

# Probing Through-Bond Connectivities and Through-Space Distances in Solids by Magic-Angle-Spinning Nuclear Magnetic Resonance

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**Abstract:** The application of a new solid-state NMR technique, total through-bond correlation spectroscopy, and of an improved scheme for total through-space correlation spectroscopy to structure determination in organic solids is explored. Both experimental techniques are employed under high-resolution conditions using magic-angle sample spinning (MAS). The results from a test sample (calcium acetate monohydrate) show that through-space and through-bond connectivities can be probed separately. The information contained in the two experiments is complementary and the combination of both results can lead to improved structure determination schemes in biomolecular and material-science applications.

## Introduction

The methodology for the determination of the molecular geometry and topology by solid-state NMR spectroscopy is rapidly emerging and complete structures of biomolecules determined by solid-state NMR have been published (e.g. ref 1). Much of this work is motivated by the enormous success of NMR structure determination of dissolved molecules, in particular of biological macromolecules.<sup>2,3</sup> Most commonly, the structure from liquid samples is obtained by combining experimental results that probe the through-bond connectivity (e.g. COSY<sup>4</sup> or TOCSY<sup>5,6</sup>) with results that probe the through-space distances (e.g. NOESY<sup>7</sup>). These pulse schemes rely on polarization or coherence transfer during the mixing period of a two-dimensional experiment. The work presented here is concerned with the possibility of applying similar schemes in the solid state. In the solid, further structural and assignment information can be extracted if the anisotropic properties of the chemical shielding or the dipolar interaction are exploited. Here we will concentrate on “isotropic” spectra.

In the solid state, much progress has recently been reported toward structure determination using the through-space interactions. In contrast to liquid-state spectroscopy, the proton spectrum of solids is usually insufficiently resolved and we focus here on <sup>13</sup>C spectroscopy in uniformly enriched samples. Extension to <sup>15</sup>N, <sup>31</sup>P, and other spin 1/2 nuclei is obvious. A number of methods are available that reestablish the dipolar interaction, which is usually suppressed by magic-angle sample

spinning (MAS). Then, polarization transfer caused by the dipole interaction can be observed. One approach to do so is a rapid reorientation of the axis of sample rotation away from the magic angle for the duration of the mixing time.<sup>8–10</sup> A second approach leaves the sample-spinning axis at the magic angle. For a specific spin pair the dipolar interaction can be reintroduced by setting the rotor frequency equal to or equal to a submultiple of the isotropic chemical-shift difference (rotational resonance).<sup>11–13</sup> Total through-space correlation spectroscopy (TOSSY) reintroduces simultaneously all dipolar connectivities in a single two-dimensional experiment. TOSSY employs, during the mixing time, a pulse sequence (usually rotor synchronized) that leads to broadband polarization transfer under MAS conditions.<sup>14–25</sup>

Recently, we have proposed an alternative total correlation experiment that monitors through-bond connectivities.<sup>26</sup> In this

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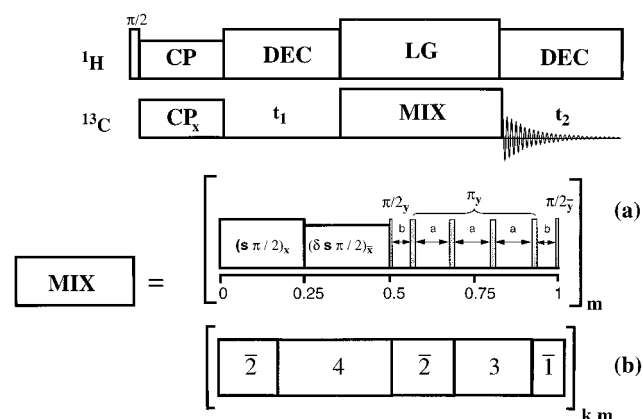
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**Figure 1.** Experimental schemes for two-dimensional TOSSY (a) and TOBSY (b) experiments. After cross polarization (CP) with radio frequency fields matched for 5 ms at 50 kHz, the  $t_1$  evolution took place under on-resonance proton decoupling at 120 kHz field strength. A mixing period of 20 ms followed under simultaneous proton Lee–Goldburg decoupling at 150 kHz applied radio frequency field strength. The proton radio frequency offset for the Lee–Goldburg condition was carefully optimized for best performance. For all experiments the carbon carrier was placed in the center of the carboxylic region of the spectrum. During acquisition, on-resonance proton decoupling at 120 kHz was used. The pulse sequence during the mixing period differs for the two experiments: for TOSSY, the dipolar recoupling scheme (a) was used. The mixing consisted of an integer number  $m$  basic RIL–ZQT cycles each of them covering one MAS period  $T_R$ . A carbon radio frequency field strength of 109 kHz was used and the amplitude attenuation parameter  $\delta$  was set to 0.9. The separation of the refocusing pulses during the second half of one rotor period in units of the flip angle of a radio frequency pulse of the same length is given by  $a(s) = ((s/4) - 1)(\pi/\omega_1)$  and  $b(s) = ((s/8) - 1)(\pi/\omega_1)$ , respectively. For TOBSY the pulse scheme (b) was used where the composite radio frequency pulse  $R = 24231^{35}$  (1 stands for a  $\pi/2$  pulse, 2 for a  $\pi$  pulse, and so forth) is repeated  $k = 8$  times per rotor cycle. Because the mixing Hamiltonian is isotropic, the desired component of the polarization after  $t_1$  must be selected either by a  $z$ -filter or by an appropriate difference experiment.<sup>36</sup> For all experiments described, the MAS spinning speed was set to 4.53 kHz. The measurements were performed on a 400-MHz Chemagnetics Infinity spectrometer using a standard 3.2 mm double-resonance MAS probehead tuned to minimize asymmetric phase transients.

experiment, abbreviated by TOBSY (total through-bond correlation spectroscopy), the dipolar interaction as well as the chemical shifts are suppressed to allow for polarization transfer mediated by the scalar  $J$  coupling.

In this contribution, improved versions of both schemes will be applied to a test sample and the complementarity of the information will be demonstrated.

### Pulse Schemes

TOBSY and TOSSY experiments follow the same general experimental scheme shown in Figure 1. Only the mixing time depends on the specific type of experiment. For TOBSY, the dipolar interactions must be minimized, for TOSSY, they must be maximized. Due to the long mixing times needed to explore small couplings, efficient proton decoupling is essential for both experiments to prevent a decay of the sum polarization. Furthermore, effective decoupling is crucial to suppress possible “false” proton-driven spin-diffusion cross peaks<sup>26–28</sup> in the TOBSY experiment (vide infra). Here we apply a two-stage

scheme where proton homonuclear Lee–Goldburg decoupling<sup>29</sup> eliminates the  $^1\text{H}–^1\text{H}$  interactions and the remaining (and now heterogeneous)  $^1\text{H}–^{13}\text{C}$  interactions are removed by a combination of sample rotation and  $^{13}\text{C}$  radio frequency irradiation.<sup>26,30,31</sup>

For the TOSSY experiment, we require (i) that the cross-peak intensity reflects the intermolecular distance and is not considerably influenced by spectral quantities, e.g. the chemical shifts, and (ii) that the polarization transfer takes place through zero-quantum transitions that conserve the sum polarization of the spin system. Then, the polarization exchange is, for spin systems with an extended coupling network, described by a kinetic matrix. The RIL–ZQT scheme<sup>20</sup> (see Figure 1a) has been optimized for that purpose and shall be used in the following for TOSSY experiments. In the delta-pulse limit and to zero-order average Hamiltonian approximation, the RIL dipolar mixing Hamiltonian in circular frequency units is given by<sup>20</sup>

$$H = \sum_{k < l} \frac{3\mu_0\gamma_k\gamma_l\hbar}{8\pi^2\sqrt{2}r_{kl}^3} \sin(2\theta) \sin(\varphi) (3I_{kz}I_{lz} - \bar{I}_k\bar{I}_l) \quad (1)$$

The polar angles  $\theta$  and  $\varphi$  orient the internuclear vector  $\vec{r}_{kl}$  with respect to the MAS rotation axis. All dipolar-coupled spins in the sample contribute to the sum in (1) and their resonance lines are connected by cross peaks in a TOSSY spectrum. The RIL cycle must be applied rotor synchronously but no particular ratio  $s$  (see Figure 1a) between carbon radio frequency field strength  $\omega_1$  and MAS frequency  $\omega_r$  must be obeyed as long as  $\omega_1$  is approximately an order of magnitude larger than  $\omega_r$ . This condition can typically be fulfilled in practice.

In the TOBSY experiment,<sup>26</sup> shown in Figure 1b, one attempts to completely suppress the dipolar interaction as well as the chemical-shielding terms. Then one can observe polarization transfer under the scalar mixing Hamiltonian (again to zero-order average Hamiltonian theory):

$$H = 2\pi \sum_{m < n} J_{mn} \bar{I}_m \bar{I}_n \quad (2)$$

The  $J_{mn}$  denote the scalar coupling constants among the bonded spins ( $m, n$ ). In practice, the TOBSY experiment leads to cross peaks between all spins that are coupled through scalar couplings (directly or relayed) that exceed a certain threshold. Spectral resolution of the  $J$  coupling in the  $^{13}\text{C}$  spectrum is not necessary. The basic pulse cycle, shown in Figure 1b, must be applied  $k = 6, 8$  (or multiples) times per rotor cycle to obtain the Hamiltonian of eq 2. A ratio of  $k = 4$  leads to an average Hamiltonian that contains no dipolar terms but still contributions from the chemical-shift anisotropy. We have found by numerical simulations that, for  $^{13}\text{C}$  spectroscopy, the field strength on the observe channel should be (or exceed) 100 kHz to efficiently suppress the isotropic and anisotropic chemical-shift contributions. The information contents of the TOBSY spectrum corresponds to that of the TOCSY spectrum of a dissolved compound.

Finally, it should be mentioned that, in principle, the TOSSY spectra can also contain cross-peak intensity caused by  $J$  interactions. In practice this is, however, not problematic because of the larger size (usually by an order of magnitude) of the dipolar interaction.

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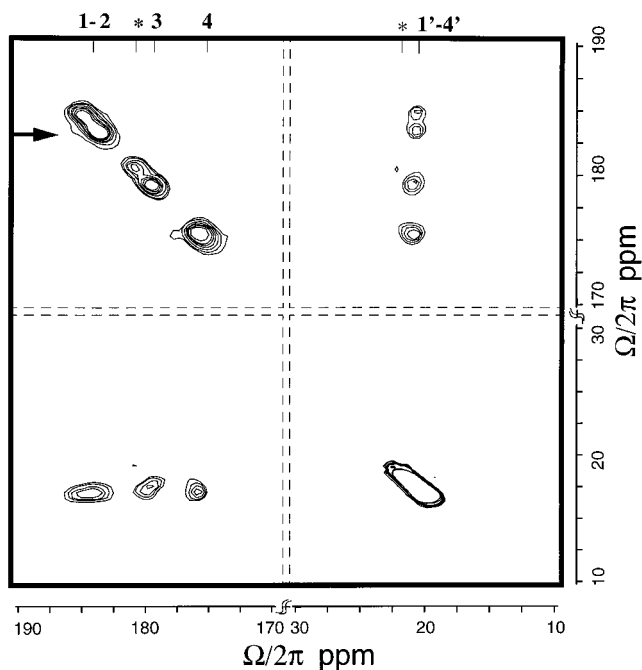
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**Figure 2.** TOBSY spectrum of the doubly-labeled monohydrate of calcium acetate. Only the carboxylic and aliphatic regions of the total 2D spectrum are shown, the rest of the spectrum is empty. Cross peaks are found within pairs of carboxylic and aliphatic resonances indicating intramolecular transfer only. 512  $t_1$  experiments were performed with 16 scans each. The mixing time was set to 20 ms. Further experimental parameters are given in the legend of Figure 1. Contour levels are shown for constant intervals between 2 and 15% of the maximal signal intensity. The signals marked by an asterisk are assigned to a second crystal form present as a contamination.

### Experimental Section

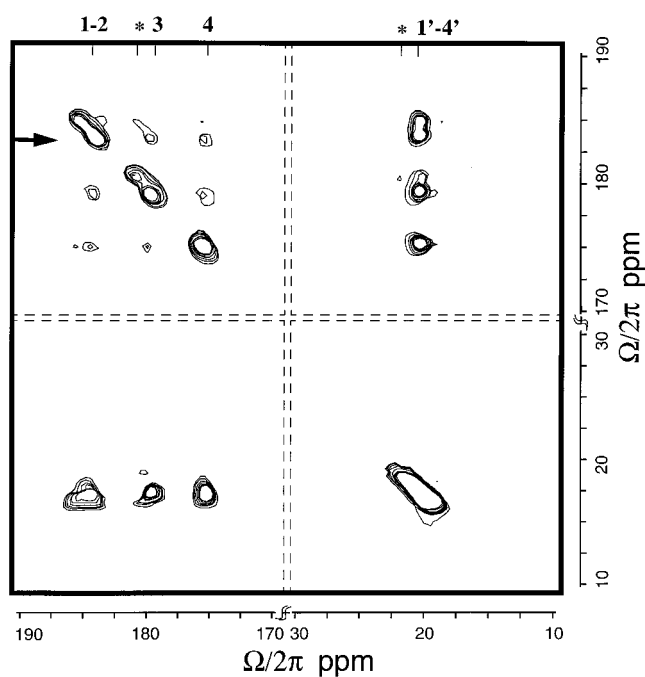
TOBSY and TOSSY experiments were performed on a powder sample of doubly  $^{13}\text{C}$  labeled calcium acetate monohydrate ( $\text{Ca}(\text{CH}_3\text{-COO})_2 \cdot \text{H}_2\text{O}$ ). This compound crystallizes in a triclinic structure with four molecules per unit cell.<sup>32</sup> For a control sample with  $^{13}\text{C}$  at natural isotopic abundance, the four crystallographically distinct carboxylic carbons were fully resolved while the four methyl resonances show partial overlap. For the fully labeled sample, the spectral resolution is slightly lower (due to unresolved  $J$  couplings and further broadening mechanisms) and the methyl resonances are not resolved anymore. Two of the four resonances in the carboxylic region of the spectrum are fully resolved in the TOBSY and TOSSY spectra of Figures 2 and 3, respectively, while two of the resonances show partial overlap. In addition, we found an additional smaller resonance at 181 ppm, most probably due to contamination with another crystal form (marked by an asterisk in the figures).

### Discussion

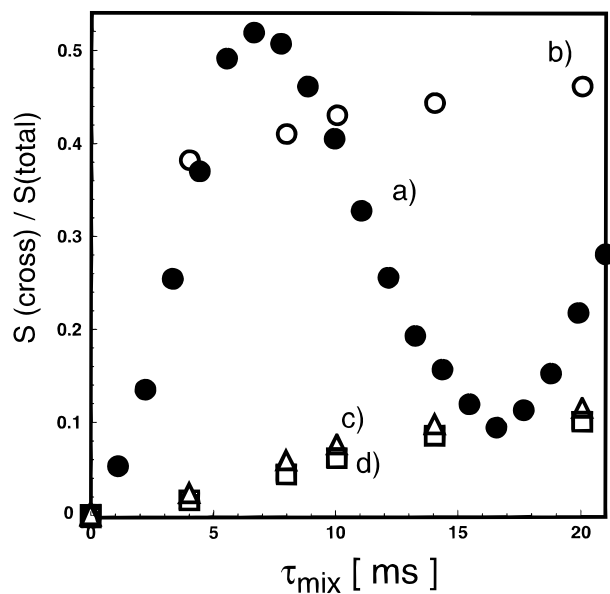
In the TOBSY spectrum (Figure 2) intramolecular cross peaks for each of the resolved crystallographically non-equivalent molecules are found while no intermolecular cross peaks are detected even at a mixing time of 20 ms (see Figure 2). The intensity of the TOBSY cross peaks as a function of the mixing time, for the cross peak involving the carboxylic resonance labeled by 4 (see Figure 2), is shown in Figure 4 (curve a). An almost undamped oscillatory time-dependence is observed. The same behavior is found for all four cross peaks. The observed oscillation frequencies,  $60 \pm 10$  Hz for all bonded pairs, are in good agreement with the expected size for the  $^{13}\text{C}$ - $^{13}\text{C}$   $^1J$  coupling that, in liquid phase, was determined to be 52 Hz.<sup>33</sup>

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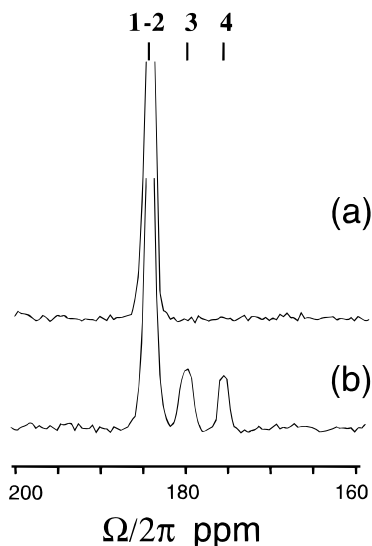


**Figure 3.** TOSSY spectrum of the same sample as used in Figure 2. Intramolecular as well as intermolecular cross peaks are detected. The mixing time was 20 ms. Further experimental parameters and contour level settings are chosen as described in the legends to Figures 1 and 2.



**Figure 4.** Curve a: intensity of the TOBSY cross peak  $S(\text{cross})$  between peak 4 and 4' (see Figure 2) as a function of the mixing time. The intensity is normalized to the total signal intensity of one spin pair  $S(\text{total})$  which amounts to  $1/4$  of the total spectral intensity. An oscillatory transfer behavior is observed. Curve b: TOSSY buildup for the same pair using the pulse scheme of Figure 1. Curves c and d: intermolecular TOSSY cross-peak buildup between pair [2,3] and pair [2,4], respectively.

The oscillatory behavior suggests that the cross peaks are actually caused by  $J$  interactions and not by insufficiently suppressed dipolar interactions. Possible unwanted (dipolar) contributions are expected to grow monotonous as a function of the mixing time. An upper limit to the unwanted cross-peak intensity can be estimated from the first minimum of the oscillation in Figure 4. Possible "false" spin-diffusion cross peaks must be smaller, at 17 ms mixing, than 0.1. The same value is reached, by  $J$  transfer, after approximately 1.5 ms



**Figure 5.** One-dimensional cross sections from the spectra of Figures 2 and 3 at the positions indicated by an arrow. For the TOBSY spectrum (a), no indication for intermolecular cross peaks is found, in accordance with expectations. For the TOSSY spectrum (b), however, intermolecular cross peaks are clearly present, again in accordance with expectations.

(Figure 4). Possible unwanted peaks grow, therefore, slower by at least an order of magnitude than the  $J$  cross peaks, despite the fact that the  $J$  interaction itself is smaller than the dipolar interaction by about an order of magnitude. Therefore, the overall selectivity can be estimated to be equal or better than two orders of magnitude. Most probably, the remaining intensity of the TOBSY cross peaks at 17 ms is predominantly caused by a distribution of oscillation frequencies due to chemical-shielding and radio frequency inhomogeneity effects and the actual suppression of dipolar contributions is even higher. Even at the longest mixing time investigated, 20 ms, no indications of cross peaks are seen between the carboxylic resonances of different molecules in the same unit cell. One of the relevant traces through the 2D spectrum at 20 ms mixing time is shown in Figure 5a). The noise level amounts to about 1% of the highest signal intensity and any "false" cross peaks must have intensities below the 1% level. These spectra were obtained using Lee–Goldburg decoupling at 150 kHz applied radio frequency field strength during mixing. In order to investigate the effect of less efficient proton decoupling during mixing on the magnitude of unwanted cross peaks, TOBSY experiments with the same parameters as used for the spectrum of Figure 5a) were performed but the proton radio frequency field strength was varied. The intensity at the position of the "false" cross peaks was monitored. Only for fields lower than about 75 kHz were "false" peaks detected (noise level again 1%). For an radio frequency field strengths of only 50 kHz, their intensity amounted to 1.7% of the highest signal intensity (or 0.03 on the scale of Figure 4).

The TOSSY experiment of Figure 3 reveals intermolecular cross peaks within the carboxylic region, in addition to the

intramolecular cross peaks. These cross peaks reflect through-space polarization transfer between the crystallographically distinct molecules in the unit cell. The next neighbor  $^{13}\text{CO}$ – $^{13}\text{CO}$  distances range from 3.9 to 5 Å and lead therefore, within the mixing time of 20 ms, to significant dipolar cross peaks. In these experiments, the cross-peak buildup is approximately exponential (see Figure 4, curves b to d). As to be expected, the TOSSY cross-peak buildup among the chemically bonded  $^{13}\text{CO}$ – $^{13}\text{CH}_3$  spins (see Figure 4, curve b) is much faster than the intermolecular  $^{13}\text{CO}$ – $^{13}\text{CO}$  transfer (see Figure 4 curves c and d). At 20 ms mixing time, the intermolecular cross peaks approach 10% of the total intensity and are much stronger than possible "false" cross peaks in TOBSY. In Figure 5b a cross section through the TOBSY data set is displayed, taken at the same position as the TOSSY cross section in Figure 5a. The presence and absence of intermolecular cross peaks is clearly seen. For the directly bonded carbon spins as well as for the intermolecular interactions, we can therefore conclude that the TOBSY spectra are virtually free from interference from dipolar coupling and that only through-bond interactions give rise to cross peaks under the experimental conditions discussed.

### Conclusions

The results presented show that through-space and through-bond connectivities can be probed separately in high-resolution solid-state NMR. The complementary information contained in the two experiments has been the basis of many successful applications to structure determination in liquid-state spectroscopy and we hope that the combined application of these two schemes to solid samples will be fruitful for biomolecular and material-science studies.

For applications to more complex spin systems further issues like sensitivity and spectral resolution become relevant. In this respect the TOBSY experiment is expected to perform very similarly to the TOSSY experiment which has been successfully applied in relatively complex systems.<sup>34</sup> The maximum distances and the extend of the through-bond connectivities that can be probed by TOSSY and TOBSY are presently limited to about 5 Å and three-bond connectivity, respectively. This limitation is caused by the decay of the sum polarization of the  $^{13}\text{C}$  (or  $^{15}\text{N}$ ) nuclei due to imperfect proton decoupling. Further investigations to improve this aspect of the experiment are presently under way.

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