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Chemical shift correlation via RFDR: Elimination of resonance offset effects

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Abstract

It is shown that it is possible to effectively execute RFDR experiments with adiabatic inversion pulses and obtain resonance offset compensation that is superior to what can be achieved by conventional rectangular pulses. Employing 40-μs tanh/tan adiabatic pulses at a power level of ∼38 kHz and a spinning speed of 12 kHz it is demonstrated that the range of resonance offset compensation achieved is sufficient to generate, via a single experiment, homonuclear chemical shift correlation spectra in the entire ^{13}C chemical shift range in peptides/proteins at the currently available field strengths.

Chemical shift correlation spectroscopy is one of the techniques increasingly used for resonance assignments, a pre-requisite for the NMR characterisation of biomolecular structures. 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. Chemical shift correlation between low γ nuclei can be achieved in solid state NMR making use of either through-bond scalar (Baldus and Meier, 1996; Hardy et al., 2001) or through-space dipolar couplings (Bennett et al., 1994; Griffin, 1998; Dusold and Sebald, 2000). In view of the larger magnitude of the dipolar coupling strength, chemical shift correlation under MAS can be conveniently achieved via dipolar interactions. A variety of recoupling procedures has been developed recently for inhibiting the spatial averaging of dipolar couplings under MAS (Bennett et al., 1994; Griffin, 1998; Baldus et al., 1998; Dusold and Sebald, 2000). Recoupling sequences form the critical building block in 2D chemical shift correlation experiments based on dipolar couplings. The quality of the 2D chemical shift spectra obtained is dependent on the efficacy of the dipolar recoupling sequence employed. Radio Frequency Driven Recoupling (RFDR) with longitudinal magnetization exchange is a simple and yet a powerful homonuclear dipolar recoupling technique which is finding increased usage for achieving chemical shift correlation (Bennett et al., 1992, 1998; Griffiths et al., 1994; Boender et al., 1995; Gilchrist et al., 2001; van Rossum et al., 2002). It has been successfully employed recently for the assignment of backbone and sidechain carbons in uniformly labelled proteins at high magnetic fields (McDermott et al., 2000; Pauli et al., 2000, 2001).

In RFDR, homonuclear dipolar recoupling is achieved via the application of rotor-synchronised 180° pulses as shown in Figure 1 for the case of one inversion pulse per rotor period. The phases of the 180° pulses are cycled to achieve efficient recovery of single spin magnetization and to generate an effective dipolar recoupling Hamiltonian during the mixing period (Bennett et al., 1998). For efficient recoupling, the 180° pulses employed should also be effective in the presence of resonance offsets, RF inhomogeneity, etc.

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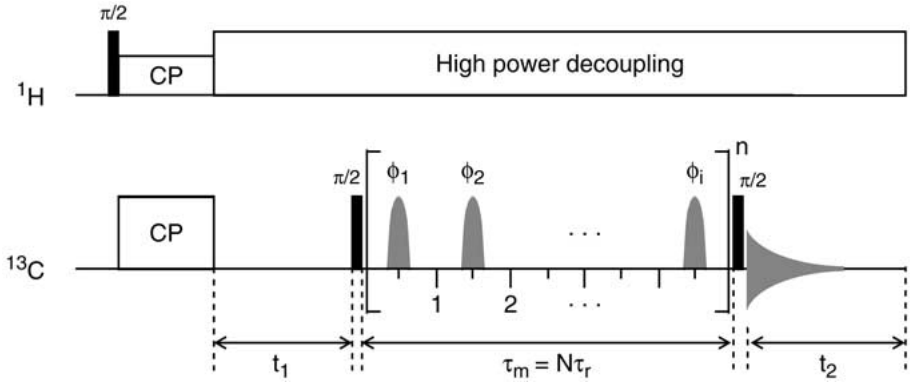


Figure 1. RFDR pulse sequence employed in this work. Homonuclear dipolar recoupling is achieved by applying an inversion pulse at the centre of each rotor period. The phase of the RF pulses are cycled as xy-8 (xyxyyxyx) for square/adiabatic 180° pulses and as p5$\overline{\text{p5}}$ (p5 = 0°, 150°, 60°, 150°, 0°) for adiabatic inversion pulses. Frequency switching in the ^{13}C channel, if needed, was effected before CP and at the beginning of τ_m. $\pi/2$ pulses are indicated by filled rectangles.

An important restriction on the RFDR experiment is that the RF field strengths employed in the recoupling and decoupling channels should be sufficiently mismatched to avoid rapid depolarisation of the carbon magnetisation during the mixing time (Bennett et al., 1992, 1998; Sodickson et al., 1993; Ishii et al., 1995; Sun et al., 1997; Zaborowski et al., 1999; Ishii, 2001). The advantages in carrying out the RFDR experiment with a ^{1}H decoupling field three times as strong as the ^{13}C RF field is clearly seen from the detailed study of Bennett et al. (1998). Typical ^{1}H decoupling field strengths available in a 4 or 5 mm probe are of the order of ∼90–100 kHz. Hence, to avoid interference between the RF fields during the mixing time, it is necessary to use only low power for the ^{13}C inversion pulses (∼30–40 kHz). The usage of such low recoupling power leads to an insufficient inversion bandwidth with conventional rectangular 180° pulses and thereby compromises the efficacy of broadband chemical shift correlation, especially at high fields. At field strengths corresponding to a ^{1}H frequency of 750–900 MHz, it is necessary to have 180° pulses with a large inversion bandwidth (∼35–40 kHz) to avoid loss of polarization due to imperfect mixing and to achieve chemical shift correlation in the entire ^{13}C spectral width of ∼180 ppm. To some extent, the phase cycling of the inversion pulses minimises cumulative signal losses due to resonance offset and other pulse errors (Bennett et al., 1992; Sodickson et al., 1993). However, when the isotropic chemical shifts span a large spectral range and when one is dealing with low power rectangular inversion pulses, the conventional xy phase cycling (Gullion et al., 1990) of the recoupling pulses may not be sufficient at high fields to achieve efficient recoupling in the entire spectral range. One can, in principle, overcome the limited inversion bandwidth of the low power rectangular pulses by carrying out two seperate experiments: one experiment to achieve chemical shift correlation only in the aliphatic region (Pauli et al., 2000, 2001) and another for the aromatic region and to *possibly* link the backbone $^{13}C^{\alpha}$ and $^{13}C'$ spins. For example, Pauli et al. (2001) obtained ^{13}C chemical shift correlation spectra of the aliphatic region of the α-Spectrin SH3 domain via RFDR and employed a proton driven spin diffusion experiment for linking the C^{α} to the C' spins. Both techniques were also employed by McDermott et al. (2000) in their study of BPTI. However, it would be highly efficient if all correlations could be achieved in one single RFDR experiment. With rectangular inversion pulses, this is only achievable when large ^{1}H decoupling field strengths of the order of ∼150 kHz are available which would then permit the usage of higher ^{13}C recoupling RF field strengths. Such high decoupling field strengths are generally only available with MAS probes of very small rotor diameter (∼2.5–3 mm). As typically only a fraction of the sample can be packed into such rotors, the net gain in sensitivity due to improved decoupling may get compromised by the small rotor volume (McDermott et al., 2000). Additionally, when dealing with a system where the labelled sample has to be diluted with unlabelled sample to avoid intermolecular interactions, it is necessary to use larger rotor volumes to obtain sufficient signal to noise ratio in a reasonable amount of time. In this context, we are currently exploring the potential of RFDR with adiabatic inversion pulses (Baum et al., 1985; Kupce and Freeman, 1995, 1996; Tannus and

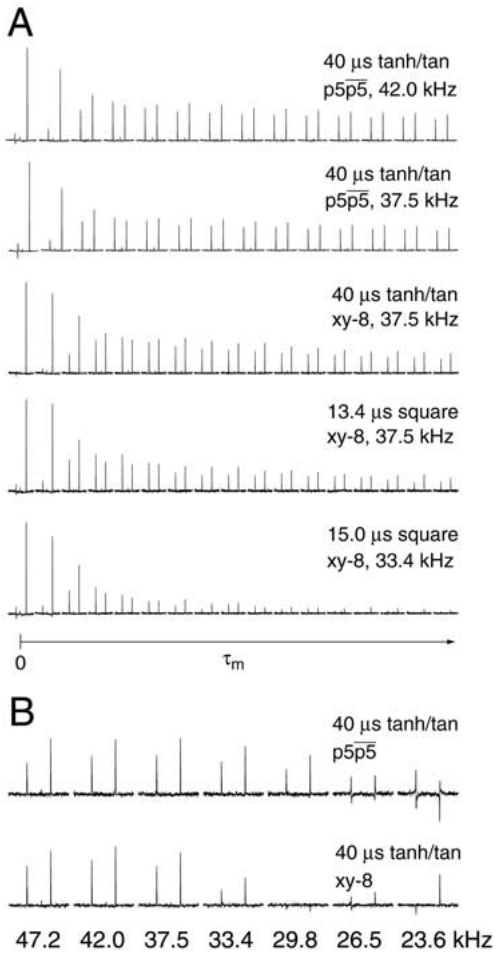


Figure 2. (A) ^{13}C longitudinal magnetization exchange spectra of doubly labelled NaAc as a function of τ_m obtained at 12 kHz spinning speed via the sequence given in Figure 1. The spectra were generated employing the inversion pulse parameters indicated. Starting with $\tau_m = 0$, the mixing time is incremented either in steps of 10 or 8 rotor periods (833 μs/666 μs) depending on the phasing scheme employed. At time $\tau_m = 0$ the methyl carbon signal is prepared along the z axis by selective cross polarization. For achieving effective selective cross polarization to the methyl carbons, the ^{13}C frequency was kept on resonance with respect to the $^{13}CH_3$ line during a short cross polarization time of 50 μs. During the mixing period; the carrier was then switched back to the centre of the $^{13}CH_3$ and $^{13}CO_2^-$ lines. (B) ^{13}C recoupling RF power dependence of the longitudinal magnetization exchange spectrum of NaAc obtained at a mixing time of ∼5 ms employing the adiabatic pulse parameters and phasing schemes indicated.

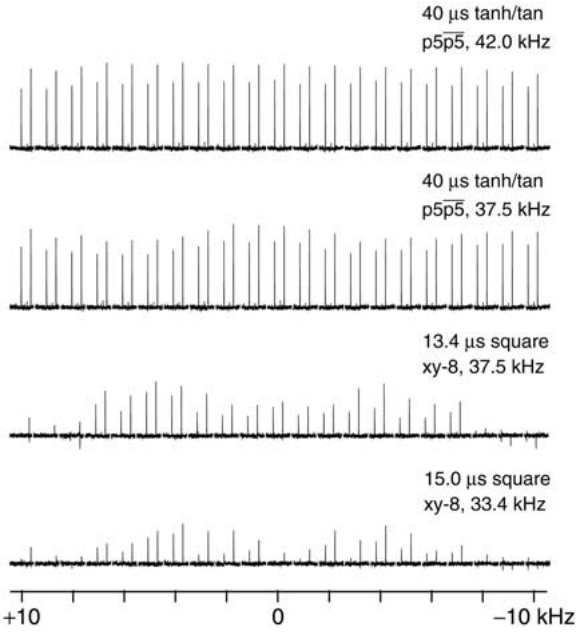


Figure 3. The resonance offset dependence of the longitudinal magnetization exchange spectrum of NaAc. These spectra were generated at a mixing time of ∼5 ms employing the inversion pulse parameters indicated. The ^{13}C frequency at the start of the selective cross polarization was kept on resonance with respect to the $^{13}CH_3$ line. The resonance offset (from the centre of the two lines) was varied at the start of the RFDR mixing period over a range of +10 to −10 kHz in steps of 1 kHz.

Garwood, 1996; Hwang et al., 1998; Heise et al., 2000; Leppert, 2001; Heise, 2001; Leppert et al., 2002) to achieve ^{13}C chemical shift correlation in the entire ∼180 ppm range (∼30–40 kHz) at high fields when the available ^{1}H decoupling power is limited to ∼90–100 kHz. From our initial experimental studies, it is seen that it is possible to effectively execute RFDR experiments with adiabatic inversion pulses and obtain resonance offset compensation which is far superior to what can be achieved with conventional rectangular pulses.

Figure 1 shows the RFDR pulse sequence employed in this work. All experiments were performed at room temperature on a 500 MHz wide bore Varian $^{\text{UNITY}}$*INOVA* solid state NMR spectrometer equipped with a 5 mm DOTY supersonic triple resonance probe and a waveform generator for pulse shaping. Cross-polarization under Hartmann-Hahn matching conditions was employed and all spectra were collected under high power ^{1}H decoupling (∼89 kHz). Typical ^{1}H and ^{13}C 90° pulse widths were 2.8 and 5.3 μs, respectively. All measurements were carried out at a spinning speed of 12 kHz employing undiluted, uni-

formly labelled samples. In this study we have mainly employed a tanh/tan adiabatic inversion pulse (Hwang et al., 1998) constructed from the following adiabatic half passage and its time reversed half passage:

$$\omega_1(t) = \omega_1(\max)\tanh(\xi 2t/T_p),$$
$$\Delta\omega(t) = \Delta\omega_{max}[\tan(\kappa(1-2t/T_p))]/\tan(\kappa),$$

where $\xi = 10$, $\tan(\kappa) = 20$ and $0 \leq t \leq T_p/2$. The adiabatic pulses employed in this work had an R value, representing the product of the pulse bandwidth and the pulse length (Hwang et al., 1998), of 60. The frequency sweep is implemented in the spectrometer hardware as a phase modulation, $\phi(t) = \int \Delta\omega(t)dt$. From our recent studies it is seen that, with the typical ^{13}C transmitter power available, it is possible to generate tanh/tan inversion pulses of short duration and with good broadband inversion characteristics (Leppert et al., 2002). In all experiments we have employed a 40-μs adiabatic pulse. As at the spinning speed of 12 kHz this pulse occupies a significant fraction of the rotor period we first assessed the efficacy of dipolar recoupling with adiabatic recoupling pulses via 1D RFDR experiments employing a doubly labelled sample of sodium acetate (NaAc). This sample represents an ideal two spin system with a large separation ($\sim$20 kHz) between the $^{13}CH_3$ and $^{13}CO_2^-$ resonances similar to what is expected between the $^{13}C^{\alpha}$ and $^{13}C'$ resonances in a peptide backbone at high fields. Figure 2 shows longitudinal exchange spectra as a function of the RFDR mixing time τ_m, employing different RF phasing schemes and pulse parameters. By selective cross polarization it was ensured that only the z magnetization of the methyl carbons was present at $\tau_m = 0$. Suitable phase cycling was also applied to the 90° RF pulses to select the desired coherence transfer pathway (Boender et al., 1995). The RFDR performance with adiabatic inversion pulses was then assessed by monitoring the exchange of longitudinal magnetization. Experiments with adiabatic pulses were carried out employing both the conventional xy-8 phasing scheme as well as the p5$\overline{\text{p5}}$ (p5 = 0°, 150°, 60°, 150°, 0°) phasing scheme (Tycko et al., 1985; Fujiwara and Nagayama, 1988). For comparison, data obtained with rectangular 180° pulses are also given in Figure 2A. It is seen that the initial rate of magnetization transfer from the methyl to the carboxyl carbons, a measure of the efficacy of dipolar recoupling, is not substantially different in the experiments carried out with rectangular or adiabatic inversion pulses, even though the adiabatic pulses have a much longer duration. However, at longer mixing times the performance of RFDR with adiabatic inversion pulses is far superior to that seen with rectangular 180° pulses. Cumulative signal losses present with rectangular inversion pulses due to imperfect mixing are substantially reduced with the use of adiabatic pulses. With adiabatic pulses, the usage of the p5$\overline{\text{p5}}$ phasing scheme is found to lead to a slightly better performance at longer mixing times compared to the xy-8 phasing scheme. This improvement is also seen (Figure 2B) when the RFDR performance is monitored as a function of the recoupling RF power. From Figure 2B, which shows longitudinal exchange spectra of NaAc at $\tau_m \cong 5$ ms, it is seen that the minimum RF power that is required to achieve satisfactory RFDR performance depends on the phasing scheme employed. With p5$\overline{\text{p5}}$, a ^{13}C recoupling power of $\sim$37.5 kHz should suffice to obtain satisfactory performance with a 40 μs tanh/tan pulse. If compensation for H_1 inhomogeneity is needed, it is necessary to use higher recoupling power. However, as the RF carrier frequency is being swept during the course of an adiabatic pulse, the interference between the decoupling and the recoupling fields is expected to be effective only during a small portion of the total time when the carrier frequency is near resonance. Hence, the usage of slightly higher recoupling power may not lead to any substantial degradation in the RFDR performance (Figure 2). Figure 3 shows the resonance offset dependence (with respect to the centre of the two lines) of the longitudinal magnetization exchange spectrum of NaAc at $\tau_m \cong 5$ ms in a range of +10 to −10 kHz. Considering that the two ^{13}C lines are separated by 20 kHz, an effective resonance offset compensation over a bandwidth of 40 kHz is obtained with adiabatic pulses. As expected, the performance of RFDR with conventional rectangular pulses is poor and hence it would necessary to take recourse to band selective chemical shift correlation spectra at high fields when sufficient ^{1}H decoupling power is not available. With adiabatic pulses, however, it should be possible to achieve 2D ^{13}C chemical shift correlation in the entire 180 ppm range in a single experiment even at high fields. To confirm this, we have carried out 2D RFDR experiments employing a uniformly labelled sample of histidine. The spectra were generated with adiabatic and rectangular inversion pulses at different RF power levels, mixing times and pulse widths. The frequency of the ^{13}C recoupling pulses was kept either at the centre between the $^{13}C'$ and $^{13}C^{\beta}$ resonances or 10 kHz away from the centre. 2D spectra with 10 kHz offset (for clarity, only the spectral region of interest is shown) and a few representative ω_1 cross sections are

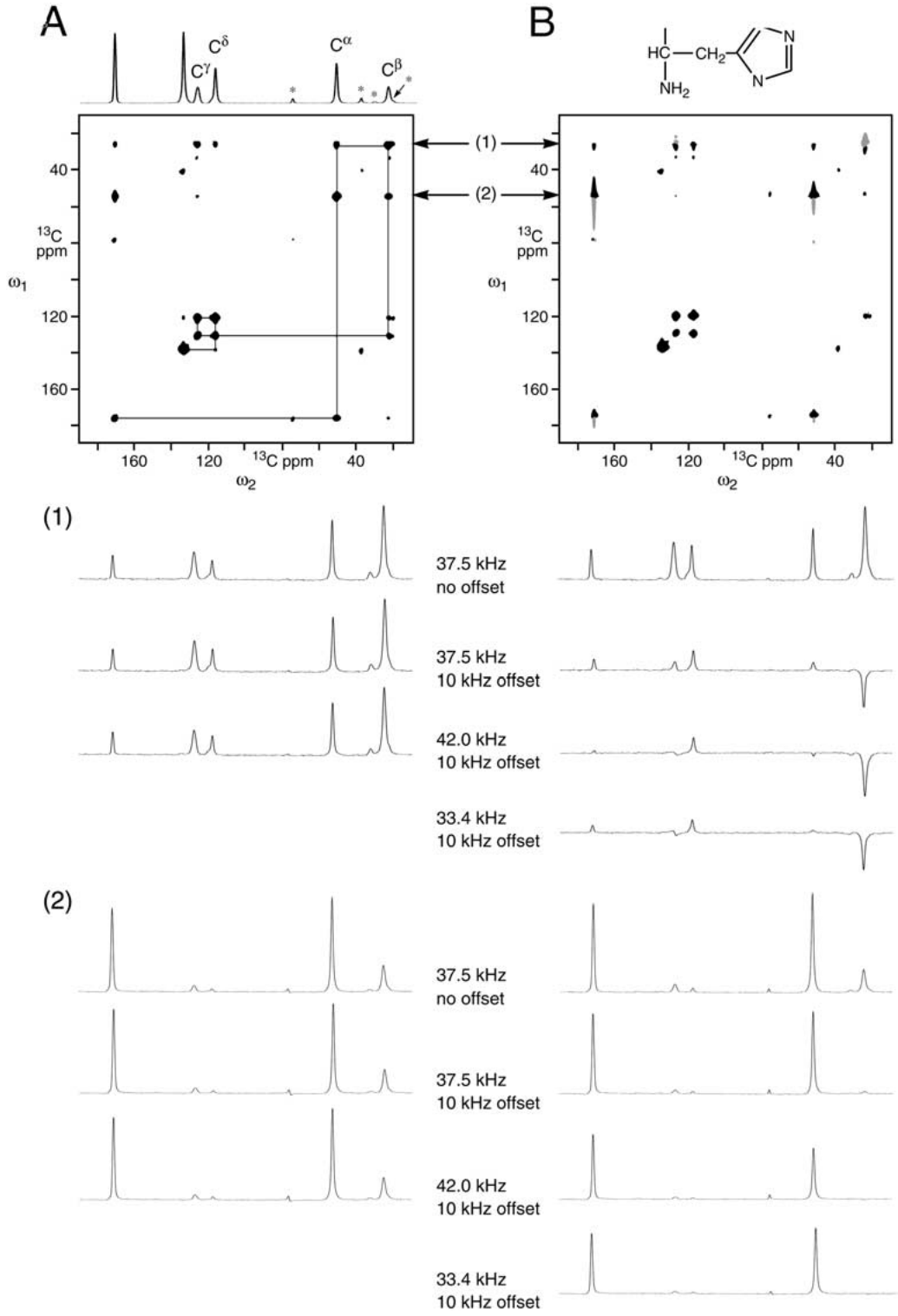
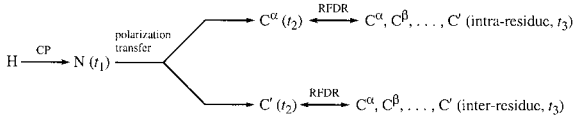


Figure 4. 2D RFDR spectra (zoomed plots) and spectral cross sections of uniformly labelled histidine at a spinning speed of 12 kHz employing ^{13}C recoupling RF pulse parameters indicated. The frequency positions at which the cross sections were taken from the 2D plots are indicated. The data have been obtained with (A) adiabatic (τ_m = 1.66 ms, p5$\overline{\text{p5}}$ phasing scheme) and (B) rectangular (τ_m = 2.0 ms, xy-8 phasing scheme) inversion pulses, respectively, employing a spectral width of 50 kHz in both dimensions, 256 t_1 increments, 8 scans per increment and a recycle delay of 2 s. The corresponding spectra and cross sections have been plotted at the same threshold/vertical scale. The 2D RFDR spectra shown at the top were obtained by keeping the ^{13}C RF carrier at 10 kHz away from the middle of the $^{13}C'$ and $^{13}C^{\beta}$ lines during τ_m. The spectral cross sections were taken from corresponding 2D spectral data. With rectangular 180° pulses, spectra were generated employing pulse widths of 15.0 μs at 33.4 kHz, 13.4 μs at 37.5 kHz and 13.4 μs at 42.0 kHz γH_1. In the spectral projection shown at the top some of the spinning sidebands are indicated with asterisks. In the 2D spectra, peaks with negative intensities are shown in grey.

given in Figure 4. It is seen from the spectral cross sections, that when the ^{13}C carrier frequency is kept at the middle of the $^{13}C'$ and $^{13}C^{\beta}$ resonances, it is possible to achieve satisfactory chemical shift correlation in the entire ^{13}C spectral range (∼18.5 kHz) employing conventional rectangular 180° pulses of 13.4 μs duration (Figure 4B). However, when the frequency of the recoupling pulses is offset by 10 kHz, the 2D RFDR spectrum generated with rectangular 180° pulses shows, even at the short mixing time used, severe intensity and phase distortions arising out of the insufficient excitation bandwidth of the inversion pulses. With rectangular 180° pulses these problems get further aggravated with larger pulse widths and when there is an error in the inversion pulse width calibration (or H_1 inhomogeneity). As expected, RFDR with adiabatic inversion pulses shows superior performance (Figure 4A), confirming our earlier conclusion that it is possible to achieve resonance offset compensation over a range of ∼40 kHz by employing tanh/tan adiabatic pulses of 40 μs duration (Figure 3). This has also been confirmed at longer mixing times (data not shown). It is seen from our work that with adiabatic inversion pulses ^{13}C chemical shift correlations in the entire 180 ppm range can be obtained via a single 2D RFDR experiment at the currently available field strengths (750–900 MHz), even when only limited ^{1}H decoupling power is available.

It is worth mentioning that although the potential of RFDR with adiabatic pulses has been illustrated employing 40 μs tanh/tan adiabatic pulses, it should be possible to employ adiabatic pulses of other shapes and, spinning speed permitting, durations. Similarly, the p5 phasing scheme employed in this work is one of several phasing schemes that can be employed successfully for adiabatic inversion of longitudinal magnetisations. In view of the encouraging RFDR results obtained with adiabatic inversion pulses we are currently assessing quantitatively, via numerical simulations and experimental measurements, the relative efficacy of RFDR with different phasing schemes, adiabatic pulse shapes and durations. The ability to achieve 2D chemical shift correlation in the entire ^{13}C spectral range at high fields also opens up some potentially interesting possibilities for achieving sequential resonance assignments in uniformly labelled peptides/proteins. For example, it may be possible to achieve sequential ^{13}C and ^{15}N resonance assignments with RFDR via a single 3D N-C-C experiment involving the following magnetization transfer pathways:



Several dipolar recoupling schemes such as DREAM (Verel et al., 2001; Detken et al., 2001) and fpRFDR (Ishii, 2001) have been successfully demonstrated recently for obtaining through space chemical shift correlation data in uniformly labelled samples at very high spinning speeds. However, with many biological systems it may not always be possible to employ very high spinning speeds, e.g., to avoid sample dehydration and hence, in this study we have only employed a moderate spinning speed of 12 kHz. However, in cases where it is possible to employ higher spinning speeds, it should be feasible to employ adiabatic pulses of shorter duration and, in fact, we have obtained encouraging initial results in this direction. Alternatively, in situations where the adiabatic pulse duration becomes very significant in relation to the rotor period it should be possible to constructively use the spin evolution during the pulse (Ishii, 2001). If it is sufficient to employ lower spinning speeds, it should be also possible to advantageously use adiabatic pulses of longer duration at reduced recoupling power levels without degrading the efficacy of RFDR. Work in these directions is in progress and the results of these investigations will be reported elsewhere.

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