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Efficient CH-Group Selection and Identification in ^{13}C Solid-State NMR by Dipolar DEPT and ^{1}H Chemical-Shift Filtering

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Abstract: A new spectral-editing technique for solid-state nuclear magnetic resonance (NMR), based principally on the different dipolar-dephasing properties of CH and CH_2 multiple-quantum (MQ) coherence, yields pure C−H spectra with overall efficiencies of up to 14%. The selection is based on dephasing of methylene heteronuclear MQ coherence by the second proton and can be considered essentially as a solid-state, slow-magic-angle-spinning version of the distortionless enhancement by polarization transfer (DEPT) experiment. A short dipolar transfer and inverse gated decoupling suppress quaternary-carbon resonances, and T_1-filtering reduces methyl signals. Applications to amorphous polymers with long, flexible sidegroups demonstrate excellent suppression of the signals of partially mobile methylene groups, consistent with simulations and superior to existing methods. CH selection in various model compounds and a humic acid confirms the robust nature and good sensitivity of the technique. Distinction of NCH and CCH groups, which have overlapping ^{13}C chemical-shift ranges, is achieved by combining dipolar DEPT with ^{1}H isotropic-chemical-shift filtering. In the humic acid, this permits unequivocal assignment of the methine resonance near 53 ppm to NCH groups.

Introduction

Separation of CH_3, CH_2, CH, and quaternary-C signals, known as spectral editing, is of significant interest in ^{13}C nuclear magnetic resonance (NMR) characterization of complex organic materials. It is routinely used in solution NMR with resolved lines, where the efficient attached-proton test (APT) or distortionless enhancement by polarization transfer (DEPT) procedures are applied.[1] In solids, these *J*-coupling-based techniques are applicable only to crystalline systems with long transverse relaxation times and resolved spectral lines.[2] Despite various developments based on short-time cross-polarization (CP) dynamics,[3−6] in noncrystalline solids clean selection has been achieved at standard magic-angle-spinning (MAS) rates only for CH_2 groups and quaternary carbons. The CH and CH_3 group signals are edited by linear combinations of various spectra with partial selectivity. In addition, signal intensities have remained rather low: Signal-to-noise ratios in the selective spectra, as compared to the full cross-polarization (CP)/MAS signal, are typically around 10%,[7] with 25% reported in the most favorable cases.[4] Furthermore, the techniques are also sensitive to reduction of C−H dipolar couplings by motional averaging. Recently, selective suppression of CH_2 signals was achieved by Saalwächter et al.[8] and De Vita and Frydman,[9] on the basis of solid-state dipolar INEPT[9] (also termed recoupled polarization transfer, REPT[8]) at high MAS spinning speeds. While it achieves clean suppression of CH_2 peaks in rigid segments, the absolute CH signal intensities are low due to the inherent sample-volume limitations of high-speed MAS rotors. In addition, the signals of mobile CH_2 groups are not suppressed well; this has in fact been used to characterize CH_2 mobility.[10]

In this paper, we introduce a new, robust approach for suppressing CH_2 and quaternary-carbon signals while retaining CH peaks with up to 14% efficiency. The technique is based on the invariance of C−H multiple-quantum (MQ) coherence under the C−H dipolar coupling, while the corresponding coherence in a CH_2 group is dephased efficiently by the second proton. Because this approach is not based on specific coupling strengths, it is applicable even in the presence of partial motional averaging. The method can be used as a filter after cross polarization from protons, but the highest efficiency is achieved by incorporating the CH_2 suppression into a solid-state dipolar DEPT transfer from ^{1}H to ^{13}C, without standard cross polarization. Like the dipolar INEPT[9]/REPT[8] method, this transfer also achieves further suppression of CH_2 signals. The pulse sequence

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resembles DEPT in solution NMR,[1,11] with a final ^1H pulse of 90° flip angle. Nevertheless, new technical tricks have to be employed, such as C−H multiple-quantum excitation and reconversion for time periods much shorter than a rotation period; this reduces quaternary-carbon and CH_3 signals and also minimizes the duration of the pulse sequence. In agreement with simulations, experiments on amorphous polymers with flexible sidegroups demonstrate excellent CH_2 suppression and CH selection, even under conditions where standard dipolar dephasing fails due to segmental mobility.

While CH signals near 70 and 35 ppm can be assigned unambiguously to OCH(C,C) (i.e., C bonded to O, H, and two C's) and CCH(C,C) groups, respectively, those near 53 ppm can be due to NCH(C,C) or CCH(C,C) moieties. While traditional spectral-editing methods have not distinguished different CH groups, the dipolar DEPT pulse sequence is easily combined with a ^1H isotropic-chemical-shift filter[12] that selects NCH/OCH versus CCH(C,C) signals. Applications of the new methods to model compounds and a humic acid are presented.

Experimental Section

Samples. Cholesteryl acetate and (−)-2,3:4,6-di-*o*-isopropylidene-2-keto-L-gulonic acid were purchased from Sigma-Aldrich. Amorphous poly(*n*-butyl methacrylate), PnBMA, poly(cyclohexyl methacrylate), PcHMA, and semicrystalline isotactic poly(1-butene), iPB, were purchased from Scientific Polymer Products (sp^2, Ontario, New York). The crystallites of iPB were in their well-ordered equilibrium modification, known as form I. The peat humic acid was obtained from a peat in Amherst, MA. The extraction and detailed characterization of this base-soluble organic fraction have been described elsewhere.[13] All samples extended over the full 1-cm length of the radio frequency (rf) coil. While a few filled the whole rotor, in most cases a simple spacer made of compacted Teflon tape was placed at the bottom of the rotor to prevent parts of the sample from being placed outside the rf coil. The sample volume was 250−300 μL.

NMR Parameters. Experiments were performed in a Bruker DSX400 spectrometer at 100 MHz for ^{13}C, using a Bruker 7-mm magic-angle spinning double resonance probehead at spinning frequencies of 5.762 or 4 kHz. The ^{13}C 90° pulse length was 4 μs. For the ^1H homonuclear decoupling, the radio frequency power was relatively high, corresponding to a 3.15-μs 90° pulse. Because of the relatively short duration of this high-power irradiation (<500 μs of ^1H pulses), as compared to standard heteronuclear decoupling, the probehead showed no degradation of performance throughout the 1-year period during which these pulse sequences were applied occasionally. The pulse length in the semiwindowless MREV-8 was 3.3 μs. For sideband suppression, four-pulse TOSS was applied before detection. For characterizing segmental mobility, ^1H wide-line separation (WISE) experiments were performed at 4-kHz spinning frequency, with ^{13}C decoupling during t_1[14] and with 70-μs Lee−Goldburg (LG) CP[15] to avoid ^1H spin diffusion and minimize intersegmental cross polarization. In t_1, 40 3-μs increments were used.

Theoretical Background

Heteronuclear C−H Coherence. The principal idea for distinguishing CH groups from other CH_n moieties, in particular

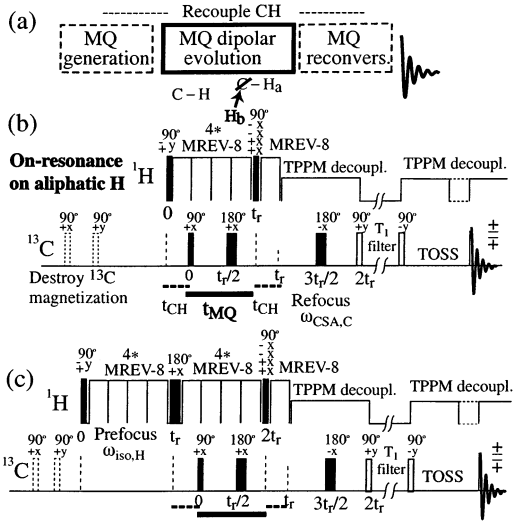

Figure 1. (a) Block diagram of the CH_2-suppression approach. The heteronuclear multiple-quantum (MQ) coherence of C−H spin-pairs is unaffected by the C−H dipolar coupling, while that of CH_2 groups is dephased. To prevent MAS from refocusing the C−H dipolar interaction, it needs to be recoupled by a suitable pulse sequence. (b) Shorter pulse sequence without proton isotropic-shift (p)refocusing. The 90° pulses for coherence transfer are the thin filled rectangles, while the filled rectangles of twice the width are the 180° pulses that recouple the C−H interactions and refocus the chemical shift. Each of the five cycles of semiwindowless MREV-8 homonuclear decoupling is shown as an open block. The first and the last of these provide excitation and reconversion, respectively, of the C−H heteronuclear coherence. During the three central MREV-8 cycles, the heteronuclear multiple-quantum coherence evolves under the C−H dipolar coupling. The four 180° pulses of TOSS before detection are not shown explicitly. Optional gated decoupling for dipolar dephasing at the end of TOSS is indicated by dashed lines. A crucial four-step phase cycle of the proton pulses and of the receiver is indicated. (c) Full pulse sequence with ^1H isotropic-shift "prefocusing". Note the $t_r/4$-shifted rotation synchronization of the ^1H and ^{13}C pulses.

CH_2 groups, is the following: Heteronuclear multiple-quantum coherence $S_{x/y}I_{x/y}$ (with x/y denoting any combination of x or y) of an S−I (i.e., C−H) spin-pair is invariant under the spin-pair dipolar coupling $H_{IS} = \hbar\omega_{CH}S_zI_z$, because $[H_{IS}, S_{x/y}I_{x/y}] = 0$. In contrast, for a CH_2 group with its two protons A and B, $S_{x/y}I_{x/yA}$ does not commute with, and is therefore quickly dephased by, the second term of $H_{IS} = \hbar\omega_{CH,A}S_zI_{zA} + \hbar\omega_{CH,B}S_zI_{zB}$. Thus, the pulse sequence needs to excite heteronuclear MQ dipolar coherence, let it evolve under the heteronuclear dipolar coupling, and reconvert the remaining heteronuclear coherence into observable ^{13}C magnetization. This is shown schematically in Figure 1a. A quantitative treatment of the spin-state evolution under the pulse sequence is given in the Appendix.

DEPT Transfer and Chemical-Shift Refocusing. In principle, C−H heteronuclear coherence can be generated after cross polarization from ^1H magnetization. However, it turns out that a pulsed transfer similar to DEPT, REPT,[8] or CH TEDOR[16] is more favorable. It combines the $^1H-^{13}C$ coherence transfer and two methods of CH_2 suppression in one step and makes it easy to remove all effects of the chemical-shift anisotropies.

Saalwächter and Spiess,[8] and also De Vita and Frydman,[9] have shown that the REPT or solid-state dipolar INEPT technique is useful for reduction of CH_2 signals. The excitation starts with transverse ^1H magnetization, while the reconversion of the heteronuclear coherence ends with ^{13}C magnetization. For a C−H spin-pair, the regular $\sin^2\Phi_{CH}$ modulation is

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obtained, with the phase Φ_{CH} resulting from the action of the C−H dipolar coupling during a period t_{CH}. In contrast, for a CH_2 group, during excitation the protons evolve in the field of the ^{13}C spin, while in the reconversion period, the ^{13}C evolves in the field of the two protons. Thus, the two periods are not identical, and the modulation function is not $\sin^2\Phi_{CH}$ but goes to zero at long recoupling times.

In designing the pulse sequence, care must be taken to refocus the isotropic and anisotropic chemical shifts of both carbons and protons. In the pulse sequence of Figure 1b, the dipolar evolution period t_{CH}, the MQ dipolar dephasing of duration $t_r - t_{CH}$, and the reconversion period t_{CH} are obvious, but the ^1H isotropic chemical shift evolution of duration t_r is not refocused. If only signals relating to protons in a narrow chemical-shift range, for example, aliphatic protons, are of interest, these can be set on resonance, and the isotropic shift evolution vanishes. In Figure 1c, a broadband sequence is shown which (p)refocuses ^1H isotropic shifts by a 180° pulse preceded by a rotation period of homonuclear decoupling. The removal of chemical shifts is facilitated by "shifted synchronization" of the transfer: As indicated in Figure 1c, the start of rotation synchronization of the ^{13}C pulses is shifted by t_{CH} relative to the ^1H pulses. Until the first ^{13}C pulse is applied, the ^{13}C part of the coherence is along z, so that it is unaffected by the ^{13}C chemical shift. Similarly, the second ^1H 90° pulse, which starts reconversion, makes the proton part of the relevant coherence longitudinal ($S_{x/y}I_z$), which commutes with the ^1H chemical shift and is therefore unaffected by it.

Short-Time Recoupling. To generate the heteronuclear coherence predominantly for protonated carbons, the recoupled periods should each be only $t_{CH} \approx 40\ \mu s$; this is similar to short-time cross polarization, which suppresses signals of unprotonated carbons. The time of 40 μs is shorter than a rotation period ($t_{CH} = t_r/4$). Most established recoupling techniques work for integer multiple of full rotation periods,[17] or in the quasi-static short-time regime. Here, we use a simple, clean alternative suitable for intermediate recoupling times.

In the pulse sequences of Figure 1b and c, the starting times of the excitation and reconversion periods are separated by t_r, and so are their ending times. During each t_{CH}-period, the coherence is modulated by the same phase factor $\sin\Phi(t_{CH})$ with $\Phi(t_{CH}) = {}_0\int^{t_{CH}} \omega_{CH}(t)\ dt$, where $\omega_{CH}(t)$ is the instantaneous heteronuclear coupling frequency. Thus, due to correct timing, the magnetization detected acquires an all-positive $\sin^2\Phi(t_{CH})$ modulation. In ref 18, a similar case in homonuclear double-quantum NMR is seen for specific timings, which produce rotational echoes in t_1.

Dipolar Dephasing of CH_2 Coherence. Without the ^{13}C 180° pulse at $^1/_2t_r$ after the start of the MQ coherence evolution, the dephasing period would be $t_r - t_{CH}$. Because the dephasing over a whole rotation period vanishes, this corresponds to $-t_{CH}$ of dephasing, which is too short for efficient suppression of CH_2 MQ-coherence. By means of the 180° pulse on ^{13}C, at a time t_{180} after the ^{13}C 90° pulse, the C−H interaction is better recoupled. The recoupling is strongest if the pulse is placed in the center of the MQ dephasing period, $t_{180} = (t_r - t_{CH})/2$. The dephasing function can be optimized to minimize CH_2 signals

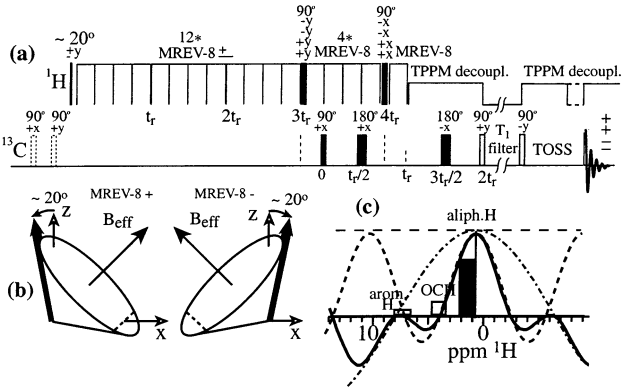

Figure 2. (a) Pulse sequence for dipolar DEPT with ^1H chemical-shift filtering. This represents the sequence of Figure 1b preceded by three rotation periods of chemical-shift filtering. (b) Schematic representation of the chemical-shift filter and the phase cycle to remove effects of transverse ^1H magnetization components at the end of the filter time. By inverting all phases in the MREV-8 phase sequence (MREV-8+ to MREV-8-), the transverse component of the effective field B_{eff} is inverted. This inverts the undesirable transverse components at the end of the chemical-shift filter, and the corresponding undesirable signals cancel in alternate scans, while those of the z-components add up. (c) Thick line: Filter function of the pulse sequence in (a) in the ^1H chemical-shift range, when the ^1H irradiation is on-resonance at 0.8 ppm. This is the product of the filter characteristic after $3t_r = 512\ \mu s$ of chemical-shift filtering (dashed line) and after $1t_r$ of chemical-shift evolution during the dipolar DEPT part of the pulse sequence (dash−dotted line). The peak positions of nonpolar aliphatic protons (filled bar) and of OCH/NCH as well as aromatic protons (open bars) are indicated in the figure.

over a wide range of coupling strengths by varying t_{CH} and t_{180}, using numerical simulations as shown below. The total phase at the end of the MQ dipolar-dephasing period is $\Phi_{MQ} = -2\Phi(t_{CH} + t_{180}) + \Phi(t_{CH})$.

Suppression of Quaternary-C Signals. The scheme of Figure 1c provides efficient CH_2 suppression and leaves the C−H signals as the dominant peaks in the spectrum. However, signals of quaternary carbons and CH_3 groups are detected at a relative intensity of ca. 40% (see spectrum of Figure 3c). The reason is that a quaternary ^{13}C and a ^1H separated by two bonds can also behave like a spin-pair, with a dipolar coupling of ca. 4 kHz (see below for the discussion of CH_3 groups). Because of the weaker C−H coupling, a shorter recoupling time could reduce these long-range signals. However, it reduces the one-bond transfer efficiency as well, and an efficient homonuclear decoupling period shorter than one cycle of MREV-8 proved difficult to implement.

Therefore, we use a quaternary-carbon suppression scheme by difference, without scaling, whose essence was originally introduced by Wu and Zilm.[4] A second spectrum is recorded with essentially the same pulse sequence, except for an added dipolar-dephasing delay of 40 μs immediately before detection (indicated dashed in Figure 1c). The resulting spectrum contains no methine carbon signals, while retaining most of the unprotonated and a significant fraction of the methyl signals. This residual spectrum is then subtracted from the CH_2-free spectrum (without any scaling). In the difference spectrum, the unprotonated carbon signals are reduced to an insignificant level. Given that the unprotonated carbon signals appear originally with about 40% efficiency relative to CH signals, and the dipolar dephasing leaves ca. 90% of their intensity, the residual quaternary-carbon signal is only about $0.1 \times 40\% = 4\%$. The price of this procedure is a decrease of the final signal-to-noise

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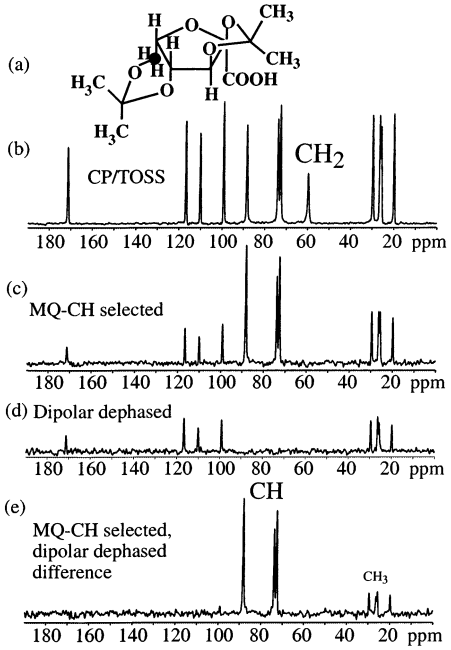

Figure 3. Demonstration of CH spectral editing on a crystalline model compound, with the molecular structure shown in (a); the CH_2 group in the molecule is highlighted. (b) Regular CP/TOSS spectrum. (c) Spectrum without CH_2 signals, due to the multiple-quantum coherence dephasing. No T_1-filter was used. (d) Similar as in (c), but with 40 μs of dipolar dephasing during TOSS before detection. (e) Methine-only spectrum obtained as the dipolar-dephasing difference of spectrum c minus spectrum d. Total number of scans for (e) (sum of (c) and (d)): 256; recycle delay: 3 s. The overall efficiency as compared to the full spectrum in (b) is 14%.

ratio per unit time by a factor of 2, because both the noise and the measurement time are doubled. This cost could be reduced by instead recording a dipolar-dephased spectrum after short CP and scaling it empirically to subtract out the quaternary-carbon signals. However, this procedure would require undesirable empirical scaling of spectra.

Reduction of CH_3 Signals. The approach described here should lead to efficient dephasing of $S_{x/y}I_{x/y}$ coherence in any multiproton CH_n group, including CH_3. This would indeed be true for immobile CH_3 groups; if the three C−H couplings were not equal, the modulation factors in eq 3d of the Appendix would be independent, and the methyl signal would essentially vanish. However, the fast rotational jumps of methyl groups at ambient temperature make the three C−H couplings equal. The dipolar fields of two protons with "opposite spin directions"[19] cancel, and part of the CH_3 coherence appears like that of an isolated C−H spin-pair (with a C−H dipolar coupling reduced by $-1/3$). In particular, the MQ-coherence dephasing in the field of the two other protons has a long-term final value of $1/2$, not of zero, because the dipolar fields partially cancel. The full calculation in the Appendix shows that the CH_3 signals are suppressed by a factor of ca. $1/3$ relative to the methine signals. In many samples, the suppression can be improved on the basis of the often short T_{1C} relaxation time of CH_3 groups, which is a result of their fast rotational jumps: A simple T_{1C} filter of 0.5−1.5 s duration reduces most CH_3 signals to an insignificant level.

^{1}H Chemical-Shift Filtering. As discussed in more detail below, the dipolar DEPT experiment can be combined with ^{1}H chemical-shift filtering, see Figure 2, to distinguish different types of methine groups with similar ^{13}C chemical shifts. The principle of the ^{1}H chemical-shift filter is described in ref 12. In short, as indicated in Figure 2b, ^{1}H magnetization off resonance by $\Delta\omega$ precesses around the effective field of the MREV-8 homonuclear decoupling sequence, on a cone for a time t_{csf}. This modulates the z-magnetization by $\cos^2(\Delta\omega t_{csf})$; a prepulse can be used to widen the cone and broaden the region of low intensity, without significant reduction of the on-resonance signal. The total filter function of the pulse sequence in Figure 2a, which also includes a $\cos(\Delta\omega t_r)$ modulation due to the dipolar DEPT itself, is shown in Figure 2c. Improvements as compared to ref 12 include selection of the on-resonance component and a phase cycle, indicated in Figure 2b, that alternates the effective field of MREV-8 so that signals from transverse ^{1}H magnetization components are subtracted out.

The parameters of the chemical-shift filter, mainly the ^{1}H on-resonance frequency and the duration of the prepulse, can be fine-tuned on the sample of interest itself, using the full signal of all CH_n groups. The ^{1}H filter profile is detected via the carbon spectrum after cross polarization. To avoid blurring of the selection of specific protons by spin diffusion or long-range ^{1}H−^{13}C transfer, a short Lee−Goldburg cross polarization period of ca. 70 μs was used. Because of ^{1}H isotropic-chemical-shift-dependent intensity modulation, the chemical-shift-filtered dipolar DEPT spectrum is less quantitative than the regular dipolar DEPT spectrum, but NCH versus CCH(C,C) peak identification can be achieved reliably.

Results and Discussion

Demonstration of the CH Selection Procedure. The procedure for obtaining a CH-only spectrum based on MQ-dephasing is demonstrated in Figure 3. The crystalline model compound, (−)-2,3:4,6-di-*o*-isopropylidene-2-keto-L-gulonic acid, see structure in (a), contains one CH_2 and three CH groups per molecule, as well as four quaternary and four methyl sites. The full CP/TOSS spectrum with 12 lines is shown in Figure 3b. After MQ-dephasing using the pulse sequence of Figure 1c, the CH_2 peak is completely removed, and the quaternary-carbon and methyl signals are strongly reduced relative to the CH lines, see Figure 3c. This already permits identification of CH and CH_2 signals, because all lines are resolved. The detection efficiency of the CH signals here is 30%.

With 40 μs of dipolar dephasing added into the MQ-dephasing pulse sequence directly before detection, the quaternary-carbon signals are retained almost fully, and the methyl carbons are retained at a level of ca. 40% (Figure 3d). Subtracting this spectrum from that in Figure 3c therefore yields a spectrum free of CH_2 and quaternary-carbon signals, Figure 3e. It is dominated by the CH peaks, with residual CH_3 signals at a 15%-level. The methyl signals could be reduced further by T_{1C} relaxation during the z-filter delay in the sequence of Figure 1c. The overall efficiency (sensitivity of CH signals per unit time) of the spectrum in Figure 3e relative to the full CP/TOSS spectrum in Figure 3b is 14%.

CH Selection in Cholesteryl Acetate. To enable comparisons with other spectral-editing techniques, we have applied MQ-based CH selection to cholesteryl acetate, which has also been used as a test compound for spectral editing in refs 4 and 6. The molecular structure is shown in Figure 4a. The CP/TOSS

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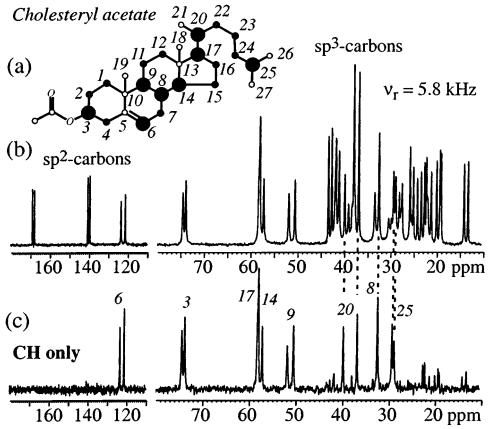

Figure 4. Methine-selection in cholesteryl acetate. (a) Molecular structure, with methine carbons highlighted as large filled circles, methylenes as small filled circles, and methyl and quaternary carbons as open circles. (b) Full TOSS spectrum at $\nu_r = 5762$ Hz. Note that the sp^2- and sp^3-carbon regions are shown on different scales. (c) Corresponding CH-only spectrum. The residual CH_2 and CH_3 signals are significantly smaller than those in the corresponding CP-based spectral-editing spectra of refs 3 and 6. A $T_{1,C}$ filter of 0.5-s duration was used to reduce the CH_3 signal intensities. A total of 1024 scans were acquired with a recycle delay of 4 s (80 min total experiment time).

spectrum in Figure 4b shows a large number of lines, many of which appear as distinct pairs, indicating that there are two chemically inequivalent molecules. The CH-only spectrum, Figure 4c, obtained after MQ-dephasing and subtraction of the dipolar-dephased spectrum clearly identifies the CH signals. Residual CH_3 signals appear at a low level, comparable to that in CP-based spectral editing.[4,6] In making this comparison, also note that in contrast to the CP-based methods, our approach does not involve any empirical scaling of spectra. Slightly more aggressive data processing, with scaling-up of the dipolar-dephased dipolar DEPT spectrum by a factor of ca. 1.2, could be used easily to reduce the residual CH_3 signals.

Applications to Partially Mobile Systems. Many polymers and other organic molecules contain partially mobile CH_2 groups. In this section, we will demonstrate the superior ability of dipolar DEPT, applied at a 4-kHz spinning frequency, to suppress these signals while selecting CH peaks.

Fast anisotropic mobility will reduce the C−H dipolar coupling by motional averaging; this can be summarized by a reduced dipolar coupling constant $\bar{\delta}_{CH}$. In Figure 5, the signal intensity for CH and CH_2 groups is plotted as a function of $\bar{\delta}_{CH}$. The ideal spectral-editing sequence would produce zero intensity for CH_2 groups and quaternary carbons, and an intensity of unity for CH sites. In Figure 5a, the curves for dipolar DEPT at $\nu_r = 5.8$ kHz are shown; this condition was used for the spectra of Figures 3 and 4, and also for several spectra shown below. For rigid segments, good CH selection will be achieved under these conditions; however, the CH_2 suppression at reduced coupling strengths is only mediocre. With a longer rotation period, for example, of 250 μs, which corresponds to a spinning frequency of 4 kHz, the time for the CH_2 dephasing is longer. Figure 5b shows that at 4 kHz indeed excellent CH_2 suppression is predicted over the full range of coupling strengths. Moderate motional narrowing results in small (<12%) negative CH_2 signals, which are easily distinguished from the positive CH lines. At a motional narrowing of $\bar{\delta}_{CH}/\delta_{CH} = 0.6$, the CH_2 signal intensity vanishes. The low maximum at $\bar{\delta}_{CH} = 0.3\delta_{CH}$ is reduced even further by subtraction of the

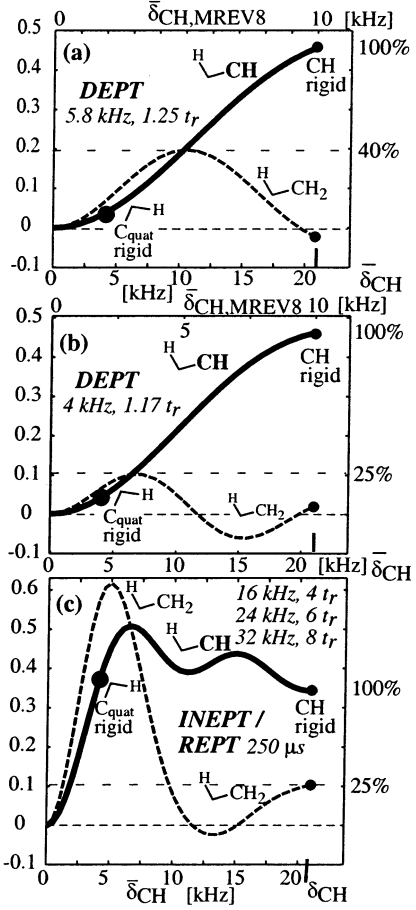

Figure 5. Comparison of dipolar DEPT with REPT/INEPT in rigid and in partially mobile systems. The signal intensity is plotted as a function of the motionally averaged CH coupling strength, $\bar{\delta}_{CH}$. Both couplings in a CH_2 group are scaled equally. A two-bond coupling, of 4 kHz in the rigid limit, is included in all simulations, rigorously for CH groups and by simple cosine modulation for CH_2 groups. (a) Simulation for the 5.8-kHz spinning frequency and pulse timing used for the experimental spectra. In the rigid limit, CH_2 groups and quaternary carbons are well suppressed (black dots). (b) Dipolar DEPT optimized for broad CH_2 suppression, at a spinning speed of 4 kHz, which is suitable for aliphatic CH signals at 100 Hz/ppm. The excitation time was $t_{CH} = t_r/6 = 42$ μs, the ^{13}C recoupling 180° pulse placed at $t_{180} = t_r/3 = 83$ μs from the first ^{13}C 90° pulse; these timings are easily implemented with six MREV-8 cycles per rotation period. The CH_2 signals remain small over a wide range of coupling strengths and never exceed 25% of the rigid-limit CH signal. (c) For comparison, dipolar INEPT/REPT curves with a relatively long dephasing time of 250 μs. The rigid-limit CH_2 and quaternary-carbon signals are seen to be undesirably large, at 25% and 100% of the rigid-limit CH signal, while the CH signal has already been visibly dephased by two-bond C−H couplings. Mobile CH_2 groups with 5-kHz CH couplings would produce large artifact peaks. Note that the C−H couplings are not scaled down by MREV-8 here, so 250 μs in REPT corresponds to 500 μs in dipolar DEPT.

dipolar DEPT spectrum with gated decoupling, as we will show below. The curves show that signals of highly mobile CH group will not be retained significantly. However, it is clear that generally a mobile CH group with a dipolar coupling comparable to the rigid-limit two-bond coupling (4 kHz) is difficult to distinguish from an unprotonated aliphatic carbon in a rigid segment, which has the same C−H coupling strength. If the dipolar DEPT experiment is to be applied to liquid crystals, lipids, or other highly mobile soft organic matter with strongly reduced $\bar{\delta}_{CH}$ values, the timings should be scaled up by at least a factor of 2; that is, a spinning frequency of 2 kHz or less should be used.

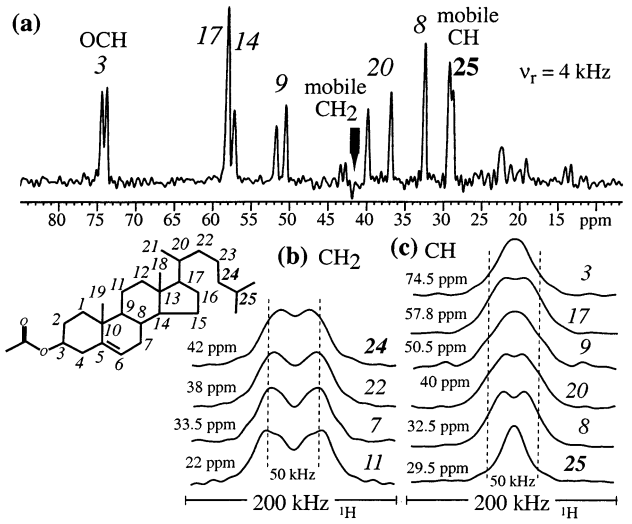

Figure 6. (a) Application of dipolar DEPT for CH signal selection in cholesteryl acetate at a spinning frequency of 4 kHz, with inverse dipolar dephasing of 40-μs duration. Signal positions of partially mobile CH_2 and CH groups are indicated. Measurement time: 2.6 h (2048 scans). (b,c) Characterization of segmental mobility by ^1H wide-line separation ^{13}C NMR, with ^{13}C decoupling during t_1 and 70-μs LGCP. ^1H cross sections of (b) CH_2 groups and (c) CH groups. Line narrowing indicates segmental mobility. The ^{13}C ppm values of the cross sections are indicated.

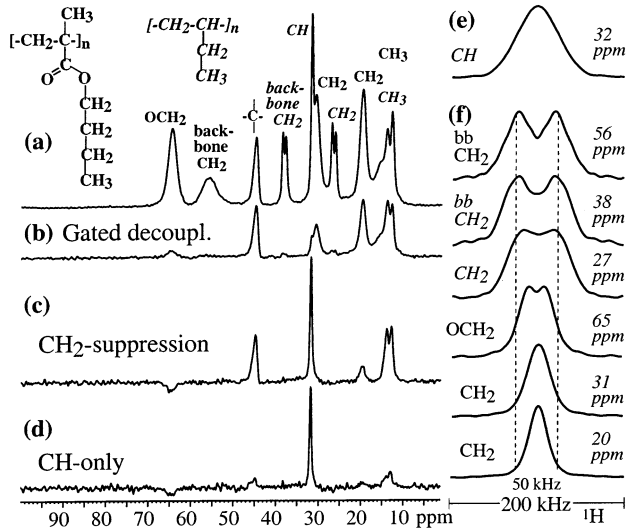

Figure 7. Application of dipolar DEPT for CH signal selection in a mixture of ca. 4/5 amorphous poly(*n*-butyl methacrylate), PnBMA, which contains several methylenes of varying mobility, and 1/5 semicrystalline isotactic poly(1-butene), iPB, which contains one CH site. The labels for iPB are printed in italics. Spinning frequency: 4 kHz, recycle delay: 2 s. (a) For reference, regular CP spectrum with 70-μs contact time. (b) Spectrum after 40-μs gated decoupling for dipolar dephasing. The mobility of the methylenes near the chain end is so high that this standard spectral-editing method does not work properly, failing to suppress the methylene signals. (c) Dipolar DEPT spectrum using the conditions of Figure 5b and a 0.5-s T_1 filter. Good CH_2 suppression is achieved despite the segmental mobility. (d) CH-only spectrum after inverse dipolar dephasing of 30-μs duration. Total measuring time for this spectrum: 2.8 h. (e,f) Characterization of segmental mobility by ^1H WISE ^{13}C NMR. In the ^1H cross sections shown, line narrowing indicates segmental mobility. The ^{13}C ppm values of the cross sections are indicated. (e) CH group. (f) CH_2 groups.

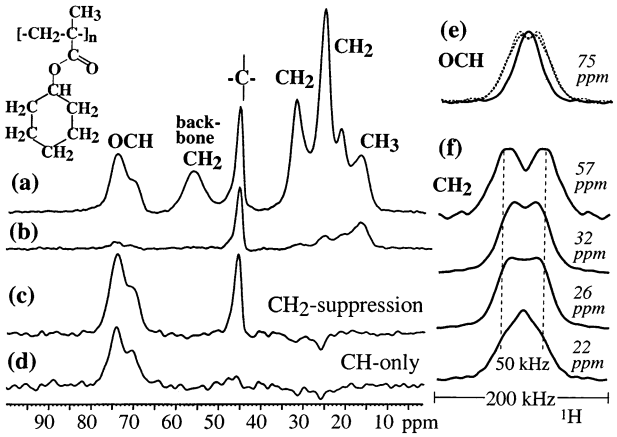

Figure 8. Application of dipolar DEPT for CH signal selection in poly(cyclohexyl methacrylate), PcHMA, an amorphous polymer with a partially mobile sidegroup that includes an OCH group, measured at a spinning frequency of 4 kHz, with 1.5-s recycle delays. (a) For reference, regular CP spectrum with 70-μs contact time. (b) Spectrum after 40-μs gated decoupling for dipolar dephasing. Suppression of some protonated-carbon signals is incomplete due to segmental mobility. (c) Dipolar DEPT spectrum using the conditions of Figure 5b and a 0.5-s T_1 filter. Good CH_2 suppression is achieved. (d) CH-only spectrum after inverse dipolar dephasing. Total measuring time for this spectrum: 2.3 h. (e,f) Characterization of segmental mobility by ^1H WISE ^{13}C NMR. Line narrowing indicates segmental mobility. The ^{13}C ppm values of the cross sections are indicated. (e) The OCH group (full line), as compared with rigid-CH-group signals in cholesteryl acetate (dashed lines), exhibits line narrowing indicating significant segmental motions. (f) CH_2 groups.

It has been suggested that similar dephasing of mobile-CH_2 signals could be achieved by dipolar INEPT.[9] However, a simulation of the symmetric REPT/INEPT experiments, Figure 5c, does not confirm this. It indicates that the CH_2 signals for some mobile groups will be very strong, exceeding those of the rigid CH sites. This has in fact been exploited for characterizing the mobility of CH_2 groups, see Figure 10 of ref 10. In the rigid limit, the CH_2 signal has just reached a secondary maximum, at ca. 25% of the CH signal, providing insufficient CH_2 suppression. In addition, the quaternary-carbon signals will be significantly stronger than those in dipolar DEPT. At the intermediate REPT dephasing time of 250 μs considered here, the CH detection efficiency has already been reduced significantly by the 4-kHz two-bond coupling included in all of the simulations of Figure 5. This shows that longer dephasing times, which would provide better CH_2 suppression, will reduce the efficiency of the REPT experiment unacceptably. Note that in the closely related simulation of REPT time curves shown in Figure 12a of ref 8, the effects of long-range C−H couplings have been neglected. Dipolar DEPT is superior to REPT/INEPT because it provides a dephasing factor exclusively for dephasing CH_2 signals, as a result of the MQ dipolar evolution. In the REPT method, the dephasing factor is tied to the CH signal reconversion and therefore cannot be optimized independently. In asymmetric REPT, the reconversion period is prolonged, but only at the expense of the CH signal, see Figure 12b of ref 8.

Figures 6−8 show experimental demonstrations of dipolar DEPT, obtained at a 4-kHz spinning frequency, that confirm the simulation of Figure 5b; in fact, they show that highly mobile-CH_2 signals will be suppressed even better than is predicted in Figure 5, due to the inverse gated decoupling that we use. To characterize the mobility of the various segments, we have recorded ^1H wide-line separation (WISE) NMR spectra[20] with ^{13}C decoupling during t_1[14] and with 70-μs Lee−Goldburg CP[15] that prevents intersegmental spin diffusion and minimizes intersegmental CP.

(20) Schmidt-Rohr, K.; Clauss, J.; Spiess, H. W. *Macromolecules* **1992**, *25*, 3273−3277.

Figure 6, which shows an application of 4-kHz dipolar DEPT and WISE NMR to cholesteryl acetate, enables comparison of dipolar DEPT with CP-based spectral editing: In Figure 7c of ref 4 and Figure 6b of ref 6, "CH-only" spectra of the same material contain residual CH_2 signals at a high (~50%) intensity level, near 42 ppm. The slight narrowing of the ^1H spectrum, obtained as a cross section from the WISE, see Figure 6b, indicates that this methylene group, which is located near the end of the aliphatic tail of the molecule, has detectable but limited mobility. In the dipolar DEPT spectrum at 5.8 kHz, Figure 4, it produces a small positive signal; at 4 kHz, Figure 6a, it is well suppressed, producing only a very small negative peak. Comparison with the negative region in the curve of Figure 5b suggests a $\bar{\delta}_{CH}$ of ca. 15 kHz. This indicates that the CP-based spectral-editing methods, which fail to suppress this signal,[4,6] are sensitive even to relatively minor motional averaging of the dipolar couplings. The neighboring CH group, which resonates at 29.5 ppm, is not fully suppressed by 40-μs of gated decoupling and also shows motional narrowing, see Figure 6c, but is nevertheless detected with good sensitivity in the dipolar DEPT spectrum of Figure 6a.

Several more highly mobile CH_2 groups are present in the side chain of poly(*n*-butyl methacrylate), PnBMA, an amorphous polymer with a reported glass-transition temperature of ca. 19 °C. Figure 7a shows the ^{13}C spectrum of this polymer, mixed with semicrystalline isotactic poly(1-butene), iPB; the iPB was added because PnBMA does not contain a CH group. The mobility of the CH_2 groups in the side chain is obvious in the 40-μs gated-decoupled spectrum, Figure 7b, where these signals are not completely dephased. Correspondingly, the WISE cross sections of the CH_2 groups, Figure 7f, show that all sites in the side chain are partially mobile. As expected, the motional narrowing is more pronounced toward the end of the side chain. Note also that the ^{13}C spectrum in Figure 7a shows progressive narrowing of the CH_2 signals down the side chain, due to conformational averaging of isotropic chemical shifts.

Figure 7c shows the dipolar DEPT spectrum of these polymers. The rigid-CH_2 signals of the chain backbones are completely suppressed. The OCH_2 group shows a small negative peak, consistent with the simulated curve of Figure 5b. The signal of the next CH_2 group in the side chain, near 30 ppm, is completely removed; this matches well with the central zero-crossing of the CH_2-curve in Figure 5b. The most mobile CH_2 group, at 20 ppm, produces a small positive peak, as expected according to Figure 5b. This peak, as well as the quaternary- and methyl-carbon signals, are removed by the subtraction of the dipolar DEPT spectrum with 30-μs gated decoupling (not shown here), to yield a clear CH-only spectrum, see Figure 7d.

The mobile-CH_2 signal at 20 ppm in the spectra of Figure 7c and d demonstrates that suppression of highly mobile CH_2-group signals in our CH-only spectrum is even better than the curve of Figure 5b indicates, due to the inverse dipolar dephasing. The signal level in Figure 7c corresponds to the calculation in Figure 5b; due to the motional averaging of the CH couplings, this signal dipolar-dephases only partially, see Figure 7b. The signal of the mobile CH_2 group is therefore removed when the dipolar-dephased DEPT spectrum is subtracted from the spectrum of Figure 7c to yield the final CH-only spectrum of Figure 7d. Any CH_2 groups of even higher mobility will also be doubly suppressed in this way.

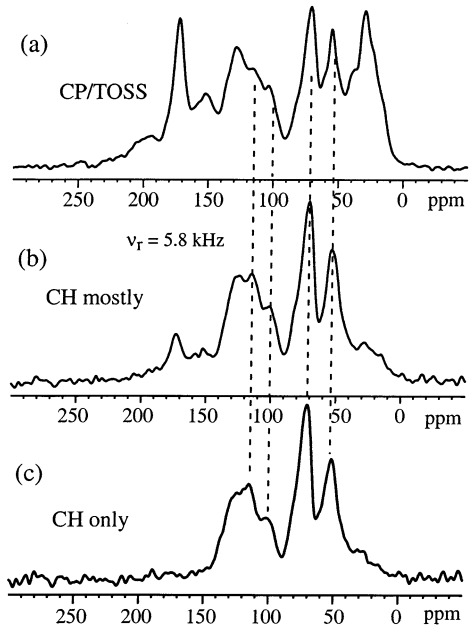

Figure 9. Application of CH spectral editing to a peat humic acid. (a) Full CP/MAS spectrum at ν_r = 5762 Hz. (b) Spectrum after multiple-quantum dephasing, with CH_2 removed, and C_{quat} and CH_3 reduced. (c) CH-only spectrum after subtraction of a spectrum with 40 μs of dipolar dephasing during TOSS. A $T_{1,C}$ filter of 0.5-s duration was used to reduce the CH_3 signal intensities. A total of 6144 scans were acquired with a recycle delay of 0.5 s (3.4 h total experiment time).

To demonstrate that the experiment retains signals of partially mobile CH groups, Figure 8 shows the application of dipolar DEPT to poly(cyclohexyl methacrylate), PcHMA, another amorphous polymer with a partially flexible sidegroup. The CP/TOSS spectrum, Figure 8a, again shows peaks that are several ppm broad, which increases the measuring time by an order of magnitude as compared to crystalline model compounds. The broadening is due to isotropic-chemical-shift dispersion as a result of the large number of conformational and packing environments in the amorphous material. As with PnBMA, standard 40-μs gated decoupling fails to remove the protonated-carbon signals completely, as a result of segmental mobility, see Figure 8b. Correspondingly, cross sections from WISE spectra, displayed in Figure 8e, show significant motional narrowing of the OCH proton line in PcHMA, as compared to CH signals in the core of cholesteryl acetate. Despite this mobility of the CH group, the CH-selection by dipolar DEPT works cleanly, Figure 8c and d. The small residual negative CH_2 signals match the expectation according to the line narrowing in the WISE spectra of Figure 8e and the curve in Figure 5b.

In summary, the data of Figures 5−8 demonstrate that our CH-selection method works even in samples that are so mobile that standard dipolar dephasing by gated decoupling of 40-μs duration fails to remove protonated-carbon signals.

CH Selection on a Humic Acid. Figure 9a−c shows the CP spectrum, the C−H mostly, and the CH-only spectrum, respectively, of a peat humic acid. The highest signal in the CH-selective spectrum is the resonance at 70 ppm. The corresponding peak in the full spectrum, Figure 9a, is predominantly from OCH groups, because OCH_2 and OCH_3 groups resonate more upfield, and dipolar-dephasing experiments have shown that the quaternary OC fraction is small. The CH-only spectrum reveals a peak centered at 53 ppm that is completely

unresolved in the nonselective CP spectrum. Its assignment to NCH groups is proven below by means of ^1H chemical-shift filtering. The relatively small fraction of nonpolar CH intensity upfield from 40 ppm is noteworthy. The overall nonpolar carbon fraction (see Figure 9a) is more than 8 times larger. Because dipolar dephasing has shown that quaternary nonpolar carbons are also rare, the major fraction of nonpolar aliphatic sites must be CH_2 and CH_3 groups. The ratio of the sp^3-hybridized CH signals in the CH-only spectrum is nearly quantitative. This is confirmed by the flat excitation profile of sp^3-hybridized CH resonances in the cholesteryl acetate spectrum of Figure 4c. The humic acid has been sufficiently purified that differential signal loss due to paramagnetic counterions is insignificant.[13,21] The main problem of quantification in CP/MAS studies of humic acids, loss of signals of unprotonated sp^2-hybridized-carbons, is irrelevant in CH-only spectra. The aromatic C−H signals are ca. 25% underrepresented in the CH-only spectrum; at the relatively high field and 5.8-kHz spinning frequency used here, TOSS reduces aromatic signals due to their large chemical shift anisotropies.

The aliphatic C−H signals observed here are typical of various peat, grassland, and tropical paddy-soil humic acids that we have investigated with the C−H selection technique. In contrast, a strong peak observed near 30 ppm in the CP-based CH-only spectrum of a peat humic acid in ref 22 is probably due to partially mobile CH_2 groups that are incompletely suppressed by CP-based spectral editing.

Simplified Sequence for Aliphatic C−H. As discussed above (see Figure 1b), the CH-selection pulse sequence can be shortened by one rotation period of semiwindowless MREV-8 decoupling if there is no proton isotropic shift/offset evolution. For aliphatic protons, this can be achieved by setting their signals on resonance. The pulse sequence with a reduced duration of the period with transverse proton magnetization, Figure 1b, increases the efficiency of the CH-selection sequence by a factor of ca. 1.3 for aliphatic CH. The overall efficiency for aliphatic CH signals is 13% relative to the regular TOSS spectrum obtained within the same length of measuring time.

Chemical-Shift-Filtered Dipolar DEPT. In CH-only spectra, the assignment of peaks between 65 and 85 ppm to OCH(C,C) and of peaks between 25 and 45 ppm to CCH(C,C) sites is unambiguous. However, signals between 45 and 60 ppm can arise from NCH or CCH(C,C) groups (see examples below). Therefore, the assignment of the peak near 53 ppm in the spectrum of the humic acid, Figure 9c, is not certain. To resolve this ambiguity, we can use differences in the ^1H chemical shifts, which are ca. 4 ppm for NCH protons and 1.5−2.5 ppm for CCH(C,C) protons. Because the dipolar DEPT experiment already involves homonuclear ^1H decoupling, setting up a ^1H chemical-shift filter[12] is quite straightforward; some details have been described above, and the pulse sequence is shown in Figure 2a.

Figure 10 demonstrates this combination of methods. Out of the full spectrum of *N*-acetyl valine shown in Figure 10a, dipolar DEPT preceded by a ^1H chemical-shift filter centered near 0.5 ppm, with a filter characteristic as shown in Figure 2c, retains the CCH(C,C) signal (and also a significant fraction of the *N*-acetyl CH_3 signal, which has a long T_1 relaxation time) but

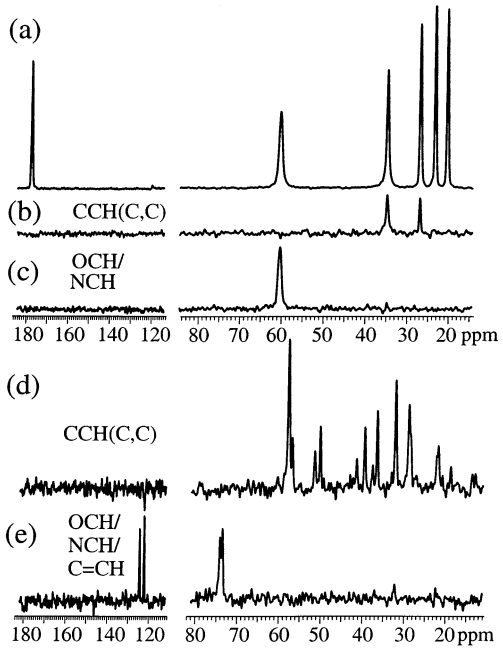

Figure 10. Dipolar DEPT combined with ^1H chemical-shift filtering. (a) Full ^{13}C spectrum of *N*-acetyl valine for reference. (b) Spectrum of the aliphatic CCH(C,C) group and the residual acetyl CH_3 signal in *N*-acetyl valine. (c) Spectrum of OCH/NCH groups in *N*-acetyl valine. (d) Spectrum of aliphatic CCH(C,C) groups in cholesteryl acetate. (e) Spectrum of OCH/NCH (and C═CH) groups in cholesteryl acetate. Peaks of CH groups near 60 ppm are shown to be due to CCH(C,C) in cholesteryl acetate and due to NCH in *N*-acetyl valine. Measuring times: 12 min for (b) and (c) each, 2 h for (d) and (e) each.

suppresses the NCH peak, see Figure 10b. Conversely, Figure 10c shows that dipolar DEPT with a ^1H chemical-shift filter centered at 5 ppm retains the NCH signal while suppressing the CCH(C,C) signal cleanly. In Figure 10d, a signal in cholesteryl acetate at the same ^{13}C chemical shift (∼60 ppm) as the NCH signal of *N*-acetyl valine is proven to be from a CCH(C,C) group, using dipolar DEPT with a 0.5-ppm-centered ^1H chemical-shift filter. The complementary experiment, with aliphatic-proton suppression by a 5-ppm-centered chemical-shift filter before the dipolar DEPT, is seen to suppress all CH peaks upfield from 60 ppm, but retains the OCH and C═CH signals. The total duration of the chemical-shift filter and dipolar DEPT MREV-8 decoupling periods is only $4.25t_r = 750\ \mu s$, which is less than double that of the "prefocused" dipolar DEPT of Figure 1c.

Chemical-Shift-Filtered Dipolar DEPT Applied to a Humic Acid. The CH-only spectrum of the humic acid in Figure 9c has revealed a strong peak at 53 ppm. Its chemical shift is consistent with only two types of CH groups: (i) NCH sites in peptide or *N*-acetyl groups, and (ii) nonpolar CH in highly branched aliphatics or in aliphatic rings; according to increment schemes, a downfield shift of ca. 6 ppm occurs for each β-substituent of a CH group, from a starting value of 23.5 ppm (see Table 3.5 of ref 23). Examples of either type of resonance are seen in Figure 10 for *N*-acetyl valine and cholesteryl acetate, respectively.

As shown for those model compounds in Figure 10, NCH and CCH(C,C) sites can be clearly distinguished by ^1H chemical-shift filtering. Figure 11 demonstrates the effect of

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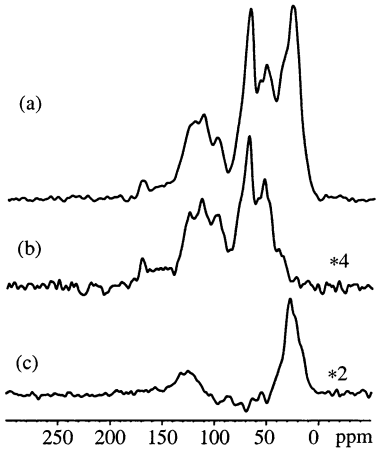

Figure 11. Demonstration of chemical-shift filtering in the peat humic acid of Figure 9. (a) For reference, full spectrum at a CP time of 70 μs. (b), (c): ^{1}H chemical-shift-filtered LGCP spectra, with $3t_r$ filters centered at (b) ca. 5 ppm and (c) ca. 0.5 ppm. The filter function in (c) corresponds to the dashed curve in Figure 2c.

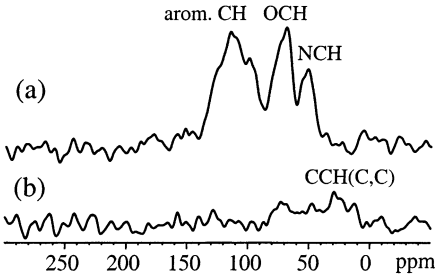

Figure 12. Dipolar DEPT combined with ^{1}H chemical-shift filtering applied to a peat humic acid, to identify the nature of the CH signal around 53 ppm in Figure 9c. (a) Spectrum of OCH, NCH, and aromatic CH groups, obtained by dipolar DEPT preceded by a ^{1}H chemical-shift filter centered near 5 ppm. The strong ^{13}C signal near 53 ppm must be due to NCH groups. (b) Spectrum of CCH(C,C) groups, obtained by dipolar DEPT preceded by a ^{1}H chemical-shift filter centered near 0.5 ppm. Only a minor contribution to the 53-ppm ^{13}C signal is observed. Measuring time: 9 h for either spectrum.

chemical-shift filtering on the full ^{13}C spectrum of the peat humic acid; recording such spectra makes it easier to fine-tune the center frequency and prepulse duration of the chemical-shift filter before it is combined with the dipolar DEPT experiment. To avoid cross talk between nonpolar aliphatic protons and other protons or OCH/NCH carbons, we used a short, 70-μs Lee−Goldburg cross polarization period. The prepulse flip angle needed for good selectivity in the humic acid was rather large, ca. 40°, while a smaller flip angle provided good results in the crystalline model compounds. We speculate that the difference may be due to the faster T_2 relaxation, driven by interaction with paramagnetic centers, in the humic acid. If longitudinal and transverse magnetization components relax differently during the chemical-shift filter, deviations from the motion on the idealized precession cone may arise.

The ^{1}H chemical-shift-filtered dipolar DEPT spectra of Figure 12, with the same chemical-shift filter settings as for the CP spectra in Figure 11, prove that the methine peak at 53 ppm is mostly due to NCH sites. In Figure 12a, the ^{1}H chemical-shift filter is centered near 5 ppm, suppressing CCH(C,C) signals. In this spectrum, the ^{13}CH peak at 53 ppm is strong. In contrast, with a ^{1}H chemical-shift filter retaining only CCH(C,C) peaks, the overall signal is low, including that near 53 ppm. The peak at that frequency is thus clearly identified as being predominantly due to NCH, not CCH(C,C) groups. As a cross-check, one notes that the sum of the two spectra of Figure 12 shows good agreement with the full CH-only spectrum in Figure 9c. The assignment of the 53-ppm peak to NCH groups has also been confirmed by SPIDER experiments,[24] which yield spectra selectively of the carbons bonded to nitrogen. For humic substances, the present dipolar DEPT method provides more quantitative information on the intensity of the NCH peak, because the technique is less susceptible to selective T_2 dephasing than is SPIDER NMR. In addition, dipolar DEPT has the advantage of requiring only ^{13}C−^{1}H double-resonance, rather than ^{14}N−^{13}C−^{1}H triple-resonance equipment.

Comparison with DEPT in Solution NMR. The pulse sequence of the dipolar DEPT experiment resembles that of standard *J*-coupling-based DEPT in solution NMR. Nevertheless, the adaptation of the sequence to solid-state NMR is not completely trivial. To avoid cancellation of the dipolar coupling by MAS, the simultaneous ^{1}H and ^{13}C pulses in regular DEPT must be displaced with respect to each other in the magic-angle-spinning version. Because of this shift in the pulse timing, dipolar DEPT may appear to be intermediate between DEPT and INEPT/REPT, where the second ^{1}H pulse and first ^{13}C 90° pulse are applied simultaneously. Nevertheless, the crucial role of multiple quantum coherence in both DEPT and our solid-state NMR experiment, but not in INEPT, reveals the closer similarity between DEPT and our method. In *J*-DEPT, heteronuclear coherence is generated during the period before simultaneous ^{13}C 90° and ^{1}H 180° pulses; in dipolar DEPT, due to refocusing of the dipolar coupling by MAS, the ^{13}C 90° pulse is displaced from the ^{1}H 180° pulse by $t_{CH} = t_r/4$, and the heteronuclear coherence is generated during this short time period. Nevertheless, the period t_r before the ^{1}H 180° pulse in Figure 1c is still necessary to remove ^{1}H isotropic chemical-shift modulation by the time when the second ^{1}H 90° pulse is applied.

Another solid-state NMR modification is the ^{13}C 180° pulse that is applied to improve dipolar recoupling during the evolution of the MQ coherence. Furthermore, a similar MAS-imposed requirement is the above-mentioned separation of excitation and reconversion of the heteronuclear coherence by an integer number of rotation periods ($1t_r$ in our case), which is necessary to produce an all-positive dipolar modulation factor.

In solution NMR, $t_{CH} = 1/J_{CH}$ provides complete conversion of coherence from single-spin to heteronuclear coherence, and from two-spin heteronuclear to three-spin heteronuclear coherence in CH_2 groups during the MQ evolution.[1] In contrast, in the dipolar experiment the cosine-modulated unconverted terms (see the Appendix) must also be considered. For this reason, variation of the flip angle of the last ^{1}H pulse as is customary in regular DEPT to select CH_2 and CH_3 groups[1] will not work well in solid-state NMR. Another obvious difference between the solid-state and solution-NMR sequences is that ^{1}H homonuclear dipolar decoupling by multiple-pulse irradiation must be applied. Only at higher spinning speeds, which can be reached only with smaller rotors of reduced sensitivity, does magic-angle spinning provide the necessary homonuclear decoupling.[8,9]

Comparison with Previous Solid-State NMR Techniques for CH Selection. More than previous CH spectral-editing

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schemes, the MQ-based selection introduced here relies on the difference between the C−H spin-pair and the three-spin CH_2 system. Other approaches, in particular those relying on cross-polarization dynamics, are mostly based on differences between effective proton dipolar fields seen by the ^{13}C spin. In such a scheme, a partially mobile CH_2 group with its reduced CH couplings will resemble a CH group, showing, for instance, a similarly delayed CP inversion behavior.[4] An example of a strong residual CH_2 artifact peak in CP-based spectral editing, at >50% of the CH signals, can be seen in Figure 6b of ref 6 or Figure 7c of ref 4; our study showed, see Figure 6, that the mobility of this group in cholesteryl acetate is not even particularly high (motional narrowing factor of ca. 0.8). In dipolar DEPT at 5.8-kHz spinning frequency, this CH_2 signal is small; at 4 kHz it is effectively suppressed. The simulation of Figure 5b and the applications to polymers with various mobile CH_2 groups, see Figures 7 and 8, show excellent CH_2 suppression over a wide range of motionally averaged C−H couplings in 4-kHz dipolar DEPT, while large mobile-CH_2 artifacts, as well as strong quaternary-carbon signals, are expected in REPT/INEPT, see Figure 5c. The first null point of the CH_2 signal that is crucial in the REPT/INEPT method depends on the C−H coupling strengths. Therefore, signals of mobile CH_2 groups are not well suppressed. This has in fact been exploited for characterizing the mobility of CH_2 groups, see Figure 10 of ref 10. Signals of mobile CH_2 groups, and even of unprotonated sp^2-hybridized carbons, are also observed in the CP-based "CH-only" spectra of ref 22. In contrast, the dipolar DEPT CH selection sequence works even when the mobility is so high that standard dipolar dephasing by 40-μs gated decoupling fails to suppress the signals of CH and CH_2 groups, see Figures 7 and 8.

As compared to methods such as REPT[8,9] that require high-speed, small-volume rotors, our method usually provides significantly better sensitivity, being applicable in the high-speed range of relatively large (7-mm diameter) rotors. For instance, the sensitivity of CH groups in the Bruker 7-mm system is 2.5 times better than that of 4-mm rotors, corresponding to 6-fold savings in spectrometer time. Note also that refs 8 and 9 demonstrate only selective CH_2-suppression, which is easier to achieve and for which our method has up to 30% efficiency. The CH-only selection, which is indispensable for systems such as humic acids with heavy peak overlap, reduces the sensitivity by a factor of 2.

Perspective. In summary, the MQ-based method presented here combines good suppression of signals of both rigid and mobile CH_2 groups with relatively good absolute sensitivity, being applicable to relatively large rotors. Once the sequence has been implemented, the only parameters to be set are the power levels to achieve the correct 90° and 180° pulse lengths of ^1H and ^{13}C, which is part of any routine setup of CP/TOSS NMR. In other words, the multiple-pulse irradiation is so short that no tedious CRAMPS tune-up is required. In half a dozen measuring periods over a time period of a year, we have applied this method successfully to various samples of natural organic matter. Replacing semiwindowless MREV-8 with superior homonuclear decoupling sequences, such as the frequency-switched Lee−Goldburg (FSLG) scheme,[25,26] might improve the efficiency further. The easy combination with ^1H chemical-shift filtering for distinguishing different types of CH groups is a new feature in spectral-editing methodology.

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Summary

A robust method for CH spectral editing has been introduced. It is based on dipolar dephasing of heteronuclear multiple-quantum coherence. This leaves C−H spin-pair coherence unaffected, while the heteronuclear coherence of CH_2 groups is dephased quickly, even in the presence of partial mobility. A DEPT coherence transfer, which is asymmetric for a CH_2 group, further reduces methylene signals. The sequence resembles DEPT, although pulse timings are modified to achieve recoupling of CH dipolar interactions. The residual signals of quaternary carbons, and partially also of methyl groups, are removed by subtracting out a spectrum with dipolar dephasing before detection. No empirical scaling factors are needed in the data processing, in contrast to CH spectral editing based on CP dynamics. The overall efficiency of 10−14% compares favorably with that of other CH spectral-editing methods.[7] At a spinning frequency of 4 kHz, suppression of signals of partially mobile CH_2 signals is excellent even in cases where standard 40-μs gated decoupling fails, and significantly better than with previous spectral-editing methods. This has been predicted theoretically and confirmed by experiments on amorphous polymers with flexible sidegroups. Residual OCH_3 signals at a relative level of ca. 20% are the potentially most problematic residual nonmethine signals. However, T_{1C} filtering can usually reduce these further to an acceptable level. The application to a humic acid demonstrates that the method performs well for challenging samples. To distinguish different types of methines with similar carbon chemical shifts, the dipolar DEPT method has been combined with ^1H chemical-shift filtering. Within 0.74 ms of total homonuclear ^1H decoupling, CCH(C,C) residues are clearly distinguished from other methines, in particular NCH groups. In the humic acid, this confirms that the methine peak near 53 ppm is predominantly due to NCH moieties.

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Appendix: Full Calculations of CH_n Signals

Above, the effects of the pulse sequences of Figure 1b and c were discussed only in a semiquantitative manner. Calculations of the evolution of CH_n groups are relatively straightforward, because the various heteronuclear dipolar coupling terms in the Hamiltonian commute.[27] The chemical shifts do not need to be considered; because of their commutation with the heteronuclear coupling, the chemical-shift evolution and refocusing occur independently.

In the following, a product-operator calculation of the density operator (i.e., state of the spin system) is performed in four steps: excitation, MQ evolution, reconversion, and identification of the observable terms at the start of detection. The calculation is restricted to the relevant terms of the density operator selected by the phase cycle. For each step, the terms retained at the start of the following step are highlighted in bold. The rotations due to the 90° pulses before and after the MQ evolution periods

(26) Bielecki, A.; Kolbert, A. C.; de Groot, H. J. M.; Griffin, R. G.; Levitt, M. H. *Adv. Magn. Reson.* **1990**, *14*, 111−124.
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have been taken into account by changes of indices between steps. At the end of each calculation, the signal intensity for the long-time powder average is given. Note that the *z*-axis of the ^{1}H reference frame is the effective field direction of MREV-8,[27] which is along (1, 0, 1) in the standard rotating frame. The *y*-axis of the ^{1}H reference frame coincides with the *y*-axis of the standard ^{1}H rotating frame.

CH:

$$\text{Excitation:}\quad \rho(t_{CH}) = I_x c + \mathbf{2S_z I_y}\, \boldsymbol{s} \tag{1a}$$

$$\text{MQ evolution:}\quad \rho(t_{CH} + t_{MQ}) = \mathbf{2S_y I_y}\boldsymbol{s} \quad \text{unchanged} \tag{1b}$$

$$\text{Reconversion:}\quad \rho(2t_{CH} + t_{MQ}) = 2S_y I_z sc - \mathbf{S_x}\boldsymbol{s^2} \tag{1c}$$

$$\text{Observable:}\quad \rho_{obs}(2t_{CH} + t_{MQ}) = -S_x s^2 \rightarrow 0.5 \tag{1d}$$

CH_2:

Excitation:

$$\rho(t_{CH}) = I_{xA}c_A + I_{xB}c_B + \mathbf{2S_z(I_{yA}}\boldsymbol{s_A} + \mathbf{I_{yB}}\boldsymbol{s_B}) \tag{2a}$$

MQ evolution:

$$\rho(t_{CH} + t_{MQ}) = \mathbf{2I_{yA}}\boldsymbol{s_A}(\mathbf{S_y}\boldsymbol{c'_B} - 2I_{zB}S_x s'_B)) + \mathbf{2I_{yB}}\boldsymbol{s_B}(\mathbf{S_y}\boldsymbol{c'_A} - 2I_{zA}S_x s'_A) \tag{2b}$$

Reconversion:

$$\rho(2t_{CH} + t_{MQ}) = \mathbf{2[I_{zA}}\boldsymbol{s_A c'_B} + \mathbf{I_{zB}}\boldsymbol{s_B c'_A}](S_y(c_A c_B - 4I_{zA}I_{zB}s_A s_B) - \mathbf{2S_x(I_{zA}}\boldsymbol{s_A c_B} + \mathbf{I_{zB}}\boldsymbol{c_A s_B})) \tag{2c}$$

Observable:

$$\rho_{obs}(2t_{CH} + t_{MQ}) = -S_x({s_A}^2 c_B c'_B + {s_B}^2 c_A c'_A) \rightarrow \mathbf{0} \tag{2d}$$

CH_3:

Excitation:

$$\rho(t_{CH}) = (I_{xA} + I_{xB} + I_{xC})c + \mathbf{2S_z(I_{yA} + I_{yB} + I_{yC})s} \tag{3a}$$

MQ evolution:

$$\rho(t_{CH} + t_{MQ}) = \mathbf{2}\boldsymbol{s}\{\mathbf{S_y}[\boldsymbol{c'^2}(\mathbf{I_{yA} + I_{yB} + I_{yC}}) - 4s'^2(I_{yA}I_{zB}I_{zC} + I_{yB}I_{zC}I_{zA} + I_{yC}I_{zA}I_{zB})] - S_x 2c's'[I_{yA}(I_{zB} + I_{zC}) + I_{yB}(I_{zC} + I_{zA}) + I_{yC}(I_{zA} + I_{zB})\} \tag{3b}$$

Reconversion:

$$\rho(2t_{CH} + t_{MQ}) = \mathbf{2(I_{zA} + I_{zB} + I_{zC})}\boldsymbol{sc'^2} \{S_y[c^3 - 4(I_{zA}I_{zB} + I_{zA}I_{zC} + I_{zB}I_{zC})s^2 c] - \mathbf{S_x}[\boldsymbol{c^2 s}\mathbf{2(I_{zA} + I_{zB} + I_{zC})} - 8I_{zA}I_{zB}I_{zC}s^3]\} \tag{3c}$$

Observable:

$$\rho_{obs}(2t_{CH} + t_{MQ}) = -S_x 3s^2 c^2 c'^2 \rightarrow 3/16 = 0.1875 \tag{3d}$$

Here, $c = \cos\Phi(t_{CH})$ and $s = \sin\Phi(t_{CH})$, where $\Phi(t_{CH})$ is the phase acquired under the action of the CH coupling during the heteronuclear-coherence excitation and also the reconversion period, both of duration t_{CH}. Similarly, $c' = \cos\Phi_{MQ}$ and $s' = \sin\Phi_{MQ}$, where Φ_{MQ} is the phase acquired by the MQ coherence under the CH dephasing. Note that the phases of a CH_3 group are reduced by $-1/3$ relative to those in rigid CH or CH_2 groups. The MQ evolution resembles the regular CH dipolar evolution of a CH_{n-1} group, because a given multiple-quantum coherence term commutes with the C−H dipolar coupling of the same proton. When comparing with the calculation for regular DEPT,[1] note that the standard DEPT timings are chosen as $1/J_{CH}$ so that all of the cosine coefficients (c, c', etc.) are zero, while all of the sine coefficients are unity. Dipolar-coupling frequencies are orientation dependent; therefore, with dipolar DEPT in an unoriented sample, no single time can be found where all cosine coefficients are zero and all sine coefficients are unity.

The CH_2 signal of eq 2d contains two essentially independent terms (c_B and c'_B) that are each very small in the powder average and therefore suppress the signal very efficiently, see Figure 5b. The c'_B term reflects the dipolar dephasing of the multiple-quantum coherence, while the other term is due to the asymmetry of the excitation, where each proton evolves in the field of the carbon, and the reconversion, where the carbon evolves in the field of the two protons.[8]

In the calculations of the CH_3 coherences, we have used that the C−H couplings of the three protons are equal, due to motional averaging by fast rotational jumps of the methyl group. As described above, the nonvanishing signal intensity in eq 3d can be explained by partial cancellation of the C−H dipolar fields of two of the protons.

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