A Structural Two-Ring Version of a Tubular Stack of β-Rings in Crystals of a Cyclic D,L-Hexapeptide

MICHELE SAVIANO, LAURA ZACCARO, ANGELA LOMBARDI,
CARLO PEDONE and BENEDETTO DI BLASIO*
Dipartimento di Chimica, Università di Napoli Federico II, Via Mezzocannone 4, 80134 Napoli, Italy

and

XICHENG SUN and GIAN P. LORENZI*
Institut für Polymere, ETH-Zentrum, 8092 Zürich, Switzerland

Abstract. An X-ray analysis of single crystals (from MeOH) of cyclo(-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) has been carried out. The analysis reveals that the molecules of the cyclopeptide occur in the crystals with two slightly different, almost hexagonal backbone conformations of the β-type, and that pairs of molecules with the same conformation interact through their nonmethylated face, forming dimeric units (units A and B) with six interannular H-bonds. This kind of pairing reproduces well that expected for a two-ring element in a stack of antiparallel β-rings. The X-ray analysis has also revealed the presence in the A units of two water molecules, each at one of two equivalent sites located on the 3-fold axis of the units and equidistant from the center of gravity, and the presence in the B units of one water molecule at the center of the units. This provides experimental support for the idea that stacks of β-rings can serve as molecular channels.

Key words: D,L-hexapeptide, cyclic; crystal structure; stacks, tubular; β-rings; channels.

1. Introduction

Assembling macrocycles into tubular structures is a promising approach for such ambitious objectives as the development of artificial channels with selective permeabilities, the attainment of one-dimensional arrays of ions or metal atoms, and the creation of new porous molecular solids [1–3]. The macrocycles can be connected by covalent bonds, for instance by anchoring them onto a rigid polymer support [4,5]. Macrocycles with some potential for self-association through noncovalent interactions are more convenient, however. An interesting class of macrocycles with this potential is that of cyclic oligopeptides formed by an even number (n) of residues in an alternating D- and L-sequence. These oligo-D,L-peptides, as pointed out by De Santis *et al.* [6], should tend to adopt a β-type conformation (macrocycles in this conformation will henceforth be referred to as β-rings) and self-aggregate through n interannular H-bonds to yield hollow, axial stacks. Previous work [7] on cyclic oligo-D,L-peptides derived from valine, leucine and phenylalanine has shown that these peptides do indeed form very stable aggregates. However, it has

---

* Authors for correspondence.

not yet been demonstrated that these aggregates have the expected tubular structures. With the aim of modeling these structures with a simpler system we have synthesized cyclo (-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) (**1**), a cyclic oligo-D,L-peptide which can dimerize but cannot form multimolecular stacks because of the N-methyl groups. In this communication we present crystallographic results showing that **1** in the crystal state forms two-β-ring units with six interannular H-bonds of exactly the same kind as foreseen for a tubular stack of antiparallel β-rings.

2. Experimental

2.1. SYNTHESIS

1 [mp 288–9°C; ^{1}H NMR (25°C, CDCl$_3$, 2.8–20.2 mg/mL): δ 7.37 (d, 3H, amide), 4.90 (t, 3H, (L)C$^\alpha$H), 4.85 (m, 3H, (D)C$^\alpha$H), 3.01 (s, 9H, NMe), 1.70 (m, 3H, (L)C$^\gamma$H), 1.61 (m, 6H, (L)CH$_2$), 1.48 (m, 6H, (D)CH$_2$), 1.39 (m, 3H, (D)C$^\gamma$H), 1.00 and 0.91 (2d, 2 × 9H, (L)CH$_3$), 0.95 and 0.89 (2d, 2 × 9H, (D)CH$_3$)] was obtained by cyclization of the linear precursor by using diphenyl phosphoryl azide [8] as condensing agent. The details of the synthesis will be reported elsewhere [9]. The single crystals used for the X-ray analysis were obtained from a solution in methanol (Fluka Chemie AG, puriss. p.a.) by slow evaporation of the solvent in air.

2.2. X-RAY ANALYSIS

The X-ray experiments were carried out with crystals at room temperature (22°C) on a CAD4 Enraf/Nonius Turbo diffractometer equipped with a MicroVax 3100 Server using graphite-monochromated radiation and pulse height discrimination. Oscillation and Weissenberg photographs were taken for a preliminary determination of the crystal symmetry and space group. Lattice parameters, obtained by a least-squares procedure from the setting angles for 25 reflections lying in the Θ range of 21–29°, are listed along with other pertinent crystal and intensity measurement data in Table I. Intensity data were collected using variable-speed (2.5 to 6.5°/min) $\omega - 2\Theta$ scans, with a scan angle $\Delta\omega = (1.1 + 0.15 \tan \Theta)°$. Background counts were taken in an additional area of $\Delta\omega/4$, on both sides of the main scan, using the same scan speed. Prescan runs were made at a speed of 5°/min. The intensities of three standard reflections were monitored at the beginning, every 2h of exposure time and at the end. Although the maximum deviation from the average reached 4%, the intensities of these reflections showed no systematic changes. Data were corrected for Lorentz and polarization effects. A Θ-dependent correction for absorption was applied using the method of Walker and Stuart [10]. The correction factors from the DIFABS program were 0.85(min), 1.25(max) and 0.995 (ave). In a range of 1 to 70° of Θ 3045 reflections were collected; 1909 of which having a

TABLE I. Crystallographic data for **1**

Molecular formula	$C_{39}H_{72}N_6O_6 \cdot 3/4H_2O$
Molecular weight	734.56
Crystal shape	Prism (from MeOH)
Crystal system	Trigonal
Space group	$P312$
Z (molecules/unit cell)	4
a (Å)	15.864(3)[a]
c (Å)	20.694(3)[a]
V (Å^3)	4510.1
D_{calc} (g/cm^3)	1.082
Measured reflections	3045
Observed reflections (with $I > 3\sigma(I)$)	1909
μ (mm^{-1})	0.540
R factor	0.085
Weighting scheme used	unit weights
No. of parameters refined	328
S	2.056
Max. and min. height in final ΔF synthesis (e Å^{-3})	0.4; –0.2
Temperature (K)	292
Radiation (Å)	1.5418 (CuK$_\alpha$)

[a] The figure in parentheses is the standard deviation in the least significant digit.

net intensity greater than $3.0\sigma(I)$ were considered observed and used for further calculations.

The structure was solved by straightforward application of the direct methods as programmed in SIR92 [11]: all atoms in the two independent dipeptide units were located in the E-map. Subsequent difference Fourier maps revealed the presence in the cell of three oxygen atoms located on a 3-fold axis that were refined with the occupancy factors set to 0.167. Furthermore, the C^δ carbon atoms of the side chain of the L-MeLeu residue of one independent unit were found to be distributed in two different positions and these atoms were introduced in the refinement calculations with half occupancy. All nonhydrogen atoms with anisotropic thermal parameters were refined by full-matrix least-squares procedures, minimizing the function $\sum w(F_o - F_c)^2$. Weights, w, were assigned to the data as $w = 1/\sigma^2(F_o)$, $\sigma(F_o)$ being based on counting statistics. The hydrogen atoms were introduced in their stereochemically expected positions with an isotropic temperature factor equal to the B_{eq} of the heavy atom to which they are linked. Their parameters were kept fixed. Refinements converged to a final R factor of 0.085 and R_w of 0.075 using anisotropic temperature factors for the nonhydrogen atoms. Refinement was ended when the shifts in the atomic coordinates and temperature factors were less than

1/5 and 1/3 of the corresponding standard deviations, respectively. All refinements were carried out using the SDP package [12]. Atomic scattering factors for all atomic species were calculated from Cromer and Waber [13]. Table II reports the final atomic parameters for all nonhydrogen atoms; the numbering of the atoms follows the recommendations of the IUPAC-IUB Commission.

3. Results and Discussion

1 crystallizes in the trigonal space group $P312$ with a total content of four cyclopeptide and three water molecules per unit cell (Table I). The cyclopeptide molecules, each consisting of three conformationally identical dipeptide units, occur in two different conformations (molecules A and molecules B). The molecules A are disposed with their dipeptide units around a 3-fold axis parallel to the c axis located at $x = -0.333$ and $y = 0.333$; the molecules B around a similar axis at $x = 0.333$ and $y = 0.667$. Thus, the cyclopeptide molecules retain a 3-fold axis as a crystallographic element of symmetry and the asymmetric unit in the cell is constituted by two dipeptide units. Bond lengths and bond angles are in good agreement with literature data; their estimated standard deviations are on the average smaller than 0.02 Å and 1.5°. Molecular models of the molecules A and B are shown in Figure 1. The molecules differ not only in the conformation of the backbone but also in that of the side chains: while molecules A exhibit values of the torsion angle χ^1 ($N-C^\alpha-C^\beta-C^\gamma$) for the D-Leu and L-MeLeu residues close to those of ideal *gauche* conformations (60° for g^+ and $-60°$ for g^-), molecules B show values corresponding to *trans* conformations for both residues. The relevant torsion angles for the two kinds of molecules are reported in Table III. As found in cyclo(-D-Val-L-Val-D-Val-L-Val-D-Val-L-Val-) and cyclo(-D-Phe-L-Phe-D-Phe-L-Phe-D-Phe-L-Phe-) [14], the backbone dihedral angles of **1** fall in the β-regions of the Ramachandran plot (for L-residues) and of the corresponding map for D-residues. Thus, as the molecular models of Figure 1 show, the molecules have an almost hexagonal backbone geometry with *trans*-peptide bonds almost perpendicular to the plane of the hexagon, and the $C^\alpha-C^\beta$ bonds almost parallel to it. The distances between C^α atoms across the ring are in the range of 7.57–7.63 Å, those between peptide planes facing each other about 6.50 Å. The β-conformation of the residues also causes the N-methyl and C=O groups of the L-residues to be all located on one side and the NH and C=O groups of the D-residues on the other side of the ring. Molecules of the same conformation, symmetry-related by a 2-fold axis perpendicular to the 3-fold axis, are connected in two dimeric units (units A and B) through their nonmethylated face by six hydrogen bonds. This type of pairing of rings corresponds exactly to the one that would characterize a stack of antiparallel β-rings of a cyclo-D,L-hexapeptide [6]. Data regarding length and linearity of the interannular H-bonds in the dimeric units are reported in Table IV.

The X-ray analysis has revealed the presence of cocrystallized water molecules with the oxygen atoms located on the 3-fold symmetry axes of the dimeric units

TABLE II. Positional parameters for a dipeptide unit of the molecules A and B[a,b]

Atom	x	y	z	$B(\text{Å}^2)$
Molecule A				
N_1	−0.3077(4)	0.1305(4)	0.6428(3)	3.8(2)
C_1^{Me}	−0.3254(8)	0.1041(7)	0.7124(4)	7.0(3)
C_1^{α}	−0.2094(6)	0.1708(6)	0.6157(4)	4.3(2)
C_1^{β}	−0.1667(6)	0.1025(6)	0.6298(4)	5.2(2)
C_1^{γ}	−0.2270(6)	0.0010(6)	0.6087(5)	6.2(3)
$C_1^{\delta 1}$	−0.1750(8)	−0.0541(9)	0.6263(7)	9.4(4)
$C_1^{\delta 2}$	−0.2446(8)	−0.0053(9)	0.5353(5)	9.3(4)
$C_1^{\prime}$	−0.1444(6)	0.2733(6)	0.6431(4)	4.7(2)
O_1	−0.1087(5)	0.2843(6)	0.6978(3)	8.2(3)
N_2	−0.1261(4)	0.3466(5)	0.6061(3)	4.4(2)
C_2^{α}	−0.0740(6)	0.4460(7)	0.6279(4)	4.8(2)
C_2^{β}	0.0330(7)	0.4983(7)	0.6009(5)	6.5(3)
C_2^{γ}	0.0978(8)	0.4584(7)	0.6258(6)	7.7(3)
$C_2^{\delta 1}$	0.189(1)	0.4991(9)	0.5828(8)	12.7(5)
$C_2^{\delta 2}$	0.124(1)	0.482(1)	0.6952(8)	14.1(6)
$C_2^{\prime}$	−0.1221(6)	0.5005(6)	0.6047(4)	5.6(2)
O_2	−0.1454(5)	0.4945(6)	0.5443(3)	10.2(2)
$Ow1^c$	−0.333	0.333	0.470(1)	12.0(9)
$Ow2^c$	−0.333	0.333	0.315(2)	12.3(9)
Molecule B				
N_1	0.1613(4)	0.4370(5)	0.8595(3)	4.3(2)
C_1^{Me}	0.1484(7)	0.4439(7)	0.7892(4)	7.3(3)
C_1^{α}	0.2167(5)	0.3911(5)	0.8828(4)	4.1(2)
C_1^{β}	0.1670(7)	0.2860(7)	0.8628(5)	5.2(3)
C_1^{γ}	0.2163(7)	0.2293(7)	0.8815(6)	7.0(3)
$C_1^{\delta 1}$	0.227(1)	0.2248(9)	0.9414(8)	13.2(6)
$C_1^{\delta 2}$	0.164(1)	0.1298(8)	0.849(1)	16.7(6)
$C_1^{\prime}$	0.3226(6)	0.4481(6)	0.8553(4)	4.4(2)
O_1	0.3393(5)	0.4470(5)	0.7993(3)	7.3(2)
N_2	0.3925(5)	0.4969(5)	0.8991(3)	4.1(2)
C_2^{α}	0.4946(6)	0.5506(6)	0.8807(4)	4.4(2)
C_2^{β}	0.5496(6)	0.5033(6)	0.9108(6)	7.1(3)
C_2^{γ}	0.654(1)	0.547(1)	0.9045(8)	16.3(7)
$C_2^{\delta 1 d}$	0.709(1)	0.512(2)	0.946(1)	11.3(7)
$C_2^{\delta 1 e}$	0.718(1)	0.627(2)	0.876(2)	13.3(9)
$C_2^{\delta 2 d}$	0.656(2)	0.535(2)	0.846(1)	7.9(7)
$C_2^{\delta 2 e}$	0.672(2)	0.475(2)	0.869(2)	16.2(9)
$C_2^{\prime}$	0.5360(5)	0.6537(6)	0.9039(3)	3.8(2)
O_2	0.5419(5)	0.6694(4)	0.9610(3)	5.8(2)
$Ow3^c$	0.333	0.667	1.0	12.4(6)

[a] Anisotropically refined atoms are given in the form of the isotropic équivalent displacement parameter defined as:

$$(4/3) * [a^2 * B(1,1) + b^2 * B(2,2) + c^2 * B(3,3) + ab(\cos \gamma) * B(1,2) + ac(\cos \beta) * B(1,3) + bc(\cos \alpha) * B(2,3)].$$

[b] The figures in parentheses are the standard deviations in the least significant digits.

[c] Occupancy factor 0.167.

[d] and [e] Occupancy factor 0.5.

Molecule A

Molecule B

Fig. 1.   Molecular models of the molecules A and B of **1** with the atomic numbering scheme. The molecule B represented is that with $\chi^{2,1} = -166.6°$ and $\chi^{2,2} = -66.9°$ (Table III).

TABLE III. Relevant torsion angles ($^\circ$) for molecules A and B

Torsion angle		Molecule A		Molecule B	
		D-Leu	L-MeLeu	D-Leu	L-MeLeu
$C'_{i-1}-N_i-C_i^\alpha-C_i'$	(φ)	136.3	−108.4	125.2	−125.2
$N_i-C_i^\alpha-C_i'-N_{i+1}$	(ψ)	−138.8	102.2	−112.8	112.4
$C_i^\alpha-C_i'-N_{i+1}-C_{i+1}^\alpha$	(ω)	−174.7	174.1	−176.8	178.0
$N_i-C^\alpha-C_i^\beta-C_i^\gamma$	(χ^1)	65.0	−54.5	−175.0	178.6
$C_i^\alpha-C_i^\beta-C_i^\gamma-C_i^{\delta_1}$	$(\chi^{2,1})$	−166.0	−179.8	−166.6[a]	60.2
				0.0[b]	
$C_i^\alpha-C_i^\beta-C_i^\gamma-C_i^{\delta_2}$	$(\chi^{2,2})$	70.2	−59.8	−66.9[a]	171.7
				−118.4[b]	

[a] and [b] This side-chain conformation is exhibited by 50% of the D-Leu residues of molecules B.

TABLE IV. Intermolecular H-bonds in crystalline 1

Molecule	Ring/Ring (NH...O=C)		Ring/Water (OH...O=C)	
	Distance (Å)	Angle ($^\circ$)	Distance (Å)	Angle ($^\circ$)
	(N...O)	(N...O=C)	(O...O)	(O...O=C)
A	3.15	161	2.81	108
			3.19	130
B	3.02	153		

A (Ow1 and Ow2) and of the dimeric units B (Ow3). Ow1 occupies one of two equivalent sites at a distance of 0.6 Å from the center of gravity of the units A and Ow2 occupies one of two equivalent sites at a distance of 3.81 Å from the same center. Ow1 is H-bonded to the C=O groups involved in the interannular H-bonding, its distance from the oxygens of the two rings being 2.81 Å and 3.19 Å respectively (Table IV), but Ow2 is too far away to form H-bonds with these groups. Ow3 is at the center of gravity of the dimeric units B, namely at the intersection of the 2-fold and 3-fold symmetry axes. Despite its location, however, there are no significant H-bonding interactions with the C=O groups for Ow3, since the orientation of these groups in the dimeric units B – they are almost perpendicular to the plane of the rings – determines too large an Ow3–O=C distance (3.38 Å). Stereoviews of a dimeric unit A with the water molecules in one of the two possible relative placements and of a dimeric unit B with the water molecule are shown in Figure 2. The molecular packing, as seen down the c axis, is presented in Figure 3.

It is important to note that the number and position of the cocrystallized water molecules can be different. Specifically, an X-ray diffraction analysis performed at a low measurement temperature on the same crystal to which the above results apply has not given any evidence of the presence of water in dimer B, and an analysis performed at room temperature on a crystal obtained from MeOH in a different
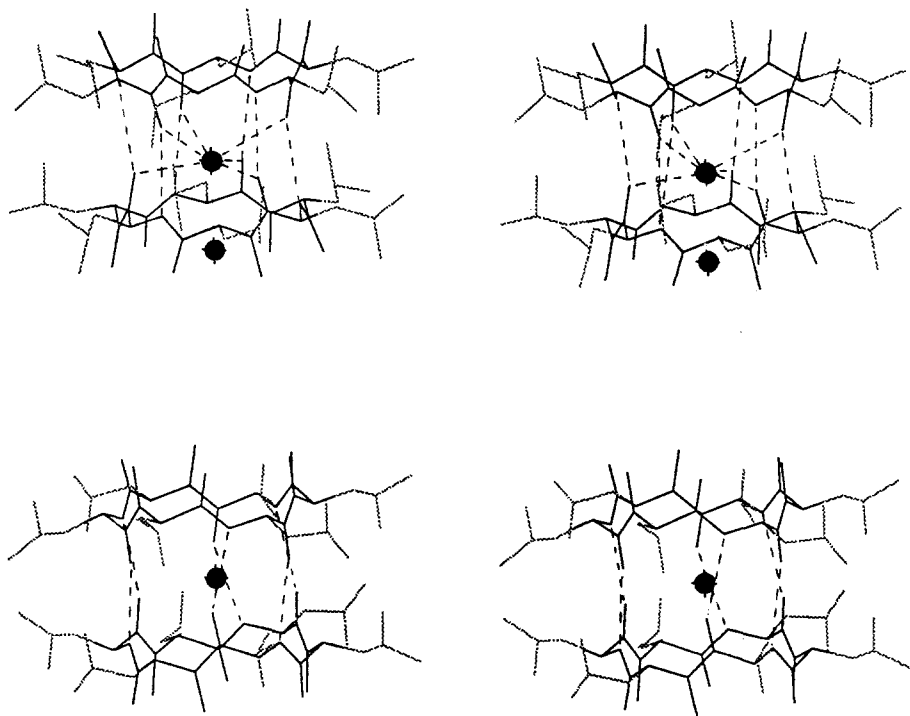
Fig. 2.   Stereoviews of a dimeric unit A (top) and B (bottom) with the respective molecules of cocrystallized water (filled circles). Dashed lines represent H-bonds.

crystallization batch has revealed the presence of a single water molecule at the center of gravity of each dimer. While further studies are needed in order to better understand this particular behavior, these results provide evidence for different modes of interaction in the cell between peptide rings and water molecules.

4. Conclusions

By showing the occurrence in crystals of **1** of units formed by two β-rings H-bonded face-to-face to one another, the results obtained confirm the validity of the theoretical predictions concerning the conformational tendencies of D,L-alternating cyclooligopeptides [6]. Furthermore, by showing that the dimeric units occurring in the crystals include molecules of water they support the idea that, under appropriate conditions, stacks of β-rings can act as channels for water and other small molecules or ions. With regard to this aspect, the observation that in the dimeric units A the water molecules can occupy different equivalent and nonequivalent sites is interesting since it is probably by moving through successive sites with varied interaction characteristics that species would migrate through such channels.
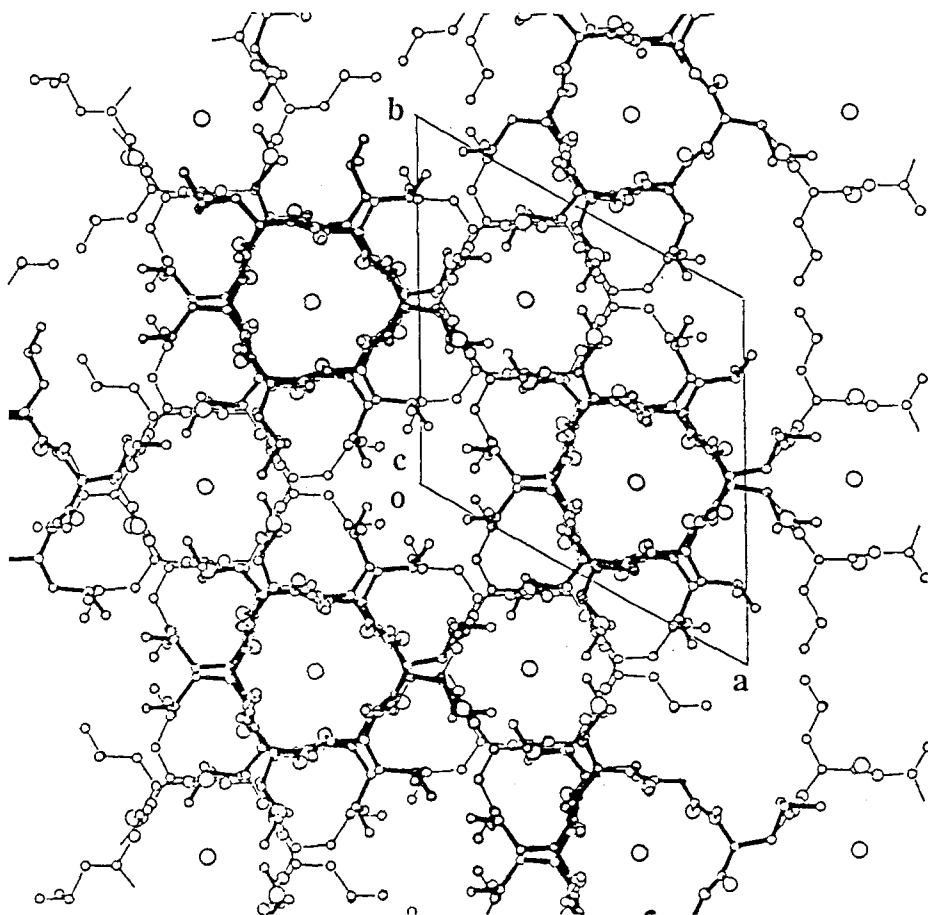
Fig. 3.   Molecular packing of **1** as seen along the *c* axis.

Acknowledgement

References

1. J.-M. Lehn, J. Malthête, and A.-M. Levelut: *J. Chem. Soc., Chem. Commun.* 1794 (1985).
2. J. Malthête, A.-M. Levelut, and J.-M. Lehn: *J. Chem. Soc., Chem. Commun.* 1434 (1992).
3. J. Zhang and J.S. Moore: *Polymer Preprints* **34** (1), 132 (1993).
4. M.F.M. Roks and R.J.M. Nolte: *Macromolecules* **25**, 5398 (1992).
5. N. Voyer: *J. Am. Chem. Soc.* **113**, 1818 (1991).
6. P. De Santis, S. Morosetti, and R. Rizzo: *Macromolecules* **7**, 52 (1974).
7. L. Tomasic and G.P. Lorenzi: *Helv. Chim. Acta* **70**, 1012 (1987).
8. T. Shioiri, K. Ninomiya, and S. Yamada: *J. Am. Chem. Soc.* **94**, 6203 (1972).

9. X. Sun and G.P. Lorenzi: *Helv. Chim. Acta.* **77** (1994) (in press).
10. N. Walker and D. Stuart: *Acta Crystallogr.* **A39**, 158 (1983).
11. A. Altomare, M.C. Burla, M. Camalli, G. Cascarano, G. Giacovazzo, C. Gugliardi, and G. Polidori: *J. Appl. Crystallogr.* **27** (1994) (in press).
12. Enraf Nonius: *Structure Determination Package*, Enraf Nonius, Delft, The Netherlands (1979).
13. D.T. Cromer and J.T. Waber: *International Tables for X-ray Crystallography*, Vol. 4, Table 2.2 B, p. 99, The Kynoch Press Birmingham, England (1984). Distr. by Kluwer Academic Publishers, Dordrecht, The Netherlands.
14. V. Pavone, E. Benedetti, B. Di Blasio, A. Lombardi, C. Pedone, L. Tomasic, and G.P. Lorenzi: *Biopolymers* **28**, 215 (1989).