considered unobserved (with $I \leq 2.0\sigma(I)$). For peptide II the number of independent reflections was 2451 with 42 unobserved. For both compounds the intensity data were scaled by their respective standard reflection. During the data collections for both peptides, the scale factor for each changed by less than 2.5%. Lorentz and polarization corrections were applied. No absorption correction was made.

Structure Determination and Refinement. Structures of both compounds were solved by direct methods using the program MULTAN.[18b] In peptide I, the phases of 250 reflections with the highest E values ($E \leq 1.53$) were generated by tangent formulas[18a] and refined. The largest peaks in the E map revealed the structure of the hexapeptide. The atomic parameters were refined isotropically to an R factor ($R = [\Sigma |(|kF_o| - |F_c|)|/\Sigma |kF_o|]$) of 0.165 for all reflections. Further refinement even with anisotropic thermal parameters improved the situation only a little ($R = 0.155$). A difference Fourier map was calculated at this stage. The map showed a large peak ($e = 4.9$ e/Å³) near the screw axis at ($x = 0$, $z = \frac{1}{2}$). This was taken to be a water molecule, an assumption proved to be true by subsequent refinements. In the following cycles the structure refined very smoothly to an R factor of 0.075. A difference Fourier at this stage revealed all the

Table I. Crystal Data

	peptide I	peptide II
formula	$C_{15}H_{24}O_6N_6 \cdot H_2O$	$C_{15}H_{24}O_6N_6 \cdot 2H_2O$
fw	402.4	420.4
crystal system	monoclinic	orthorhombic
cell parameters at -135 °C	$a = 12.312$ (3) Å	$a = 9.274$ (3) Å
	$b = 4.959$ (2) Å	$b = 24.357$ (16) Å
	$c = 15.876$ (6) Å	$c = 9.168$ (3) Å
	$\beta = 99.07$ (4)	$V = 2070.9$
	$V = 957.2$	$\rho_{calcd} = 1.348$ g cm^{-3}
	$\rho_{calcd} = 1.396$ g cm^{-3}	
space group	$P2_1, Z = 2$	$P2_12_12_1, Z = 4$
intensity data		
no. of independent reflections	2225	2451
no. unobserved	242	42
$2\theta_{max}$	150°	150°
radiation	Cu Kα	Cu Kα
crystal size	0.60 × 0.09 × 0.03 mm	0.57 × 0.35 × 0.18 mm

Table II. Positional Parameters of Nonhydrogen Atoms in Peptide I

atoms	10^5x	10^4y	10^5z
O_1	17 181 (17)	13 673 (5)	17 800 (12)
O_2	24 651 (14)	11 667 (5)	44 382 (11)
O_3	56 541 (16)	8 937 (5)	37 052 (14)
O_4	78 180 (21)	14 737 (5)	29 458 (14)
O_5	67 141 (16)	9 819 (5)	5 170 (12)
O_6	42 917 (15)	11 274 (5)	17 808 (12)
O_w	2 841 (17)	12 280 (5)	47 012 (16)
N_1	31 918 (17)	8 687 (5)	8 323 (13)
N_2	20 218 (17)	9 386 (5)	22 393 (13)
N_3	36 122 (18)	11 449 (6)	34 617 (13)
N_4	64 881 (18)	12 826 (6)	41 772 (15)
N_5	79 200 (18)	10 266 (5)	27 395 (13)
N_6	61 282 (18)	8 239 (6)	16 939 (14)
C_1^α	22 632 (21)	10 530 (7)	7 825 (16)
C_1'	20 018 (20)	11 343 (7)	16 536 (16)
C_1^β	12 567 (22)	9 198 (7)	2 655 (17)
C_2^α	17 017 (20)	9 922 (6)	30 703 (16)
C_2'	26 384 (21)	11 073 (6)	37 126 (15)
C_2^β	12 540 (24)	7 374 (7)	34 270 (17)
C_3^α	45 272 (22)	12 702 (7)	40 032 (17)
C_3'	56 021 (21)	11 292 (7)	39 370 (15)
C_4^α	76 005 (21)	11 901 (7)	41 186 (18)
C_4'	78 123 (21)	12 432 (7)	32 186 (16)
C_5^α	79 282 (22)	10 479 (6)	18 268 (16)
C_5'	68 549 (21)	9 493 (6)	12 952 (16)
C_5^β	89 101 (22)	9 017 (8)	15 697 (18)
C_6^α	50 808 (22)	7 286 (7)	12 489 (17)
C_6'	41 622 (21)	9 296 (6)	13 078 (16)

hydrogen atom positions. Hydrogen parameters were refined isotropically and refinement was discontinued when the maximum parameter shift was less than 0.4 of its corresponding standard deviation. The final R factor for the compound was 0.0445 for all 2225 reflections.

For peptide II, all 29 nonhydrogen atoms including two oxygens of water molecules were obtained from MULTAN. These atoms were refined first isotropically and then at a later stage with anisotropic thermal parameters to an R factor of 0.065. A difference Fourier map was calculated. All the hydrogen atoms except two belonging to atom C_2^β were easily located from this map. Hydrogen parameters were refined isotropically. The refinement was discontinued when the parameter shifts for nonhydrogen atoms were less than 0.35 of their corresponding standard deviations. The final R factor for all 2225 reflections was 0.0373.

Table III. Positional and Thermal Parameters for Hydrogen Atoms in Peptide I

atom	10^3x	10^3y	10^3z	B, Å^2
$H(N_1)$	318 (3)	741 (8)	48 (2)	2.6 (7)
$H(N_2)$	217 (2)	773 (8)	213 (2)	2.4 (7)
$H(N_3)$	369 (2)	1114 (8)	297 (2)	1.6 (7)
$H(N_4)$	640 (3)	1453 (10)	425 (2)	4.0 (10)
$H(N_5)$	786 (3)	866 (10)	301 (2)	3.9 (9)
$H(N_6)$	624 (2)	815 (8)	224 (2)	2.0 (7)
$H(C_1^\alpha)$	239 (2)	1215 (8)	52 (2)	2.2 (7)
$H(C_2^\alpha)$	113 (2)	1128 (8)	302 (2)	2.2 (7)
$H(C_3^\alpha)_1$	461 (2)	1444 (7)	384 (2)	1.1 (6)
$H(C_3^\alpha)_2$	438 (2)	1281 (8)	457 (2)	1.5 (6)
$H(C_4^\alpha)_1$	767 (3)	1001 (8)	427 (2)	2.6 (8)
$H(C_4^\alpha)_2$	811 (2)	1284 (8)	451 (2)	2.0 (7)
$H(C_5^\alpha)$	796 (2)	1242 (7)	172 (2)	1.1 (6)
$H(C_6^\alpha)_1$	490 (3)	565 (8)	149 (2)	2.7 (8)
$H(C_6^\alpha)_2$	512 (3)	696 (9)	67 (2)	3.2 (8)
$H(C_1^\beta)_1$	112 (2)	743 (9)	52 (2)	2.7 (8)
$H(C_1^\beta)_2$	62 (2)	1034 (8)	23 (2)	2.5 (8)
$H(C_1^\beta)_3$	139 (2)	879 (7)	-33 (2)	1.6 (6)
$H(C_2^\beta)_1$	183 (3)	604 (10)	352 (2)	4.0 (10)
$H(C_2^\beta)_2$	67 (2)	663 (9)	301 (2)	2.3 (7)
$H(C_2^\beta)_3$	100 (3)	777 (9)	396 (2)	3.7 (9)
$H(C_5^\beta)_1$	887 (3)	902 (2)	97 (2)	2.9 (8)
$H(C_5^\beta)_2$	963 (3)	989 (9)	184 (2)	3.4 (9)
$H(C_5^\beta)_3$	897 (2)	727 (8)	175 (2)	2.2 (7)
$H(O_w)_1$	100 (3)	1224 (10)	468 (2)	4.4 (10)
$H(O_w)_2$	13 (4)	1391 (12)	491 (3)	6.0 (12)

Table IV. Positional Parameters of Nonhydrogen Atoms in Peptide II

atoms	10^5x	10^5y	10^5z
O_1	-33 445 (18)	50 953 (6)	33 242 (21)
O_2	16 211 (18)	49 691 (7)	38 069 (19)
O_3	11 343 (16)	64 174 (5)	59 815 (15)
O_4	20 854 (16)	75 013 (5)	81 178 (16)
O_5	-26 810 (19)	77 188 (7)	81 129 (18)
O_6	-26 250 (15)	61 991 (5)	57 882 (15)
O_{w_1}	1 558 (18)	66 529 (8)	31 607 (19)
O_{w_2}	-18 709 (21)	60 636 (6)	87 521 (18)
N_1	-33 890 (18)	65 562 (6)	36 607 (17)
N_2	-15 906 (19)	56 973 (7)	26 939 (20)
N_3	-234 (18)	54 401 (6)	51 119 (19)
N_4	22 125 (18)	60 491 (6)	79 642 (18)
N_5	4 568 (18)	68 901 (6)	89 591 (18)
N_6	-13 420 (18)	71 589 (6)	66 628 (18)
C_1^α	-40 224 (20)	60 384 (7)	31 953 (22)
C_1'	-29 352 (22)	55 681 (7)	30 850 (22)
C_2^α	-4 635 (25)	52 870 (9)	25 071 (25)
C_2'	4 627 (23)	52 197 (8)	38 742 (23)
C_3^α	8 287 (22)	54 460 (7)	64 221 (22)
C_3'	14 217 (19)	60 140 (7)	67 417 (21)
C_4^α	28 889 (21)	65 667 (7)	83 890 (21)
C_4'	17 647 (20)	70 292 (7)	84 464 (19)
C_5^α	-6 506 (22)	72 985 (8)	91 984 (21)
C_5'	-16 556 (22)	74 073 (7)	79 198 (22)
C_6^α	-22 767 (24)	71 768 (8)	53 938 (22)
C_6'	-27 668 (20)	66 004 (7)	49 676 (20)
C_1^β	-47 928 (25)	61 133 (8)	17 365 (25)
C_2^β	4 759 (34)	54 352 (17)	11 953 (30)
C_4^β	41 811 (22)	67 099 (9)	74 596 (26)

In all least-squares refinements the quantity $\Sigma w_F(|kF_o| - |F_c|)^2$ was minimized where weight w_F was defined as $w_F = 1/\sigma_F^2$ and σ_F was obtained from intensity statistics.[19] The scattering factors for O, N, and C atoms were taken from International Tables for X-ray Crystallography[20] and those for hydrogen atoms were from Stewart, Davidson, and Simpson.[21]

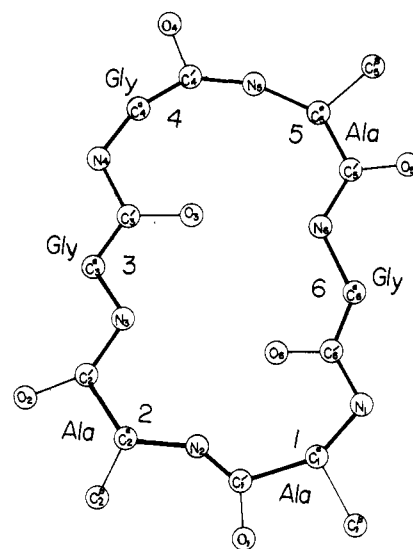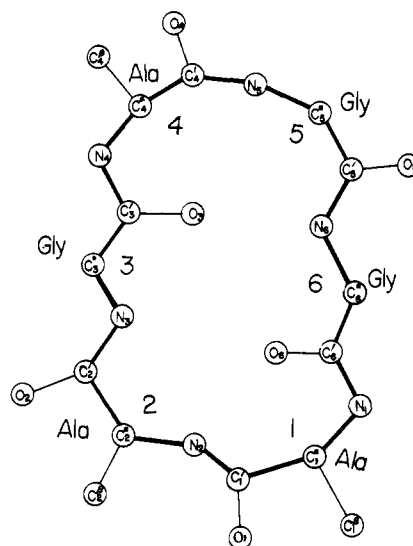**Table V.** Positional and Thermal Parameters of Hydrogen Atoms in Peptide II

atom	10^3x	10^3y	10^3z	B, Å^2
H(N$_1$)	−336 (3)	679 (1)	319 (3)	2.0 (5)
H(N$_2$)	−133 (3)	600 (1)	261 (3)	2.1 (5)
H(N$_3$)	−82 (3)	559 (1)	526 (3)	2.5 (5)
H(N$_4$)	252 (3)	572 (1)	844 (3)	2.8 (6)
H(N$_5$)	34 (3)	650 (1)	907 (3)	3.3 (6)
H(N$_6$)	−54 (3)	695 (1)	664 (3)	1.7 (5)
H(C$_1^\alpha$)	−475 (3)	590 (1)	391 (3)	1.9 (5)
H(C$_2^\alpha$)	−86 (3)	497 (1)	237 (3)	2.8 (6)
H(C$_3^\alpha$)$_1$	160 (3)	521 (1)	629 (3)	2.8 (6)
H(C$_3^\alpha$)$_2$	19 (3)	531 (1)	726 (3)	2.5 (5)
H(C$_4^\alpha$)	322 (3)	649 (1)	944 (3)	2.6 (5)
H(C$_5^\alpha$)$_1$	−124 (3)	718 (1)	1019 (3)	3.0 (6)
H(C$_5^\alpha$)$_2$	−15 (3)	769 (1)	941 (3)	2.7 (6)
H(C$_6^\alpha$)$_1$	−314 (3)	741 (1)	554 (3)	3.2 (6)
H(C$_6^\alpha$)$_2$	−168 (3)	731 (1)	445 (3)	3.1 (6)
H(C$_1^\beta$)$_1$	−406 (3)	625 (1)	107 (4)	4.2 (7)
H(C$_1^\beta$)$_2$	−555 (3)	638 (1)	188 (4)	3.7 (7)
H(C$_1^\beta$)$_3$	−541 (3)	576 (1)	158 (4)	3.6 (6)
H(C$_2^\beta$)	113 (5)	516 (2)	113 (5)	7.1 (11)
H(C$_4^\beta$)$_1$	493 (4)	645 (1)	755 (4)	3.8 (7)
H(C$_4^\beta$)$_2$	465 (3)	706 (1)	769 (3)	3.0 (6)
H(C$_4^\beta$)$_3$	390 (3)	669 (1)	662 (3)	3.2 (6)
H(O$_{w_1}$)$_1$	83 (4)	688 (2)	277 (5)	5.6 (7)
H(O$_{w_1}$)$_2$	53 (3)	651 (1)	401 (3)	2.5 (7)
H)O$_{w_2}$)$_1$	−206 (4)	612 (1)	786 (4)	4.0 (9)
H(O$_{w_2}$)$_2$	−202 (4)	569 (1)	887 (4)	4.4 (5)

Figure 1. Atom labeling scheme in peptide I. Bold numerals indicate the amino acid residues.

Figure 2. Atom labeling scheme in peptide II.

Description and Discussion of the Structures

The final atomic parameters for peptide I are given in Table II (nonhydrogen atoms) and in Table III (hydrogen atoms) and the corresponding parameters for peptide II are listed in Tables IV and V. Nonhydrogen atoms in both molecules are labeled according to the IUPAC–IUB convention[22] and the labeling schemes are shown in Figures 1 and 2. Hydrogen atoms are designated according to the atoms to which they are bonded. Bond lengths and angles calculated on the basis of the final parameters are listed in Tables VI through IX.

The bond distances and bond angles in the two molecules are quite normal. The average values of each of the bond lengths and angles in two peptides are within one standard deviation of each other. These average values, in turn, are in fair agreement with the corresponding values observed in other small peptides, both cyclic[12,23,24] and linear[25] (Table X). However, there are small but systematic differences in the $C_j'-N_{j+1}$ and the C_j'=O distances in cyclic and linear peptides. In cyclic peptides, the average $C_j'-N_{j+1}$ distance is 0.014 Å longer and the average C_j'=O distance is about 0.008 Å shorter than the corresponding distances in linear peptides. The present structures do not show any significant difference in the average value of the $N_jC_j^\alpha C_j'$ angle from those in linear peptides, as was reported in the case of *cyclo*-(4-Gly-2-D-Ala-).[12]

The configurations of two peptide molecules are shown in stereodrawings given in Figures 3 and 4. Their conformational angles, ϕ_j, ψ_j, and ω_j, are listed in Tables XI and XII. The convention followed is the one established by the IUPAC–IUB Commission on Biochemical Nomenclature.[22] All peptide units in the two molecules are in the trans conformation. The maximum deviation of ω_j from 180° is 11.2° in peptide I and 6.6° in peptide II. Conformations about the $C_j^\alpha-C_j'$ bonds (angle ψ_j) in the two molecules are different. In peptide II, the two residues from N$_3$ to N$_4$ and from N$_6$ to N$_1$ are near the trans conformation with ψ_j near 180°, while the other four are near

Table VI. Bond Lengths of Nonhydrogen Atoms in Peptide I[a]

bond	$j = 1$	$j = 2$	$j = 3$	$j = 4$	$j = 5$	$j = 6$	av	rms deviation, Å
1. N$_j$C$_j^\alpha$	1.456	1.460	1.445	1.461	1.454	1.448	1.454	0.006
2. C$_j^\alpha$C$_j^\beta$	1.525	1.523			1.520		1.523	
3. C$_j^\alpha$C$_j'$	1.523	1.525	1.515	1.515	1.532	1.522	1.522	0.006
4. C$_j'$O$_j$	1.233	1.240	1.229	1.223	1.231	1.230	1.231	0.004
5. C$_j'$N$_{j+1}$	1.342	1.335	1.335	1.335	1.329	1.343	1.337	0.005

[a] Standard deviations for bond distances range from 0.003 to 0.004 Å.

Table VII. Bond Lengths of Nonhydrogen Atoms in Peptide II[a]

bond	$j=1$	$j=2$	$j=3$	$j=4$	$j=5$	$j=6$	av	rms deviation, Å
1. $N_jC_j^\alpha$	1.455	1.456	1.438	1.461	1.447	1.452	1.452	0.007
2. $C_j^\alpha C_j^\beta$	1.527	1.528		1.511			1.522	
3. $C_j^\alpha C_j'$	1.529	1.528	1.517	1.536	1.521	1.527	1.526	0.006
4. $C_j'O_j$	1.232	1.237	1.234	1.225	1.229	1.240	1.233	0.005
5. $C_j'N_{j+1}$	1.335	1.334	1.342	1.344	1.334	1.334	1.337	0.004

[a] Standard deviations for bond distances range from 0.002 to 0.003 Å.

Table VIII. Bond Angles in Peptide I[a]

angle	$j=1$	$j=2$	$j=3$	$j=4$	$j=5$	$j=6$	av	rms dev, deg
1. $C_{j-1}'N_jC_j^\alpha$	120.7	121.1	122.4	122.3	121.7	122.3	121.8	0.7
2. $N_jC_j^\alpha C_j'$	113.2	112.9	111.6	107.9	112.6	111.7	111.7	1.8
3. $N_jC_j^\alpha C_j^\beta$	108.8	110.5			111.3		110.2	
4. $C_j'C_j^\alpha C_j^\beta$	109.5	110.0			110.7		110.1	
5. $C_j^\alpha C_j'O_j$	120.1	119.2	123.1	120.5	118.9	122.3	120.7	1.5
6. $C_j^\alpha C_j'N_{j+1}$	117.0	118.2	113.6	116.4	118.3	115.0	116.4	1.7
7. $O_jC_j'N_{j+1}$	122.7	122.6	123.2	122.9	122.7	122.7	122.8	0.2

[a] Standard deviations range from 0.2 to 0.3°.

Table IX. Bond Angles in Peptide II[a]

angle	$j=1$	$j=2$	$j=3$	$j=4$	$j=5$	$j=6$	av	rms dev, deg
1. $C_{j-1}'N_jC_j^\alpha$	120.5	122.7	121.9	120.8	121.4	123.3	121.8	1.0
2. $N_jC_j^\alpha C_j'$	113.7	112.4	111.7	110.5	116.0	110.8	112.5	1.9
3. $N_jC_j^\alpha C_j^\beta$	110.0	109.9		112.9			110.9	
4. $C_j'C_j^\alpha C_j^\beta$	109.9	110.5		112.9			111.1	
5. $C_j^\alpha C_j'O_j$	119.0	120.0	122.6	121.0	118.1	122.5	120.5	1.7
6. $C_j^\alpha C_j'N_{j+1}$	117.2	117.7	114.7	116.1	117.0	115.6	116.4	1.0
7. $O_jC_j'N_{j+1}$	123.8	122.3	122.6	122.8	125.0	121.8	123.1	1.1

[a] Standard deviations range from 0.1 to 0.2°.

Table X. Comparison of Average Values of Bond Lengths and Angles in Small Peptides

bond no. (as defined in Table VI)	peptide I	peptide II	small cyclic peptides			small linear peptides[d]
			a	b	c	
1	1.454	1.452	1.460	1.477	1.449	1.455
2	1.523	1.522			1.528	
3	1.522	1.526	1.515	1.536	1.503	1.510
4	1.231	1.233	1.232	1.229	1.237	1.240
5	1.337	1.337	1.338	1.344	1.341	1.325
angle no. (as defined in Table VIII)						
1	121.8	121.8	122.5	124.2	120.6	122.0
2	111.7	112.5	113.1	105.2	114.5	111.7
3	110.2	110.9				
4	110.1	111.1				
5	120.7	120.5	121.0	120.0	121.1	120.5
6	116.4	116.4	115.6	116.6	116.6	116.0
7	122.8	123.1	123.0	123.2	122.2	123.5

[a] Reference 12. [b] Reference 23. [c] Reference 24. [d] Reference 25.

the cis conformation with ψ_j lying between −6.3 and +37.7°. In peptide I, however, ψ_j has a much greater spread. In peptide II, the conformational angles for residues 1, 2, and 3 are nearly equal in magnitude and opposite in sign to those in residues 4, 5, and 6. This indicates that aside from the CH_3 groups, the peptide II molecule possesses an approximate center of symmetry. Peptide I does not possess such pseudosymmetry.

β Turns. In both molecules there are two intramolecular NH···O hydrogen bonds of the type 4→1. In each molecule, these bonds connect amide and carbonyl groups of two glycyl residues. The overall conformations of *cyclo*-(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly-) and (L-Ala-L-Ala-Gly-L-Ala-Gly-Gly-) are quite similar to those of *cyclo*-(4-Gly-2-D-Ala-)[12] and to the most prevalent form of *cyclo*-(6-Gly-).[2] However, some
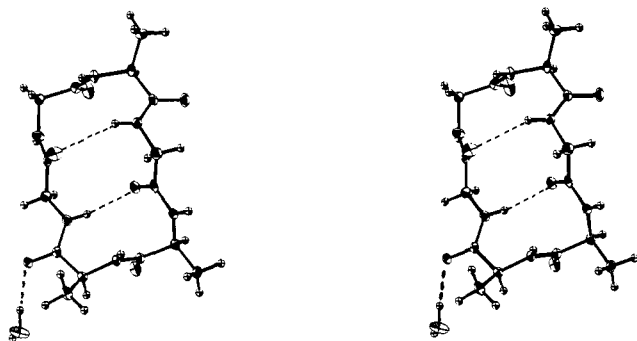
Figure 3. Stereoview of the single molecule of peptide I. Intramolecular hydrogen bonds are shown by dashed lines.
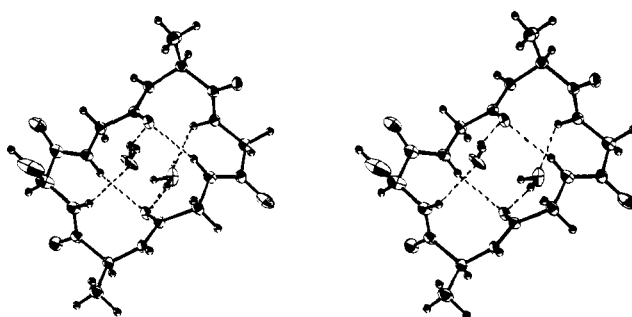

Figure 4. Stereoview of the single molecule of peptide II. Intramolecular hydrogen bonds (including those with water molecules) are shown by dashed lines.
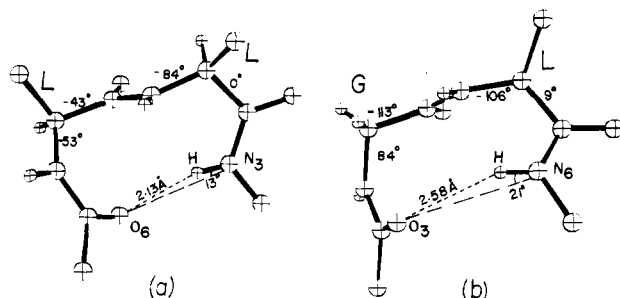
Table XI. Conformational Angles for Peptide I

	$j = 1$	$j = 2$	$j = 3$	$j = 4$	$j = 5$	$j = 6$
ϕ_j	−53.1	−84.2	+139.2	+84.2	−105.8	−96.4
ψ_j	−43.0	−0.2	+158.5	−112.8	−9.4	+173.0
ω_j	−175.3	+175.6	−176.6	+168.8	+178.6	−175.7

Table XII. Conformational Angles for Peptide II

	$j = 1$	$j = 2$	$j = 3$	$j = 4$	$j = 5$	$j = 6$
ϕ_j	−62.0	−95.4	+105.7	+53.8	+90.9	−119.0
ψ_j	−32.5	+13.7	+179.1	+37.7	−6.3	−167.2
ω_j	−178.2	−173.9	+178.2	+174.0	+173.4	−174.1

Figure 5. Two β turns in peptide I. L stands for L-alanyl and G stands for glycyl. Conformational angles are rounded off to nearest whole numbers.
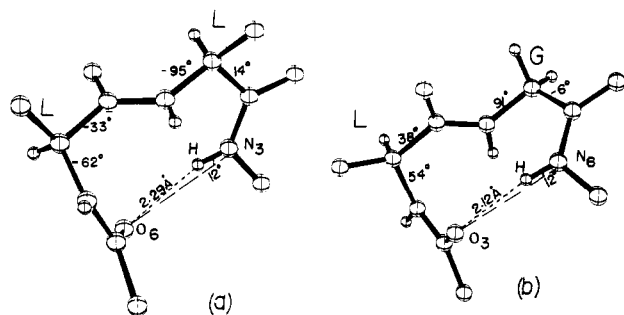

Figure 6. Two β turns in peptide II.

conformational features of the so-called β turns observed in the present structures are quite different from those observed in other reported cyclic peptide structures.

The two β turns of peptide I molecule are shown in Figures 5a and 5b along with their relevant conformational angles and hydrogen bond dimensions. The β turns of peptide II are shown in Figures 6a and 6b. In both molecules, all alanine residues occupy corners.

The conformational features of the four β turns are further illustrated by means of conformational maps given in Figures 7a and 7b. These maps were drawn following the procedure set up by Venkatachalam.[4] To describe some of the conformational features observed in the present structures, we have adopted a system of designation of the β turns based on the original classification of Venkatachalam.[4] The present system is related to earlier classifications in the following way:

proposed designation	Venkatachalam[4]	Karle[26]
$\beta(I)$	conformation type I	4→1 of type I (trans)
$\beta(II)$	conformation type II	4→1 of type II (trans)
$\beta(I')$	conformation type I'	4→1 of type I (trans)
$\beta(II')$	conformation type II'	4→1 of type II (trans)

In the conformation maps, each β turn is represented by a vector in (ϕ,ψ) space. The vector starts at (ϕ_{i+1},ψ_{i+1}) and ends at (ϕ_{i+2},ψ_{i+2}), where subscripts refer to amino acid residues at corners, and a hydrogen bond connects residues i and $i + 3$. The areas bounded by the dotted lines are the calculated allowed regions for (ϕ_{i+1},ψ_{i+1}) conformational angles, and areas bounded by dashed lines are calculated allowed regions for (ϕ_{i+2},ψ_{i+2}) angles. For a normal β turn with a proper hydrogen bond (lying within the limits of Venkatachalam's parameters) this will be represented by a vector starting from a dotted-line area and ending in the dashed-lined area. Figure 7a shows the allowed regions for $\beta(I)$ and $\beta(II)$ and Figure 7b shows the allowed regions for $\beta(I')$ and $\beta(II')$ turns.

The two β turns in the peptide I molecule are represented by vector A and vector B, while the two turns of the peptide II molecule are represented by vector C and vector D (Figures 7a and 7b). Of the four β turns, the two with only alanyl residues at the corners are of the type $\beta(I)$. The other two with mixed residues at the corners (GL in peptide I and LG in peptide II; G = glycyl, L = L-alanyl) belong to type $\beta(II')$ and $\beta(I')$, respectively. Peptide I is probably the only example where two different types of β turns are seen to exist in the same molecule in a crystalline state. In a recent report of spectroscopic studies, Walter, Wyssbrod, and Glickson[27] mentioned the possibility of the presence of both $\beta(I)$ and $\beta(II)$ within the same molecule. The conformational angles of $\beta(II')$ in peptide I compare well with those of $\beta(II')$ observed in valinomycin,[26] with DL (D = D-amino acid residue) at the corners of the turn. The present structure, therefore, provides experimental proof of a theoretical prediction[4] that a D residue can be replaced by a glycine residue. The $\beta(II')$ in the present structure is stabilized by an extremely weak hydrogen bond (H···O = 2.58 Å, H–N–O = 21°). In fact, such a situation was not considered to be a hydrogen bond by Venkatachalam. This is why the foot of the vector B in Figure 7b lies outside the al-
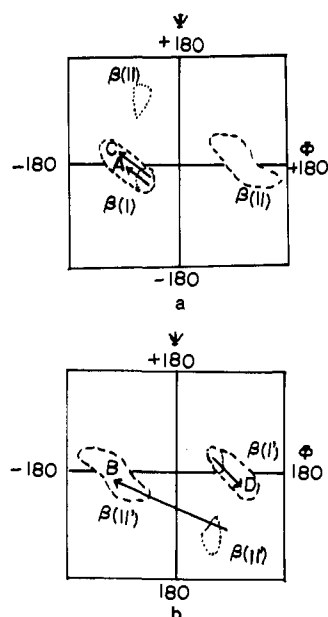
Figure 7. (a) Conformational map showing the calculated[4] allowed regions for β(I) and β(II) turns. (b) Conformational map showing calculated allowed regions for β(I') and β(II') turns. For β turns designations see text.

Table XIII. Conformational Parameters for Planarity in Peptide I[a]

peptide unit	1	2	3	4	5	6
ω_1	−175.3	175.6	−176.6	168.8	178.6	−175.7
ω_2	174.6	−175.4	169.0	−176.4	−175.9	172.6
ω_3	0.1	−3.1	5.2	−7.1	−3.6	6.9
ω_4	−0.7	3.3	−12.8	−0.5	6.3	−10.0
χ_C	4.6	−1.3	−1.8	−4.1	2.2	−2.6
χ_N	−5.4	7.7	−16.2	10.7	7.7	−14.3
τ	179.7	180.1	176.2	176.2	181.4	178.4

[a] $\chi_C = (\omega_1 - \omega_3 + \pi)$, mod 2π. $\chi_N = (\omega_2 - \omega_3 + \pi)$, mod 2π. $\tau = \frac{1}{2}(\omega_1 + \omega_2)$, $|\omega_1 - \omega_2| < \pi$.

lowed region. Another relevant point of interest in this part of the molecule is the environment of the amide nitrogen, N_6. This is described later in some detail.

In the peptide II molecule, both the β turns are stabilized by strong hydrogen bonds. β(I) with LL at the corners has normal conformational angles. The presence of β(I') in this molecule with LG at the corners is quite an unexpected occurrence and has not been reported before. Normally, β(I') occurs in a turn with two D residues at the corners, as in *cyclo*-(4-Gly-2-D-Ala-) or with two G residues at the corners, as in cyclohexaglycyl.[26]

Planarity. The individual peptide units in the two molecules are approximately planar. The root mean square (rms) deviations of atoms from the least-squares planes through the peptide units are within 0.01 Å. In both the molecules, the successive peptide units are nearly perpendicular to each other. The average dihedral angle between the planes of adjacent peptide units is 74° in peptide I. The corresponding angle in peptide II is 77°. To test the nonplanarity of the amide groups arising from out-of-plane bending of the bonds attached to C' and N, the conformational angles, ω_1, ω_2, ω_3, ω_4, χ_N, χ_C, and τ as defined by Winkler and Dunitz,[28] were calculated for both the molecules. These angles are given in Tables XIII and XIV. $|\chi_C|$ for both the peptides are quite small (average values: 3° for peptide I and 2° for peptide II). These values indicate that out-of-plane bending at the C' atoms in both molecules is

Table XIV. Conformational Parameters for Planarity in Peptide II[a]

peptide unit	1	2	3	4	5	6
ω_1	−178.2	−173.9	178.2	174.0	173.4	−174.1
ω_2	−174.9	−173.4	−171.3	173.7	177.7	−171.9
ω_3	0.1	5.9	−5.6	−2.0	−7.3	4.6
ω_4	6.8	6.8	12.4	−10.4	−1.6	9.5
χ_C	1.7	0.2	3.8	−4.0	0.7	1.3
χ_N	5.0	0.7	14.3	−4.3	5.0	3.5
τ	183.5	186.4	183.4	173.8	175.5	187.0

[a] See footnote *a* in Table XIII.

Table XV. Hydrogen Bond Parameters in Peptide I

bond	donor D	acceptor A[a]	D–H, Å	H⋯A, Å	length D–H⋯A, Å	angle D–H–A, deg
a	N_3–H⋯	O_6(i)	0.81	2.14	2.92	161.6
b	N_6–H⋯	O_3(i)	0.85	2.58	3.35	152.0
c	O_w–H⋯	O_2(i)	0.89	1.92	2.80	168.5
d	O_w–H⋯	O_w(ii)	0.91	1.88	2.78	175.9
e	N_2–H⋯	O_1(iii)	0.86	2.14	2.93	153.3
f	N_5–H⋯	O_4(iii)	0.91	1.95	2.77	148.8
g	N_1–H⋯	O_5(iv)	0.85	2.05	2.89	169.6
h	N_4–H⋯	O_2(v)	0.86	2.55	3.04	116.7
i[b]	N_6–H⋯	O_4(iii)	0.85	2.69	3.16	116.5

[a] (i) x, y, z. (ii) $\bar{x}, \frac{1}{2} + y, 1 - z$. (iii) $x, y - 1, z$. (iv) $1 - x, -\frac{1}{2} + y, \bar{z}$. (v) $1 - x, \frac{1}{2} + y, 1 - z$. [b] See text.

Table XVI. Hydrogen Bond Parameters in Peptide II

bond	donor D	acceptor A[a]	D–H, Å	H⋯A, Å	length D–H⋯A, Å	angle D–H–A, deg
a	N_3–H⋯	O_6(i)	0.83	2.29	3.10	164.0
b	N_6–H⋯	O_3(i)	0.90	2.12	2.99	163.5
c	O_{w_1}–H⋯	O_3(i)	0.91	1.91	2.80	164.0
d	N_2–H⋯	O_{w_1}(i)	0.79	2.16	2.87	150.2
e	O_{w_2}–H⋯	O_6(i)	0.85	1.98	2.83	174.0
f	N_5–H⋯	O_{w_2}(i)	0.96	2.33	2.96	122.4
g	O_{w_1}–H⋯	O_5(ii)	0.91	1.88	2.78	174.3
h	O_{w_2}–H⋯	O_1(iii)	0.93	2.01	2.86	151.9
i	N_1–H⋯	O_4(iv)	0.71	2.15	2.85	170.5
j	N_4–H⋯	O_2(v)	0.95	1.90	2.81	161.7

[a] (i) x, y, z. (ii) $\frac{1}{2} + x, \frac{3}{2} - y, 1 - z$. (iii) $-\frac{1}{2} - x, 1 - y, \frac{1}{2} + z$. (iv) $-\frac{1}{2} + x, \frac{3}{2} - y, 1 - z$. (v) $\frac{1}{2} - x, 1 - y, \frac{1}{2} + z$.

negligible. $|\chi_N|$ values, on the other hand, are quite appreciable with an average of 10.2° in peptide I, and 5.4° in peptide II. The rather significant out-of-plane bending at the N atoms, and comparatively smaller bending at C', seem to be characteristic of all peptides as noted by Ramachandran, Lakshminarayanan, and Kolaskar.[29] τ angles in the two molecules are different, being systematically smaller in peptide I than in peptide II. Of the 12 peptide linkages, the nonplanarity is least in the two Ala-Ala linkages.

Hydrogen Bonds. In both structures hydrogen bonding schemes are quite extensive and efficient. They utilize all the C=O and NH groups and provide each water molecule three bonds each. Tables XV and XVI list all the hydrogen bond parameters.

There are eight hydrogen bonds in the peptide I structure. Of the two intramolecular bonds, N_3–H⋯O_6 is quite strong, while N_6–H⋯O_3 is extremely weak. The other NH and OC groups in the molecule are involved in four intermolecular
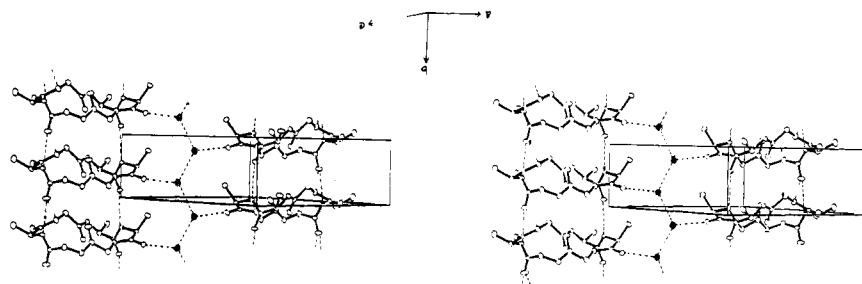
Figure 8. Stereoview of partial packing scheme in peptide I structure. The water molecules are indicated by dashed circles. Hydrogen bonds are indicated by dashed lines.
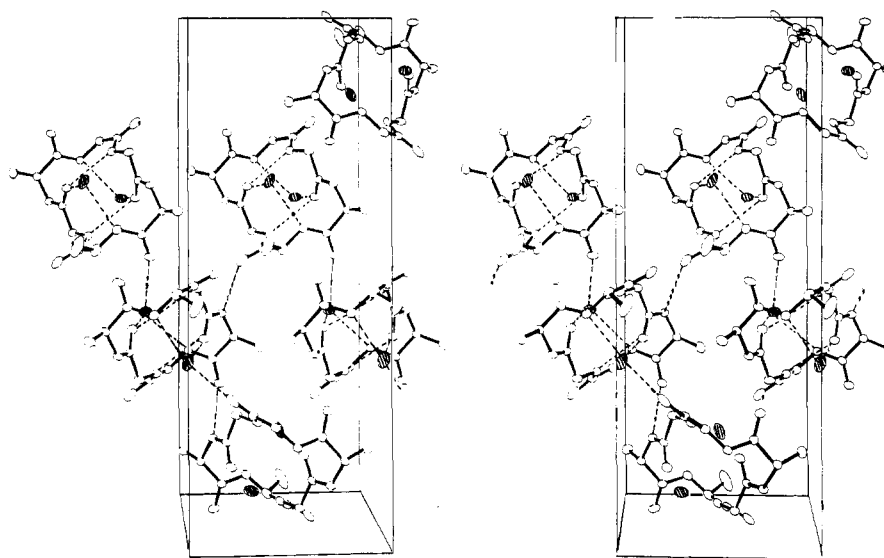

Figure 9. Stereoview of the packing scheme in peptide II. Water molecules are indicated by dashed lines.

hydrogen bonds. The water molecule is hydrogen bonded to only one atom (O_2) of the peptide molecule. Water molecules related by a screw axis at ($x = 0, z = \frac{1}{2}$) are held together by strong hydrogen bonds. As a result, an unending chain of water molecules is formed along the b axis. The atom N_6 has two short contacts, one intramolecular with O_3, and the other intermolecular with O_4. The overall geometry of N_6 is shown below:
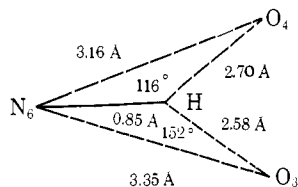
Although the $N_6 \cdots O_4$ distance of 3.16 Å is much shorter than the $N_6 \cdots O_3$ distance of 3.35 Å, the $H \cdots O_4$ distance of 2.7 Å excludes the possibility of bifurcated hydrogen bonding between the atoms N_6, O_3, and O_4.

The hydrogen bonding scheme in the peptide II structure is dominated by two water molecules. Of the ten hydrogen bonds present in the structure, six involve water molecules. Each water is hydrogen bonded to an amide and a CO group of the same peptide molecule. The intramolecular hydrogen bonds, N_3-$H \cdots O_6$ and N_6-$H \cdots O_3$ are both quite strong. There are only two intermolecular $NH \cdots OC$ hydrogen bonds in this structure.

Crystal Structures. The crystal structures of the two compounds are quite different. Peptide I crystallizes in the monoclinic space group $P2_1$ with an unusually short b axis (4.959 Å). The crystal structure of the compound consists of stacks of molecules lying on top of each other forming a flat-

tened cylinder with b as the cylinder axis. Unlike the slanted cylinder formation observed in the case of tetrapeptide, L-Ser(O-t-Bu)-β-Ala-Gly-L-β-As(OMe),[24] the cylinder in the present structure is approximately vertical. Within each cylinder, molecules on top of each other are connected by two fairly strong N-H$\cdots$O hydrogen bonds. These bonds link N_2 and N_5 of one molecule to O_1 and O_4 of the neighboring molecule. The distance between the adjacent planes of the 18-membered ring is approximately equal to the length of the b axis. This distance is considerably shorter than the expected interplanar distance (5.5 Å) in synthetic cylindrical peptides.[30] Figure 8 gives the stereoview of the part of the whole packing scheme in the peptide I structure. Neighboring cylinders related by a screw axis at ($x = 0, z = \frac{1}{2}$) are held together by the water chain. Peptide cylinders shown in Figure 8 are linked to other neighboring cylinders (not shown in the figure) by direct hydrogen bonds (bonds g and h in Table XV).

Peptide II crystallizes in the orthorhombic space group $P2_12_12_1$ and does not possess a short axis. The overall packing scheme (shown in Figure 9) does not lead to any cylinder formation. Most of the amide and carbonyl groups in this structure are occupied by water molecules. In contrast, in the peptide I structure, more amide and carbonyls are available for direct contacts with neighboring molecules leading to a completely different crystal structure.

Cycloisomers. The two cycloisomeric molecules under investigation are found to be dimensionally similar, but they differ significantly in their conformations, planarity, and crystal structures.

Hexapeptides may be broadly classified into two groups: (1) those which possess two normal 4→1 intramolecular N-H$\cdots$O hydrogen bonds, and have nearly centrosymmetric backbone

structures like cyclohexaglycyl, *cyclo*-(-4-Gly-2-D-Ala-), and some other synthetic hexapeptides,[8] and (2) those with a single intramolecular hydrogen bond and asymmetric backbone structures. Hexapeptides belonging to the latter group mostly appear as part of more complex molecules like ferrichrysin, ferrichrome A, and ferrichrome. Conformational features of the peptide II molecule are consistent with group 1 peptides. Peptide I, on the other hand, with one normal and one very weak intramolecular hydrogen bond, seems to form an intermediate structure between the two groups.

The crystal-structure data alone cannot provide the proper explanation for the conformational differences observed in the two cycloisomers. It has been observed[31] that major conformational changes in peptides can be a result of intermolecular hydrogen bonding in the crystalline state. The state of hydration in the two molecules is different. The synthesized peptide II contains two water molecules. These water molecules are involved in multiple intramolecular cross-linkages (Figure 4). Such bondings obviously have affected the overall geometry of the peptide II molecule. The involvement of two water molecules in intramolecular linkages in peptide II is strikingly similar to the situation in *cyclo*-(-4-Gly-2-D-Ala-), where two water molecules are involved in a very similar hydrogen bonding scheme. The conformational features of both peptide II and *cyclo*-(-4-Gly-2-D-Ala-) are very similar, in spite of the fact that one contains three L-alanyls and the other two D-alanyls. It may be concluded, therefore, that the intramolecular cross-linking with water molecules determines the conformational features in peptide II and *cyclo*-(-4-Gly-2-D-Ala-). The water molecule in peptide I was included during recrystallization. It is only involved in intermolecular hydrogen bonding and probably has very little effect on the conformation of the 18-membered ring. Only further structural data of cycloisomers (preferably dehydrated) can give us greater insight into the subject.

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