NMR Studies of Configuration and Tautomeric Equilibria in Nitroacridine Antitumor Agents

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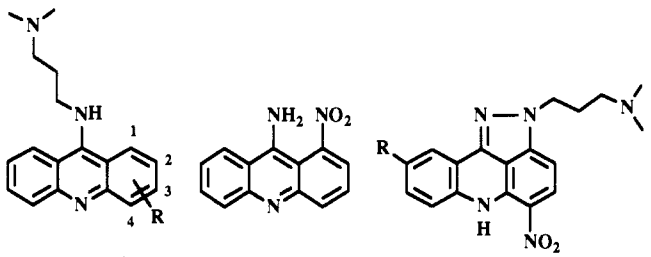
The solution configurations of the 1-nitroacridine nitracrine (a clinically used anticancer agent and experimental hypoxia-selective cytotoxin) and its nitro isomers were determined, as both free bases in $CDCl_3$ and as monocations (chromophore free base) and dications in D_2O, by high-resolution proton magnetic resonance spectroscopy. The free bases of the 1-, 2-, and 3-nitro isomers exist in the aminoacridine configuration in $CDCl_3$, while the 4-nitro isomer appears to exchange slowly between the aminoacridine and iminoacridan configurations. As cations at pH 2 in D_2O, all four isomers exist in the aminoacridine configuration. When the pH is increased to 7–8 to form the free bases of the nitroacridine chromophores, the 2- and 3-nitro isomers retain the aminoacridine configuration, but the 1- and 4-nitro isomers convert to the iminoacridan configuration. These results are relevant to the ongoing discussion of aminoacridine–iminoacridan tautomerism of these acridine derivatives in solution.

The nitroacridine derivative nitracrine (1) was originally developed as an antitumor agent by Ledochowski and co-workers,[1-3] who screened a large number of 9-[(dialkylamino)alkyl]acridines against the sarcoma 180 tumor line and showed the unique effects of a 1-nitro group. The markedly superior activity of the 1-nitro derivatives led to further studies being focused almost entirely on these compounds, and particularly on nitracrine itself. The unique biological properties of the 1-nitro derivatives have been attributed to the fact that crystallographic studies have shown these compounds[4,5] (but not the 2- or 3-nitro isomers)[6] to exist in the iminoacridan rather than the aminoacridine tautomeric form (Figure 1), with the nitro group twisted significantly out of coplanarity with the ring. It has been suggested[5] that such a conformation interacts with DNA (following reductive metabolism) to form particularly cytotoxic lesions.



1 : R = 1-NO_2
2 : R = 2-NO_2
3 : R = 3-NO_2
4 : R = 4-NO_2
5 : R = H

6

7 : R = H
8 : R = OMe

While the clinical use of nitracrine as a classical cytotoxic antitumor agent[7] has not been maintained, recent work has shown the compound to have a range of other biological properties. It is a very potent hypoxia-selective cytotoxin in vitro,[8] with some substituted analogues also showing this property in vivo.[9] It is also a very effective radiosensitizer of hypoxic mammalian cells.[10] The discovery of these additional properties of nitracine has prompted a reevaluation of the isomeric nitroacridines (2–4). Recent studies have shown that hypoxia-selective cytotoxicity is also exhibited by the 4-isomer (4)[11] and by a series of side-chain variants of the 1-isomer (where the best correlation between activity appeared to be with the proportion of iminoacridan tautomer present[12]). In addition, all four isomers have been shown to have radiosensitizing ability.[13]

Table I. ^{17}O NMR Data for Compounds 1–4

compd	chemical shift, ppm	torsion angle (Θ), deg
1	605	42
2	567	7
3	582	11
4	623	65

This revival of interest in the isomeric nitroacridines, and the apparent relationship between biological properties and configuration, has prompted us to undertake an NMR examination of their structure and tautomeric properties in solution, in order to provide comparative physicochemical data upon which to base structure–activity relationships.

Results and Discussion

Nitro Group Torsion Angles: ^{17}O NMR. Existing crystal structure data for nitracrine (1)[4,5] show that severe nonbonded interactions exist between the side chain and nitro group, resulting in a butterfly-shaped acridine ring system and an out-of-plane twist of 60° for the nitro group in the solid state. In contrast, the 2-isomer (2) has a planar ring system and coplanar nitro group.[6] No crystal structures have been reported for the 3- and 4-isomers.

To determine whether these solid-state structural properties were retained in solution, we studied the ^{17}O NMR spectra of the four isomeric nitroacridines (1–4), as the free bases. Balakrishnan and Boykin[14] have shown that, for benzenoid systems, nitro group ^{17}O chemical shifts

(1) Radzikowski, C; Ledochowski, A.; Hrabowska, M.; Horowska, B.; Stefanska, B.; Konopa, J.; Morowska, E. *Arch. Immunol. Ther. Exp.* **1969**, *17*, 99.
(2) Radzikowski, C.; Ledochowski, A.; Hrabowska, M.; Stefanska, B.; Horowska, B.; Konopa, J.; Morowska, E.; Urbanska, M. *Arch. Immunol. Ther. Exp.* **1969**, *19*, 219.
(3) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In, *Molecular Aspects of Anti-cancer Drug Action*; Neidle, S., and Waring, M. J., Eds.; MacMillan: London, 1983; pp 1–34.
(4) Dauter, Z.; Bogucka-Ledochowska, M.; Hempel, A.; Ledochowski, A.; Kosturkiewicz, A. *Rocz. Chem.* **1976**, *50*, 1573.
(5) Stekowski, J. J.; Kollat, P.; Bogucka-Ledochowska, M.; Glusker, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 2067.
(6) Hempel, A.; Hull, S. E.; Dauter, Z.; Bogucka-Ledochowska, M.; Konitz, A. *Acta Crystallogr.* **1979**, *B35*, 477.
(7) Warwas, M.; Narezewska, B.; Dobryszycka, W. *Arch. Immunol. Ther. Exp.* **1977**, *25*, 235.
(8) Wilson, W. R.; Denny, W. A.; Twigden, S. J.; Baguley, B. C.; Probert, J. C. *Br. J. Cancer* **1984**, *49*, 215.
(9) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 31.
(10) Roberts, P. B.; Anderson, R. F.; Wilson, W. R. *Int. J. Radiat. Biol.* **1987**, *51*, 641.
(11) Denny, W. A.; Wilson, W. R.; Atwell, G. J.; Boyd, M.; Pullen, S. M.; Anderson, R. F. NATO Advanced Study Series 413, in press.
(12) Denny, W. A.; Wilson, W. R.; Atwell, G. J.; Anderson, R. F. *J. Med. Chem.*, in press.
(13) Roberts, P. B.; Denny, W. A.; Wakelin, L. P. G.; Anderson, R. F.; Wilson, W. R. *Radiat. Res.*, in press.
(14) Balakrishnan, P.; Boykin, D. W. *J. Org. Chem.* **1986**, *50*, 3661.

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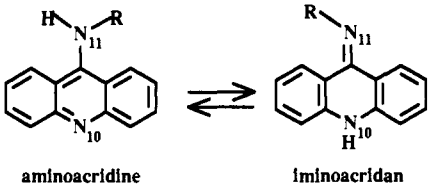


Figure 1. Tautomerism in 9-substituted acridines.

can be related to the nitro group torsion angle Θ (the extent to which it deviates from coplanarity with the aromatic ring) by the equation $\Theta = 1.29\delta - 739$. Although the ^{17}O resonances for the nitroacridines were significantly broader than that of nitrobenzene (used as a standard), the compounds fell quite clearly into two groups (Table I). The 2- and 3-nitro isomers (**2**, **3**) had resonances close to that of nitrobenzene, with twist angles Θ determined by the equation of 7° and 11°, respectively. Given the accuracy of the measurement, these are probably not significantly different from zero, implying that the nitro group in these isomers lies essentially coplanar. In contrast, the value of 42° calculated for nitracrine (1) and that of 64° for the 4-isomer (4) suggest severe out-of-plane distortion of the nitro group in these compounds. The ^{17}O NMR data reported here are in good agreement with the crystal-structure data for the 1- and 2-isomers with respect to nitro group twist angles, suggesting that the same tautomeric forms are present both in the solid state and in solution.

Imino-Amino Tautomerism: ^1H and ^{13}C NMR. Crystallographic studies of a number of nitroacridines have also provided information about the structural features governing the aminoacridine–iminoacridan tautomerism observed with these compounds (Figure 1). A crystal structure of nitracrine monohydrochloride (with the charge on the dimethylamino chain) shows a C9–N11 bond length (between the chromophore and the side chain) of 1.313 Å, considerably shorter than that for other 9-aminoacridines[6,15–17] and consistent with an iminoacridan tautomer. A later summary of several crystallographic studies showed that, for nitroacridines to adopt the iminoacridan form in the solid state, the chromophore had to be uncharged and possess both 1-nitro and 9-alkyl substituents.[5] Thus the dication of nitracrine (1) and the free bases of both the 2-isomer (**2**)[6] and 9-amino-1-nitroacridine (**6**)[5] (without a 9-alkyl substituent) all crystallize as the aminoacridine tautomer.

Evidence that the imino tautomer also persists in solution is more indirect, but has been provided by the kinetics of hydrolysis of nitro-substituted 9-aminoacridines.[18] These rates decrease with rising pH for 1-nitroacridines (as the more hydrolysis-resistant imino form is adopted) but increase with rising pH for isomeric 3-nitroacridines. One NMR study has been carried out[19] where the results of ^1H and ^{13}C NMR spectra of the monohydrochloride salt of nitracrine measured at various pH levels were interpreted as implying the existence of the imino tautomer.

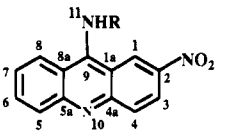
To provide direct comparative evidence for the conformation of the isomeric nitroacridines (1–4) in solution, we undertook detailed ^1H NMR studies in both $CDCl_3$ and D_2O. Resonances H5–H8 were expected to be generally the most diagnostic of amino or imino tautomer, since they would be least affected by the presence of the nitro group and most affected by the presence or absence of a proton at N10. It was therefore first necessary to assign these unambiguously, particularly since some conflict exists in the literature about the assignment of the aromatic protons in 9-aminoacridines.[20–22] This was carried out by using various 2D techniques, including a COLOC experiment, to establish long range C–H coupling for **2** in $CDCl_3$ (see Table II for data pertaining to compound **2**). In this compound, the bridgehead carbon at position 9 was easily assigned, and long-range (three-bond) C–H coupling was observed between this carbon and the proton resonances at δ 9.15 and 8.12 ppm, which could therefore be assigned to the protons at positions 1 and 8 (Table II). This allowed complete assignments for protons H5 to H8, which were in agreement with the original work of Kokko and Goldstein.[20] Once this was settled, other assignments followed, using 2D experiments (COSY and NOESY) and comparison with previous work.[19–22] In $CDCl_3$ solution, coupling of the N11 (amine) proton resonance to the α-CH_2 side-chain protons could also be observed.

A number of compounds, including the nitracrine isomers (1–4), were then studied in detail in $CDCl_3$ solvent (Table III). Pyrazoloacridine **7** and its 7-methoxy analogue **8** were used as examples of acridines fixed in the iminoacridan configuration. The 7-methoxy compound allowed unambiguous assignment of resonances H5–H8 (Table III), and calculations of the expected resonances for pyrazoloacridine (without the OMe group) were in good agreement with those observed. A cross peak between the N10 (imine) proton and H5 in the NOESY spectrum was also seen (see Figure 2).

The 2- and 3-nitro isomers (**2** and **3**), the desnitro isomer (**5**), and 1-nitro-9-aminoacridine (**6**) are known to exist in the aminoacridine configuration in the solid state[5] and are widely assumed to exist in this form in solution also. Assignment of all aromatic proton resonances for these compounds was accomplished with the aid of COSY experiments (Table III). COSY experiments also showed coupling of the N11 proton to the α-CH_2 of the side chain, confirming the aminoacridine configuration in solution (Figure 3).

Having established the expected pattern of chemical shifts for the H5 to H8 protons in closely-related com-

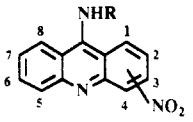
Table II. ^1H and ^{13}C NMR Data for Compound **2**



position	chemical shifts, ppm ^1H	chemical shifts, ppm ^{13}C	position	chemical shifts, ppm ^1H	chemical shifts, ppm ^{13}C
1a		112.5	5	7.97	129.5
1	9.15	122.8	6	7.69	131.6
2		140.8	7	7.29	122.5
3	8.27	122.8	8	8.12	124.2
4	7.90	130.2	8a		114.9
4a		151.2	9		154.5
5a		151.5			

(15) Talacki, R.; Carrell, H. L.; Glusker, J. P. *Acta Crystallogr.* **1974**, *B30*, 1044.
(16) Courseille, C.; Geoffre, S.; Busetta, B. *Cryst. Struct. Commun.* **1980**, *9*, 287.
(17) Pett, V. B.; Rossi, M.; Glusker, J. P.; Stezowski, J. J.; Boguc-ka-Ledochowska, M. *Bioorg. Chem.* **1982**, *11*, 443.
(18) Skonieczny, S. *Heterocycles* **1980**, *14*, 985.
(19) Wieczorkiewicz, T.; Lubas, B.; Siatecki, Z.; Kozlowski, H. *Acta Biochim. Pol.* **1983**, *30*, 71.
(20) Kokko, J. P.; Goldstein, J. H. *Spectrochim. Acta* **1963**, *19*, 1119.
(21) Acheson, R. M. In *Acridines*; Wiley-Interscience: New York 1973; p 687.
(22) Martin, R. F.; Kelly, D. P. *Aust. J. Chem.* **1979**, *32*, 2537.

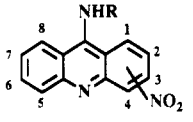
Table III. Aromatic Proton Resonances for the Free Bases of Compounds 1–7 in $CDCl_3$



compd	chemical shift, ppm									confign
	H1	H2	H3	H4	H5	H6	H7	H8	NH	
1		8.11	7.51	7.81	8.00	7.69	7.38	7.91	9.23	amino[a,b]
2	9.15		8.27	7.90	7.97	7.69	7.29	8.12	10.04	amino[a,b]
3	8.24	7.93		8.91	8.04	7.70	7.36	8.13	9.08	amino[a,b]
4 (major)	8.54	7.09	8.36		7.22	7.47	7.16	7.93	10.52	imino[a]
4 (minor)	8.27	7.23	7.89		8.02	7.65	7.31	8.01	9.01	amino[a,b]
5	8.01	7.22	7.59	8.05	8.05	7.59	7.22	8.01	8.17	amino[a,b]
6		8.21	7.63	7.69	8.03	7.78	7.52	7.96	5.82	amino[b]
7		6.57	7.94		7.31	7.43	7.27	8.04	10.29	imino[c,d]
8		6.65	8.01		7.34	7.11		7.55	10.41	imino[c,d]

[a] Inferred from the chemical shift data. [b] Determined by COSY; see supplementary material. [c] Determined NOESY; see supplementary material. [d] Locked in the iminoacridan configuration.

Table IV. Aromatic Proton Resonances for the Isomeric Nitroacridines in D_2O at Varying pH



compd	pH	chemical shift, ppm								confign[a]
		H1	H2	H3	H4	H5	H6	H7	H8	
1	2		8.24	7.99	8.04	7.81	7.99	7.62	8.27	amino
	7		7.37	7.50	7.36	7.29	7.55	7.18	7.81	imino
2	5	9.32		8.63	7.85	7.82	8.04	7.65	8.39	amino
	7	8.89		8.32	7.56	7.63	7.88	7.51	8.15	imino
3	5	8.53	8.19		8.53	7.80	8.02	7.63	8.36	amino
	7	8.11	7.85		8.17	7.63	7.84	7.48	8.08	amino
4	5	8.97	7.67	8.75		7.99	8.05	7.67	8.39	amino
	7	8.30	7.13	8.03		7.34	7.61	7.30	7.84	imino
7	5		6.39	7.48		6.98	7.32	7.22	7.55	imino

[a] Inferred from chemical shift data.

pounds of independently confirmed iminoacridan or aminoacridine configurations, we then studied the four isomeric nitroacridines (1–4). The ^1H NMR spectrum of 1 showed H5 to H8 resonances which were very similar to those of the known aminoacridines discussed above, particularly that of H5 at δ 8.00 ppm (Table III). Coupling of the N11 (amine) proton to the α-CH_2 of the side chain was observed, directly confirming an aminoacridine structure for the free base of 1 in dilute $CDCl_3$ solution. Through-space interaction was observed between the amine proton and H-8 in a NOESY experiment. Similar experiments also confirmed the existence of the aminoacridine configuration for compounds **2**, **3**, and **5** (bearing an alkyl side chain), since in dilute $CDCl_3$ solutions (0.01–0.05 M) coupling was observed between the α-CH_2 protons of the aliphatic side chain and the amine proton attached to N11. Increasing the concentration above ca. 0.05 M led to broadening and eventual disappearance of the NH peak but little change in the other resonances, except for the 1-nitro compound 1. In this case increasing the concentration to 0.1 M led to an upfield shift of the aromatic protons, particularly that of H5. Earlier experiments using such concentrated solutions caused us to previously[11] assign the iminoacridan configuration to the free base of 1 in $CDCl_3$. However, the present results (both chemical shift and COSY) clearly show that, at least in dilute $CDCl_3$, this compound exists in the aminoacridine configuration.

The NMR spectrum of 4 was more complex. At least two species could be observed, in unequal proportions. The aromatic resonances of protons H5–H8 in the major component were similar to those of pyrazoloacridine **7**, particularly that of H5 at δ 7.72 ppm, suggesting the imino configuration. NOESY experiments confirmed that the major component was the iminoacridan, since a cross peak was observed between the proton on N10 and H5 of the aromatic system. The minor component could, from COSY results, be the aminoacridine tautomer, suggesting a slow exchange between the two configurations for this particular compound.

We then studied the spectra of compounds 1–4 in D_2O solution at three different pHs. Since[13] the pK_as of the nitroacridine chromophores in these compounds lie between 6 and 7.5, the compounds will exist as dications (with both the chromophore and side chain charged) at pH 2, but essentially as monocations (chromophore uncharged) at pH 7–8. The NMR of nitracrine itself (1) at varying pHs has been examined by Wieczorkiewicz,[19] but no comparative studies between the isomers 1–4 have been done. The HCl salts of these compounds were dissolved in D_2O to give solutions of pH ca. 5, and the pH of samples was adjusted to either ca. 2 or ca. 8. ^1H NMR spectra were measured for all three samples in each case, and COSY experiments were also carried out where needed for assignment. The ^1H spectrum of the HCl salt of pyrazoloacridine **7** was also measured, to provide the resonance positions for a compound in the iminoacridan configuration. The data are shown in Table IV. For compounds 2–4 there was little difference between spectra taken at pH 2 and 5, since the pK_as of the chromophores are ca.

7 and they will be protonated at both pHs. Generally, only the data at pH 5 are given. There was a difference between pH 2 and 5 for nitracrine, since the pK_a of the chromophore is 6.2, and the data for pH 2 are given (Table IV). At the lower pH, where all the compounds are dications, the H5 resonance in particular occurred at very similar positions (7.99–7.81 ppm). On addition of NaOH to raise the pH to ca. 7–8, all the chemical shifts moved upfield. For compounds **2** and **3**, which exist in the aminoacridine configuration as free bases in $CDCl_3$, the average $\Delta\delta$ for the H5–H8 aromatic protons was ca. 0.2 ppm, due primarily to deprotonation of the chromophore. For **1** and **4** the overall decrease in δ was generally greater, but was particularly large for both the H5 and H8 protons (Table IV), with the H5 resonance position moving close to that seen for H5 in the model iminoacridan compound **7** (Table IV). On this evidence, it seems likely that both the 1- and 4-nitro isomers adopt the iminoacridan configuration as free bases in aqueous solution.

Conclusions

9-(Alkylamino)acridines can exist in either the aminoacridine or iminoacridan configuration, and correlations between structure and configuration adopted in the solid state have been elucidated.[5] The apparent relationship between biological properties and configuration have made it important to see whether the same configurations are adopted in solution. We have shown that use of a variety of 2D ^1H and ^{13}C NMR techniques allows the unambiguous assignment of the H5 to H8 aromatic proton resonances for a number of 9-(alkylamino)acridines, and that the chemical shifts of these protons can be used to assign configurations in solution.

Dilute solutions of the free bases of the 1-, 2-, and 3-nitro isomers of 9-(alkylamino)acridine in $CDCl_3$ exist in the aminoacridine configuration (although in concentrated solutions the 1-nitro isomer[1] may be in the imino form), while the 4-nitro isomer appears to exchange slowly between both imino and amino forms. All four isomers exist in the aminoacridine conformation as cations at pH 2 in D_2O. When the pH of these D_2O solutions is increased to 7–8, the 2- and 3-nitro isomers (**2** and **3**) retain the aminoacridine configuration, but the 1- and 4-nitro isomers (**1** and **4**) convert to the iminoacridan configuration. This is the most significant finding, since the 1-nitro isomer of nitracrine (**1**) has by far the lowest aqueous pK_a of the four isomers (6.21). Under physiological conditions it will therefore exist primarily as the free base, and thus in the iminoacridan configuration. This may be a factor in its extraordinary potency compared with the isomers **2**–**4**.[13]

Experimental Section

Syntheses of nitroacridines **1**–**6** have been reported previously.[23] Pyrazoloacridines **7** and **8** were kindly provided by Dr. Les Werbel of Warner-Lambert/Parke-Davis. NMR studies were carried out on a Bruker AM-400. For D_2O studies, the dihydrochloride salt of a compound (2 mg) was dissolved in D_2O (1 mL) to give a solution of pH ca. 5, and this was adjusted to either ca. 2 or ca. 7–8 by addition of 0.1 N aqueous HCl or NaOH.

^1H and ^{13}C NMR spectra were recorded at 22 °C with a Bruker 400 AM (^1H, 400.13 MHz; ^{13}C, 100.62 MHz) spectrometer equipped with an ASPECT 3000 data system using a 5-mm $^1H/^{13}C$ dual probe. Samples of 0.05 M ($CDCl_3$) or 0.02 M solutions (D_2O) were used with TMS internal reference ($CDCl_3$) or trimethylsilyl tetradeuteriopropionate (TSP) external reference (D_2O). Data were collected and processed with the DISNMR program. Resonances were assigned by selective decoupling and the simulation program PANIC, available in the Bruker software.

1D Spectra. Typical ^1H FT conditions were as follows: pulse width, 3 μs; flip angle, 30°; sweep width, 6024.096 Hz; data points, 32K; acquisition time, 2.72 s; resolution, 0.37 Hz per point. ^{13}C conditions for BB decoupled and DEPT NMR experiments were as follows: 90° pulse, 5.5 μs; decoupler pulse width, 15 μs; sweep width, 25 000 Hz; data points, 64K; acquisition time, 1.31 s; resolution, 0.76 Hz per point.

2D Spectra. All two-dimensional spectra (COSY, NOESY, and heteronuclear shift correlation) were obtained with Bruker software. COSY[24] and NOESY spectra were acquired with 32 scans over 1024 data points and 4310.345 Hz for each of the 256 values of evolution time (t_1). The FIDs were zero-filled and a sine-bell window function was used before FT. Data were symmetrized prior to plotting. For NOESY spectra, a mixing time of 1.0 s was randomly modulated by ± 2%.

^1H–^{13}C correlation spectra were acquired with XHCORRD[25-27] with 192 scans over 1024 data points and 11 363.636 Hz for each of the 256 values of evolution time. The spectral width in the second dimension was 3787.878 Hz. An unshifted sine-bell window function was used before FT. Long-range ^1H–^{13}C chemical shift correlation spectra were obtained with the COLOC[28] pulse sequence. The FIDs were acquired with 320 scans over 2048 data points and 14 285.714 Hz for each of 128 values of evolution time. The spectral width in the second dimension was 3787.878 Hz. An unshifted sine-bell window function was used before FT.

Natural-abundance ^{17}O spectra were measured at 54.23 MHz in 10-mm tubes with a tuneable broad-band probe. Solutions were ca. 100 mM in CH_3CN, containing 10% CD_3CN to provide a lock signal. The probe temperature was 70 °C, and the spectra were referenced to external D_2O at 70 °C, over a spectral width of 8000 Hz, using 4K data points zero-filled to 8K. The pulse width was 15.0 μs, with an acquisition time of 0.25 s. Signal-to-noise ratios of 2–3 to 1 and peak widths of 700–1000 Hz were obtained with 300 000–800 000 scans and an exponential line broadening of 10–20 Hz. Under these conditions, the ^{17}O resonance of nitrobenzene occurred at δ 575 ppm (lit.[14] value δ 576 ppm).

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Supplementary Material Available: 2D NOESY spectrum of compound (**8**) and 2D COSY spectrum of compound (**2**) (3 pages). Ordering information is given on any current masthead page.

(23) Ledochowski, A.; Stefanska, B. *Ann. Soc. Chim. Pol.* **1966**, *40*, 301.
(24) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(25) Bax, A. *J. Magn. Reson.* **1983**, *53*, 517.
(26) Rutar, V. *J. Magn. Reson.* **1984**, *58*, 306.
(27) Wilde, J. A.; Bolton, P. H. *J. Magn. Reson.* **1984**, *59*, 343.
(28) Kessler, H.; Griesling, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 337.