The investigation of lipids, polymers, and other organic materials has been greatly advanced by solid-state NMR utilizing anisotropic nuclear interactions, such as dipolar and quadrupolar couplings. In particular, the quadrupolar 2H interaction has proved to be a useful local probe of molecular order, structure, and dynamics.\(^1\) In rigid solids, the 2H quadrupolar coupling reflects the angle between the C-\(^{1}\)H bond and the external magnetic field, while in mobile systems, the motionally narrowed spectrum provides the order parameter of the C-\(^{1}\)H bond.\(^1\) The necessity of 2H labeling can be avoided by measuring 13C-\(^{1}\)H dipolar couplings, which yield similar orientational information. Overlapping dipolar spectra of the various sites in a complex molecule can be separated by two-dimensional (2D) NMR techniques, specifically separated local field (SLF) NMR.\(^4\)\(^-\)\(^7\) However, traditional SLF techniques do not produce dipolar powder spectra with simple splittings for CH2 or CH3 groups.\(^6\) Only recently, proton-detected local field (PDLF) NMR, which involves the detection of proton spins evolving in the dipolar field of a single 13C nucleus, has made it possible to obtain clear Pake-type C-\(^{1}\)H dipolar powder spectra.\(^4\)\(^-\)\(^9\)

In this Communication, we demonstrate the measurement of C-\(^{1}\)H dipolar coupling constants \(\delta_0\) including the signs, for a number of sites in a liquid crystalline lipid, lecithin. 2D PDLF NMR with switched-angle spinning (SAS) provides the dipolar coupling period, in which \(\delta_0\) and \(\Omega_0\) have opposite signs.\(^1\)\(^1\)\(^1\) Since the rigid value of dipolar coupling \(\delta_0\) is negative, lecithin, a lipid abundant in biological membranes, was chosen in this study since C-\(^{1}\)H couplings have been measured for \(\approx 20\) sites\(^1\)\(^2\) in phospholipids and can thus be used to estimate the accuracy of the dipolar couplings. Egg yolk lecithin, a phosphocholine with 16:0, 18:1, and 18:2 acyl chains, was hydrated with D\(_2\)O [70:30 (w/w)] and freeze-thawed to yield a uniform aqueous dispersion.

Figure 1 displays the pulse sequence designed to exhibit the sign of the heteronuclear coupling as the sign of the corresponding 13C magic-angle spinning (MAS) peak intensity. At sufficiently short \(t_1\), due to \(\sin(\omega t_1) \approx \omega t_1\), the sign of the heteronuclear coupling \(\omega\) is manifested as the sign of the signal. The technique is termed DISTINCT (Dipolar Sine Term by Indirect-Coupling Transformation). In the first part of the experiment, rapid off-magic-angle spinning (OMAS) scales the dipolar couplings by a factor \(P_2 = 1/2(3\cos^2 \theta - 1)\), where \(\theta\) is the angle between the spin axis and the external field. Scaled C-\(^{1}\)H couplings are selectively retained under homonuclear 1H decoupling and a pair of 180° pulses. From 1H magnetization, the C-\(^{1}\)H interactions create heteronuclear coherences that are modulated with the sine of the sum of the J and dipolar C-\(^{1}\)H couplings. Having survived the hop time in the form of heteronuclear two-spin order \(S_2\), these sine-modulated terms are converted, following a 180° pulse before the hopping period, into detectable 13C magnetization under the action of the isotropic heteronuclear J coupling and then detected under 1H decoupling. Thus, the sine-modulated C-\(^{1}\)H couplings determine the sign of the peaks in the resulting 13C MAS spectrum.
Figure 2. DISTINCT spectra of lecithin in the L0 phase at scaling factors (a) \(P_2 = +0.2\) and (b) \(P_2 = -0.2\). Each peak sign reflects the sign of the sum of the corresponding J coupling and \(P_2\)-scaled dipolar coupling constant. The \(^{13}C\) MAS signals have been assigned by Husted et al. The experiments were carried out at 75.74 MHz for \(^{13}C\) using a 7-mm sample rotor spinning at 2080 Hz in a home-built SAS probe head. Measuring times were 8 and 4 h.

The coupling strength is significantly smaller (narrowing factor \(< 0.02\)) than expected from \(^{2}H\) NMR (0.045) of fully hydrated bilayers. This may be due to lower hydration of our sample.

Since the dipolar frequency depends on the angle \(\beta\) between the director and the rotor axis according to \(1/2(3 \cos^2 \beta - 1)\), both positive and negative dipolar frequencies occur due to the isotropic distribution of bilayer orientations. By incrementing \(t_1\) in the pulse sequence of Figure 1, we obtained a 2D DISTINCT spectrum (not shown) which establishes that the powder pattern maximum determines the sign in the 1D DISTINCT spectrum. Taking into account that the frequency of the powder-pattern maximum, \(-1/2\beta_D\), has a sign opposite that of the dipolar coupling constant, the \(\Delta \phi\) of the acyl sites is found to be positive, i.e., their narrowing factors are negative.

To measure the strengths of the dipolar couplings \(\Delta \phi\) and complete the sign determination, we have taken 2D PDLF SAS spectra with the pulse sequence of Nakai and Terao, where initially \(^{1}H\) magnetization evolves under \(^{13}C-^{2}H\) dipolar and J couplings at \(\theta = 54.74^\circ\). After cross-polarization (CP) from \(^{1}H\) to \(^{13}C\) and a hop to \(\theta = 54.74^\circ\), the \(^{13}C\) MAS spectrum is observed during the detection period. This results in a 2D spectrum where the \(^{13}C-^{2}H\) Pake patterns are separated according to the isotropic \(^{13}C\) chemical shifts. In the spectra of Figure 3, taken with \(P_2\) scaling factors of \(\pm 0.2\), \(^{2}H\) splittings between 200 and 1300 Hz are observed. Upon inversion of \(P_2\) from \(-0.2\) to \(+0.2\), splittings increase or decrease according to the relative signs of \(J\) and \(\Delta \phi\). For splittings exceeding twice the \(J\) coupling, the average of the splittings at opposite \(P_2\) values is \(|\Delta \phi|\), while their difference is the \(J\) coupling. In Figure 4, the dipolar couplings with their signs are summarized and compared with \(^{2}H\) NMR order parameters from the literature. Due to scaling factors from sample rotation and homonuclear decoupling, the dipolar couplings are less precise than the \(^{1}H\) results. The \(^{13}C-^{2}H\) couplings are hardly affected by the acyl chain composition, except in the double bond region and at the end of the acyl chains. This can be concluded from \(^{13}C-^{2}H\) dipolar couplings measured in DMPC, as well as from \(^{2}H\) data. The signs of the \(^{13}C-^{2}H\) values determined here are important for testing structural models of the lipid and providing complete order tensor information.

Figure 3. PDLF 2D \(^{13}C-^{2}H\) spectra of lecithin with a schematic of the lecithin molecule. (a) and (b) Spectrum taken with \(P_2 = -0.2\); measuring time, 12 h. (a) Acyl region. (b) Headgroup and glycerol regions. (c) Similar to (b), but with \(P_2 = +0.2\). The increase of the \(C_a\) splitting relative to that in (c) verifies the positive dipolar order parameter (narrowing factor) of \(C_a\), while the decrease for \(C_1, C_2\), and \(C_3\) indicates negative order parameters. The peak in the center of the \((CH_3)_2\) dipolar pattern, which is enhanced at longer CP times, results from the weak dipolar interactions of the specific methylene carbon with the protons of neighboring CH_2 groups. CP time was 0.5 ms; MREV-8 scaling factor, from the J splittings, was 0.5 \(\pm 0.05\).

Figure 4. Motionally averaged \(^{13}C-^{2}H\) dipolar couplings \((\mathbf{B})\), including signs, for various segments in the lecithin molecule, compared with values of \(^{2}H\) quadrupolar couplings \((\Delta \omega)\) from the literature. For clarity, the \(^{2}H\) couplings, whose signs are unknown, are shown with the same signs as those of the corresponding dipolar couplings. On the right, the dipolar coupling constants are given (without scaling by sample rotation). The narrowing factors on the left axis are equivalent to \(S_{C-H}\) bond order parameters. Most CH_2 groups display only one splitting, but for certain CH_2 groups at the junction of the glycerol and acyl regions, inequivalent \(^{13}C-^{2}H\) couplings can be observed. In the acyl region, only the order parameters for the saturated chains are shown for clarity.

The methods described here are expected to increase the amount of structural and dynamical NMR data on lipids, liquid crystals, and oriented polymers, supplementing deuteron NMR.

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