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A STRUCTURAL AND CONFORMATIONAL STUDY OF 8-*p*-NITROCINNAMYL-3-PROPIONYL-3,8-DIAZABICYCLO[3.2.1]-OCTANE, SELECTIVE AGONIST OF μ-OPIOID RECEPTORS

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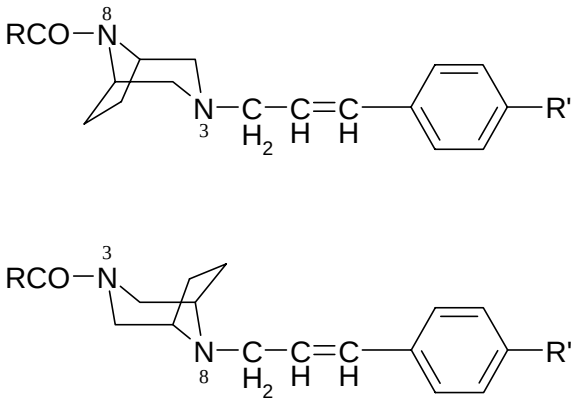
Abstract- 8-*p*-Nitrocinnamyl-3-propionyl-3,8-diazabicyclo[3.2.1]octane (**2b**) and its isomer (**1b**), having the nitrogen substituents exchanged, have been compared by X-Ray structural analysis. The different orientation of the cinnamyl chain in the two derivatives has been discussed and related to the pharmacological properties.

Pain is a very complex and dynamic process, involving multiple, interrelated neurotransmitter-neuromodulator systems. The discovery of compounds that can safely treat both acute and chronic pain without the side effects of drug dependency is highly desirable in pain management. In the course of our studies in this field, we evidenced the analgesic properties of a series of 3,8-diazabicyclo[3.2.1]octane (DBO) derivatives. In particular, the essential requirements for the activity of this class seemed to be the presence of a propionyl group at N8 and a cinnamyl chain at N3 (compound **1a**).[1] Further studies showed that inversion of the N3 and N8 substituents led to a compound (**2a**), which was about 5 times less potent.[1] Later on it was evidenced that the analgesic properties of this class were related to a selective affinity towards μ-opioid receptors.[2] In addition it was shown that the insertion of a substituent on the phenyl ring could greatly modulate the biological profile. In particular, the presence of a *para*-nitro group led to compounds (**1b)** and (**2b),** which showed higher in vitro μ/δ-selectivity with respect to the unsubstituted analogues, accompanied by *in vivo* better analgesic properties and minor side effects. However, contrary to what seen in the unsubstitued models, in this case reversion of the substituents of **1b** led to a more active compound (**2b**).[3] It should also be noted that compound (**2b)** (ED_{50}= 0.16 mg/Kg ip in the hot-plate test in mice) induced tolerance after 13 days of repeated treatment, while for **1b** (ED_{50}= 0.44 mg/Kg) and morphine (ED_{50}= 5 mg/Kg) a complete tolerance was seen after seven and three days, respectively. The conformational studies and the X-Ray structures correlated to their biological properties for **1b**, **1c** and **1d** have been previously reported.[4] In this paper we describe parallel investigations on the **2b** derivative (Scheme 1).

RESULTS AND DISCUSSION

X-Ray structure determination

Scheme 1



Compound	R	R'
1a	ethyl	H
1b	ethyl	*p*-NO_2
1c	*n*-propyl	*p*-NO_2
1d	*t*-butyl	*p*-NO_2
2a	ethyl	H
2b	ethyl	*p*-NO_2

The molecular structure of **2b** is shown in Figure 1. The piperazine ring is in a chair conformation and the aralkenyl chain is equatorially oriented. The mobility of the latter is represented by the different orientation found with respect to its isomer having the nitrogen substituent exchanged (**1b**).

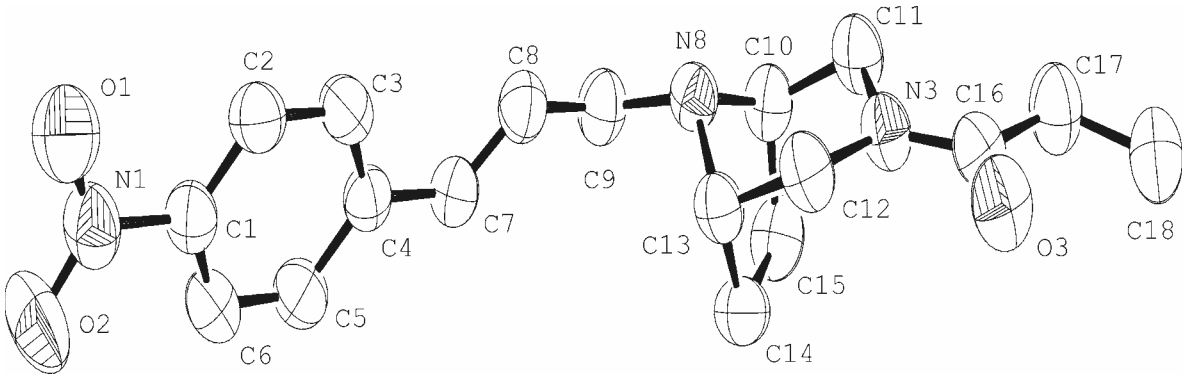


Figure 1. An ORTEP representation of **2b**. Ellipsoids are at 50% of probability.

Table 1. Selected torsion angles(°) for **1b**, **1c**, and **2b**

		1b[a]	**1c**[a]	**2b**
τ_1	C(13)-N(8)-C(9)-C(8)	176(3)	-173.1(5)	177.3(2)
τ_2	N(8)-C(9)-C(8)-C(7)	127(3)	-132.9(7)	-129.7(2)
τ_3	C(9)-C(8)-C(7)-C(4)	171(3)	180.0(6)	-176.9(2)
τ_4	C(8)-C(7)-C(4)-C(5)	178(3)	170.7(7)	176.6(2)

[a]) For **1b** and **1c** is N(3), due to the reversion of the substituents.

Figure 2 is a superimposition of the two structures. In table 1 are reported the significant torsion angles in the two compounds and in the related **1c**. It is worth to observe as in the parent compounds having the

ethyl moiety replaced by *n*-propyl (**1c**) or *t*-butyl (**1d**) there is an opposite orientation of the aralkenyl chain. Quite unexpectedly, **1c** carrying the *p*-nitro substituted phenyl side chain at the position 8 shows an almost equal orientation of **2b** which belongs to the inverted series (see figure 3 where the molecular structures of **1b**, **1c**, **1d** and **2b** are superimposed).

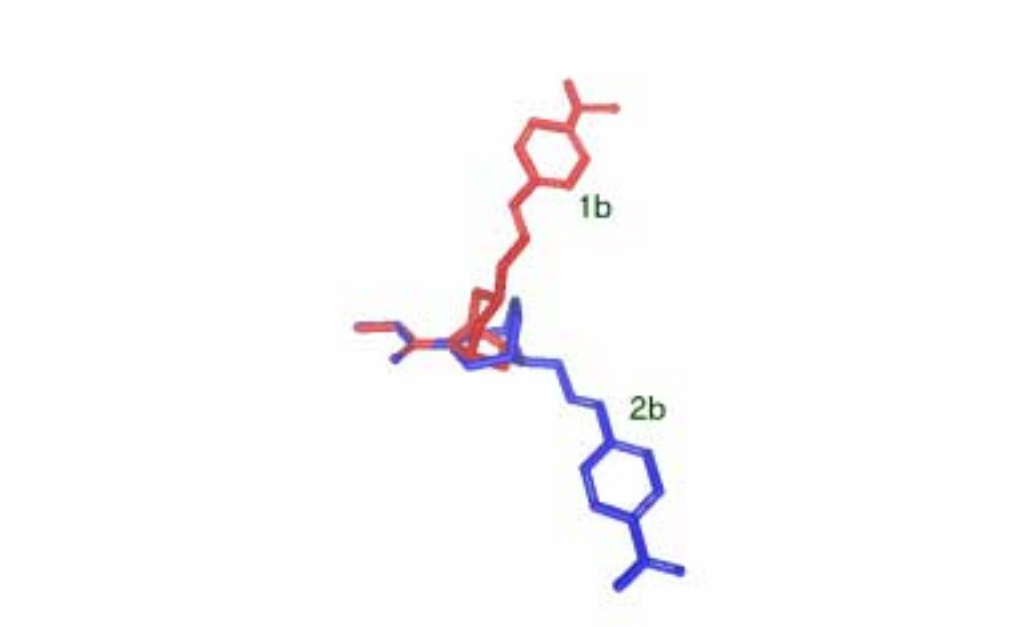


Figure 2. Relative positions of the cinnamyl chains in the two isomers (**1b**) and (**2b**).

The mean plane calculated over the four carbons of the piperazine ring shows coplanarity of the atoms, while the two nitrogens are apart from this plane, -0.878(2)Å N(8) and 0.480(2)Å N(3).

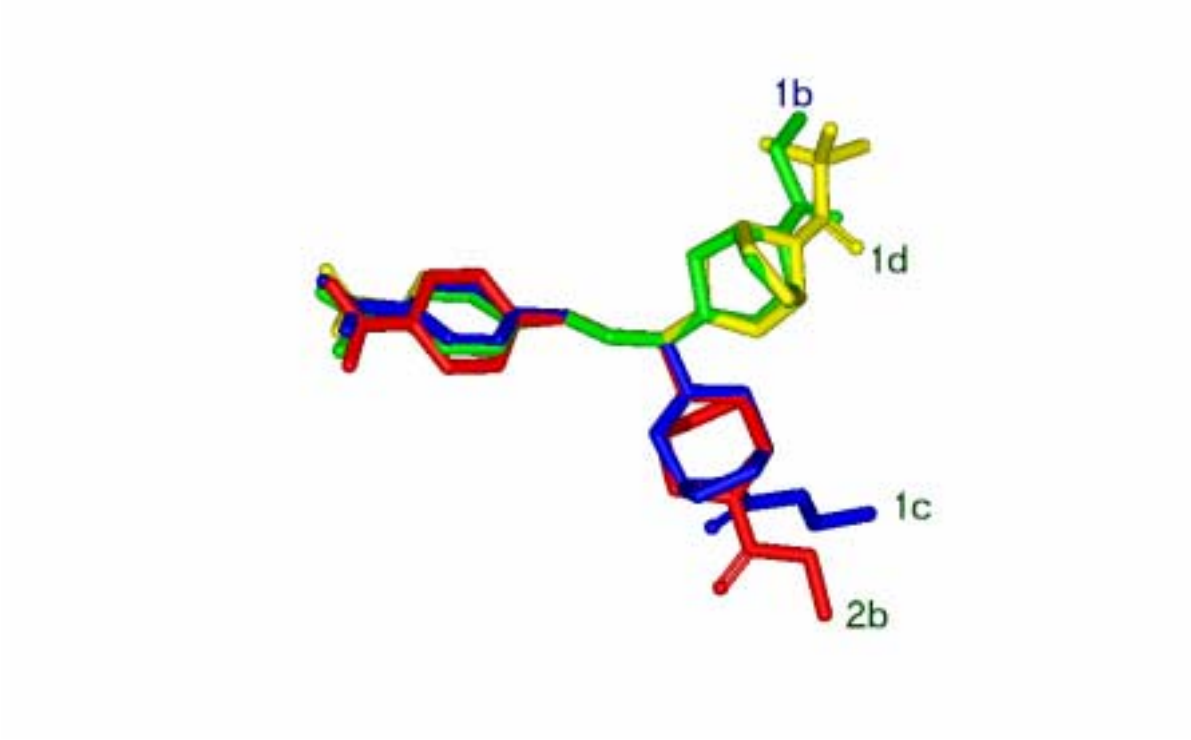


Figure 3. Superimposition of the structures (**1b**), (**1c**), (**1d**) and (**2b**).

The smaller deviation of N(3) could be related to the steric hindrance of the ethylene bridge present on the same side. In fact in **1b** the different location of the bridge allows a more symmetrical arrangement of the nitrogens with out of plane distances 0.704(9)Å for N(3) and –0.766(9)Å for N(8).

It is known that the nitrogen linked to a carbonyl group has a considerable double bond character due to the sp^2 hybridisation of the nitrogen, as in the present structure where N(3)-C(16) bond distance is 1.349(3) Å. This allows a planar arrangement of the N-C(=O)-C group (the range of the deviations from the best mean plane are –0.007(2)Å for C(16) to 0.002(2)Å for O(3)). The tertiary amine nitrogen N(8) instead being sp^3 hybridised presents a normal N-C single bond character (see Table 2).

The molecule, according to an MM2 theoretical study,[5] can in principle adopt two chair conformations with the substituents at the "amine" nitrogen either axial or equatorial. When the chain is equatorially oriented it has a higher degree of mobility then axially, due to steric reasons and in any case it seems uninfluent on the conformation of the bicyclic part as verified by the crystal structure of **2b** where the cinnamyl groups is equatorially oriented but with opposite orientation with respect to **1b**.

Table 2. Selected geometrical parameters for **2b**.

Bond distances (Å)			bond angles (°)	
N(1)	O(1)	1.215(3)	O(1)-N(1)-O(2)	122.9(3)
N(1)	O(2)	1.221(3)	N(3)-C(16)-O(3)	121.2(2)
N(1)	C(1)	1.468(3)	N(3)-C(16)-C(17)	118.0(2)
N(3)	C(11)	1.467(3)	N(8)-C(9)-C(8)	111.8(2)
N(3)	C(12)	1.472(3)	O(3)-C(16)-C(17)	120.7(2)
N(3)	C(16)	1.349(3)	C(4)-C(7)-C(8)	127.8(2)
N(8)	C(9)	1.464(3)	C(7)-C(8)-C(9)	123.7(2)
N(8)	C(10)	1.474(3)	C(10)-N(8)-C(13)	100.0(1)
N(8)	C(13)	1.473(3)	C(10)-C(15)-C(14)	103.8(2)
O(3)	C(16)	1.226(3)	C(11)-N(3)-C(12)	116.1(2)
C(4)	C(7)	1.469(3)	C(11)-N(3)-C(16)	124.1(2)
C(7)	C(8)	1.315(3)	C(13)-C(14)-C(15)	103.4(2)
C(8)	C(9)	1.500(3)		

The benzene ring is almost coplanar with the double bond C7-C8 as in **1b**, as shown by the torsion angle (τ_4, see Table 1). The *p*-nitro group is not coplanar with the benzene ring with deviations from the best mean plane of –0.024(2)Å N(1), -0.178(2)Å O(1) and 0.093(2)Å O(2). The *n*-propionyl substituent is rotated according to the torsion angles C(11)-N(3)-C(16)-O(3) of 168.9(2)° and C(12)-N(3)-C(16)-C(17) of 180.0(2)° as in **1b**. The crystal packing is characterized by π interactions among the *p*-nitrophenyl moieties that are regularly stacked at a distance of about 3.4Å (Figure 4). The molecular cohesion is also

reinforced by two intermolecular contacts involving the carbonyl oxygen C(10)-H(10)···O(3)' of 2.46(2)Å (' at 1+x, y, z and an angle of 155(1)°) and the oxygen of the nitro group C(13)-H(13)···O(1)" of 2.53(2)Å (" at –x, -y+z, -z+1 and an angle 156(2)°).

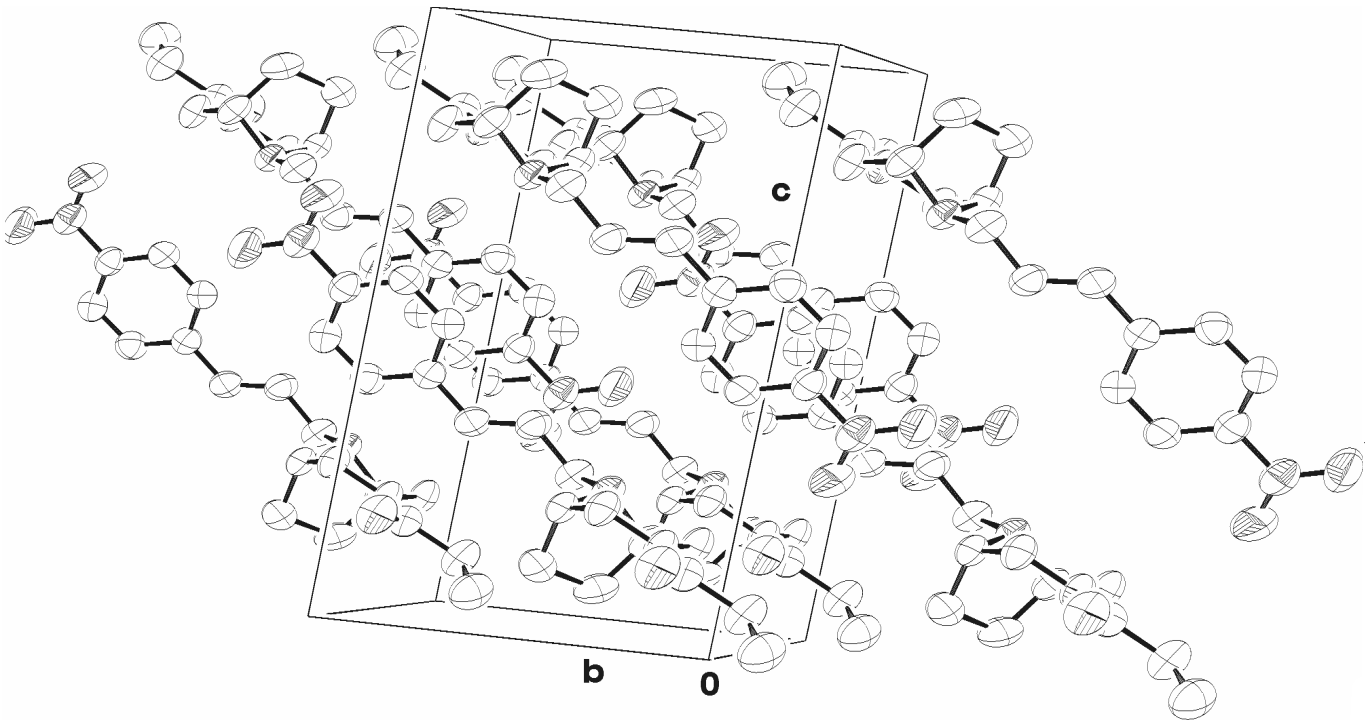


Figure 4. Crystal packing of **2b**.

Biological Results

The results of a binding study[3] give a slightly different affinity towards μ opioid receptors for **2b** compared to **1b** and morphine (Ki=5.1 nM, 25 nM and 2.5 nM respectively), while their affinity towards δ and κ receptors is negligible.

Theoretical Calculations

The conformational analysis on **2b** was made on the basis of the crystallographic results using the TINKER/SCAN[7] module to detect low-energy conformations, applying an energy window of 3.0 Kcal/mol. The resulting geometries were successively analysed using semiempirical methods (MOPAC[8]). In all calculations, the 8-nitrogen was considered protonated in order to simulate the physiological conditions, and the analysis was extended to the conformers with an axial arrangement of the aralkenyl chain in order to investigate the energy differences between the axial and equatorial structures. In addition, a comparison with the results obtained for **1b** having an inverted attachment of the cynnamyl moiety showed for the two conformations a difference in energy very low with ΔE of 2.31 Kcal for **1b** and 2.12 Kcal for **2b**, in agreement with the reduced steric hindrance of the ethylene bridge in both. A dihedral

driver analysis of τ_2 (MOPAC) on the **1b** and **2b** equatorial conformers showed as the energy minima are in agreement with the τ_2 values (see Table 1) resulting from the X-Ray analysis. The only difference between the **1b** and **2b** derivatives is in the distribution of the energy levels, being the difference in energy between the higher and lower values of 11.49 Kcal for **1b** and 2.33 Kcal for **2b**, suggesting for the first a reduced conformational freedom with respect to the second. For both **1b** and **2b** we have obtained some structures with the piperazine ring in boat conformation, all characterized by intramolecular hydrogen bond interaction between N(8) and O(3), leading to a low boat-chair energy difference (ΔE=1.17 and 1.77 Kcal for **1b** and **2b** respectively). In Table 3 are compared the significant distances for the pharmacophore groups in **1b** and **2b** for the ligand-receptor interactions obtained from X-Ray analysis and theoretical calculations. The inverted attachment of the cinnamyl moiety in **2b** with respect to **1b** induces a lengthening of the d_1 distance in **2b**, while for d_2 and d_3 the effect is opposite even though the reduction of the distance is more evident for d_3 for the chair conformation.

Table 3. Significant distances of the pharmacophore groups in **1b** and **2b.**

	d_1	d_2	d_3
2b X-ray	4.95	8.69	5.98
Chair	4.98	8.55	5.87
1b[a] X-ray	4.37	8.86	6.22
Chair	4.58(4.35)	8.79(8.85)	6.06(6.11)

[a]) in **1b** the position of the bridge is close to N(3).

The Connolly surfaces obtained with MEP (Molecular Electrostatic Potential analysis), (see Figure 5) show two negative electrostatic potential zones in the same position for **1b** and **2b**, but in the latter they are more defined. These results could be related to the difference in the values of the affinity constants.[1]

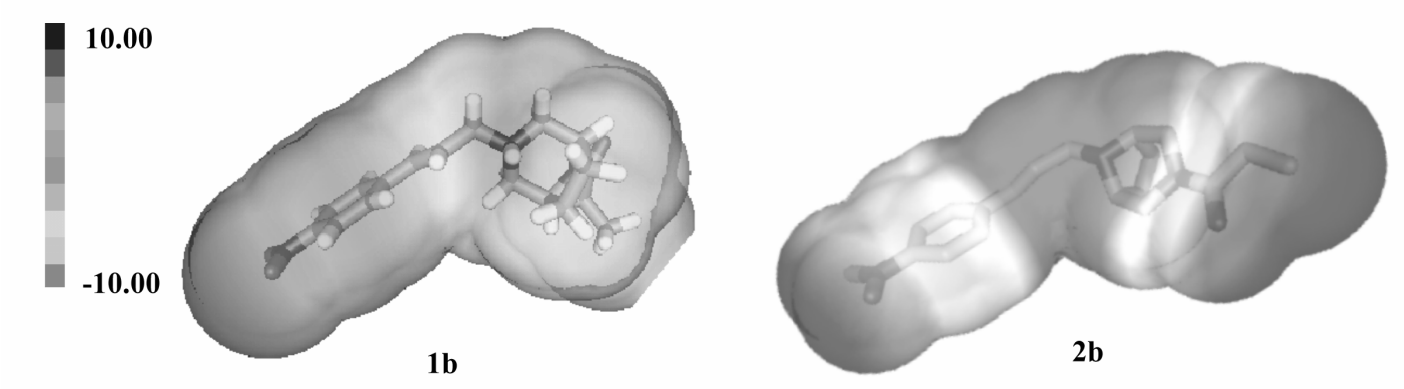


Figure 5. MEP Connolly surfaces[9] for **1b** and **2b.**

CONCLUSIONS

The compounds (**1b)** and (**2b)** show affinity towards the μ-receptors but with different orientation of the cinnamyl chain. The molecular geometry of **2b** is instead close to that of the compound (**1c)**. In the latter hydrogen bond interactions with water molecules which in principle could be responsible for observed molecular geometry are present in the crystal cell. In **2b** the packing is dominated by the molecular stacking of the phenyl rings (not present in **1b**). Binding studies[3] on **2b** and its isomer (**1b)** demonstrated a different potency in inducing physical dependence. The opposite orientation of the aralkenyl chain in **1b** and **2b** could be partially related to slightly different interactions with the receptor sites producing the different side effects, confirming the hypothesis of Pasternak *et al*.[6] on the existence of two functionally distinct μ receptor subtypes μ_1 and μ_2 where the latter should be responsible for dependence.

Table 4: Crystallographic data for **2b**

Empirical Formula	**$C_{18}H_{23}N_3O_3$**	**Crystal size (mm)**	**0.10 x 0.10 x 0.08**
Molecular weight	**329.40**	***Temperature (°C)***	**23**
Crystal system	**triclinic**	***Radiation (λ MoKα, Å)***	**0.71069**
Space group	$P\bar{1}$	***Diffractometer/scan***	**Enraf-Nonius**
Cell parameters (Å)			**CAD-4(ω/2θ)**
a	**7.257(2)**	***Scan width***	**1.2+0.35 tan ϑ**
b	**8.174(2)**	***Data Collected***	**(-9,10,0) to (9,10,19)**
c	**14.999(3)**	***θ range (°)***	**2.5-28**
α(°)	**93.33(2)**	***Reflections collected***	**3867**
β(°)	**92.80(1)**	***Reflections observed I>2σ(I)***	**3648**
γ(°)	**102.36(1)**	***R***	**0.06**
Volume ($Å^3$)	**865.8(1)**	***wR (on F^2)***	**0.14**
D_{calc} (Mg m^{-3})	**1.26**	**Gof. (F^2)**	**1.17**
Z	**2**		

$$R = \Sigma\left(\left|Fo\right| - \left|Fc\right|\right) \Big/ \Sigma\left|Fo\right|; \qquad wR = \left(\Sigma\, w\left(Fo^2 - Fc^2 \right)^2 \Big/ \Sigma\, wFo^2 \right)^{1/2}$$

EXPERIMENTAL

Chemistry

The synthesis and chemical properties of compound (**2b)** were previously reported.[2]

X-Ray Crystallography

A summary of the data collection and refinement process is in Table 4[¥]. The orientation matrix and cell dimensions were determined by least squares refinement of the angular positions of 20 reflections. The H atoms were located in difference Fourier synthesis and introduced at the observed positions. The structure was refined by full-matrix least squares using anisotropic temperature factors for non H atoms and isotropic for the H atoms. The programs used for structure solution and refinement were respectively MULTAN82,[10] PARST,[11] and SHELX76.[12] ORTEP[13] was used for the drawings.

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[¥] Supplementary material: Crystallographic data (excluding structure factors) for the crystal structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. 174071. Copies of the data can be obtained free of charge on application To the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (Fax: +44-(0) 12 23-33 60 33 or e-mail: deposit@ccdc.cam.ac.uk). The list of Fo/Fc-data is available from the author up to one year after the publication has appeared.

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