Journal of Magnetic Resonance **143,** 382–386 (2000)
doi:10.1006/jmre.1999.1987, available online at http://www.idealibrary.com on IDEAL®

Enhanced Suppression of Residual Water in a “270” WET Sequence

Shanmin Zhang, Xianbin Yang, and David G. Gorenstein

Sealy Center for Structural Biology and the Department of Human Biological Chemistry and Genetics,
University of Texas Medical Branch, Galveston, Texas 77555-1157

Received September 8, 1999; revised November 12, 1999

In certain water suppression experiments, the residual water, which comes from a region away from the center of the RF coil and experiences a much smaller flip angle than the designed one, may appear. The residual water in the WET sequence can be reduced significantly by using a composite $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ pulse, which de-excites molecules experiencing a small flip angle. The composite pulse, however, has two null excitation points near on resonance, causing a severe loss of spectrum intensity and baseline distortion toward the null points. Since the residual water experiences a very small flip angle, it can be treated as a linear spin system; i.e., the intensity of the residual water is proportional to the pulse strength and width. Based on this principle, the residual water can be reduced dramatically by replacing the 90° pulse in the “270” WET sequence with a 270° pulse for one out of every four scans, without noticeable loss of intensity and baseline distortion. © 2000 Academic Press

Key Words: **residual water suppression; WET sequence; composite pulse; excitation profile; linear response.**

INTRODUCTION

In NMR, many pulse sequences for water suppression use narrow selective RF pulses with the water peak on resonance and with a desired flip angle, defined as (*1*)

$$\vartheta = \int_0^{\mathrm{pw}} f_1(t)\,dt, \qquad [1]$$

where $f_1(t)$ and pw are the RF pulse strength and width, respectively. Equation [1] is adequate only for water molecules close to the center of the RF coil. For those molecules further away from the center region, the resonance frequency can be shifted substantially in response to the inhomogeneity of the external magnetic field ($\mathbf{B}_0$) and the pulse strength experienced by these molecules can be reduced significantly, resulting in an off-resonance excitation and a much smaller flip angle than the desired one (ϑ). Therefore, a residual water signal, which may spread out in a quite broad range, will appear in the spectrum. To overcome this problem, a composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ (*2*) is used in the WET sequence (*3*, *4*), which reduces residual water dramatically. However, the composite pulse also distorts the excitation profile severely especially in the downfield region due to the off-resonance effect.

Since the residual water experiences a very small flip angle, the corresponding spin system can be treated as a linear system; i.e., the residual signal is proportional to the RF pulse strength and width. Based on this principle, we show that the residual water can be reduced by replacing the 90° pulse with a 270° pulse of opposite phase for one out of every four scans in the new “270” WET sequence (Fig. 1). After four scans, the residual water excited by the 90° pulse is accumulated three times and is then subtracted from the one by the 270° pulse, while the desired signals are added four times.

In this paper the excitation by the composite $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ pulse is simulated using the Bloch equations and the two nearest null excitation points are calculated, which explains the nonsymmetric excitation by the composite pulse. Comparison is made between the experimental results afforded by a 270° pulse in the “270” WET sequence and those from a composite pulse and a single 90° pulse. The residual water in the WET-NOESY (*3*) and WATERGATE (*5*) is also discussed.

THE EXCITATION BY THE COMPOSITE $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ PULSE

It was shown that the composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ (*2*) is quite effective in eliminating signals experiencing a flip angle smaller than 30° or so under on-resonance conditions. Therefore, it is useful in removing residual water near on resonance. However, the composite pulse has a nonsymmetric and quite limited excitation bandwidth (Fig. 2d) as a result of the two nonsymmetric null excitation points located at offset $\Delta_1 = -0.8080 f_1$ and $\Delta_2 = 0.5475 f_1$, respectively, which can be understood by the following analysis.

The excitation profile by a single 90° pulse is shown in Fig. 2a, which can be obtained analytically (*6*). It is, however, quite tedious to derive the profile analytically for two consecutive 90° pulses of different phases. Nevertheless, one can always get the two-pulse excitation with computer simulation using the Bloth equations (*7*, *8*) as plotted in Fig. 2b, which shows a null excitation point at $\Delta_1 = -0.8080 f_1$. This null point can be revealed by calculating the composite rotations by the two consecutive pulses $90^\circ_x 90^\circ_y$.

1090-7807/00 $35.00

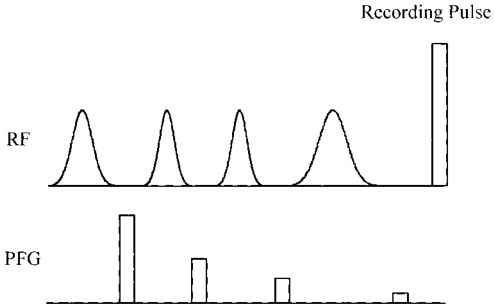

FIG. 1. The WET sequence with relative flip angles (of the selective pulses) 81.4°, 101.4°, 69.3°, and 161.0° to alleviate the problem caused by the inhomogeneity of the RF pulses and spin-lattice relaxation. In order to have similar selective regions, all four selective pulses have the same pulse strength, which also simplifies the calibration of the WET experiment. The pulse angles are adjusted by the relative pulse widths rather than the power levels. To reduce residual water, the last 90° recording pulse can be replaced by a composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$, or a 90° pulse for the first three scans and a 270° pulse for the fourth scan.

It is well known that for a spin-1/2 system an arbitrary rotation around an axis **n** and with a rotation angle φ can be expressed as (*9*)

$$R_{\mathbf{n}}(\varphi) = e^{-i(\varphi \mathbf{n}\cdot\boldsymbol{\sigma}/2)} = \cos\left(\frac{\varphi}{2}\right) - i(n_x\sigma_x + n_y\sigma_y + n_z\sigma_z)\sin\left(\frac{\varphi}{2}\right), \qquad [2]$$

where σ_x, σ_y, and σ_z are the three Pauli matrices. The two consecutive rotations by $90^\circ_x 90^\circ_y$ can be calculated readily,

$$\begin{aligned}
&\left[\cos\left(\frac{\varphi}{2}\right) - i(n_y\sigma_y + n_z\sigma_z)\sin\left(\frac{\varphi}{2}\right)\right] \\
&\quad\times\left[\cos\left(\frac{\varphi}{2}\right) - i(n_x\sigma_x + n_z\sigma_z)\sin\left(\frac{\varphi}{2}\right)\right] \\
&= \cos^2\left(\frac{\varphi}{2}\right) - n_z^2\sin^2\left(\frac{\varphi}{2}\right) \\
&\quad - i\left[\left(n_x\cos\left(\frac{\varphi}{2}\right) + n_yn_z\sin\left(\frac{\varphi}{2}\right)\right)\sigma_x\right. \\
&\quad + \left(n_y\cos\left(\frac{\varphi}{2}\right) + n_xn_z\sin\left(\frac{\varphi}{2}\right)\right)\sigma_y \\
&\quad \left. + \left(2n_z\cos\left(\frac{\varphi}{2}\right) - n_xn_y\sin\left(\frac{\varphi}{2}\right)\right)\sigma_z\right]\sin\left(\frac{\varphi}{2}\right), \qquad [3]
\end{aligned}$$

where the effective rotation angle $\varphi = (\sqrt{f_1^2 + \Delta^2}/f_1) \times 90^\circ$, the three components of the unit vector **n,** $n_x = n_y = f_1/\sqrt{f_1^2 + \Delta^2}$, $n_z = \Delta/\sqrt{f_1^2 + \Delta^2}$, and Δ is the offset.

Equation [3] shows that the coefficients of σ_x and σ_y are identical and if they become zero for a particular φ or Δ the combined rotation is around the z axis, resulting in a null excitation. This null point can be calculated simply by setting the coefficient of σ_x equal to zero, which leads to

$$-\frac{\sqrt{1 + \lambda^2}}{\lambda} = \tan(\sqrt{1 + \lambda^2} \times 45^\circ), \qquad [4]$$

with a solution of $\lambda = \Delta_1/f_1 = -0.8080$. This null point agrees well with the computer simulated one using the Bloch equations (Fig. 2b). Similarly, the second pair of the composite pulse $90^\circ_{-x}90^\circ_{-y}$ will also form a null point that corresponds to the same offset $\Delta_1/f_1 = -0.8080$ as the first one. Therefore, the null point remains after the entire four 90° pulses as shown in Fig. 2d.

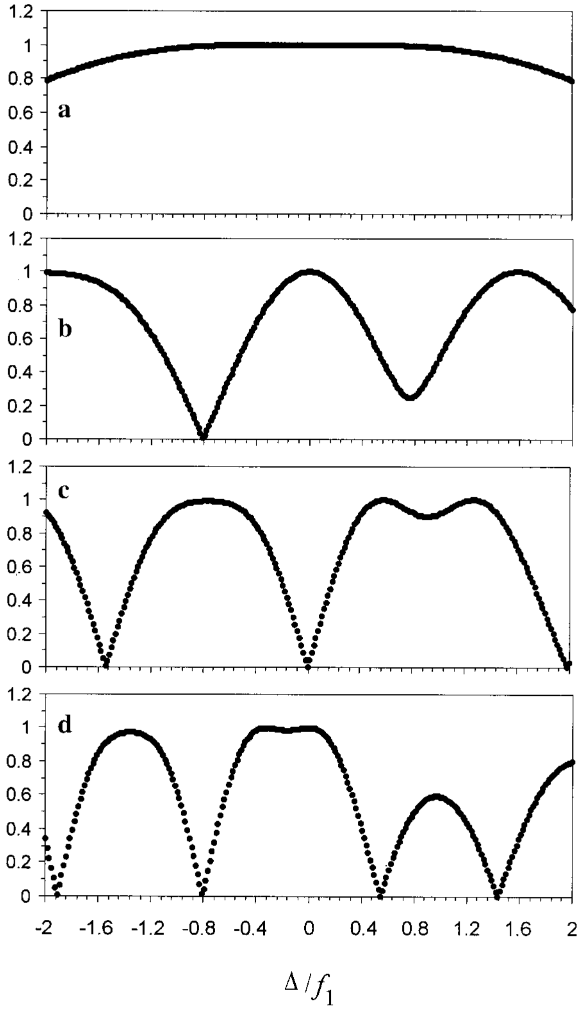

FIG. 2. The excitation profile ($\sqrt{M_x^2(\Delta/f_1) + M_y^2(\Delta/f_1)}$) simulated with the Bloch equations for a single 90°_x pulse (a), a composite pulse of $90^\circ_x90^\circ_y$ (b), a composite pulse of $90^\circ_x90^\circ_y90^\circ_{-x}$ (c), and a composite pulse of $90^\circ_x90^\circ_y90^\circ_{-x}90^\circ_{-y}$ (d).

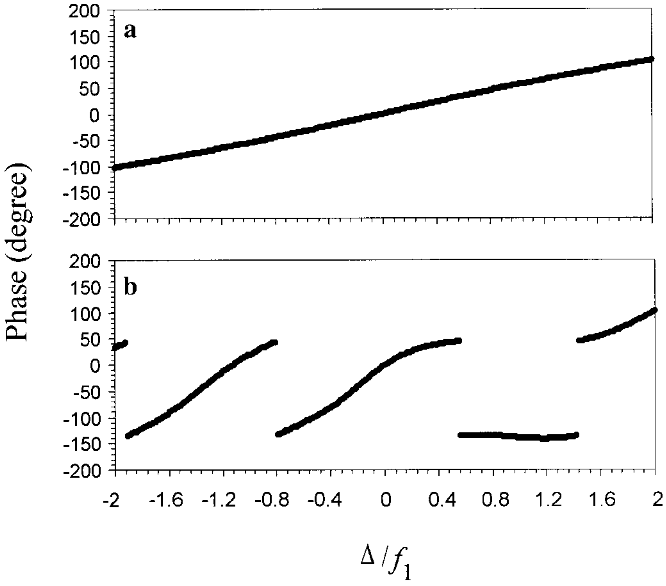

FIG. 3. The phases of the excitation profiles by a single 90° pulse (a) and by a composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ (b). A nonlinear phase distortion with a phase inversion at each null excitation point is introduced by the composite pulse.

By setting the coefficient of σ_z equal to zero, we found another equation,

$$2\lambda\sqrt{1+\lambda^2} = \tan(\sqrt{1+\lambda^2} \times 45^\circ), \qquad [5]$$

with a solution of $\lambda = \Delta_2/f_1 = 0.5475$. For this particular offset Δ_2, the composite rotation is around an axis in the x–y plane having a 45° phase with respect to the x axis. Similarly, one can show that the combined rotation by the second pair of pulses has an opposite rotation axis but the same rotation angle as the first combined rotation for this particular offset Δ_2. After the four pulses the overall rotation for Δ_2 is then zero, leading to a second null point that again agrees well with the result from the Bloch equations (Fig. 2d).

The second null point Δ_2 is located on the side opposite to Δ_1 and is near to on resonance. It is therefore more noticeable in experiments, especially at high magnetic fields where the signals have a higher frequency dispersion.

Due to off-resonance symmetry, one can show that the two null points at Δ_1 and Δ_2 change positions if the composite pulse alters its relative phases from a phase counterclockwise composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ to a clockwise composite pulse $90^\circ_x 90^\circ_{-y} 90^\circ_{-x} 90^\circ_y$.

The composite pulse also introduces a nonlinear phase distortion as shown in Fig. 3, which cannot be corrected by a first-order phase correction. In addition, a phase inversion occurs at each null point (Figs. 2, 3), which is a general phenomenon in NMR and will be discussed elsewhere.

THE “270” WET SEQUENCE USING A 270° PULSE

The WET sequence (Fig. 1) is an efficient water suppression scheme, which is able to reduce the water signal to the order of 10^{-5}. In the sequence, a selective RF pulse is applied to rotate the water magnetization to the x–y plane. A strong pulse field gradient along the z axis is then followed, which dephases the magnetization in the x–y plane. The small remaining z component magnetization is further reduced by the other three pairs of the selective pulses and pulse field gradients. In order to reduce the effects of the inhomogeneity of the RF pulse and the spin–lattice relaxation of the sample, the four selective RF pulses have different rotation angles (*3*). The four pulse field gradients also have different strengths to avoid gradient echoes.

We used four scans in the “270” WET sequence. In the first three scans, a hard 90° pulse is used as usual. In addition to the desired signals, it also accumulates three times the residual water that experiences a much smaller flip angle. In the fourth scan, a 270° pulse, which replaces the 90° pulse and has an opposite phase, is then substituted without changing the receiver phase. It yields the same desired signals as the 90° pulse does under strong pulse conditions. In addition, it also yields three times the intensity of the residual water under the linearity condition since the pulse is three times longer than the 90° pulse. The fourth FID is then added to the previous three FIDs. The desired signals are added but the residual water is subtracted.

EXPERIMENTAL

The experiments were performed on a Varian Unit-Plus 750-MHz NMR instrument with a sample of 1.3 mM chicken egg white lysozyme in 90% H_2O/10% D_2O at 25°C.

Figure 4a shows a spectrum obtained using a normal WET sequence (Fig. 1) with a single 90° recording pulse. The spectrum is accumulated four times with CYCLOPS phase cycling (*10*). A strong broad residual water remains in the middle of the spectrum due mainly to the residual water and the radiation damping effect (*1*), a problem of increasing concern at very high fields.

The residual water is reduced dramatically as shown in Fig. 4b by using the composite $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ pulse (*2*), which de-excites the water molecules experiencing a small flip angle. The remaining water is also much narrower since the residual water away from the center region of the RF coil that tends to be shifted by the inhomogeneity of the magnetic field is partially eliminated. Therefore, some peaks close to the water are partially resolved. However, the peaks suffer severe loss of intensities toward the null point at $\Delta_2 = 0.5475 f_1$.[1] In addi-

---

[1] During the course of our study, a similar result was also reported independently by Dr. Stephen H. Smallcombe (Varian NMR Instruments) at the 1999 Varian users meeting in Orlando.

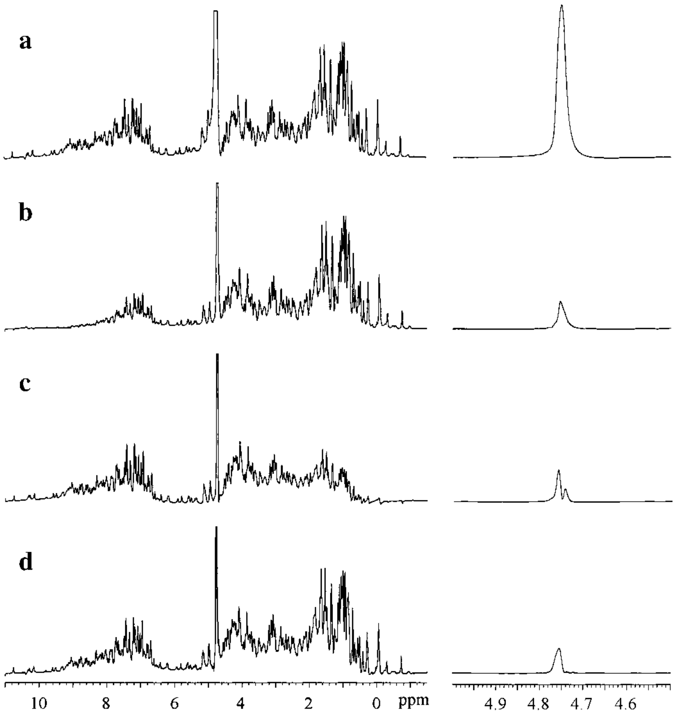

FIG. 4. Spectra of lysozyme obtained using the WET sequence with a 90° recording pulse (a), with a composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ (b), with a composite pulse $90^\circ_x 90^\circ_{-y} 90^\circ_{-x} 90^\circ_y$ (c), and with a 90° pulse (the first three scans) and a 270° pulse (the fourth scan; "270" WET) (d). All the spectra are carefully and separately phased. The full scale of water regions of the corresponding spectra are shown on the right. All the selective pulses have a Gaussian shape with pulse widths of 8.29, 10.33, 7.06, and 16.4 ms, which correspond to flip angles of 81.4°, 101.4°, 69.3°, and 161.0°, respectively. The four-pulse field gradient width is 2 ms and strengths are 30, 15, 7.5, and 3.75 G/cm, respectively. The last 90° pulse width is 7.3 μs and the corresponding pulse strength $f_1 = 34.3$ kHz.

tion, the baseline of the spectrum is also partially distorted toward the null.

Figure 4c shows the spectrum obtained by the phase clockwise composite pulse $90^\circ_x 90^\circ_{-y} 90^\circ_{-x} 90^\circ_y$. The intensity loss is shifted to the upfield region since the two null points Δ_1 and Δ_2 change their locations as discussed above.

The "270" WET spectrum obtained with a 270° pulse in the fourth scan is shown in Fig. 4d. The residual water is reduced as dramatically as in the composite pulse sequence but the desired signal intensities and the baseline of the spectrum are comparable to the results by the single 90° pulse (Fig. 4a).

The excitation profile generated by the 270° pulse has two symmetric null points located at $\Delta_2 = -\Delta_1 = 0.8819 f_1$, which are further from on resonance than that in the composite pulse and will cause less reduction in the signal intensity. In addition, the 270° pulse introduces a smaller baseline distortion than that by the composite pulse. Besides, the distortion has only one-fourth the effect on the final accumulated spectrum since the 270° pulse is used only once in every four scans.

As an application of the "270" WET sequence we show suppression of multiple lines. Figure 5a is the proton spectrum of 1-octene with some impurities, which needed to be identified. To see the smaller peaks the main 1-octene peaks have to be suppressed. It can be achieved by using five frequency-shifted (*8*) selective pulses linked sequentially rather than using a single selective pulse suppressing multiple lines simultaneously that may introduce severe interference leading to a poor suppression. The relative intensities of some impurity peaks are enhanced significantly as shown in Fig. 5b.

CONCLUSIONS

The residual water, which experiences a much smaller flip angle under normal pulse strength, can be treated as a linear system under RF pulse excitation. It can be reduced dramatically by the "270" WET sequence. The composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$, which de-excites the water molecules experiencing a small flip angle, also reduces residual water considerably. However, it causes a severe intensity loss toward the second null point $\Delta_2 = 0.5475 f_1$ introduced by cancellation of the two composite rotations around the two opposite axes in the x–y plane. Another null excitation point at Δ_1 caused by the two composite rotations around the z axis is further from on resonance and it usually causes less intensity distortion. The two null points are nonsymmetric with respect to the on resonance due to the different origins as discussed above. The locations of Δ_1 and Δ_2 will change if the relative phases of the composite pulse alter from counterclockwise to clockwise, moving the intensity loss from the downfield to the upfield region. Since both null points are proportional to the RF field

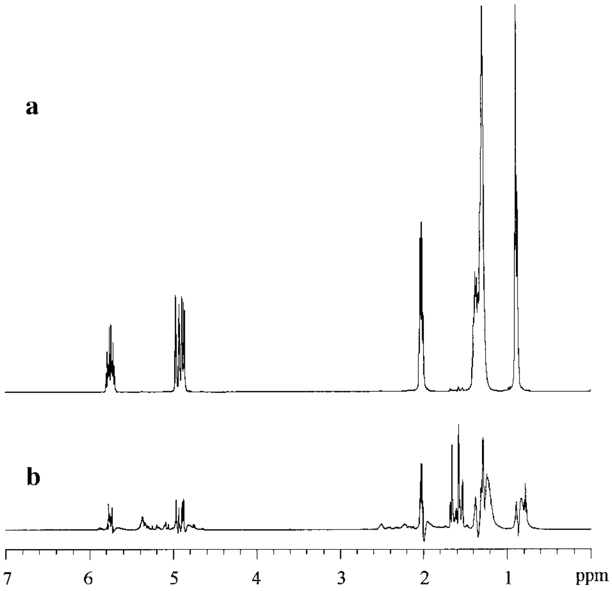

FIG. 5. Proton spectrum of 1-octene with some impurities (a). The peaks from the impurities are enhanced by the "270" WET sequence with five frequency-shifted selective pulses linked sequentially (b).

strength f_1, the intensity distortion, in theory, can be alleviated by using a RF field of sufficient strength and moving the two null points further outside of the spectral window. However, at very high fields this becomes problematic. In addition, the composite pulse $90^\circ_x 90^\circ_y 90^\circ_{-x} 90^\circ_{-y}$ also introduces baseline distortion.

The modified "270" WET sequence with residual water suppression can also be extended for suppression of multiple solvent lines, if the single line selective pulses are replaced by multiple line selective pulses, which is quite useful in LC NMR as demonstrated by Smallcombe *et al.* (*4*). Similar to the suppression of residual water discussed above, all the residual solvent signal will also be suppressed.

In the WET-NOESY experiment, the residual water created by all the pulses prior to the last one is dephased by the gradient pulses in the mixing time. The phases of the last pulse and the receiver are $x\ x\ y\ y$ and $x\ -x\ y\ -y$, respectively (showing only the first four phases). The residual water from the first two scans cancels since the pulse phase remains the same in the first two scans while the phase of the receiver changes sign. Similarly, the residual water will also cancel for the following two scans. Therefore, the residual water is canceled for every two scans in the WET-NOESY experiment and there is no need to replace the last 90° pulse with a 270° pulse.

In the WATERGATE sequence (*5*), the residual water created by the last three pulses (two selective 90° pulses and one hard 180° pulse) is dephased by the last gradient pulse and no spin echo is formed for the residual water due to a small flip angle. Therefore, it is not necessary to modify the pulse sequence as well.

Our "270" WET method for suppressing residual water may be used in multidimensional experiments where substantial residual water signals are excited by the last 90° pulse and the receiver phases are synchronized with the phase of the last pulse.

ACKNOWLEDGMENTS

This research was supported by NIH (AI27744), NIEHS (ES06676), the Welch Foundation (H-1296), the Lucille P. Markey Foundation, and the Sealy and Smith Foundation. Building funds were provided by NIH (1CO6CA59098). The sample of 1-octene was provided by Dr. Claire B. Conboy from the Dow Chemical Company.

REFERENCES

1. A. Abragam, "Principles of Nuclear Magnetism," Oxford Univ. Press, Oxford (1961).
2. A. Bax, *J. Magn. Reson.* **65,** 142 (1985).
3. R. J. Ogg, P. B. Kingsley, and J. S. Taylor, *J. Magn. Reson. B* **104,** 1 (1994).
4. S. H. Smallcombe, S. L. Patt, and P. A. Keifer, *J. Magn. Reson. A* **117,** 295 (1995).
5. M. Piotto, V. Saudek, and V. Sklenář, *J. Biomol. NMR* **2,** 661 (1992).
6. R. R. Ernst, G. Bodenhausen, and A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Clarendon Press, Oxford (1987).
7. F. Bloch, *Phys. Rev.* **70,** 460 (1946).
8. S. Zhang and D. G. Gorenstein, *J. Chem. Phys.* **105,** 5659 (1996).
9. J. J. Sakurai, "Modern Quantum Mechanics," Addison–Wesley, Reading, MA (1985).
10. D. I. Hoult, *Prog. NMR Spectrosc.* **12,** 41 (1978).