

# Efficient Deuterium–Carbon REDOR NMR Spectroscopy

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**Phase modulated pulses for deuterium recoupling in <sup>2</sup>H–<sup>13</sup>C REDOR NMR spectroscopy have been introduced to improve dephasing of the detected <sup>13</sup>C nuclei. The deuterium inversion properties of phase modulated recoupling pulses have been studied experimentally on L-alanine-2-*d*<sub>1</sub> and theoretically using average Hamiltonian theory and exact simulations of the equation of motion of the density matrix. The best <sup>13</sup>C dephasing was observed when XYXYX (PM5) deuterium recoupling pulses were applied. A comparison to the 90°–180°–90° (CPL) composite pulse scheme revealed an improvement of recoupling on the order of 2.5. Simple CW recoupling pulses of the same length of PM5 and CPL pulses showed the weakest <sup>13</sup>C dephasing. Simulations have shown that the <sup>2</sup>H recoupling efficiency of PM5 REDOR experiments approach the very efficient REAPDOR results. However, in our case a REAPDOR study of L-alanine-2-*d*<sub>1</sub> resulted in a significant decrease of the <sup>13</sup>C signal intensity due to pulse imperfections of <sup>13</sup>C  $\pi$ -pulses. The new PM5-REDOR technique has been employed to study the torsion angle between C1/2 and C5 in ethylmalonic acid-4-*d*<sub>2</sub>.**

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## INTRODUCTION

Determining the positions of hydrogens in molecules is important for understanding structural and functional properties of organic solids. Higher order biomolecular structures rely on the existence of hydrogen bonds to support supramolecular assemblages (1) and the folding of proteins and polypeptides (2). Although determining the exact localization of hydrogen atoms can provide essential information for understanding structural properties of macromolecules, it is still a severe analytical challenge. The application of X-ray diffraction techniques is in many cases not feasible because of insufficient X-ray scattering from the small hydrogen atoms. Scattering techniques are in particular restricted when studying amorphous or disordered powder samples. A possible alternative technique is solid-state NMR (3, 4).

Solid-state NMR of deuterium-labeled samples enables structural investigation of selected hydrogen positions, regardless of the morphology of the studied system. Deuterium NMR signals are dominated by quadrupolar couplings and are very

sensitive to local dynamic properties, e.g., torsional oscillations (5), exchange processes (6–9), and paramagnetic interactions with ions in metal coordination complexes (10–12). These effects can assist in the characterization of deuterons in organic solids in terms of bond distances and angles. Further, the dipolar coupling strength between deuterium atoms and their adjacent X nuclei (e.g., the spin-half  $X = {}^{13}\text{C}$ ) can provide direct geometrical information about intra- or intermolecular distances between the coupled nuclei (13–18).

Heteronuclear dipolar <sup>2</sup>H–X NMR spectroscopy in combination with magic angle spinning (MAS) enables the detection of a set of dipolar couplings between a deuterium and its neighboring X nuclei, resulting in a collection of internuclear distances. Such distances when spanning over several bonds can be evaluated in terms of dihedral angles, constraining molecular conformations. Some solid-state NMR techniques that reintroduce <sup>2</sup>H–X dipolar couplings to X-spin MAS spectra are rotational echo double resonance (REDOR) (19–22), transfer of populations in double resonance (TRAPDOR) (23–25), rotational echo adiabatic passage double resonance (REAPDOR) (26–28), dipolar exchange assisted recoupling (29), and simultaneous frequency and amplitude modulation of the <sup>2</sup>H radiofrequency (30, 31). Most applications of <sup>2</sup>H recoupling have been performed using REDOR (13–18, 32–34).

In REDOR spectroscopy dipolar recoupling in an AX spin pair is achieved when rotor synchronized  $\pi$ -pulses are applied both to the observed RF channel of X and to the unobserved A channel. To elucidate molecular conformations via REDOR relatively large distances must be determined. Detection of the corresponding weak heteronuclear dipolar couplings requires that the observed X signal must be detectable over a long REDOR evolution period. For example, dipolar couplings weaker than 100 Hz require that the signal is measurable over an evolution time that exceeds 25 ms. During this evolution time the signal can decrease due to relaxation processes and pulse imperfections. Thus the number of pulses on the observed channel must be minimized and a strong signal-to-noise ratio is necessary in particular for experiments on low abundant spins. Furthermore the dipolar recoupling and REDOR decay rates must be optimized. A strong REDOR decay rate shortens the necessary REDOR evolution time and makes the detection

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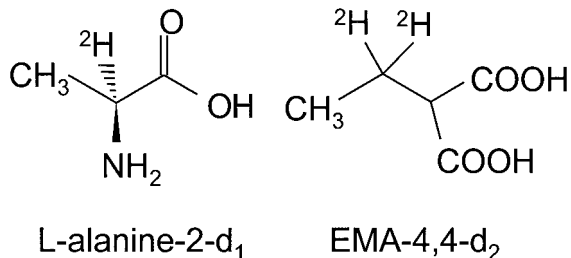


FIG. 1. Deuterated sites of alanine and ethylmalonic acid (EMA).

of weak dipolar interactions more feasible. Thus, improvements of the recoupling pulses have to be employed when the standard REDOR  $\pi$ -pulses are not sufficient.

If one of the AX spins is deuterium its signal detection is complicated by the quadrupole interaction. The  $^2\text{H}$ -MAS spectra can display 20–40 rotational sidebands at a moderate spinning frequency of  $\sim 5$  kHz. Thus, when deuterium  $\pi$ -pulses are applied during  $^2\text{H}$ -X REDOR experiments the intensity of these spectra diminishes and phase distortions occur. Moreover the presence of the quadrupole interaction makes it complicated to simulate  $^2\text{H}$ -MAS spectra that are excited by proton–deuterium CPMAS (35).

With these considerations in mind we prefer to detect the X-spin signal, while applying most recoupling pulses to the deuterium channel. Thus, improved inversion efficiency of the pulses on the deuterium spins is needed since the broad quadrupolar spectrum limits the frequency range of inversion of standard  $\pi$ -pulses of moderate RF power (30–40 kHz for commercial triple tune probes).

Improvement of the inversion characteristics of deuterium recoupling pulses was achieved recently by the introduction of composite pulse schemes (16). It was demonstrated that by replacing the  $\pi$ -pulses in  $^2\text{H}$ -X REDOR experiments with  $(\pi/2)-(\pi)-(\pi/2)$  composite pulses the REDOR decay of the X-signals was significantly increased. This enables detection of weaker dipolar couplings and longer distances (e.g., spanning over three single bonds for the detection of a dihedral angle).

Here we present  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments that employ phase modulated recoupling pulses on the deuterium spins. The advantage of phase modulated REDOR is compared with REAPDOR which relies on the adiabatic inversion of deuterium spins (26). In the following section we first derive a zero-order average REDOR Hamiltonian and define a set of parameters that measures the efficiency of a REDOR experiment. Then in conjunction with a numerical study the  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiment is optimized by adjusting the pulse length and modulation period of the deuterium recoupling pulses. To demonstrate the advantage of the modulation approach REDOR results of L-alanine-2- $d_1$  and ethylmalonic acid-4- $d_2$  (EMA) are shown (Fig. 1).

## THEORETICAL BACKGROUND

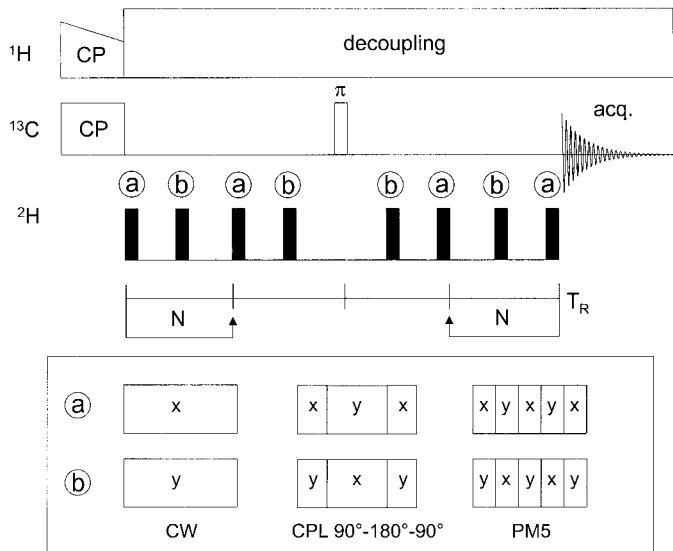
Many triple-frequency MAS probes of commercial solid-state NMR spectrometers do not provide sufficient RF power to invert deuterium powder spectra. Although most of the  $\pi$ -pulses in a  $^{13}\text{C}$  detected  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiment could have been applied on the carbon spins, we prefer not to do so to prevent dephasing of the signal intensities due to pulse imperfections and cross-polarization effects. Thus we have tried to improve the REDOR decay efficiency by modifying the deuterium pulses. Two different concepts of RF pulse modulation have been investigated. In the first approach the standard deuterium  $\pi$ -pulses were replaced by composite pulses. It was shown (16) that the  $90_x-90_y-90_x$  and  $90_x-180_y-90_x$  pulse schemes improve the  $^{13}\text{C}$ -REDOR decay considerably. Evaluation of an average Hamiltonian for these experiments led to the conclusion that these composite pulses reduce the size of the double-quantum matrix elements and cause an increase of the REDOR decay rate of powder samples.

The second approach, presented in this publication, optimizes the REDOR decay by fast amplitude and phase modulation of the deuterium pulses. It has been shown that double sideband modulation (36–38) of an on-resonance pulse can excite and invert spin-1 quadrupolar spins in single crystals and powder samples. Such pulses excite the two quadrupolar satellite lines of single crystallites simultaneously. Selective inversion can be achieved by matching the frequency sidebands of a pulse to the transition frequencies of the deuterons in nonspinning powders. In rotating solids the quadrupole interactions become time dependent and the effective quadrupolar frequencies of all crystallites change during the pulse. This prevents a constant match condition of the pulse modulation frequency to particular quadrupolar interactions and complicates the spin evolution during the RF irradiation. In some cases the evolution of spin-1 nuclei can be described by an adiabatic process (26, 27, 39). However, that is certainly not the case for those deuterons in a powder that have effective quadrupole frequency of the same order of magnitude as the RF intensity. For spins with large quadrupole frequencies adiabatic population and coherence transfer processes during MAS experiments have led to a variety of techniques, such as the TRAPDOR, REAPDOR, RIAC-, and FAM-MQMAS experiments (23, 26, 40, 41).

### The Average REDOR Hamiltonian

To investigate the effect of the modulated RF pulses on the deuterium spins we calculate the average dipolar Hamiltonian of one rotor period in a  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiment, as shown in Fig. 2. During this period a deuterium spin with  $S = 1$  and a carbon spin  $I = \frac{1}{2}$  experience a Hamiltonian of the form

$$H(t) = \omega_D(t)2I_zS_z + \frac{1}{3}\omega_Q(t)(3S_z^2 - 2) + H_{\text{RF}}(t), \quad [1]$$



**FIG. 2.** Pulse sequence used for  $^{13}\text{C}$ - $^2\text{H}$  REDOR experiments. All three pulse schemes were applied in a XY-4 phase alternation with an overall pulse length of 28  $\mu\text{s}$ .

where  $\omega_D(t)$  and  $\omega_Q(t)$  are the time-dependent frequency coefficients of the heteronuclear dipolar and quadrupolar tensors, respectively. These coefficients are a function of the magnitudes and orientations of the tensor components of the individual crystallites in the sample. Explicit expressions for the time-dependent coefficients  $\omega_Q(t)$  as well as for  $\omega_D(t)$  can be found elsewhere (42). The magnitude of  $\omega_Q(t)$  is given by the quadrupolar coupling constant. The  $\omega_D(t)$  coefficients are determined by the dipolar interaction that is inversely proportional to the third power of the interatomic distance ( $r_{IS}$ ). The orientational dependence of  $\omega_Q(t)$  and  $\omega_D(t)$  is a function of the Euler angles ( $\alpha, \beta, \gamma$ ) of the quadrupole tensor and the polar angles ( $\theta_D, \varphi_D$ ) of the dipolar vector, defined with respect to a frame fixed to the sample rotor (3, 6). The  $H_{\text{RF}}(t)$  term in Eq. [1] represents the two modulated pulses (Fig. 2) applied to the  $S$ -spin during the rotor period.

The response of the  $I$ -spin to this Hamiltonian can be evaluated by transforming it to an interaction representation, defined by the  $S$ -spin part of the Hamiltonian,

$$\tilde{H}_D(t) = 2\omega_D(t)I_z U_S^{-1}(t)S_z U_S(t), \quad [2]$$

with

$$U_S(t) = T \exp \left\{ -i \int_0^t d\tau (1/3 \omega_Q(\tau)(3S_z^2 - 2) + H_{\text{RF}}(\tau)) \right\}. \quad [3]$$

This transformation operates only on the  $S$ -spin and does not

affect the  $I$ -spin signal. Thus the  $I$ -signal at times  $NT_R$ , with  $T_R = 2\pi/\omega_R$ , where  $\omega_R$  is the spinning frequency and  $N$  is an integer, is proportional to

$$S^{\text{REDOR}}(NT_R) = 2/3 \text{Tr} \{ \tilde{U}_D^N(T_R) I_x \tilde{U}_D^{-N}(T_R) I^- \} \quad [4]$$

with

$$\tilde{U}_D(T_R) = \exp \left\{ -i \int_0^{T_R} dt \tilde{H}_D(t) \right\}. \quad [5]$$

The transformation of  $S_z$  in Eq. [2] can generate all possible time-dependent bilinear terms  $S_p S_q$ , and the interaction frame Hamiltonian can therefore contain terms that are proportional to trilinear operators  $I_z S_p S_q$ , with  $p, q = x, y, z$ . To estimate the influence of the quadrupole interaction on the effect of the RF pulses we can replace the time-dependent interaction Hamiltonian in Eq. [2] with its zero-order average Hamiltonian

$$\bar{H}_D = 1/T_R \int_0^{T_R} dt \tilde{H}_D(t). \quad [6]$$

This time-independent Hamiltonian can be represented as a  $6 \times 6$  matrix in the manifold of spin states  $\{|m_I, m_S\rangle\}$ , with  $m_S = 1, 0, -1$  and  $m_I = \alpha, \beta$ , with a general form

$$\bar{H}_D = \begin{pmatrix} 1/2\bar{h} & 0 \\ 0 & -1/2\bar{h} \end{pmatrix}, \quad [7]$$

where  $\bar{h}$  is a  $3 \times 3$  matrix, according to  $\bar{H}_D = \bar{h} I_z$ . Diagonalization of  $\bar{h}$  results in a diagonal matrix  $\bar{\lambda} = \bar{d}^{-1} \bar{h} \bar{d}$  with three elements  $\lambda_i$ ,  $i = 1, 2, 3$ , and

$$\bar{D}^{-1} \bar{H}_D \bar{D} = \bar{\Lambda} = \begin{pmatrix} \frac{1}{2} \bar{\lambda} & 0 \\ 0 & -\frac{1}{2} \bar{\lambda} \end{pmatrix}. \quad [8]$$

The  $I$ -signal in Eq. [4] can now be approximated, relying solely on the zero-order average Hamiltonian, by

$$\begin{aligned} S^{\text{REDOR}}(NT_R) &= 2/3 \text{Tr} \{ \exp\{-i\bar{\Lambda}NT_R\} I_x \exp\{i\bar{\Lambda}NT_R\} I^- \} \\ &= 1/3 (\cos(\lambda_1 NT_R) + \cos(\lambda_2 NT_R) \\ &\quad + \cos(\lambda_3 NT_R)), \end{aligned} \quad [9]$$

where we replaced Eq. [5] with  $\bar{D} \exp\{-i\bar{\Lambda}T_R\} \bar{D}^{-1}$  and used the fact that  $\bar{D}$  commutes with the components of  $\bar{I}$ . The diagonal matrix elements of  $\bar{\lambda}$  are  $\lambda_1, \lambda_2, \lambda_3$ . Thus the REDOR decay signal of a single crystallite is determined by the eigen-

values of the average Hamiltonian  $\bar{H}_D$ . For ideal  $\pi$ -pulses  $\lambda_2 = 0$  and  $\lambda_1 = -\lambda_3$  for all crystallites, resulting in a powder  $I$ -signal that decays to  $\frac{1}{3}$ . For all other types of pulses this is not the case and an increase of one of the three  $\lambda$ -parameters, by modification of the RF pulse shapes, improves the  $I$ -spin REDOR decay. Evaluation of the  $\lambda_i$ 's for the different crystallites, with orientations defined by the Euler angles  $(\alpha, \beta, \gamma)$ , can give us an overall picture of the efficiency of the REDOR dephasing. One way of representing the efficiency of a REDOR pulse sequence is to calculate numerically, following Eqs. [2] to [8], the powder averaged values of the eigenvalues of  $\bar{\lambda}$ :

$$\bar{\lambda}_i(\beta) = \left| \frac{1}{4\pi^2} \sin \beta \int_0^{2\pi} \int_0^{2\pi} d\alpha d\gamma \lambda_i(\alpha, \beta, \gamma) \right|, \quad [10]$$

as a function of the angle  $\beta$ , with  $\lambda_1 < \lambda_2 < \lambda_3$  for each crystallite. Maximization of these parameters can provide a method for optimization of the REDOR pulses.

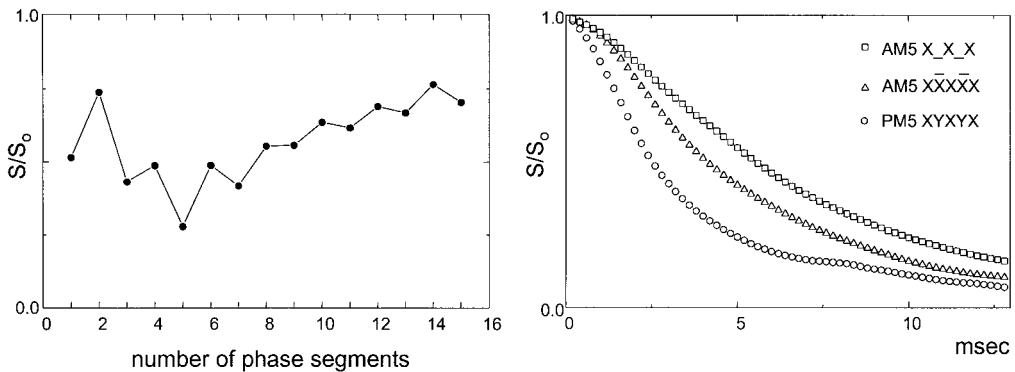
### Numerical Simulations

The  $\bar{\lambda}$ -parameters as well as the REDOR decay functions were simulated by using a computer program that takes into account all experimental parameters, such as the  $^2\text{H}$  and  $^{13}\text{C}$  RF field strengths, pulse lengths and phases, the deuterium quadrupole tensor parameters, the spinning speed, and the strength and orientation of the dipolar tensor. For the  $\bar{\lambda}$ -parameters in Eq. [10] the interaction Hamiltonians (Eq. [2]) and their zero-order averages (Eq. [6]) were calculated for each single crystallite of the powder by a stepwise integration of Eqs. [3] and [6] with constant time steps  $\Delta\tau = 0.25 \mu\text{s}$ . For the REDOR signals a similar stepwise integration was performed to calculate the spin evolution operators  $U_D(T_R)$ . Insertion in Eq. [4] resulted in the REDOR signals of each crystallite and powder decay curves were obtained by the addition of these signals from 300 (for fitting of data) or 1154 (for the representation of the final results) crystallites (43, 44). Off-resonance and chem-

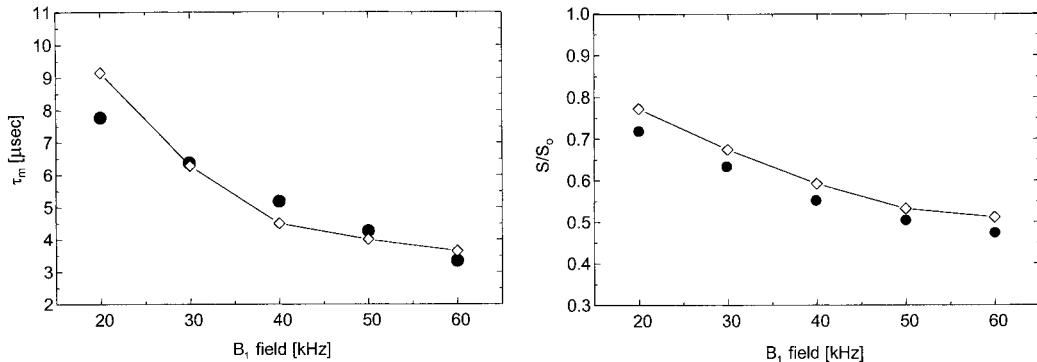
ical shift anisotropy terms in the Hamiltonian were neglected during the simulations. Fitting of the experimental data to a set of optimal parameters, such as the magnitude of the dipolar vector and its polar angles, was accomplished by requiring at least 70% of the data to be confined between two extreme simulated results for two sets of parameters. These parameters determine the error bars of fitting parameters.

### Phase Modulated RF Pulses

Amplitude modulation (AM) of the  $B_1$  field on the deuterons can be accomplished by periodically alternating the phase of an RF pulse of constant intensity. Such an AM pulse mimics a sinusoidal modulated RF field with a typical modulation frequency equal to  $\omega_m = \pi/\tau_m$ , with  $\tau_m$  the length of each pulse segment with constant amplitude. Fourier transformation of such an AM ( $X\bar{X}$ ) pulse has frequency components at  $n\omega_m$ , with  $n = \pm 1, \pm 2, \dots$ . Addition of a central frequency component at  $n = 0$  can be accomplished by adding a constant value to the time-dependent amplitude of the pulse. If this addition has the same phase ( $X$ ) as the rest of the pulse and its strength is equal to the amplitude of the  $X$ -pulse segments we obtain an AM pulse of the form  $X_X$ , consisting of short  $X$ -pulses with delays of zero intensity between them. However, if the addition points in the  $Y$ -direction of the rotating frame one gets a pulse that successively makes an angle of  $\pi/4$  and  $-\pi/4$  with the  $X$ -direction. A rotation of this pulse by  $\pi/4$  results in a phase modulated (PM) pulse ( $XY$ ), consisting of pulse segments pointing in the  $x$ - and  $y$ -direction. All three pulses  $X\bar{X}$ ,  $X_X$ , and  $XY$  with equal pulse segments  $\tau_m$  have Fourier components  $\omega_m$  that act on the deuterium spins. Experimental and numerical studies of the effect of these pulses, with intensities varying between 30 and 50 kHz, have shown that the  $XY$  pulses result in the best REDOR decay curves. For most of the crystallites the experimental RF field strength is not sufficiently weak for the spins to undergo population inversions during the level crossings when  $\omega_Q(t) \sim \omega_m$  and  $\omega_Q(t) \sim 0$ . Furthermore these studies indicate that the pulses containing



**FIG. 3.** Left: simulated dependence of the REDOR effect after 4.8 msec on the number of phase segments in a PMn-REDOR experiment. Calculations were performed with a 28- $\mu\text{s}$  overall pulse length, a  $B_1$  of 35 kHz and a spinning speed of 5 kHz. Since the strongest dipolar dephasing is found for five phase segments PM5 recoupling pulses were applied in subsequent experiments. Right: simulated dipolar decay curves corresponding to different modulated  $^2\text{H}$  pulses.



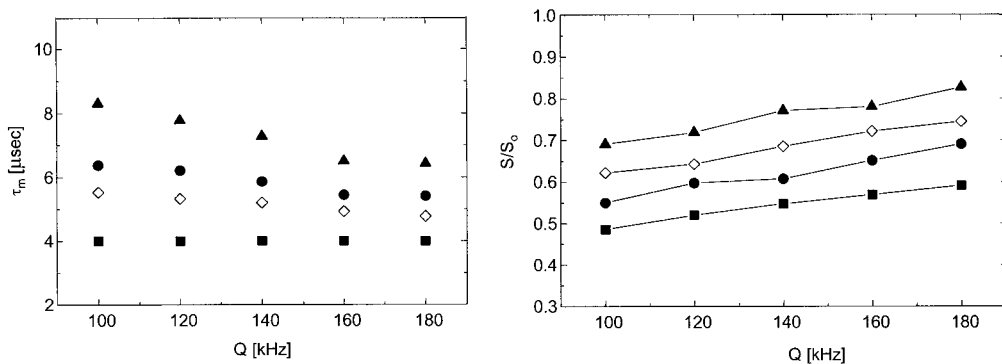
**FIG. 4.** Simulated dependence of the phase modulation period on the RF field strength available for the deuterium spins during PM5-REDOR experiments (left). The simulated points ( $\bullet$   $f_R = 5$  kHz;  $\diamond$   $f_R = 10$  kHz) refer to the setup of PM5 recoupling pulses, which provide the strongest REDOR effect. Right: magnitude of the REDOR effect ( $S/S_0$ ) for the same RF field dependence after 2-ms evolution.

both  $x$ - and  $y$ -components perform better than single phase pulses, just as in the case of the composite pulse (CPL) schemes (16). Thus in the following we restrict our discussion to PM pulses.

To accomplish sufficient REDOR dephasing by applying two deuterium pulses per rotor cycle the PM pulses must be significantly shorter than half the rotor period. To do so for RF amplitudes that are less than 50 kHz we have chosen five time segments per pulse (PM5). Pulses of the type PM $n$  with  $n$  values different from 5 were considered but did not give better results on our spectrometer. To demonstrate this some typical PM $n$  REDOR decay curves  $S(t)/S_0(t)$  were calculated for an RF intensity of 35 kHz and a spinning frequency equal to 5 kHz and are shown in Fig. 3. Here  $S(t)$  and  $S_0(t)$  are the  $S$ -spin signals as a function of the length of the REDOR sequence in Fig. 2 with and without deuterium pulses, respectively. These calculations for pulses with an overall length of 28  $\mu\text{s}$  reveal that odd numbers of phase segments induces a stronger REDOR decay than even numbers. In Fig. 3 a comparison between  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments with  $X\bar{X}$ ,  $X_X$ , and  $XY$  pulses is also shown. In all REDOR experiments the  $XY$ -4

phase cycling scheme is applied. Thus in the case of the PM5 pulses the first pulse in each rotor cycle has the form  $XYXYX$  and the second pulse  $YXYXY$ . The  $X\bar{X}$  and  $X_X$  pulses are therefore  $X\bar{X}X\bar{X}X$  and  $Y\bar{Y}Y\bar{Y}Y$ , and  $X_X X_X$  and  $Y_Y Y_Y$ , respectively.

The optimization of the PM5 pulses can be done experimentally or numerically by changing the length  $\tau_m$  of the pulse segments and the RF intensities  $\nu_1$ . For  $\nu_1$  values between 20 and 60 kHz the maximal REDOR decay functions  $S(t)/S_0(t)$  have been found by changing  $\tau_m$ . The results of a calculation for a  $\nu^Q = \omega^Q/2\pi$  value equal to 125 kHz and for spinning frequencies 5 and 10 kHz are shown in Fig. 4. The optimal PM5 pulse lengths vary from 45 to 16  $\mu\text{s}$  for increasing  $\nu_1$ . The REDOR curves decay to 0.75 and 0.5 at  $t = 2$  ms for  $\nu_1$  equal to 20 and 60 kHz. The dependence of the optimal  $\nu_1$  and  $\tau_m$  values on the quadrupolar frequency  $\nu^Q$  is shown in Fig. 5. Here it can be seen that for  $\nu_1 = 50$  kHz the best  $\tau_m$  is insensitive to changes in the quadrupolar frequency. For smaller values of the RF amplitude optimization of  $\tau_m$  can help to improve REDOR dephasing. It is therefore advisable to maximize the RF field intensity and to adjust the length of the



**FIG. 5.** Dependence of the PM5-modulation period on the strength of the  $^2\text{H}$  quadrupolar coupling for  $B_1 = 20$  kHz ( $\blacktriangle$ ), 35 kHz ( $\bullet$ ), and 50 kHz ( $\blacksquare$ ) with 5 kHz spinning speed and  $B_1 = 35$  kHz ( $\diamond$ ) with 10 kHz spinning speed. The right hand side of the figure shows the REDOR effects ( $\tau_{\text{REDOR}} = 2$  ms) for the same experimental parameter of RF intensity and spinning speed as a function of the quadrupolar coupling.

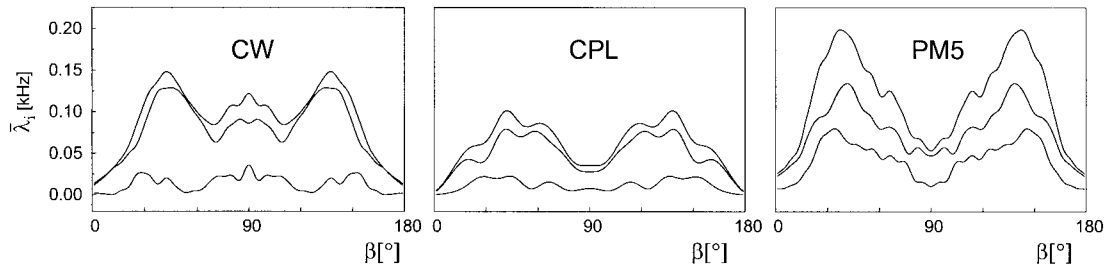


FIG. 6. The elements  $\bar{\lambda}_i(\beta)$  (Eq. [10]) for CW,  $90^\circ$ - $180^\circ$ - $90^\circ$  CPL and PM5 recoupling.

segments accordingly. Small changes in  $\tau_m$  do not change the REDOR curves much, thus it is not necessary to set this time with a precision of less than  $1 \mu\text{s}$ . The triple tuned probe of our spectrometer can sustain a maximum RF power level on the deuterium channel corresponding to a  $\nu_1$  value of about 40 kHz. Thus the experimental PM5 pulse segments were chosen between 5 and  $6 \mu\text{s}$ .

#### CW and CPL versus PM REDOR

To compare the REDOR efficiency of the new PM5 recoupling pulses with the  $90^\circ$ - $180^\circ$ - $90^\circ$  CPL and CW pulses, the  $\bar{\lambda}$  parameters in Eq. [10] were calculated for the three schemes shown in Fig. 2 with identical overall pulse lengths of  $28 \mu\text{s}$  and amplitudes of 35 kHz. The orientation of the dipolar vector was set collinear to the main axis of the quadrupole tensor. In Fig. 6 the three  $\bar{\lambda}_i$  parameters of the three REDOR experiments are shown as a function of the  $\beta$  Euler angle. In all cases all three parameters are different from zero, resulting in a total REDOR decay below  $\frac{1}{3}$  even for the finite intensity CW pulses. The figure reveals further that the parameters of the PM5 recoupling pulses are larger than those of the other pulses. Whereas in the CW and CPL case the three parameters are similar, the PM5 pulses show a significant increase of all  $\bar{\lambda}_i$ 's. In particular the increase of the smallest parameter indicates that in actual PM5-REDOR experiments the  $S(t)/S_0(t)$  will decay far beyond the  $\frac{1}{3}$  level. This is in agreement with experimental observations discussed in the next sections.

## EXPERIMENTAL

All experiments were performed on a Bruker DSX 300-MHz machine using a Bruker standard 7-mm triple-resonance HP probe with an insert for  $^{13}\text{C}/^2\text{H}$  observation and irradiation. Variable amplitude CPMAS was used to excite the carbon spins with a ramped proton-locking field, changing from 100 to 50% maximum intensity within 1.5 ms, and a rectangular constant field on the  $^{13}\text{C}$  spins. After the CP mixing time the protons were decoupled by a CW RF field. In the CW-, CPL-, and PM5-REDOR experiments one single  $\pi$ -pulse of  $10 \mu\text{s}$  was applied to the  $^{13}\text{C}$  spins in the middle of the pulse sequence as indicated in Fig. 2. For the reference REDOR signals  $S_0(t)$  no deuterium pulses were applied to the spin system. The

REDOR recoupling experiments were performed by applying two pulses per rotor cycle, either as rectangular CW pulses,  $90^\circ$ - $180^\circ$ - $90^\circ$  composite pulses, or PM5 pulses in a mirror symmetric XY alternation as shown in Fig. 2. In the REAPDOR experiment two  $\pi$ -pulses per rotor cycle were applied in the  $^{13}\text{C}$  channel with an XY-4 phase cycling scheme. These were followed by an additional  $\pi$ -pulse in the middle of the rotor cycle with a phase equal to the phase of the CP mixing pulse on the carbons. After these pulses the adiabatic inversion pulse on the deuterium channel with a length of half a rotor cycle was given to the sample around the rotational echo position, and another  $\pi$ -pulse followed by an XY-4 sequence was applied (27). For the reference REAPDOR signals the deuterium pulse was omitted from the pulse sequence.

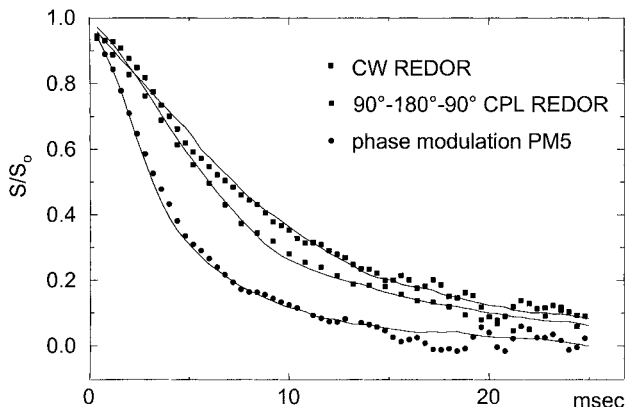
All MAS recoupling experiments have been accomplished with a rotation frequency of 5 kHz. During the experiments the deviations in the rotation frequency were below 3 Hz. The quadrupolar frequencies of ethylmalonic acid and L-alanine were determined both by static powder measurements, using a  $(\pi/2)_x - (\pi/2)_y$  solid echo sequence, and by detecting single FID deuterium MAS signals at a spinning frequency of 3 kHz. The  $^2\text{H}$  quadrupolar parameters were evaluated by fitting simulated spectra to the experimental ones.

L-Alanine-2- $d_1$  was purchased from Cambridge Isotope Laboratories and investigated without further treatment.

## RESULTS

### $^2\text{H}$ - $^{13}\text{C}$ REDOR of L-Alanine

To compare the effect of the phase modulated recoupling pulses (PM5) in  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments with CW and CPL ( $90^\circ$ - $180^\circ$ - $90^\circ$ ) pulses the  $S(t)/S_0(t)$   $^{13}\text{C}$  signals of natural abundant  $\text{C}_\beta$  in singly deuterated L-alanine (2- $d_1$ ) are shown in Fig. 7 for all three pulse schemes. The deuterium at the  $\text{C}_\alpha$  position displays a typical quadrupolar coupling strength of 125 kHz. The corresponding dipolar decay curves for the C1 carbon (not shown) gave similar results. Because we are mainly interested in larger  $^{13}\text{C}$ - $^2\text{H}$  distances, the REDOR signals of the directly bonded  $\text{C}_\alpha$  are not discussed here. These signals were completely dephased after 4 ms of REDOR evolution.



**FIG. 7.** Experimental and simulated (line graphs) dipolar decay curves of the different  $^{13}\text{C}$ - $^2\text{H}$  REDOR experiments for  $\text{C}_\beta$  of alanine. The pulse time for deuterium irradiation was identical in all three cases ( $28 \mu\text{s}$  per recoupling pulse).

To compare the effects of CW, CPL ( $90^\circ$ - $180^\circ$ - $90^\circ$ ), and PM5 recoupling pulses on REDOR intensities the overall pulse length of each pulse in the  $^2\text{H}$  channel was made equal for all three schemes. A recoupling pulse length of  $28 \mu\text{s}$  was chosen. The  $90^\circ$  pulse condition was adjusted to  $7 \mu\text{s}$ , ensuring that the overall length of CPL  $90^\circ$ - $180^\circ$ - $90^\circ$  pulses was  $28 \mu\text{s}$ . The intensity of all pulses was  $35.6 \text{ kHz}$ . For the PM5 recoupling pulses five segments of  $5.6 \mu\text{s}$  were combined. We found an improvement in the CW-REDOR decay when the deuterium pulses were longer than the length of a  $\pi$ -pulse. For that reason the pulses in the CW-REDOR experiment were also equal to  $28 \mu\text{s}$ , corresponding to a  $2\pi$ -pulse.

Figure 7 shows large differences in the efficiency of deuterium pulses among the three REDOR experiments. The weakest dipolar decay belongs to the REDOR experiment with simple rectangular CW pulses. The CPL recoupling of deuterium causes a stronger signal decay, which can be attributed to a combined effect of reduction of double quantum terms and the appearance of single quantum terms in the average toggling frame Hamiltonian, according to (16). However, the strongest dipolar recoupling is achieved using PM5 pulses.

The results in Fig. 7 were fitted to simulated REDOR decay curves taking into account the quadrupolar interaction and the dipolar coupling strength. A best fit for all dipolar dephasing curves was found for a dipolar coupling strength of  $(500 \pm 40) \text{ Hz}$ , corresponding to an  $^2\text{H}$ - $^{13}\text{C}_\beta$  distance of  $(2.10 \pm 0.05) \text{ \AA}$ . The  $^2\text{H}$ - $^{13}\text{C}_\beta$  distance obtained from the REDOR NMR is in good agreement with known crystal data (45). The angle  $\theta_D$  between the main principal axis of the quadrupolar tensor and the dipolar vector  $\vec{r}_{\text{C}_\beta\text{-}^2\text{H}}$  was not relevant within the error range of our simulations.

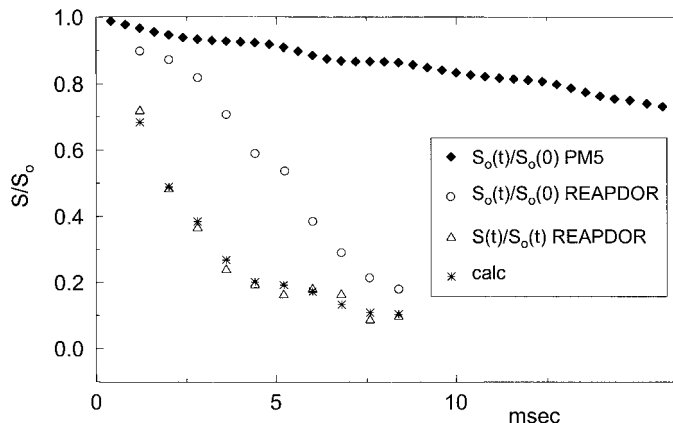
### $^{13}\text{C}$ - $^2\text{H}$ REAPDOR of *L*-Alanine

Recently the REAPDOR method was introduced to improve the methodology for measuring distances between quadrupolar

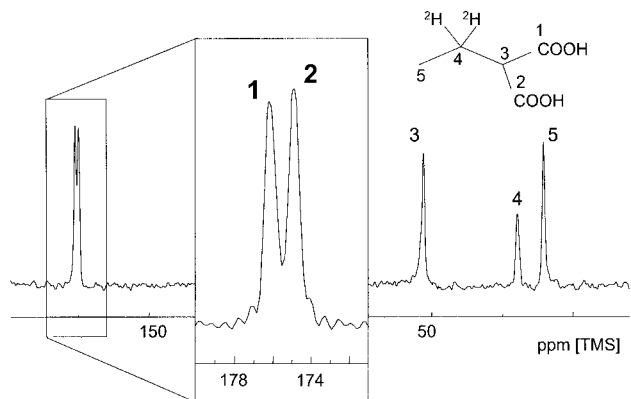
and spin- $\frac{1}{2}$  nuclei (26–28). This technique combines a standard REDOR experiment with the adiabatic TRAPDOR dephasing (23–25). In the  $^{13}\text{C}$ - $^2\text{H}$  REAPDOR experiment all  $\pi$ -pulses are applied on the carbon nucleus except for a long RF pulse on the deuteron in the middle of the pulse sequence. Here we show a comparison between a REAPDOR and a PM5-REDOR experiment on the  $^{13}\text{C}_\beta$ - $^2\text{H}$  spin pair in *L*-alanine-2- $d_1$ . While in the REAPDOR case all  $\pi$ -pulses are applied on the carbon channel, in PM5-REDOR only one  $\pi$ -pulse is applied on this channel.

In Fig. 8 the natural abundance methyl  $^{13}\text{C}_\beta$  reference signals  $S_0(t)/S_0(0)$  of the REAPDOR and PM5-REDOR experiments are shown, together with the  $S(t)/S_0(t)$  REAPDOR results. The REAPDOR reference experiment consists of rotor synchronized  $\pi$ -pulses on the  $^{13}\text{C}$  channel, following an XY-4 cycle scheme, without the presence of the adiabatic deuterium pulse. The REAPDOR  $S_0(t)/S_0(0)$  decays within 5 ms toward a value that is less than 0.2, much faster than the simple Hahn-echo PM5-REDOR reference signal. This significant difference is due to the imperfections of the finite  $\pi$ -pulses in the observation channel and possible cross-polarization effects during these pulses. Whereas the PM5-REDOR reference signal is mainly influenced by  $T_2$  relaxation, which is about 100 ms for the methyl carbon, the REAPDOR reference signal completely vanishes after 12–15 ms. Although the REAPDOR signal exhibits a stronger decay than the PM5-REDOR signal, the measurements of the latter can be extended to longer times. This advantage is particularly significant when simulations are performed to obtain weak dipolar interactions corresponding to long internuclear distances.

The experimental REAPDOR  $S(t)/S_0(t)$  data in Fig. 8 are compared with simulated results from a straightforward calculation which takes into account the dipolar parameters deduced from the PM5-REDOR experiments.



**FIG. 8.** REAPDOR experiment on alanine ( $\text{C}_\beta$ ). Normalized experimental data points ( $\Delta$ ) and simulation ( $*$ ). The reference REAPDOR experiment ( $\circ$ ) displays a strong signal decay due to pulse imperfections in the  $^{13}\text{C}$  observation channel unlike the reference of PM5 REDOR ( $\blacklozenge$ ) which shows solely a normal  $T_2$  decay (the reference signals  $S_0$  are normalized to be comparable).

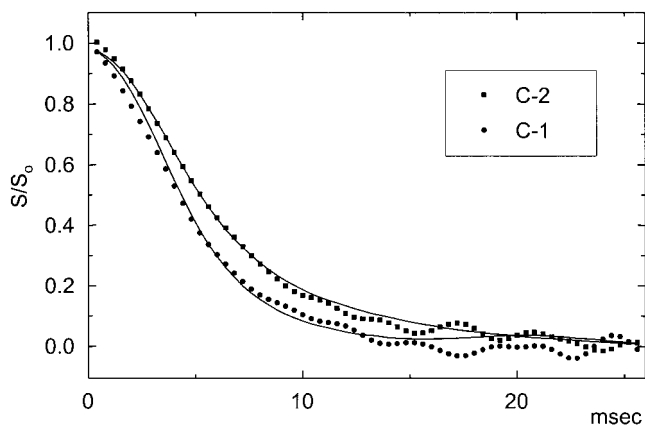


**FIG. 9.**  $^{13}\text{C}$ -CPMAS spectrum of ethylmalonic acid (EMA) with resonance assignments. The dipolar decay of the carbonyl signals (C1,2) has been observed in the course of a PM5-REDOR experiment in order to evaluate the torsion angle  $\varphi$  (C1/2-C3-C4-CH<sub>3</sub>).

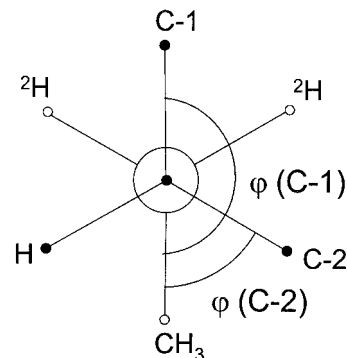
#### PM5-REDOR on Ethyl Malonic Acid

$^{13}\text{C}$ - $^2\text{H}$  REDOR experiments employing PM5 recoupling pulses were also applied to ethylmalonic acid (4,4- $d_2$ ), doubly deuterated at the C4 position (Fig. 1). In Fig. 9 the natural abundance  $^{13}\text{C}$  CPMAS spectrum of EMA is displayed with an enlarged sector showing the two resonance lines of the carbonyl carbons (C1,2). PM5-REDOR data of these two carbons were detected. Their analysis should provide all necessary information to determine the molecular conformation of EMA by means of the dihedral angle  $\varphi$  (C1,2-C3-C4-C5).

The PM5-REDOR results of the C1 and C2 carbonyl carbons are shown in Fig. 10. The pulse segments of the PM5 pulse were like in the previous experiments equal 5.6  $\mu\text{s}$ . The stronger REDOR decay belongs to the carbonyl carbon (C1) that is low field shifted with respect to C2. The numerical analysis of these REDOR decay functions have been achieved by employing a three-spin model ( $^{13}\text{C}$ - $^2\text{H}_2$ ). The quadrupolar coupling strength of the two deuterium labels equals 125 kHz,



**FIG. 10.** Experimental and simulated (solid lines)  $^2\text{H}$ -PM5-REDOR decay curves of the carbonyl carbons (C1/2) of EMA.



**FIG. 11.** Conformations of EMA elucidated by  $^2\text{H}$ -PM5-REDOR corresponding to a torsion angle  $\varphi(\text{C}2) = -60^\circ$ . The REDOR distance constraints do not distinguish between  $\varphi(\text{C}2) = -60^\circ$  and  $\varphi(\text{C}2) = +60^\circ$ , whereas the X-ray analysis predicts the depicted structure.

similar to L-alanine-2- $d_1$ . The significant molecular parameter of C1,2- $^2\text{H}_2$  distances and polar angles  $\theta_D^{1,2}$  were deduced keeping the C4- $^2\text{H}_2$  moiety fixed with the two quadrupolar tensors pointing in the C4- $^2\text{H}$  directions. The torsion angle  $\varphi$  was stepwise varied as a molecular degree of freedom, thus constraining the C1,2- $^2\text{H}_2$  distances and the polar angles  $\theta_D^{1,2}$ . In this way  $\varphi$  could be determined relative to C1 and C2 with  $\varphi(\text{C}1) = 180^\circ \pm 20^\circ$  and  $\varphi(\text{C}2) = \pm 70^\circ \pm 20^\circ$  (Fig. 11). Corresponding dipolar couplings, distances, and polar angles  $\theta_D^{1,2}$  are summarized in Table 1.

#### DISCUSSION

Carbon detected  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments on molecules labeled only at specific proton positions require an accurate accumulation of the weak natural abundance carbon signals. For that reason it is desirable to minimize the number of pulses on the carbon channel of the spectrometer. We therefore applied all pulses for dipolar recoupling on the deuterium channel and gave only one refocusing  $\pi$ -pulse to the  $^{13}\text{C}$  spins. We have shown in this publication that the use of phase modulated recoupling pulses (PM $n$ ) on the deuteriums provides a dipolar decay that approaches the efficient REAPDOR dephasing result. While REAPDOR could cause a serious decrease of the  $^{13}\text{C}$  signal intensity when the carbon pulses are relatively weak,

**TABLE 1**  
Molecular Parameter Used in Simulations of the  $^2\text{H}$ - $^{13}\text{C}$  REDOR Experiments and Comparison to X-Ray Diffraction Data

$^{13}\text{C}$ site	$D_{\text{C-D}}$ (Hz)	$r_{\text{C-D}}$ ( $\text{\AA}$ )	$\theta_D$ ( $^\circ$ )	$\varphi$ ( $^\circ$ ) NMR	$\varphi$ ( $^\circ$ ) X-ray <sup>a</sup>
1	250	2.64	65	180 (20)	180
1	250	2.74	65		
2	270	2.67	70	$\pm 70$ (20)	60
2	120	3.33	25		

<sup>a</sup> Reference (47).



due to lack of RF power in the triple tuned probe, the PM $n$ -REDOR experiments are not disturbed by these recoupling pulses. In our  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments the dipolar dephasing could be monitored during an evolution period of 25 ms. In contrast, with the same NMR probe the REAPDOR evolution period could not be longer than 7 ms when  $S_0(t)$  became almost zero (Fig. 8). This observation has been confirmed by calculations of the  $S_0(t)$ -REAPDOR signals, which were dephased by the  $^{13}\text{C}$ -pulses in the same experimental order. It should be mentioned, however, that for the recoupling of carbon–deuterium interactions with strong quadrupolar frequencies ( $>120$  kHz) and very weak  $B_1$  field strengths ( $\sim 20$  kHz), the dipolar dephasing is stronger for REAPDOR than for any PM $n$  experiment. As soon as the experimental setup allows the application of multiple  $^2\text{H}$  pulses of the order of 25–30  $\mu\text{s}$  with RF power around 30–40 kHz the REAPDOR and the PM5 REDOR experiments become comparable. Under these conditions, the latter should be preferred to enable the detection of long REDOR decays making it possible to observe weaker interactions and longer distances.

The efficiency of PM5-REDOR compared to other dephasing techniques was shown by presenting results from an L-alanine sample singly deuterated at the  $\text{C}_\alpha$  position. REDOR decay curves of the methyl carbon were fit to simulated data and gave good agreement with experimental results. These calculations were sensitive to the  $^{13}\text{C}$ - $^2\text{H}$  distance while changes of the polar angle  $\theta_D$  had only a small effect on the REDOR decay. For example, a variation of  $\theta_D$  from  $0^\circ$  to  $90^\circ$  caused a slightly stronger calculated dephasing curve which could be compensated by a reduction of the dipolar coupling from 0.52 to 0.48 kHz. These values are within the error range of  $\pm 0.04$  kHz found for a 0.50-kHz dipolar coupling strength. The corresponding distance of  $(2.10 \pm 0.05)$  Å agrees well with earlier reported X-ray crystal data (45).

The new PM5-REDOR technique resulted also in valuable structural information of a three-spin  $^{13}\text{C}$ - $^2\text{H}_2$  system in doubly deuterated EMA. The torsion angles  $\varphi(\text{C1},2)$  between the C1–C3 and C2–C3 bond and the C4–C5 bond directions (Fig. 11) were determined by means of the dipolar decay curves of C1 and C2. Figure 10 shows the experimental and simulated PM5-REDOR data for these two carbons. To calculate the three-spin REDOR decay curves as a function of  $\varphi$ , all molecular simulation parameters, i.e., distances and polar angle  $\theta_D^{1,2}$ , were deduced a priori from the torsional constraint. The C1/2–C3–C4–C5 single bond lengths as well as fixed bond angles were taken from related crystal data of diethylmalonic acid (46). This strategy might be applied to  $^2\text{H}$ - $^{13}\text{C}$  REDOR experiments on  $\text{C}_\beta$ -deuterated peptides to determine the dihedral angles  $\varphi$  and  $\Psi$  based on expected bond lengths in peptide backbones. An exact fit of the three-spin PM5-REDOR data of EMA using torsional constraints resulted in dihedral angles  $\varphi(\text{C1}) = 180^\circ \pm 20^\circ$  and  $\varphi(\text{C2}) = \pm 70^\circ \pm 20^\circ$ . Figure 11 displays the conformation of EMA following from NMR experiments. Since through-space distances are employed to

measure a rotation around the C3–C4 bond the direction of rotation is ambiguous. However, in the case of  $\varphi(\text{C1})$  a unique solution was obtained because of the extreme value of the trans conformation. For  $\varphi(\text{C2})$  the direction of rotation cannot be determined by NMR alone. From the crystal structure of EMA (47) it follows that  $\varphi(\text{C1})$  equals  $180^\circ$  and that  $\varphi(\text{C2})$  is  $-60^\circ$  following a counterclockwise rotation. This agrees well with our  $^2\text{H}$ - $^{13}\text{C}$  REDOR NMR results.

Further, PM5-REDOR on EMA allowed an assignment of the isotropic chemical shifts to molecular conformations. Figure 10 depicts a stronger dipolar interaction for C1, which exhibits a low field shift, than for C2. Thus C1 must be closer to the deuterons, placing the low field shifted carbon in a trans position relative to the methyl group and the more shielded carbon (C2) is in a gauche position ( $\pm 60^\circ$ ). This agrees with the  $\gamma$ -gauche effect which has long been known as an influence of the position of a  $\gamma$ -neighbored heavy atom to the chemical shift of a  $^{13}\text{C}$  nucleus (48). While the  $\gamma$ -gauche effect predicts a low field shift of the carbon with a gauche conformation in the order of 5 ppm, the C2 in EMA exhibits a weaker shift of approximately 2 ppm. This may be attributed to the relatively high shielding of a carbonyl carbon causing less sensitivity to conformational features.

## CONCLUSION

A new way of efficient deuterium recoupling in  $^2\text{H}$ - $^{13}\text{C}$  REDOR NMR has been reported. It has been shown that the use of phase modulated  $^2\text{H}$  pulses, including five phase segments (PM5), gives stronger dipolar dephasing than CW-recoupling, amplitude modulated  $\bar{X}\bar{X}\bar{X}\bar{X}\bar{X}$ , and  $\bar{X}\bar{X}\bar{X}$ , as well as  $90^\circ$ – $180^\circ$ – $90^\circ$  CPL REDOR pulses. It was further shown theoretically and experimentally, using singly deuterated L-alanine, that careful setup of the XY deuterium pulses, including the length of their phase segments and the RF field strength, provides a REDOR dipolar recoupling that approaches the efficient REAPDOR dephasing results for quadrupole interactions in the range of 80 to 200 kHz. While in REAPDOR experiments the reference signal can dephase fast due to  $^{13}\text{C}$  pulse imperfection and CP processes, PM5-REDOR does not cause any unnecessary decay of the reference signal. This allows measurements over long REDOR evolution periods, needed for the detection of weak dipolar couplings. The PM5-REDOR dephasing signal of singly deuterated L-alanine was observed for 25 ms. For doubly deuterated EMA we demonstrated the use of the PM5-method to measure torsion angles. In the three-spin REDOR case of EMA the torsion angle  $\varphi$  was verified by an evaluation of individual dipolar decays for both carbonyl carbons. The detection of relatively weak  $^2\text{H}$ - $^{13}\text{C}$  dipolar interactions by PM5-REDOR, combined with the distance and torsional constraints of the molecular parameters, can also be extended to investigate peptide folding.

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