Crystal and Molecular Structure of Complex between *cyclo*(L-Prolylglycyl)$_4$ and RbSCN

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Abstract: The cyclic octapeptide *cyclo*(L-prolylglycyl)$_4$ yields, with rubidium thiocyanate in acetone–water, crystals having the formula *c*(L-Pro-Gly)$_4$·RbSCN·3H$_2$O. Three-dimensional x-ray crystal structure analysis has shown that two of these formula units are related by a crystallographic twofold axis as a dimer. All peptide bonds are trans in the *c*(L-Pro-Gly)$_4$ moiety. The rubidium cation has a distorted octahedral environment consisting of four glycyl carbonyl oxygens from one cyclic peptide of the dimer, one glycyl carbonyl oxygen from the other cyclic peptide of the dimer, and one oxygen from a water molecule. The space group is $P4_12_12$, and there are eight formula weights in a unit cell having dimensions $a = b = 13.787$ and $c = 38.974$ Å. For the 1309 observed independent x-ray diffraction maxima ($I \geq 2\sigma$) the value of $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ is 0.092.

Some of the biological functions of the naturally occurring cyclic peptides are related to their structural conformations. Of the antibiotics,[1-5] such as gramacidin S, antamanide, ilamycin, ferrichrome, and alamethicin, the last two also have a role in ion transport.[6,7] Antamanide also forms complexes with alkali cations,[8] although it has no effect on ionic balance in systems of cell membranes. On the other hand, gramicidin S has no internal cavity in its structures,[9,10] and does not strongly bind metal cations. Selectivities in cation binding are closely related to the detailed conformations of these cyclic peptides. Hence, these conformations have been studied in solutions[11-16] and in the crystalline states.[17-19]

Among the amino acids which are present in cyclic peptides, proline and glycine play special roles. While the relatively rigid proline ring limits conformations, proline is unique in that the energy difference is small between the cis and trans forms of the peptide bond to its N atom. Also, glycine frequently facilitates a reverse turn because it alone among amino acids has H in place of a bulkier R group. For these reasons the synthesis of synthetic cyclic peptides, especially those containing Pro and Gly, is likely to elucidate structural aspects of biologically interesting cyclic peptides. A number of such studies have been under way by Blout and co-workers.[20-23] Early studies of *c*(*cyclo*)-(L-Pro)$_3$ have established the all cis conformation in the crystal[24] in general agreement with the results in solution,[20] except for moderate differences in the torsion angles $\phi(C_\alpha\text{–N})$ and $\psi(C_\alpha\text{–C})$ of the polypeptide backbone.[24c] Later studies included the synthesis of *c*(L-Pro-Gly)$_n$ for $n = 2, 3, 4, \ldots$, in the expectation that retention of some axial symmetry and restriction to Pro and Gly might simplify conformational analysis by NMR methods. Moreover, for larger n the cyclic peptides were expected to develop central cavities in which carbonyl oxygens and other ligands may bond metal ions. We now comment on this structure–function relationship for $n = 2$, 3, and 4.

An asymmetric conformation found in an NMR study[26] in solution for *c*(L-Pro-Gly)$_2$ has a cis-trans-cis-trans peptide backbone, in which the two Pro-Gly bonds are trans and the two Gly-Pro bonds are cis, but the C_α–CO bonds of the two Pro residues differ: one is trans′ while the other is cis′.

The *c*(L-Pro-Gly)$_3$ molecule has been predicted[27] to have all peptide bonds in the trans conformation on the basis of an energy-minimization study, in which ϕ and ψ angles were varied. This same conformation has been established in nonpolar solvents from studies of circular dichroism and NMR spectra.[28,29] This conformation has C_3 symmetry, and is stabilized by three 1 ← 3 hydrogen bonds in γ turns. In polar solvents, however, *c*(L-Pro-Gly)$_3$ has one cis Gly-Pro bond, and the C_3 symmetry no longer exists. Cations bind to an all trans conformation in which these hydrogen bonds are broken. Ratios are 1:1 between cations Li^+, Na^+, K^+, Rb^+, or Ca^{2+} and *c*(L-Pro-Gly)$_3$, but Mg^{2+} forms three different complexes in ratios Mg^{2+}/*c*(L-Pro-Gly)$_3$ of 1/2, 1/1, and 2/1.

The all-trans conformation has been indicated[30] for *c*(L-Pro-Gly)$_4$ from NMR spectra. Complexes with Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Mg^{2+}, and Ba^{2+} are in cation/peptide ratios of 1/1 in water and of 1/1 and 1/2 in acetonitrile. Also in acetonitrile, Na^+ forms complexes in ratios of 1/1, 1/2, and 2/1. The present x-ray diffraction study was made on the Rb^+ complex of *c*(L-Pro-Gly)$_4$. We shall show that in the crystal of [*c*(L-Pro-Gly)$_4$·RbSCN·3H$_2$O]$_2$ the ratio of Rb^+/peptide is 2/2, an unexpected result when compared with the studies noted above for solutions. Analysis for sulfur gave one S atom per molecule. We also find that all peptide bonds are trans in this dimer.

Structure Determination. Single crystals of *c*(L-Pro-Gly)$_4$·RbSCN·3H$_2$O were grown from acetone solution by C. M. Deber. The initial crystal data are shown in Table I. A measured density of about 1.5 g/cm^3 was obtained from our very limited sample. Unit cell dimensions were determined from scattering angles measured on the Picker FACS-1 automatic diffractometer with the use of Cu Kα radiation. These dimensions and errors were determined by least-squares methods.

Two crystals of the sizes 0.50 × 0.38 × 0.17 mm and 0.30 × 0.35 × 0.12 mm were used to collect two sets of intensity data on the Picker FACS automatic diffractometer. Absorption corrections were made based on accurate measurements of the dimensions of crystal faces. The usual Lorentz and polarization corrections were then made. A list of 2200 unique reflections was obtained after minimizing[31]

$$R = \sum_{hi,hj} W_{hij}(\ln S_i I_{hi} - \ln S_j I_{hj})^2$$

where S_i is the scale factor for the ith set, I_{hi} is the intensity of I_h of set i, W_{hij} is $(\sigma_{hi}^2 + \sigma_{hj}^2)^{-1}$, and σ_{hi} is the statistical (counting) error in I_{hi}. The value of

$$R_c = \sum_h |I_{hi} - I_{hj}| / \sum_h |I_{hi} + I_{hj}|$$

was 0.061 over all observed data. The 1309 reflections for which $I \geq 2\sigma$ comprise only 30% of the theoretically observable diffraction maxima. Only these data were included in the least-squares refinement. This limitation of data owing to the somewhat poor quality of the crystals is probably the cause of the relatively high standard deviations of the final bond distances and angles.

The position of the Rb cation was found at (0.75, 0.42, 0.79) from an analysis of the sharpened Patterson function. However, vectors belonging to S atoms were not immediately lo-

Table I. Crystal Data

Compd	c(L-Pro-Gly)$_4$·RbSCN·3H$_2$O
Mol wt	814.3
Crystal system	Tetragonal
Unit cell	$a = b = 13.787 \pm 0.001$ Å
	$c = 38.974 \pm 0.007$ Å
Space group	$P4_12_12$
Density	$d_{calcd} = 1.456$ g/cm^3
$F(000)$	3392 electrons
μ(Cu Kα)	31.214 cm^{-1}

cated. Initial phases, computed from the rubidium cation, followed by successive refinements of three-dimensional electron density maps, lead to the locations of 44 atoms of the cyclic peptide, the SCN anion, and three water molecules. The S atom, found at (0.20, 0.93, 0.83), yields an Rb–S vector at (0.05, 0.01, 0.04) in Patterson space, very close to the origin. For this reason we did not recognize it in the Patterson function. Distinction between N and C was made on the basis of location of the proline rings.

At an early stage of the refinement, the difference electron density map appeared to show some disorder of the SCN$^-$ group. Later the positions of all the 40 hydrogen atoms of the cyclic peptide were computed by assuming a C–H bond distance of 1.05 Å and an N–H bond distance of 0.98 Å, and were compared with the difference electron density map. Seventeen positions coincided with expected peaks, and the others were in positive regions. These calculated hydrogen positions were included in the structure factor calculation, but not in the further refinement. Using weights $w = 1/|F_o|$ for $|F_o| \geq 15$ and $w = \sqrt{1/15|F_o|}$ for $|F_o| < 15$, we refined to $R = 0.092$. At this stage, the S—C≡N anion still had poor bond distances and an abnormal angle (S–C = 1.99 Å, C≡N = 0.83 Å, and ∠S—C≡N = 140°). Attempts to treat the SCN anion as disordered failed to improve the model, so results are presented for the ordered model. On the final difference electron density map, no electron densities were greater than 0.5 e/Å^3 near the Rb cation site and 0.4 e/Å^3 elsewhere.

Results and Discussion

Positional parameters are given in Table II for nonhydrogen atoms. Thermal parameters and approximate hydrogen coordinates are available in the supplementary material.

The conformation of the cyclic peptide molecule c(L-Pro-Gly)$_4$ is shown in Figure 1 as an ORTEP stereoscopic drawing which also gives the standard numbering system[32] of the atoms. The bond distance and angles of the cyclic peptide molecule are listed in Table III and compared with the usual peptide bond distances and angles as given by Corey and Pauling.[33] The average standard deviation of the bond distances and angles for the peptide backbone is 0.03 Å and 2°, respectively, and 0.04 Å and 3° for the remainder of the peptide molecule. There is nearly perfect planarity within experimental errors (dev ≤0.03 Å) in two of the peptide units. The proline rings are somewhat puckered, similar to the distortions found in other proline-containing cyclic peptides.[24,34,35] The conformational angles ω_i, ϕ_i, and ψ_i are listed in Table IV. Clearly, all peptide bonds are trans in agreement with the NMR study. The Pro C_3^α–C_3–O_3 bond ($\psi_3 = 8°$) is cis′, the Pro C_7^α–C_7O_7 bond ($\psi_7 = -132°$) is trans′, and the Pro C_5^α–C_5O_5 bond ($\psi_5 = -147°$) is essentially trans′. In spite of the cyclic nature of the peptide, no axial symmetry (C_4 or C_2) is maintained.

The rubidium cation nearly at the center of the cyclic peptide ring is coordinated to six oxygens: O_2, O_4, O_6, and O_8 of the glycyl carbonyl groups of the cyclic peptide, O_6 of another cyclic peptide related by a twofold crystallographic axis along the diagonal direction of xy plane, and an oxygen of a water molecule W_1. Through four coordination bonds, Rb–O_6, Rb–O_6′, Rb′–O_6, and Rb′–O_6′, two cyclic peptide molecules, related by the twofold symmetry noted above, are bridged together (Figure 2). Each rubidium cation has a distorted octahedral environment. This coordination geometry is described in Table V. The angle between planes $O_2O_4O_6O_8$ and $O_2'O_4'O_6'O_8'$ is 6°. The Rb–O bond distances vary from 2.78 to 3.02 Å, in fair agreement with the sum of the ionic radius of Rb$^+$ (1.48 Å) and the van der Waals radius of oxygen (1.40 Å). The bond angles also appear to be normal as compared with those in other rubidium complexes.[36-38] In particular the structure of the Rb salt of *N*-(purin-6-ylcarbamoyl)-L-threonine tetrahydrate has a six-coordinated Rb ion.[38] However, the interatomic distance between Rb$^+$ and O_1 is 3.23 Å, which is 0.35 Å longer than the added radii sum (2.88 Å); a weak seventh coordination bond of Rb$^+$–O_1 might exist. The

Table II. Final Positional Parameters of the Complex with Standard Deviations in Parentheses

Atom	x	y	z
N_1	1.015 (2)	0.252 (2)	0.8172 (6)
C_1^α	1.070 (2)	0.331 (2)	0.8042 (5)
C_1^β	1.164 (3)	0.322 (3)	0.8213 (7)
C_1^γ	1.164 (3)	0.222 (3)	0.8397 (8)
C_1^δ	1.065 (3)	0.198 (3)	0.8470 (8)
C_1	1.016 (3)	0.431 (3)	0.8099 (10)
O_1	0.950 (1)	0.433 (2)	0.8308 (4)
N_2	1.060 (2)	0.500 (2)	0.7922 (7)
C_2^α	1.033 (2)	0.601 (2)	0.7994 (10)
C_2	0.939 (2)	0.621 (3)	0.7785 (6)
O_2	0.886 (2)	0.569 (2)	0.7633 (4)
N_3	0.924 (2)	0.725 (2)	0.7831 (6)
C_3^α	0.851 (2)	0.767 (2)	0.7611 (9)
C_3^β	0.878 (3)	0.869 (3)	0.7603 (8)
C_3^γ	0.940 (3)	0.885 (2)	0.7899 (10)
C_3^δ	0.995 (3)	0.802 (3)	0.7973 (8)
C_3	0.743 (3)	0.753 (3)	0.7769 (6)
O_3	0.677 (2)	0.792 (2)	0.7635 (5)
N_4	0.741 (2)	0.716 (2)	0.8094 (6)
C_4^α	0.644 (2)	0.709 (3)	0.8259 (7)
C_4	0.584 (2)	0.630 (2)	0.8060 (8)
O_4	0.607 (1)	0.573 (2)	0.7845 (4)
N_5	0.492 (2)	0.628 (2)	0.8209 (5)
C_5^α	0.414 (2)	0.563 (2)	0.8059 (6)
C_5^β	0.321 (3)	0.595 (2)	0.8217 (10)
C_5^γ	0.359 (3)	0.637 (3)	0.8559 (8)
C_5^δ	0.450 (3)	0.683 (2)	0.8480 (7)
C_5	0.445 (2)	0.461 (2)	0.8155 (6)
O_5	0.474 (2)	0.437 (2)	0.8458 (5)
N_6	0.422 (2)	0.386 (2)	0.7924 (6)
C_6^α	0.418 (2)	0.287 (3)	0.8031 (8)
C_6	0.520 (2)	0.241 (3)	0.7963 (8)
O_6	0.587 (1)	0.280 (1)	0.7818 (4)
N_7	0.529 (2)	0.151 (2)	0.8080 (4)
C_7^α	0.607 (2)	0.090 (3)	0.7965 (8)
C_7^β	0.570 (3)	−0.013 (2)	0.8042 (8)
C_7^γ	0.503 (3)	−0.002 (3)	0.8325 (8)
C_7^δ	0.452 (2)	0.093 (2)	0.8236 (7)
C_7	0.699 (3)	0.111 (3)	0.8152 (10)
O_7	0.698 (2)	0.125 (3)	0.8467 (8)
N_8	0.783 (2)	0.124 (2)	0.8013 (6)
C_8^α	0.874 (4)	0.148 (4)	0.8160 (11)
C_8	0.928 (3)	0.224 (2)	0.8013 (9)
O_8	0.892 (2)	0.266 (2)	0.7771 (4)
Rb	0.7450 (2)	0.4210 (2)	0.7911 (1)
S	0.206 (1)	0.934 (2)	0.8256 (6)
C	0.278 (3)	0.861 (5)	0.7901 (18)
N	0.318 (5)	0.871 (6)	0.7749 (11)
W_1	0.676 (2)	0.461 (2)	0.8567 (7)
W_2	0.486 (2)	0.771 (2)	0.7482 (5)
W_3	0.854 (2)	0.599 (2)	0.8533 (4)

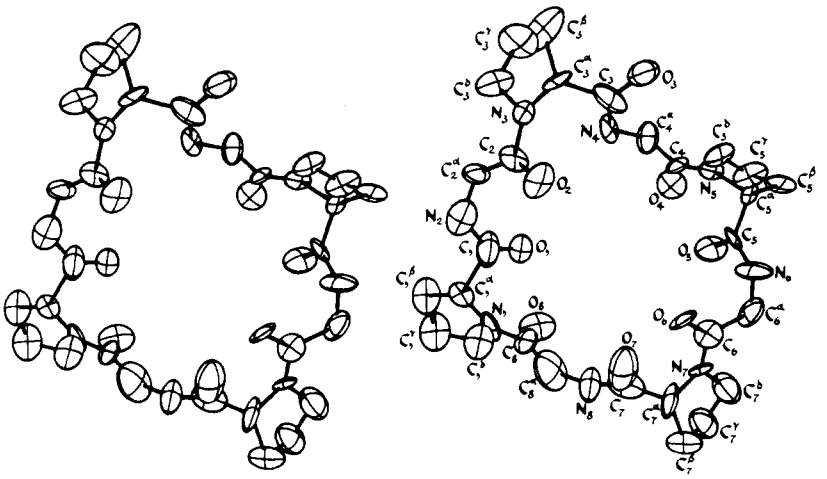

Figure 1. Stereoscopic showing of the c(L-Pro-Gly)$_4$ molecule and numbering scheme.

Table III. Bond Distances (Å) and Bond Angles (deg) of the c(L-Pro-Gly)$_4$ Molecule

i^b =	1	2	3	4	5	6	7	8	Standard[a]
$C_{i-1}N_i$	1.40	1.32	1.44	1.37	1.39	1.41	1.32	1.29	1.325
$N_iC_i^{\alpha}$	1.41	1.47	1.44	1.48	1.52	1.43	1.44	1.42	1.455
$C_{i-1}C_{i-1}^{\alpha}$	1.42	1.58	1.55	1.63	1.57	1.51	1.57	1.49	1.51
$C_{i-1}O_{i-1}$	1.22	1.22	1.18	1.17	1.19	1.28	1.21	1.24	1.24
$C_i^{\alpha}C_i^{\beta}$	1.47		1.46		1.48		1.54		
$C_i^{\beta}C_i^{\gamma}$	1.55		1.45		1.55		1.44		
$C_i^{\gamma}C_i^{\delta}$	1.43		1.40		1.44		1.54		
$N_iC_i^{\delta}$	1.54		1.55		1.43		1.46		
$C_{i-1}N_iC_i^{\alpha}$	121	118	115	116	120	122	120	131	122
$N_iC_i^{\alpha}C_i$	112	106	111	108	104	108	112	118	111
$C_{i-1}^{\alpha}C_{i-1}O_{i-1}$	118	117	132	119	132	123	126	120	120.5
$C_{i-1}^{\alpha}C_{i-1}N_i$	119	110	103	114	107	117	114	126	116
$O_{i-1}C_{i-1}N_i$	124	132	124	125	121	118	120	114	123.5
$N_iC_i^{\alpha}C_i^{\beta}$	104		103		106		103		
$C_iC_i^{\alpha}C_i^{\beta}$	115		111		115		112		
$C_i^{\alpha}C_i^{\beta}C_i^{\gamma}$	106		106		100		105		
$C_i^{\beta}C_i^{\gamma}C_i^{\delta}$	107		111		106		102		
$C_i^{\gamma}C_i^{\delta}N_i$	99		98		106		103		
$C_{i-1}N_iC_i^{\delta}$	125		129		132		126		
$C_i^{\alpha}N_iC_i^{\delta}$	114		112		108		111		

[a] See ref 33. [b] When $i = 1$, $i - 1 = 8$.

Table IV. Standard Conformation Parameters[a] (deg)[32]

	i							
	1	2	3	4	5	6	7	8
ϕ_i (C_{α}-N)	69	−83	84	68	72	−93	81	−131
ψ_i (C-C)	−168	−173	8	−178	−147	174	−132	175
ω_i (peptide)	−168	169	175	175	−158	164	178	−178

[a] The convention followed is that by the IUPAC-IUB Commission on Biochemical Nomenclature. Specifically, ϕ_i refers to the torsional angle of the sequence of atoms $C_{i-1}N_iC_i^{\alpha}C_i$, ψ_i to the sequence of $N_iC_i^{\alpha}C_iN_{i+1}$, and ω_i to the sequence of $C_i^{\alpha}C_iN_{i+1}C_{i+1}^{\alpha}$. The coordinates (Table II) are for c(D-Pro-Gly)$_4$.

interatomic distances between Rb^+ and O_3, O_5, O_7, W_2, or W_3 are all greater than 3.75 Å; it is not likely that they form any coordination bond. Within a dimer, the Rb^+–Rb^+ distance of 4.54 Å is larger than the Rb^+–Rb^+ distance of 3.96 Å reported for Rb–F crystal.[39] Therefore, no significant electrostatic repulsion is expected to exist. The standard deviations of bond distances and angles involving Rb are 0.02 Å and 1°, respectively. This mode of bonding is a new feature of the interactions between cations and cyclic peptides. In a preliminary study of the crystal structure of c(L-Pro-Gly)$_4$·CsSCN complex we have also found a similar dimer. Thus both for Rb^+ and Cs^+ the ratio of M^+/peptide is 2/2[40] in the crystalline state, so far not detectable from the NMR or CD results found in solution. The SCN^- groups are located in the cavities between the cyclic peptide–Rb^+ complexes. The water molecule W_1 specifically links the Rb^+ and thiocyanate ions.

There is no intrahydrogen bond within the cyclic peptide itself, but there are four hydrogen bonds connecting the two cyclic peptides of the dimer. All three water molecules participate in both intra- and intercomplex hydrogen bonding.

Table V. Coordination Geometry around Rubidium

A. Bond	Distance, Å	Bond	Distance, Å
$Rb-O_2$	3.02	$Rb-O_8$	2.99
$Rb-O_4$	2.84	$Rb-W_1$	2.78
$Rb-O_6$	2.95	$Rb-O_6'$	2.86
B. Angle	**Deg**	**Angle**	**Deg**
O_8-Rb-O_6	90	O_6-Rb-O_6'	76
O_8-Rb-O_4	164	O_4-Rb-O_2	84
O_8-Rb-O_2	89	O_4-Rb-W_1	73
O_8-Rb-W_1	123	O_4-Rb-O_6'	82
O_8-Rb-O_6'	83	O_2-Rb-W_1	115
O_6-Rb-O_4	89	O_2-Rb-O_6	75
O_6-Rb-O_2	152	W_1-Rb-O_6'	151
O_6-Rb-W_1	90		

Within a monomer, water molecule W_1 not only bonds to Rb^+, but also forms hydrogen bonds to O_5 and water molecule W_3, whereas water molecule W_2 forms hydrogen bonds to O_3 and N of SCN^- group. Finally, water molecule W_3 forms hydrogen bonds to O_1, N_4 and W_1. We note that the four glycyl carbonyl oxygens which are involved in coordination bonding are not hydrogen bonded, and that one prolyl carbonyl oxygen, O_7, which forms a trans′ Pro $C_7^{\alpha}-C_7-O_7$ bond, does not form a hydrogen bond either. However, between molecules in the dimer units, W_1 forms a hydrogen bond to the S atom transformed into coordinates of $(\frac{1}{2}-x, \frac{1}{2}-y, \frac{7}{4}-z)$, W_2 to N_2 of $(1-y, 2-x, \frac{3}{2}-z)$, S atom to W_1 of $(\frac{1}{2}+x, \frac{3}{2}-y, \frac{7}{4}-z)$, and N_2 atom to W_2 of $(2-y, 1-x, \frac{3}{2}-z)$. Through all these hydrogen bonds, molecules are linked together along the x, y, and z direction. It is also worth mentioning that O_3 of the cis′ Pro $C_3^{\alpha}-C_3-O_3$ bond is involved in forming the H-bond bridge, via water molecule W_2, to link molecules in different cells. We present these hydrogen bonds in Figure 2 and list their distances and angles in Table VI.

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Supplementary Material Available: the complete list of observed and calculated structure factors, the thermal parameters of nonhydrogen atoms, and the approximate hydrogen coordinates (13 pages). Ordering information is given on any current masthead page.

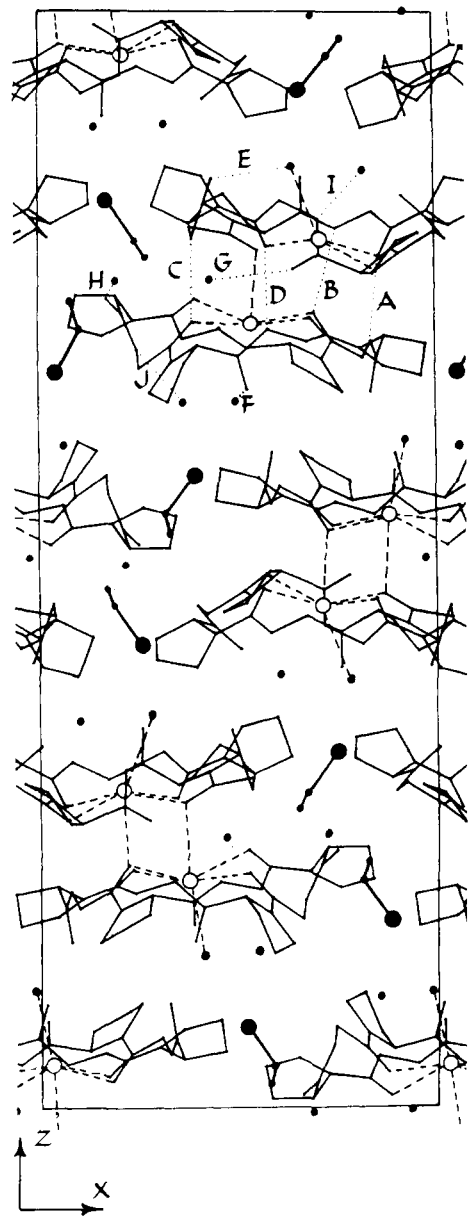

Figure 2. The packing of the c(L-Pro-Gly)$_4$·RbSCN·3H$_2$O complexes in the unit cell. Broken lines represent coordination bonds and dotted lines hydrogen bonds within one dimer. A = O_8 - - - N_8', B = N_8 - - - O_8', C = N_6 - - - O_4', D = O_4 - - - N_6', E = O_5 - - - W_1, F = O_5' - - - W_1', G = O_3 - - - W_2, H = O_3' - - - W_2', I = N_4 - - - W_3, and J = N_4' - - - W_3'.

Table VI. Hydrogen Bonds

	Distance, Å	Angle, deg	Symmetry transformation
	Intramonomeric		
O_1- - -W_3	2.79		
O_3- - -W_2	2.72		
O_5- - -W_1	2.83		
N_4H- - -W_3	2.82	153	
$N(SCN^-)$- - -W_2	2.88		
W_1- - -W_3	3.11		
	Intradimeric		$1-y, 1-x, \frac{3}{2}-z$
O_4- - -HN_6'	2.99	158	
O_8- - -HN_8'	3.12	156	
N_6H- - -O_4'	2.99	158	
N_8H- - -O_8'	3.12	156	
	Interdimeric		
W_1- - -S	3.00		$\frac{1}{2}+x, \frac{1}{2}-y, \frac{7}{4}-z$
W_2- - -HN_2	2.81	152	$1-y, 2-x, \frac{3}{2}-z$

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Absolute Configuration of (+)-Cyclophosphamide. A Crystal and Molecular Structure Determination by X-Ray Diffraction

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Abstract: The crystal and molecular structure of enantiomerically homogeneous cyclophosphamide ($C_7H_{15}N_2O_2PCl_2$) has been determined by x-ray diffraction with the absolute configuration being established by the anomalous dispersion of the Cl and P atoms. It is found that the dextrorotatory enantiomer of cyclophosphamide ($[\alpha]_D^{20}$ 2.3° (*c* 3.0, methanol)) has the *R* configuration at phosphorus. The compound crystallized in the rhombohedral space group *R*3 with the three molecules in the cell related by the threefold axis forming a trimeric unit by NH···O=P hydrogen bonding. Cell parameters are a = 10.520 (5) Å and α = 108.9 (1)°. The conformation of the enantiomerically homogeneous cyclophosphamide as compared to the racemate differs mainly in the orientation of one of the chloroethyl chains.

Cyclophosphamide (2-[bis(2-chloroethyl)amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide, **1**) is a widely used anticancer drug which is prepared synthetically and administered clinically in racemic form (Cytoxan).[2] The broad spectrum of activity[3] exhibited by **1** has led to considerable interest in its metabolism, and a substantial amount of chemical and biochemical data supports the degradative pathway shown in Scheme I. Fragmentation of enzymatically produced 4-hydroxycyclophosphamide (**2**) and/or its putative aldehyde tautomer, aldophosphamide (**3**), affords acrolein and phosphoramide mustard (**4**), generally regarded as the ultimate DNA cross-linking agent. Competing enzymatic conversion of **2** and/or **3** into 4-ketocyclophosphamide (**5**) and carboxyphosphamide (**6**) is associated with drug detoxification.[4] Since biological systems normally exhibit a marked enantiomeric selectivity, it was expected[5] and recently found[6] that the antipodal forms of **1** exhibit significantly different therapeutic indices[7] with (−)-**1** being more effective against PC6 mouse