Synthesis and Structure of N^1-*e*-Benzo-4,7,13,16,21,26-hexaoxa-1,10-diazabicyclo[8.8.8]hexacos-23-yl-N^2-phenylthiourea. Derivative of a Bifunctional Complexing Agent

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A cryptand with an aminobenzo group 4,7,13,16,21,24-hexaoxa-5,6-(4′-aminobenzo)-1,10-diazabicyclo-[8.8.8]hexacosane, has been synthesized as a bifunctional complexing intermediate for investigation of the labeling of proteins with metal radionuclides. This amine, although unstable, can be prepared as the stable stannic salt. The free base was converted to the corresponding phenylthiourea by 1) reaction with phenylisothiocyanate and 2) conversion to its isothiocyanate followed by reaction with aniline. The thiourea structure was confirmed by X-ray crystallographic analysis.

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Cryptands and their corresponding metal ion complexes, cryptates, are well known [1,2], however, only a few examples have been prepared suitably derivatized for attachment to macromolecules [3,4,5]. Our interest in cryptands having the functionality to covalently bind to proteins arises from their potential utility as radionuclide carriers for medical diagnosis and therapy. Synthetic control of the cryptand cage size allows the use of a variety of metals for introduction into the cavity. Some very large metals do not form stable complexes with the more common chelating agents used for protein labeling [6]. Since the higher linear energy transfer radiations are associated with the higher Z number elements, these radionuclides are of particular interest for cancer therapy. Although this use of cryptates has been suggested [7], only one example of cryptate-protein bioconjugates is found in the literature [8]. Gansow prepared the lanthanum and praseodymium cryptates of 4,7,13,18,21-pentaoxa-5,6-(4′-aminobenzo)-1,10-diazabicyclo[8.5.8]tricosane and subsequently coupled them to ribonuclease A by diazotization.

The amine **5** first reported here was synthesized by a similar route used for the preparation of the aminobenzocryptand described above [9]. Initially it was prepared by catalytic hydrogenation of the corresponding nitro compound but was sufficiently unstable to make characterization difficult. Apparently sensitive to both light and air, the amine darkens upon standing. In addition its trihydrochloride salt is extremely deliquescent. Reduction of the nitro precursor with stannous chloride [10] provided an amine complex of the tin salt that was quite stable. The free amine could be extracted from a basic slurry of the tin salt. An important extension of previous work is the conversion of an aminocryptand to its isothiocyanate **6**. This intermediate was not isolated but converted directly to the aniline thiourea, N^1-*e*-benzo-4,7,13,16,21,26-hexaoxa-1,10-diazabicyclo[8.8.8]hexacos-23-yl-N^2-phenylthiourea **7**. This material had identical spectral properties to those of the thiourea obtained from the reaction of the amine with

Reaction Scheme

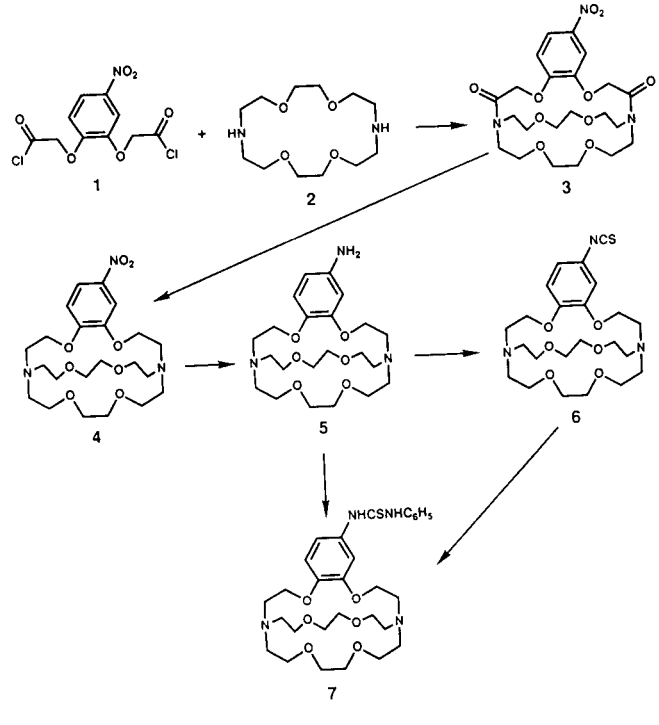

Figure 1

phenylisothiocyanate. The thiourea was isolated as its sodium chloride cryptate and its structure was demonstrated conclusively by X-ray crystallography.

The isothiocyanate derivative of the aminobenzocryptand reported here may produce bioconjugates with greater retention of biological function than those resulting from diazonium salt coupling reactions since milder conditions are employed [11]. The preparation of this isothiocyanate affords medical researchers a tool for exploiting biologically significant proteins for diagnosis and therapy.

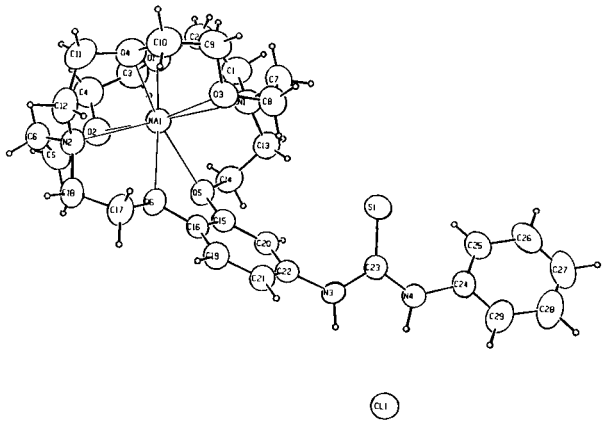

Figure 2

EXPERIMENTAL

Syntheses.

Melting points were obtained on a Thomas Hoover Uni-melt melting point apparatus and are uncorrected. A Nicolet 5DXB FTIR spectrophotometer was used to acquire ir spectra and results are expressed in cm^{-1}. Proton magnetic resonance spectra were collected with a Bruker WM-360 pulsed FT and an IBM NR/80 FTNMR spectrophotometers. Results are reported in ppm (δ). Mass spectra were obtained from VG Analytical ZAB 250HF and Nermag R10-10C mass spectrometers for Cl and FAB results, respectively. Reagents were reagent grade or better. "Dry" solvents were prepared by the methods reported in Perrin and Armarego [12]. The tlc was conducted on DC-Plastikfolien Kieselgel 60 (Merck) strips which were dried at 100° before use. Water and aqueous solutions were derived from in-line Millipore Milli-RO-15 and Super-Q water systems.

4,7,13,16,21,24-Hexaoxa-5,6-(4'-nitrobenzo)-1,10-diazabicyclo-[8.8.8]hexacosane-2,9-dione (**3**).

A 2 ℓ, 3-neck flask equipped with 2 graduated addition funnels, nitrogen inlet, and magnetic stirrer was charged with 1 ℓ of dry toluene and cooled in an ice bath. A solution of 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane **2** (2.62 g, 10.0 mmoles, Aldrich) in 125 ml of dry toluene and 1.0 g (15.0 mmoles) of triethylamine was added to one of the addition funnels. To the other, was added a dry toluene solution (125 ml) of 3.08 g (10.0 mmoles) of 1,2-bis-(oxyacetyl chloride)-4-nitrobenzene (**1**) prepared as previously reported [9]. The solutions in the addition funnels were added to

Table 1

Positional Parameters and Their Estimated Standard Deviations

Atom	x	y	z	B(A2)
CL1	0.95927(8)	0.22733(7)	0.10250(5)	4.66(3)
CL3	0.2166(3)	0.1250(3)	0.8686(2)	13.1(1)
CL3'	0.2692(3)	0.1784(2)	0.8966(2)	8.97(7)
CL4	0.4279(2)	0.0978(2)	0.8894(2)	9.09(8)
CL4'	0.3483(3)	0.0350(2)	0.8533(1)	9.54(8)
CL5	0.1690(2)	0.7311(1)	0.1043(1)	11.37(7)
CL6	-0.1385(3)	0.6437(2)	0.0430(1)	12.03(9)
CL6'	-0.2325(6)	0.5315(3)	-0.0015(2)	10.9(2)
S2	0.51492(9)	0.25683(7)	0.49769(5)	4.44(3)
NA1	0.7239(1)	0.48831(8)	0.27756(7)	3.73(3)
O1	0.4407(2)	0.3465(2)	0.1384(1)	4.39(7)
O2	0.7964(2)	0.6789(2)	0.2788(1)	4.92(7)
O3	0.6103(2)	0.2605(2)	0.2474(1)	4.20(6)
O4	0.7048(2)	0.3389(2)	0.1468(1)	4.75(7)
O5	0.7565(2)	0.6093(1)	0.4302(1)	3.63(6)
O6	1.0240(2)	0.6902(2)	0.4433(1)	3.81(6)
N1	0.3802(2)	0.2691(2)	0.2516(2)	3.97(8)
N2	1.0533(3)	0.6959(2)	0.3080(1)	4.04(8)
N3	0.8517(3)	0.5738(2)	0.6610(1)	3.83(8)
N4	0.6634(3)	0.4253(2)	0.6840(1)	3.90(8)
C1	0.2411(3)	0.2191(3)	0.1776(2)	5.0(1)
C2	0.2572(3)	0.1997(3)	0.1004(2)	4.9(1)
C3	0.4656(3)	0.4588(3)	0.1560(2)	5.6(1)
C4	0.6560(4)	0.6013(3)	0.1899(2)	5.8(1)
C5	0.9879(4)	0.8191(3)	0.3204(2)	5.6(1)
C6	1.0574(4)	0.7766(3)	0.2848(2)	5.1(1)
C7	0.3200(3)	0.1302(3)	0.2183(2)	4.7(1)
C8	0.4899(3)	0.1868(3)	0.2684(2)	4.8(1)
C9	0.5353(4)	0.1423(3)	0.1525(2)	5.4(1)
C10	0.6768(3)	0.2302(3)	0.1417(2)	5.5(1)
C11	0.8476(4)	0.4361(3)	0.1427(2)	5.9(1)
C12	1.0407(3)	0.5937(3)	0.2416(2)	5.6(1)
C13	0.4230(3)	0.3555(3)	0.3432(2)	4.3(1)
C14	0.5833(3)	0.5360(2)	0.4059(2)	3.94(8)
C15	0.8469(3)	0.6220(2)	0.4936(2)	3.19(8)
C16	0.9953(3)	0.6673(2)	0.5012(2)	3.21(8)
C17	1.1850(3)	0.7543(3)	0.4538(2)	4.9(1)
C18	1.2198(3)	0.8194(3)	0.4099(2)	5.2(1)
C19	1.0942(3)	0.6824(2)	0.5621(2)	3.84(9)
C20	0.8060(3)	0.5964(2)	0.5484(2)	3.45(9)
C21	1.0483(3)	0.6518(2)	0.6149(2)	3.81(9)
C22	0.9038(3)	0.6082(2)	0.6081(2)	3.60(9)
C23	0.6798(3)	0.4228(2)	0.6180(2)	3.30(8)
C24	0.5146(3)	0.2928(2)	0.6697(2)	3.74(9)
C25	0.3237(4)	0.1691(3)	0.5954(2)	4.9(1)
C26	0.1891(4)	0.0489(4)	0.5900(2)	6.6(2)
C27	0.2448(5)	0.0561(3)	0.6606(2)	7.4(2)
C28	0.4343/(5)	0.1804(3)	0.7355(2)	7.21(1)
C29	0.5682(4)	0.2987(3)	0.7397(2)	5.6(1)
C31	0.3219(4)	0.1144(3)	0.9286 (2)	6.6(1)
C32'	-0.011(2)	0.677(2)	0.023(1)	11.6(6)*
C32	-0.0244(9)	0.6265(6)	0.0070(4)	9.7(3)

Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: (4/3) * [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab(cos gamma)*B(1,2) + ac(cos beta)*B(1,3) + bc(cos alpha)*B(2,3)]

Table 2

Bond Distances in Angstroms

Atom 1	Atom 3	Distance	Atom 1	Atom 2	Distance	Atom 1	Atom 3	Distance
O1	C2	1.432(5)	C4	C3	1.487(7)	C18	N2	1.473(5)
O1	C3	1.410(5)	C5	O2	1.429(5)	C18	C17	1.499(6)
O2	C4	1.422(5)	C5	C6	1.493(7)	C19	C16	1.379(5)
O2	C5	1.429(5)	C6	N2	1.476(5)	C19	C21	1.381(5)
O3	C8	1.428(5)	C6	C5	1.493(7)	C20	C15	1.371(5)
O3	C9	1.442(5)	C7	N1	1.468(5)	C20	C22	1.393(5)
O4	C10	1.427(5)	C7	C8	1.514(6)	C21	C19	1.381(5)
O4	C11	1.420(6)	C8	O3	1.428(5)	C21	C22	1.377(5)
O5	C14	1.438(5)	C8	C7	1.514(6)	C22	N3	1.429(5)
O5	C15	1.378(4)	C9	C3	1.442(5)	C22	C20	1.393(5)
O6	C16	1.372(4)	C9	C10	1.472(7)	C22	C21	1.377(5)
O6	C17	1.431(4)	C10	O4	1.427(5)	C23	S2	1.678(4)
N1	C1	1.468(5)	C10	C9	1.472(7)	C23	N3	1.365(5)
N1	C7	1.468(5)	C11	O4	1.420(6)	C23	N4	1.350(4)
N1	C13	1.453(5)	C11	C12	1.499(7)	C24	N4	1.419(5)
N2	C6	1.476(5)	C12	N2	1.464(6)	C24	C25	1.381(6)
N2	C12	1.464(6)	C12	C11	1.499(7)	C24	C29	1.376(6)
N2	C18	1.473(5)	C13	N1	1.453(5)	C25	C24	1.381(6)
N3	C22	1.429(5)	C13	C14	1.498(6)	C25	C26	1.384(6)
N3	C23	1.365(5)	C14	O5	1.438(4)	C26	C25	1.384(6)
N4	C23	1.350(4)	C14	C13	1.498(6)	C26	C27	1.378(8)
N4	C24	1.419(5)	C15	O5	1.378(4)	C27	C26	1.378(8)
C1	N1	1.468(5)	C15	C16	1.408(5)	C27	C28	1.371(8)
C1	C2	1.512(6)	C15	C20	1.371(5)	C28	C27	1.371(8)
C2	O1	1.432(5)	C16	O6	1.372(4)	C28	C29	1.381(7)
C2	C1	1.512(6)	C16	C15	1.408(5)	C29	C24	1.376(6)
C3	O1	1.410(5)	C16	C19	1.379(5)	C29	C28	1.381(7)
C3	C4	1.487(7)	C17	O6	1.431(4)			
C4	O2	1.422(5)	C17	C18	1.499(6)			

Numbers in parentheses are estimated standard deviation is the least significant digits.

the reaction flask at the same rate over 5 hours. Stirring was continued for 12 hours after the addition was complete. The triethylamine hydrochloride was removed by filtration and the filtrate evaporated *in vacuo*. The pasty residue was dissolved in 20 ml of chloroform:benzene (1:1) and dried with 10 g of neutral alumina. Chromatography over a column of 50 g of alumina with chloroform:toluene (1:2) afforded a fluffy white material which was recrystallized from toluene and provided 2.18 g (4.38 mmoles, 44%) of **3** mp 132-134°; ms (Cl, ammonia): m/z 497; ^{1}H nmr (deuteriochloroform): 7.83 (dd, 1H), 7.78 (d, 1H), 7.01 (d, 1H), 5.65-4.76 (d x 4, 4H), 4.38-2.77 (m, 24H), 3.79-3.53 (m x 2, 28H).

Anal. Calcd. for $C_{22}H_{31}N_3O_{10}$: C, 53.11; H, 6.28; N, 8.45. Found: C, 53.27; H, 6.15; N, 8.29.

4,7,13,16,21,24-Hexaoxa-5,6-(4′-nitrobenzo)-1,10-diazabicyclo-[8.8.8]hexacosane (**4**).

A procedure similar to that of Brown and Heim [13] was followed. To a 500 ml 3-neck flask filled with nitrogen inlet, reflux condenser, dropping funnel, and ice bath was added 25 ml of borane:tetrahydrofuran complex (1.0 *M*, 25 mmoles). To the dropping funnel was added 2.96 g (5.68 mmoles) of **3** in 30 ml of dry tetrahydrofuran. The addition was completed in 30 minutes. The nitrogen inlet and ice bath were removed and the solution was heated to reflux for 1 hour. The flask was allowed to cool and 3 ml of water and 25 ml of 6*N* hydrochloric acid were added. The tetrahydrofuran was distilled at atmospheric pressure and the remaining liquid was removed *in vacuo*. The light tan paste was recrystallized from methanol as the dihydrochloride of **4** (2.68 g, 4.94 mmoles, 87%), mp 100-101°; ms: (Cl, ammonia) m/z 470; ^{1}H nmr (deuterium oxide): 8.05 (dd, 1H), 7.94 (d, 1H), 7.24 (d, 1H), 4.63-4.58 and 3.91-3.75 (m x 2, 4H), 3.79-3.53 (m x 2, 28H).

Anal. Calcd. for $C_{22}H_{35}N_3O_8 \cdot 2HCl \cdot 1.5H_2O$: C, 46.40; H, 7.08; N, 7.38. Found: C, 46.61; H, 7.11; N, 7.02.

4,7,13,16,21,24-Hexaoxa-5,6-(4′-aminobenzo)-1,10-diazabicyclo-[8.8.8]hexacosane (**5**).

To a 25 ml conical flask was placed 100 mg (0.184 mmole) of **4** dihydrochloride, 251 mg stannous chloride dihydrate (1.11 mmoles) and 0.5 ml of concentrated hydrochloric acid. After mixing, the slurry in the flask was heated in a boiling water bath for 5 minutes. The flask was cooled and 2 ml of tetrahydrofuran was added. The white residue was filtered and washed with 4 ml of acetone. After drying the product weighed 130 mg. This material was not further characterized, but was assumed to be the stannic salt of the amine [10]. Upon treatment with 1 ml of concentrated ammonium hydroxide, extraction with chloroform (2 x 4 ml), dry-

Table 3

Bond Angles in Degrees

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C2	O1	C3	113.3(3)	O2	C5	C6	112.9(4)	C16	C19	C21	120.9(3)
C4	O2	C5	114.6(3)	N2	C6	C5	111.9(4)	C15	C20	C22	121.0(3)
C8	O3	C9	113.0(3)	N1	C7	C8	111.0(3)	C19	C21	C22	119.7(3)
C10	O4	C11	113.2(3)	O3	C8	C7	112.6(3)	N3	C22	C20	119.4(3)
C14	O5	C15	117.7(3)	O3	C9	C10	108.4(4)	N3	C22	C21	120.8(3)
C16	O6	C17	118.3(3)	O4	C10	C9	109.3(4)	C20	C22	C21	119.7(3)
C1	N1	C7	112.0(3)	O4	C11	C12	111.9(3)	S2	C23	N3	122.6(3)
C1	N1	C13	111.4(3)	N2	C12	C11	113.6(4)	S2	C23	N4	125.6(3)
C7	N1	C13	111.6(3)	N1	C13	C14	111.8(3)	N3	C23	N4	111.8(3)
C6	N2	C12	112.1(3)	O5	C14	C13	111.9(3)	N4	C24	C25	124.0(4)
C6	N2	C18	110.1(3)	O5	C15	C16	114.9(3)	N4	C24	C29	116.5(4)
C12	N2	C18	111.5(3)	O5	C15	C20	126.1(3)	C25	C24	C29	119.3(4)
C22	N3	C23	123.7(3)	C16	C15	C20	119.0(3)	C24	C25	C26	119.7(4)
C23	N4	C24	129.0(3)	O6	C16	C15	114.0(3)	C25	C26	C27	120.3(5)
N1	C1	C2	112.3(3)	O6	C16	C19	126.4(3)	C26	C27	C28	120.2(5)
O1	C2	C1	113.3(3)	C15	C16	C19	119.6(3)	C27	C28	C29	119.3(5)
O1	C3	C4	108.5(4)	O6	C17	C18	106.7(3)	C24	C29	C28	121.1(5)
O2	C4	C3	108.3(4)	N2	C18	C17	113.3(4)				

Numbers in parentheses are estimated standard deviations in the least significant digits.

ing over sodium sulfate, and evaporation of the solvent, 78 mg (0.170 mmole) of viscous reddish amber oil **5** was obtained; tlc (methanol:chloroform; 1:4) Rf = 0.33; ms: (Cl, ammonia) m/z 440; ^{1}H nmr (deuterium oxide): 7.21-7.01 (m, 3H), 4.53 (m, 2H), 3.92-3.58 (m, 30H).

N^1-*e*-Benzo-4,7,13,16,21,26-hexaoxa-1,10-diazabicyclo[8.8.8]hexacos-23-yl-*N*^{2}-phenylthiourea (**7**).

Method 1.

The isolation of 408 mg (0.927 mmole) of free amine **5** from 612 mg of its stannic salt was carried out as described above. To the flask containing the amine was added a stir bar and 2 ml of methanol containing 167 mg (1.24 mmoles) of phenylisothiocyanate. A solid began forming within 20 minutes. The mixture was stirred overnight and the solid was filtered and washed with 4 ml of methanol:isopropyl ether (1:1). After drying the crude thiourea **7** weighed 337 mg (0.586 mmole, 63%), mp 165-170° dec; ir (neat): 1521, 1507, 1226, 1101; ^{1}H nmr (deuteriochloroform): 7.80-6.61 (m, 8H), 4.17-3.96 (m, 4H), 3.67-3.2 (m, 4H), 3.67-3.28 (m, 16H), 3.03-2.44 (m, 12H); ms: (FAB, xenon) m/z 597.2723, Calcd. for M + sodium: 597.2723. An analytical sample recrystallized twice from dichloromethane:isopropyl ether melted at 168-170° dec [14].

Anal. Calcd. for $C_{29}H_{42}O_6N_4S\cdot NaCl\cdot 0.5CH_2Cl_2$: C, 52.86; H, 6.36; N, 8.22; S, 4.70. Found: C, 52.83; H, 6.46; N, 8.08; S, 4.86.

Method 2.

One hundred eleven mg (0.252 mmole) of amine **5** was obtained by extraction as described from 140 mg of the stannic salt and recovered in a 25 ml conical flask. To the flask containing the amine was added a stir bar, 1 ml of chloroform, 0.5 ml of saturated aqueous lithium carbonate, 0.5 ml of water, and 100 μl of thiophosgene. The flask was stoppered and the contents stirred. The reaction could be conveniently monitored by fluorescamine [15]. After 2 hours the *p*H of the aqueous phase was about 6 and the chloroform layer was removed by aspiration. The aqueous layer was washed with 2 ml of chloroform. Aniline (50 mg, 0.537 mmole) in 2 ml of 2-propanol was added to the flask followed by the dropwise addition of saturated lithium carbonate (approximately 250 μl) until the solution was slightly basic. The resulting solution was stirred for 2 hours. The solvents were then removed *in vacuo* and the residue was mixed with 1 ml of concentrated ammonium hydroxide and extracted with (2 x 3 ml) of chloroform. The chloroform solution was dried over sodium sulfate and evaporated to near dryness. The addition of 10 ml of isopropyl ether afforded 70 mg (0.122 mmole, 48%) of crude **7**, mp 154-162° dec. Spectral properties of this material were identical to those of the product obtained in method 1.

II. X-ray Crystallographic Analysis.

Recrystallization of the crude material obtained by method 2 from dichloromethane:isopropyl ether afforded the crystals for X-ray crystallographic analysis. A colorless lath (0.25 x 0.35 x 0.52 mm) was encapsulated in a glass capillary and mounted on an Enraf-Nonius CAD4 automated diffractometer. Cell dimensions for the chosen triclinic cell were determined from 46 centered reflections with θ between 7.5 and 12.5° and were a = 12.321(1), b = 15.481(1), c = 19.893(2) Å, α = 114.52(1), β = 99.67(1), and γ = 131.42(1)°. The molecular formula was determined to be $C_{29}H_{42}O_6N_4S\cdot NaCl\cdot 2CH_2Cl_2$. Diffraction data were collected between 2.0 and 25.0° θ (λ = Mokα) using $\theta-2\theta$ scans with a scan width of 0.9 + 0.35 tan θ and a scan speed in the range 2.0-5.0°/minute. All computer calculations were performed with the Enraf-Nonius supplied SDP crystallographic set of computer programs. Lorentz and polarization corrections were applied to the 9289 data. Corrections for fluctuations in intensities of the four reference reflections were less than 1.1%. An emperical absorption correction based on psi scans was made. The maximum

correction was 6.5%; the average correction was 2.5%. Averaging equivalent reflections resulted in 6340 unique reflections with 4007 having $F > 2.0\ \sigma_F$. The internal agreement for equivalent reflections was 2.5%. The structure was solved by direct methods and refined by full-matrix least-squares methods. All hydrogen atoms (except two on one of the two included dichloromethane solvent molecules) were located in difference density maps. All non-hydrogen atoms (except one half-occupancy solvent carbon atom) were refined anisotropically. Hydrogen atoms were set to ideal positions, *i.e.*, atom to H distance = 0.95 Å for tetrahedral or trigonal positions, with $B_H = 1.1\ B_{iso}$ of the attached atom. The refinement converged to $R_F = 0.048$ and $wR_F = 0.069$, where $w = [(\sigma_F)^2 + (0.04\ F)^2]^{-1}$. The final shiftmax/ESD was 0.11. The highest peak in the difference map was 0.356 $e^-/Å^3$ and the deepest valley was 0.281 $e^-/Å^3$; both occurred near chlorine atoms in the disordered solvent molecules. Positional parameters are given in Table 1. Bond distances and bond angles are provided in Tables 2 and 3, respectively.

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