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Crystal and Solution Forms of a Cyclic Heptapeptide, Pseudostellarin D[1)]

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Abstract : Crystal and solution forms of a cyclic heptapeptide, pseudostellarin D, cyclo(Gly-Tyr-Gly-Pro-Leu-Ile-Leu), were examined by spectroscopic evidences. A single crystal X-ray analysis showed that a revised structure, pseudostellarin D possesses a type II β-turn between Leu^7 and Gly^1, and a type I β-turn between Pro^4 and Leu^5. One transannular 4→1 hydrogen bond between Ile^6-NH and Gly^3-CO and two bifurcated hydrogen bonds between Tyr^2-NH and Ile^6-CO, and Gly^3-NH and Ile^6-CO were observed, forming a classical β-bulge unit in the crystal. The dominant solution conformation analyzed by high field NMR employing vicinal coupling constant, temperature dependence of NH protons, and ROESY spectrum was, on the whole, homologous to that observed in the solid state.

Introduction

Cyclic peptides are fairly and widely distributed natural products exhibiting a wide variety of biological functions[2] and have often been used as models for the studies of structural features of proteins.[3] In addition, cyclic peptide molecules are quite flexible, and assume a number of different stable conformations. The conformation can be affected by the polarity of the solvent, intra- and intermolecular hydrogen bonds, presence of complexing ions, nature of the side chains, and in crystalline state occasionally by packing forces. Since the chemical and biological activities of cyclic peptides are intimately related to the conformation of the peptide, it is essential to establish the factors which determine the dominating conformation. Conformational studies on cyclic peptides have been made mostly on synthetic and naturally occurring cyclic pentapeptides[4] and hexapeptides[5]. Octa and larger cyclic peptides have also been studied.[6] However, there have been no systematic examinations of cyclic heptapeptides reported.

A tyrosinase inhibitory cyclic heptapeptide, pseudostellarin D, has been isolated from the roots of *Pseudostellaria heterophylla*,[7] which is a well known Chinese traditional medicine used as a lung and spleen tonic.[8] The sequence of pseudostellarin D analyzed by incorrect HMBC correlations due to the very close chemical shifts of two amide protons at Gly and Tyr residues (δ 8.14 and 8.15, respectively) should be revised by X-ray analysis in this paper.

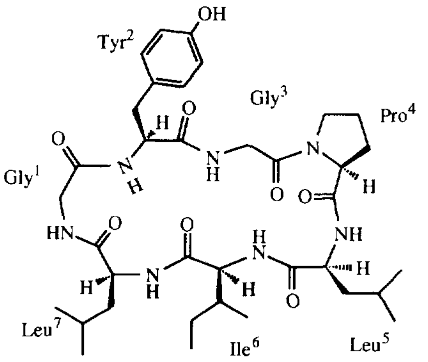

Figure 1. Structure of pseudostellarin D; One of the Gly residues was provisionally numbered as a first amino acid.

So far, only several naturally occurring cyclic heptapeptides have been reported, such as ilamycin B_1,[9] a dolastatin 3 analogue,[10] cycloheptasarcosine,[11] rhizonin A,[12] and evolidine[13] which all being characterized by X-ray analyses, hymenamide[14] and phakellistatin.[15] It appeared that the hydrogen bondings play important roles in determining the conformation of cyclic peptides of moderate size to which X-ray analysis is applicable. The crystal structure revealed by X-ray analysis demonstrates one possible conformation, whereas in solution, cyclic peptides often take multiple conformations and informations on the conformation are an average conformation.

In the present paper, in order to identify the specific functional groups which determine the overall conformation of the cyclic heptapeptide, pseudostellarin D, and to elucidate the relationships between the sequence and the three-dimensional structure of pseudostellarin D, conformational analysis using X-ray and high field NMR was undertaken.

Solid state conformation

A single crystal of pseudostellarin D, colourless needles, mp. 177 - 179 °C, was grown from CH_3CN-MeOH-H_2O solution and analyzed by X-ray diffraction technique. The lattice constants and intensity data collection were obtained on a Rigaku AFC7R automated diffractometer. Because the crystals deteriorated rapidly upon drying, they were sealed in a thin-walled glass capillary containing the mother liquor. The structure was solved by direct method and refined by least-squares methods to the final R factors of 0.064. The crystal displayed the symmetry of the orthorhombic space group $P2_12_12_1$ with 4 formula units in the unit cell. Unit cell dimensions were a=15.798 Å, b=28.963 Å, and c=9.605 Å, and there were one CH_3CN and one H_2O molecules in one asymmetric unit.

Crystallographic data are given in Table 1. Figure 2 shows a perspective view of the backbone of pseudostellarin D which is a cyclic heptapeptide consisting solely of L-amino acid residues. The present results showed that the proposed structure of pseudostellarin D[7] should be revised to cyclo(Gly-Tyr-Gly-Pro-Leu-Ile-Leu). The peptide bonds generally assume the common planar trans conformation. Table 4 shows the backbone dihedral angles in this compound. In Figure 3, the ϕ and ψ-angles along the skeleton ring of pseudostellarin D are summarized in the Ramachandran plots.

The (ϕ, ψ)-values of all residues lie within the allowed region of the Ramachandran plot, except for Ile^6 residue. The (ϕ, ψ)-value of Ile^6, whose NH and CO are involved in intramolecular hydrogen bonds, shows that Ile^6 lies almost outside the permissible region for the usual amino acid,[16] whereas the other residues fall generally into the β-sheet region in the diagram. One of the carbon atoms (C64) in Ile^6 was disordered and distributed to the two positions with occupancy factor of 0.478 : 0.522 (C64 : C64'). The distortions in ω angles over 10 degrees were not observed. In addition, the torsion angles (θ, χ^1, χ^2, χ^3 and χ^4) around the proline ring indicate that the prolyl conformation at residue 4 is of C$_2$-C$^\gamma$-endo form.[17]

The averaged backbone bond distances (N_i-C_i^α 1.458, C_i^α-C_i 1.510, C_i-O_i 1.241, and C_i-N_{i+1} 1.329) and angles (C_{i-1}-N_i-C_i^α 121.8°, N_i-C_i^α-C_i, 112.2°, C_i^α-C_i-N_{i+1}, 117.6°, C_i^α-C_i-O_i 119.7°, and O_i-C_i-N_{i+1} 122.6°) are within the permissible ranges for peptides[18] and suggest that the crystal of this conformation is not under unusual strain.

Cyclic peptide backbone rings are constrained with turns and these turns are often stabilized by intramolecular hydrogen bonds, which have been implicated in the bioactivity of several naturally occurring peptides. Such structures may provide good models for the studies of various possible types of turns containing intramolecular hydrogen bonds. The crystal structure of pseudostellarin D contains three intramolecular NH···O hydrogen bonds between Ile^6-NH and Gly^3-CO, between Tyr^2-NH and Ile^6-CO, and between Gly^3-NH and Ile^6-CO [HN6---O3 of 2.33 Å, HN2---O6 of 2.50 Å, and HN3---O6 of 2.02 Å as

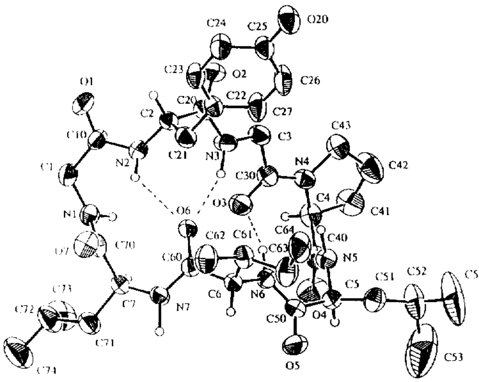

Fig. 2. A ORTEP perspective view of the crystal structure of pseudostellarin D; Only amide and α hydrogens (small circles) are included and dashed lines indicate intramolecular hydrogen bonds. The disordered atom (C64') was omitted.

Table 1. Crystal data of pseudostellarin D

Empirical Formula	$C_{38}H_{60}N_8O_9$ (772.94)
Color, Habit	colorless, prismatic
Dimensions	0.50 × 0.20 × 0.15 mm
System	orthorhombic
Lattice Type	Primitive
Dcalc	1.168 g/cm^3
Lattice Parameters (Å)	a=15.798(6) b=28.963(6) c=9.605(5) V=4395(3)Å^3
Space Group	$P2_12_12_1$
Z value	4
Final R value (R_w)	0.064 (0.082)

Table 2. Intramolecular hydrogen bonds of pseudostellarin D.

From	To	Distance (Å)		Angle (°)
		O...N	O...HN	O...HN
N6	O3	3.40 (0.01)	2.33	162.3
N2	O6	2.93 (0.01)	2.50	102.5
N3	O6	3.03 (0.01)	2.02	149.5

shown in Table 2]. Of them, the amide hydrogens of Ile^6 and Tyr^2 were considered to be involved in weaker hydrogen bonds than the other, because of the distance (3.40Å) of N6...O3 and the angle (102.5°) of O6...H-N2, respectively. These 3 hydrogen bonds constitute an antiparallel β-sheet structure. As a consequence, in the molecule, two β-turns are formed by the residues 7 → 1 and 4 → 5. The Leu^7 → Gly^1 turn is type II β-turn formed by the intramolecular hydrogen bond between Tyr^2-NH and Ile^6-CO. The other turn, Pro^4 → Leu^5 serve a type I β-turn.

The backbone conformation of pseudostellarin D contains a β bulge unit, which is defined as a region formed by two consecutive β-type hydrogen bonds, including two residues on one strand opposite a single residue on the other strand. The β-bulges are extremely rare in parallel β structure. Bulges are not unusual in protein structures, such as PEC-60,[19] trypsin,[20] and immunoglobulin,[21] and are considered to characterize the secondary structure of proteins. A classical β-bulge as originally defined by Richardson,[22] displays a pair of convergent hydrogen bonds from N-H's of adjacent residues on one strand to C=O of the residue opposite. Side chains of all three residues are on the same side of the β-sheet. These features results in a bending of the β-sheet and may affect its directionality. The classical β-bulge is formed by an approximate α-helical conformation (on average ϕ=-100°, ψ=-45°) in residue 1 and a normal β conformations (on average ϕ=-140°, ψ=160°) in residue 2. Cyclic heptapeptides, ilamycin B1 and evolidine, which possess the same structural motif with one cis proline amide bond, are known to have the classical β-bulge unit. By using of computational simulation,[13] Eggleston et al. demonstrated the possibility of β-bulge unit in cyclic heptapeptides without a cis peptide bond. However, this is the first paper which unequivocally demonstrated the presence of a β-bulge unit in a cyclic heptapeptide with all trans amide conformation by X-ray analysis.

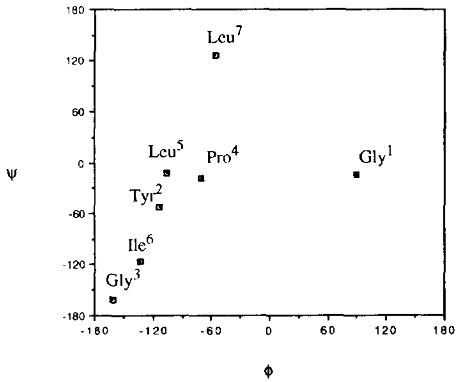

Figure 3. Ramachandran plot of the crystal of pseudostellarin D

Solution state conformation

Only one set of signals were observed in [^{2}H$_6$]DMSO. Conformation of pseudostellarin D in [^{2}H$_6$]DMSO was analyzed by comparing such NMR data as vicinal coupling constant, temperature dependence of NH protons,[23] and ROESY spectrum,[24] with those in the crystal structure.

The temperature dependence of the chemical shifts of the amide protons in [^{2}H$_6$]DMSO was recorded in ten intervals over the range 300 - 330 K, the result being shown in Table 3. Since the hydrogens, exposed to the solvent, exhibited larger temperature coefficient (>4 ppb/K) than intramolecularly hydrogen-bonded hydrogens (<3 ppb/K),[23] the temperature coefficient indicated the presence of three intramolecular hydrogen bonds between Ile^6-NH and Gly^3-CO, between Tyr^2-NH and Ile^6-CO, and Gly^3-NH and Ile^6-CO, as in the case of crystal conformation of pseudostellarin D.

Of the three, the temperature dependence of Tyr^2 suggested that this NH was relatively involved in a weaker hydrogen bond than those of Gly^3-NH and Ile^6-NH. The NH of Ile^6 was, on the other hand, considered to be involved in a strong intramolecular hydrogen bond. The temperature coefficient of Gly^1 and Leu^7 are quite large, >5 ppb/K, suggesting that the amide hydrogens are not intramolecularly hydrogen bonded in this solvent. The coefficient (3.3 ppb/K) of Leu^5 is in the region which falls between the two categories: the rather low value for non-hydrogen bonded NH may be due to the steric shielding from the solvent by the side chains of Pro^4 and/or Leu^5.

In ROESY spectrum, the strong ROE enhancements between Leu^7-Hα and Gly^1-NH, between Gly^1-NH and Gly-Hα, and between Gly^1-NH and Tyr^2-NH indicate the presence of type II β-turn conformation between Leu^7 and Gly^1. In addition, the no ROE observed between Gly^1-NH and Leu^7-NH is indicative of the type II β-turn between Gly^1 and Leu^7. On the other hand, the type I β-turn conformation between Pro^4 and Leu^5 was implied by a strong ROE between Leu^5-NH and Pro^4-Hδ, a medium ROE between Leu^5-NH and Leu-Hα, and a weak ROE between Leu^5-NH and Pro^4-Hα. These conformational relationships are what are observed in the crystal conformation, and other ROEs indicated by arrows in Fig. 4 are also noted in the crystal conformation.

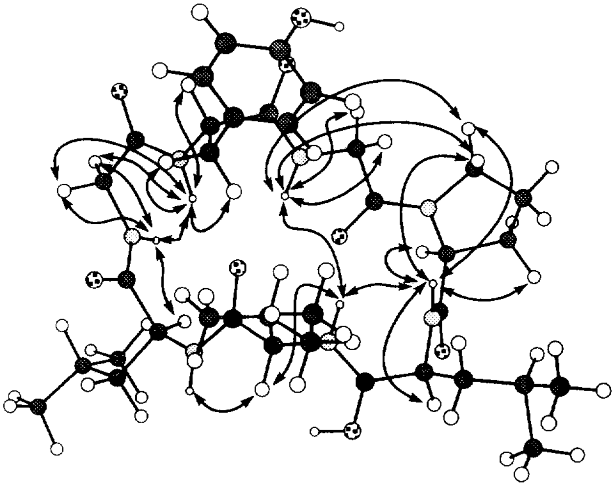

Fig. 4 Perspective view of pseudostellarin D according to the X-ray analysis; The arrows show the ROE relationships in [$^2H_6$]DMSO.

Table 3 Temperature coefficients (-dδ/dT × 10^3 ppm/K) of NH chemical shifts of pseudostellarin D in ten intervals over the range 300 - 330 K in [$^2H_6$]DMSO.

	Gly^1	Tyr^2	Gly^3	Leu^5	Ile^6	Leu^7
pseudostellarin D	6.7	3.0	2.3	3.3	-0.7	7.0

Table 4. Backbone dihedral angles (ϕ) in pseudostellarin D, calculated from X-ray structure and vicinal NH-CαH coupling constants (Hz), and comparison for evolidine crystal structure.

residues	Hz	ϕ angle(°)[a]	pseudostellarin D[b]			residues	evolidine[c]		
			ϕ	ψ	ω		ϕ	ψ	ω
Gly[1]	4.0, 8.5	**90**, 103	89	-14	-172	Pro	-93	13	2
Tyr[2]	10.5	**-108**, -132	-113	-53	180	Val	-95	-16	-176
Gly[3]	d		-162	-162	172	Asn	-159	151	-172
Pro[4]			-71	-18	179	Leu	-54	-31	180
Leu[5]	9.4	**-97**, -143	-106	-12	-173	Ser	-100	2	180
Ile[6]	9.0	-94, **-146**	-134	117	-173	Phe	-157	74	-179
Leu[7]	2.1	**-52**, 172	-56	126	-180	Leu	-65	151	-175

The calculated ϕ angles shown by bold letters are close to those calculated by X-ray structure

[a] Calculated by using the Karplus-Bystrov equation: $^3J_{HN\alpha}=9.4\cos^2|60-\phi|-1.1\cos|60-\phi|+0.4$ for non-Gly residues and and $\Sigma^3J_{HN\alpha2}=6.0\cos^2\phi-1.5\cos\phi+12.5\sin^2\phi$ for Gly residue.

[b] dihedral angles calculated by X-ray structure of pseudostellarin D

[c] dihedral angles calculated by X-ray structure of evolidine[13]

[d] The NH signal of Gly[3] was observed as broad singlet.

The torsion angles can be calculated from the NH-CαH coupling constant experimentally obtained by ^{1}H NMR spectrum by using functions that relate these values. The J values and one set of the corresponding dihedral angles, ϕ, calculated by the Karplus type equation proposed by Bystrov *et al.*,[25] (Table 4) are approximately the same as those calculated by X-ray structure (Bold letters in Table 4 are closely corresponded to X-ray data).

On the basis of the foregoing evidences in the NMR spectra, its conformation in solution state was considered to be generally the same as that in the solid state. Miller et al. suggested that the conformation of cyclic heptapeptides is principally controlled by the chirality of the amino acids.[26] The crystal and solution conformations of the cyclic heptapeptide, pseudostellarin D, with all L amino acids and trans amide bonds, are characterized by two β-turns (type I and type II) incorporating a classical β-bulge unit. These conformational characteristics may be favorable features for cyclic heptapeptides consisting of all L amino acids, even when the peptide contains a cis amide bond, as demonstrated by evolidine. The dihedral angles of evolidine[13] in the crystal structure are compared with those of pseudostellarin D in Table 4. Evolidine and pseudostellarin D assume similar backbone conformations as illustrated in Fig. 5. In addition, though the types of β-turns are different from each other (evolidine: types I and VI, pseudostellarin D: types I and II), the pattern of three intramolecular hydrogen bonds is almost the same. To examine if these can be applied generally to the conformations of cyclic heptapeptides, conformational studies of other cyclic heptapeptides are in progress.

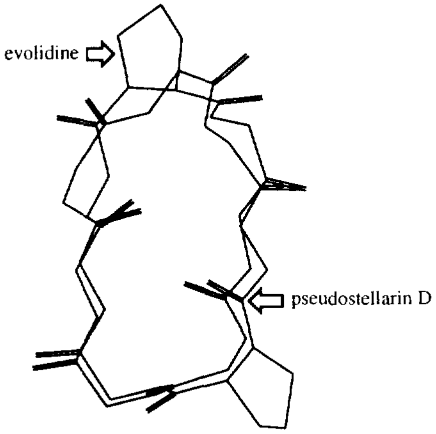

Fig. 5 A superimposed stereostructures of crystal state pseudostellrin D and evolidine; All hydrogens and side chains except that of Pro are omitted.

Experimental

General Details. - ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers (AM400 and AM500) at 300K and processed on a Bruker data station with an Aspect 3000 computer. ROESY experiment was made with a mixing time of 90 msec. The NMR coupling constants (J) are given in Hz.

Materials. - Pseudostellarin D was prepared from roots of *Pseudostellaria heterophylla,* as described in our previous paper.[7]

X-ray analysis of pseudostellarin D. - A colorless prismatic crystal of $C_{38}H_{60}N_8O_9$ having approximate dimensions of 0.50 × 0.20 × 0.15 mm was sealed in a thin-walled glass capillary. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a 12kW rotating anode generator. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 14 carefully centered reflections in the range $40.09<2\theta<40.85°$ corresponded to a primitive orthorhombic cell with dimensions: a=15.798(6), b=28.963(6), c=9.605(5) Å, V=4395(3) Å^3. For Z=4 and F.W.=772.94, the calculated density is 1.17 g/cm^3. The systematic absences of: h00: $h \neq 2n$, 0k0: $k \neq 2n$, 00l: $l \neq 2n$, uniquely determined the space group to be: $P2_12_12_1$(#19). The data were collected at 23 ± 1°C by using the ω-2θ scan technique to a maximum 2θ value of 110.1°. The omega scans of several intense reflections, performed prior to the data collection, had an average width at half-height of 0.22° with a take-off angle of 6.0°. Scans were made in the range (1.20 + 0.30 tan θ)° at a speed of 16.0° / min (in omega). The weak reflections ($I < 10.0\sigma(I)$) were rescanned (maximum of 12 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 1.0 mm, the crystal to detector distance was 235 mm, and the computer controlled detector aperture was set at 3.0 × 6.0 mm (horizontal × vertical).

Of the 3177 reflections which were collected, 3173 were unique (R_{int}=0.288). The intensities of three representative reflections were measured after every 100 reflections. No decay correction was applied. The linear absorption coefficient, μ, for Cu-Kα radiation was 6.9 cm^{-1}. An empirical absorption correction based on azimuthal scans of several reflections was applied to give transmission factors ranging from 0.87 to 0.99. The data were corrected for the Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient=9.91887e-07).

The structure was solved by direct methods[27] and expanded by using Fourier techniques.[28] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1956 observed reflections ($I>3.00\sigma(I)$) and 507 variable parameters and converged to unweighted and weighted agreement factors of R=0.064, R_w=0.082, respectively. The standard deviation of an observation of unit weight was 1.51. The weighting scheme was based on counting statistics and included a factor (p=0.100) to downweight the intense reflections. Plots of $\Sigma\omega(|Fo|-|Fc|)^2$ versus $|Fo|$, reflection order

Table 5 Atomic coordinates and B_{eq} of pseudostellarin D

atom	x	y	z	B_{eq}
O(1)	0.3061(4)	0.1480 (3)	0.1299 (8)	8.2 (2)
O(2)	0.5500 (4)	0.1604 (3)	-0.0395 (6)	7.3 (2)
O(3)	0.6609 (4)	0.0644 (2)	0.3145 (6)	7.1 (2)
O(4)	0.8380 (6)	0.0030 (2)	0.5111 (9)	9.4 (2)
O(5)	0.7903 (5)	0.1297 (4)	0.7940 (6)	9.4 (2)
O(6)	0.5495 (3)	0.1289 (2)	0.4724 (5)	5.9 (1)
O(7)	0.3496 (5)	0.1513 (2)	0.5994 (8)	8.0 (2)
O(20)	0.6358 (4)	0.3635 (3)	-0.1095 (10)	9.8 (2)
O(100)	0.4906 (6)	0.0330 (3)	0.3250 (1)	11.4 (3)
N(1)	0.3621 (4)	0.0898 (2)	0.4542 (7)	5.7 (2)
N(2)	0.4258 (4)	0.1503 (2)	0.2546 (6)	5.8 (2)
N(3)	0.5984 (5)	0.1343 (3)	0.1681 (7)	6.2 (2)
N(4)	0.7799 (5)	0.0593 (2)	0.1895 (6)	5.6 (2)
N(5)	0.8407 (4)	0.0782 (2)	0.4563 (7)	5.5 (2)
N(6)	0.7166 (4)	0.1303 (2)	0.5928 (6)	5.6 (1)
N(7)	0.5149 (4)	0.1176 (2)	0.7008 (6)	5.3 (1)
N(100)	0.8211 (10)	0.1713 (4)	0.2830 (1)	11.8 (4)
C(1)	0.3046 (5)	0.1093 (3)	0.3470 (1)	6.9 (2)
C(2)	0.4701 (5)	0.1820 (3)	0.1623 (8)	5.1 (2)
C(3)	0.6683 (5)	0.1101 (3)	0.1056 (8)	6.2 (2)
C(4)	0.8108 (6)	0.0215 (3)	0.2780 (1)	6.3 (2)
C(5)	0.8581 (5)	0.0950 (3)	0.5974 (9)	5.9 (2)
C(6)	0.6467 (5)	0.1572 (3)	0.6442 (7)	5.3 (2)
C(7)	0.4412 (5)	0.0899 (3)	0.6675 (7)	5.2 (2)
C(10)	0.3460 (5)	0.1370 (3)	0.2336 (9)	5.8 (2)
C(20)	0.5421 (5)	0.1574 (3)	0.0884 (9)	5.5 (2)
C(21)	0.4984 (6)	0.2246 (3)	0.2380 (9)	6.1 (2)
C(22)	0.5353 (5)	0.2627 (3)	0.1477 (9)	5.5 (2)
C(23)	0.4852 (5)	0.2927 (3)	0.0780 (1)	6.7 (2)
C(24)	0.5183 (6)	0.3264 (3)	-0.0090 (1)	7.9 (3)
C(25)	0.6038 (5)	0.3289 (3)	-0.0270 (1)	6.9 (2)
C(26)	0.6558 (6)	0.3000 (5)	0.0490 (2)	10.8 (4)
C(27)	0.6201 (6)	0.2660 (4)	0.1280 (2)	9.8 (3)
C(30)	0.7030 (5)	0.0753 (3)	0.2114 (8)	5.9 (2)
C(40)	0.8300 (5)	0.0341 (3)	0.4253 (10)	5.9 (2)
C(41)	0.8888 (8)	0.0035 (4)	0.2000 (1)	8.8 (3)
C(42)	0.9087 (9)	0.0388 (6)	0.1000 (2)	12.1 (5)
C(43)	0.8348 (6)	0.0663 (3)	0.0681 (10)	7.1 (2)
C(50)	0.7837 (6)	0.1185 (3)	0.6652 (9)	6.4 (2)
C(51)	0.9337 (6)	0.1273 (3)	0.5980 (1)	7.0 (2)
C(52)	1.0175 (8)	0.1061 (4)	0.5670 (2)	10.6 (4)
C(53)	1.0490 (1)	0.0850 (1)	0.7090 (6)	26.0 (1)
C(54)	1.0850 (1)	0.1378 (10)	0.5580 (4)	22.0 (1)
C(60)	0.5653 (5)	0.1334 (3)	0.5989 (8)	5.3 (2)
C(61)	0.6490 (5)	0.2069 (3)	0.5898 (10)	6.2 (2)
C(62)	0.5704 (7)	0.2323 (4)	0.6370 (1)	8.4 (3)
C(63)	0.7320 (7)	0.2311 (4)	0.6310 (2)	9.0 (3)
C(64')*	0.7430 (2)	0.2440 (1)	0.7690 (3)	10.6 (9)
C(64)	0.7410 (1)	0.2719 (8)	0.5730 (3)	10.2 (8)
C(70)	0.3820 (5)	0.1137 (3)	0.5682 (10)	6.0 (2)
C(71)	0.3942 (6)	0.0771 (4)	0.8026 (10)	7.0 (2)
C(72)	0.3236 (8)	0.0424 (5)	0.7860 (1)	9.3 (3)
C(73)	0.3540 (1)	-0.0033 (6)	0.7350 (2)	12.6 (5)
C(74)	0.2730 (1)	0.0387 (7)	0.9130 (2)	14.8 (6)
C(101)	0.8374 (8)	0.2089 (4)	0.2550 (1)	8.4 (3)
C(102)	0.8582 (9)	0.2559 (4)	0.2080 (2)	9.9 (3)

* see text

in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.53 and -0.18 e/Å^3, respectively. Neutral atom scattering factors were taken from Cromer and Waber.[29] Anomalous dispersion effects were included in Fcalc[30]; the values for Δf' and Δf" were those of Creagh and McAuley.[31] The values for the mass attenuation coefficients are those of Creagh and Hubbel.[32] All calculations were performed by using the teXsan[33] crystallographic software package of Molecular Structure Corporation.

The refined fractional atomic coordinates are shown in Table 5. The bond lengths, bond angles, hydrogen-atom coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

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