

# Efficient Deuterium–Carbon REDOR NMR Spectroscopy

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Received September 22, 1999; revised March 9, 2000

**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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