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Broadband Homonuclear Correlation Spectroscopy at High Magnetic Fields and MAS Frequencies

Gaël De Paëpe, Marvin J. Bayro, Józef Lewandowski, and Robert G. Griffin*

Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received August 29, 2005; E-mail: rgg@mit.edu

Structural studies of amyloid,[1] membrane,[2] and nanocrystalline peptides and proteins[3,4] are currently being performed with high-resolution solid-state NMR, and the initial step in the investigation of these systems is assignment of the spectra.[5,6] Spectral assignments of ^{13}C and ^{15}N backbone and side chain resonances, and subsequently ^{13}C−^{13}C and ^{13}C−^{15}N distance and torsion angle measurements, are accomplished by recoupling dipolar interactions during magic angle spinning (MAS) experiments,[7−14] and a large number of broadband homonuclear recoupling techniques have been introduced which successfully accomplish this goal.[8,15−18] However, essentially all of these experimental techniques were developed and function at low magnetic fields ($B_0 \leq 12$ T, ≤ 500 MHz) and spinning frequencies ($\omega_r/2\pi \leq 10$ kHz). Thus, for reasons detailed below, they are not applicable to experiments performed at high magnetic fields ($B_0 \sim 16-21$ T, 700−900 MHz) and elevated spinning frequencies ($\omega_r/2\pi \sim 10-30$ kHz), where structural studies are performed. Here we report the development of a novel dipolar recoupling scheme, cosine modulated (CM) adiabatic recoupling (CMAR), that performs broadband homonuclear dipolar recoupling at $\omega_r/2\pi$ ranging from 10 to 30 kHz and high B_0 and provides a means to perform spectral assignments. *The scheme has the interesting property that it simultaneously recouples the ^{13}C's and decouples the ^{1}H spins; thus, there is no ^{1}H decoupling present during the mixing period.*

It is axiomatic that MAS experiments at high B_0 require high $\omega_r/2\pi$ to attenuate rotational sidebands from the increased shift anisotropies and, therefore, to fully manifest the available resolution and sensitivity of the spectra. However, most recoupling techniques do not function well at high $\omega_r/2\pi$ because the ratio of the ^{13}C nutation frequency (γB_1) to $\omega_r/2\pi$ (denoted as N) is typically 5 (SPC-5[17]), 7 (POST-C7[15]), or even 8.5 (DRAWS[8]) times $\omega_r/2\pi$, and concurrently, the ^{1}H nutation frequency should be ≥ 3 times the ^{13}C γB_1.[17] The high radio frequency (rf) powers required to satisfy these constraints result in sample heating and challenge the technical integrity of commercial MAS probes.

These limitations stimulated the development of sequences with a reduced N.[17,18] In addition, dipolar recoupling using an *adiabatic sweep* of the Hamiltonian spin system and an attenuated rf field on the recoupling channel was recently introduced.[16,19] While the latter approach concurrently reduces the rf power requirements at high MAS frequencies, it introduces a sensitivity to isotropic and anisotropic chemical shift offsets, which are amplified at high B_0.[16,19] Finally, recent studies showed that some double quantum (DQ) recoupling sequences remain efficient in the *absence of ^{1}H decoupling* at moderate MAS rates (12−20 kHz)[20,21] or in the very high MAS regime (30−60 kHz).[22,23]

The dipolar recoupling scheme reported here allows one to observe broadband homonuclear chemical shift correlations at high B_0, over the typical range of MAS frequencies available (10−30 kHz). This new scheme, CMAR, combines rapid cosine modulation

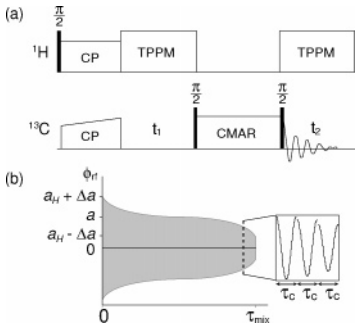

Figure 1. (a) Two-dimensional ^{13}C−^{13}C correlation pulse sequence used with CMAR recoupling sequence. Note that no ^{1}H decoupling rf field is applied during the mixing time. (b) ^{13}C rf phase modulation applied during the recoupling period.

of the ^{13}C rf phase together with an adiabatic sweep of the cosine modulation amplitude, without ^{1}H decoupling. It is illustrated schematically in Figure 1 and functions as follows.

Recently, it was shown that CM[24] of the phase of the ^{1}H *decoupling* rf leads to the reintroduction of the ^{1}H−^{1}H couplings through a DQ homonuclear rotary resonance (HORROR)[7] mechanism.[25] The recoupling mechanism functions in a *modulation frame* (MF) defined by the modulation frequency and the mean axis of the irradiation.[24,25] This suggests that the application of CM irradiation to the ^{13}C spin system can be employed to perform homonuclear recoupling of the ^{13}C−^{13}C interactions and concurrently perform heteronuclear decoupling of the ^{1}H−^{13}C interactions. Furthermore, in this frame, effects of ^{13}C chemical shift anisotropies and rf inhomogeneity are attenuated, leading to efficient DQ excitation even at high B_0.

The HORROR[7] condition in the MF[25] is defined by the following two equations:

$$a_{\mathrm{H}} = \frac{\nu_{\mathrm{r}}}{\nu_1}; \frac{\nu_{\mathrm{c}}^{\mathrm{H}}}{\nu_1} = 1 - \frac{1}{4}\left(\frac{\nu_{\mathrm{r}}}{\nu_1}\right)^2$$

where a_{H} is the amplitude of the modulation (rad), $\nu_{\mathrm{c}}^{\mathrm{H}}$ the frequency (Hz) of the modulation, ν_{r} the MAS frequency, and ν_1 the ^{13}C nutation frequency. Under these conditions, the rf irradiation can be expressed in the MF as a static rf irradiation of amplitude $\nu_{\mathrm{eff}} \approx a_{\mathrm{H}}\nu_1/2$ that matches half the MAS frequency. This cosine modulated rotary recoupling (CMRR) experiment is being explored as a part of a homonuclear recoupling technique for low γ spins.

The CMAR scheme reported in this Communication is an extension of this new recoupling scheme, in which an adiabatic sweep of the modulation amplitude a through the CMRR matching condition a_{H} is performed. The modulation frequency ν_{c} is constant throughout the sweep of the amplitude a. As shown in Figure 1b, this irradiation scheme consists of a fast cosine phase modulation

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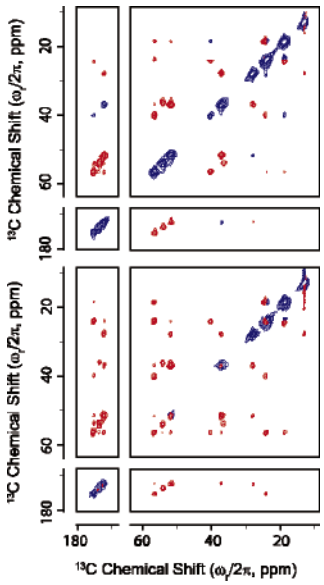

Figure 2. Two-dimensional correlation spectrum of the tripeptide 100% [U-^{13}C, ^{15}N] N-f-MLF-OH using $CM_{3.5}AR$ recoupling at 28.6 kHz MAS and ~100 kHz of ^{13}C rf field strength using (top) 2 ms and (bottom) 5 ms mixing time, with no ^{1}H decoupling. The evolution and acquisition periods used 83 kHz of TPPM decoupling, and 512 points were collected in the direct and 128 points in the indirect dimension, with 8 scans per transient.

envelope inside an adiabatic function (typically a tangent). The slow variation of the cosine phase modulation amplitude leads to an adiabatic passage through the recoupling condition. The spin dynamics induced by this rf phase sweep in the MF is analogous to the rf amplitude sweep through the HORROR condition carried out in the rotating frame with the DREAM experiment.[16,19,26] The adiabatic process involved does not rely on the exact value of the dipolar coupling and, thus, constitutes a robust and efficient approach for correlation experiments in multiply labeled samples. Furthermore, recoupling in the MF permits the use of CMAR without concurrent ^{1}H decoupling enabling its application at high MAS rates.

The experimental realization of CMAR as an efficient homonuclear correlation technique at high $\omega_r/2\pi$ is illustrated in Figure 2, which shows ^{13}C−^{13}C correlation spectra of a sample of the tripeptide [U-^{13}C, ^{15}N] N-f-MLF-OH,[13] at $\omega_r/2\pi = 28.6$ kHz and a magnetic field of 17.6 T (750 MHz). γB_1 (^{13}C) = 100 kHz and no ^{1}H decoupling was employed. This data set was collected using a 2.5 mm, triple-channel Bruker probe.

These high $B_0/\omega_r/2\pi$ spectra present correlations for all directly bonded spins, whose isotropic chemical shifts span ~35 kHz, indicating the efficient broadband performance of the CMAR recoupling scheme. These correlation spectra also demonstrate one of the remarkable features of this recoupling technique, namely, the fact that high intensity rf recoupling pulses can be safely applied during the mixing time because there is no concurrent proton decoupling irradiation.

In summary, we have introduced a novel adiabatic DQ recoupling scheme. The advantages of this new technique are several-fold. First, the experiment can be performed with a ^{13}C rf field of sufficient strength to uniformly recouple the entire ^{13}C spectrum at high B_0, resulting in true broadband behavior for the CMAR experiment. This characteristic represents an improvement over the DREAM experiment, in which the mean amplitude of the sweep is only half the MAS frequency. The recently developed DREAM-C7[27] sequence addresses this issue, but is limited to low MAS frequencies (<15 kHz) due to high rf power requirements. With CM_pAR, the optimal ratio (p) of the modulation frequency (approximately the rf field strength) to the MAS frequency lies in the range of 3 to 8. The exact value is a compromise between direct interference between the MAS and rf averaging (small p value) and a small modulation amplitude a_H (high p value). The spectra in Figure 2 demonstrate that $CM_{3.5}AR$ functions efficiently at high MAS frequencies (28.6 kHz) with $p = 3.5$. Furthermore, without ^{1}H irradiation, higher rf fields can be applied to the ^{13}C's, allowing more efficient recoupling at high MAS rates and magnetic fields. These characteristics extend the range of applicability of homonuclear recoupling techniques to high-resolution conditions and should be of major interest for structure determination of biomolecules. A detailed description of the spin dynamics associated with the CMAR recoupling mechanism is currently in preparation.

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Supporting Information Available: Additional figures. This material is available free of charge via the Internet at http://pubs.acs.

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