High-Field and High-Speed CP-MAS ^{13}C NMR Heteronuclear Dipolar-Correlation Spectroscopy of Solids with Frequency-Switched Lee–Goldburg Homonuclear Decoupling

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In this Communication, we demonstrate that the use of frequency–switched Lee-Goldberg irradiation during the proton evolution of fast-spinning 2-D heteronuclear (^{1}H–^{13}C) correlation spectroscopy strongly enhances the proton resolution.

Heteronuclear correlation spectroscopy is an important tool for resolution enhancement in multidimensional NMR spectroscopy. For solids, line narrowing in the proton dimension is generally achieved by application of CRAMPS techniques to suppress the strong homonuclear dipolar interactions between the abundant protons (*1–11*). However, in high-speed MAS NMR research of, for instance, biological systems, the use of multiple-pulse techniques to study the proton chemical-shift dispersion is limited for several reasons. First, the application of multiple-pulse techniques usually requires cycle times that are short compared to the rotor period. This effectively puts a restriction on the spinning speeds that can be used in practice and the efficacy of the MAS averaging. It also limits the possibilities for application of CRAMPS techniques in high-field MAS, where high spinning speeds will be required to obtain sufficient resolution in multidimensional spectra. For instance, the WAHUHA-4, which has the shortest cycle time, performs well only at moderately high spinning rates. More-elaborate CRAMPS sequences require longer cycle times, and the possibilities for application in high-speed MAS will be even less favorable. In addition, biological MAS NMR research often must be performed at low temperatures, which makes the use of tuned-up multiple-pulse sequences impractical.

Recently, it was demonstrated that, from straightforward 2-D high-speed MAS heteronuclear (^{1}H–^{13}C) CP/WISE spectra collected in a high magnetic field and without any homonuclear decoupling scheme during the proton evolution, ^{1}H–^{13}C correlations and proton chemical shifts in the solid state can be obtained directly (*12*). In high field, dipolar interactions are truncated by the increased chemical-shift dispersion. The resolution enhancement is quite remarkable for protons which are subject to the largest chemical shifts.

On the other hand, the resolution in the upfield region is not optimal, due to a combination of strong homonuclear dipolar couplings and small shift dispersion for most aliphatic protons. To obtain good proton resolution over the entire spectrum, the application of a technique to suppress the homonuclear dipolar interactions between the abundant protons is necessary.

It was shown by Lee and Goldburg that the application of an RF field off-resonance according to $\Delta \mathrm{LG}/\omega_1 = \frac{1}{2}\sqrt{2}$ produces an effective field in the rotating frame inclined at the magic angle $\theta_\mathrm{m} = \tan^{-1}(\sqrt{2})$ to the static field $\mathbf{H}_0$. With Lee–Goldburg (LG) irradiation, the spins precess rapidly around this magic-angle axis, which effectively results in a cancellation of the first two terms of the ''dipolar alphabet'' in the tilted rotating frame (*13*). It has been shown that when the LG irradiation is both frequency- and phase-switched (FS-LG) after successive periods $\tau = (2\pi/\omega_1)\sqrt{2/3}$, corresponding with the time to complete a 2π rotation around the tilted axis, the efficiency of decoupling is significantly improved (*14, 15*).

FS-LG can be used for homonuclear decoupling in a heteronuclear correlation experiment, since it is not necessary to observe the protons directly. The LG cycle time is the inverse of the precession rate along the (1, 1, 1) axis. For moderately high RF power, the rate is typically 10 μs, which is short compared to the MAS period, even for high spinning speeds. Modern NMR spectrometers enable rapid coherent switching of both frequency and phase of the RF during a pulse, and the FS-LG sequence is relatively straightforward to implement. Since the only parameters that need to be adjusted are the offset frequency ΔLG and the duration of the successive frequency intervals τ, the sequence is robust and can easily be readjusted if necessary.

The pulse sequence we used in this study to collect 2D heteronuclear correlation spectra is depicted in Fig. 1. The sequence starts with a $(\pi/2 + \theta_\mathrm{m})_y$ pulse on the protons, directly followed by a train of frequency- and phase-switched LG pulses in the xz plane. After the evolution
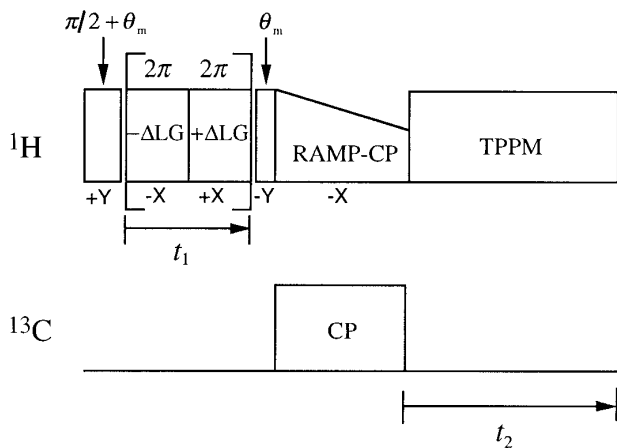
FIG. 1. Pulse sequence for the 2D heteronuclear correlation spectra, with frequency-switched Lee–Goldburg irradiation during the evolution.

period, the proton magnetization is turned back by a single magic angle y pulse, which will turn any component perpendicular to the LG pulse back into the xy plane, and which will bring the spin-locked component along the LG-pulse back to the z axis.

It can be shown that in the spin-pair approximation, the homonuclear dipolar interaction can be expanded into five terms (*13, 16*). The secular term

$$\mathcal{H}_{\text{dip}}^{0} = \sum_{j<k} B_{jk}(I_{jz}I_{kz} - \tfrac{1}{3}\mathbf{I}_j \cdot \mathbf{I}_k)$$

with

$$B_{jk} = \left(\frac{\mu_0}{4\pi}\right) \frac{3\gamma^2\hbar}{2} \frac{(1 - 3 \cos^2\theta_{jk})}{r_{jk}^3}$$

forms the major source of line broadening (*17*) and should be attenuated for high-resolution spectroscopy.

In a high magnetic field, the chemical-shift dispersion will be considerable, which effectively truncates the $\mathcal{H}_{\text{dip}}^{0}$ term (*12*). When the shift dispersion increases, the mutual overlap of the proton lines diminishes, which reduces the dipolar couplings. This effect is nonlinear, since a decreased coupling will also give rise to additional line narrowing.

Second, the application of an off-resonance RF field $H^{\text{RF}}(t) = 2H_1\cos \omega t$ yields, in the off-resonance rotating frame, i.e., the frame rotating with angular frequency ω along the z axis, a truncated Hamiltonian

$$H_{\text{R}} = \sum_i \left\{ \Delta\omega_i I_z^i - \gamma\left(H_0 - \frac{\omega}{\gamma}\right)I_z^i - \gamma H_1 I_x^i \right\} + \mathcal{H}_{\text{dip}}^{0},$$

with the chemical-shift dispersion, $\Sigma_i \, \Delta\omega_i I_z^i$, included explicitly. H_{R} can be transformed to a tilted rotating frame

(*18*), with the z axis along the direction of the effective field $\mathbf{H}_e = H_1\mathbf{e}_x + [H_0 - (\omega/\gamma)]\mathbf{e}_z$, yielding

$$\tilde{\mathcal{H}}_{\text{R}} = \sum_i \{ (\Delta\omega_i\cos \theta + \omega_e)\tilde{I}_z^i - \Delta\omega_i\sin \theta\tilde{I}_x^i \}$$

$$+ \sum_{M=-2}^{2} \lambda_M(\theta) \, \tilde{\mathcal{H}}_{\text{dip}}^{M},$$

according to Fig. 2. The explicit form of the terms $\lambda_M(\theta) \, \tilde{\mathcal{H}}_{\text{dip}}^{M}$ can be found, for instance, in Ref. (*13*). $\tilde{\mathcal{H}}_{\text{dip}}^{0}$ remains as the only secular term.

In FS-LG, ω_e is switched between $+\Delta$LG$/\cos \Theta$ and $-\Delta$LG$/\cos \Theta$. Subsequent transformation to the frame rotating with ω_e along the z axis is performed using the propagator

$$\mathbf{U}_e(t) = \prod_i \exp(-i\omega_e t\tilde{I}_z^i),$$

and leads to the two time-dependent Hamiltonians

$$\begin{cases} \tilde{\mathcal{H}}_{\text{R,e}}^{+\Delta\text{LG}}(t) = \mathbf{U}_e^{-1}(t) \tilde{\mathcal{H}}_{\text{R}}\mathbf{U}_e(t) \\ \tilde{\mathcal{H}}_{\text{R,e}}^{-\Delta\text{LG}}(t) = \mathbf{U}_e(t) \tilde{\mathcal{H}}_{\text{R}}\mathbf{U}_e^{-1}(t) \end{cases},$$

for $\omega_e = \Delta$LG$/\cos \Theta$, and

$$\tilde{\mathcal{H}}_{\text{R,e}}^{\pm\Delta\text{LG}}(t) = \sum_i \{ (\Delta\omega_i\cos \theta \pm \omega_e)\tilde{I}_z^i$$

$$- \Delta\omega_i\sin \theta(\tilde{I}_x^i \cos \omega_e t \mp \tilde{I}_y^i \sin \omega_e t) \}$$

$$+ \lambda_0(\theta) \, \tilde{\mathcal{H}}_{\text{dip}}^{0} + \sum_{\substack{M=-2 \\ M \neq 0}}^{2} \exp(\pm M\omega_e t)\lambda_M(\theta) \, \tilde{\mathcal{H}}_{\text{dip}}^{M}.$$

When the off-resonance frequency ω is set to a Lee–Goldburg condition, $\omega = \gamma(H_0 \pm \tfrac{1}{2}\sqrt{2}H_1)$, the $\tilde{\mathcal{H}}_{\text{dip}}^{0}$ vanishes due to the $\lambda_0(\theta) = \tfrac{1}{2}(3 \cos^2\theta - 1)$ dependence (*13*). The remaining linewidth originates from the nonsecular terms $\tilde{\mathcal{H}}_{\text{dip}}^{\pm1}$ and $\tilde{\mathcal{H}}_{\text{dip}}^{\pm2}$ (*13*). These nonsecular terms will be trun-
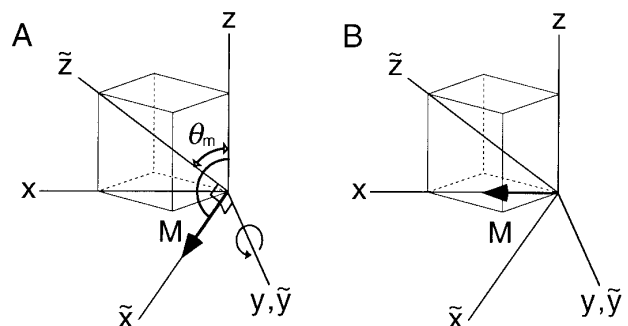

FIG. 2. (A) Starting with a $(\pi/2 + \theta_{\text{m}})_y$ pulse, the component along the z axis is zero, and (B) any component in the plane perpendicular to the $\tilde{z}$ axis is selected by the $\theta_{\text{m,y}}$ back pulse.
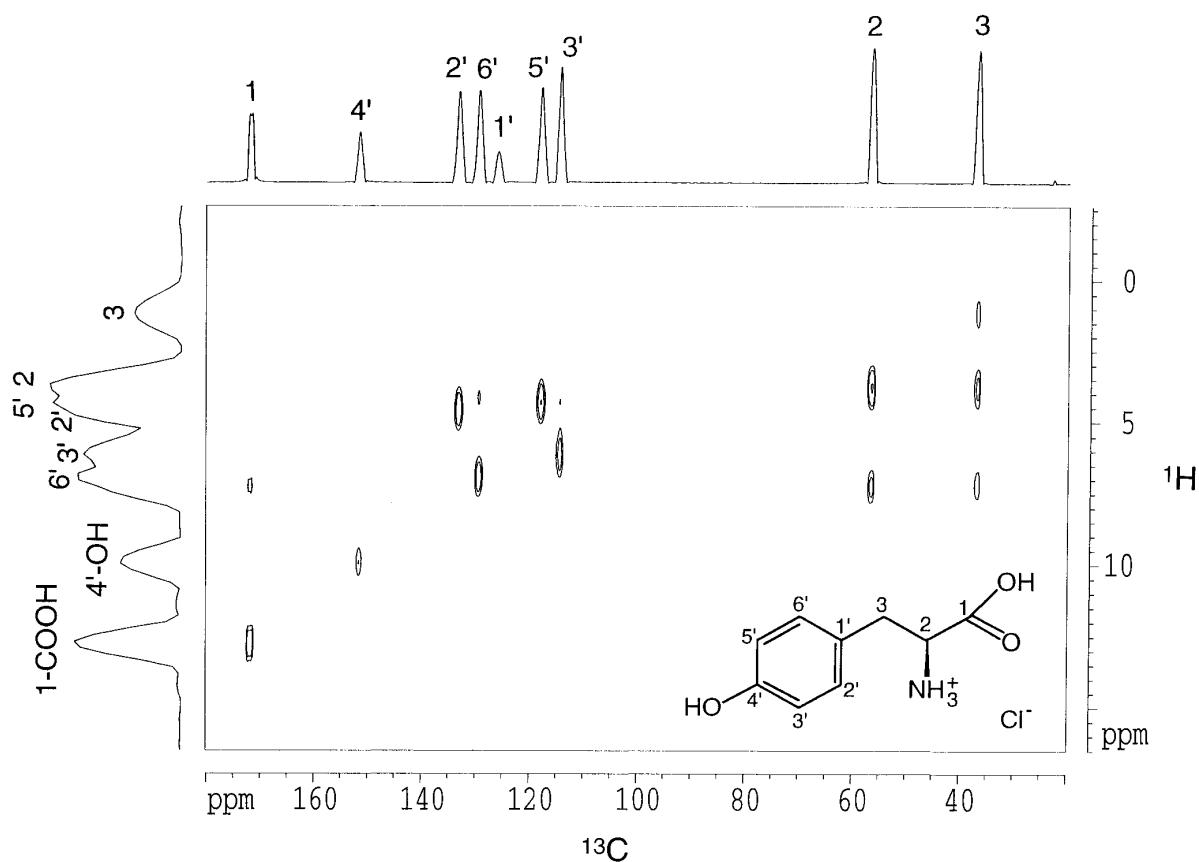
FIG. 3. Two-dimensional heteronuclear (^{1}H–^{13}C) FS-LG decoupled correlation spectrum recorded from [U-^{13}C] L-tyrosine with a spinning speed of 13.0 kHz. The assignment of the signals is indicated in the projections. The scheme depicts the IUPAC numbering for the L-tyrosine · HCl salt.

cated when the tilted effective field $H_e = \omega_e/\gamma$ is large, i.e., $(\gamma H_e)^2 \gg |\tilde{H}_{dip}^M|^2$. The truncation will be most effective for the $\tilde{H}_{dip}^{\pm 2}$ terms which have a $2\omega_e$ time dependence. However, it is important to realize that all five terms $\tilde{H}_{dip}^M$ in the tilted rotating frame originate from the single term H_{dip}^0 in the rotating frame. This implies an additional advantage when H_{dip}^0 is attenuated by a high static field, since in that case the terms $\tilde{H}_{dip}^M$ are also reduced. Hence, high fields and Lee–Goldburg decoupling are thus co-operative homonuclear line-narrowing mechanisms.

In two successive FS-LG periods, the chemical-shift dispersion will be scaled by the factor $\cos \theta = 1/\sqrt{3}$. The time-dependent transverse part will be truncated. Any additional dispersion due to $\omega_e \tilde{I}_z$ will be refocused by the sign reversal in the successive periods. By setting $\omega_e \tau = 2\pi$, both tilted doubly rotating frames will coincide with the tilted rotating frame at the beginning and at the end of each evolution period. Therefore, the proton magnetization will effectively evolve in the plane perpendicular to the tilted $\tilde{z}$ axis. To optimize sensitivity, it is important to start the evolution in the plane perpendicular to the $\tilde{z}$ axis by giving the $(\pi/2 + \theta_m)_y$ pulse, and to select a component in this plane at the

end of the evolution period by the θ_m back pulse, cf. Figs. 1 and 2.

Solid-state cross-polarization magic-angle-spinning (CP/ MAS) FS-LG decoupled dipolar-correlation spectra were obtained in a moderately high field of 9.4 T with the pulse sequence in Fig. 1, using a solid-state DMX-400 NMR spectrometer equipped with a 4 mm double-resonance MAS probe (Bruker, Karlsruhe, Germany). The sample volume was restricted to the middle of the rotor to improve the RF homogeneity. Since the Hartmann–Hahn matching and corresponding efficiency of CP magnetization transfer is very sensitive to RF power instabilities at high MAS frequencies, we used a ramped-amplitude CP sequence (RAMP-CP) (19) to restore a broader matching profile. During the carbon acquisition (t_2), the protons are decoupled from the carbons by using a TPPM decoupling scheme, which improves the high-field ^{13}C resolution considerably (20). The phases of the entire FS-LG sequence and the two magic angle pulses are varied in a TPPI scheme to simulate phase-sensitive detection (21). The duration τ of the successive FS-LG pulses was optimized to 9.15 μs. The CP contact time should be kept short to avoid relayed homonuclear

spin-diffusion-type processes in both proton and uniformly labeled carbon spin reservoirs during CP. The contact time for the CP was 500 μs. The phase modulation angle for the TPPM decoupling was set to 15.0°, and the flip-pulse length was optimized to 5.8 μs to yield optimal ^{13}C resolution. The magic-angle pulse length was 1.8 μs.

The method was applied to a sample of [U−^{13}C] L-tyrosine, using a high spinning speed of 13.0 kHz. The 2-D heteronuclear correlation spectrum and its projections are shown in Fig. 3. The proton–carbon correlations are well resolved, also in the aliphatic region of the spectrum. The overall sensitivity is good, due to the high spinning speed used for the experiment. The proton lines are also partially resolved in the F_1 projection, which underlines the good overall performance of the FS-LG decoupling during t_1. The strongest correlations in Fig. 3 are between carbons and directly bonded protons. Both intramolecular and through-space intermolecular correlations with distant protons occur at lower levels of cross-peak intensity. Mutual correlations between 1-COOH, 2-H, and 3-H$_2$ are observed at 7.2 ppm in the ^{1}H dimension and could originate from the NH$_3^+$ moiety.

It is thus concluded that heteronuclear dipolar-correlation spectroscopy using FS-LG homonuclear decoupling performs well at high spinning speeds and in high fields. The resolution enhancement that can be obtained in this way offers promising possibilities for the development of assignment strategies and structure refinement using uniformly or multispin-enriched biological samples, in high or ultrahigh fields.

ACKNOWLEDGMENT

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