

^{13}C – ^1H dipolar-assisted rotational resonance in magic-angle spinning NMR

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Abstract

A new ^{13}C – ^{13}C recoupling mechanism which occurs under magic-angle spinning (MAS) is presented. The mechanism can basically be attributed to rotational resonance (R^2), but the conventional R^2 condition is modified by a recoupled ^{13}C – ^1H dipolar interaction. The ^{13}C – ^1H recoupling is attained by ^1H rf irradiation fulfilling a rotary-resonance condition. The present method does not have the drawbacks associated with rf irradiation on ^{13}C and is applicable for band-selective recoupling between carbonyl/aromatic carbons and aliphatic carbons. The ^{13}C – ^{13}C recoupling mechanism under ^{13}C – ^1H recoupling is theoretically explained and is experimentally demonstrated using *N*-acetyl[1,2- ^{13}C] DL-valine and uniformly ^{13}C , ^{15}N -labeled glycylisoleucine. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Recently, we proposed a new ^{13}C – ^{13}C recoupling mechanism under magic angle spinning (MAS) referred to as resonant interference recoupling (RIR) [1]. In RIR, the FSLG- $m\overline{2mm}$ [2] sequence is applied to ^1H spins under MAS to produce modulation-induced ^{13}C – ^1H dipolar sidebands for a ^{13}C spin coupled to ^1H . The spectral overlap necessary to ensure energy conservation for efficient polarization transfer between two ^{13}C spins is realized between a spinning sideband of one ^{13}C spin and a modulation-induced sideband of the other ^{13}C spin and vice versa. The polarization-transfer mechanism is thus the combination of rotor-driven and ^1H -driven [3]. While the ^{13}C – ^{13}C recoupling methods employing rf irradiation on ^{13}C cause a fast decay of ^{13}C magnetization due to

inhomogeneity of the rf irradiation and a short relaxation time under an rf field, RIR does not because it requires no rf irradiation on ^{13}C .

In this work, we present a new ^{13}C – ^{13}C recoupling method under MAS without employing ^{13}C rf irradiation. Similarly to RIR, the new method utilizes the ^{13}C – ^1H dipolar interaction, and is again classified into the combined rotor-driven and ^1H -driven mechanism. The ^{13}C – ^1H dipolar interaction is recovered by CW irradiation on ^1H with which the ^1H rf-field intensity ν_1 satisfying the rotary-resonance condition $\nu_1 = n\nu_R$ ($n = 1$ or 2) [4]. Contrary to RIR, the flip-flop term of the ^1H – ^1H dipolar interaction is not averaged to be zero and the recoupled ^{13}C – ^1H dipolar interaction is time independent. Hence, the ^{13}C lines are broadened by both ^{13}C – ^1H and ^1H – ^1H dipolar interactions. The spectral overlap between the two relevant ^{13}C spins is realized between a spinning sideband of one ^{13}C spin and the ^{13}C – ^1H dipolar pattern of the other ^{13}C spin and vice versa.

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2. Theory

We consider a system composed of two dipolar-coupled ^{13}C spins (S_1 and S_2) and one ^1H spin (I). It will be shown later that the homogeneous broadening originated from the other ^1H spins increases the efficiency of the ^{13}C – ^{13}C polarization transfer. However, since they are not directly related to the ^{13}C – ^{13}C recoupling mechanism, the other ^1H spins are not explicitly treated. For simplicity, we neglect the chemical shift anisotropy (CSA) interactions, and assume that the rf field with the intensity ν_1 is applied to the I spin on resonance. Further, we assume that the ^{13}C – ^1H interaction for the second S spin (S_2) is negligible. In the double rotating frame, the total spin Hamiltonian consists of the Zeeman interaction \mathcal{H}_Z , the ^{13}C – ^{13}C dipolar interaction $\mathcal{H}_D^{SS}(t)$, and the ^{13}C – ^1H dipolar interaction $\mathcal{H}_D^{IS}(t)$ as

$$\mathcal{H} = \mathcal{H}_Z + \mathcal{H}_D^{SS}(t) + \mathcal{H}_D^{IS}(t), \quad (1)$$

with

$$\mathcal{H}_Z = \Delta_1 S_{z1} + \Delta_2 S_{z2} + \nu_1 I_x,$$

$$\mathcal{H}_D^{SS}(t) = d(t) \left\{ S_{1z} S_{2z} - \frac{1}{4} (S_{1+} S_{2-} + S_{1-} S_{2+}) \right\},$$

$$\mathcal{H}_D^{IS}(t) = D(t) S_{1z} I_z, \quad (2)$$

where Δ_n is the resonance offset of the S_n spin, and

$$\begin{aligned} d(t) &= d_0 \{ g_1(\alpha_d, \beta_d) \cos(2\pi\nu_R t + \gamma_d) \\ &\quad + g_2(\alpha_d, \beta_d) \cos(4\pi\nu_R t + 2\gamma_d) \}, \\ D(t) &= D_0 \{ G_1(\alpha_D, \beta_D) \cos(2\pi\nu_R t + \gamma_D) \\ &\quad + G_2(\alpha_D, \beta_D) \cos(4\pi\nu_R t + 2\gamma_D) \}. \end{aligned} \quad (3)$$

Here, d_0 and D_0 are the dipolar coupling constants for ^{13}C – ^{13}C and ^{13}C – ^1H , respectively, ν_R is an MAS frequency, and $(\alpha_d, \beta_d, \gamma_d)$ and $(\alpha_D, \beta_D, \gamma_D)$ are the Euler angles describing the orientation of the S_1 – S_2 and the S_1 – I internuclear vectors in the rotor-fixed frame, respectively.

There are two rotary-resonance conditions: $\nu_1 = n\nu_R$ with $n = 1, 2$. With the $n = 1$ rotary-resonance condition, the total Hamiltonian is trans-

ferred into the interaction frame defined by the unitary transformation

$$U = \exp(2\pi i \nu_1 I_x t). \quad (4)$$

Only the ^{13}C – ^1H dipolar interaction is affected by this transformation, and the following zeroth-order average Hamiltonian is obtained:

$$\overline{\mathcal{H}_D^{IS}} = \pm D_1 S_z, \quad (5)$$

with

$$D_1 = \frac{1}{4} D_0 G_1(\alpha_D, \beta_D) \cos \gamma_D. \quad (6)$$

Here, we replaced I_z by $\pm 1/2$. D_1 represents the recovered ^{13}C – ^1H dipolar coupling. The total Hamiltonian is further transformed by the unitary transformation

$$U = \exp[2\pi i \{ (\Delta_1 \pm D_1) S_{1z} + \Delta_2 S_{2z} \} t]. \quad (7)$$

The ^{13}C – ^{13}C dipolar interaction $\mathcal{H}_D^{SS}(t)$ in this new frame is given by

$$\begin{aligned} \widetilde{\mathcal{H}_D^{SS}}(t) &= d(t) \left[S_{1z} S_{2z} - \frac{1}{4} \{ S_{1+} S_{2-} \exp(2\pi i \Delta t) \right. \\ &\quad \left. + S_{1-} S_{2+} \exp(-2\pi i \Delta t) \} \right], \end{aligned} \quad (8)$$

with

$$\Delta = \Delta_1 \pm D_1 - \Delta_2. \quad (9)$$

In the conventional rotational resonance (R^2) [5–12], ^1H decoupling applied eliminates D_1 , leading to the R^2 recoupling condition of $\Delta_1 - \Delta_2 = m\nu_R$ ($m = \pm 1, \pm 2, \dots$). In the present case, the non-zero ^{13}C – ^1H dipolar coupling modifies the conventional R^2 condition as

$$\Delta_1 \pm D_1 - \Delta_2 = m\nu_R. \quad (10)$$

This corresponds to a situation that one of the spinning sidebands of S_2 overlaps the ^{13}C – ^1H dipolar powder pattern of S_1 . We refer to this recoupling as the dipolar-assisted rotational resonance (DARR) recoupling. It should be mentioned here that similar DARR recoupling can take place also under the $n = 2$ rotary-resonance condition. Further, the other heteronuclear dipolar recoupling methods, such as MORE [13] and FSLG- $m\overline{2mm}$, can also be used for DARR. As will be mentioned below, however, DARR under FSLG- $m\overline{2mm}$ is significantly inefficient, indicating

that ^1H – ^1H coupling is indispensable for efficient DARR.

3. Experimental

The NMR experiments were carried out using a Chemagnetics CMX-300 spectrometer operating at the resonance frequency of 75.5 MHz for ^{13}C with a CP/MAS probe (Doty Sci.) for a 5 mm rotor. The decoupling ^1H frequency was chosen to be ca. 2 ppm downfield from tetramethylsilane (TMS) and its intensity was 83 kHz. For the FSLG- $m\overline{2mm}$ sequence, we adopted the $m = 2$ sequence (FSLG-242), and the ^1H rf-field intensity ν_1 was set to 86.7 kHz for a spinning speed of 12 kHz and the ^1H mean frequency for the FSLG- $m\overline{2mm}$ sequence was 4000 Hz downfield from TMS [2].

4. Results and discussion

Fig. 1a,b shows ^{13}C CP/MAS spectra of N -acetyl[1,2- ^{13}C] DL-valine ($^{13}\text{C} \sim 99\%$) (a) with and (b) without ^1H CW decoupling. The spinning frequency ν_R is 12.0 kHz, which deviates by ca. 3.2 kHz from the R^2 condition between $^{13}\text{C}=\text{O}$ and ^{13}CH appearing at ~ 4.4 kHz and at ~ -4.4 kHz, respectively. Fig. 1c,d demonstrates ^{13}C CP/MAS spectra observed (c) under ^1H irradiation with the $n = 1$ rotary-resonance condition ($\nu_1 = \nu_R$) and (d) under FSLG-242 with the ^{13}C – ^1H dipolar recoupling condition [2], showing that, under either irradiation, spectral overlap occurs between the ^{13}CH lineshape and one of the spinning sidebands of $^{13}\text{C}=\text{O}$.

We made 1D polarization-transfer experiments for N -acetyl[1,2- ^{13}C] DL-valine at $\nu_R = 12$ kHz as follows. The longitudinal ^{13}C magnetization is created by a 90° pulse after CP, and the ^{13}C magnetization of ^{13}CH is selectively inverted by a soft pulse. After a certain mixing time τ , the resulting magnetization is observed by applying the second 90° pulse. For the mixing, three different ^1H irradiation schemes were tried, namely, no rf field, CW with $\nu_1 = \nu_R$, and FSLG- $m\overline{2mm}$. The first is the conventional ^1H -driven polarization transfer, while the second and the third correspond

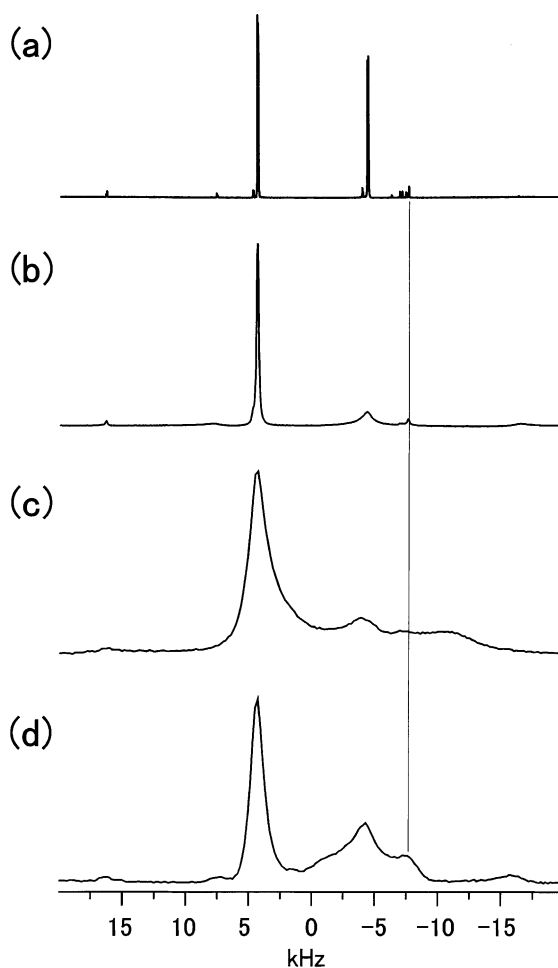


Fig. 1. ^{13}C CP/MAS spectra of N -acetyl[1,2- ^{13}C] DL-valine at $\nu_R = 12.0$ kHz: with ^1H CW decoupling (a), without ^1H decoupling (b), with ^1H irradiation for ^{13}C – ^1H recoupling by rotary resonance ($\nu_1 = \nu_R = 12$ kHz) (c) and by the FSLG-242 sequence (d). The vertical line shows the position of a spinning sideband of $^{13}\text{C}=\text{O}$.

to polarization transfer under DARR with and without ^1H – ^1H coupling, respectively. Fig. 2 shows the mixing-time dependence of the normalized magnetization for $^{13}\text{C}=\text{O}$ and ^{13}CH . It is shown that the polarization transfer under the ^1H CW irradiation satisfying the rotary-resonance condition ($\nu_1 = \nu_R$), that is, DARR with ^1H – ^1H coupling, is most efficient. On the other hand, in spite of the fact that spectral overlap between a sideband of $^{13}\text{C}=\text{O}$ and the ^{13}CH lineshape is larger under FSLG-242 than under no rf field (Fig.

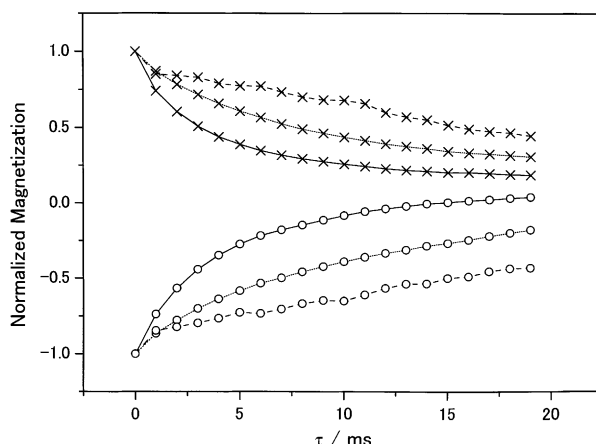


Fig. 2. Mixing-time dependence of the normalized magnetization of $^{13}\text{C}=\text{O}$ (\times) and ^{13}CH (\circ) in *N*-acetyl[1,2- ^{13}C] DL-valine at $\nu_{\text{R}} = 12.0$ kHz. The ^{13}CH magnetization is selectively inverted before the mixing time. Three different ^1H irradiation conditions during the mixing time were examined: (1) no irradiation (dotted line), (2) FSLG-242 (broken line), and (3) irradiation with the $n = 1$ rotary-resonance condition (solid line). The lines are drawn for eye guidance.

1), the polarization transfer is least efficient under FSLG-242. This shows the indispensable role of the ^1H – ^1H dipolar interaction for efficient DARR, which can be explained as follows. The spectral overlap for DARR occurs only for S_1 – S_2 pairs satisfying the DARR condition (Eq. (10)). The homogeneous broadening of the S resonances due to the non-zero ^1H – ^1H interaction relaxes the DARR condition, thus increasing the number of carbons to be recoupled.

The 1D polarization-transfer experiments by CW irradiation with various ν_1 during τ were made at $\nu_{\text{R}} = 12$ kHz. The apparent polarization-transfer rates k for various ν_1 were deduced for $^{13}\text{C}=\text{O}$ by fitting the initial decay in the polarization-transfer curve to a single exponential function $\exp(-k\tau)$, which are shown in Fig. 3. The plot shows the two maxima at $\nu_1 = 12$ and 24 kHz with similar k values, which correspond to the $n = 1$ and 2 rotary-resonance conditions, respectively. The maximum around the $n = 1$ condition is significantly broad, indicating that slight misadjustments of the ^1H rf intensity as well as the spinning frequency are not crucial. This apparent tolerance would alleviate the precise settings of experimental conditions often required for other experiments.

Since the orientations of the ^{13}C – ^1H vectors with the ^{13}CH carbons whose resonances appear at

a spinning sideband of $^{13}\text{C}=\text{O}$ differ for the different rotary-resonance conditions, the number of the ^{13}CH spins satisfying the DARR condition should be increased by using the two rotary-resonance conditions alternatively. Therefore, polarization-transfer efficiency would be improved by alternating the ^1H rf-field intensity between $\nu_1 = \nu_{\text{R}}$ and $\nu_1 = 2\nu_{\text{R}}$ in a period comparable to the characteristic correlation time of ^{13}C – ^{13}C polarization transfer. Since the ^{13}C – ^{13}C dipolar interaction is in the order of a few kHz, an alternation period of 0.25–0.5 ms would be suitable. The polarization-transfer curves observed under ^1H irradiation with the constant rf intensity satisfying the $n = 1$ or 2 rotary-resonance condition and with the alternating intensities between the two conditions for 0.25 ms are shown in Fig. 4. The curve observed for the alternation period of 0.5 ms is similar to that of 0.25 ms (not shown). While the two curves taken under each of the two rotary-resonance conditions are similar, the improvement by the alternation of them is appreciable, and it is thus recommended to alter the ^1H intensity for more efficient polarization transfer. We refer to the single and the alternating matching schemes as the ^{13}C – ^1H dipolar-assisted rotational resonance with single and alternate matching (DARRSM and DARRAM), respectively. The observed improve-

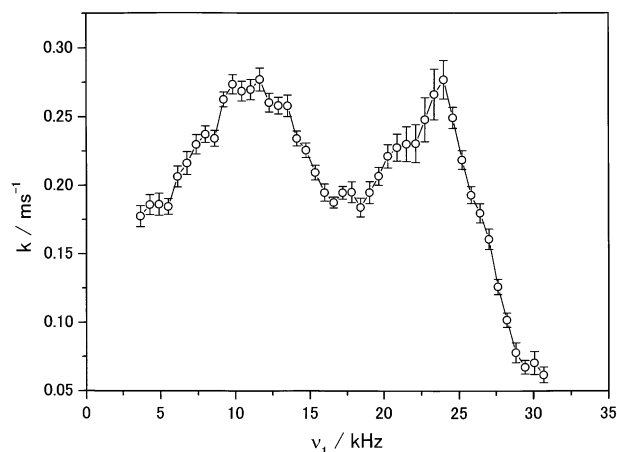


Fig. 3. Dependence of the polarization-transfer rate (k) on the ^1H rf-field intensity ν_1 of $^{13}\text{C}=\text{O}$ in N -acetyl[1,2- ^{13}C] DL-valine at $\nu_R = 12$ kHz. The rates were deduced from the polarization-transfer curves similar to those shown in Fig. 2 which were obtained under CW irradiation at various ν_1 . The apparent rates were obtained by fitting the initial decay in the polarization-transfer curve to a single exponential function $\exp(-k\tau)$. The line is drawn for eye guidance.

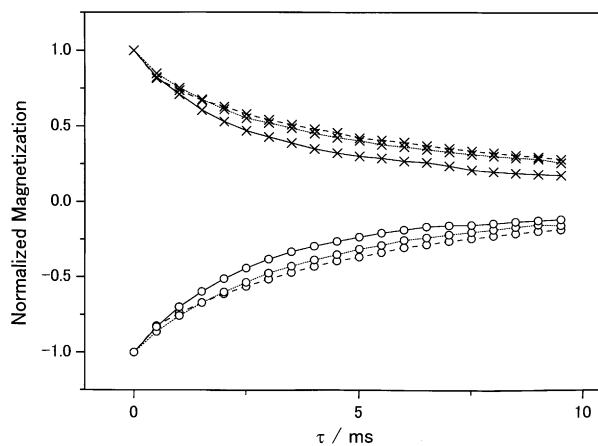


Fig. 4. Mixing-time dependence of the normalized magnetization of $^{13}\text{C}=\text{O}$ (\times) and ^{13}CH (\circ) in N -acetyl[1,2- ^{13}C] DL-valine at $\nu_R = 12.0$ kHz. The ^{13}CH magnetization is selectively inverted before the mixing time. The dotted line denotes the curve observed under the constant rf intensity satisfying the $n = 1$ rotary-resonance condition and the broken line is that satisfying the $n = 2$ rotary-resonance condition. The solid line denotes the curve observed under the alternating ^1H rf-field intensities between the $n = 1$ and the $n = 2$ rotary-resonance conditions for every 0.25 ms. The lines are drawn for eye guidance.

ment in DARRAM further motivates us to employ other ^{13}C – ^1H recoupling methods such as MORE [13] in conjunction with rotary resonance. Such experiments are currently undergoing and will be published elsewhere.

Similarly to RIR and R^2 , DARR does not have the drawbacks associated with rf irradiation on ^{13}C : it allows the mixing time to be long

without requiring any critical adjustments. Further, while R^2 broadens the relevant ^{13}C lines, the advantage of high resolution can fully be appreciated for RIR and DARR. RIR is frequency-selective recoupling, while DARR shows band-selective nature because of the homogeneous broadening: for a commonly used static magnetic field and a spinning speed of 10–30

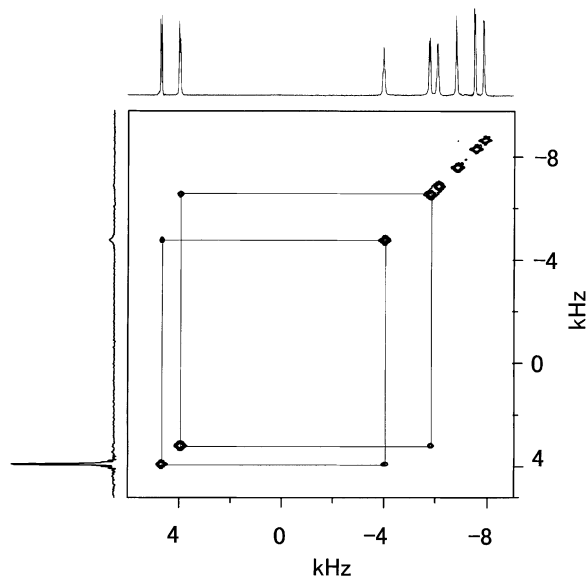


Fig. 5. The contour plot of the 2D ^{13}C – ^{13}C shift-correlation spectrum of uniformly ^{13}C , ^{15}N -labeled glycyllisoleucine with the mixing time of 3 ms. At the top is shown the 1D spectrum, and the side spectrum is the cross-section at the $^{13}\text{C}=\text{O}$ peak (~ 4.4 kHz) of the isoleucine residue. During the mixing time, ^1H irradiation to enhance ^{13}C – ^{13}C polarization transfer by DARRAM was applied with the alternation period of 0.5 ms. The spinning frequency was 15.0 kHz. The solid lines connect paired peaks assigned to the directly bonded $\text{C}=\text{O}$ and C_α carbons and the corresponding off-diagonal peaks.

kHz, DARR can be used to recouple carboxyl/carbonyl/aromatic carbons and aliphatic carbons. To appreciate this, a 2D ^{13}C – ^{13}C shift-correlation spectrum of uniformly ^{13}C , ^{15}N -labeled glycyllisoleucine ($^{13}\text{C} \sim 99\%$) were observed (Fig. 5) using the conventional 2D exchange pulse sequence. The spinning frequency ν_R was set to 15.0 kHz to avoid R^2 , and DARRAM with the alternation period of 0.5 ms was applied during the mixing time of 3 ms. In Fig. 5, only the cross-peaks between directly bonded $\text{C}=\text{O}$ and C_α [14,15] are appreciable as indicated by the solid lines, showing the band-selective nature of DARR. Additional cross-peaks are expected for a longer mixing time, which can be extended to ~ 1 s due to the low power requirement for DARR. These features would make DARR useful for the structural study of peptides. ^{13}C – ^{13}C polarization transfer at much longer mixing times, as well as comparison of transfer efficiencies under RIR and DARR, are currently being examined and will be published elsewhere.

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