COMMUNICATIONS

Total Correlation Spectroscopy in the Solid State. The Use of Scalar Couplings to Determine the Through-Bond Connectivity

M. BALDUS AND B. H. MEIER

*NSR-Center for Molecular Structure, Design and Synthesis, Laboratory of Physical Chemistry,
University of Nijmegen, Toernooiveld 6525 ED Nijmegen, The Netherlands*

A two-dimensional NMR experiment (*1, 2*) that elucidates the carbon–carbon through-bond connectivity in the solid state is described. The experiment takes place under high-resolution conditions employing magic-angle spinning (MAS) and makes it possible to obtain, in the solid state, the information that, in liquid state, is obtained from a TOCSY experiment (*3*). TOCSY is widely used for the determination of through-bond connectivity in liquid-state spectroscopy. The solid-state version of this experiment, presented here, is expected to further enhance the applicability of NMR to structure elucidation in solids.

It is well known that much of the success of NMR structure determination of organic and biological molecules in the liquid state (*4*) is based on the combination of through-bond information, often extracted from COSY (*5*) or TOCSY spectra, and through-space information, usually derived from a NOESY experiment (*6*). The combination of the solid-state through-bond experiment introduced here and MAS through-space correlation experiments, now emerging in the literature (*7–16*), is therefore highly desirable.

The isotropic part of the homonuclear *J* coupling (typically about 50 Hz between two directly bonded carbons) is usually approximately 40 times smaller than the dipolar coupling between the nuclei. Therefore, *J* couplings have, so far, played only a minor role in solid-state spectroscopy. Structure information is usually obtained from the dipolar interaction only. The dipolar interaction, described by a second-rank tensor, is averaged out by magic-angle sample spinning and must be reintroduced by specially designed pulse sequences (*7–16*).

Here, we follow a different strategy and exploit the *J*-coupling interaction. It should be noted that, in the solid state, the *J* coupling and the dipolar coupling appear explicitly in the system Hamiltonian and therefore dipolar-correlated spectra have also been denoted by the term TOCSY in the literature. To avoid confusion, we use, in this Communication, the terms total through-bond-correlation spectroscopy (TOBSY) if the transfer is mediated by *J* interactions and total through-space-correlation spectroscopy (TOSSY) if dipolar couplings are employed.

To design a TOBSY experiment, the homo- and heteronuclear dipolar interaction as well as the chemical shifts must be suppressed during the mixing time of the experiment (for the experimental scheme, see Fig. 1) such that the isotropic *J* coupling is the dominant interaction in the Hamiltonian

$$H \approx H_J = 2\pi \sum_{i<j} J_{ij} \mathbf{I}_i \cdot \mathbf{I}_j. \qquad [1]$$

Here, the sum runs over all pairs of coupled spins. This Hamiltonian is identical to the TOCSY Hamiltonian and it is well known that it leads to a net polarization transfer as well as to antiphase signals (*3*) in the resulting 2D spectra. The net polariza-
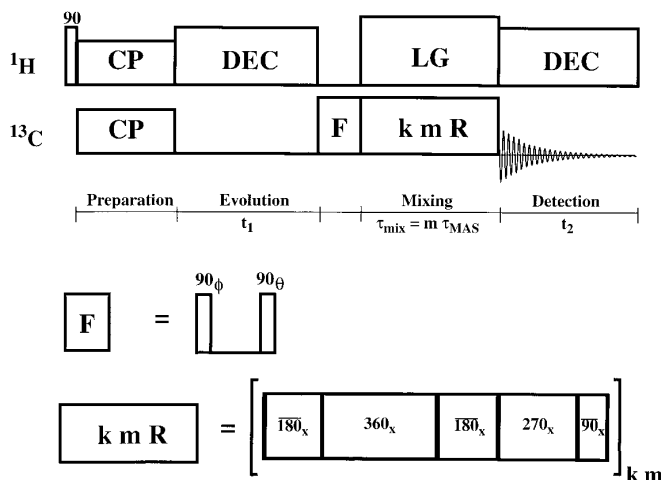

FIG. 1. Two-dimensional TOBSY pulse scheme. During CP, Hartmann–Hahn cross polarization takes place (2 ms with fields matched at 75 kHz). During the t_1 evolution period, on-resonance proton decoupling at 100 kHz field strength is used. The phases ϕ and θ of the *z* filter F (*23*) were set to select pure *x*, *y*, or *z* magnetization at the beginning of the mixing time (for *z* selection, the second pulse is shifted to the end of the mixing time). *J*-induced polarization transfer then occurs during the mixing time $\tau_{\text{mix}} = m\tau_{\text{MAS}}$ (where $m = 1, 2, 3, 4 \cdots$) under simultaneous Lee–Goldburg (LG) proton decoupling at 120 kHz RF field strength. The mixing scheme R is applied such that $k = 4$ or $k = 8$ R cycles cover one rotor period τ_{MAS}, resulting in a 102 kHz ^{13}C RF field in a MAS spinning speed of 8.5 and 4.25 kHz, respectively. For detection during t_2, on-resonance proton decoupling at 100 kHz was used. All experiments were performed on a 400 MHz Chemagnetics Infinity spectrometer using standard Chemagnetics 3.2 and 4 mm double-resonance MAS probeheads.

tion transfer (e.g., $I_{1x} \rightarrow I_{2x}$) leads always to positive in-phase cross peaks while the anti-phase signals (e.g., originating from the transfer $I_{1x} \rightarrow I_{1y}I_{2z} - I_{1x}I_{2y}$) lead to dispersion signals in the detection period and integrate to zero over the entire multiplet. These anti-phase components do not contribute to the signal at time $t_2 = 0$ but evolve into observable coherence in the course of the detection time under the influence of the J interaction. In solid state, where the J interaction is often not resolved in the detection period, these anti-phase components are not observable. Under these conditions, the TOBSY experiment leads to pure-absorption lineshapes in the 2D spectrum.

We assume for the following discussion that the heteronuclear interaction is fully suppressed by the decoupling scheme. We shall return to this point later. The homonuclear interactions that must be suppressed in the mixing time of the experiment to achieve an effective Hamiltonian of the form given in Eq. [1] include the isotropic chemical shift H_{CS}, the chemical-shift anisotropy, H_{CSA}, and the dipolar interaction H_D. The symmetry of these interactions with respect to rotations in spin space (RF pulses) and in real space (sample spinning) is listed in Table 1. It is immediately obvious that, in principle, it is possible to craft a Hamiltonian as given in Eq. [1] because the symmetry properties distinguish the J coupling from all other terms. Ramamoorthy *et al.* (*17*) have shown by numerical simulation that the polarization transfer in solids can in fact be dominated by J interactions for a particular choice of pulse sequence and spin system. Here we aim for a pulse scheme that guarantees through-bond transfer and suppresses through-space transfer for a wide range of spin-system parameters. In particular, we request that the zeroth- and first-order average Hamiltonians (*18*) do not contain any dipolar contributions.

Fast magic-angle spinning, for example, removes the spatial second-rank interactions H_{CSA} and H_D while rotor-synchronous π pulses in the delta-pulse limit (or a sufficiently strong continuous-wave spin lock) eliminate the rank 1 spin term of the chemical shift, H_{CS}. It can easily be shown that the combination of the spatial and spin-averaging techniques mentioned above leads, in zeroth-order average Hamiltonian theory, to the desired Hamiltonian

$$\bar{H}^{(0)} = H_J. \qquad [2]$$

TABLE 1
Transformation Properties of the Relevant Interactions Present in the Spin Hamiltonian

Interaction	Rank of the interaction tensor under rotations	
	In real space	In spin space
H_D (dipolar)	2	2
H_{CSA} (anisotropic chemical shift)	2	1
H_{CS} (isotropic chemical shift)	0	1
H_J (isotropic J coupling)	0	0

TABLE 2
Intensity of the Magnetization-Transfer Cross-Peaks in the Initial-Rate Two-Dimensional Experiment of Fig. 1[a]

Hamiltonian	Initial condition		
	x	y	z
Theoretical predictions			
$\mathcal{H}_J$ (J coupling)	$+a$	$+a$	$+a$
$\mathcal{H}_D$ (full dipolar)	$+b/\sqrt{2}$	$-b$	$-b$
$I_1^+I_2^+ + I_1^-I_2^-$ (flop–flop)	$-c$	0	0
$I_1^+I_2^- + I_1^-I_2^+$ (flip–flop)	$+c$	0	0
Experimental results			
TOBSY (J coupling) (%)	$+8 \pm 4$	$+9 \pm 4$	$+15 \pm 5$
TOSSY (dipolar coupling) (%)	$+3 \pm 1$	-4 ± 1	-3 ± 1

[a] For the theoretical predictions, the intensity is listed in arbitrary units denoted by a, b, and c for the different Hamiltonians. For all interactions, an effective quantization direction along the x axis of the normal rotating frame is assumed. For the experimental results, obtained from a doubly ^{13}C-labeled zinc acetate sample, the cross-peak intensity, compared to the total intensity of the 2D spectrum, is given. For the RIL—ZQT (*13*) scheme (TOSSY), a mixing time of 0.4 ms was used while for the TOBSY transfer a contact time of 1.5 ms was chosen.

The zeroth-order Hamiltonian is a good approximation only in the limiting case of fast spinning. In practice, the spinning speed does not greatly exceed the isotropic and anisotropic chemical shifts. The simple pulse schemes mentioned above are therefore, for most spin systems, not sufficient to suppress the unwanted terms efficiently enough. The pulse scheme given in Fig. 1
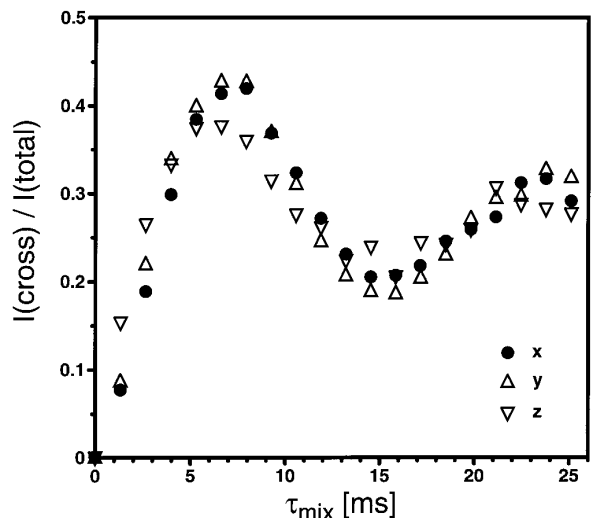

FIG. 2. Cross-peak intensity, normalized by the total intensity of the spectrum, for a doubly ^{13}C-labeled zinc acetate sample as a function of the mixing time. The initial condition for the TOBSY mixing cycle was selected to be x ($\bullet$), y ($\triangle$), or z magnetization ($\triangledown$). The spinning speed and carbon RF field strength were set to 4.3 and 103 kHz, respectively. For y and z initial conditions, the phase of the RF irradiation was incremented by 180° after each full rotor cycle to compensate for RF inhomogeneity (see text). The data were acquired as a selective slice out of the 2D spectrum in a 1D experiment.
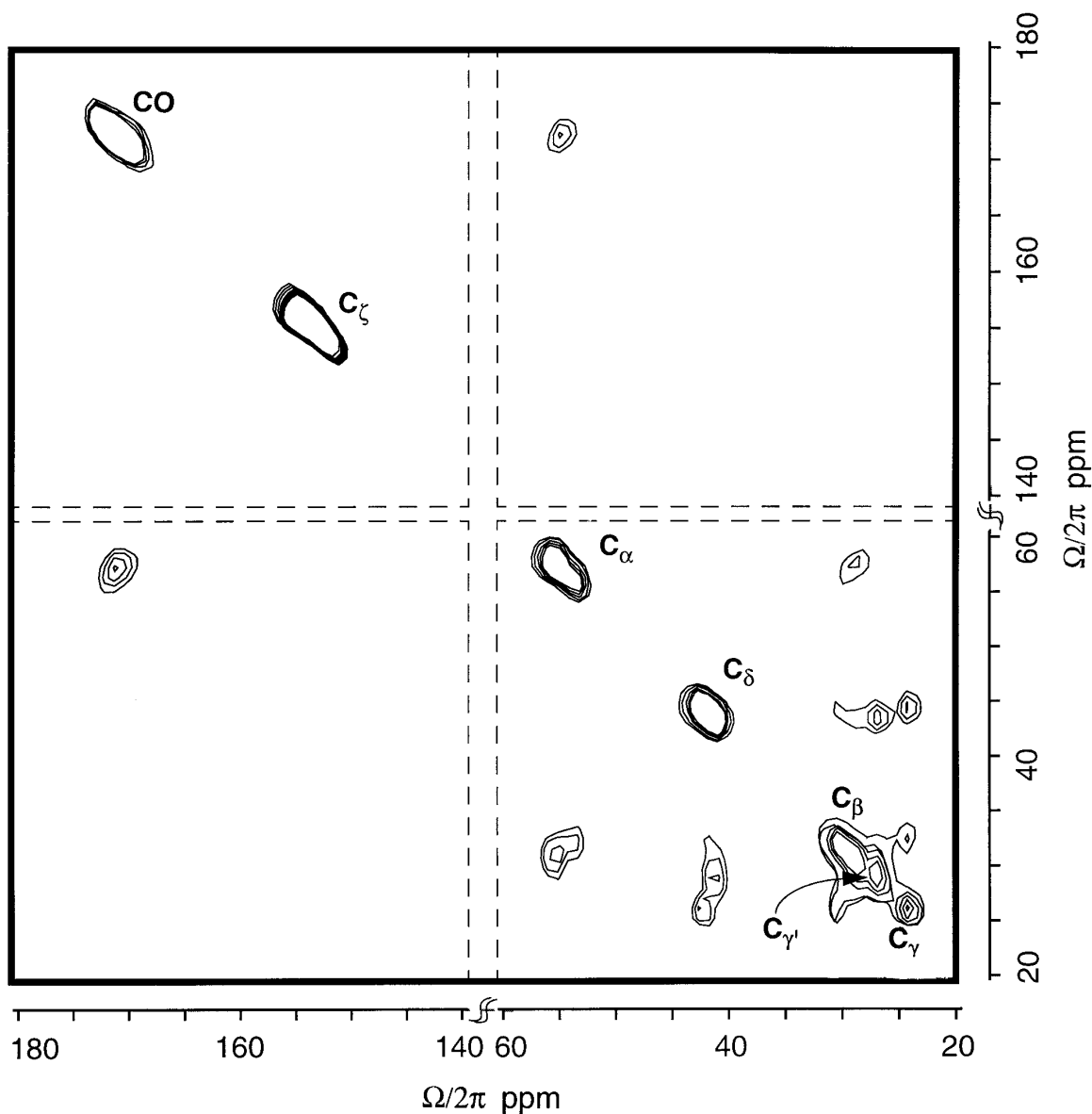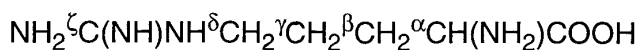
$NH_2{}^{\zeta}C(NH)NH^{\delta}CH_2{}^{\gamma}CH_2{}^{\beta}CH_2{}^{\alpha}CH(NH_2)COOH$

FIG. 3. TOBSY initial-rate spectrum from a fully (^{13}C, ^{15}N)-labeled arginine powder sample after a mixing time of 3 ms. Only the aromatic and aliphatic regions of the total 2D spectrum are shown. All one-bond correlations are represented through cross peaks. The spinning speed was set to 8.5 kHz used for four R cycles per MAS period. A total of 256 t_1 experiments averaging eight scans in the t_2 dimension was performed, leading to a 256 × 512 data matrix. Contour levels are chosen at constant intervals between 5 and 15% of the maximal signal intensity.

employs composite π pulses of the form R = $\overline{2}4\overline{2}3\overline{1}$ where 1 stands for a $\pi/2$ pulse, 2 for a π pulse, and so forth. Overlined pulses are applied along the $-x$ direction of the rotating frame, the other pulses along the x direction. This pulse scheme has been developed in the context of the WALTZ pulse sequences (*19*) which are known to yield good compensation for chemical-shift offsets. Because the TOBSY scheme combines MAS with multiple-pulse irradiation, care must be exercised that none of the interactions, eliminated by the MAS alone, are reintroduced. In fact, it turns out that the WALTZ8 pulse sequence R$\overline{\overline{R}}$R$\overline{R}$, when combined with MAS (one or two WALTZ8 cycles per

rotor cycle resulting in $k = 4$ or $k = 8$), reintroduces CSA terms and dipolar terms into the zeroth-order Hamiltonian. The simplified pulse sequence of Fig. 1 with $k = 8$ R pulses per rotor cycle leads to the desired zeroth-order average Hamiltonian. Also in first-order AHT, no dipolar or CS contributions arise. Compensation for RF inhomogeneity can be added by changing the phase of the RF irradiation after each full rotor cycle but is not always desirable because the RF inhomogeneity can eliminate undesired components of the density operator (*3*). For $k = 4$, the dipolar interaction is averaged out, but a contribution from H_{CSA} to $\overline{H}^{(0)}$ remains. In practice, this is sometimes tolerable.
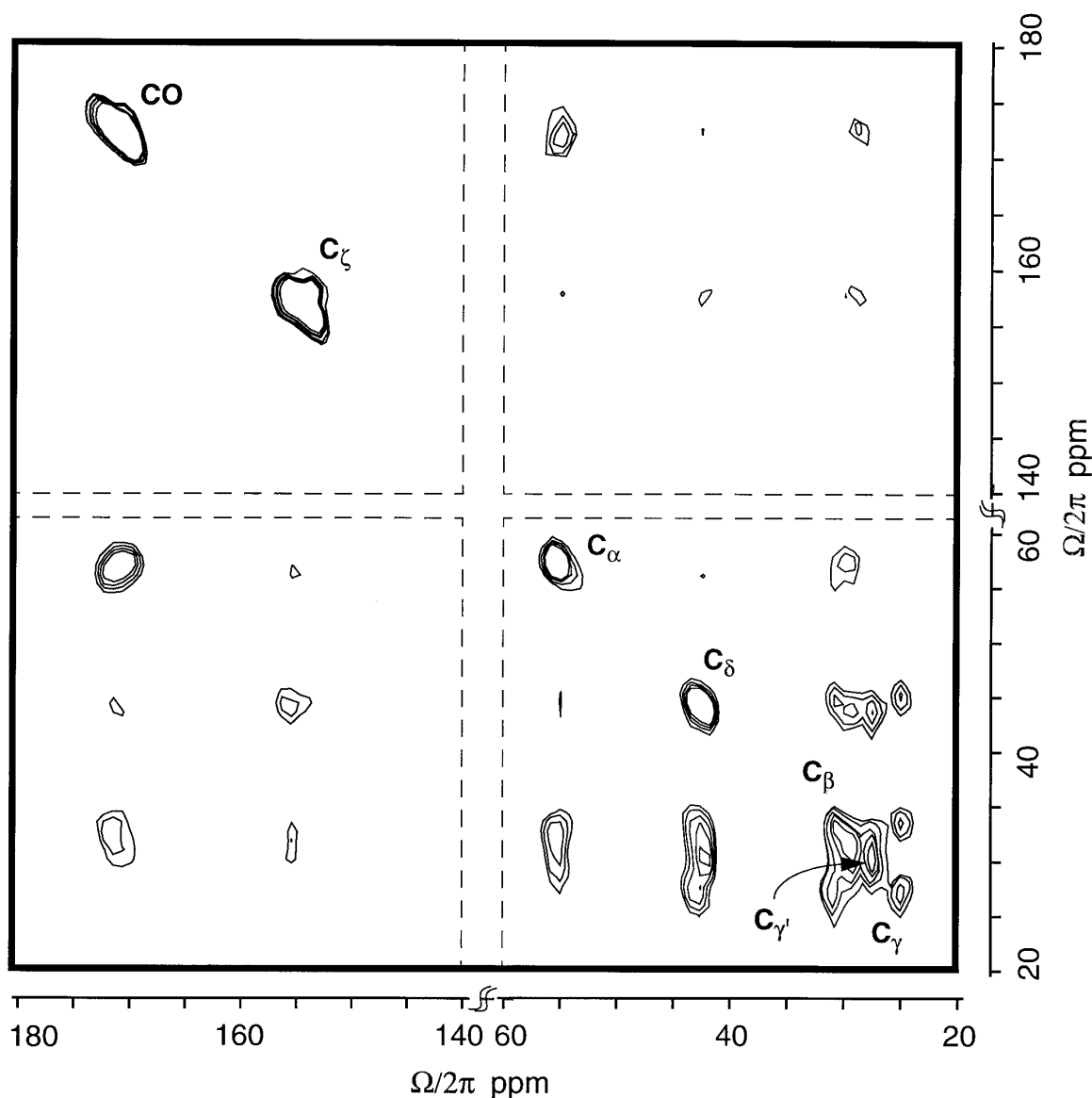
FIG. 4. TOBSY spectrum on the arginine powder sample of Fig. 3 after a mixing time of 15 ms and using the experimental conditions described in the legend to Fig. 3. Here, 16 scans in the detection dimension were accumulated. In addition to the one-bond cross peaks of Fig. 3, now also long-range and indirect (^{13}C, ^{13}C) couplings are observed. Contour-level settings are described in the legend of Fig. 3.

It is a fact that, whenever a reasonably strong J coupling between nuclei is found, there is also a much stronger homonuclear dipolar coupling present. Numerical calculations not described in detail in this Communication show that the pulse sequence of Fig. 1 (4R or 8R per MAS cycle) is able to scale the effective dipolar couplings far below the J couplings for typical parameter values of a ^{13}C spectrum. Furthermore, these simulations indicate that no significant changes occur if the multipulse sequence is not applied exactly rotor synchronous.

Nevertheless, an experimental confirmation that the transfer actually stems from J interactions and not from a poorly suppressed dipolar interaction seems appropriate. To do so, we again make use of the symmetry properties of Table 1. The J coupling interaction, H_J, is isotropic and its polarization- and

coherence-transfer properties are independent of the direction of the initial magnetization. The dipolar interaction, in contrast, is a traceless tensor of rank 2 and the sign of the transferred magnetization, in initial rate, is positive for polarization transfer (initial condition along the effective quantization direction which is the I_x direction of the usual rotating frame) and negative for coherence transfer (magnetization along I_y and I_z of the rotating frame). In higher-order average Hamiltonian theory, dipolar contributions of different symmetry can appear. The most important ones are also included in Table 2, which compares the predicted sign of the initial-rate polarization transfer between two spins, for initial conditions along the x, y, and z directions in the rotating frame, with experimental results obtained using a doubly ^{13}C-labeled zinc acetate sample and the

pulse sequence of Fig. 1. Furthermore, the results from a control experiment with the TOSSY sequence RIL—ZQT (*13*) are given in Table 2. The experimental behavior corroborates the presence of TOBSY transfer for the sequence of Fig. 1 and TOSSY transfer for the RIL—ZQT sequence and shows positive and negative coherence-transfer signals for TOBSY and TOSSY, respectively. We have, furthermore, checked the cross-peak intensities listed in Table 2 under different experimental parameter settings (RF carrier, spinning speed) and have found no significant changes. The buildup of the cross-peak intensity for longer mixing times in the two-spin system of zinc acetate is shown in Fig. 2. Again, the isotropic behavior in spin space predicted for J transfer is observed.

Ideally, an oscillatory behavior with a period of 17 ms would be expected for the data in Fig. 2. The maximum transfer would reach unity if expressed by the ratio of cross-peak intensity and total intensity. Experimentally, an oscillation with the predicted frequency is observed. Imperfections of the pulse sequence attenuate the purely oscillatory behavior, and the achieved transfer efficiency is lower than expected for the isolated two-spin system.

So far, we have assumed that the proton spins are completely decoupled from the carbons and have neglected all heteronuclear interactions. For finite RF fields, nonideal decoupling may result and can manifest itself by (a) ^{13}C $T_{1\rho}$ relaxation which compromises the signal-to-noise ratio of the ^{13}C spectra and leads to intensity distortions; and (b) proton-driven spin diffusion between dipolar-coupled ^{13}C. This effect, observed by Sachleben *et al.* (*20*) in laboratory-frame experiments with low-power on-resonance proton decoupling could lead to ''false'' cross peaks in TOBSY spectra. Proton-driven spin diffusion is an incoherent effect which would lead to an exponential time evolution of the cross-peak amplitude. We have recently shown in another context (*21*) that proton homonuclear decoupling using the Lee–Goldburg (*22*) decoupling scheme leads to excellent heteronuclear decoupling under MAS and we apply this scheme also in the present context (see Fig. 1). It is easily seen from Fig. 2 that, under our experimental conditions, coherent J transfer takes place and incoherent contributions may be neglected. A more detailed discussion of possible incoherent contributions will be presented elsewhere. Having verified that the TOBSY pulse sequence leads to pure J transfer, we apply it to a sample of fully $^{15}N–^{13}C$ labeled arginine. The TOBSY spectrum for a short mixing time (3 ms) and the assignment of the resonance lines is given in Fig. 3. Only the directly bonded ^{13}C nuclei are connected by sizeable J cross peaks, as expected for the initial-rate regime. On the basis of this spectrum, we can immediately resolve the assignment problem of the five resolved resonances in the aliphatic region of our sample to the only four chemically distinct aliphatic carbons in the molecule. The resonance C_γ is obviously split into two signals. In a control sample of natural-abundance arginine, only one of the two C_γ resonances is found. We assign the two resonances to two different crystal forms being present in our labeled sample. For a longer mixing time of 15 ms, all cross peaks (except those between C_γ and $C_{\gamma'}$) are found (see Fig. 4) as expected for a total correlation spectrum. These data demonstrate the feasibility of TOBSY in the solid state.

The TOBSY cross-peak pattern of our simple test molecule is quite similar to the TOSSY pattern (*13*) obtained from the same sample. Nevertheless, the information content is different because through-bond and through-space connectivities, respectively, are detected.

We expect that TOBSY spectroscopy will be useful for structure determination of organic, inorganic, and biological molecules in crystalline and amorphous phase as well as in membranes. No spectrally resolved J couplings are required to perform the TOBSY experiment.

REFERENCES

1. R. R. Ernst, G. Bodenhausen, and A. Wokaun, ''Principles of Nuclear Magnetic Resonance in One and Two Dimensions,'' Clarendon Press, Oxford, 1987.

2. R. R. Ernst, *Angew. Chem.* 104, 817 (1992).

3. L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.* 53, 512 (1983).

4. K. Wüthrich, ''NMR of Proteins and Nucleic Acids,'' Wiley–Interscience, New York, 1986.

5. W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.* 64, 2229 (1976).

6. J. Jeener, B. H. Meier, P. Bachmann, and R. R. Ernst, *J. Chem. Phys.* 71, 4546 (1979).

7. B. H. Meier and W. L. Earl, *J. Am. Chem. Soc.* 109, 7937 (1987).

8. R. Tycko and G. Dabbagh, *Chem. Phys. Lett.* 173, 461 (1990).

9. T. Gullion and S. Vega, *Chem. Phys. Lett.* 194, 423 (1992).

10. A. E. Bennett, J. H. Ok, R. G. Griffin, and S. Vega, *J. Chem. Phys.* 96, 8624 (1992).

11. J. H. Ok, R. G. S. Spencer, A. E. Bennett, and R. G. Griffin, *Chem. Phys. Lett.* 197, 389 (1992).

12. T. Fujiwara, A. Ramamoorthy, K. Nagayama, K. Hioka, and T. Fujito, *Chem. Phys. Lett.* 212, 81 (1993).

13. M. Baldus, M. Tomaselli, B. H. Meier, and R. R. Ernst, *Chem. Phys. Lett.* 230, 329 (1994).

14. N. C. Nielsen, H. Bildsoe, H. J. Jakobsen, and M. H. Levitt, *J. Chem. Phys.* 101, 1805 (1994).

15. Y. K. Lee, N. D. Kurur, M. Helmle, O. G. Johannessen, N. C. Nielsen, and M. H. Levitt, *Chem. Phys. Lett.* 242, 304 (1995).

16. T. Fujiwara, K. Sugase, M. Kainosho, A. Ono, A. Ono, and H. Akutsu, *J. Am. Chem. Soc.* 117, 11351 (1995).

17. A. Ramamoorthy, T. Fujiwara, and K. Nagayama, *J. Magn. Reson. A* 104, 366 (1993).

18. U. Häberlen, ''High Resolution NMR in Solids: Selective Averaging,'' Academic Press New York, 1968.

19. A. J. Shaka, J. Keeler, and R. Freeman, *J. Magn. Reson.* 53, 313 (1983).

20. J. R. Sachleben, L. Emsley, S. Caldarelli, M. Bardet, G. Bodenhausen, and A. Pines, 35th ENC Conference, Post WP205, Pacific Grove, 1994.

21. M. Baldus, D. G. Geurts, S. Hediger, and B. H. Meier, *J. Magn. Reson. A* 118, 140 (1996).

22. M. Lee and W. I. Goldburg, *Phys. Rev. A* 140, 1261 (1965).

23. O. W. Sørensen, M. Rance, and R. R. Ernst, *J. Magn. Reson.* 56, 527 (1984).