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Two-Dimensional $^{13}C-^{13}C$ Correlation Spectroscopy with Magic Angle Spinning and Dynamic Nuclear Polarization

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The range of applicability of solid-state NMR (SSNMR) experiments is often limited by low signal-to-noise, due in part to the necessity of observing low-γ, low-abundance nuclei. Magic angle spinning(MAS)[1,2] dramatically increases the resolution and sensitivity of SSNMR by averaging chemical shift anisotropies and dipolar couplings. Nevertheless, low sensitivity remains an issue. In this communication we show that dynamic nuclear polarization (DNP)[3] can be used to enhance the signal intensities in MAS experiments at 5 T. Signal enhancements of up to 23 have been obtained at 85−90 K using a custom-designed high-power gyrotron. The extended stability of MAS/DNP experiments at low temperature is demonstrated with ^{1}H-driven ^{13}C spin-difusion experiments on the amino acid proline. These $^{13}C-^{13}C$ chemical shift correlation spectra are the first two-dimensional MAS/DNP experiments performed at high field (>1.4 T). In conjunction with previous results,[4,5] these experiments suggest that DNP will be a viable sensitivity-enhancement technique for structural studies of a number of chemical, physical and biological systems.

The sensitivity of SSNMR can be increased by 2 to 3 orders of magnitude with DNP, a technique that transfers the high Boltzmann polarization of unpaired electrons to nuclei. This polarization transfer is driven by microwave irradiation at or near the electron Larmor frequency. Until recently, the applications of DNP to SSNMR have primarily involved polymers and carbonaceous materials at low fields (<1.4 T).[6,7] Given that the NMR spectral resolution increases with magnetic field, the extension of DNP techniques to higher fields, and consequently higher microwave frequencies, is clearly desirable. Progress at higher fields has been limited primarily by a paucity of microwave sources operating at frequencies corresponding to fields employed in current superconducting NMR spectrometers (28 GHz/T or 140 GHz for DNP experiments at 5 T).

Cyclotron resonance masers (gyrotrons) developed for plasma fusion experiments operate in a frequency range amenable to high-field DNP experiments. They are typically designed for high output power, kW to MW, and short pulses, μs, whereas continuous-wave (CW) DNP experiments require long pulses, s, or CW operation with ∼1−100 W of microwave power. We have obtained large DNP signal enhancements, up to 185 for static experiments at 15 K, using a 140 GHz gyrotron modified for DNP applications.[8] The polarization transfer occurred via thermal mixing[3,9] with 4-amino-TEMPO, a nitroxide radical, doped in glycerol/water, a cryoprotectant. We have shown that this solvent system is applicable to a wide range of biological samples.[5] We have also reported DNP signal enhancements of up to ∼50 on the MAS spectra of arginine and the 18.7 kD protein T4 lysozyme both with 4-amino-TEMPO in glycerol/water at 50 K.[4] These results clearly demonstrated that high-field DNP can be combined with MAS for high-resolution spectroscopy, but they were limited to relatively short one-dimensional experiments due to the low stability and high cost of MAS with cryogenic helium.

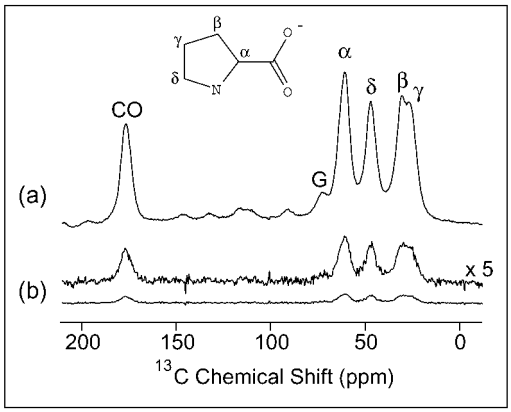

Figure 1. ^{13}C CP-MAS experiment on U-$^{13}C-^{15}N$ proline (120 mg/mL) in glycerol/water (60/40, v/v) doped with 40 mM 4-amino-TEMPO with (a) and without (b) DNP, at 92 K and 4.57 kHz spinning frequency. Thirty-two transients were averaged with a 5 s recycle delay. The gyrotron was on CW, providing 0.6 W of microwave power at the sample. The DNP signal enhancement is equal to 15. The glycerol natural abundance signal is labeled as "G" and the low-intensity peaks between the carbonyl and glycerol resonances are spinning sidebands.

An attractive alternative is to perform MAS at liquid nitrogen temperatures—in the range of 80−100 K. Higher microwave powers and longer acquisition times are then required since the DNP efficiency decreases with increasing temperature. Accordingly, the 140 GHz gyrotron was modified based on a 250 GHz device that was designed specifically for DNP applications and has been operating with 5−10 W of CW output power.[10] The changes allowed for quasi-CW operation with output powers of up to ∼12 W. The low-temperature MAS probe uses cold nitrogen gas as both the cooling and spinning gas. The microwaves are transmitted through a waveguide that ends approximately 1 mm from the NMR coil. The NMR performance is unaffected by the waveguide. The probe and upgraded gyrotron will be described in detail in a separate publication.

High-resolution spectra of U-$^{13}C-^{15}N$-proline in 40 mM 4-amino-TEMPO/glycerol/water with and without DNP are shown in Figure 1. A signal enhancement of 15 is measured at 92 K with 0.6 W of microwave power at the sample. This corresponds to a factor of

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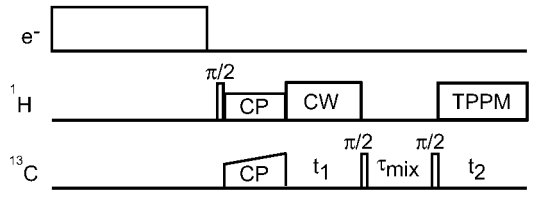

Figure 2. DNP-enhanced ^{1}H-driven spin-difusion pulse sequence. Microwave irradiation (139.66 GHz from the gyrotron) polarizes ^{1}H (211.62 MHz), and the enhanced polarization is transferred to ^{13}C via cross polarization, CP.[15] Transverse ^{13}C magnetization evolves during an incremented delay, t_1, followed by a $\pi/2$ pulse that creates longitudinal magnetization before the mixing period, τ_{mix}. ^{1}H decoupling is removed during τ_{mix}, and magnetization exchange occurs via ^{1}H-driven spin diffusion. A second $\pi/2$ pulse returns the magnetization back to the transverse plane before acquisition with TPPM decoupling.[16] Typical acquisition parameters are 70 kHz field strength for the ^{1}H $\pi/2$ pulse and decoupling, 40 kHz for CP (600 μs), 50 kHz for the ^{13}C $\pi/2$, 6.4 μs TPPM pulse length and 14° phase difference. The samples are placed in a pre-cooled probe (~120 K).

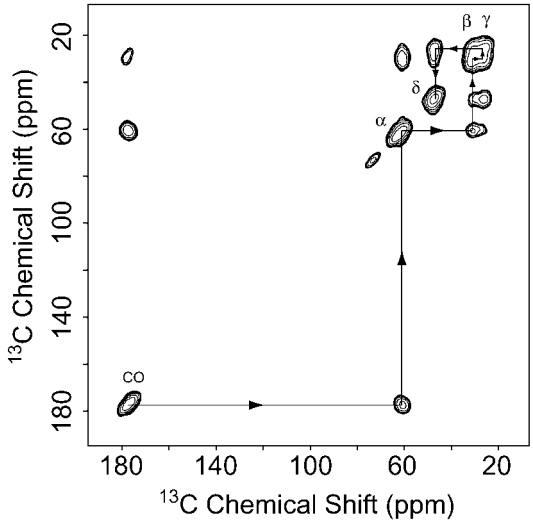

Figure 3. Spin-diffusion experiment on U-^{13}C−^{15}N proline (120 mg/mL) in glycerol/water with 40 mM 4-amino-TEMPO. The mixing time was 5 ms. The gyrotron was pulsed on for 2 s with a recycle delay of 3 s. The signal enhancement is 9. 56 slices were acquired in t_1 with 80 μs dwell time, and eight transients were averaged for each slice. The temperature was stable at 90.0 ± 0.5 K, the spinning frequency at 4.55 kHz ± 20 Hz, and the microwave power at 0.5 ± 0.05 W.

225 decrease in signal acquisition time. The low-temperature spinning apparatus and gyrotron can operate in a stable mode for extended periods of time as is required for 2D experiments such as homonuclear chemical shift correlation spectroscopy. This experiment, depicted in Figure 2, consists of a period of microwave irradiation followed by a ^{1}H-driven spin-diffusion mixing period and acquisition during decoupling. A 2D DNP-enhanced spin-diffusion spectrum of U-^{13}C−^{15}N-proline at 90 K and 4.5 kHz spinning frequency is shown in Figure 3. The mixing time was 5 ms and strong cross-peaks to the nearest neighbors are seen for each resonance. This experiment was performed with the gyrotron pulsed on for 2 s and off for 3 s, and the total acquisition time was ~1.5 h. The DNP signal enhancement, measured from the first t_1 point with and without DNP, is equal to 9. A similar experiment was performed with a 30 ms mixing time, and as expected, full exchange was observed between all the sites.

The line widths for proline frozen in glycerol/water (60/40, v/v) doped with 40 mM 4-amino-TEMPO are larger than for the polycrystalline powder. This increased line width is primarily due to inhomogeneous broadening from structural disorder in the frozen water/glycerol matrix. Aqueous glycerol solutions form a glass upon cooling and have been successfully used as a cryoprotectant for biological samples at low temperatures.[11] However, if the freezing rate is slow, the local formation of ice crystals that cause disorder can occur. The extent of crystallization depends on the solvent composition and cooling rate, both of which are currently under investigation. These studies will include rapid freeze−quench techniques, that have been shown to minimize the formation of ice domains and improve the spectral resolution in SSNMR.[12] A second source of line broadening is the electron−nuclear dipolar interaction. Comparisons of MAS spectra for 1-^{13}C glycine in glycerol/water with and without TEMPO indicate that the line broadening arising from the electron source is small (~50 Hz for 40 mM 4-amino-TEMPO) because the electron−nuclear dipolar couplings are partially averaged by MAS. Approaches that will minimize this broadening are also under investigation.

In summary, we have shown that high-field DNP can be combined with MAS experiments to obtain enhanced sensitivity. Significantly larger signal enhancements should be attainable after technical improvements that are currently in progress to lower the sample temperature and increase the microwave power. The stability of the high-power gyrotron and MAS with cold nitrogen have been demonstrated with ^{13}C−^{13}C chemical shift correlation experiments on proline in glycerol/water doped with 4-amino-TEMPO. Correlation spectra indicate connectivity between nuclear spins and hence are powerful tools for resonance assignments, which is the first step in structural studies. The second step is the acquisition of structural parameters—distances and torsion angles—that provide information about the three-dimensional structure. In MAS experiments, structural constraints are often measured with recoupling techniques that require synchronization of the rf pulses and spinning frequency.[13,14] The spinning frequency is now stable enough to combine rotor-synchronized recoupling sequences with DNP at 90 K.

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