

# Selective Homonuclear Polarization Transfer in the Tilted Rotating Frame under Magic Angle Spinning in Solids

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Received January 30, 1997; revised May 7, 1997

**An application of the R2TR method (1995, *Chem. Phys. Lett.* 232, 424) to selective homonuclear polarization transfer under magic angle spinning is proposed. It is shown that, for a spinning speed fast enough to remove the maximum homonuclear dipolar coupling constant  $\omega_D$  involved, the flip-flop and flop-flop mechanisms are suitable for recoupling the spins with a chemical shift difference larger than  $\omega_D$  and a difference comparable to or smaller than  $\omega_D$ , respectively. It is also shown that, for fast polarization transfer, the off-resonance frequencies should be much higher than the RF intensity in the flip-flop condition, while for the flop-flop condition, the off-resonance frequencies should be much lower than the RF intensity. Some one- and two-dimensional experiments are proposed by utilizing the capability of the R2TR method to abruptly switch on and off the recoupling condition, and are demonstrated for triply  $^{13}\text{C}$ -enriched L-alanine. The mixing time required for population transfer was found to be ca. 0.5 ms for the methine and methyl  $^{13}\text{C}$  spins separated by 1.5 Å and ca. 5 ms for the methyl and the carboxyl carbons separated by 2.5 Å. The experimental results and theoretical simulations show that selective polarization transfer is achieved when the difference in the isotropic chemical shifts between the relevant pair of spins and a neighboring spin is more than 1000 Hz.** © 1997 Academic Press

a single sample owing to its high resolution. To recover a particular dipolar interaction in a multiple-spin system, a selective recoupling method under MAS fast enough to remove all the other dipolar interactions simultaneously is required. When we desire to measure several internuclear distances, only one multiply labeled sample must be prepared in a selective recoupling approach, whereas a nonselective method requires many differently pair-labeled samples.

Another advantage of using a selective recoupling method in a multiple-spin system is that it enables us to perform some useful correlation experiments. For example, a two-dimensional (2D) correlation spectrum between two dipolar powder patterns can give the relative orientation of the two dipolar principal-axis systems, which is directly related to the inter-bond angle. Such a spectrum in the A–B–C–D system can be obtained by selectively recoupling the A–B dipolar interaction in the  $t_1$  period, the B–C dipolar interaction in the mixing period for the polarization/coherence transfer, and the C–D dipolar interaction in the  $t_2$  period. In such correlation NMR, however, it is required to abruptly switch a selectively recoupled dipolar interaction to another at the time of a change of the period.

For homonuclear dipolar interactions, the rotational resonance ( $R^2$ ) (6–16) has been used as a simple selective recoupling method. In the  $R^2$  method, selection of a pair of spins is made by adjusting an integral multiple of the MAS frequency  $\omega_R$  to the difference of the chemical shifts of the two spins. Since it is not easy to switch the spinning speed abruptly, the application of the  $R^2$  method to a correlation experiment is difficult. Moreover, when one desires to recouple the dipolar interaction between the carbons with a small chemical shift difference using the  $R^2$  method, the MAS frequency must be lowered; however, the other dipolar interactions cannot perfectly be decoupled by slow MAS, thus leading a poor selectivity. So far, only a few selective recoupling methods using RF pulses have been proposed for heteronuclear dipolar interactions (17) and for homonuclear dipolar interactions (18–20). Recently, we showed that selective recoupling among homonuclear spins can be achieved by the RF irradiation that satisfies one of the fol-

## INTRODUCTION

Recently, considerably ingenuity has been brought to the development of solid-state NMR techniques for structural determination. In solid-state NMR, the internuclear distance between two spins can be deduced from the strength of the dipolar interaction. So far, several techniques have been proposed for measuring internuclear distances either under magic angle spinning (MAS) or without MAS. The application of MAS is not a prerequisite for the determination of the distance in a two-spin system, and techniques based on the Hahn-echo sequence for a stationary powdered sample are developed for homonuclear (1–4) and heteronuclear (5) two spins. Much of the usefulness of MAS is appreciated in the case of a multiple-spin system, where MAS provides the possibility of determining more than one distance using

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lowing conditions, namely,  $\Sigma = n\omega_R$ ,  $\Delta = n\omega_R$ , and  $\omega_{eX} = n\omega_R$ , where  $\Sigma$  and  $\Delta$  are the sum and the difference of the strength of the effective field of the two spins ( $X = I$  and  $S$ ), respectively (21). We referred to these conditions as the R2TR (the rotational resonance in the tilted rotating frame) conditions. One of the significant features of the R2TR method is that we can switch dipolar interactions to be recoupled from one to another without any appreciable delay by changing conditions of the RF irradiation. Also, we can often find an R2TR condition to recouple a particular dipolar interaction between spins even with a small chemical shift difference under MAS fast enough to remove all the other dipolar interactions. Thus, the R2TR method should be suitable for correlation experiments.

In this work, we demonstrate that the R2TR method can be used for selective polarization transfer among homonuclear spins. The mixing-time dependence of the polarization transfer and the chemical shift difference required for a selective transfer are investigated. Further, 2D selective  $^{13}\text{C}$ - $^{13}\text{C}$  homonuclear shift-correlation experiments are demonstrated.

## THEORY

### *Homonuclear Dipolar Recoupling in the Tilted Rotating Frame under MAS*

In this section we give a brief theory of the R2TR phenomenon to the extent necessary for the present study. We consider a dipolar-coupled homonuclear two-spin  $I$ - $S$  system in a rotating powdered sample under a continuous wave RF irradiation along the  $x$  axis of the rotating frame. For simplicity, we neglect the chemical shift anisotropies. The homonuclear dipolar interaction in the tilted rotating frame,  $H_D(t)$ , can be written as

$$H_D(t) = D(t) \{ AI_z S_z + B(I_+ S_- + I_- S_+) + RI_z S_{\pm} + SI_{\pm} S_z + Q(I_+ S_+ + I_- S_-) \}. \quad [1]$$

$D(t)$  is the time-dependent spatial part and can be written as

$$D(t) = \sum_{n=\pm 1, \pm 2} \omega_{Dn} \exp(in\omega_R t), \quad [2]$$

with

$$\begin{aligned} \omega_{D\pm 1} &= \frac{3d}{4} \sin 2\theta_{\text{MAS}} \sin 2\theta_D \exp(\pm i\phi_D) \\ &= \omega_{d1} \exp(\pm i\phi_D) \end{aligned} \quad [3]$$

and

$$\begin{aligned} \omega_{D\pm 2} &= \frac{3d}{4} \sin^2 \theta_{\text{MAS}} \sin^2 \theta_D \exp(\pm 2i\phi_D) \\ &= \omega_{d2} \exp(\pm 2i\phi_D), \end{aligned} \quad [4]$$

where  $\theta_{\text{MAS}}$  is the magic angle,  $(\theta_D, \phi_D)$  are the polar angles defining the direction of the  $I$ - $S$  vector in a rotor-fixed frame, and  $d = (\mu_0/4\pi)(\gamma^2 \hbar/r^3)$  is the dipolar coupling constant for the internuclear distance  $r$ . The coefficients  $A$ ,  $B$ ,  $R$ ,  $S$ , and  $Q$  in Eq. [1] are functions of the angle  $\beta_W$  between the  $z$  axis of the rotating frame (i.e., the direction of the static field) and the  $Z$  axis of the tilted rotating frame (i.e., the direction of the effective field) for the  $W$  spin ( $W = I$  or  $S$ ). Among them, only the  $B$  and  $Q$  terms are relevant in this study:

$$B = -\frac{1}{8}(1 + \cos \beta_I \cos \beta_S - 2 \sin \beta_I \sin \beta_S) \quad [5]$$

and

$$Q = \frac{1}{8}(1 - \cos \beta_I \cos \beta_S + 2 \sin \beta_I \sin \beta_S). \quad [6]$$

$H_D(t)$  is then transferred into the interaction frame by using the unitary operator

$$U_{\text{eff}}(t) = \exp(-i\omega_{eI} I_z t) \exp(-i\omega_{eS} S_z t), \quad [7]$$

where  $\omega_{eW}$  is the strength of the effective field for the  $W$  spin. The result is

$$\begin{aligned} H_D^*(t) &= U_{\text{eff}}^{-1}(t) H_D(t) U_{\text{eff}}(t) \\ &= \sum_{n=\pm 1, \pm 2} \omega_{Dn} [ AI_Z S_Z \exp(-in\omega_R t) \\ &\quad + B[I_+ S_- \exp\{i(n\omega_R + \Delta_{\text{eff}})t\} \\ &\quad + I_- S_+ \exp\{i(n\omega_R - \Delta_{\text{eff}})t\}] \\ &\quad + R[I_Z S_+ \exp\{i(n\omega_R + \omega_{eS})t\} \\ &\quad + I_Z S_- \exp\{i(n\omega_R - \omega_{eS})t\}] \\ &\quad + S[I_+ S_Z \exp\{i(n\omega_R + \omega_{eI})t\} \\ &\quad + I_- S_Z \exp\{i(n\omega_R - \omega_{eI})t\}] \\ &\quad + Q[I_+ S_+ \exp\{i(n\omega_R + \Sigma_{\text{eff}})t\} \\ &\quad + I_- S_- \exp\{i(n\omega_R - \Sigma_{\text{eff}})t\}]]. \end{aligned} \quad [8]$$

The spatial part of  $H_D^*(t)$  is modulated by MAS with the spinning frequency  $\omega_R$ , while the spin part is modulated by the effective field with the frequencies of  $\Sigma_{\text{eff}} = \omega_{eI} + \omega_{eS}$ ,  $\Delta_{\text{eff}} = \omega_{eI} - \omega_{eS}$ ,  $\omega_{eI}$ , and  $\omega_{eS}$ . When one of the conditions  $\Sigma_{\text{eff}}$ ,  $\Delta_{\text{eff}}$ ,  $\omega_{eI}$ , and  $\omega_{eS} = m\omega_R$  ( $m = \pm 1$  or  $\pm 2$ ) is met, the time dependence of the spatial part and the spin part in  $H_D^*(t)$  is partially canceled with each other to give a static dipolar interaction  $\overline{H_D^*}$ . For example, when  $\Delta_{\text{eff}} = \omega_R$ , Eq. [8] becomes

$$\begin{aligned}
H_D^*(t) = & B(\omega_{D-1}I_+S_- + \omega_{D+1}I_-S_+) \\
& + \sum_{n=\pm 1, \pm 2} B[\omega_{D+n}I_+S_- \exp\{i(n\omega_R + \Delta_{\text{eff}})t\} \\
& + \omega_{D-n}I_-S_+ \exp\{i(-n\omega_R - \Delta_{\text{eff}})t\}] \\
& + \sum_{n=\pm 1, \pm 2} \omega_{Dn}[AI_ZS_Z \exp(-in\omega_R t) \\
& + R[I_ZS_+ \exp\{i(n\omega_R + \omega_{\text{es}})t\} \\
& + I_ZS_- \exp\{i(n\omega_R - \omega_{\text{es}})t\}] \\
& + S[I_+S_Z \exp\{i(n\omega_R + \omega_{\text{el}})t\} \\
& + I_-S_Z \exp\{i(n\omega_R - \omega_{\text{el}})t\}] \\
& + Q[I_+S_+ \exp\{i(n\omega_R + \Sigma_{\text{eff}})t\} \\
& + I_-S_- \exp\{i(n\omega_R - \Sigma_{\text{eff}})t\}]. \quad [9]
\end{aligned}$$

Except for the first time-independent term, all the other time-dependent terms are dropped off at the zeroth order, and the resulting zeroth-order average Hamiltonian becomes

$$\begin{aligned}
\overline{H_D^*} &= B(\omega_{D-1}I_+S_- + \omega_{D+1}I_-S_+) \\
&= B\omega_{\text{dl}}\{I_+S_- \exp(-i\phi) + I_-S_+ \exp(+i\phi)\}. \quad [10]
\end{aligned}$$

It may be helpful to further transfer the Hamiltonian as follows:

$$\begin{aligned}
\overline{H_D^{**}} &= \exp\left(i\phi \frac{I_Z - S_Z}{2}\right) \overline{H_D^*} \exp\left(-i\phi \frac{I_Z - S_Z}{2}\right) \\
&= B\omega_{\text{dl}}(I_+S_- + I_-S_+). \quad [11]
\end{aligned}$$

Nielsen *et al.* (18) proposed double-quantum homonuclear rotary resonance (2Q-HORROR) for the recovery of homonuclear dipolar interactions under MAS. They applied a RF field with the intensity of  $\omega_1 = \frac{1}{2}\omega_R$  that is much stronger than the chemical shift difference; it corresponds to a special case of the R2TR condition  $\Sigma_{\text{eff}} = m\omega_R$ .

So far, we have neglected the chemical shift anisotropy (CSA). It is true that the CSA interaction would affect the lineshape of cross peaks of a correlation spectrum, but in the following we show that its effect on the polarization transfer is not significant. The unitary operator (Eq. [7]) may be modified to incorporate the CSA interaction as

$$\begin{aligned}
U_{\text{eff}}(t) &= \exp(-i\omega_{\text{el}}I_Z t) \exp(-i\omega_{\text{es}}S_Z t) \\
&\times \exp\{-i\Phi(t)(I_Z - S_Z)t\}, \quad [12]
\end{aligned}$$

where

$$\Phi(t) = \int_0^t \Delta^{\text{ani}}(t) dt \quad [13]$$

with

$$\begin{aligned}
\Delta^{\text{ani}}(t) &= (C_1^I \cos \beta_I - C_1^S \cos \beta_S) \cos \omega_R t \\
&+ (C_2^I \cos \beta_I - C_2^S \cos \beta_S) \cos 2\omega_R t \\
&+ (S_1^I \cos \beta_I - S_1^S \cos \beta_S) \sin \omega_R t \\
&+ (S_2^I \cos \beta_I - S_2^S \cos \beta_S) \sin 2\omega_R t \quad [14] \\
&\equiv C_1 \cos \omega_R t + C_2 \cos 2\omega_R t + S_1 \sin \omega_R t \\
&+ S_2 \sin 2\omega_R t, \quad [15]
\end{aligned}$$

where  $C_i^W$  and  $S_i^W$  are coefficients given by the principal values and orientation of the CSA tensor of the  $W$  spin in the rotor-fixed coordinate system (22). After lengthy but straightforward calculations, one obtains

$$\begin{aligned}
\overline{H_D^*} &= \sum_{N=-\infty}^{\infty} I_N B\omega_{\text{dl}} \{I_+S_- \exp(-i\phi) \\
&+ I_-S_+ \exp(+i\phi)\}, \quad [16]
\end{aligned}$$

where  $I_N$  is the  $N$ th spinning sideband intensity of the CSA difference tensor in the tilted rotating frame given by a product of Bessel functions:

$$\begin{aligned}
I_N &= \sum_{p+q+2s+2r=N} \sum \sum J_p(C_1/\omega_R) J_q(S_1/\omega_R) J_s(C_2/2\omega_R) \\
&\times J_r(S_2/2\omega_R) (i)^{q+r} (-1)^{p+s} \\
&\times \exp\{-i(2S_1 + S_2)/2\omega_R\}. \quad [17]
\end{aligned}$$

The recoupled dipolar interaction is, thus, reduced by an additional fluctuation of the effective field due to the CSA interaction. This reduction is not significant when the spinning frequency is much larger than the anisotropies. More detailed discussion and corrections due to higher-order average Hamiltonians will be reported in a forthcoming paper.

#### Polarization Transfer in the Tilted Rotating Frame under MAS

When the condition  $\Delta$ , i.e.,  $\Delta_{\text{eff}} = m\omega_R$ , is satisfied, the flip-flop term in Eq. [8] becomes time independent:

$$\begin{aligned}
\overline{H_D^*} &= B\omega_{\text{dl}} \{I_+S_- \exp(-im\phi) \\
&+ I_-S_+ \exp(+im\phi)\}. \quad [18]
\end{aligned}$$

This causes an energy-conserving zero-quantum transition, and the sum of the magnetizations along the individual effective fields,  $M_{IZ} + M_{SZ}$ , is conserved. The total propagator in the tilted rotating frame can be written as

$$U(t) = \exp(-i\omega_{eI}I_z t)\exp(-i\omega_{eS}S_z t)\exp(-i\overline{H}_D^* t). \quad [19]$$

It may be worth pointing out that the polarization transfer mechanism by the R2TR method is similar to the conventional heteronuclear cross-polarization (CP) mechanism under MAS. When a modified Hartmann–Hahn condition of  $\omega_{1I} - \omega_{1S} = m\omega_R$  ( $m$  is an integer) (23–25) is met, the propagator for the CP process under MAS in the double rotating frame can be written as

$$U(t) = \exp(-i\omega_{1I}I_x t)\exp(-i\omega_{1S}S_x t)\exp(-i\overline{H}_d^{IS} t), \quad [20]$$

where  $\omega_{1I}$  and  $\omega_{1S}$  are the spin-locking fields applied for the  $I$  spin and the  $S$  spin, respectively, and  $\overline{H}_d^{IS}$  is the heteronuclear dipolar interaction averaged over a rotor cycle:

$$\overline{H}_d^{IS} \propto I_y S_y + I_z S_z. \quad [21]$$

Polarization transfer between the  $I$  and  $S$  spins is caused by the heteronuclear flip-flop term  $\overline{H}_d^{IS}$  along the  $x$  axes of the double rotating frame. In this case, the sum of the magnetizations along the respective  $x$  axes of the double rotating frame,  $M_{Ix} + M_{Sx}$ , is conserved. The similarity between Eqs. [19] and [20] shows that the present magnetization transfer experiment by the R2TR method can be understood as a selective homonuclear Hartmann–Hahn cross-polarization experiment under MAS.

In the conventional CP method, one applies a  $90^\circ_y$  pulse to the abundant spin prior to spin locking the magnetization along the  $x$  axis. Similarly, in the polarization transfer from the  $I$  spin to the  $S$  spin by the R2TR method, one should apply a  $\beta_I$ -degree pulse along the  $y$  axis of the rotating frame for spin locking the  $I$  magnetization. If we do not apply such a *trim* pulse, only the component of the magnetization in the direction of the effective field will be used for the polarization transfer, the magnetization perpendicular to the effective field being lost by the  $T_{2\rho}$  process. Note here that the polarization transferred to the  $S$  spin is also stored along its effective field. Therefore, to utilize the transferred  $S$  polarization for a further purpose, one should again take the angle  $\beta_S$  into account.

A simple remedy of the nuisance of applying the  $\beta$ -degree pulses is to irradiate a RF field far off resonance with  $\omega_1 \ll \Delta\omega_W$  ( $W = I, S$ ), where  $\Delta\omega_W$  is an offset frequency for the  $W$  spin. This setting makes both angles  $\beta$  for the  $I$  and  $S$  spins small, virtually eliminating the necessity of the  $\beta$  angle pulses. Therefore, we can spin lock the polarization nearly along the  $z$  axis of the rotating frame by just applying a mixing field. The far off-resonance irradiation is also desirable in the condition  $\Delta$  because the coefficient  $B$  has its

maximum when  $\beta_I = \beta_S = 0$ , thus ensuring the most efficient polarization transfer.

When the chemical shift difference is small, a slow spinning speed is required to satisfy the condition  $\Delta_{\text{eff}} = m\omega_R$ . However, slow MAS suffers from two drawbacks. First, it yields many crowded spinning sidebands as well known, making the spectrum very complex and lowering the signal-to-noise ratio. The other problem is that usually  $^{13}\text{C}$ – $^{13}\text{C}$  dipolar couplings cannot be decoupled by slow MAS, leading to a poor selectivity of dipolar recoupling. For example, in order to decouple the  $^{13}\text{C}$ – $^{13}\text{C}$  dipolar interaction for a typical C–C single-bond length of 1.54 Å, the spinning speed must be much faster than the dipolar constant of 2100 Hz. Therefore, we need a condition to recouple the dipolar interaction between the carbons with a small chemical shift difference under a sufficiently high-speed MAS. When it is difficult to find the condition  $\Delta$  for such a high spinning speed, we may utilize another condition  $\Sigma$  for this purpose, because the small difference of resonance frequencies can be effectively expanded under the condition  $\Sigma$ .

Under the R2TR condition  $\Sigma$ , i.e.,  $\Sigma_{\text{eff}} = m\omega_R$ , the recoupled flop-flop term in Eq. [8] can be written as

$$\overline{H}_D^* = Q\omega_{dm} \{ I_+ S_+ \exp(-im\phi) + I_- S_- \exp(+im\phi) \}. \quad [22]$$

This causes an energy-conserving double-quantum transition, so that the difference of the magnetizations along the respective  $Z$  axes,  $M_{Iz} - M_{Sz}$ , is conserved. In this case,  $Q$  is desirable to be as large as possible for the efficient transfer. In contrast to the coefficient  $B$ , the coefficient  $Q$  has the maximum at  $\beta_I = \beta_S = 90^\circ$ . Since we are considering a case of a small chemical shift difference, it is easy to make  $\beta_W$  close to  $90^\circ$  by setting a RF field intensity to satisfy  $\Delta\omega_W \ll \omega_1$  ( $W = I, S$ ). Further, it is advisable to apply the RF field at the middle of the two resonance frequencies to use the RF power most efficiently. Then, both effective fields  $\omega_{eI}$  and  $\omega_{eS}$  are close to the respective  $x$  axes of the double rotating frame. Therefore, we may apply a  $90^\circ_y$  hard pulse for spin locking and can regard the polarization transfer as taking place along the  $x$  axes.

Under the other R2TR conditions, i.e.,  $\omega_{eI} = m\omega_R$  and  $\omega_{eS} = m\omega_R$ ,

$$\overline{H}_D^* = S\omega_{dm} \{ I_+ S_z \exp(-im\phi) + I_- S_z \exp(+im\phi) \} \quad [23]$$

and

$$\overline{H}_D^* = R\omega_{dm} \{ I_z S_+ \exp(-im\phi) + I_z S_- \exp(+im\phi) \}, \quad [24]$$

respectively, are recovered. These do not conserve the energy of the system and are not useful for the polarization/coherence transfer.

In the above discussions, only two spins are considered. For a multiple-spin system, when the chemical shift differences between the spins are sufficiently large, one may choose a RF field that satisfies an R2TR condition  $\Delta$  or  $\Sigma$  for a particular spin pair without fulfilling any R2TR conditions for the other spin pairs. The selectivity depends on the chemical shift differences; it will be discussed quantitatively afterward.

## EXPERIMENTAL

Triply  $^{13}\text{C}$ -enriched L-alanine ( $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$ ), which has been kindly supplied by Professor M. Kainosyo at Tokyo Metropolitan University, was used in all experiments. The NMR experiments were performed by using a Chemanetics CMX-300 spectrometer operating at a resonance frequency of 75.49 MHz for  $^{13}\text{C}$  with a homebuilt MAS probe. The outer diameter of the  $\text{Al}_2\text{O}_3$  rotor is 5.0 mm and the sample length is 8 mm. The contact time for CP is 2 ms and the recycling delay time between successive scans is 4 s. The RF field strength for high-power proton decoupling and CP is about 63 kHz.

For simulations, a multistep method was used, in which one rotor period was divided into  $\sim 40$ –200 segments during which the Hamiltonian is assumed to be time independent. All the calculations have been performed for a three-spin system with chemical shift tensors. The chemical shift tensor determined by a single-crystal NMR study (26) and the molecular geometry obtained by a neutron diffraction study (27) were used for calculations. Since the direction cosines reported for the chemical shift tensor of the methyl carbon (26) do not give a consistent Euler rotation matrix, we multiply the direction cosines of the  $\sigma_{22}$  vector by  $-1$ . The Euler angles of the chemical shift tensor of the methyl carbon with respect to the crystal-axis system thus obtained are  $\alpha = -140.0^\circ$ ,  $\beta = -77.4^\circ$ , and  $\gamma = 52.9^\circ$ . A similar correction of the tensor has also been reported by Schaefer and colleagues (28).

## PULSE SEQUENCES

To investigate the mixing-time dependence and the selectivity of the R2TR polarization transfer, we have performed some 1D experiments using the pulse sequence shown in Fig. 1. For 1D observation of selective polarization transfer in a three  $^{13}\text{C}$  spin ( $I$ - $S$ - $L$ ) system, the initial state of the magnetizations is prepared as  $M_{IZ} = M_{0I}$ ,  $M_{SZ} = 0$ , and  $M_{LZ} = M_{0L}$ , where  $M_{0W}$  denotes the amount of the cross-polarized magnetization of the spin  $W$ . To achieve this initial state, a flip-back  $90^\circ$  hard pulse is applied to  $^{13}\text{C}$  after CP to restore all  $^{13}\text{C}$  magnetizations along the  $z$  axis, and then the  $S$  magne-

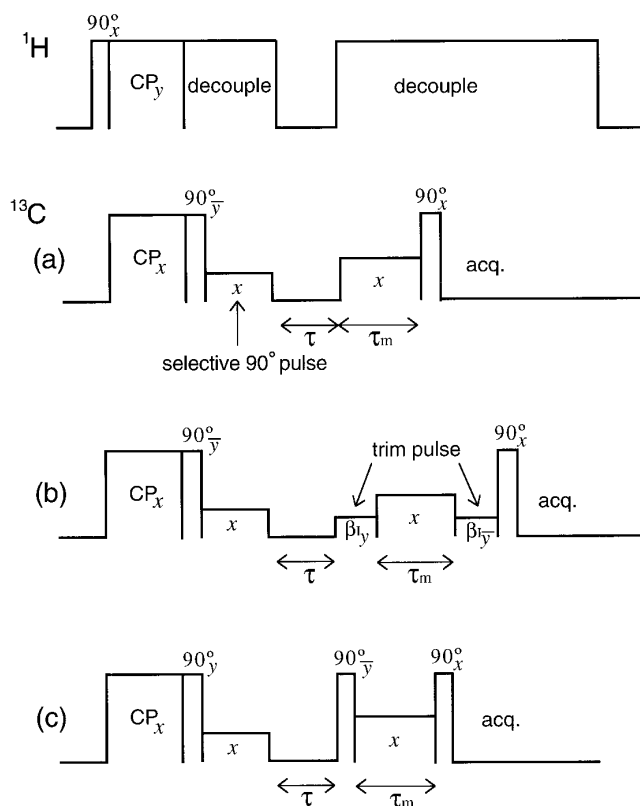
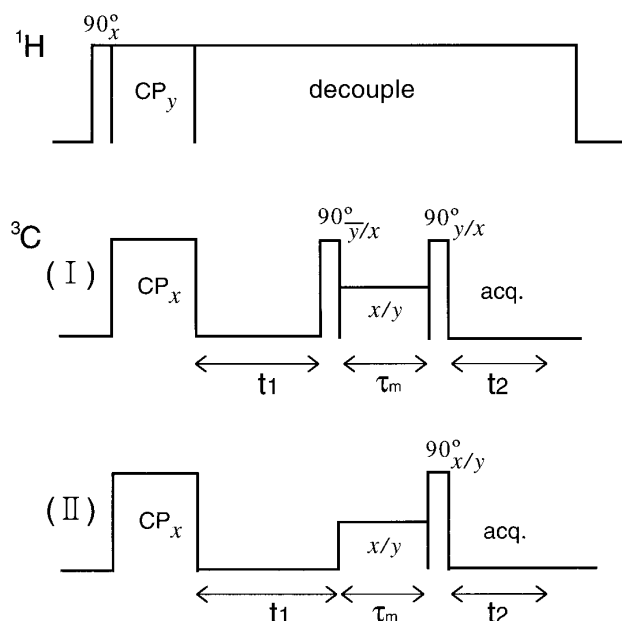


FIG. 1. Pulse sequences for one-dimensional selective homonuclear polarization transfer experiments. The pulse sequence for  $^1\text{H}$  is shown at the top and the sequences (a)–(c) are for  $^{13}\text{C}$ .

tization, to which magnetization will be transferred from the  $I$  spin, is destroyed by an application of a selective  $90^\circ S$  pulse followed by a dephasing time  $\tau$ . Ideally, the selective polarization transfer has no influence on the third spin  $L$ .

As discussed in the previous section, it is desirable to incorporate a trim pulse to align the  $z$  magnetization of the  $I$  spin along the  $Z$  axis of the  $I$  spin. For the condition  $\Delta$ , we examine the effect of the trim pulse by comparing the results obtained with and without the trim pulse, i.e., using sequences (b) and (a), respectively. In experiments under the condition  $\Sigma$ , we apply a  $90^\circ_y$  hard pulse instead of a trim pulse to spin lock all  $z$  magnetizations along the  $x$  axis (sequence (c)).

Next, a RF field satisfying the R2TR condition  $\Delta$  or  $\Sigma$  is applied for a mixing time  $\tau_m$ . After a mixing time longer than  $T_{2\rho}$ , only the magnetizations parallel to their respective effective fields survive. In sequence (b), the second trim pulse restores  $M_{IZ}$  back to the  $z$  axis of the rotating frame, and a hard  $90^\circ$  pulse is applied for the acquisition. Since the trim pulse does not work for the rest of the spins, certain amounts of the  $S$  and  $L$  magnetizations will be lost. In sequences (a) and (c), no trim pulses are applied for the acquisition, so that certain amounts of the magnetizations will be lost. To minimize the magnetization loss, the angle  $\beta$  should be close to 0 for the condition  $\Delta$  or  $90^\circ$  for the



**FIG. 2.** Pulse sequences for two-dimensional selective homonuclear shift-correlation experiments. The pulse sequence for  $^1\text{H}$  is shown at the top and the sequences (I) and (II) are for  $^{13}\text{C}$ .

condition  $\Sigma$ . Fortunately, this condition is the same as the above-mentioned irradiation condition for fast transfer.

At the limit of  $\Delta\omega_W \rightarrow \infty$ , however, the R2TR condition  $\Delta$  becomes identical to the conventional  $R^2$  condition, causing line broadening due to the  $R^2$  phenomenon in the observation period. Therefore, the spinning frequency  $\omega_R$  should be slightly deviated from the  $R^2$  condition to avoid the broadening. Practically, a deviation of 1000 Hz from the exact rotational resonance condition is sufficient for suppression of the  $R^2$  phenomenon.

In sequences (a) and (b), the phase of the final  $90^\circ$  pulse is not very important because the  $Z$  axis lies close to the  $z$  axis of the rotating frame. In sequence (c), the final  $90^\circ$  pulse is applied to choose one of the  $x$ ,  $y$  components to facilitate the data analysis.

Figure 2 shows two pulse sequences used for 2D selective homonuclear shift-correlation experiments. Sequence (I) is used for the condition  $\Delta$ . A  $90^\circ$  pulse at the end of the  $t_1$  period is used to select one of the  $x$ ,  $y$  components. During the exchange period  $\tau_m$ , a RF field satisfying the condition  $\Delta$  is applied. Then, a  $90^\circ$  pulse is used to read out the NMR signal in the  $t_2$  period. Sequence (II) is used for the condition  $\Sigma$ , in which a RF field fulfills the condition  $\Sigma$ . In this sequence, the effective fields lie close to the  $xy$  plane during the mixing time  $\tau_m$ . The  $90^\circ$  pulse after the mixing time is used to select one of the  $x$ ,  $y$  components.

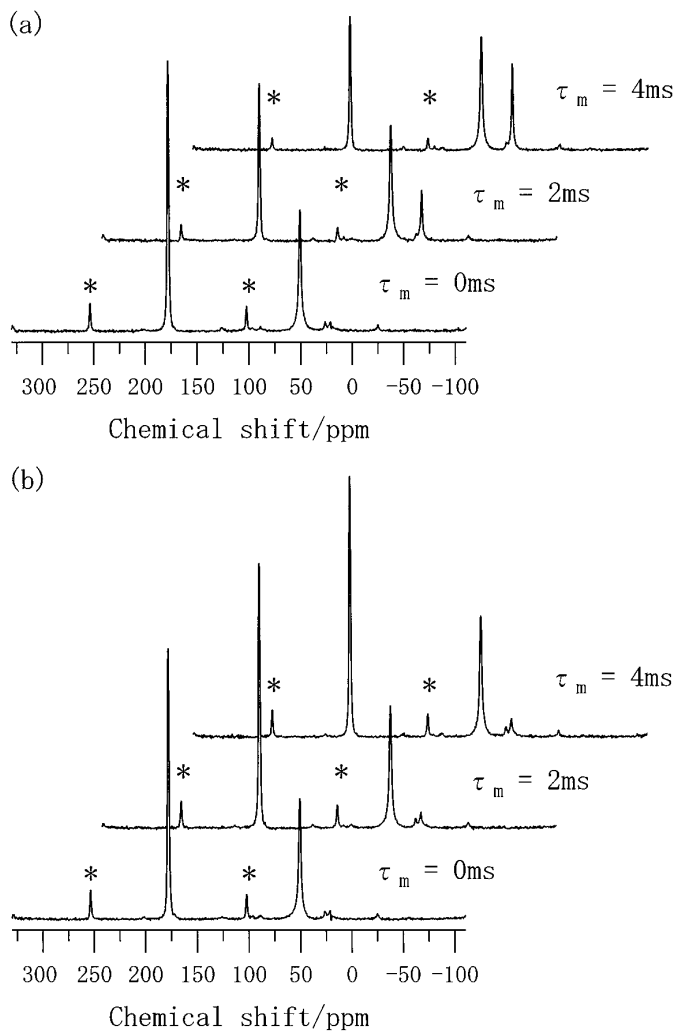
## RESULTS AND DISCUSSION

### The Flip-Flop Transfer under the Condition $\Delta$

The chemical shift difference between the carboxyl carbon ( $I$  spin) and the methyl carbon ( $S$  spin) is 11,905 Hz; in all

experiments described in this section, an R2TR condition  $\Delta_{\text{eff}} = 2\omega_R$  for this pair of spins was satisfied during the mixing time by irradiating the RF field with an intensity of 4000 Hz at a frequency downfield by 9000 Hz from the resonance frequency of the carboxyl carbon, and adjusting  $\omega_R$  to be 5718 Hz. Then, the resonance offsets of the carboxyl, the methine, and the methyl carbons are  $-9000$ ,  $-18,565$ , and  $-20,905$  Hz, respectively, being larger than the intensity of the RF field. Thus, the effective field of each carbon lies close to the  $z$  axis. Further, none of the R2TR conditions are satisfied for the pairs including the methine carbon.

Figure 3a shows the spectra obtained by using sequence

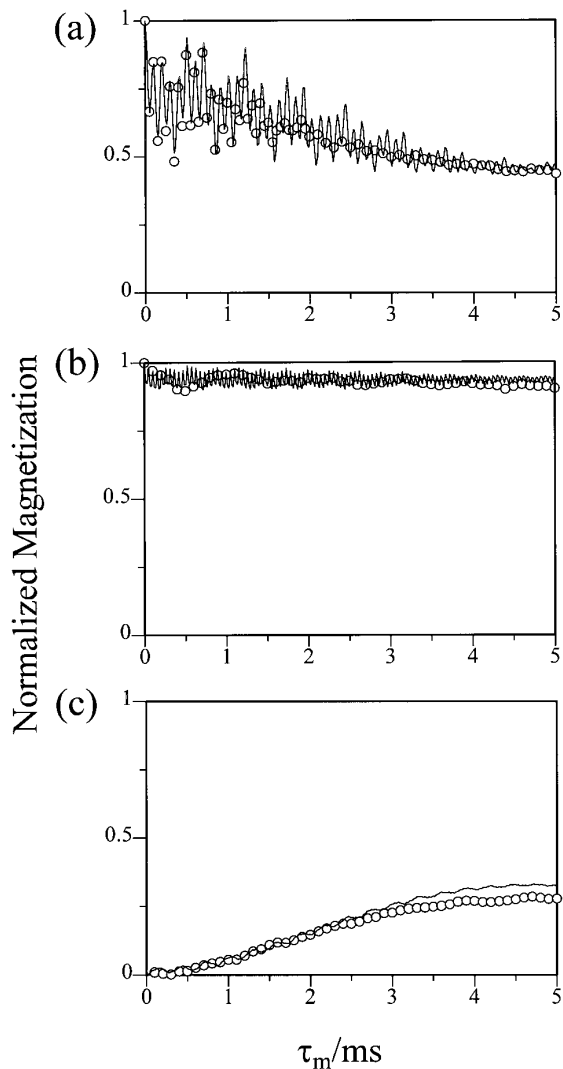


**FIG. 3.**  $^{13}\text{C}$  CPMAS NMR spectra of triply  $^{13}\text{C}$ -enriched L-alanine as observed using sequence (a) in Fig. 1 under magic angle spinning with a speed of  $\omega_R = 5718$  Hz. A selective  $90^\circ$  pulse is applied to the methyl carbon for saturating the signal. (a)  $H_1 = 4000$  Hz is applied at 296.7 ppm for a mixing time  $\tau_m$  to satisfy a R2TR condition  $\Delta_{\text{eff}} = 2\omega_R$  for the carboxyl and the methyl carbons. (b) No RF field was applied ( $H_1 = 0$  Hz). The peaks marked by asterisks denote spinning sidebands.

(a) in Fig. 1 with  $\tau_m = 0, 2,$  and 4 ms. A selective  $90^\circ$  pulse is applied to the methyl carbon for saturating the signal. When  $\tau_m = 0$  ms, the methyl peak at 20 ppm is negligibly small, and the intensities of the other two peaks (the carboxyl peak at 178 ppm and the methine peak at 51 ppm) are nearly the same as those taken by using the conventional CP/MAS method, showing the initial state of  $M_{IZ} = M_{OI}$  for the carboxyl carbon,  $M_{SZ} = 0$  for the methyl carbon, and  $M_{LZ} = M_{OL}$  for the methine carbon. With increasing mixing time, the carboxyl peak intensity decreases, and simultaneously the methyl peak intensity increases, while no apparent change occurs for the methine peak. For comparison, in Fig. 3b we show the spectra obtained without RF irradiation during the mixing time under the same MAS frequency. Note that no apparent change in the three peak intensities was observed for the change in the mixing time, showing that the observed intensity change in Fig. 3a was brought about by the RF irradiation satisfying the condition  $\Delta$  for the carboxyl and the methyl carbons.

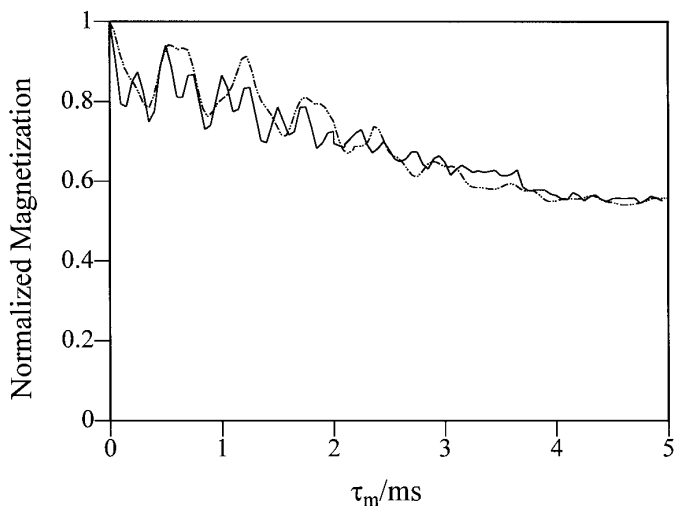
Figure 4 shows the mixing-time dependence of the individual  $^{13}\text{C}$  magnetizations after saturation of the methyl carbon signal, which is observed using pulse sequence (a), together with the simulated results. The observed intensities of the carboxyl and methine carbons at  $\tau_m = 0$  were normalized to be 1.0. The methyl intensities at various  $\tau_m$  were normalized to the intensity taken by a CP control experiment. Except for the initial rapid oscillation of the carboxyl  $^{13}\text{C}$  magnetization and slight decay due to relaxation, the sum of the  $^{13}\text{C}$  magnetizations of the carboxyl and the methyl carbons,  $M_{OI} + M_{OS}$ , is shown to be conserved, thus giving evidence that the polarization transfer is caused by the flip-flop motion as expected. These results also show that a mixing time of 5 ms is enough for the polarization transfer between two carbons separated by 2.5 Å. The oscillation observed for the carboxyl magnetization is due to the precession of  $M_{OI}$  around the effective field. The methine  $^{13}\text{C}$  magnetization shows only a small oscillation, because the effective field makes only  $12^\circ$  with the  $z$  axis. The oscillations of the magnetizations around their effective fields decay due to the transverse relaxation. The reason that the observed decay is faster than the calculated one is presumably due to the precession-frequency distribution caused by the RF field inhomogeneity. This damping reduces the magnetization useful for the polarization transfer by a factor of  $\cos \beta_I$  (in the present case,  $\cos \beta_I = 0.914$ ). For the  $S$  magnetization (the methyl carbon), no appreciable initial oscillatory behavior was observed, because it is created along the effective field  $\omega_{eS}$  by the polarization transfer.

Figure 5 shows the mixing-time dependence of the carboxyl  $^{13}\text{C}$  magnetization observed using sequence (b). As expected, the oscillation of the magnetization around the effective field disappears and the signal amplitude is somewhat enhanced owing to the presence of the trim pulse; however, it still shows an oscillation. The Fourier transforms



**FIG. 4.** Experimental (O) and simulated (—) mixing-time dependence of the normalized magnetizations of (a) the carboxyl, (b) the methine, and (c) the methyl carbons after saturation of the methyl carbon signal under the condition  $\Delta_{\text{eff}} = 2\omega_R$  satisfied between the carboxyl and the methyl carbons. The experiments were performed using pulse sequence (a) in Fig. 1 with the same parameters as described in the legend to Fig. 3.

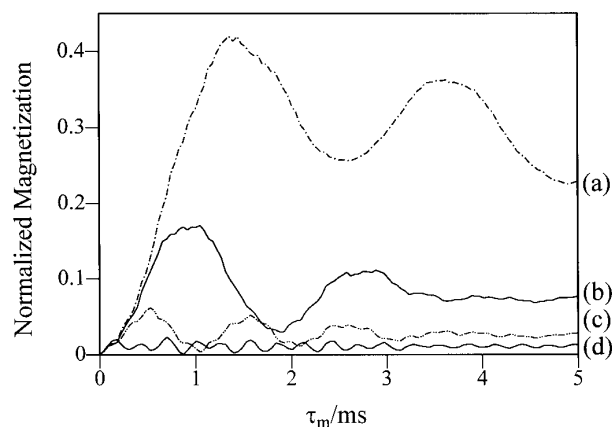
(not shown here) of these oscillations involve broad peaks at 4060 and 1730 Hz. These frequencies are given approximately by  $|\omega_{eI} - n\omega_R|$  ( $\sim 4131$  Hz for  $n = 1$  and 1587 Hz for  $n = 2$ ). The numerical simulation shows that these extra frequency components of  $\omega_{eI} - n\omega_R$  appear when we include the anisotropic chemical shift interaction. These can be understood as the spinning sidebands in the tilted rotating frame. For the simulated one, the main frequency component is 1630 Hz, while for the experimental one, another frequency component of 4060 Hz is still appreciable. The experimental and the simulated behavior of the carboxyl magnetization shown in Fig. 5 do not agree with each other in details for this discrepancy. The numerical simulation



**FIG. 5.** Experimental (—) and simulated (---) mixing-time dependence of the normalized magnetization of the carboxyl carbon after saturation of the methyl carbon signal under the condition  $\Delta_{\text{eff}} = 2\omega_R$  satisfied between the carboxyl and the methyl carbons. The experiments were performed using pulse sequence (b) in Fig. 1.

showed that this discrepancy can be attributed to a slight deviation from the exact  $\beta_I$  angle, and the inhomogeneity of the RF field.

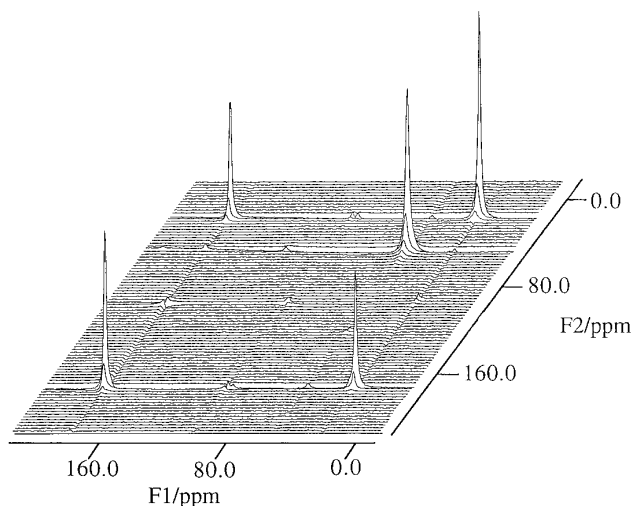
The observed methine  $^{13}\text{C}$  magnetization is virtually independent of the mixing time as seen in Figs. 3a and 4b. This result confirms that the dipolar interaction between the carboxyl and the methyl carbons could selectively be recoupled in spite of the fact that the recoupled dipolar coupling is below one-fourth the other couplings; the stronger couplings remain completely decoupled by MAS. This apparent selectivity may have been enhanced by the particular initial state of the magnetizations, because, at the beginning of the mixing time, there is no polarization gradient between the carboxyl and the methine carbons. To examine selectivity more quantitatively, we simulated the magnetization transfer with the initial state of  $M_{Iz} = M_{0I}$ ,  $M_{Sz} = 0$ , and  $M_{Lz} = 0$ . Further, to examine how large the chemical shift difference between  $S$  and  $L$  should be to achieve high selectivity, we assumed several isotropic chemical shift values for the methine carbon, keeping its chemical shift anisotropy constant. Figure 6 shows the calculated mixing-time dependence of the methine  $^{13}\text{C}$  magnetization for four chemical shift differences between the methyl and the methine carbons: 0, 500, 1000, and 2340 Hz. From Fig. 6, we can conclude that the chemical shift difference should be larger than 1000 Hz for selective transfer. In general, however, the selectivity of the R2TR method may be slightly poor as compared to the  $\text{R}^2$  method, because the difference of the effective field intensities is always smaller than the difference of the isotropic chemical shift values. When more than two lines have nearly equal chemical shift differences, however, the conventional  $\text{R}^2$



**FIG. 6.** Simulated mixing-time dependence of the methine carbon magnetization under the same conditions as described in the legend to Fig. 3, excluding the initial  $S$  magnetization and the isotropic chemical shift of the methine carbon:  $M_{Sz} = 0$ , and the assumed chemical shift relative to the methyl carbon is: (a) 0 Hz, (b) 500 Hz, (c) 1000 Hz, and (d) 2340 Hz.

technique cannot selectively recover a particular dipolar coupling, while the R2TR method can. Also, when the chemical shift difference between the spins to be recoupled is comparable to or smaller than the maximum  $^{13}\text{C}-^{13}\text{C}$  dipolar coupling constant, selectivity of the  $\text{R}^2$  method is poor, while it causes no problems for the R2TR method.

Figure 7 shows a 2D selective homonuclear shift-correlation spectrum taken by using sequence (I) in Fig. 2. The condition  $\Delta_{\text{eff}} = 2\omega_R$  was realized by the RF irradiation described above with a mixing time of 4 ms. The cross peaks are observed between the carboxyl and methyl carbons. The signs of the cross peaks are positive, showing that the ex-



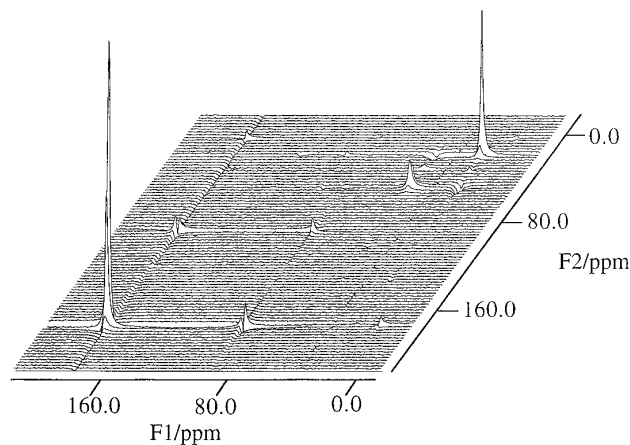
**FIG. 7.** Stack plot of the 2D selective homonuclear shift-correlation spectrum observed using pulse sequence (I) in Fig. 2 under a R2TR condition  $\Delta_{\text{eff}} = 2\omega_R$  satisfied between the carboxyl and methyl carbons with the parameters described in the legend to Fig. 3. The mixing time is 4 ms.



change takes place by the flip-flop process. No apparent cross peaks are observed for the pairs including the methine carbon, clearly showing that the dipolar interaction between the carboxyl and methyl carbons can selectively be recoupled.

### The Flop-Flop Transfer under the Condition $\Sigma$

The chemical shift difference between the methine carbon ( $I$  spin) and the methyl carbon ( $S$  spin) is ca. 2340 Hz; in all the experiments in this section we adopted an R2TR condition  $\Sigma_{\text{eff}} = \omega_R$  for this pair of spins, which was realized

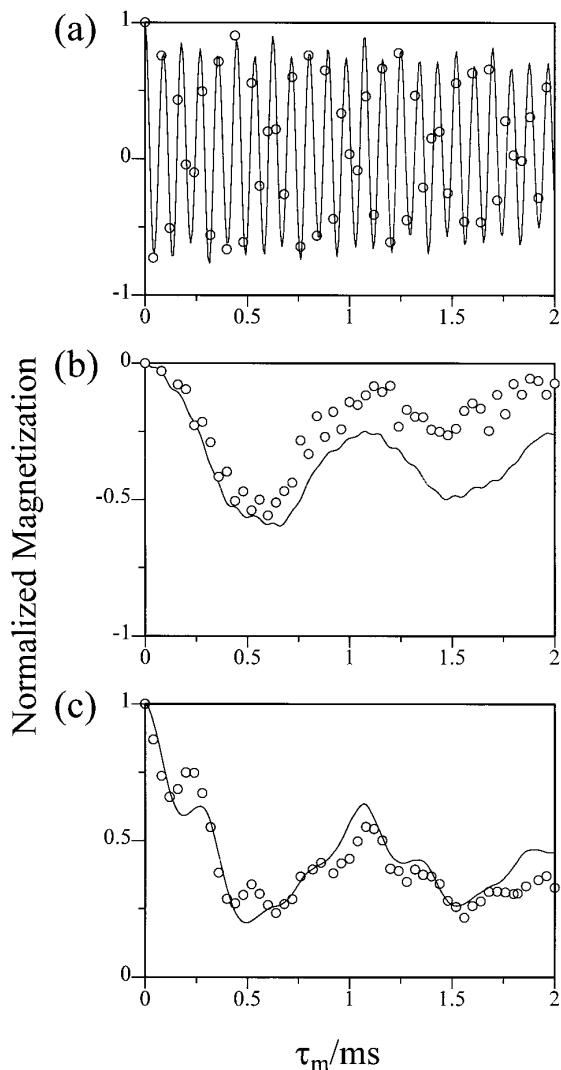


**FIG. 9.** Stack plot of the 2D selective homonuclear shift-correlation spectrum observed using pulse sequence (II) in Fig. 2 under a R2TR condition  $\Sigma_{\text{eff}} = \omega_R$  satisfied between the methine and methyl carbons with the parameters described in the legend to Fig. 8. The mixing time is 1 ms.

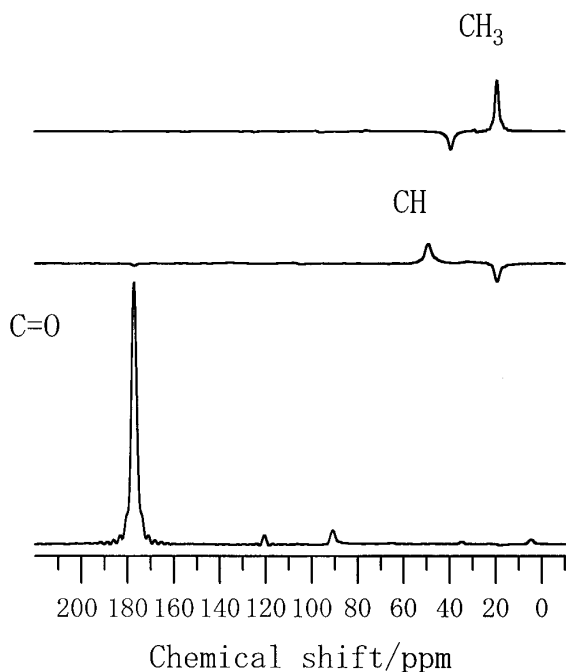
by irradiating the RF field with an intensity of 3032 Hz at the middle of the resonance frequencies of the methine and the methyl carbons, and adjusting  $\omega_R$  to be 6500 Hz. None of the R2TR conditions are satisfied for the pairs including the carboxyl carbon. The resonance offsets of the carboxyl, the methine, and the methyl carbons are 10,735, 1170, and  $-1170$  Hz, respectively. The offsets of the methyl and the methine carbons are smaller than the intensity of the RF field. Thus the effective fields of the methine and the methyl carbons are oriented close to the  $x$  axis, and we observe transverse magnetizations along the  $x$  axis. A  $90_x^\circ$  pulse is applied after the mixing time to eliminate the  $y$  components.

Figure 8 shows the mixing-time dependence of the magnetizations observed by using sequence (c) in Fig. 1 with the simulations. The initial state of the magnetizations was set as  $M_{IX} = M_{OI}$  for the methyl carbon,  $M_{SX} = 0$  for the methine carbon, and  $M_{LX} = M_{OL}$  for the carboxyl carbon. The observed intensities of the carboxyl and methyl carbons at  $\tau_m = 0$  were normalized to be 1.0. The methine intensities at various  $\tau_m$  were normalized to the intensity taken by a CP control experiment. Figure 8 indicates that the difference between the magnetizations of the methine and the methyl carbons,  $M_{OI} - M_{OS}$ , is conserved, thus providing evidence that the coherence transfer is caused by the flop-flop motion as expected. It also shows that a mixing time of 0.5 ms is adequate for the polarization transfer between two carbons separated by 1.5 Å. Because the carboxyl carbon is irrelevant to the present R2TR condition, only the precession around the effective field was observed.

Figure 9 shows a 2D selective homonuclear shift-correlation spectrum observed by using the pulse sequence (II) in Fig. 2 with a mixing time of 1 ms. The  $\omega_1$  spectra sliced at the methyl, the methine, and the carboxyl  $^{13}\text{C}$  resonances in the  $\omega_2$  dimension are also shown in Fig. 10. The cross peaks



**FIG. 8.** Experimental ( $\circ$ ) and simulated (—) mixing-time dependence of the normalized magnetizations of (a) the carboxyl, (b) the methine, and (c) the methyl carbons after saturation of the methine signal under a R2TR condition  $\Sigma_{\text{eff}} = \omega_R$  satisfied between the methine and the methyl carbons by applying the RF field with an intensity of 3032 Hz at the middle of them under magic angle spinning with a speed of 6500 Hz. The experiments were performed using pulse sequence (c) in Fig. 1.



**FIG. 10.** The cross sections of the 2D spectrum in Fig. 9 at the resonance frequencies of the methyl (top), the methine (middle), and the carboxyl (bottom) carbons in the  $\omega_2$  dimension.

appear between the methine and the methyl  $^{13}\text{C}$  signals with negative amplitudes. The negative amplitude is a feature of 2D cross peaks caused by the flop-flop exchange process. The same amplitudes decrease or increase at the same time in the flop-flop process. No apparent cross peaks were observed for the pairs including the carboxyl carbon. This example shows that even for a small chemical shift difference the R2TR method may provide satisfactory selectivity, removing  $^{13}\text{C}$ - $^{13}\text{C}$  dipolar broadening as well as spinning sidebands by fast MAS.

### CONCLUSION

We have shown that the R2TR method can be applied to polarization transfer between a particular pair of spins in a multiply labeled spin system. Since the selection of a pair of spins is made by RF irradiation, quick switching of the pair recoupled is possible. This feature makes the R2TR approach superior to the conventional  $\text{R}^2$  method in 2D selective homonuclear correlation NMR, because in the latter selection must be made by a technically demanding and time-consuming method of spinning-speed switching (16). In fact, the two recoupling conditions described in the text (Figs. 7 and 9) can be realized under a common spinning speed of, e.g., 9000 Hz, with the same resonance offset and RF intensities of 12,350 Hz for the former and 4345 Hz for the latter, so that the pair recoupled can be quickly switched by changing both the RF intensity and the offset. Another

advantage of the R2TR method over the  $\text{R}^2$  method is that we can find a proper recoupling condition regardless of the magnitude of a chemical shift difference for a spinning speed fast enough to eliminate spinning sidebands as well as  $^{13}\text{C}$ - $^{13}\text{C}$  dipolar couplings; in the flip-flop condition, a large chemical shift difference can effectively be scaled down to equal  $n\omega_R$ , while a small one can effectively be expanded by applying the flop-flop condition. For fast polarization transfer, the off-resonance frequencies should be much higher and lower than the rf field intensity in the flip-flop and flop-flop conditions, respectively. These settings also reduce the necessity of trim pulses for maximizing the signals. The selectivity achieved by the R2TR method, however, may be insufficient to select a pair of spins, one of whose chemical shifts is close to that of any other spin. In such cases, the use of a static magnetic field as high as possible is desirable.

### ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Science and Culture of Japan.

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