$^{13}C-^{13}C$ Correlations and Internuclear Distance Measurements with 2D-MELODRAMA

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Abstract: Under magic-angle spinning (MAS), the 2D-MELODRAMA pulse sequence reintroduces homonuclear dipolar couplings, thereby allowing internuclear correlations to be determined. The evolution of the density matrix under the average Hamiltonian established under the 2D-MELODRAMA pulse sequence has been evaluated, and closed-form expressions for the dependence of cross-peak intensity as a function of the dipolar mixing time are presented. In the case of sodium proprionate-2-3-$^{13}C_2$ (>90% ^{13}C) it is shown that analysis of cross-peak intensity vs mixing times allows estimates of dipolar coupling with an accuracy comparable with the 1D-MELODRAMA experiment. The potential for simultaneous multiple internuclear correlations and distance measurements in spin systems for which there is not a large difference in resonance frequencies is illustrated with glucose-$^{13}C_6$ (36% uniformly labeled). It is shown that multiple correlations for each ring carbon can be detected and good estimates of the corresponding internuclear distances can be extracted from analysis of cross-peak intensities as a function of the dipolar recoupling time.

Introduction

Significant progress has been made during the past decade in developing nuclear magnetic resonance (NMR) multidimensional correlation spectroscopy to investigate molecular structure in amorphous or polycrystalline samples. These techniques combine magic-angle spinning (MAS),[1,2] which achieves a "high resolution" spectrum, with rotor-synchronized rf pulse trains which reintroduce the dipolar couplings that provide structural information. The experiments may be classified as heteronuclear (e.g. REDOR/TEDOR,[3,4] RFDRCP[5]) and homonuclear (e.g. rotational resonance,[6,7] SEDRA,[8] RFDR,[9,10] DRAMA[11,12] and its variants, DRAWS[13,14]). Because the direct dipolar coupling scales as the inverse cube of the internuclear separation, there is the potential for measurement of longer distances with a higher degree of accuracy and precision than is attainable with solution-state NOE NMR studies. A frequent criticism of many of these techniques is that multiple distances must be measured one at a time, which is expensive and time-consuming. To address the latter issue, two-dimensional variants have been proposed which allow chemical shift correlations with concomitant internuclear distance information.[15,16] Carbohydrates present a particularly difficult problem since chemical synthesis is frequently difficult and isolation from natural sources leads to small quantities. These practical concerns make it essential that distances be measurable between multiple sites (multisite labeling) and on relatively small samples. Because the chemical shift dispersion of resonances is often small (<40 ppm for ^{13}C) in carbohydrate systems, spectral resolution is of significant concern. Additionally, this chemical shift range makes carbohydrate systems challenging in that the frequency differences may be too small for sequences such as RFDR[9,10] and too large for DRAMA[11,12] and its variants. MELODRAMA,[17] by contrast, is simple to analyze, insensitive to the range of resonance offsets likely to occur in many carbohydrates, and allows accurate homonuclear distance measurements as well as homonuclear chemical shift correlation in an amino acid and a peptide. Thus its application to carbohydrate systems appears attractive. However, no detailed analysis of the dependence of 2D-cross-peak intensity on the values of dipolar coupling has been reported. In this study, we describe the theoretical dependence of the 2D-MELODRAMA cross-peak intensity on the dipolar recoupling time (mixing time). The analysis has been applied to cross-peak intensities of 2D spectra of sodium proprionate-2-3-$^{13}C_2$ (>90% ^{13}C), and compared with the corresponding 1D-

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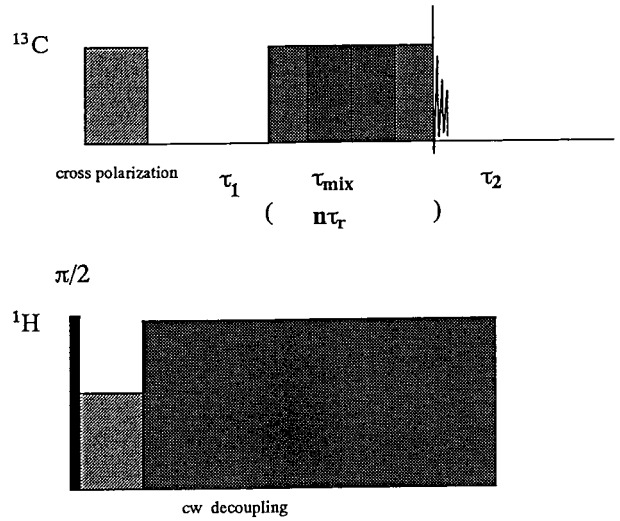

Figure 1. 2D-MELODRAMA pulse sequence. During the interval t_1, evolution is under the chemical shift Hamiltonian. Reintroduction of the homonuclear dipolar coupling by the MELODRAMA sequence during the interval t_{mix} gives rise to coherence transfer from spin I to spin S. During the interval t_2, evolution is under the chemical shift Hamiltonian. Phase cycling of the rf pulses is as given by Sun et al.[17]

MELODRAMA results. Application to glucose-$^{13}C_6$ (36% uniformly labeled) demonstrates that sufficient resolution may be achieved to detect multiple correlations among the ring carbons and to estimate distances between sites with good accuracy. Furthermore it is shown that such measurements can be made on small amounts of material (5 mg in the case of the glucose sample).

Materials and Methods

Materials. Sodium proprionate-2-3-$^{13}C_2$ (90% ^{13}C) was purchased from MSD Isotopes (Montréal, Québec). D-Glucose-$^{13}C_2$ (36.6% ^{13}C) was obtained from ISOTEC Inc. (Miamisburg, OH).

NMR Spectroscopy. All spectra were recorded on a Varian Unity-400 spectrometer at a ^{13}C resonance frequency of 100.1 MHz. The 1D- and 2D-MELODRAMA sequence is shown in Figure 1 and was performed as described by Sun *et al.*[17] Samples consisted of 15 mg of labeled sodium proprionate and 5 mg of glucose in a 5 mm zirconia rotor with Kel F end caps. No attempt was made to confine the sample to the center of the rotor as suggested by Sun *et al.*[17] Spectra were acquired with use of a Doty 5 mm MAS probe with fiber optic spin rate detection (Doty Scientific, Columbia, SC). ^{1}H-^{13}C cross-polarization (0.75 ms contact time) and MELODRAMA spin-lock were performed with a ^{13}C γB_1 of 25 kHz at a spinning rate of 5 kHz ($N = 5$). A ^{1}H cw-decoupling field strength of ~80 kHz was used during the t_1, mixing, and acquisition periods. The spinning rate was controlled to within ±5 Hz with a Bruker spin-rate controller (Bruker Instruments, Billerica, MA). 2D spectra were acquired with 1 K complex data points in the t_2 dimension and 64 to 100 t_1 increments and a spectral width in both dimensions of 15 kHz and 5188 Hz, for sodium proprionate and glucose, respectively. The recycle time was set at ~5T_1 for protons (20 s for sodium proprionate and 80 s for d-glucose). A sine-bell squared apodization was used prior to a 1K × 1K complex Fourier transform. Peak heights were determined by standard Varian software routines.

Theory

The total spin Hamiltonian in the rotating frame for a homonuclear spin pair (I,S) undergoing MAS can be expressed in terms of the I and S spin chemical shift (*CS*) interactions, the dipolar coupling (D) interaction, and the rf field interaction as

$$H(t) = H_{rf} + H_{int} \tag{1}$$

$$H_{rf} = \omega_1(I_x + S_x) \tag{2}$$

$$H_{int} = H_{CS} + H_D \equiv \omega_I(t)I_z + \omega_S(t)S_z + \omega_D(t)(3I_zS_z - \mathbf{I}\cdot\mathbf{S}) \tag{3}$$

Each of the terms in the internal spin Hamiltonian of eq 3 is time dependent, and the explicit form of this time dependence imposed by MAS on the internal spin interactions λ ($\lambda \equiv CS$ or D) can be compactly expressed as[17]

$$\omega_\lambda(t) = \omega_{iso}^\lambda + \sum_{m=-2}^{2} \omega_\lambda^m(\alpha,\beta,\gamma)e^{-im\omega_r t} \tag{4}$$

where ω_{iso}^λ is the isotropic chemical shift, and the $\omega_\lambda^m(\alpha,\beta,\gamma)$'s are the components of the respective λ interaction tensors in the rotor frame. If the Hamiltonian in the toggling (interaction) frame[18] is cyclic, according to average Hamiltonian theory (AHT),[18] the evolution of the spin system under H_{int} and a periodic pulse sequence can be viewed as being generated by an effective, constant Hamiltonian:[17]

$$\overline{\tilde{H}(t)} = \sum_{p,q=x,y,z}\sum_{m=0}^{2} [a_{p,m}(I_p\omega_{CS,m}^I + S_p\omega_{CS,m}^S) + \omega_{D,m}a_{pq,m}I_pS_q] \tag{5}$$

where

$$\tilde{H}(t) = U_{rf}^{-1}H_{int}(t)U_{rf} \tag{6}$$

Under the MELODRAMA sequence,[17] where the cycle time is four rotor periods ($\tau_c = 4\tau_r$), Sun et al.[17] have shown that for *integer* values of the ratio $N = \omega_{rf}/\omega_r$, only the $a_{pq,1}$ of eq 5 survive, and in particular, for *even N*, the average Hamiltonian takes the form

$$\overline{\tilde{H}(t)} = D_N(I_yS_y - I_xS_x) \tag{7}$$

$$= D_NI_x^{1,4} \tag{8}$$

where the residual dipolar coupling D_N can be expressed in terms of the dipolar coupling constant ω_D as[17]

$$D_N = \omega_D\frac{6N^2\sin(2\beta)\cos(\gamma)}{\sqrt{2}\pi(4N^2-1)} \tag{9}$$

where

$$\omega_D = \frac{\mu_0}{4\pi}\frac{\hbar\gamma_I\gamma_S}{r_{IS}^3} \tag{10}$$

The fact that the MELODRAMA cycle time is four rotor periods ($\tau_c = 4\tau_r$) would suggest that for two different mixing periods, $n_1\tau_r$ and $n_2\tau_r$, each expressed as some integer multiple n of the rotor period, only durations which are congruent modulo 4, i.e., $n_1 \equiv n_2$ *mod* 4, should be considered. However, our calculations of the functional dependence of the coefficients a_p and a_{pq} in eq 5 on the parameter $s = \omega_{rf}/\omega_r$ show that there is a simple relationship between the duration of the mixing period

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(expressed as an integral multiple of the rotor period) and the average Hamiltonian.[19,20] If the mixing time is $t_c = nt_r$, the average Hamiltonian can be expressed as

$$\overline{\overline{H}}(t) = \frac{n}{4} D_N I x^{1,4} \tag{11}$$

with corresponding signals given by

$$\langle I_z + S_z \rangle(t) = \frac{1}{4\pi} \int_0^{\pi} \mathrm{d}\beta \sin\beta \int_0^{2\pi} \mathrm{d}\gamma \cos\left[\frac{n}{4} D_N(\beta,\gamma) t\right] \tag{12}$$

As an example, from eq 11, we see that for a basic MELODRAMA pulse sequence of one rotor period in duration, the average Hamiltonian would be scaled down by a factor of 4. In considering the effects of MELODRAMA dipolar mixing in a two-dimensional homonuclear ($^{13}C-^{13}C$) chemical shift correlation experiment,[17] we develop an expression for the cross-peak intensity following a mixing period of n MELODRAMA cycles. Referring to Figure 1, and assuming an initial condition of $I_y + S_y$ following cross-polarization, through the chemical shift evolution periods of duration t_1 and t_2, and the MELODRAMA mixing period of duration t_m, we focus on the fate of magnetization originating on the I spin. Chemical shift evolution during periods t_1 and t_2 is governed by the Hamiltonian

$$H = \omega_I I_z + \omega_S S_z \tag{13}$$

while the appropriate form of the Hamiltonian for an initial state of $I_y + S_y$ (odd N) during a MELODRAMA mixing time t_m ($t_m = 4t_r$) is[17]

$$\overline{\overline{H}}(t) = D_N(I_z S_z - I_x S_x) \tag{14}$$

During t_1

$$I_y \xrightarrow{\omega_I t_1 I_z} I_y\, \omega_I t_1 - I_x \sin \omega_I t_1 \tag{15}$$

As we now keep track of I spin magnetization through successive periods of MELODRAMA mixing, we consider only the contribution from the first term of eq 15; it is easily shown that the second term of this equation makes no off-diagonal contribution. During the first MELODRAMA mixing period of duration t_m, using product operator techniques[19,21] and the fact that I_xS_x, I_yS_y, and I_zS_z commute with each other, we find that the term in I_y evolves under the action of Hamiltonian of eq 14 as:

(1 cycle)

$$I_y \cos\omega_I t_1 \xrightarrow{\overline{\overline{H}} t_m} I_y \cos\omega_I t_1 \cos^2(D_N t_m/2) - I_x S_z \cos\omega_I t_1 \sin(D_N t_m) - I_z S_x \cos\omega_I t_1 \sin(D_N t_m) - S_y \cos\omega_I t_1 \sin^2(D_N t_m/2) \tag{16}$$

$$= I_y \cos\omega_I t_1 \left[\frac{1}{2}(1 + (D_N t_m))\right] - I_x S_z \cos\omega_I t_1 \sin(D_N t_m) - I_z S_x \cos\omega_I t_1 \sin(D_N t_m) - S_y \cos\omega_I t_1 \left[\frac{1}{2}(1 - \cos(D_N t_m))\right] \tag{17}$$

Before we consider the effects of further MELODRAMA cycles, we pause to note parenthetically the origin of cross-peaks from this expression. During an acquisition period t_2 following one such MELODRAMA mixing period, each of the four terms in eqs 16 and 17 evolve under the chemical shift Hamiltonian of eq 13, leading to the following expression for the density operator ρ:

$$\begin{aligned}\rho = {} & I_y \cos(\omega_I t_1) \cos(\omega_I t_2) \cos^2(D_N t_m/2) \\ & - I_x \cos(\omega_I t_1) \sin(\omega_I t_2) \cos^2(D_N t_m/2) \\ & - I_x S_z \cos(\omega_I t_1) \cos(\omega_I t_2) \sin(D_N t_m) \\ & - I_y S_z \cos(\omega_I t_1) \sin(\omega_I t_2) \sin(D_N t_m) \\ & - I_z S_x \cos(\omega_I t_1) \cos(\omega_S t_2) \sin(D_N t_m) \\ & - I_z S_y \cos(\omega_I t_1) \sin(\omega_S t_2) \sin(D_N t_m) \\ & - S_y \cos(\omega_I t_1) \cos(\omega_S t_2) \sin^2(D_N t_m/2) \\ & + S_x \cos(\omega_I t_1) \sin(\omega_S t_2) \sin^2(D_N t_m/2)\end{aligned} \tag{18}$$

The $+I_y$ term in this expression corresponds to a *positive* diagonal peak, the $-S_y$ term corresponds to a *negative* cross-peak, and the remaining antiphase terms are unobservable and do not contribute any intensity. According to this expression, the complex magnetization recorded during the acquisition period would be given by

$$\begin{aligned}M_I^+(t) &\propto Tr[\rho(I_x + iI_y)] \\ &= Tr[(I_y \cos(\omega_I t_2) - I_x \sin(\omega_I t_2))(I_x + iI_y)] \times \cos(\omega_I t_1) \cos^2(D_N t_m/2) \\ &= -2 \cos(\omega_I t_1) \cos^2(D_N t_m/2) \exp[i(\omega_I t_2 - \pi/2)]\end{aligned} \tag{19}$$

$$\begin{aligned}M_S^+(t) &\propto Tr[\rho(S_x + iS_y)] \\ &= 2 \cos(\omega_I t_1) \sin^2(D_N t_m/2) \exp[i(\omega_S t_2 - \pi/2)]\end{aligned} \tag{20}$$

If instead there is a second consecutive MELODRAMA mixing period of duration t_m, each of the four terms in eq 17 then evolve under the action of Hamiltonian of eq 14 as follows:

$$I_y \xrightarrow{\overline{\overline{H}} t_m} I_y \cos^2(D_N t_m/2) - I_x S_z \sin(D_N t_m) - I_z S_x \sin(D_N t_m) - S_y \sin^2(D_N t_m/2) \tag{21}$$

$$-I_x S_z \xrightarrow{\overline{\overline{H}} t_m} - I_x S_z \cos^2(D_N t_m/2) - \frac{1}{2} I_y \sin(D_N t_m/2) \cos(D_N t_m/2) - \frac{1}{2} S_y \sin(D_N t_m/2) \cos(D_N t_m/2) + I_z S_x \sin^2(D_N t_m/2) \tag{22}$$

(19) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Skelton, N. J. *Protein NMR Spectroscopy (Principles and Practice)*; Academic Press: San Diego, 1996; pp 73−93.

(20) Waugh, J. S. *J. Magn. Reson.* **1982**, *50*, 30−49.

(21) Slichter, C. *Principles of Magnetic Resonance*; Springer, New York, 1990; pp 344−350.

$$-I_zS_x \xrightarrow{\bar{\bar{H}}\,t_m} -I_zS_x\cos^2(D_Nt_m/2) - \frac{1}{2}S_y\sin(D_Nt_m/2)\cos(D_Nt_m/2) - \frac{1}{2}I_y\sin(D_Nt_m/2)\cos(D_Nt_m/2) + I_xS_z\sin^2(D_Nt_m/2) \quad (23)$$

$$-S_y \xrightarrow{\bar{\bar{H}}\,t_m} -S_y\cos^2(D_Nt_m/2) + I_xS_z\sin(D_Nt_m) + I_zS_x\sin(D_Nt_m) + I_y\sin^2(D_Nt_m/2) \quad (24)$$

These evolution equations can be used to obtain, after some simplification, the following expression for the Hamiltonian after 2 MELODRAMA cycles:

(2 cycles)

$$I_y\cos\omega_It_1 \xrightarrow{2\bar{\bar{H}}\,t_m} I_y\cos\omega_It_1\left[\frac{1}{2}(1+\cos(2D_Nt_m))\right] - I_xS_z\cos\omega_It_1\sin(D_Nt_m) - I_zS_x\cos\omega_It_1\sin(D_Nt_m) - S_y\cos\omega_It_1\left[\frac{1}{2}(1-\cos(2D_Nt_m))\right] \quad (25)$$

Similarly we find after three and four consecutive MELODRAMA mixing periods, each of duration t_m, the following expressions for the respective Hamiltonians:

(3 cycles)

$$I_y\cos\omega_It_1 \xrightarrow{3\bar{\bar{H}}\,t_m} I_y\cos\omega_It_1\left[\frac{1}{2}(1+\cos(3D_Nt_m))\right] - I_xS_z\cos\omega_It_1\sin(3D_Nt_m) - I_zS_x\cos\omega_It_1\sin(3D_Nt_m) - S_y\cos\omega_It_1\left[\frac{1}{2}(1-\cos(3D_Nt_m))\right] \quad (26)$$

(4 cycles)

$$I_y\cos\omega_It_1 \xrightarrow{4\bar{\bar{H}}\,t_m} I_y\cos\omega_It_1\left[\frac{1}{2}(1+\cos(4D_Nt_m))\right] - I_xS_z\cos\omega_It_1\sin(4D_Nt_m) - I_zS_x\cos\omega_It_1\sin(4D_Nt_m) - S_y\cos\omega_It_1\left[\frac{1}{2}(1-\cos(4D_Nt_m))\right] \quad (27)$$

Referring to eq 18, we see that each of the S_y terms in eq 17 and eqs 25−27 gives rise to cross-peaks in the two-dimensional chemical shift correlation experiment[17] of Figure 1.

As an alternative to the tedious calculation of the Hamiltonian for each increment of the mixing period by one MELODRAMA cycle, the expressions of eqs 21−24 can be used to derive simple recursion relations for the coefficients of the terms of I_y, $-S_y$, and $-I_xS_z$ (or $-I_zS_x$) after n MELODRAMA cycles. If we denote $C1_n$, $C2_n$, and $C3_n$ as the coefficients of the terms in I_y, $-S_y$, and $-I_xS_z$ (or $-I_zS_x$) respectively, in the Hamiltonian after the nth MELODRAMA cycle, we derive the coupled recursion relations

$$C1_n = \cos^2(D_Nt_m/2) * C1_{n-1} + \sin^2(D_Nt_m/2) * C2_{n-1} - \frac{1}{2}\sin(D_Nt_m) * C3_{n-1} \quad (28)$$

$$C2_n = \sin^2(D_Nt_m/2) * C1_{n-1} + \cos^2(D_Nt_m/2) * C2_{n-1} + \frac{1}{2}\sin(D_Nt_m) * C3_{n-1} \quad (29)$$

$$C3_n = \sin(D_Nt_m) * (C1_{n-1} - C2_{n-1}) + \cos(D_Nt_m) * C3_{n-1} \quad (30)$$

with $C1_0 = 1$, $C2_0 = 0$, and $C3_0 = 0$. Straightforward matrix techniques can now be used to solve these coupled recursion relations for the coefficients as

$$C1_n = \frac{1}{2}[1+\cos(nD_Nt_m)] \equiv \cos^2 n\theta \quad (31)$$

$$C2_n = \frac{1}{2}[1-\cos(nD_Nt_m)] \equiv \sin^2 n\theta \quad (32)$$

$$C3_n = \sin(nD_Nt_m) \equiv \sin 2n\theta \quad (33)$$

where

$$\theta = D_Nt_m/2 \quad (34)$$

The recursion relations of eqs 28−30 can be formulated in matrix form as

$$\mathbf{z}_n = \mathbf{A}\mathbf{z}_{n-1} \quad (35)$$

where

$$\mathbf{z}_n = \begin{bmatrix} C1_n \\ C2_n \\ C3_n \end{bmatrix} \quad (36)$$

$$\mathbf{z}_{n-1} = \begin{bmatrix} C1_{n-1} \\ C2_{n-1} \\ C3_{n-1} \end{bmatrix} \quad (37)$$

$$\mathbf{A} = \begin{bmatrix} \cos^2\theta & \sin^2\theta & -\sin\theta\cos\theta \\ \sin^2\theta & \cos^2\theta & \sin\theta\cos\theta \\ 2\sin\theta\cos\theta & -2\sin\theta\cos\theta & \cos^2\theta - \sin^2\theta \end{bmatrix} \quad (38)$$

This set of coupled finite difference equations which define the recursion coefficients $\{C1_n, C2_n, C3_n\}$ can be solved by using standard techniques.[22] We find

$$\mathbf{z}_n = \mathbf{A}^n\mathbf{z}_0 \quad (39)$$

where

$$\mathbf{z}_0 = \begin{bmatrix} C1_0 \\ C2_0 \\ C3_0 \end{bmatrix} \equiv \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \quad (40)$$

Writing out the matrix elements of eq 39, using an * to denote irrelevant elements, we note that

(22) Jordan, D. W.; Smith, P. *Mathematical Techniques*; Oxford University Press: Oxford, 1994; pp 583−599.

$$\begin{bmatrix} \star \\ C2_n \\ \star \end{bmatrix} = \begin{bmatrix} \star & \star & \star \\ \mathbf{A}^n_{21} & \mathbf{A}^n_{22} & \mathbf{A}^n_{23} \\ \star & \star & \star \end{bmatrix} \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \tag{41}$$

and thus $C2_n$, the coefficient that determines the amplitude of the cross-peak intensity after n MELODRAMA cycles, is simply given by

$$C2_n = \mathbf{A}^n_{21} \tag{42}$$

It remains to find the explicit matrix elements of $\mathbf{A}^n$, for which purpose we use induction to show that

$$\mathbf{A}^n = \begin{bmatrix} \star & \star & \star \\ \sin^2 n\theta & \cos^2 n\theta & \sin n\theta \cos n\theta \\ \star & \star & \star \end{bmatrix} \tag{43}$$

where, as above, the starred elements are irrelevant in this case for the induction argument. Equation 38 shows that the equality of eq 43 is satisfied for $n = 1$; we assume it is satisfied for $n = k - 1$, and use this fact to show that eq 43 is then satisfied for $n = k$. Therefore, suppose

$$\mathbf{A}^{k-1} = \begin{bmatrix} \star & \star & \star \\ \sin^2 (k-1)\theta & \cos^2(k-1)\theta & \sin(k-1)\theta \cos(k-1)\theta \\ \star & \star & \star \end{bmatrix} \tag{44}$$

It follows then from eqs 38 and 44 that $\mathbf{A}^k = \mathbf{A}^{k-1}\mathbf{A}$ can be expressed as the matrix product

$$\begin{bmatrix} \star & \star & \star \\ \sin^2 (k-1)\theta & \cos^2(k-1)\theta & \sin(k-1)\theta \cos(k-1)\theta \\ \star & \star & \star \end{bmatrix} \times \begin{bmatrix} \cos^2\theta & \sin^2\theta & -\sin\theta\cos\theta \\ \sin^2\theta & \cos^2\theta & \sin\theta\cos\theta \\ 2\sin\theta\cos\theta & -2\sin\theta\cos\theta & \cos^2\theta - \sin^2\theta \end{bmatrix} \tag{45}$$

and so for example

$$\mathbf{A}^k_{21} = \sin^2(k-1)\theta\cos^2\theta + \cos^2(k-1)\theta\sin^2\theta + 2\sin(k-1)\theta\cos(k-1)\theta\sin\theta\cos\theta \tag{46}$$

$$= \{\sin(k-1)\theta\cos\theta + \cos(k-1)\theta\sin\theta\}^2 \tag{47}$$

$$= \{\sin[(k-1)\theta + \theta]\}^2 \tag{48}$$

$$= \sin^2(k\theta) \tag{49}$$

Similarly, from eq 45, we find

$$\mathbf{A}^k_{22} = \cos^2(k\theta) \tag{50}$$

$$\mathbf{A}^k_{23} = \sin(k\theta)\cos(k\theta) \tag{51}$$

and so

$$\mathbf{A}^k \begin{bmatrix} \star & \star & \star \\ \sin^2 k\theta & \cos^2 k\theta & \sin k\theta \cos k\theta \\ \star & \star & \star \end{bmatrix} \tag{52}$$

which completes the induction argument.

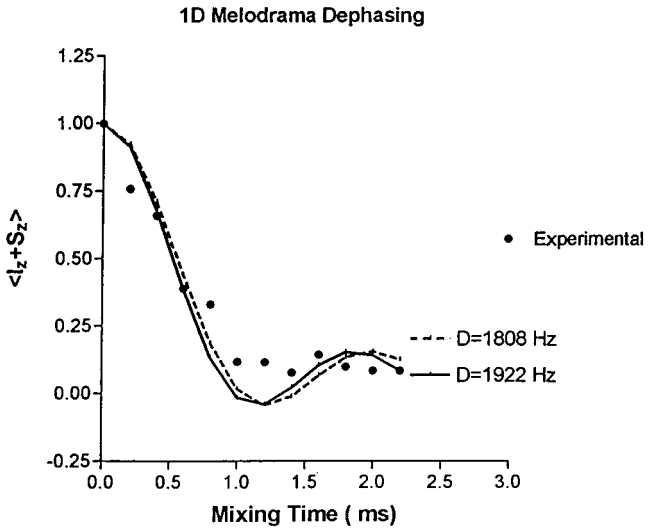

Figure 2. Total intensity of the ^{13}C resonances of the CH_2-CH_3 carbons of sodium proprionate-2-3-$^{13}C_2$ with use of the 1D-MELODRAMA pulse sequence. The spinning rate was 5 kHz, and the ratio of ^{13}C radio frequency field strength to the spinning rate was 5 ($N = 5$). Filled circles are the experimental data points, and the solid and dashed lines are fits of eq 12 to the data with values of dipolar coupling, D_N, of 1922 and 1808 Hz, respectively.

Data Analysis

Inspection of eq 12 reveals that, because of the nature of the average Hamiltonian established by the MELODRAMA sequence, fitting of experimental data as a function of the dipolar recoupling time, t_m, reduces to a dependence on one parameter, D_N. In simple isolated two-spin systems such as sodium proprionate-2-3-$^{13}C_2$, the diagonal and cross-peak intensities of 2D-MELODRAMA spectra as a function of the number of rotor periods of dipolar recoupling may be fit directly according to eqs 31 and 32. However, for multispin systems it is desirable to have the level of isotopic labeling low (<20% uniform labeling) to ensure that the MELODRAMA experiment is dominated by *isolated* two-spin interactions. Thus the total diagonal intensity associated with each label position, C_i, represents the sum of the diagonal intensity for each C_i-C_j spin pair, which for each pair is given by eq 31. As a result of this superposition of diagonal peaks, it is not practical to fit either diagonal peak intensities or absolute cross-peak intensities. In such cases relative cross-peak intensities may be fit by minimizing an error function of the form:

$$Error = [W_{ij}\,(R_{(calc)i} - R_{(exp)i})/R_{(exp)i}]^2 \tag{53}$$

where $R_{(calc)i}$ is the ratio of the calculated cross-peak intensity $I_{i(calc)}(\tau_j)$ at a mixing time of τ_j to that of the reference calculated cross-peak intensity $I_{i(calc)}(\tau_1)$ at a mixing time of τ_1, $R_{(exp)i}$ is the ratio of the corresponding experimental cross-peak intensities, and W_{ij} is the signal-to-noise ratio of cross-peak $I_i(\tau_j)$ relative to that of $I_i(\tau_1)$. The powder averaged signal $I_{i(calc)}(\tau_j)$ is calculated according to:

$$I_{i(calc)}(\tau_1) = \frac{1}{4\pi}\int_0^{\pi}\int_0^{2\pi} I_{i(calc)}(\beta,\gamma)(\tau_1)\sin\beta\,d\beta\,d\gamma \tag{54}$$

$I_{i(calc)}(\beta,\gamma)(\tau_1)$ is the cross-peak intensity associated with a particular crystallite orientation, where β and γ are the usual polar and azimuthal angles relating the orientation of the internuclear dipolar vector to that rotor axis. $I_{i(calc)}(\beta,\gamma)(\tau_1)$ is

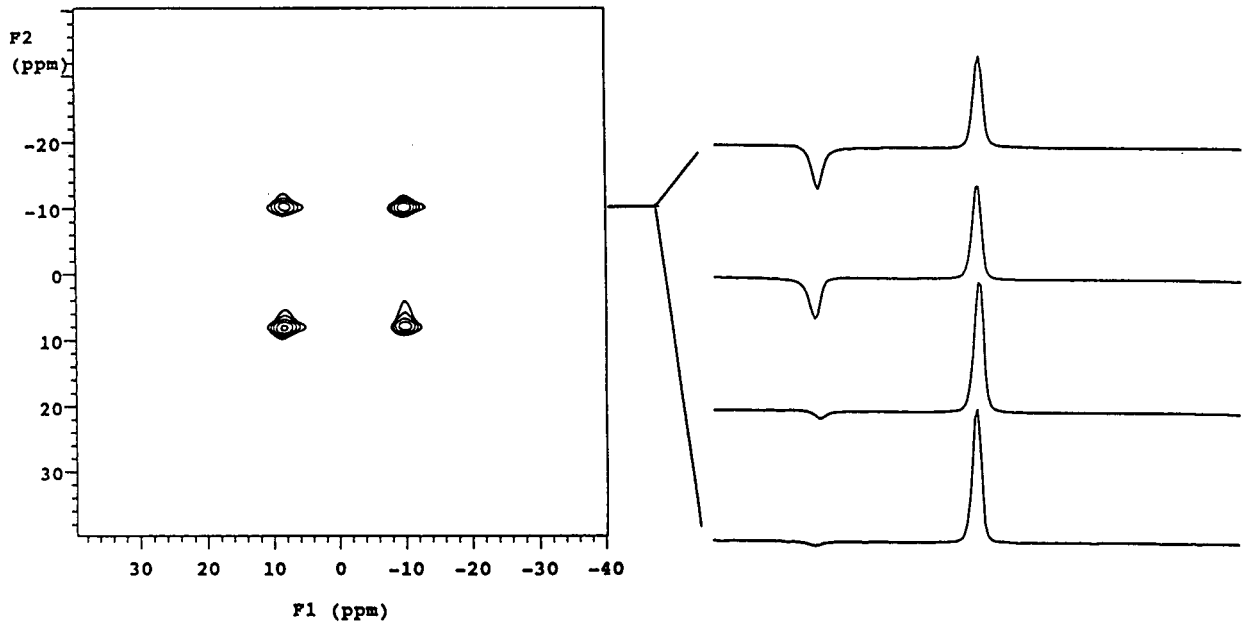

Figure 3. 2D-MELODRAMA ^{13}C NMR spectra of sodium proprionate-2-3-$^{13}C_2$. Left, contour plot for mixing time of 1 ms. Right, traces along F_1 axis at the CH_3 resonance showing the cross-peak intensity at the CH_2 chemical shift as a function of the mixing time: from bottom to top, 0.2, 0.4, 1.0, and 2.0 ms, respectively. Data were acquired at a spinning rate of 5 kHz, and the ratio of the ^{13}C radio frequency field strength to the spinning rate was 5 ($N = 5$).

given by eq 32 where D_N is given by eq 9. The error function represents a one-parameter fit that may be minimized by using the golden routine.[23]

Results

Sodium Proprionate-2-3-$^{13}C_2$. Application of the 1D-MELODRAMA sequence to sodium proprionate-2-3-$^{13}C_2$ (90% ^{13}C) under conditions where the ratio $\gamma B_1(^{13}C)/\nu_r$, N, is equal to 5 for increasing number of rotor periods gives rise to the expected dipolar dephasing curve of Figure 2. The total peak intensity ($I_y + S_y$) was fit to eq 13 of Sun *et al.*[17] to give a dipolar coupling of 1865 ± 57 Hz corresponding to a C−C bond length of 1.59 ± 0.03 Å, in good agreement with the 1.54 Å expected for this type of C−C bond. Figure 3 shows results of the 2D-MELODRAMA chemical shift correlation experiment as a function of the mixing time. As the dipolar interaction is reintroduced for longer periods of time, the transfer of coherence between the methyl and methylene carbons becomes more complete. Evaluation of the density matrix under the average dipolar recoupling Hamiltonian (eq 11) gives the cross-peak intensity at a mixing time t_m as

$$I_{cross} = \frac{1}{8\pi}\int_0^{\pi}\int_0^{2\pi}\{-1 + \cos[D_N(\beta,\gamma)\tau_m]\}\sin\beta\,d\beta\,d\gamma \quad (55)$$

and a diagonal peak intensity given by

$$I_{diags} = \frac{1}{8\pi}\int_0^{\pi}\int_0^{2\pi}\{1 + \cos[D_N(\beta,\gamma)\tau_m]\}\sin\beta\,d\beta\,d\gamma \quad (56)$$

yielding a total spectral intensity I

$$I = I_{diags} + I_{cross} = \frac{1}{4\pi}\int_0^{\pi}\int_0^{2\pi}\cos[D_N(\beta,\gamma)\tau_m]\sin\beta\,d\beta\,d\gamma \quad (57)$$

(23) Bock, K.; Thogersen, H. In *Annual Reports on NMR Spectroscopy*; Webb, G. A., Ed.; Academic Press: San Diego, 1996; Vol. 13, pp 2−49.

It is expected that the projection of the 2D-MELODRAMA spectrum onto the F_1 or F_2 axis would be equivalent to the corresponding 1D dipolar-dephased spectrum. Thus the total spectral intensity of a trace from the 2D-MELODRAMA spectrum through a cross-peak corresponds to the dephasing curve arising from the 1D-MELODRAMA[17] sequence. Figure 4 shows the predicted dependence of the diagonal, cross-peak, and total peak intensities on the mixing time for two values of the dipolar coupling constant and at a $\gamma B_1/\nu_r$ ratio (N) of 5. The diagonal and cross-peak intensities approach equal values which are of opposite sign. As expected the total signal intensity arising from one of the spins corresponds to the normal 1D-MELODRAMA dephasing curve. In principle, the analysis of the buildup of cross-peak intensity as a function of mixing time is straightforward and depends only on the magnitude of D_N. In an isolated two-spin system that is 100% isotopically enriched this is true since the absolute intensity of the diagonal may be obtained at $t_m = 0$ and used to normalize cross-peak intensities. Additionally, for each value t_m, T_2 effects may be compensated since

$$I = I_{diags} - I_{cross} \quad (58)$$

$$= I_0\exp(-t_m/T_2) \quad (59)$$

where

$$I_{diags} = I_0\exp(-t_m/T_2)\frac{1}{8\pi}\int_0^{\pi}\int_0^{2\pi}\times \{1 + \cos[D_N(\beta,\gamma)\tau_m]\}\sin\beta\,d\beta\,d\gamma \quad (60)$$

$$I_{cross} = I_0\exp(-t_m/T_2)\frac{1}{8\pi}\int_0^{\pi}\int_0^{2\pi}\times \{-1 + \cos[D_N(\beta,\gamma)\tau_m]\}\sin\beta\,d\beta\,d\gamma \quad (61)$$

where I_0 is the initial intensity at $t_m = 0$. This may be particularly important when D_N is comparable to $1/\pi T_2$. For more complicated and perhaps more interesting situations in which there are more than two sites labeled at ≪100%, it is

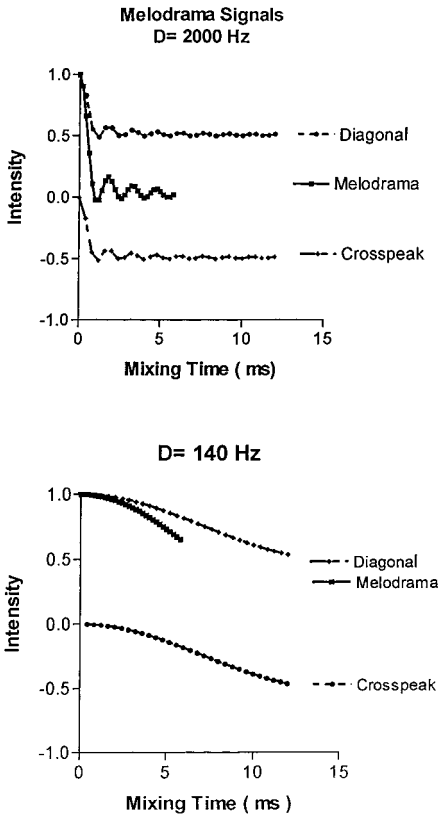

Figure 4. The simulated 2D-MELODRAMA signal intensities as a function of the dipolar coupling, D_N, and the dipolar recoupling time, t_{mix}. Intensities were calculated according to eqs 55, 56, and 57 as a powder average with a ratio of the radio frequency field strength to the spinning rate of 5 ($N = 5$). Top: $D_N = 2$ kHz; bottom: $D_N = 140$ Hz. The values of D_N were selected to represent the range of values of D_N expected to occur in glucose.

more difficult to analyze the buildup of the absolute cross-peak intensity as a function of mixing time since the value of I_0 for each site involved in a two-spin interaction may not be readily determined. An alternative is to express the cross-peak intensity at several values of t_m relative to that at a specific value of the mixing time:

$$R^i_{cross} = I^i_{cross}(t^i_m)/I^j_{cross}(t^j_m) \qquad (62)$$

where $I^i_{cross}(t^i_m)$ and $I^j_{cross}(t^j_m)$ are cross-peak intensities at mixing times t^i_m and t^j_m, respectively. In this case, t^j_m is a mixing time for which the absolute cross-peak intensity is near maximal and most reliable (best signal-to-noise ratio). Figure 5 shows results of the best fit of the relative cross-peak intensities of both sets of peaks in the sodium proprionate-2-3-$^{13}C_2$ 2D-MELODRAMA spectra (Figure 3) to D_N according to eq 53, which gave $D_N = 1516 \pm 56$ Hz. In an alternative approach based on eqs 31 and 32, a similar fit of the ratio of cross-peak to diagonal intensity gave a value of 1508 Hz for D_N. The former results give a C_2-C_3 bond length of 1.7 ± 0.02 Å. The accuracy of the measured distance by 2D-MELODRAMA appears to be less than that obtained by the 1D experiment but is still within 10% of the actual value. This may in part arise from the use of peak ratios derived from 2D cross-peak intensities whose accuracy is limited by the signal-to-noise ratio. The results show that on a small amount of material (15 mg), 2D-MELODRAMA can reveal homonuclear chemical shift correlations and permits the measurement of internuclear distances with good accuracy.

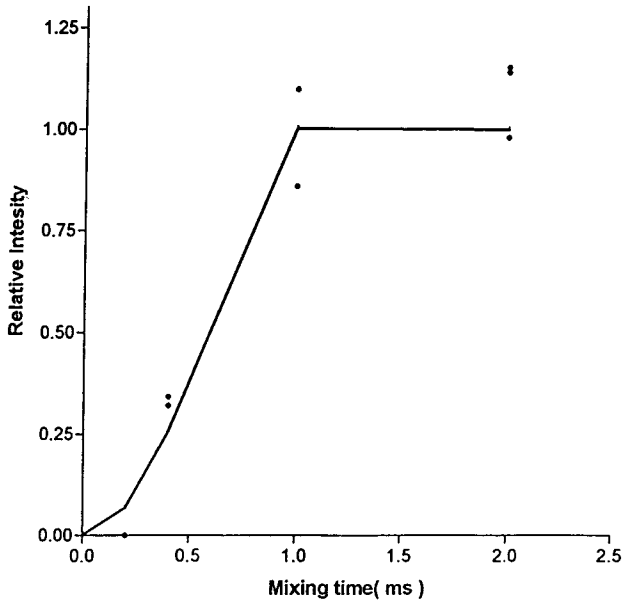

Figure 5. Best fit of the relative intensity of the 2D-MELODRAMA cross-peak intensity at several values of the mixing time, t_{mix}, for sodium proprionate-2-3-$^{13}C_2$. The cross-peak intensities were taken from the spectra shown in Figure 3. All cross-peak intensities were referenced to that for $t_{mix} = 2$ ms. Data fit was according to eq 53. Solid line best fit with $D_N = 1516 \pm 56$ Hz (the error corresponds to the standard deviation in the D_N values when the peak heights or peak integrals were used in the fits and also when either cross-peak intensity at $t_{mix} = 2$ ms was used for referencing).

Glucose-$^{13}C_6$. A criticism of distance measurements by solid-state NMR techniques is that typically such measurements are made on pairs of sites, and then only on one pair at a time. This task can certainly be time consuming, if not costly as well. The 2D-MELODRAMA sequence has been shown to be capable of revealing multiple homonuclear chemical shift correlations in peptides having more than two sites labeled with ^{13}C at >90% enrichment.[17] Carbohydrate systems represent a difficult class of biomolecules for such studies because chemical synthesis is difficult and costly and the ^{13}C chemical shift range is often small. Thus it is highly desirable in these cases to be able to incorporate multiply labeled sites either through chemical or biochemical synthesis to make simultaneous multiple chemical shift correlations and distance measurements on small amounts of material. To reduce multi-spin effects and maximize isolated two-spin correlations, it is expected that the level of uniform isotopic labeling must be low (e.g. 10−20%). To test the viability of the 2D-MELODRAMA experiment for determining accurate multiple internuclear distances for carbohydrates, measurements were made on 5 mg of glucose uniformly ^{13}C labeled at 36.6%. It was expected that at this level of isotopic labeling, the assumption of isolated spin pairs may be problematic for some sites. Some positions (e.g. C3 and C5) exhibit a small chemical shift difference (<0.5 ppm), a situation typically found in carbohydrates. Thus the sample is a stringent test of the sequence's ability to resolve correlations and distances between sites having a small range of shifts on a small sample (≈5 mg). Figure 6a shows that in the 1D spectrum of glucose, all resonances for the six carbons are resolved. Resonances may be assigned based on those of glucose in aqueous solution,[23] and these assignments are consistent with C−C correlations revealed by the 2D-MELODRAMA spectra. Figure 6b shows ^{13}C 2D-MELODRAMA spectra and corresponding traces at the anomeric carbon frequency (C1) at several mixing times. At short mixing times, only correlations between C1 and ring

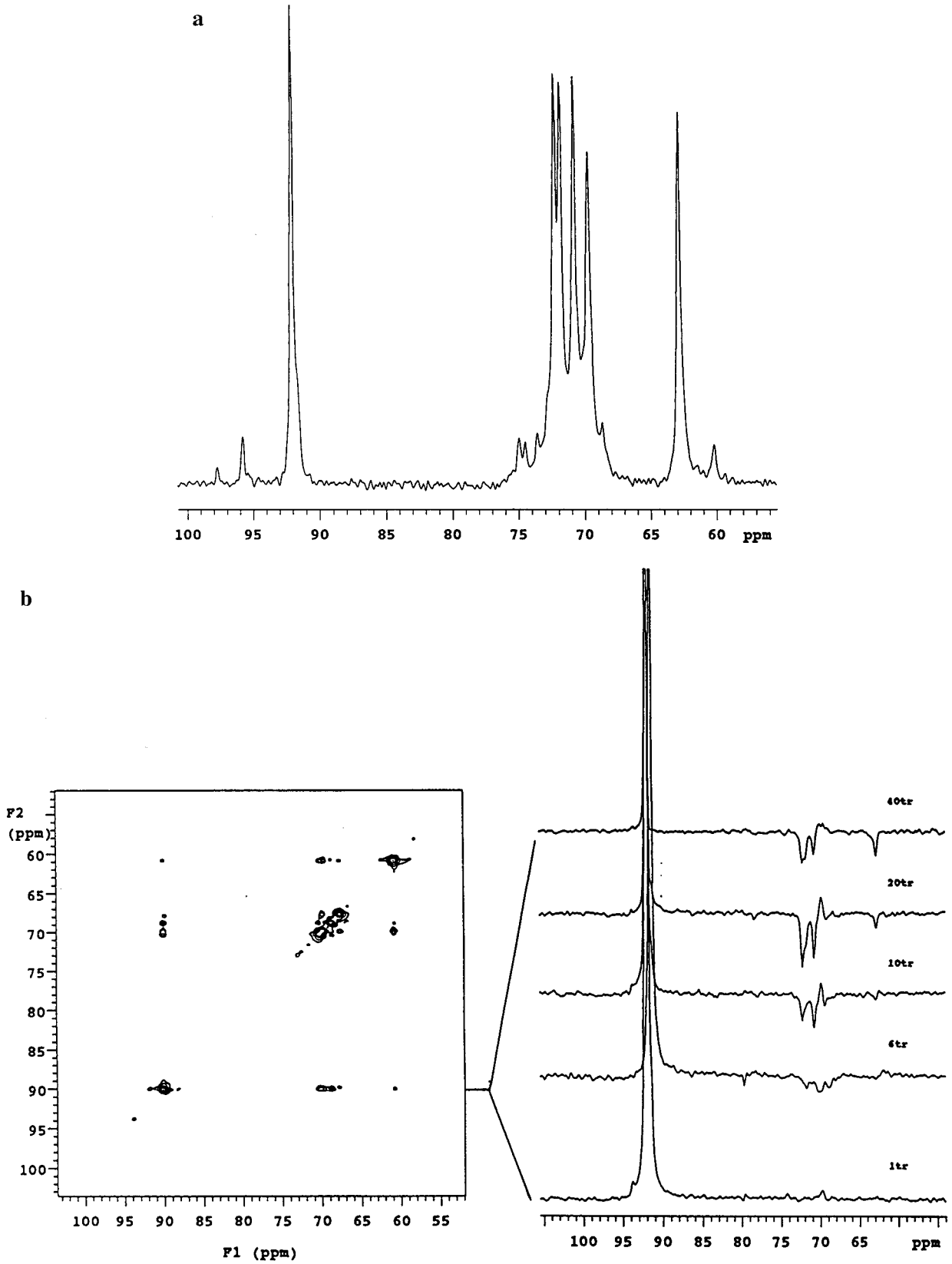

Figure 6. (a) 1D ^{13}C MAS NMR spectrum of glucose-$^{13}C_6$ showing spectral resolution. Small peaks are the β-anomer of glucose. (b) 2D-MELODRAMA ^{13}C NMR spectra of glucose-$^{13}C_6$. Left, contour plot for $t_{mix} = 4$ ms. Right, traces along F_1 axis at the C1 resonance showing the cross-peak intensity at the C2 to C6 carbon chemical shifts as a function of the t_{mix}: from bottom to top, 0.2, 1.2, 2.0, 4.0, and 8.0 ms, respectively. Data were acquired at a spinning rate of 5 kHz, and the ratio of ^{13}C radio frequency field strength to the spinning rate was 5 ($N = 5$).

positions <2 Å are detected. At longer mixing times correlations between C1 and all other carbons are detectable. Of interest is that the signal-to-noise ratio is sufficient to detect C−C correlations even between relatively distant carbons. On the basis of the cross-peak intensity predicted by eq 32 at the measured mixing times, a qualitative analysis of the data would allow C−C correlations to be grouped into short (<2 Å), medium (2−3 Å), and long (>3 Å). Inspection of traces through

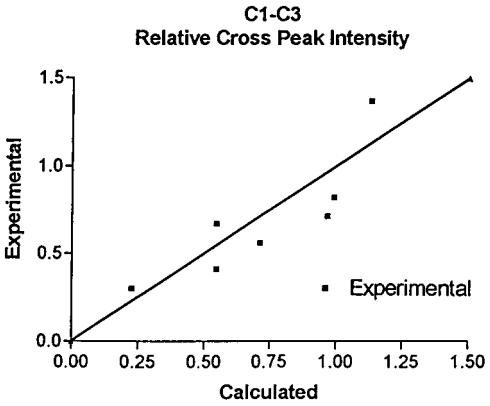

Figure 7. Best fit of the relative intensity of the 2D-MELODRAMA ^{13}C cross-peak intensity for the C1−C3 pair of glucose-$^{13}C_6$ at several values of t_{mix}. The cross-peak intensities were taken from the spectra shown in Figure 6. All cross-peak intensities were referenced to that for $t_{mix} = 2$ and 6 ms. Data fit was according to eq 53 with $D_N = 515$ Hz. The solid line represents that for exact correspondence between calculated and experimental intensities.

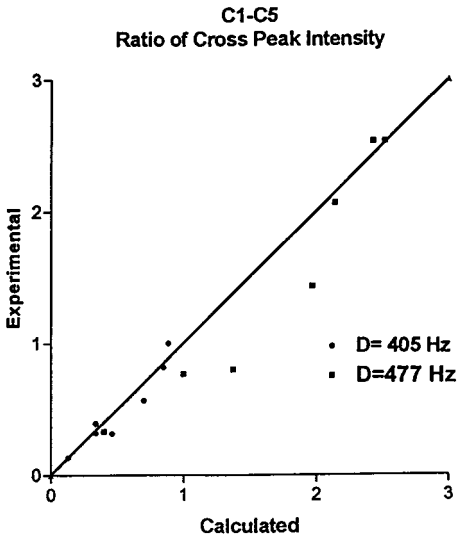

Figure 8. Best fit of the relative intensity of the 2D-MELODRAMA ^{13}C cross-peak intensity for the C1−C5 pair of glucose-$^{13}C_6$ at several values of t_{mix}. The cross-peak intensities were taken from the spectra shown in Figure 6. All cross-peak intensities were referenced to that for $t_{mix} = 2$ ms (●) and 6 ms (□). Data fit was according to eq 53 with the indicated values of D_N. The solid line represents that for exact correspondence between calculated and experimental intensities.

the 2D spectrum shows that spectral resolution is not sufficient to monitor all C−C correlations (data not shown). Since the diagonal peak is relatively large and has opposite sign to that of the associated cross-peak, overlap of the two makes detection of cross-peaks difficult, and quantitation of intensities impossible. For this reason, the 2D spectrum does not appear to be symmetric about the diagonal. In principle, analysis of the cross-peak intensity at one or more mixing times as described above should permit the evaluation of the entire distance matrix relating all inter-carbon separations. However, unlike a simple two-spin system, the multiply labeled glucose presents several problems. First, the diagonal represents a superposition of diagonals associated with each two-spin subsystem so that normalization of cross-peak intensity is difficult. Second, since the expected magnitudes of dipolar couplings range from 2 kHz to <100 Hz, it is difficult to optimally define the buildup of all cross-peak intensities without recourse to what may in some cases be impractical experimental times. Finally, for the long mixing times required when small dipolar couplings are being measured, it is difficult to directly estimate intensity corrections for T_2 effects by the approach discussed above. For the glucose data, the buildup of cross-peak intensity as a function of mixing time was analyzed according to eq 53 where the intensity at each value of the mixing time is expressed relative to the intensity at a particular value of the mixing time. Results of typical fits are shown in Figures 7 and 8. Internuclear distances derived from the estimated dipolar couplings for the glucose sample are shown in Table 1. For comparison the corresponding distances derived from a neutron diffraction study of methyl α-D-glucospyranoside[24] are also given in Table 1. Due to spectral overlap some internuclear distances could not be determined such as the short C3−C4 (1.53 Å). In general there is good agreement between distances estimated from the dependence of cross-peak intensities on mixing time and those derived from single-crystal studies. However, there are some notable discrepancies. While correlations between positions having internuclear distances greater than 3 Å are observed, the derived distances appear to be shorter than expected. A possible source of this inaccuracy is that long mixing times are required to properly define the cross-peak buildup curves. As a result, transverse relaxation effects could lead to cross-peak intensities that are significantly less than expected. Since the fitting procedure uses relative cross-peak intensities (where experimental and calculated cross-peak intensities are referenced with respect to that from a long mixing time) the latter effect would give the appearance of a cross-peak buildup rate greater than expected for the true internuclear distance. This appears to be the most likely explanation for the short C1−C6 and C3−C6 distances. In contrast, short distances are measured as being 10−20% longer. This is similar to the result obtained with

Table 1. Glucose $^{13}C-^{13}C$ Distances (in Å) from 2D-MELODRAMA Cross-Peak Intensities[a,b]

position	C1	C2	C3	C4	C5	C6
C1	0.00	1.83 ± 0.02	2.48 ± 0.1	ND	2.60 ± 0.07	2.89 ± 0.10
	0.00	*1.53*	*2.49*	*2.87*	*2.38*	*3.67*
C2	2.00 ± 0.04	0.00	1.8 ± 0.04	1.81 ± 0.17	ND	ND
	1.53	*0.00*	*1.52*	*2.49*	*2.87*	*4.24*
C3	2.34 ± 0.05	1.61 ± 0.1	0.00	ND	ND	3.08 ± 0.16
	2.49	*1.52*	*0.00*	*1.53*	*2.51*	*3.87*
C4	ND	1.81 ± 0.17	ND	0.00	1.8 ± 0.03	2.6 ± 0.01
	2.87	*2.49*	*1.53*	*0.00*	*1.53*	*2.52*
C5	2.61 ± 0.03	ND	ND	1.98 ± 0.1	0.00	2.2 ± 0.05
	2.38	*2.87*	*2.51*	*1.53*	*0.00*	*1.52*
C6	3.03 ± 0.01	ND	ND	2.66 ± 0.2	ND	0.00
	3.67	*4.24*	*3.87*	*2.52*	*1.52*	*0.00*

[a] Errors represent standard deviations among fits for which different cross peaks were used in referencing relative cross-peak buildup. [b] Values in italics are calculated from the crystal structure of methyl α-D-glucopyranoside.[24] ND = Not determined either because of spectral overlap or absence of corresponding cross-peak.

sodium proprionate for which the C−C distance measured by 2D-MELODRAMA was ∼10% greater than expected. Sun *et al.*[17] have noted that rf inhomogeneity reduces the efficiency of the reintroduction of homonuclear dipolar recoupling by the MELODRAMA sequence, which would be manifest as an apparently smaller dipolar coupling. In the present case this seems unlikely since for the sodium proprionate sample, 1D-MELODRAMA gave close agreement between the measured and expected internuclear distance. Inspection of Figure 4 and simulations of cross-peak intensity (data not shown) as a function of mixing time show that, for a spinning speed of 5 kHz and a dipolar coupling of 2 kHz, the buildup of cross-peak intensity is essentially complete after 8 rotor cycles. Thus for the glucose case the experimental cross-peak buildup may not be as well defined for short distances (1.5 Å) as for longer distances, leading to less accuracy in estimating the dipolar coupling. In cases where both short and long distances are expected, a possible strategy is to use higher spinning rates so that better definition of cross-peak buildup may be obtained. In the present study, a spinning rate of 5 kHz was the maximum stable spinning speed achievable.

Conclusions

2D-MELODRAMA allows multiple homonuclear chemical shift correlations in systems where there is not a large separation in the resonance frequencies of dipolar coupled spin pairs. In addition, analysis of the buildup of cross-peak intensity as a function of the dipolar recoupling period can provide estimates of internuclear separations with good accuracy (within ∼10% of the true value). It has been shown that the cross-peak intensity has a simple dependence on the value of the dipolar coupling and the mixing time, and that analysis of experimental results amounts to a simple one-parameter fit of the data. While there are a number of well-established techniques for distance measurements in peptides and proteins, the 2D-MELODRAMA approach discussed in this study appears to hold particular promise for 3D structural analysis of carbohydrate systems. Since carbohydrates are notoriously difficult to crystallize, the 2D-MELODRAMA approach certainly offers an alternative to single-crystal X-ray crystallography for structural analysis with Ångström resolution. One of the frequently used methods of establishing conformation of glucoconjugates is the combination of NMR spectroscopy and molecular mechanics/dynamics calculations. However, there are still important concerns with the force field calculations which arise mainly from insufficiently validated parameter sets.[25] In contrast to the situation which obtains in the area of peptides and proteins, this arises in part from the lack of a sufficiently large base of known structures from which to refine force field parameter sets. Solid-state NMR distance measurements therefore hold considerable promise for expanding the database of carbohydrate structures, thereby contributing to the refinement of parameter sets for carbohydrate structure prediction.

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