



# Measurement of $^{15}\text{N}$ – $^1\text{H}$ bond lengths by rotational-echo double-resonance NMR spectroscopy

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## Abstract

A method based on rotational-echo double-resonance (REDOR) NMR is proposed to measure  $^1\text{H}$ – $^{15}\text{N}$  bond lengths under magic-angle-spinning. Protons are selectively polarized and evolve under the frequency-switched Lee–Goldburg (FSLG) homonuclear decoupling, while two  $^{15}\text{N}$   $\pi$  pulses are applied to restore  $^1\text{H}$ – $^{15}\text{N}$  dipolar interactions. Such a method relies on the  $^1\text{H}$ – $^{15}\text{N}$  dipolar couplings and requires no knowledge of their chemical shift tensors allowing one to precisely extract their couplings. This method could be applicable for measuring relatively long distances between amide protons and other nucleus such as  $^{13}\text{C}$  if applying the  $\pi$  pulses to  $^{13}\text{C}$ .

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## 1. Introduction

For many years, protons have been the source of polarization for enhancing the sensitivity of dilute spins such as  $^{13}\text{C}$  and  $^{15}\text{N}$  in distance measurements under magic-angle-spinning (MAS) condition but otherwise not an important part of the experiments [1]. Since the magnitude of nuclear dipolar interactions between two spins is proportional to their gyromagnetic ratios and is inversely proportional to the cube of their through-space distance, two dilute spins with low gyromagnetic ratios exhibit a relatively small dipolar coupling,

especially in the presence of molecular motions [2,3], so that the distance accessible for measurements becomes limited. Use of nuclei with high gyromagnetic ratio such as  $^1\text{H}$  in the distance measurements would greatly increase dipolar couplings available for detection.

Recently, several solid-state MAS NMR methods utilizing protons [4–9] have been proposed to determine  $^1\text{H}$ – $^{13}\text{C}$  or  $^1\text{H}$ – $^{15}\text{N}$  heteronuclear dipolar couplings. In solids, homonuclear dipolar couplings among abundant  $^1\text{H}$  spins are typically much greater than any other internal spin interactions and have to be suppressed in order to detect relatively weak heteronuclear dipolar couplings, such as  $^1\text{H}$ – $^{13}\text{C}$  or  $^1\text{H}$ – $^{15}\text{N}$ , which must be prevented from being averaged by MAS. Pulse sequences such as FSLG-22 [4],

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MSHOT [10], R18<sub>2</sub> [5], and T-MREV [6] have been developed to suppress strong proton homonuclear dipolar interactions and to recouple the heteronuclear dipolar interactions under MAS. However, it was shown that not only the <sup>15</sup>N–<sup>1</sup>H dipolar coupling but also the coupling to the non-directly bonded <sup>1</sup>H, the H<sup>N</sup>–N–H<sup>α</sup> angle, and/or the chemical shift anisotropy of the bonded <sup>1</sup>H contribute to the recoupled H–N dipolar spectra [5,6]. Lee–Goldburg cross-polarization (LG-CP) [7] suppressed proton dipolar interactions under Lee–Goldburg sequence [11] during which an Hartmann–Hahn condition was established at the first matching sideband i.e.,  $\omega_{\text{eff}}^{\text{H}} - \omega_1^{\text{C}} = \pm\omega_r$ , where  $\omega_{\text{eff}}^{\text{H}}$  is the proton effective spin-lock amplitude along the axis tilted 54.7° away from +Z axis,  $\omega_1^{\text{C}}$  is the rf spin-lock amplitude applied to <sup>13</sup>C, and  $\omega_r$  is the sample spinning speed. Under such a matching condition, the buildup of <sup>13</sup>C signals is in oscillatory manners depending on <sup>13</sup>C–<sup>1</sup>H dipolar couplings. Thus, LG-CP could measure weak <sup>13</sup>C–<sup>1</sup>H dipolar couplings (i.e., long <sup>13</sup>C–<sup>1</sup>H distances). However, it would be extremely difficult to fulfill the matching condition for all <sup>13</sup>C resonances, since the bandwidth of the matching sideband is very narrow under fast MAS especially when proton dipolar interactions are suppressed [12]. Dipolar filter [8] based on rotational-echo double-resonance (REDOR) NMR [13] was also suggested to dephase proton magnetization before CP. Moreover, at very high spinning speed that effectively gives rise to high-resolution <sup>1</sup>H spectra, inverse detection scheme [9] can be used to enhance <sup>15</sup>N sensitivity and to detect <sup>15</sup>N–<sup>1</sup>H bond lengths based on the REDOR recoupling method.

In this Letter, we present an alternative NMR technique for measuring <sup>15</sup>N–<sup>1</sup>H bond length under MAS condition. The method combines frequency-switched Lee–Goldburg (FSLG) homonuclear decoupling [14] with REDOR [13], a well-known technique for measuring heteronuclear dipolar interaction between two dilute spins (e.g., <sup>13</sup>C and <sup>15</sup>N). It will be shown by experiments and simulations that <sup>1</sup>H–<sup>15</sup>N dipolar couplings can be precisely measured. Both advantages and disadvantages of this technique will be further discussed.

## 2. Experimental

The experiments were performed on a Bruker DMX-300 NMR spectrometer ( $B_0 = 7$  T), equipped with a Bruker CPMAS probe with 7 mm rotors and with Larmor frequencies of 30 MHz for <sup>15</sup>N and 300 MHz for <sup>1</sup>H. About 10 mg of *N*-acetyl-<sup>15</sup>N-D,L-valine was packed in a 7 mm MAS rotor with spherical spacer and spun at 4.5 kHz (i.e.,  $\tau_R$  was 222.2  $\mu$ s). The use of such a rotor is to minimize the <sup>1</sup>H rf inhomogeneity [15], thus reducing its effect on the FSLG decoupling. The variation of the spinning speed was controlled within  $\pm 2$  Hz. FSLG is achieved by ramping the phase of the <sup>1</sup>H rf field while keeping the <sup>1</sup>H carrier frequencies unchanged [16]. A FSLG waveform for such a phase ramp was generated by a C program and transferred to the waveform generator. In the experiments, <sup>1</sup>H  $\pi/2$  and  $\pi$  pulse lengths were 4 and 8  $\mu$ s, respectively, while the rf amplitude of 55.5 kHz was used for the FSLG decoupling so that  $t_c$  was 14.7  $\mu$ s. The <sup>15</sup>N  $\pi$  pulse length was 13.0  $\mu$ s, while  $\tau_1$ ,  $\tau_2$ ,  $\tau_3$ , and  $\tau_4$  were set to 2000, 500, 50, and 50  $\mu$ s, respectively. 512 scans were used to accumulate the <sup>15</sup>N signal with a recycle delay of 2 s.

## 3. Result and discussion

As shown in Fig. 1, an initial <sup>15</sup>N magnetization is enhanced by conventional cross-polarization (CP) with a contact time of  $\tau_1$ . After the CP, the proton magnetization is flipped back to the +Z axis while the <sup>15</sup>N magnetization is spin-locked along the +X axis for a period of  $\tau_2$ , during which any <sup>1</sup>H component remaining in the  $x$ – $y$  plane is allowed to decay completely, followed by another CP contact between <sup>1</sup>H and <sup>15</sup>N, which allows for the magnetization transfer from <sup>15</sup>N to <sup>1</sup>H. When the CP contact time  $\tau_3$  is short enough, the <sup>15</sup>N magnetization is coherently transferred to its nearby <sup>1</sup>H [17,18] since the CP is mediated by <sup>1</sup>H–<sup>15</sup>N dipolar interactions. Therefore, those protons that are directly attached to the polarized <sup>15</sup>N (i.e., amide protons) become re-polarized along +X axis, while the other protons remain untouched in the +Z axis. Fig. 2 shows the <sup>1</sup>H spectra recorded

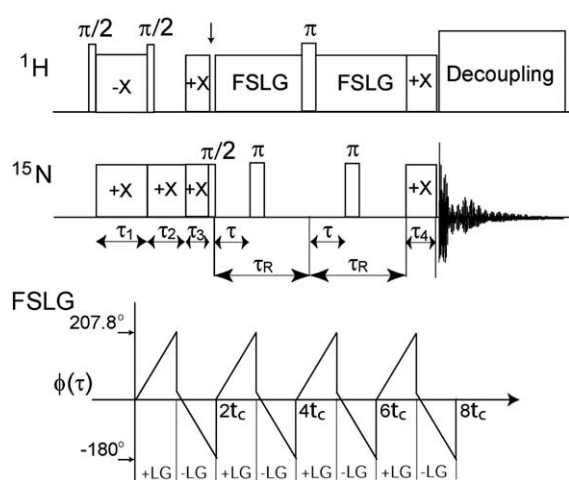


Fig. 1. Pulse sequence for measuring  $^{15}\text{N}$ - $^1\text{H}$  bond length using the rotational-echo double-resonance NMR spectroscopy. Frequency-switched Lee-Goldburg (FSLG) homonuclear decoupling is achieved by ramping the phase of the  $^1\text{H}$  rf field while keeping the  $^1\text{H}$  carrier frequency unchanged.

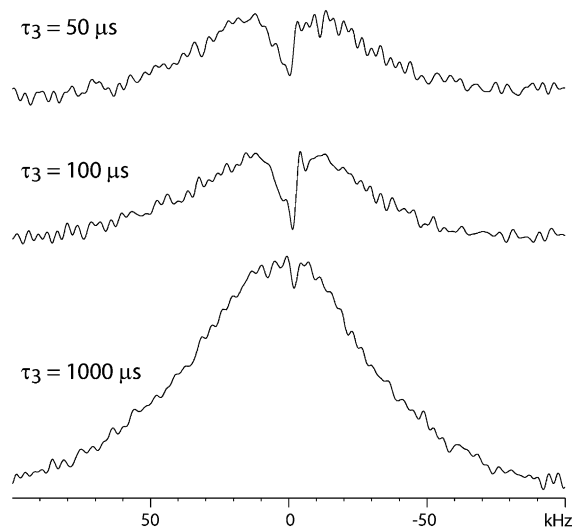


Fig. 2. Re-polarized  $^1\text{H}$  NMR spectra of  $N$ -acetyl- $^{15}\text{N}$ -D,L-valine at various contact time  $\tau_3$ .

after the second CP (indicated by an vertical arrow in Fig. 1) without applying proton homonuclear decoupling during the acquisition. When  $\tau_3$  was short ( $<100\ \mu\text{s}$ ), a 'Pake'-like doublet [19] was visible indicating that only those protons that covalently bonded to  $^{15}\text{N}$  are re-polarized. When  $\tau_3$

was long (e.g., 1 ms), the  $^1\text{H}$  spectrum became featureless implying that all protons are re-polarized. A  $\pi/2$   $^{15}\text{N}$  pulse is applied right after the second CP to flip the  $^{15}\text{N}$  magnetization back to equilibrium, while the re-polarized amide  $^1\text{H}$  magnetization evolves under FSLG [14] for two rotor periods. A  $\pi$  pulse is inserted in the middle of the  $^1\text{H}$  evolution to refocus the  $^1\text{H}$  chemical shift. It is known that at the rotational echoes generated by the sample spinning, the  $^1\text{H}$ - $^{15}\text{N}$  dipolar interactions do not contribute to the decay of the  $^1\text{H}$  signals during the evolution time of  $2\tau_R$ . However, when two  $^{15}\text{N}$   $\pi$  pulses are applied, as positioned in Fig. 1, the  $^1\text{H}$ - $^{15}\text{N}$  dipolar interactions are prevented from refocusing at the time of the rotational echoes, thus resulting in a decrease of the  $^1\text{H}$  magnetization, which is monitored indirectly by the  $^{15}\text{N}$  observations via a third CP contact  $\tau_4$ . Therefore, as in the REDOR experiments, the  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings can be precisely extracted by observing the ratio of the  $^{15}\text{N}$  signal intensities with and without the  $^{15}\text{N}$   $\pi$  pulses as a function of their position. Different from other methods [5,6], this approach relies on the  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings and requires no knowledge of the chemical shift tensors of the  $^1\text{H}$  and  $^{15}\text{N}$  spins and their relative orientation. As a result, the data analysis for extracting the dipolar couplings is rather simple. It is worth noting that the  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings will be scaled by a factor of 0.57 due to the FSLG irradiation [20].

Fig. 3 shows the  $^{15}\text{N}$  spectra of  $N$ -acetyl- $^{15}\text{N}$ -D,L-valine using the pulse sequence shown in Fig. 1. Without the  $^{15}\text{N}$   $\pi$  pulses, the full rotational-echo  $^1\text{H}$  signal is monitored (cf. Fig. 3a). The decay of the  $^1\text{H}$  signal is attributed to the transverse spin relaxation  $T_2$ , which is significantly increased by the FSLG homonuclear decoupling [20]. When the  $^{15}\text{N}$   $\pi$  pulses were applied, the observed  $^{15}\text{N}$  signal was greatly attenuated (cf. Fig. 3b), indicating that the  $^1\text{H}$ - $^{15}\text{N}$  dipolar interaction is recoupled by the  $^{15}\text{N}$   $\pi$  pulses and results in a dephase of the  $^1\text{H}$  magnetization. It is important to note that the polarization transfers between the  $^{15}\text{N}$  and  $^1\text{H}$  spins during the second and third CP contacts should be coherent enough so that the observed  $^{15}\text{N}$  signal solely represents the changes of the  $^1\text{H}$  magnetization. In our

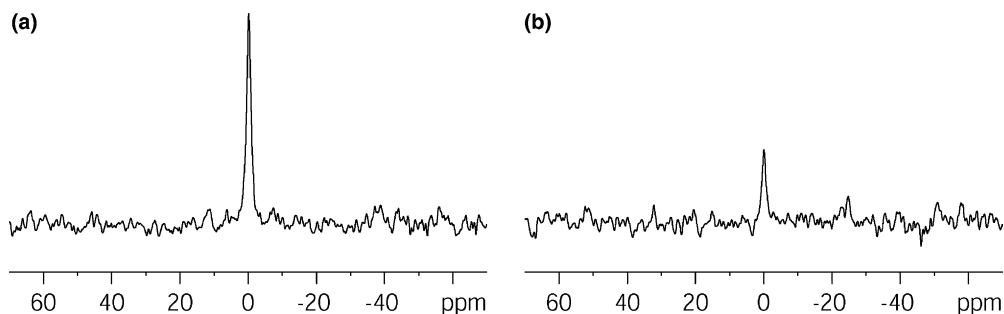


Fig. 3.  $^{15}\text{N}$  spectra of *N*-acetyl- $^{15}\text{N}$ -D,L-valine by using the pulse sequence shown in Fig. 1 without (a) and with (b) the  $^{15}\text{N}$   $\pi$  pulses, where  $\tau$  was set to 11.1  $\mu\text{s}$ .

experiments, we used a short contact time of 50  $\mu\text{s}$  for  $\tau_3$  and  $\tau_4$  to establish the coherent polarization transfers between the directly bonded  $^1\text{H}$  and  $^{15}\text{N}$  spins [17,18]. However, such a short contact time may result in low efficiency polarization transfers between  $^1\text{H}$  and  $^{15}\text{N}$ . It is worth noting that since only the amide  $^1\text{H}$  magnetization is selectively repolarized, a direct observation of the  $^1\text{H}$  magnetization [9] at the time of the rotational echoes under multiple pulse decoupling sequences [21], rather than the indirect observation via the third CP contact, can be used to enhance the sensitivity.

As in REDOR [13], two  $^{15}\text{N}$   $\pi$  pulses can be applied per rotor cycle, one in the middle and one at the end of each rotor cycle, to allow the phase angle from a weak dipolar coupling to be accumulated over many rotor cycles. In such a version, the efficiency of the homonuclear dipolar decoupling is particularly important for observation of the  $^{15}\text{N}$  signal. Although this method does not directly rely on  $^1\text{H}$   $T_2$ , it is required that the proton magnetization be monitored. For the sample of *N*-acetyl-valine, the measured  $^1\text{H}$   $T_2$  was 2.8 ms under the FSLG decoupling. It was shown [10] that at a modest rf field the decoupling efficiency of the FSLG sequences was relatively low in the presence of MAS. Symmetry-based sequences [22] such as MSHOT [10] and R18 $_2^5$  [5] could be applied so as to improve the homonuclear decoupling efficiency and to further lengthen the  $^1\text{H}$   $T_2$ , thus allowing to recouple weak heteronuclear dipolar couplings. On the other hand, the phase angle resulting from a relatively strong heteronuclear dipolar coupling, such as from the covalently

bonded N–H pair studied here, greatly depends on the position  $\tau$  of the  $\pi$  pulses within a rotor cycle, as diagrammed in Fig. 1. Fig. 4 shows the plot of the ratio of the  $^{15}\text{N}$  signal intensities with and without the  $^{15}\text{N}$   $\pi$  pulses vs  $\tau$ . The error bars in Fig. 4 were calculated by considering the noise level in the  $^{15}\text{N}$  spectra. After taking into account the finite  $^{15}\text{N}$   $\pi$  pulse length [23], the simulated curves were

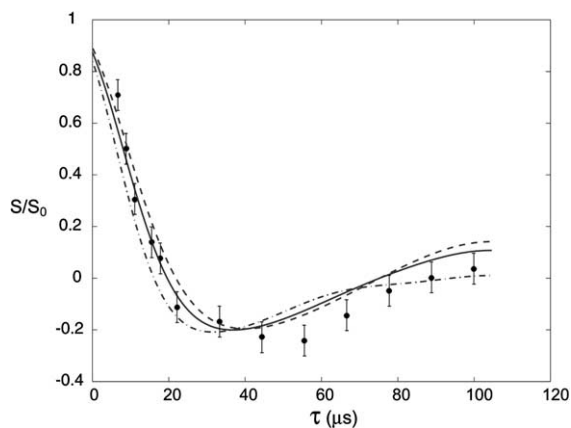


Fig. 4. Plots of the ratio of the  $^{15}\text{N}$  signal intensities with ( $S$ ) and without ( $S_0$ ) the  $\pi$  pulses on the  $^{15}\text{N}$  channel vs position  $\tau$  of the  $\pi$  pulses. The experimental data with error bars, which were calculated by considering the noise levels in the spectra, are compared to simulated curves at  $^1\text{H}$ – $^{15}\text{N}$  dipolar couplings of 6.5 (dashed line), 7.0 (solid line), and 8.0 kHz (dash-dotted line). The average  $^1\text{H}$ – $^{15}\text{N}$  dipolar coupling is  $7.25 \pm 0.75$  kHz. After considering the scaling factor of 0.57 induced by the FSLG decoupling, the actual  $^1\text{H}$ – $^{15}\text{N}$  dipolar coupling is  $12.5 \pm 1.3$  kHz, corresponding to a distance of  $99 \pm 4$  pm. The MATLAB program was used for the simulations taking into account the  $^{15}\text{N}$   $\pi$  pulse length of 13  $\mu\text{s}$ .

Table 1  
N–H dipolar coupling ( $D_{\text{NH}}$ ) and distance ( $r_{\text{NH}}$ ) in *N*-acetyl- $^{15}\text{N}$ -D,L-valine

This work		Diffraction [24]	NMR [6]
$D_{\text{NH}}/2\pi$ (kHz)	$r_{\text{NH}}$ (pm)	$r_{\text{NH}}$ (pm)	$r_{\text{NH}}$ (pm)
$12.5 \pm 1.3$	$99 \pm 4$	$95.7 \pm 0.5$	103.5

obtained with dipolar couplings of 6.5, 7.0, and 8.0 kHz, as shown in Fig. 4. Clearly, the  $^1\text{H}$ – $^{15}\text{N}$  dipolar coupling observed is  $7.25 \pm 0.75$  kHz. After considering the scaling factor of 0.57 induced by the FSLG irradiation on  $^1\text{H}$ , the actual  $^1\text{H}$ – $^{15}\text{N}$  dipolar coupling is  $12.5 \pm 1.3$  kHz, corresponding to a distance of  $99 \pm 4$  pm, which is consistent with the neutron diffraction data [24] as well as the previous NMR measurement [6], as compared in Table 1.

#### 4. Conclusion

We have demonstrated that specific protons (e.g., amide protons) can be selectively polarized and used to measure  $^{15}\text{N}$ – $^1\text{H}$  bond lengths under MAS condition using the principle of REDOR. The approach utilizes  $^1\text{H}$ , which possesses high gyromagnetic ratio, so as to increase dipolar couplings available for detection. As in REDOR, this method depends on the dipolar coupling and requires no knowledge of the chemical shift tensors. Therefore, the data analysis for extracting the dipolar coupling becomes very simple and thus reliable. Moreover, it could be applicable for measuring the distance between the amide protons and other nuclei such as  $^{13}\text{C}$  when the recoupling  $\pi$  pulses are applied to the  $^{13}\text{C}$  channel while observing the amide  $^{15}\text{N}$ . This approach is thereby capable of accessing relatively long distances in a comparison with a C–N pair because of the use of protons with high gyromagnetic ratio.

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#### References

- [1] R. Fu, T.A. Cross, *Annu. Rev. Biophys. Biomol. Struct.* 28 (1999) 235.
- [2] M. Cotten, R. Fu, T.A. Cross, *Biophys. J.* 76 (1999) 1179.
- [3] R. Fu, M. Cotten, T.A. Cross, *J. Biomol. NMR* 16 (2000) 261.
- [4] K. Takegoshi, T. Terao, *Solid State Nucl. Magn. Reson.* 13 (1999) 203.
- [5] X. Zhao, J.L. Sudmeier, W.W. Bachovchin, M.H. Levitt, *J. Am. Chem. Soc.* 123 (2001) 11097.
- [6] M. Hohwy, C.P. Jaroniec, B. Reif, C.M. Rienstra, R.G. Griffin, *J. Am. Chem. Soc.* 122 (2000) 3218.
- [7] B.-J. van Rossum, C.P. de Groot, V. Ladizhansky, S. Vega, H.J.M. de Groot, *J. Am. Chem. Soc.* 122 (2000) 3465.
- [8] X.L. Yao, K. Schmidt-Rohr, M. Hong, *J. Magn. Reson.* 149 (2001) 139.
- [9] I. Schnell, K. Saalwaechter, *J. Am. Chem. Soc.* 124 (2002) 10938.
- [10] M. Hohwy, N.C. Nielsen, *J. Chem. Phys.* 106 (1997) 7571.
- [11] M. Lee, W. Goldberg, *Phys. Rev.* 140 (1965) 1261.
- [12] S. Hediger, B.H. Meier, R.R. Ernst, *Chem. Phys. Lett.* 213 (1993) 627.
- [13] T. Gullion, J. Schaefer, *J. Magn. Reson.* 81 (1989) 196.
- [14] A. Bielecki, A.C. Kolbert, H.J.M. de Groot, R.G. Griffin, M.H. Levitt, *Adv. Magn. Reson.* 14 (1990) 111.
- [15] K. Nishimura, R. Fu, T.A. Cross, *J. Magn. Reson.* 152 (2001) 227.
- [16] R. Fu, C. Tian, H. Kim, S.A. Smith, T.A. Cross, *J. Magn. Reson.* 159 (2002) 167.
- [17] F. Tian, T.A. Cross, *J. Magn. Reson.* 125 (1997) 220.
- [18] F. Tian, R. Fu, T.A. Cross, *J. Magn. Reson.* 139 (1999) 377.
- [19] G.E. Pake, *J. Chem. Phys.* 16 (1948) 327.
- [20] A. Bielecki, A.C. Bolbert, M.H. Levitt, *Chem. Phys. Lett.* 155 (1989) 341.

- [21] J.S. Waugh, L.M. Huber, U. Haeberlen, *Phys. Rev. Lett.* 20 (1968) 180.
- [22] X. Zhao, M. Eden, M.H. Levitt, *Chem. Phys. Lett.* 342 (2001) 353.
- [23] C.P. Jaroniec, B.A. Tounge, C.M. Rienstra, J. Herzfeld, R.G. Griffin, *J. Magn. Reson.* 146 (2000) 132.
- [24] P.J. Carroll, P.L. Stewart, S.J. Opella, *Acta Crystallogr. C* 46 (1990) 243.