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# Adiabatic TOBSY in rotating solids

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**Abstract**

A MAS solid state NMR approach for achieving efficient scalar coupling mediated through-bond $^{13}$C chemical shift correlations of the aliphatic carbons in uniformly labelled peptides/proteins is described. The method involves the application of a continuous train of adiabatic inversion pulses, as in the adiabatic TOCSY experiments carried out in solution state NMR studies. While rotor synchronised application of adiabatic inversion pulses leads to dipolar correlations, it is shown here via numerical simulations and experimental measurements that asynchronous application of adiabatic pulses can facilitate the mapping of through-bond connectivities. The method employs a suitable phasing scheme for generating the desired isotropic mixing Hamiltonian and requires moderate $^{13}$C RF field strength only.

## Introduction

The potential of MAS solid state NMR in the structural characterisation of biomolecular systems was demonstrated by Castellani et al. (2002) who obtained the first protein structure, namely of the SH3 domain of α-spectrin, employing MAS NMR derived approximate distance constraints and distance geometry algorithm. As with solution state studies, the pre-requisite for any MAS NMR based structural investigation is the sequence specific assignment of resonances. The connectivity pattern seen in a 2D homonuclear chemical shift correlation spectrum permits the assignment of resonances to a specific class of residues, for example to a particular type of amino acid in a peptide or a protein. The assigned resonances are then employed for obtaining structural constraints such as internuclear distances. Although weak dipolar couplings between low γ nuclei are normally lost under magic angle spinning, recently a variety of RF pulse sequences have been proposed to inhibit such spatial averaging of weak dipolar interactions. Hence chemical shift correlation experiments between low γ nuclei can be achieved in solid state NMR making use of either through-space dipolar couplings (Bennett et al., 1994; Griffin, 1998; Dusold and Sebald, 2000) or, as in solution state NMR, through-bond scalar couplings (Baldus and Meier, 1996; Baldus et al., 1997; Hardy et al., 2001; Heindrichs et al., 2001; Chan and Brunklaus, 2001; Hardy et al., 2003). As the magnitude of the dipolar coupling strength between covalently linked $^{13}$C nuclei is much larger than the corresponding scalar coupling, dipolar recoupling methods are generally employed to obtain chemical shift correlation data under magic angle spinning conditions. Dipolar recoupling sequences have also been widely employed for obtaining structural information from selectively labelled peptides/proteins and nucleic acids. Among the various dipolar recoupling schemes reported in the literature, radio frequency driven recoupling (RFDR) (Bennett et al., 1992, 1998) with longitudinal magnetisation exchange is one of the simplest techniques that has found application recently in generating $^{13}$C chemical shift correlation data of biological systems at high fields. RFDR typically involves the rotor synchronised application of conventional rectangular 180° pulses. We have shown recently (Heise et al., 2002; Leppert et al., 2003) that superior RFDR performance employing only moderate RF field strengths

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can be achieved with adiabatic inversion pulses. The range of resonance offset compensation achieved with adiabatic pulses was found to be sufficient to generate, via a single RFDR experiment, homonuclear chemical shift correlation spectra in the entire $^{13}C$ chemical shift range in peptides/proteins at the currently available Zeeman field strengths.

Dipolar couplings reflect a through-space interaction of nuclear spins and hence, to achieve unambiguous resonance assignments via through-space correlation experiments it is necessary to employ short dipolar recoupling periods for driving polarisation/coherence transfers. Under this condition, only spins in spatial proximity (e.g., one-bond distance) are expected to lead to cross peaks in 2D shift correlation experiments. For resonance assignment purposes, it is often advantageous to obtain, besides the correlations involving directly linked nuclei, connectivities between all nuclei which are separated by one or more bonds but still belong to the same spin network, e.g., all $^{13}C$ nuclei belonging to an amino acid residue of a protein. Such total correlation experiments are routinely carried out in solution state NMR and are expected to be also useful in MAS SSNMR investigations. However, generation of total chemical shift correlation data via dipolar recoupling experiments can be problematic. Long dipolar recoupling times employed in such experiments can lead to correlations arising from weak inter-molecular and inter-residue dipolar interactions and may render unambiguous resonance assignments difficult. Total through-bond correlation spectroscopy (TOBSY), introduced by Baldus and Meier (1996), overcomes such difficulties by generating chemical shift correlations exclusively via scalar couplings. Both through-space and through-bond correlation experiments were successfully employed in the recent sequential resonance assignment study of a uniformly labelled sample of antamanide (Detken et al., 2001).

To overcome problems arising out of RF field inhomogeneity and miscalibration of the $B_1$ field strength employed, adiabatic inversion pulses based mixing sequences have been successfully demonstrated recently in solution state NMR to obtain scalar coupling mediated chemical shift correlation data in proteins (Kupce et al., 1998; Peti et al., 2000). Here, we have explored the possibilities for obtaining through-bond scalar coupling mediated $^{13}C$ shift correlation data in rotating solids. While isotropic molecular tumbling in the liquid state averages out dipolar interactions, homonuclear dipolar interactions, besides chemical shifts, must also be suppressed in the solid state for isotropic J coupling to become the dominant interaction in the Hamiltonian during adiabatic mixing. As shown below these requirements can be met for the $^{13}C$ aliphatic region of amino acid residues. The basic rationale behind our SS NMR approach is that the dipolar interactions are modulated by MAS. Hence, efficient dipolar recoupling and the generation of through-space chemical shift correlation data via adiabatic inversion pulse driven magnetisation exchange require the application of rotor synchronous 180° pulses. In contrast, scalar coupling interactions are not modulated by MAS and polarisation/coherence transfers mediated by scalar couplings are independent of the crystallite orientation. This makes it feasible to minimise dipolar recoupling and to achieve scalar coupling mediated MAS shift correlation data via asynchronous application of adiabatic inversion pulses.

## Numerical and experimental procedures

The RF pulse sequence employed for obtaining adiabatic TOBSY data is shown in Figure 1A. The pulse sequence we have employed recently (Heise et al., 2002; Leppert et al., 2003) for obtaining through-space correlation via RFDR with adiabatic pulses is also given in Figure 1B. The application of a continuous train of 180° pulses with a proper phasing scheme, with one pulse per rotor period and with each pulse centered at the middle of the rotor period (Figure 1B) can be employed to obtain through-space correlation data at high MAS frequencies. In our TOBSY studies, we have used adiabatic pulses with durations shorter than the rotor period by ∼20% and employed supercycles such as (p5)(m4), (p5)(p7)(m4) and (p5)(p9)(m4) (Tycko et al., 1985; Levitt et al., 1983; Leppert et al., 2003) to obtain the desired isotropic Hamiltonian over the spectral range of interest. The phasing schemes {0°, 0°, 180°, 180°}, {0°, 240°, 240°, 60°, 0°}, {0°, 105°, 300°, 255°, 300°, 105°, 0°} and {0°, 15°, 180°, 165°, 270°, 165°, 180°, 15°, 0°} represent the phase cycles (m4), (p5), (p7) and (p9), respectively. Experiments were carried out with an undiluted and uniformly {$^{15}N$, $^{13}C$} labelled sample of arginine hydrochloride at room temperature on a 500 MHz wide bore Varian $^{\mathrm{UNITY}}$*INOVA* solid state NMR spectrometer equipped with a 5 mm DOTY supersonic triple resonance probe and a waveform generator for pulse shaping. Cross-polarisation under

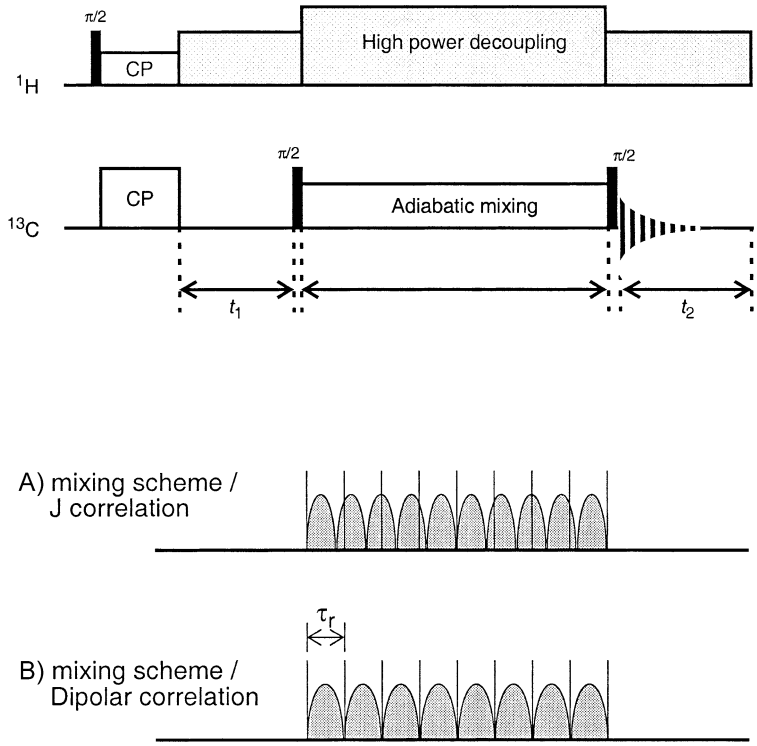


*Figure 1.* RF pulse sequence with adiabatic mixing for $^{13}$C MAS solid state NMR chemical shift correlation studies in uniformly labelled biological systems.

Hartmann-Hahn matching conditions was employed and all spectra, unless mentioned otherwise, were collected under high power $^{1}$H decoupling (∼90 kHz). Typical $^{1}$H and $^{13}$C 90° pulse widths were 2.8 and 5.5 μs, respectively. Other details are given in the figure captions.

Numerical simulations were carried out using the SIMPSON program (Bak et al., 2000) considering two spin-1/2 $^{13}$C nuclei, a Zeeman field strength of 11.7 T, typical $^{13}$C chemical shift, scalar, dipolar coupling parameters and spinning speeds indicated in the figure captions. As in our earlier studies (Heise et al., 2002; Leppert et al., 2003) tanh/tan adiabatic pulses (Hwang et al., 1998) with durations, power levels and phasing scheme as given in the figure captions were employed. A numerical assessment of the efficacy of the RF pulse sequence given in Figure 1A as shown below suggests that with moderate RF field strengths it should be possible to conveniently employ adiabatic TOBSY mixing schemes to obtain through-bond scalar coupling mediated $^{13}$C chemical shift correlations for the aliphatic side-chains of amino acids and for the sugar carbons in nucleic acids.

## Results and discussion

The results obtained from numerical simulations are given in Figure 2. In these simulations, unless mentioned otherwise, we have monitored as a function of the mixing time and as in our earlier study (Leppert et al., 2003) the magnitude of longitudinal magnetisation transferred to spin 2 starting with $z$ magnetisation on spin 1 at zero mixing time. The RF field strengths employed in these MAS solid state NMR simulations approximately correspond to the values needed for optimal response under adiabatic mixing. Figures 2A–C show the magnetisation transfer characteristics of adiabatic mixing schemes at the spinning speeds indicated. These plots were generated employing the (p5)(m4) supercycle and with different resonance offsets of the two spins from the RF carrier. Unless mentioned otherwise, $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shift and dipolar tensor parameters of alanine (Brinkmann et al., 2002 and Leppert et al., 2003) were employed in these simulations. As expected under efficient dipolar recoupling conditions, the RFDR response obtained with $\tau_p = \tau_r$ employing the sequence given in Figure 1B shows the typical fast initial rate of transfer of

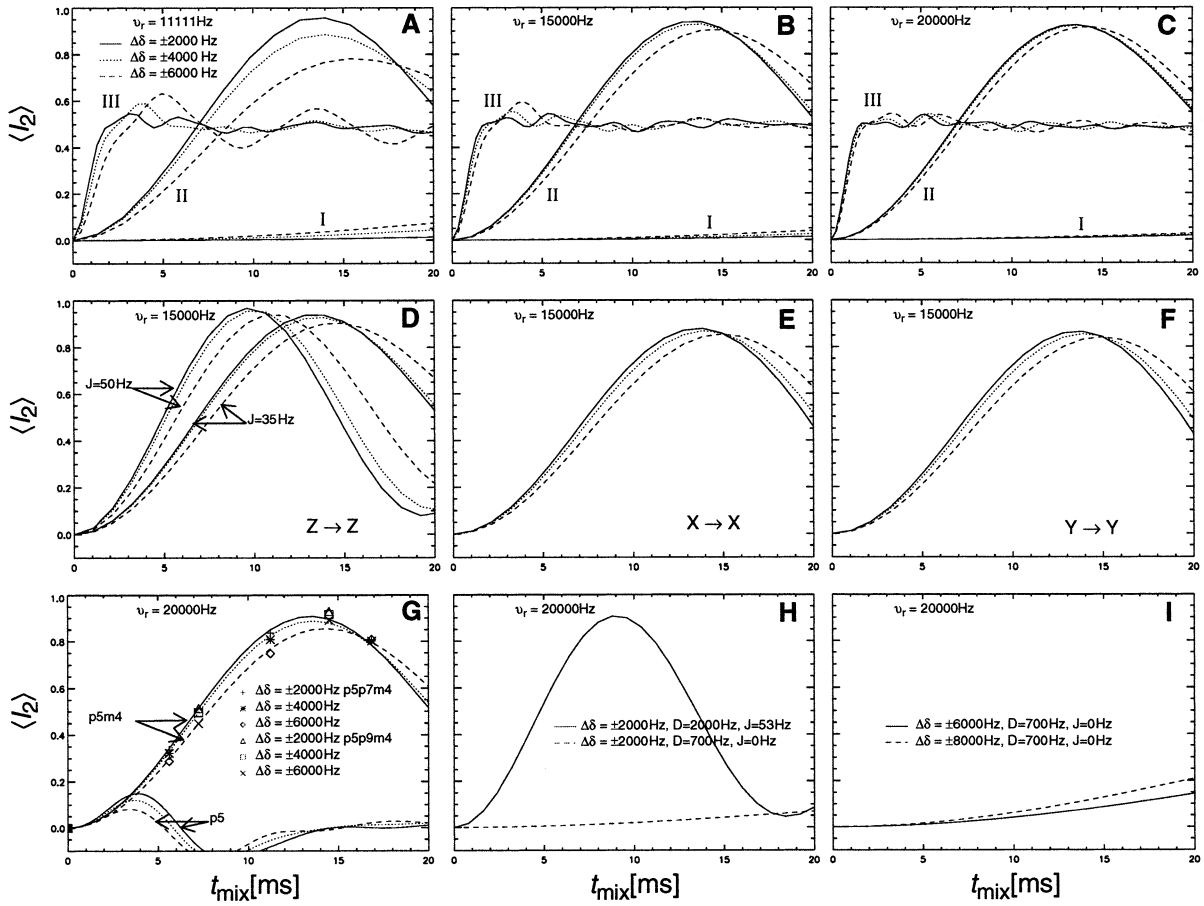


*Figure 2.* Magnitude of the transferred magnetisation on spin 2, starting with longitudinal (A-D, G-I) and transversal (E, F) magnetisation on spin 1 at zero mixing time. The TOBSY plots at the spinning speeds of 11111 Hz, 15 000 Hz and 20 000 Hz were generated with tanh/tan pulses of durations 70, 53 and 40 μs, respectively. The product of the adiabatic pulse bandwidth and the pulse length was 60. RF field strengths of 40, 50 and 60 kHz were employed in generating the TOBSY and RFDR plots at the spinning speeds mentioned above. The RF carrier was always kept at the middle of the two resonances and, unless indicated, the (p5)(m4) phasing scheme was used in these simulations. The resonance offset of the two nuclei ($\Delta\delta$) employed for obtaining the plots (A-F) are given in panel (A). The simulated plots I-III in (A-C) were generated under the following conditions: (I) TOBSY, D = 700 Hz, J = 0 Hz, $\tau_p < \tau_r$; (II) TOBSY, D = 2000 Hz, J = 35 Hz, $\tau_p < \tau_r$; (III) RFDR, D = 2000 Hz, J = 35 Hz, $\tau_p = \tau_r$. (D) Dependence of the TOBSY transfer characteristics on the scalar coupling strength ($J_{12}$) obtained employing a dipolar coupling strength ($D_{12}$) of 2000 Hz. (E, F) TOBSY transfer characteristics starting with $x$ and $y$ magnetisation on spin 1. $D_{12}$ and $J_{12}$ values of 2000 Hz and 35 Hz, respectively, were employed. (G) TOBSY transfer characteristics with different adiabatic phasing schemes and generated with $D_{12}$ and $J_{12}$ values of 2000 Hz and 35 Hz, respectively. (H) depicts the TOBSY transfer characteristics corresponding to two nuclei with large CSAs. The plot corresponding to the dipolar coupling strength of 700 Hz was generated employing the following parameters: spin 1: shift anisotropy $\delta_{aniso} = -80.43$ ppm, asymmetry parameter $\eta = 0.98$, Euler angles defining the orientation of the CS tensor in the molecular frame $\Omega_{PM} = \{99.4^\circ, 146.0^\circ, 138.9^\circ\}$; spin 2: $\delta_{aniso} = -74.5$ ppm, $\eta = 0.88$, CS tensor orientation $\Omega_{PM} = \{-0.7^\circ, 88.5^\circ, 52.5^\circ\}$, dipolar tensor orientation $\Omega_{PM} = \{0^\circ, 0^\circ, 0^\circ\}$. The plot corresponding to the dipolar coupling strength of 2000 Hz was generated employing the following parameters: spin 1,2: shift anisotropy $\delta_{aniso} = -74.5$ ppm, asymmetry parameter $\eta = 0.88$, CS tensor orientation $\Omega_{PM} = \{-0.7^\circ, 88.5^\circ, 52.5^\circ\}$, dipolar tensor orientation $\Omega_{PM} = \{0^\circ, 0^\circ, 0^\circ\}$. (I) Performance characteristics of the adiabatic TOBSY mixing scheme corresponding to two nuclei that are not directly bonded and with large CSA on one of the spins only. The parameters employed were: spin 1: shift anisotropy $\delta_{aniso} = -19.43$ ppm, asymmetry parameter $\eta = 0.98$, CS tensor orientation $\Omega_{PM} = \{99.4^\circ, 146.0^\circ, 138.9^\circ\}$; spin 2: $\delta_{aniso} = -74.5$ ppm, $\eta = 0.88$, CS tensor orientation $\Omega_{PM} = \{-0.7^\circ, 88.5^\circ, 52.5^\circ\}$, dipolar tensor orientation $\Omega_{PM} = \{0^\circ, 0^\circ, 0^\circ\}$.

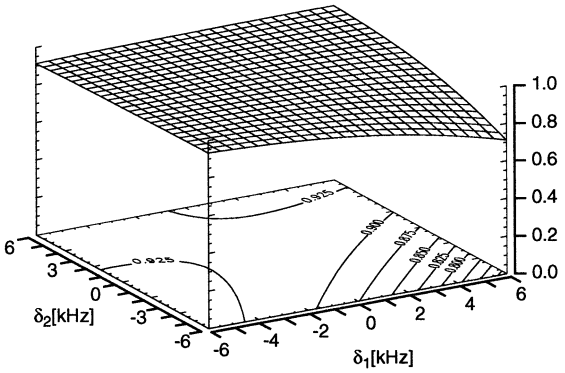


*Figure 3.* Transferred signal amplitude on the second spin ($^{13}C^{\beta}$) starting with $z$ magnetisation on spin 1 ($^{13}C^{\alpha}$) at zero mixing time, as a function of the isotropic chemical shifts $\delta_1$ and $\delta_2$ of the two nuclei. The plot was generated for a spinning speed of 20 000 Hz, mixing time of 13.6 ms, dipolar coupling strength of 2000 Hz, scalar coupling strength of 35 Hz, employing 40 μs tanh/tan inversion pulses, (p5)(m4) phasing scheme and $^{13}C$ RF field strength of 60 kHz. The $^{13}C$ RF carrier frequency was kept at the center of the spectral range. $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ anisotropic chemical shift and dipolar tensor parameters of alanine were employed as in Figure 2A.

magnetisation from one spin to another. On the other hand, the TOBSY response obtained with $\tau_p < \tau_r$ (Figure 1A) and with dipolar and scalar coupling strengths of 2000 Hz and 35 Hz, respectively, shows much slower rates of magnetisation transfer and the maxima in the transfer curves appear at $\sim$ (1/2J). Irrespective of whether the TOBSY mixing time corresponds to an integral number of rotor periods or not, it is seen from numerical simulations that an efficient transfer of magnetisation to spin 2 can be achieved after a time of $\sim$ (1/2J), as expected for evolution under an ideal isotropic mixing Hamiltonian. The TOBSY response corresponding to a weak long range dipolar interaction between remote spins was evaluated employing a smaller dipolar coupling of 700 Hz and without scalar coupling between the spins. Under this condition, no appreciable transfer of magnetisation to spin 2 is seen. It is worth mentioning that satisfactory TOBSY response could be obtained over a range of adiabatic pulse width ($\tau_p < \tau_r$) and that the theoretical simulations shown in Figure 2 were obtained for a representative case with adiabatic pulse width approximately 20% shorter than the rotor period. The plots shown in Figures 2A–C clearly indicate that with adiabatic mixing schemes it is possible to efficiently suppress weak inter-molecular and inter-residue dipolar interactions as well as to obtain, as in liquid state, scalar coupling mediated correlation spectra for the aliphatic region of amino acid residues.

The scalar coupling mediated magnetisation transfer achieved with asynchronous application of adiabatic inversion pulses is further confirmed by the response observed for two different scalar coupling strengths (Figure 2D) and by the transfer characteristics observed starting with $x$ and $y$ components of magnetisation of spin 1 (Figures 2E, F). These plots generated at a representative spinning speed of 15 000 Hz clearly show that the mixing Hamiltonian generated by the adiabatic mixing scheme employed here essentially consists of isotropic scalar coupling interactions only. The effect of different phasing schemes on the magnetisation transfer characteristics at a representative spinning speed of 20 000 Hz is shown in Figure 2G. The TOBSY performance of the simple (p5) phasing scheme appears not to be satisfactory at very high spinning speeds and the performance characteristics of the different supercycles (p5)(m4), (p5)(p7)(m4) and (p5)(p9)(m4) are apparently similar. The TOBSY performance at a spinning speed of 20 000 Hz, corresponding to two dipolar coupled $^{13}C$ spins with large CSAs, e.g., as in the case of aromatic carbons is shown in Figure 2H. Here also the transfer characteristics are as expected. However, numerical simulations carried out at lower spinning speeds (data not shown) indicate that the suppression of weak dipolar interactions is much better at high spinning speeds when the two nuclei involved in the magnetisation transfer have substantial CSAs. Suppression of weak dipolar interactions is also found to be not satisfactory (Figure 2I) when only one of the dipolar coupled spins has substantial CSA and the isotropic chemical shift separation between the two dipolar coupled nuclei is large, e.g., as in the case of the $^{13}C^{\alpha}$ and $^{13}C'$ nuclei. Also, adiabatic TOBSY leads to a lower transfer efficiency for directly bonded $^{13}C^{\alpha}$ and $^{13}C'$ nuclei (data not shown). However, these difficulties are not expected to pose any serious problems in the study of isotopically labelled proteins: firstly, RFDR with adiabatic pulses can be used at short mixing times to achieve $^{13}C$ chemical shift correlation of all directly bonded carbons in the entire spectral range (Heise et al., 2002; Leppert et al., 2003) and secondly, the TOBSY experiment is typically required, as in solution state, for achieving total chemical shift correlation of the aliphatic carbons of amino acid side chains. Figure 3 shows a representative plot of the magnitude of the transferred longitudinal magnetisation on spin 2 ($^{13}C^{\beta}$), starting with $z$ magnetisation on spin 1 ($^{13}C^{\alpha}$) at zero mixing time, and as a function of the isotropic chemical shifts $\delta_1$ and $\delta_2$ of two directly bonded nuclei. From Figure 3

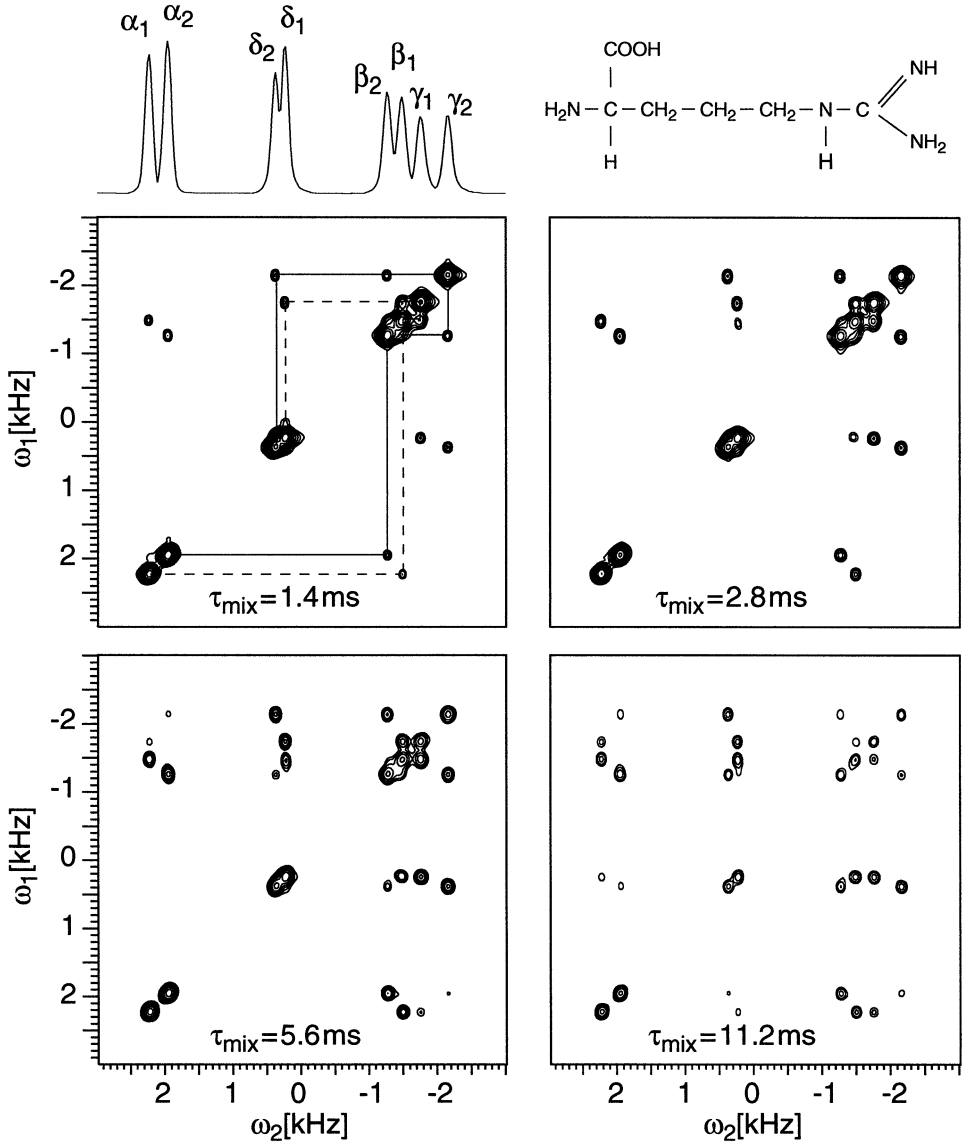


*Figure 4.* $^{13}$C TOBSY spectra of the aliphatic carbons of a fully labelled powder sample of L-arginine · HCl. Spectral data were collected as a function of the mixing time indicated employing a spinning speed of 11 111 Hz, 70 μs tanh/tan inversion pulses, (p5)(m4) phasing scheme, $^{13}$C RF field strength of ∼ 40 kHz, spectral widths, respectively, of 50 kHz and 20 kHz in the direct and indirect dimensions, 128 $t_1$ increments, 16 scans per $t_1$ increment and a recycle delay of 3 s. All spectra show correlations arising from longitudinal magnetisation transfers under adiabatic mixing and were collected by keeping the RF carrier at the center of the spectral range. The spectra are plotted at the same threshold/vertical scale.

it can be deduced that it should be possible to obtain efficiently scalar coupling mediated $^{13}$C chemical shift correlation data for the aliphatic side chains employing adiabatic TOBSY mixing schemes.

To assess experimentally the efficacy of adiabatic TOBSY, the $^{13}$C chemical shift correlation spectra of the aliphatic carbons of a fully labelled arginine powder sample were collected as a function of the mixing time at a spinning speed of 11111 Hz. Two resonances are seen for each of the arginine aliphatic carbons, probably due to the presence of two different crystal forms of arginine (Baldus et al., 1996). Figure 4 shows representative experimental spectra. Under isotropic mixing, the time dependence of $^{13}$C–$^{13}$C magnetisation transfer in different amino acid spin systems has been reported in the solution state NMR

literature (Clore et al., 1990) and the buildup of the direct and relay TOBSY cross peak intensities observed here (Figure 4) approximately correspond to what is expected from these studies. Thus, for example, at a mixing time of 1.4 ms even the intensities of the correlation peaks arising from directly bonded nuclei are very weak, with most of the signal intensities residing in the diagonal peaks. While most of the cross peaks arising from single step relay, for example $^{13}C^{\alpha} \rightarrow {}^{13}C^{\beta} \rightarrow {}^{13}C^{\gamma}$, can be seen at 5.6 ms, those arising from two step relay transfers can be only seen with larger mixing times. In view of the large mixing times and high $^{13}C$ RF field strengths employed in TOBSY experiments, efficient proton decoupling is critical to avoid interference between the $^{13}C$ and $^{1}H$ RF fields and hence signal intensity losses (Hardy et al., 2001, 2003). This is also reflected in our experimental studies. Although we have been able to only employ in our experiments a modest decoupling field strength of ∼100 kHz during adiabatic mixing, the performance of TOBSY would increase substantially with higher decoupling field strengths that is now becoming available in the current generation MAS probes.

The potential of adiabatic TOBSY mixing has been demonstrated here for the $^{13}C$ chemical shift correlation of the aliphatic side chains of amino acid residues. It is conceivable that adiabatic TOBSY mixing schemes can also be effectively employed in MAS solid state NMR studies of isotopically labelled RNAs, as the sugar carbons of nucleic acids have small CSAs and span a small chemical shift range. At the spinning speeds employed in this work we have typically used adiabatic pulses with durations smaller than the rotor period by ∼20%. At very high MAS frequencies TOBSY with adiabatic pulses having durations $\tau_p < \tau_r$ would require high RF power levels. In this context we have explored the possibilities to carry out TOBSY employing inversion pulses with durations $\tau_p > \tau_r$. Our preliminary studies in this direction are encouraging (data not shown). The recent study of Hardy et al. (2003) also clearly shows that adiabatic pulses with durations larger than the rotor period can be employed successfully in TOBSY experiments at high MAS frequencies. From the effective bandwidth seen from numerical simulations, it is apparent that adiabatic TOBSY mixing schemes will be useful also at higher Zeeman field strengths. Recently we have demonstrated (Heise et al., 2002; Leppert et al., 2003, 2004a,b) that it is possible to obtain efficient through-space MAS solid state NMR dipolar correlation data via rotor-synchronous application of adiabatic inversion pulses. From the current study it becomes clear that rotor-asynchronous application of a continuous train of adiabatic inversion pulses can be employed for $^{13}C$ TOBSY experiments on isotopically labelled biological solids.

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