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STRUCTURE OF CLATHRIDINE Zn-COMPLEX, A METABOLITE OF THE MARINE SPONGE *CLATHRINA CLATHRUS*

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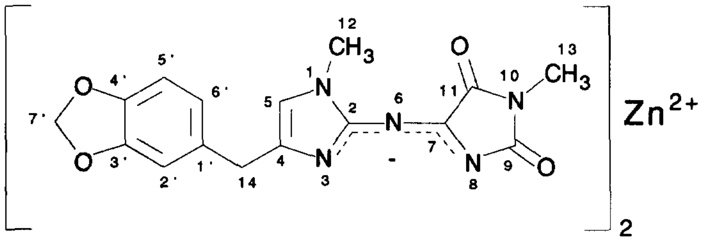
Summary: The solid state structure of the Zn-complex of clathridine (**1**), a genuine metabolite of the sponge *Clathrina clathrus*, was established by X-ray diffraction analysis. In addition, the conformation of **1** in $CDCl_3$ solution was investigated by NOE difference NMR experiments.

Recent studies on the metabolites of the encrusting sponge *Clathrina clathrus* revealed the presence of a novel imidazole-containing compound, clathridine (**2**), whose structure was assigned on the basis of spectral and chemical evidence.[1] The same sponge also contained the zinc complex of clathridine (**1**), which was proved to derive from two deprotonated clathridine molecules and one Zn^{2+} ion on the basis of spectral data. In accordance with this structural hypothesis, compound **1** was synthesized in very good yield by treatment of a CH_2Cl_2 solution of **2** with an aqueous solution of $ZnSO_4$ under stirring.

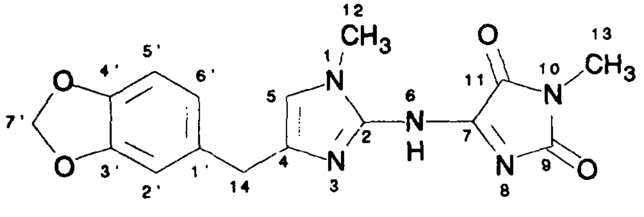


1

^{1}H-NMR data ($CDCl_3$)

H5	6.63 (s)
$H_3$12	3.80 (s)
$H_3$13	3.03 (s)
$H_2$14	3.49 and 3.35 (AB, *J*=16.1 Hz)
H2'	6.24 (s)
H5'	6.50 (d, *J*=8.2 Hz)
H6'	6.25 (d, *J*=8.2 Hz)
$H_2$7'	5.90 and 5.88 (AB, *J*=1.5 Hz)

The spectral features of **1**, requiring a chiral center in the molecule, allowed also to hypothesize a stereostructure of **2**, having a tetrahedral geometry. In fact in the ^{1}H NMR spectrum of **1**, the methylene groups at C14 and C7' of each ligand resonated as two AB systems centered at δ 5.89 and 3.42 respectively, while they appeared as two sharp singlets at δ 5.92 and 3.79 in the ^{1}H NMR spectrum of **2**. This requires that the hydrogen atoms of each methylene group are enantiotopic in **2**, while in **1** they are diastereotopic. Consequently, a planar structure, missing



2

^{1}H-NMR data ($CDCl_3$)

H5	6.52 (s)
$H_3$12	3.70'(s)
$H_3$13	3.17 (s)
$H_2$14	3.79 (s)
H2'	6.71 (s)
H5'	6.75 (d, J=8.2 Hz)
H6'	6.70 (d, J=8.2 Hz)
$H_2$7'	5.92 (s)

any chiral center, could be excluded for **1**; in contrast a tetrahedral geometry, very frequently encountered in nitrogenous zinc complexes, seemed to be highly probable. Therefore we proposed for compound **1** a structure having this geometry, with the zinc atoms linking the N3 and N8 of each clathridine unit.

Compound **1** was proved to be a genuine metabolite of the sponge. This appeared to be quite interesting on account of the important role of zinc and zinc-chelating substances in several biological systems.[2] In this paper we report the determination of solid-state structure of **1** from X-ray diffraction data, and an investigation on conformation of **1** in $CHCl_3$ solution based on NOE difference NMR experiments.

Compound **1**, synthesized from natural **2** as reported, was recrystallized from hot EtOAc. A crystal, of

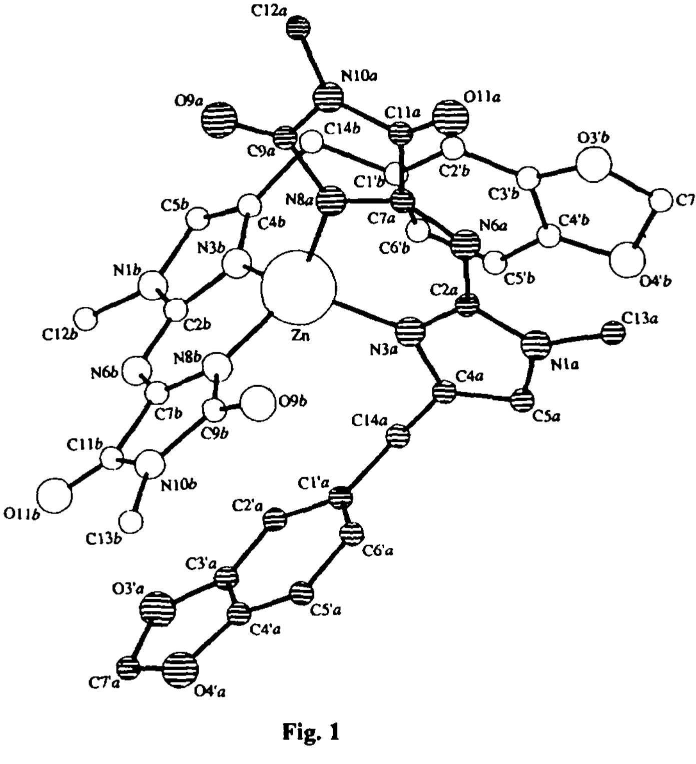


Fig. 1

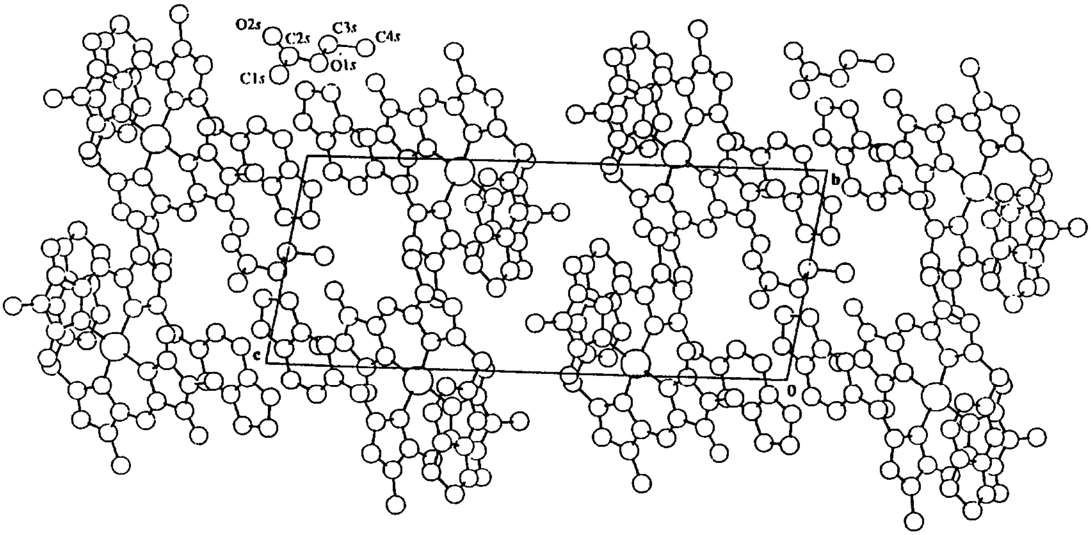


Fig. 2. A view of the crystal packing of **1** in the bc plane.Only one of the two symmetry-related positions of the solvent molecules is represented.

approximate dimension 0.15x0.13x0.20 mm, was used for X-ray analysis. A molecular model of the Zn(II) complex is reported in Fig. 1. The independent crystallographic unit within the unit cell consists of two deprotonated clathridine molecules coordinated to a Zn(II) ion, and an ethyl acetate solvent molecule statistically positioned around the centre of symmetry at 1/2, 1/2, 0. Four nitrogen atoms N3*a*, N8*a* and N3*b*, N8*b* of the independent clathridine molecules, are ligands of the Zn(II) ion in a distorted tetrahedral coordination. The Zn-N distances and the N-Zn-N angles are in the range 1.960-2.001 Å and 91°-120° respectively. The molecular structure of both clathridine molecules is folded with the piperonyl unit and the imidazol-type rings lying in nearly perpendicular planes; however the orientation of the two piperonyl planes are approximately of opposite sign. The two dihedral angles N3*a*-C4*a*-C14*a*-C1'*a*, C4*a*-C14*a*-C1'*a*-C6'*a* and N3*b*-C4*b*-C14*b*-C1'*b*, C4*b*-C14*b*-C1'*b*-C6'*b* are -77°, -26° and 67°, 45° respectively. The geometrical parameters for both molecules are similar and unexceptional. The imidazol-type rings and the piperonyl unit of the two clathridine molecules are planar within the experimental errors, the highest deviations of the atoms of the imidazol-rings and piperonyl unit from the least-square plane being 0.06 Å and 0.02 Å respectively. In the crystal there are two molecular complexes related by a centre of symmetry. The packing creates large cavities in the lattice which are filled by an ethyl acetate solvent molecule in two statistical positions related to a symmetry centre. The complexes are held together by van der Waals interactions. Only few intermolecular contacts less than 3.5 Å are present. The closest intermolecular distance is 2.94 Å between the O11*a* atom of an imidazol-type ring and the same atom related by -x+1, -y+1, -z+1.

Table 1. Positional Parameters and Their Estimated Standard Deviations for **1**.

Atom	x	y	z	B(Å²)	Atom	x	y	z	B(Å²)
Zn	0.18195(4)	0.05745(4)	0.29514(2)	3.008(8)	O11*b*	0.1908(3)	-0.3609(3)	0.1010(1)	6.19(8)
O3'*a*	-0.0774(5	-0.3044(3)	0.0204(2)	8.8(1)	N1*b*	-0.2103(3)	-0.3619(3)	0.2743(1)	4.40(7)
O4'*a*	0.1635(4)	-0.1633(3)	-0.0225(2)	8.3(1)	N3*b*	-0.0138(3)	-0.1317(2)	0.3044(1)	3.40(5)
O9*a*	0.3347(3)	-0.0069(2)	0.4184(1)	5.29(6)	N6*b*	0.0092(3)	-0.2964(3)	0.2079(1)	3.71(6)
O11*a*	0.6229(2)	0.5137(2)	0.4529(1)	4.14(5)	N8*b*	0.2474(3)	-0.0552(2)	0.2236(1)	3.27(5)
N1*a*	0.2508(3)	0.4918(2)	0.2804(1)	3.82(6)	N10*b*	0.3787(3)	-0.1155(3)	0.1398(1)	4.47(7)
N3*a*	0.1763(3)	0.2446(2)	0.2747(1)	3.06(5)	C2*b*	-0.0644(3)	-0.2588(3)	0.2599(1)	3.48(6)
N6*a*	0.3854(3)	0.4327(2)	0.3522(1)	3.01(5)	C4*b*	-0.1303(4)	-0.1595(3)	0.3496(2)	4.05(7)
N8*a*	0.3246(3)	0.1780(2)	0.3688(1)	3.03(5)	C5*b*	-0.2521(4)	-0.3012(3)	0.3312(2)	4.70(8)
N10*a*	0.4973(3)	0.2517(3)	0.4517(1)	3.53(6)	C7*b*	0.1498(3)	-0.2005(3)	0.1943(1)	3.38(6)
C2*a*	0.2730(3)	0.3857(3)	0.3042(1)	3.00(6)	C9*b*	0.3866(4)	-0.0006(3)	0.1909(2)	3.88(7)
C4*a*	0.0874(3)	0.2635(3)	0.2305(2)	3.78(7)	C11*b*	0.2358(4)	-0.2420(3)	0.1383(2)	4.21(8)
C5*a*	0.1316(4)	0.4149(3)	0.2336(2)	4.39(8)	C12*b*	-0.3022(5)	-0.5175(4)	0.2388(2)	6.2(1)
C7*a*	0.4013(3)	0.3336(3)	0.3790(1)	2.75(6)	C13*b*	0.5025(5)	0.9047(5)	0.0934(2)	7.0(1)
C9*a*	0.3798(3)	0.1248(3)	0.4130(1)	3.51(7)	C14*b*	-0.1139(4)	-0.0450(4)	0.4078(2)	4.63(8)
C11*a*	0.5241(3)	0.3849(3)	0.4328(1)	3.16(6)	C1'*b*	-0.1172(3)	0.0975(3)	0.3931(2)	3.96(7)
C12*a*	0.5837(4)	0.2383(4)	0.5032(2)	5.37(9)	C2'*b*	-0.0065(3)	0.2432(4)	0.4222(2)	3.99(7)
C13*a*	0.3349(5)	0.6588(3)	0.3004(2)	5.6(1)	C3'*b*	-0.0160(3)	0.3665(3)	0.4075(2)	3.91(7)
C14*a*	-0.0363(4)	0.1288(4)	0.1884(2)	4.75(9)	C4'*b*	-0.1271(4)	0.3522(3)	0.3641(2)	4.31(8)
C1'*a*	0.0289(4)	0.0570(4)	0.1333(2)	4.54(8)	C5'*b*	-0.2372(4)	0.2113(4)	0.3349(2)	5.24(9)
C2'*a*	-0.0654(5)	-0.0972(4)	0.1063(2)	5.5(1)	C6'*b*	-0.2311(4)	0.0842(4)	0.3504(2)	4.83(9)
C3'*a*	-0.0075(5)	-0.1564(4)	0.0545(2)	5.9(1)	C7'*b*	0.0004(5)	0.5987(4)	0.4074(2)	5.7(1)
C4'*a*	0.1343(5)	-0.0741(4)	0.0290(2)	6.0(1)	O1*s*	0.5313(6)	0.4371(5)	0.0121(3)	5.4(1)*
C5'*a*	0.2311(6)	0.0756(5)	0.0545(2)	6.7(1)	O2*s*	0.5741(7)	0.5643(7)	0.1100(3)	7.7(2)*
C6'*a*	0.1748(5)	0.1392(4)	0.1075(2)	5.6(1)	C1*s*	0.676(1)	0.380(1)	0.0816(5)	7.8(2)*
C7'*a*	0.0279(8)	-0.3104(5)	-0.0290(2)	8.9(2)	C2*s*	0.5922(8)	0.4679(8)	0.0703(4)	4.8(1)*
O3'*b*	0.0803(3)	0.5205(3)	0.4282(1)	5.27(6)	C3*s*	0.458(1)	0.525(1)	0.0013(8)	11.3(4)*
O4'*b*	-0.1012(3)	0.4961(3)	0.3552(1)	5.75(6)	C4*s*	0.461(2)	0.512(1)	-0.0739(7)	11.1(4)*
O9*b*	0.5001(3)	0.1240(3)	0.2028(1)	5.27(7)					

Numbers in parentheses are estimated standard deviations in the least significant digits. Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: 4/3 [$a^2 \cdot B(1,1) + b^2 \cdot B(2,2) + c^2 \cdot B(3,3) + ab(\cos\gamma) \cdot B(1,2) + ac(\cos\beta) \cdot B(1,3) + bc(\cos\alpha) \cdot B(2,3)$].

a and *b* are used to distinguish atoms belonging to the two clathridine units; *s* indicates the atoms of the solvent molecule.

Table 2. Bond Distances in Angstroms and Their Estimated Standard Deviations for **1**.

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
Zn	N8*a*	1.960(2)	C1'*a*	C2'*a*	1.406(4)	C4*b*	C5*b*	1.357(3)
Zn	N3*a*	2.001(3)	C1'*a*	C6'*a*	1.387(5)	C7*b*	C11*b*	1.514(5)
Zn	N8*b*	1.975(3)	C2'*a*	C3'*a*	1.368(6)	C14*b*	C4*b*	1.495(4)
Zn	N3*b*	1.980(2)	C3'*a*	C4'*a*	1.357(6)	O11*b*	C11*b*	1.208(4)
N1*a*	C2*a*	1.343(4)	C4'*a*	C5'*a*	1.370(5)	O9*b*	C9*b*	1.208(3)
N1*a*	C5*a*	1.389(4)	C5'*a*	C6'*a*	1.399(6)	C1'*b*	C14*b*	1.511(6)
N1*a*	C12*a*	1.466(3)	O3'*a*	C3'*a*	1.386(4)	C1'*b*	C2'*b*	1.398(4)
N3*a*	C2*a*	1.336(3)	O3'*a*	C7'*a*	1.425(7)	C3'*b*	C2'*b*	1.354(6)
N3*a*	C4*a*	1.386(5)	O4'*a*	C4'*a*	1.387(5)	C4'*b*	C3'*b*	1.382(5)
N6*a*	C2*a*	1.379(4)	O4'*a*	C7'*a*	1.429(5)	C4'*b*	C5'*b*	1.368(4)
N6*a*	C7*a*	1.287(4)	N1*b*	C5*b*	1.381(5)	C5'*b*	C6'*b*	1.383(6)
N8*a*	C7*a*	1.355(3)	N1*b*	C12*b*	1.469(4)	C6'*b*	C1'*b*	1.394(5)
N8*a*	C9*a*	1.385(5)	N1*b*	C2*b*	1.352(3)	O3'*b*	C3'*b*	1.379(3)
N10*a*	C11*a*	1.356(4)	N3*b*	C2*b*	1.344(3)	O3'*b*	C7'*b*	1.433(6)
N10*a*	C13*a*	1.465(5)	N3*b*	C4*b*	1.388(4)	O4'*b*	C4'*b*	1.379(4)
N10*a*	C9*a*	1.403(3)	N6*b*	C2*b*	1.367(4)	O4'*b*	C7'*b*	1.429(4)
C11*a*	C7*a*	1.528(4)	N6*b*	C7*b*	1.289(3)	C2*s*	C1*s*	1.47(2)
C5*a*	C4*a*	1.353(5)	N8*b*	C7*b*	1.364(3)	C3*s*	C4*s*	1.59(2)
C4*a*	C14*a*	1.498(4)	N8*b*	C9*b*	1.378(4)	C2*s*	O2*s*	1.23(1)
O9*a*	C9*a*	1.201(4)	N10*b*	C11*b*	1.360(3)	O1*s*	C2*s*	1.327(9)
O11*a*	C11*a*	1.207(3)	N10*b*	C9*b*	1.412(4)	O1*s*	C3*s*	1.39(2)
C14*a*	C1'*a*	1.517(5)	N10*b*	C13*b*	1.458(4)			

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

a and *b* are used to distinguish atoms belonging to the two clathridine units; *s* indicates the atoms of the solvent molecule.

Table 3. Selected Bond Angles in Degrees and Their Estimated Standard Deviation for **1**.

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
N3*a*	Zn	N8*a*	91.6(1)	N6*a*	C7*a*	N8*a*	131.4(2)
N3*a*	Zn	N3*b*	122.4(1)	Zn	N8*a*	C9*a*	127.8(2)
N3*a*	Zn	N8*b*	113.4(1)	Zn	N8*a*	C7*a*	122.8(2)
N8*a*	Zn	N3*b*	120.0(1)	Zn	N3*b*	C2*b*	122.2(2)
N8*a*	Zn	N8*b*	121.2(1)	Zn	N3*b*	C4*b*	130.8(2)
N3*b*	Zn	N8*b*	90.99(9)	N3*b*	C2*b*	N6*b*	130.8(2)
Zn	N3*a*	C2*a*	121.9(2)	C2*b*	N6*b*	C7*b*	120.4(2)
Zn	N3*a*	C4*a*	132.0(2)	N6*b*	C7*b*	N8*b*	130.7(3)
N3*a*	C2*a*	N6*a*	130.3(3)	Zn	N8*b*	C7*b*	123.4(2)
C2*a*	N6*a*	C7*a*	120.6(2)	Zn	N8*b*	C9*b*	127.9(2)

Numbers in parentheses are estimated standard deviations in the least significant digits.
a and *b* are used to distinguish atoms belonging to the two clathridine units; *s* indicates the atoms of the solvent molecule.

The solid-state geometry of the molecule lacks any element of symmetry: the tetrahedral arrangement of the imidazole moiety of the two ligands around the zinc atom is nearly symmetric (the two moieties are related through a *pseudo*-C_2 axis), but the piperonyl units, oriented to opposite sides, make the molecule asymmetric on the whole. This did not agree with the ^1H NMR spectrum, which exhibited coincident resonances for the two ligands tetrahedrally linked to the zinc atom, thus requiring a symmetrical conformational arrangement in solution. In order to ascertain whether in $CHCl_3$ solution **1** exists in a single rigid or a flexible conformation we performed several NOE difference experiments. The results are summarized in Table 4. Most of the NOEs reported are due to spatially close protons belonging to the same clathridine unit. However, the weak, but significative NOE enhancements observed for the signal at δ 6.78 (H5') on irradiation either at δ 3.53 ($H_3$12) or at δ 3.10 ($H_3$13) cannot be explained in such a way, since no reasonable conformation of clathridine accounting for the above results by intraligand interaction exists. Hence, we must assume that the observed NOEs derived from an interaction of H5' of a clathridine unit with $H_3$12 and $H_3$13 of the other one.

The co-occurrence of a weak NOE interaction of H5' with two apart located groups points to a flexible conformation of **1**, deriving from a rotation of the piperonyl unit of each ligand around the C4-C14 and C14-C1' bonds, which allows an alternating approach of H5' to $H_3$12 and $H_3$13.

Table 4. Nuclear Overhauser Enhancement Data for **1**

irradiated signal	enhanced signals (% enhancement)
H5	H2' (1.5), H12 (1.0), $H_2$14 (0.5)
H5'	H5 (1.7), H6' (6.8), H2' (4.1), $H_3$12 (0.4), $H_3$13 (0.4)
H2' and H6'	H5 (2.0), H5' (16.0), $H_2$14 (2.5)
$H_3$12	H5 (11.3), H5' (1.1), H2' (0.5)
$H_3$13	H5' (1.2)

EXPERIMENTAL

Zinc complex of clathridine: Compound 1 (50 mg) was synthesized from clathridine 2 and $ZnSO_4$ as previously reported.[1] Yellow-orange crystals (m.p. 197-198°C), in the form of small prisms, were obtained by crystallization from hot ethyl acetate solution. A crystal, of approximate dimensions 0.15 x 0.13 x 0.20 mm, suitable for X-ray diffraction analysis, was used for the unit cell determination and intensity data collection.

X-Ray Diffraction Analysis. Crystal data of Zn-complex of clathridine 1: $C_{32}H_{28}N_{10}O_8 \cdot 1/2\ C_4H_8O_2$; Mw=790.07 amu; Triclinic; **a**=9.435(2) Å, **b**=9.902(2) Å, c=21.418(3) Å, α=100.05(4)°, β=86.92(3)°, γ=116.8(8)°; Z=2; Dc=1.494 g/cm^3; Dexp=1.49 g/cm^3 ; F(000)=816; Space Group P$\bar{1}$; Cu-Kα radiation λ=1.54178 Å; μ(Cu-kα)=15 cm^{-1}.

X-ray diffraction intensities were collected with an Enraf-Nonius CAD4 diffractometer, equipped with a Microvax II digital computer, using Cu-Kα radiation and a graphite monocromator. An ω-2θ scan mode was used for the peak measurements; background counts were taken at the end of each scan. A total of 6607 independent reflections were collected in the θ range 1-70°; 6247 with $I>3\sigma(I)$ were considered observed and used in the subsequent calculations. The data were corrected for Lorentz and polaritation effects but no absorption corrections were made. The structure of the complex was solved by direct methods using the MULTAN program in the form programmed by Germain *et al.*[3] The map corresponding to the highest combined figure of merit revealed the position of 25 non hydrogen atoms. The remaining heavy atoms, together an ethyl acetate solvent molecule, were located by means of difference Fourier maps. Full matrix refinement, including anisotropic thermal parameters for non hydrogen atoms, and isotropic refinement of hydrogen atoms located in a difference Fourier maps, gave an R=0.044 and Rw=0.048. The four carbon atoms and the two oxygen atoms of the solvent molecule were refined with partial occupancy of 0.5. Refinement was ended when the maximum shift in the atomic coordinates and anisotropic temperature factors for the heavy atoms was less than 1/5 and 1/3 of the corresponding standard deviations, respectively. The atomic scattering factors, with the real and imaginary dispersion corrections for all species, were calculated according to Cramer and Waber.[4] All calculations were carried out on a Microvax II using SDP package of crystallographic programs. The final atomic coordinates, bond distances and bond angles are reported in Table 1, Table 2 and Table 3 respectively.

NMR Analysis. The ^1H NMR spectra, including NOE difference experiments, were recorded on a Bruker WM250 spectrometer in $CDCl_3$ solution. All chemical shifts are reported in ppm, using TMS as internal standard (δ=0). Nuclear Overhauser effects were determined with the aid of an Aspect 2000 microprogram using samples previously degassed by bubbling argon through the solution.

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