Pergamon

Tetrahedron, Vol. 52, No. 16, pp. 5691-5698, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00205-0

Nuclear Magnetic Resonance Investigations of Azo-Hydrazone, Acid-Base Equilibria of FD&C Yellow No. 6

Eugene P. Mazzola,* Saleh A. Turujman, Sandra J. Bell, Julie N. Barrows, John E. Bailey, Jr., Ely G. Mouchahoir, Steven Prescott, Mark D. Archer, and Mark Oliver

Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C St., S.W., Washington, DC 20204, USA

William F. Reynolds and Kathy W. Nielsen

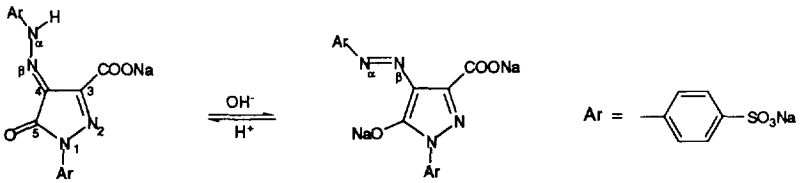
Department of Chemistry, University of Toronto
Toronto, Ontario, Canada M5S 1A1

Key words: azo-hydrazone equilibrium; dynamic NMR

Abstract: *Azo-hydrazone, acid-base equilibria were examined for the water-soluble dye FD&C Yellow No. 6 (Y6), with the observation of dynamic NMR effects near, and at, pH 12, where the dye converts from a predominantly hydrazone tautomer (acidic to moderately basic pH) to a prevailing azo-anion resonance hybrid (high pH). Semilogarithmatic pH-^{13}C chemical shift plots and a 2-bond, $^{15}N\beta$-$^{13}C_{8a}$ coupling constants vs. pH relationship both indicate that the midpoint of the azo-hydrazone equilibrium occurs at* ca. *pH 12. Potentiometric titrations further indicate that the pK_a of Y6 is* ca. *12. These data suggest that the observed NMR line broadening is due to slow proton transfer between the hydrazone-NH and water.*

INTRODUCTION

Compounds exhibiting azo-hydrazone tautomerism have been extensively investigated in a variety of organic solvent systems.[1] However, partly because of the difficulty of obtaining IR spectra of aqueous solutions, their water-soluble analogs were long neglected and have only recently begun to be studied.[2] We have earlier examined the azo-hydrazone, acid-base equilibria of FD&C Yellow No. 5 (Y5, **1**) by ^{15}N NMR



1a **1b**

Fig. 1. Hydrazone-azo equilibrium of Yellow No. 5 (Y5).

and found it to exist predominantly as a hydrazone below pH 9, as shown in the *anti*-configuration, **1a**, and almost exclusively as an azo-anion, *e.g.*, **1b**, above pH 11 (Figure 1).[2]

We now report on the investigation of the bisaryl "azo" compound FD&C Yellow No. 6, (Y6, **2**), which exhibits similar behavior. ^{15}N and ^{13}C NMR were used to characterize Y6 at a variety of pH values between 7 and 14. Total ^{13}C NMR signal assignments were made on the basis of direct and long-range heteronuclear chemical shift correlation experiments and observed couplings in the β-^{15}N-labeled isotopomer of Y6 ([β-^{15}N]-Y6).

RESULTS AND DISCUSSION

1. *^{15}N NMR Spectra and Signal Assignments*

The pH values at which ^{15}N NMR spectra were recorded were selected on the basis of preliminary ^{13}C NMR spectral results. The latter exhibited broadening of most signals between pH 11 and 13, with maximum line broadening observed at pH 12. ^{15}N NMR spectra were, therefore, determined at pH 7, where Y6 was believed to occur predominantly as a hydrazone tautomer, and at pH 14, where an azo-anion resonance hybrid would be the major contributor to its overall structure.

The ^{15}N NMR spectra of Y6, like those of Y5, exhibit significant change from pH 7 to 14. At pH 7, chemical shift values are -179.6 and -4.0 ppm (relative to $NH_4{}^{15}NO_3$). The former is typical of those reported for hydrazone sp^3-hybridized nitrogens, whereas the latter is characteristic of imino nitrogens.[3] These resonances are similar to those of Y5 at pH 7 [**1a**: -168.9 (N_α) and -11.2 ppm (N_β)][2] and were likewise assigned to the α- and β-nitrogens, respectively. Moreover, these chemical shifts strongly suggest that Y6 also exists as a hydrazone at pH 7. At pH 14, signals were observed at 111 and 106.6 ppm (also relative to $NH_4{}^{15}NO_3$), which are in the range found for azo compounds.[4] They were assigned by comparison with the chemical shift of [β-^{15}N]-Y6, which appears at 106.4 ppm. The resonance at 106.6 ppm was, therefore, assigned to the β-nitrogen. These chemical shifts similarly indicate that Y6 occurs as an azo-anion at high pH. The extremely large changes in ^{15}N chemical shifts with pH (*ca.* 290 ppm for N_α), corresponding to a major fraction of the total ^{15}N chemical shift range,[5] confirm that deprotonation in Y6 is associated with substantial electronic reorganization.

2. *^{13}C and ^{1}H NMR Spectra and Signal Assignments*

The ^{13}C NMR spectrum of Y6 at pH 7 is consistent with the previous inference that the dye exists principally as a hydrazone at this pH (Table 1). As the pH is increased to 12, most of the ^{13}C NMR signals exhibit broadening along with either upfield or downfield shifts. As stated above, maximum line broadening occurs at pH 12. As the solution pH is further increased to 14, the broadened resonances sharpen and continue their respective migrations. This dynamic behavior suggests the existence of one or more intermediate (on the NMR timescale) equilibrium processes.[6]

Table 1. ^{1}H and ^{13}C NMR Data[a] for Compounds **2a** and **2b**.

	2a[b]		**2a,2b**	**2b**[c]	
Position	^{1}H	^{13}C	FLOCK	^{1}H	^{13}C
1		129.2	H-3, H-8		134.1
2		178.9	H-3, H-4		168.2
3	5.71 d (9.5)	126.4	H-4	6.83 d (9.3)	129.3
4	6.69 d (9.5)	143.0	H-5	7.55 d (9.3)	136.4
4a		127.4	H-3, H-8		125.5
5	7.15 d (1.6)	126.8	H-7	8.05 d (2)	126.6
6		141.0	H-8		136.0
7	7.30 dd (8,1.6)	126.6	H-5	7.93 dd (8.9,2)	124.6
8	7.24 d (8)	122.4		8.73 d (8.9)	123.7
8a		134.5	H-4, H-5, H-7		133.9
1'		143.2	H-3'/5'		156.1
2'/6'	6.54 d (8.4)	117.1	H-2', H-6'	7.54 d (8.5)	121.9
3'/5'	7.32 d (8.4)	127.7	H-3', H-5'	7.75 d (8.5)	127.2
4'		140.5	H-2'/6'		141.7

[a]Chemical shifts referenced to dioxane at 3.70 ppm (^{1}H) and 67.4 ppm (^{13}C); J-values in Hz in parentheses. [b]In D_2O at pH 7. [c]In D_2O at pH 14.

^{13}C and ^{1}H NMR signals of Y6 at pH 7 (Table 1) were assigned by means of homonuclear (COSY), direct heteronuclear (HETCOR), and long-range, heteronuclear chemical shift correlation (FLOCK[7]) experiments with the distinctive 3-spin system comprising protons 5, 7, and 8 serving as a starting point. The assignment of selected ^{13}C and ^{1}H NMR signals is described below. Comparison of the ^{13}C NMR spectra of Y6 and its β-^{15}N-labeled isotopomer ([β-^{15}N]-Y6 at pH 7) revealed (i) a 5-Hz coupling in the signal at 143.2 ppm (quaternary carbon) and (ii) considerable broadening in the double-intensity resonance at 117.1 ppm for [β-^{15}N]-Y6, whereas only single lines were observed in the corresponding signals for Y6. The former resonance line was then ascribed to C-1' and the latter to C-2'/6'.

Carbons 1 and 4a could not be differentiated either by the FLOCK experiment, because both carbons display correlations with protons 3 and 8 (Table 1), or by couplings to the β-nitrogen in [β-^{15}N]-Y6, because both are less than 1 Hz. As expected, possible 2-bond correlations between C-4a and H-4 and/or H-5 were not observed because of negligible coupling between C-4a and these protons.[8] Assignment of the lower field

signals of Y6 at pH 7 and 14 (129.2 and 134.0 ppm, respectively) to C-1 and the higher field resonances (127.4 and 125.5 ppm, respectively) to C-4a was based on the following line of reasoning. Spin-lattice relaxation times, T_1, were determined at these pH values. At pH 7, the signal at 129.2 ppm had a T_1 value of 1.4 s; the value at 127.4 ppm was 0.9 s. At pH 14, the resonance at 134.0 ppm had a T_1 value of 2.3 s; the value at 125.5 ppm was 1.4 s. Examination of Dreiding models of the hydrazone and azo forms of Y6 demonstrated that C-1 is situated *ca.* 2.7 Å from H-8 and C-4a is located *ca.* 2.1 Å from both H-4 and H-5. Because of the considerable decrease in effectiveness of the dipole-dipole relaxation mechanism with increasing internuclear distance,[9] C-4a would be expected to have a shorter T_1 value than C-1. The remainder of the signals of Y6 at pH 14 (Table 1) were then assigned by means of the same chemical shift correlation experiments described above.

3. *Molecular Conformations and Configurations*

^{13}C and ^{15}N NMR have provided evidence that Y6 exists predominantly as a hydrazone at pH 7 (*vide supra*). Examination of the 2-bond coupling constants between the β-nitrogen and carbons 2 and 8a in the ^{13}C NMR spectrum of [β-^{15}N]-Y6 at this pH reveals an 8.6-Hz coupling to C-8a and essentially no coupling to C-2 (Table 2). The difference in magnitude between these two coupling constants indicates that C-8a is situated *cis* to the lone-pair electrons of the β-nitrogen and that Y6, therefore, occurs as the *syn*-hydrazone (**2a**) at pH 7.[10]

Table 2. Y6 ^{13}C-^{15}N Coupling Constants.

pH	$^2J_{N\beta C_{8a}}$	$^2J_{N\beta C_2}$
7.0	8.6	0
11.5	7.2	-[a]
12.0	5.7	-[a]
12.26	5.2	-[a]
13.0	3.5	<1
14.0	3.0	<1

[a]Could not be determined because of extensive line broadening.

Y6 has also been inferred to be present as an azo-anion species at pH 14. The corresponding couplings between the β-nitrogen and carbons 2 and 8a at this pH are the following: 3 Hz to C-8a and essentially zero to C-2 (Table 2). These data suggest that C-8a is similarly located *cis* to the β-nitrogen lone-pair electrons and that Y6 exists as the *trans*-azo compound (**2b**) at pH 14.[10]

4. *Dynamic NMR Spectra*

A final matter to be addressed concerns the nature of the interconversion of hydrazone and azo species with changing pH. As previously mentioned, Y6 exhibits significant ^{13}C NMR line broadening between pH 11 and 13. Both the 2-bond $N\beta$-C_{8a} coupling constant data and ^{13}C NMR signal broadening can be ascribed to an acid-base equilibrium involving species **2a** and **2b** depicted in Figure 2. Two experimental

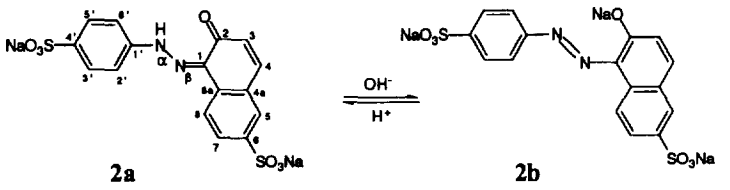


Fig. 2. Hydrazone-azo equilibrium of Yellow No. 6 (Y6).

observations support this interpretation. First, the pK_a for Y6 has been determined potentiometrically to be *ca.* 12.[11] This figure can be regarded only as approximate, however, because it lies outside the range of pK_a values normally accessible by titrations of this type.

Second, semilogarithmatic plots of the (absolute value of) log $[(\delta_{pH\ 10} - \delta)/(\delta - \delta_{pH\ 14})]$ *versus* pH,[12] which is derived from the Henderson-Hasselbalch equation,[13] have been constructed for carbons 3, 4a, and 7 over the pH range 11-13 (Table 3). The V-shaped plots (Fig. 3) which result are typical of those observed

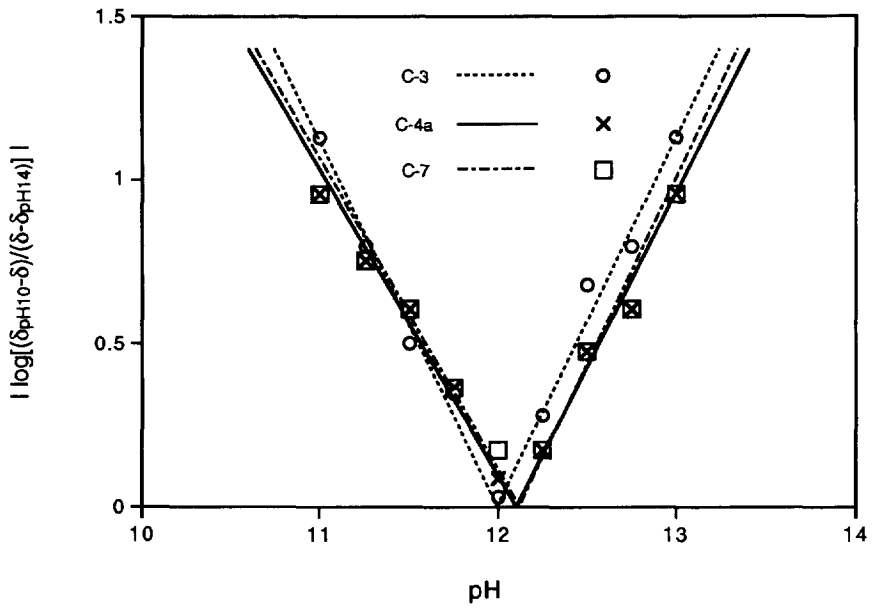


Fig. 3. pK_a Determination for Y6 using the Data of Table 3.

for acid-base systems and support the above, potentiometrically determined, pK_a value of *ca.* 12. This agreement lends further credence to the conclusion that an acid-base equilibrium exists between **2a** and **2b**. It should be noted that carbons 3, 4a, and 7 were selected for examination over those exhibiting even larger chemical shift separations, *viz.*, carbons 1, 4, 6, and 2'/6,' because their chemical shifts could be precisely determined over a range of pH values without uncertainties due to accidental signal overlap and appreciable

Table 3. Y6 pH-Chemical Shift Data

	C-3		C-4a		C-7	
pH	δ	$\log(\delta^a-\delta)/(\delta-\delta^b)$	δ	$\log(\delta^a-\delta)/(\delta-\delta^b)$	δ	$\log(\delta^a-\delta)/(\delta-\delta^b)$
10.0	126.4	-	127.4	-	126.6	-
11.0	126.6	1.130	127.3	0.954	126.4	0.954
11.25	126.8	0.796	127.2	0.753	126.3	0.753
11.5	127.1	0.497	127.1	0.602	126.2	0.602
11.75	127.3	0.347	126.9	0.368	126.0	0.368
12.0	127.9	0.030	126.6	0.087	125.8	0.176
12.25	128.3	0.279	126.3	0.176	125.4	0.176
12.5	128.8	0.681	126.0	0.477	125.1	0.477
12.75	128.9	0.796	125.9	0.602	125.0	0.602
13.0	129.1	1.130	125.7	0.954	124.8	0.954
14.0	129.3	-	125.5	-	124.6	-

[a]pH 10. [b]pH 14.

line broadening. The Y6 system is atypical in that the azo-anion conjugate base (**2b**) is considerably different structurally from the hydrazone acid (**2a**) from which it is derived. The relatively fast (although still slow on the ^{13}C NMR timescale) proton transfer between the hydrazone-NH and water was unexpected since corresponding tautomeric equilibria for organic solvent-soluble analogs of this dye typically exhibit *separate* ^{13}C NMR signals for each tautomer.[14] However, similar dynamic effects have been observed for a considerably different, nitrogen-acid system, *viz.*, a protonated *tertiary*-nitrogen aniline.[15]

The existence of signal broadening in the ^{13}C NMR spectra raises the possibility of calculating average lifetimes of the hydrazone and azo-anion species. The standard practice of variable-temperature experiments is largely precluded in these systems because of both the temperature dependence of solution pH and the difficulty of buffering over the pH range 10-14. As an approximate alternative, the linewidths of nine carbon resonances were measured at pH 10, 12, and 14. If the assumption again is made that the pK_a occurs at pH 12, these linewidths, together with the chemical shift separations, can be inserted into the following equation for the *near-fast* exchange regime:[16]

$$(T_{2'})^{-1} = N_A(T_{2_A})^{-1} + N_B(T_{2_B})^{-1} + N_A^2 N_B^2 (\omega_A - \omega_B)^2 (\tau_A + \tau_B) \quad (1)$$

where N_A and N_B are the respective mole fractions of the hydrazone and azo species, T_{2_A} and T_{2_B} are the natural linewidths, ω_A and ω_B represent the chemical shift separation, $T_{2'}$ is the linewidth at pH 12, and τ_A and τ_B are the lifetimes. Average lifetimes ($1/2\ [\tau_A + \tau_B]$) were obtained which are in relatively good agreement with each other (Table 4) and are, for the sake of consistency, also in the range of $0.1\text{-}10^{-5}$ s, where dynamic NMR effects are observed.[6c] Because the occurrence of dynamic NMR effects is dependent on the chemical shift separation of individual resonances in exchanging species, the relative amounts of these interconverting species, and their rate of exchange, it is noteworthy that the ^{15}N NMR signal for N_α (but not for N_β) is noticeably broadened at pH 14. Even though one would expect only *ca.* 1% of the conjugate acid

hydrazone to be present at pH 14, the chemical shift difference for N_α in the hydrazone and azo-anion forms is apparently so large (*vide supra*) that the effects of incomplete averaging are observed in this case.

Table 4. Y6 Azo-Hydrazone Average Lifetime Calculations.

Position	$W_{H/2}$(pH 10)	$W_{H/2}$(pH 14)	$W_{H/2}$(pH 12)	$\Delta\delta$ (Hz)	τ (ms)
5	3.4	3.3	3.3	26	-[a]
3'/5'	3.4	2.6	3.0	56	-[a]
8a	1.9	1.8	4.3	81	2.9
4'	1.9	2.1	5.2	100	2.6
4a	1.8	1.1	9.5	211	1.4
7	3.2	3.0	12.6	217	1.6
3	4.0	3.6	17.8	246	1.9
1	1.7	1.8	*ca.* 40	454	1.5
2'/6'	4.5	3.5	68	455	2.5
6	1.6	2.1	*ca.* 50	503	1.5
4	4.9	2.2	*ca.* 75	674	1.3
2	1.9	1.6	-[b]	1079	-
1'	2.1	2.8	-[b]	1256	-

[a]Not exchange broadened. [b]Too Broad to be observed.

EXPERIMENTAL

The disodium salt of 2-hydroxy-1-[(4-sulfophenyl)azo]-6-naphthalenesulfonic acid (Y6) (Hilton-Davis) was used without further purification. The β-^{15}N-labeled isotopomer ([β-^{15}N]-Y6) was prepared by diazotization of 4-aminobenzenesulfonic acid (Fisher) with $Na^{15}NO_2$ (95 at.% ^{15}N, Merck) and coupling to 6-hydroxy-2-naphthalenesulfonic acid, sodium salt (Hilton-Davis).

Proton, ^{13}C, and ^{15}N NMR spectra were recorded at 400, 100, and 40.6 MHz, respectively, in D_2O on a Varian NMR Instruments VXR-400 spectrometer (Table 1). Both proton and ^{13}C chemical shifts are reported relative to TMS. ^{15}N chemical shifts are reported relative to the $^{15}NO_3^-$ resonance; aqueous, saturated ammonium [^{15}N]nitrate solution was the external standard.

COSY NMR spectra were recorded with a spectral width of 1000 Hz in each domain and with 512 data points in the F2 dimension. A ^{1}H pulse width of 30 μs (90°) and a 1-s repetition rate were used to acquire 512 incremented proton NMR spectra of 64 scans each. Free-induction decays were processed as a 1024 X 1024 matrix with appropriate zero filling and pseudo-echo weighting.

Directly bonded, heteronuclear chemical shift correlation NMR spectra were obtained at 100.6 MHz (XL-400) with spectral widths of 3150 and 960 Hz in the carbon and proton dimensions, respectively, and with 256 data points in the ^{13}C dimension. Sixty-four incremented ^{13}C spectra of 32 scans each were acquired by using 11.5-μs (90°) ^{1}H pulse widths and a 1-s repetition rate. Free-induction decays in both dimensions were processed as a 128 X 512 matrix with appropriate zero filling and modified pseudo-echo weighting. The value $^1J(CH)$ = 165 Hz was used for calculating the delays Δ_1 and Δ_2.

Long-range, heteronuclear chemical shift correlation NMR spectra were recorded at 100.6 MHz (XL-400) with spectral widths of 7450 and 960 Hz in the carbon and proton dimensions, respectively, and with 1024 data points in the ^{13}C dimension; 192 incremented ^{13}C spectra of 256 scans each were acquired by using 11.5-μs (90°) ^{1}H pulse widths and a 1-s repetition rate. Free-induction decays in both dimensions were processed as a 512 X 2048 matrix with appropriate zero filling and modified pseudo-echo weighting. The value $^{n}J(CH) = 7.5$ Hz was used for calculating the delays Δ_1 and Δ_2, and $^{1}J(CH) = 165$ Hz was used for τ in the bilinear rotation decoupling (BIRD)[17] pulses.

REFERENCES

1. Ball, P.; Nichols, C. H. *Dyes Pigm.* **1982**, *3*, 5-26.
2. Bell, S. J.; Mazzola, E. P.; Coxon, B. *Dyes Pigm.* **1989**, *11*, 93-99.
3. Westerman, P. W.; Botto, R. E.; Roberts, J. D. *J. Org. Chem.* **1978**, *43*, 2590-2596.
4. (a) Porter, N. A.; DuBay, G. R.; Green, J. G. *J. Am. Chem. Soc.* **1978**, *100*, 920-925. (b) Duthaler, R. O.; Roberts, J. D. *Ibid.* **1978**, *100*, 4969-4973.
5. Levy, G. C.; Lichter, R. L. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; Wiley-Interscience: New York, 1979; Chapter 3.
6. (a) Gutowsky, H. S.; Holm, C. H. *J. Chem. Phys.* **1956**, *25*, 1228-1234. (b) McConnell, H. M., *Ibid.* **1958**, *28*, 430-431. (c) Drago, R. S. *Physical Methods for Chemists*, 2nd Ed.; Saunders: New York, 1992; pp 95-98.
7. Reynolds, W. F.; McLean, S.; Perpick, M.; Enriquez, R. G. *Magn. Reson. Chem.* **1989**, *27*, 162-169.
8. (a) Hansen, P. E. *Progr. Nucl. Magn. Reson. Spectrosc.* **1981**, *14*, 175-296. (b) Marshall, J. L. *Carbon-Carbon and Carbon-Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis*; VCH Publishers: Deerfield Beach, FL, 1983; pp 42-48.
9. Farrar, T. C.; Becker, E. D. *Pulse and Fourier Transform NMR*; Academic Press: New York, 1971; pp 53-58.
10. (a) Pregosin, P. S.; Randall, E. W.; White, A. I. *J. Chem. Soc. Perkin II* **1972**, 1-4. (b) Buchanan, G. W.; Dawson, B. A. *Can. J. Chem.* **1977**, *55*, 1437-1439. (c) Buchanan, G. W.; Dawson, B. A. *Org. Magn. Reson.* **1980**, *13*, 293-298.
11. Microlit Laboratories, Madison, NJ.
12. (a) Cohen, J. S.; Shrager, R. L.; McNeel, M.; Schechter, A. N. *Nature* **1970**, *228*, 642-644. (b) Finer, E. G. *Macromolecules and Solids*; in R. K. Harris, ed., *Nuclear Magnetic Resonance*, Vol. 1. The Chemical Society: London, 1972; pp 273-294. (c) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH Publishers: New York, 1987; pp 122-123.
13. Henderson, L. J. *J. Am. Chem. Soc.* **1908**, *30*, 954-960.
14. Lycka, A. *Annu. Rep. Nucl. Magn. Reson. Spectrosc.* **1993**, *26*, 247-281.
15. Scher, A. L., 26th Middle Atlantic Regional Meeting of the American Chemical Society, Alexandria, VA, Dec. 1992.
16. Drago, R. S. *Physical Methods for Chemists*, 2nd Ed.; Saunders: New York, 1992; pp 290-295.
17. Garbow, J. R.; Weitekamp, D. P.; Pines, A. *Chem. Phys. Lett.* **1982**, *93*, 504-509.

(*Received in USA* 25 *September* 1995; *accepted* 12 *February* 1996)