currently incorporating the peptide group contributions in the same way. Our limited experience with plastocyanin suggests that relatively small adjustments in a fairly "high-resolution" NMR structure can yield chemical shift estimates whose errors are no larger than those for the crystal structures considered here.

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Registry No. Lysozyme, 9001-63-2; dihydrofolate reductase, 9002-03-3; ribonuclease T-1, 9026-12-4; trypsin inhibitor, 9035-81-8; ribonuclease A, 9001-99-4; serine protease inhibitor, 37205-61-1; tendamistat, 86596-25-0.

Supplementary Material Available: Table of observed shifts, along with those calculated in the final parameter set, for all of the observations used in the calculations for the protons attached to a carbon atom (33 pages). Ordering information is given on any current masthead page.

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Double-Quantum Filtering in Magic-Angle-Spinning NMR Spectroscopy: An Approach to Spectral Simplification and Molecular Structure Determination

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Abstract: We show how simple radio-frequency pulse sequences can be used to select resonances from pairs of magnetic dipole-coupled nuclear spins and to suppress resonances from isolated spins in magic-angle-spinning (MAS) NMR experiments, thereby simplifying the spectra and providing information about internuclear distances. Non-zero average dipole–dipole couplings are generated by means of DRAMA sequences (Tycko, R.; Dabbagh, G. *Chem. Phys. Lett.* **1990**, *173*, 461-465). Double-quantum filtering techniques are then used to select the resonances of coupled spin pairs. Two experimental demonstrations of double-quantum filtering in ^{13}C MAS spectra of mixtures of organic compounds are presented, one in which the NMR signal from labeled carbon sites in a doubly ^{13}C-labeled compound ($(CH_3)_2C(OH)SO_3Na$) is selected while natural abundance signals from an unlabeled compound (*N*-acetyl-L-valine) are suppressed, and one in which natural abundance signals from a singly ^{13}C-labeled compound (methionine·HCl) are selected while signals from an unlabeled compound (*N*-acetyl-L-valine) are suppressed. The implications of these experiments, including potential applications to the simplification of MAS spectra of complex molecules, such as biopolymers, and to the determination of the structure of selected regions of complex molecules, are analyzed in detail.

Introduction

Magic angle spinning (MAS) is very widely used to obtain high resolution in nuclear magnetic resonance (NMR) spectra of polycrystalline and noncrystalline solids. High resolution is achieved because MAS has the effect of averaging out the (second-rank) orientation-dependent parts of nuclear spin interactions, principally anisotropic chemical shifts (CSA) and nuclear magnetic dipole–dipole couplings. This paper addresses two important problems with the MAS technique. First, the fact that dipole–dipole couplings are averaged out in a conventional MAS experiment also means that the structural information contained in these couplings is lost. Second, even the line narrowing that results from MAS is very often insufficient to produce a spectrum in which resonances from inequivalent nuclei are resolved, for example in ^{13}C MAS spectra of large molecules such as peptides and small proteins where there are many inequivalent nuclei or in spectra of noncrystalline materials such as amorphous synthetic polymers where the MAS lines are inhomogeneously broadened as a consequence of disorder. In such situations, it is desirable to simplify the spectrum by selecting certain resonances of interest and suppressing others.

Several groups have dealt with the first problem by devising somewhat more complicated MAS techniques that allow the retention of both the high resolution of MAS and the information contained in homonuclear[1-14] and heteronuclear[15-20] dipole–dipole couplings. In particular, we have shown[1] that homonuclear dipole–dipole couplings can be prevented from averaging out during

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MAS by the application of simple resonant radio-frequency (rf) pulse sequences in synchrony with the sample rotation. We refer to such sequences by the acronym DRAMA (dipolar recovery at the magic angle). If a DRAMA sequence is applied in one time period of a two-dimensional ^{13}C NMR experiment on a ^{13}C-labeled organic compound, for example, a two-dimensional spectrum can be obtained in which dipolar powder patterns appear along one frequency axis and isotropic shifts appear along the other.[1]

More generally, DRAMA sequences can be used to turn dipole–dipole couplings on and off at will during an MAS experiment. DRAMA sequences can therefore be incorporated into a double-quantum filtering[21-25] technique that addresses the second problem. We show in this paper that it is possible to use double-quantum filtering to pick out only those resonances that arise from dipole-coupled pairs of spins in an MAS spectrum and to suppress resonances from isolated, uncoupled spins. The ability to pick out resonances from pairs of dipole-coupled spins is potentially quite important, as it permits a simplification of MAS spectra of complex molecules and aids in the assignment of resonances and the extraction of information about molecular structures from the spectra. The applications of double-quantum filtering using DRAMA sequences are discussed below in greater detail.

Two earlier demonstrations of double-quantum filtering via dipole–dipole couplings in MAS NMR spectroscopy have been reported. Menger et al.[26] showed that double-quantum coherence can be prepared in a MAS experiment in the same manner as in a nonspinning experiment provided that the preparation of double-quantum coherences takes place in less than one sample rotation period. In practice, this limits the application of the technique to strongly coupled, i.e. directly bonded, nuclei or to very slow spinning rates. Meier and Earl[2,3] used rather elaborate "time-reversal" sequences to prevent dipole–dipole couplings from averaging out during MAS experiments. The technique described in this paper is considerably simpler. Our results are the first reported for pairs of ^{13}C nuclei that are not directly bonded, suggesting that the double-quantum filtering technique described in this paper will have wider applicability than previous techniques.

For completeness, we note that it is also possible to carry out double-quantum filtering in MAS experiments by making use of *scalar*, rather than dipole–dipole, couplings.[27] Since scalar couplings are transmitted through chemical bonds and are sufficiently strong only between directly bonded nuclei, double-quantum filtering via scalar couplings is not applicable to the experiments described below. Double-quantum coherences have also been prepared in ^{2}H MAS NMR experiments,[28,29] where the large ^{2}H *quadrupole* coupling allows single-spin, double-quantum coherences to be prepared efficiently in a period that is much less than the sample rotation period.

Theory

As shown previously,[1] the combination of MAS and the pulse sequence in Figure 1a has the effect of producing an average

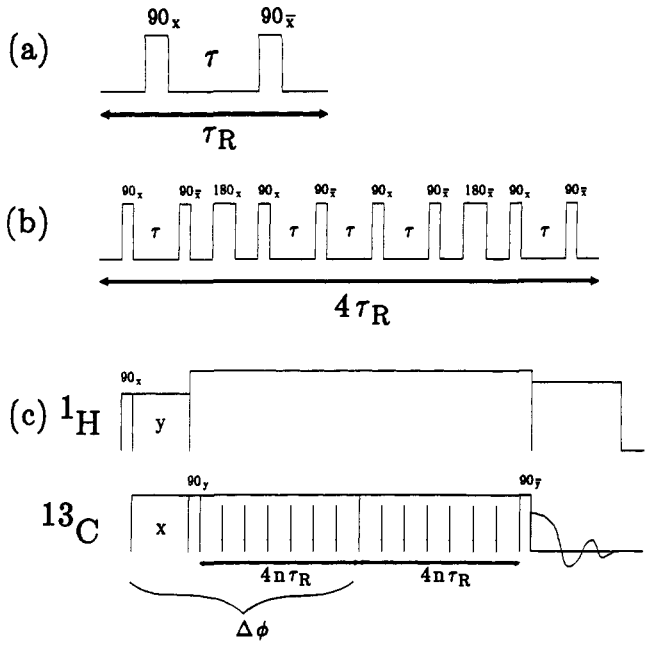

Figure 1. (a) DRAMA pulse sequence element that leads to non-zero average dipole–dipole couplings between nearby nuclei in a MAS NMR experiment. As usual, θ_α denotes an rf pulse that rotates spin angular momenta by the angle θ about the axis α in the rotating frame. τ_R is the sample rotation period. (b) DRAMA sequence based on part a designed to average out resonance offsets and chemical shift anisotropies as well. (c) Double-quantum filtering sequence used to obtain spectra in Figures 3b and 4b. Standard cross-polarization and proton decoupling are employed. The DRAMA sequence in part b is repeated n times to generate double-quantum coherences, then n times again to convert double-quantum coherences back to observable nuclear magnetization. Phase cycling is applied to the indicated ^{13}C pulses in order to cancel NMR signals that do not derive from double-quantum coherences. Signals from scans taken with the overall rf phase shift $\Delta\phi = (\pi/2)k$ and with the NMR signals multiplied by -1^k are added together, with $k = 0, 1, 2, 3$. Additional phase cycling (not shown) to cancel pulse ringdown and quadrature artifacts is also employed. Unmarked delays in parts a and b are of equal length and are adjusted so that the overall cycle times are τ_R and $4\tau_R$, respectively.

nuclear magnetic dipole–dipole coupling $\langle H_d(t)\rangle$ between spins 1 and 2 of the form

$$\langle H_d(t)\rangle = dF(\theta,\phi)(T_{zz} - T_{yy}) \quad (1)$$

$$T_{\alpha\alpha} \equiv 3I_{\alpha,1}I_{\alpha,2} - \vec{I}_1\cdot\vec{I}_2 \quad (2)$$

where d is the coupling constant (defined to be the maximum splitting in the nonspinning spectrum for a pair of spin-$^1/_2$ nuclei), F is a function of the orientation of the internuclear vector specified by angles θ and ϕ in an axis system fixed in the sample rotor, and $\vec{I}_i$ is the spin angular momentum vector operator for spin i. $\langle H_d(t)\rangle$ is the average coupling over one sample rotation period τ_R, in the limit of δ-function pulses. Figure 1b shows a variant of the sequence in Figure 1a that produces the same $\langle H_d(t)\rangle$ in the limit of δ-function pulses, but in addition averages out resonance offsets and CSA over a period $2\tau_R$ and has a vanishing first correction term to the average resonance offset over a period $4\tau_R$. Since resonance offsets and CSA are not negligible in our experiments, we use the DRAMA sequence in Figure 1b.

The complete pulse sequence used to obtain the double-quantum filtered ^{13}C MAS spectra presented below is shown in Figure 1c. Cross-polarization from protons to carbons, followed by a $\pi/2$ pulse applied at the ^{13}C frequency, creates a spin state in which ^{13}C nuclei are polarized along the z axis of the rotating frame. For a pair of spins, this state is described by a density operator proportional to $I_{z,1} + I_{z,2}$. The DRAMA sequence in Figure 1b is then applied for a time $4n\tau_R$ to generate double-quantum coherence, described by components of the density operator proportional to $I_{+,1}I_{+,2}$ and $I_{-,1}I_{-,2}$. As in other examples of multiple-quantum filtering, phase cycling is used to cancel out other

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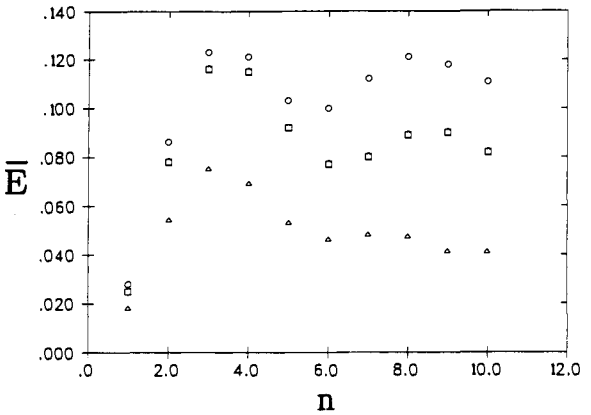

Figure 2. Calculated efficiency $\bar{E}(n)$ of the double-quantum filtering technique in Figure 1c. The efficiency is the ratio of the signal in a filtered spectrum to that in an unfiltered MAS spectrum with the same total number of scans. The calculations assume randomly oriented pairs of dipole-coupled spin-$^1/_2$ nuclei with coupling constant d = 1.0 kHz, τ_R = 250.0 μs, τ = 117.5 μs, and 10.0 μs π pulses. The two spins have axially symmetric chemical shift tensors, with unique axes parallel to the internuclear vector and with anisotropies $\Delta\sigma_i$. The resonance offsets are $\Delta\nu_i$. Circles: $\Delta\nu_1 = 0$; $\Delta\nu_2 = 0$; $\Delta\sigma_1 = 0$; $\Delta\sigma_2 = 0$. Squares: $\Delta\nu_1 = 0.5$ kHz; $\Delta\nu_2 = -0.5$ kHz; $\Delta\sigma_1 = 1.0$ kHz; $\Delta\sigma_2 = -1.0$ kHz. Triangles: $\Delta\nu_1 = 1.0$ kHz; $\Delta\nu_2 = -1.0$ kHz; $\Delta\sigma_1 = 2.0$ kHz; $\Delta\sigma_2 = -2.0$ kHz.

components of the density operator at this point. Double-quantum coherence is then converted back to polarization along z by application of the same DRAMA sequence for an additional time $4n\tau_R$. NMR signals are acquired after a final $\pi/2$ pulse. Since isolated spins cannot become involved in double-quantum coherence, their contribution to the signal is canceled out by the phase cycling. Only signal from coupled groups of spins, primarily pairs in our experiments, can survive.

The signal-to-noise ratio in the double-quantum filtered spectrum depends obviously on the efficiency of the double-quantum filter, i.e. the probability amplitude E for converting a density operator $I_{z,1} + I_{z,2}$ to a linear combination of $I_{+,1}I_{+,2}$ and $I_{-,1}I_{-,2}$ and then back to $I_{z,1} + I_{z,2}$. For a particular pair of spins, E depends on d, θ, ϕ, n, τ_R, and the details of the DRAMA sequence. Let $U(\theta,\phi,n)$ be the evolution operator for a particular DRAMA sequence and particular values of τ_R and d. Then $E(\theta,\phi,n)$ is given by

$$E(\theta,\phi,n) = -\mathrm{Tr}\{I_x^{1,4}UI_z^{1,4}U^{-1}\}\mathrm{Tr}\{I_z^{1,4}UI_x^{1,4}U^{-1}\} - \mathrm{Tr}\{I_y^{1,4}UI_z^{1,4}U^{-1}\}\mathrm{Tr}\{I_z^{1,4}UI_y^{1,4}U^{-1}\} \quad (3)$$

$$= -\tfrac{1}{4}\mathrm{Re}(U_{1,1}U_{4,1}^* - U_{4,4}U_{1,4}^*)\mathrm{Re}(U_{1,1}U_{1,4}^* - U_{4,4}U_{4,1}^*) + \tfrac{1}{4}\mathrm{Im}(U_{1,1}U_{4,1}^* + U_{4,4}U_{1,4}^*)\mathrm{Im}(U_{1,1}U_{1,4}^* + U_{4,4}U_{4,1}^*) \quad (4)$$

$$U_{1,1} \equiv \langle++|U(\theta,\phi,n)|++\rangle \quad (5)$$

$$U_{4,4} \equiv \langle--|U(\theta,\phi,n)|--\rangle \quad (6)$$

$$U_{1,4} \equiv \langle++|U(\theta,\phi,n)|--\rangle \quad (7)$$

where the "fictitious spin-$^1/_2$" operators[30,31] are defined by

$$I_z^{1,4} \equiv \frac{1}{2}(I_{z,1} + I_{z,2}) \quad (8)$$

$$I_x^{1,4} \equiv \frac{1}{2}(I_{+,1}I_{+,2} + I_{-,1}I_{-,2}) \quad (9)$$

$$I_y^{1,4} \equiv \frac{1}{2i}(I_{+,1}I_{+,2} - I_{-,1}I_{-,2}) \quad (10)$$

It is a straightforward matter to calculate $U(\theta,\phi,n)$, and hence $E(\theta,\phi,n)$, numerically for a pair of coupled spin-$^1/_2$ nuclei.[32] Averaging over θ and ϕ then gives the orientationally averaged amplitude $\bar{E}(n)$ that is the efficiency of the double-quantum filtering sequence when applied to an unoriented polycrystalline or noncrystalline sample. $\bar{E}(n)$ is the factor by which the NMR signal from coupled pairs of spins is attenuated in a double-quantum filtering experiment when compared with the signal in an unfiltered MAS experiment.

Figure 2 shows calculated values of $\bar{E}(n)$ for the sequence in Figure 1c. The maximum of $\bar{E}(n)$ for this simple sequence is about 0.12. Approaches to improving the efficiency are discussed below.

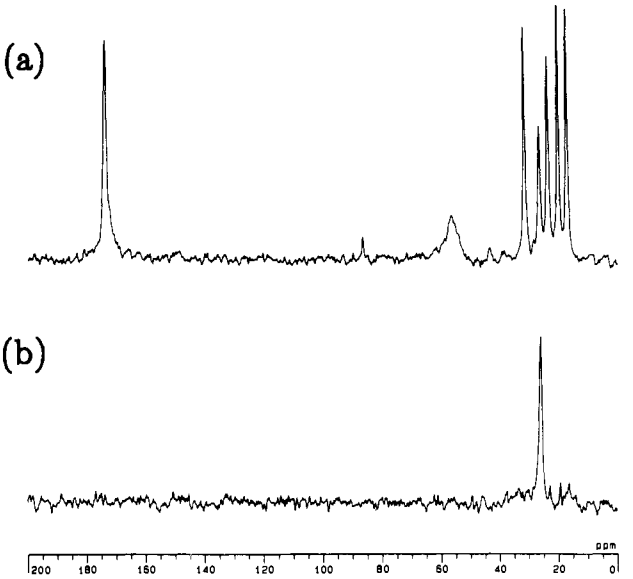

Figure 3. Unfiltered (a) and double-quantum filtered (b) ^{13}C MAS spectra at 25.4 MHz of a mixture of $(CH_3)_2C(OH)SO_3Na$, in which 5% of the molecules are ^{13}C labeled at both methyl positions, and unlabeled *N*-acetyl-L-valine: (a) 16 scans; (b) 1600 scans; τ_R = 250.0 μs, τ = 117.5 μs. Total sample weight = 266 mg. The resonance from the $(CH_3)_2C(OH)SO_3Na$ methyl carbons is selected. Natural abundance resonances are suppressed. (^{13}C carrier frequency set to 44 ppm.)

Experimental Methods

Spectra described below were obtained with a Chemagnetics CMX NMR spectrometer operating at a ^{13}C NMR frequency of 25.4 MHz. Similar results were also obtained at 100.4 MHz. A modified Doty Scientific MAS probe was used along with a home-built system for stabilizing the sample spinning rate to within 1 ppt throughout the course of an experiment. The ^{13}C rf level was set to 50 kHz. The proton decoupling level was 105 kHz during application of the DRAMA sequence at the ^{13}C frequency. This rather high level is necessary to achieve efficient decoupling while a pulse sequence is being applied to carbon spins, as previously observed.[33] Decoupling levels during the acquisition of ^{13}C signals were 73 kHz for Figure 3 and 105 kHz for Figure 4. ^{13}C pulses were delivered through an ENI 5100L-NMR amplifier; proton pulses were delivered through an ENI 550L amplifier followed by a Henry Radio 1002A amplifier tuned to 100.9 MHz. Tune-up sequences were used to set pulse lengths and minimize pulse phase transients as described previously.[32]

$(CH_3)_2C(OH)SO_3Na$ (bisulfite adduct of acetone), in which 5% of the molecules are ^{13}C labeled at both methyl positions, was prepared by reaction of a mixture of double-labeled and unlabeled acetone with $NaHSO_3$(aq), followed by precipitation with ethanol. To prepare methionine·HCl in which 50% of the molecules are ^{13}C labeled at the *S*-methyl position, a mixture of labeled and unlabeled methionine was dissolved in methanol. The desired product precipitated when HCl(g) was bubbled through the solution.

Results

Figure 3a shows the ^{13}C MAS spectrum of a mixture of 5% double-labeled $(CH_3)_2C(OH)SO_3Na$ and unlabeled *N*-acetyl-L-valine, obtained with cross-polarization. The spectrum is the result of 16 scans. The proportions of the two compounds in the mixture are adjusted so that the $(CH_3)_2C(OH)SO_3Na$ methyl resonance has about half the intensity of the *N*-acetyl-L-valine resonances.

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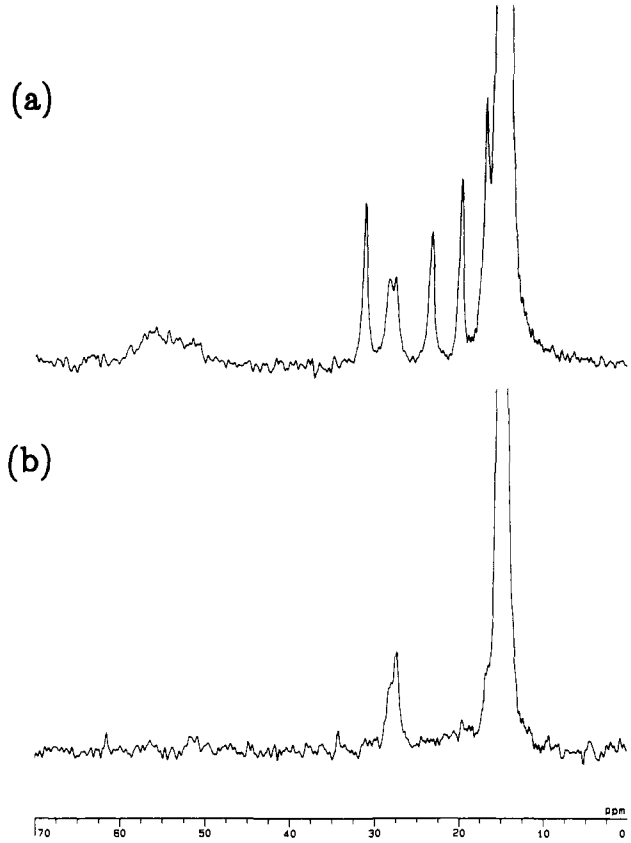

Figure 4. Unfiltered (a) and double-quantum filtered (b) ^{13}C MAS spectra at 25.4 MHz of a mixture of methionine·HCl, in which 50% of the molecules are ^{13}C labeled at the *S*-methyl position, and unlabeled *N*-acetyl-L-valine: (a) 16 scans; (b) 4800 scans; τ_R = 235.0 μs, τ = 110.0 μs. Total sample weight = 155 mg. The natural abundance resonances from the β and γ carbons of methionine·HCl are selected. Natural abundance resonances from *N*-acetyl-L-valine are suppressed. (^{13}C carrier frequency set to 34 ppm.)

Figure 3b shows a double-quantum filtered MAS spectrum of the same sample resulting from a total of 1600 scans using the sequence in Figure 1c. The *N*-acetyl-L-valine resonances are substantially suppressed, while the signal-to-noise ratio for the $(CH_3)_2C(OH)SO_3Na$ methyl resonance is about the same as in Figure 3a. A quantitative comparison of the signal intensities in filtered and unfiltered spectra, taking into account signal from unlabeled $(CH_3)_2C(OH)SO_3Na$ in unfiltered spectra, indicates an experimental efficiency $\bar{E}(2)$ = 0.105 for the conditions in Figure 3. For the labeled methyl carbons in $(CH_3)_2C(OH)SO_3Na$, d = 1.45 kHz.[33]

Figure 4a shows part of the ^{13}C MAS spectrum of a mixture of methionine·HCl, in which 50% of the molecules are labeled at the *S*-methyl position, and *N*-acetyl-L-valine. The spectrum is the result of 16 scans. The one very intense resonance is that of the labeled carbon on methionine·HCl. Natural abundance ^{13}C resonances from the methyl and methine carbons of *N*-acetyl-L-valine and the β and γ carbons of methionine·HCl are resolved. The α carbons of *N*-acetyl-L-valine and methionine·HCl contribute to the broad resonance at 55 ppm. Figure 4b shows the double-quantum filtered spectrum of the same sample resulting from a total of 4800 scans using the sequence in Figure 1c. The only natural abundance resonances that remain in Figure 4b are from the β and γ carbons of methionine·HCl. In crystalline methionine·HCl, the intramolecular (shortest) distance from the *S*-methyl carbon to the γ carbon is 2.79 Å, while that to the β carbon is 3.30 Å.[35] The corresponding coupling constants are d = 1.06 and 0.64 kHz, respectively. Since the filtering efficiency should be greater for the natural abundance ^{13}C nuclei that are more strongly coupled to ^{13}C at the labeled site, we assign the resonance at 27.4 ppm, which is more intense in Figure 4b, to the γ carbon and the resonance at 28.1 ppm to the β carbon. The apparent experimental efficiency is $\bar{E}(2)$ = 0.07 for the γ carbon, i.e. less than in the example in Figure 3, but only half of the γ ^{13}C nuclei are coupled to an *S*-methyl ^{13}C on the same molecule.

Discussion

The spectra in Figure 3 and 4 demonstrate the basic feasibility of selecting resonances from nonbonded, dipole-coupled pairs of ^{13}C nuclei in a MAS spectrum using a double-quantum filter based on a simple DRAMA sequence. Many potential applications of this technique come to mind. A few of the applications that motivate the work described in this paper are discussed in the following text.

An attractive approach to determining aspects of the conformation of a large molecule in a polycrystalline or noncrystalline solid is to isotopically label the molecule at two positions and measure the distance, or distribution of distances, between the two labels by measuring the dipole–dipole coupling between them.[1,6-8,13-20,32-34] In ^{13}C NMR spectroscopy, the natural abundance background signal interferes significantly with the signal from the labels when the molecule contains on the order of 1000 or more carbons (assuming 100% labeling and random distribution of 1 ppm wide lines over a 50 ppm interval), e.g. if the molecule is a protein of molecular weight greater than about 20 000. It then becomes desirable to suppress the natural abundance background with double-quantum filtering. The distance determination itself can be made, at least approximately, by observing the dependence of the filtered signal intensity on n, or by a more direct measurement of the coupling. As the molecular weight increases, significant signal from natural abundance ^{13}C *pairs* appears in the filtered spectrum. Natural abundance ^{13}C pairs should interfere in the filtered spectrum of a doubly labeled molecule when it contains on the order of 20 000 or more carbons (assuming that 5% of the natural abundance ^{13}C nuclei occur as pairs).

Information about the structure, composition, and dynamics of a specific region within a large molecule can also be obtained by combining double-quantum filtering with isotopic labeling at a *single* position, as suggested by the spectra in Figure 4. The label can be introduced by synthesis or biosynthesis, or by binding or reacting a small molecule with the large molecule. Examples of the latter means of introducing a label include binding of a labeled inhibitor at the active site of an enzyme or binding of a labeled hormone or neurotransmitter to a receptor. In principle at least, the double-quantum filtered spectrum will contain resolved resonances from the spatial region around the labeled site, which is likely to be the region of greatest interest, even though the unfiltered MAS spectrum may be too congested to show resolved resonances. The radius of the selected region can be varied by varying n or other parameters in the DRAMA sequence, allowing an approximate determination of the distances from the labeled to the unlabeled sites. Even at the current stage of development of the techniques, radii of 4 Å or greater are accessible in ^{13}C NMR experiments. Spin relaxation or line shape[36] measurements can be performed on the selected region, providing information about molecular dynamics. Information about the composition of the selected region, e.g. the amino acids present at the active site of an enzyme or the binding site of a receptor, is contained in the chemical shifts of the natural abundance resonances from the selected region. As the molecular weight of the molecule or complex increases, this approach to obtaining a resolved ^{13}C MAS spectrum from a spatially localized region again suffers a limitation imposed by the presence of natural abundance ^{13}C pairs. With the assumptions stated above, the natural abundance ^{13}C pairs outside of the selected region should interfere significantly with signals from the selected region when the molecule or complex contains on the order of 200 or more carbons. This size limit can

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be increased substantially in a number of ways. For example, difference spectroscopy (subtracting the double-quantum filtered spectrum of the unlabeled molecule or complex from that of the labeled molecule or complex[14]) or selective excitation of the ^{13}C label may be used to further enhance signals from the selected region relative to signals from outside of that region.

When applied to molecules with resolved or partially resolved ^{13}C MAS spectra, double-quantum filtering based on dipole–dipole couplings can provide information about internuclear distances without requiring isotopic labeling. A two-dimensional version of the technique described in this paper, analogous to the two-dimensional INADEQUATE technique used in solution NMR spectroscopy,[24,25] would reveal which pairs of resonances in the MAS spectrum arise from pairs of nuclei that are within a certain radius of one another, where the radius is determined by the parameters of the DRAMA sequence used to generate double-quantum coherences. Alternatively, the spectrum obtained by taking the difference between two one-dimensional double-quantum filtered spectra, one with selective inversion of a particular ^{13}C spin before preparation of double-quantum coherences and one without, will contain only resonances from nuclei that are within a certain radius of the inverted nucleus.

The practicality of any of the experiments outlined above depends critically on sensitivity considerations. In turn, this depends on the efficiency of the double-quantum filter. The fundamental obstacle to obtaining a filtering efficiency of unity is the orientation dependence of the dipole–dipole coupling contained in $F(\theta,\phi)$. In general, the maximum value of $E(\theta,\phi,n)$ occurs at different values of n for different values of θ and ϕ. One approach to achieving the maximum efficiency over a large range of coupling strengths at a single value of n, thereby increasing the maximum of $\bar{E}(n)$, is to construct composite DRAMA sequences in analogy to the composite sequences used by Barbara et al.[37] for broadband excitation of double-quantum coherences in nonspinning samples. An alternative approach is to carry out a numerical search[32] for DRAMA sequences that are somewhat more complicated than that in Figure 1b, using a computer to find values of pulse lengths, phases, and delays that maximize $\bar{E}(n)$. Both approaches are under investigation. As indicated in Figure 2, it is also important to evaluate the sensitivity of the sequences to resonance offsets and CSA. The decrease in $\bar{E}(n)$ with increasing offset and CSA width places limits on the spectral range and types of carbons to which the double-quantum filtering techniques can be successfully applied.

To get a quantitative estimate of the restriction imposed by sensitivity considerations on the size of the molecules that can be studied in double-quantum filtering experiments, we can begin with the conservative assumptions that the total sample size is limited to 100 mg and that it is possible to observe a resonance from no less than 10^{18} equivalent ^{13}C nuclei in an unfiltered, cross-polarized MAS experiment with an adequate signal-to-noise ratio in a single scan. In 10000 scans, it is then possible to detect the NMR signal from a single carbon site at natural abundance in a molecule with molecular weight up to about 60000. In a double-quantum filtering experiment in which a single site is labeled with the goal of obtaining the MAS spectrum of a region of the molecule localized around the labeled site, the upper limit on the molecular weight becomes about $60000\bar{E}(n)$. In a double-quantum filtering experiment on an unlabeled molecule intended to provide information about the approximate distances between nuclei that give rise to resolved resonances, the upper limit on the molecular weight is about $600\bar{E}(n)$. Of course, these limits are intended as order-of-magnitude estimates only, since the sensitivity depends on spin relaxation rates, the cross-polarization enhancement, the line widths, the field strength, the probe efficiency and filling factor, the sample temperature, etc. Nonetheless, the analysis presented above certainly indicates that double-quantum filtering can be successfully applied in structural studies of complex molecules.

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Computational Study of the Carboranylcarbenes 1-CH-1,2-$C_2B_3H_4$ and 1-CH-1,2-$C_2B_4H_5$

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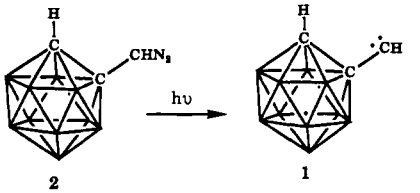
Abstract: Calculations are reported for the first two members of the carborane series, 1,2-$C_2B_3H_5$ and 1,2-$C_2B_4H_6$, with a carbene substituent at the 1-position. For the first member of the series, 1-CH-1,2-$C_2B_3H_4$, a singlet carbene attached to the cage causes a complete disruption of the cage structure. By contrast, the second member, 1-CH-1,2-$C_2B_4H_5$, is an energy minimum. On both potential energy surfaces, the global minimum is an expanded cage isostructural and isoelectronic with the next higher carborane. The relevance of the current work to the known chemistry of *o*-carboranylcarbene, 1-CH-1,2-$C_2B_{10}H_{11}$, is discussed.

Introduction

The overlap of carbene chemistry with carborane chemistry holds great potential for new and perhaps unexpected structures.[1-3] The reactive carbene center may donate a pair of electrons and insert into the carborane cage, may abstract a hydrogen atom forming an exocyclic carbon–carbon double bond, or may insert into a B–H bond forming a fused three-membered ring. Lastly, if the carbene center is attached by a flexible tether, a carbon-to-boron bridge can be formed.[3] Jones[1-3] has explored the carbene–carborane interface with the *o*-carboranylcarbene system (**1**) which is generated from a precursor diazo compound (**2**) by photolysis (eq 1).



2 **1** (1)

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