

The Direct Detection of a Hydrogen Bond in the Solid State by NMR through the Observation of a Hydrogen-Bond Mediated ^{15}N – ^{15}N J Coupling

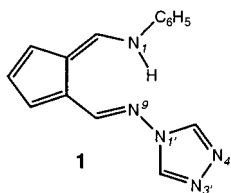
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Hydrogen bonds play a fundamental role in structural chemistry, for example, in determining the secondary structure of proteins and nucleic acids.¹ Moreover, in supramolecular chemistry, self-assembly is frequently driven by the formation of intermolecular hydrogen bonds.² However, hydrogen-bonded structures, because they by nature involve hydrogen atoms, are not easy to study using X-ray diffraction techniques. NMR, by comparison, seems to be very well placed to study hydrogen bonding. Indeed, in solution-state NMR of bio-macromolecules, it has recently been shown that J couplings can be observed across hydrogen bonds.³ This observation has generated much interest since, first, it was previously considered that scalar couplings only exist across covalent bonds, and second, the measurement of such J couplings allows the identification of hydrogen-bonded partners, as well as the quantification of hydrogen-bond strengths and geometries. In this Communication, we demonstrate, for the first time, that a *hydrogen-bond mediated J coupling* can be observed in the *solid state*. Thus, direct evidence for the existence of a hydrogen bond is provided.

In solid-state NMR, through-bond J couplings are usually one or more orders of magnitude smaller than the broadenings associated with anisotropic interactions such as the through-space dipolar coupling and the chemical shift anisotropy. Nevertheless, solid-state NMR experiments have recently been presented which utilize J couplings to establish homonuclear single-quantum (SQ)⁴ and double-quantum (DQ)⁵ as well as heteronuclear⁶ correlations. In this Communication, ^{15}N – ^{15}N J couplings in fully ^{15}N -labeled **1** (*N*-{[[5-(phenylamino)methylene]-1, 3-cyclopentadien-1-yl] methylene}-1,2,4-triazole-4-amine) are probed by means of the solid-state INADEQUATE⁵ and refocused INADEQUATE⁷ experiments. In previous studies, where the synthesis of **1** is described, $^1J(\text{N9},\text{N1}')$ and $^{2h}J(\text{N1}\cdots\text{H}\cdots\text{N9})$ equal to 11.8 and 8.6 Hz have been measured for a solution of **1** in CDCl_3 .



All spectra presented here were recorded on a Bruker DSX 500 NMR spectrometer, operating at ^{15}N and ^1H Larmor frequencies of 50.7 and 500.1 MHz, respectively, using a 4 mm magic-angle

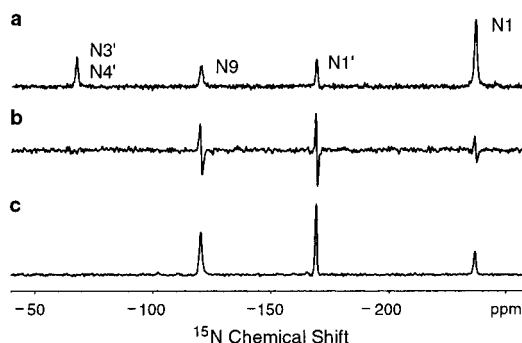


Figure 1. (a) CP MAS, (b) INADEQUATE,⁵ and (c) refocused INADEQUATE⁷ one-dimensional ^{15}N solid-state NMR spectra of **1**. The CP contact time was 3 ms in (a) and 5 ms in (b, c). A recycle delay of 3 s in (a) and 5 s in (b, c) was used. The number of co-added transients was 128 in (a) and 4096 in (b, c). The J coupling evolution period, τ ,^{5,7} was 10 ms in (b, c).

spinning (MAS) triple-resonance probe. The sample volume was restricted by flat spacers, such that approximately 20 mg of sample was used. The MAS frequency was 12.0 kHz. The ^1H and ^{15}N 90° pulse lengths were 2.5 and 5.0 μs , respectively. For cross polarization (CP) from ^1H to ^{15}N , the ^{15}N rf field was set to around 45 kHz, while a ramped rf field was applied on the ^1H channel.⁹ ^1H decoupling used the TPPM method,¹⁰ with a ^1H rf field of 100 kHz. A 32-step phase cycle was used for the INADEQUATE and refocused INADEQUATE experiments (the pulse program and phase cycle is available from our website¹¹ or by request to the authors). ^{15}N chemical shifts are referenced relative to neat liquid CH_3NO_2 , by using the ^{15}N resonance of glycine at -347.4 ppm as an external reference. To convert to the chemical shift scale frequently used in protein NMR, where the reference is liquid ammonia at -50 °C, it is necessary to add 379.5 to the given values.¹²

Figure 1a shows the ^{15}N CP MAS spectrum of **1**. On the basis of the known solution-state chemical shifts,⁸ the resonances can be assigned: -67.4 (N3', N4'), -119.9 (N9), -168.9 (N1'), -236.4 (N1). For short CP contact times, the resonance at -236.4 is dominant, since this is the only protonated nitrogen atom. The line widths (full-width at half-maximum height) are between 50 and 70 Hz and are, thus, significantly larger than the solution-state homonuclear ^{15}N J couplings, such that a simple splitting of the type observed in solution-state NMR spectra is not evident.

In an INADEQUATE experiment, DQ coherence (DQC) is created for J -coupled pairs of nuclei.^{5,13} It is to be noted that when the two nuclei of a through-bond connected isolated spin pair have the same chemical shift, neither a J splitting nor a signal in the INADEQUATE spectrum is observed.¹⁴ This is to be contrasted

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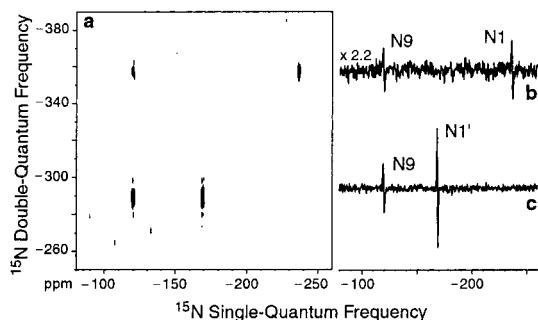


Figure 2. (a) A two-dimensional ^{15}N solid-state NMR INADEQUATE⁵ spectrum of **1**, recorded with a J coupling evolution period,⁵ $\tau = 14$ ms. The CP contact time was 5 ms, and a recycle delay of 6 s was used. A total of 64 transients were coadded for each of 128 t_1 increments of 12.5 μs . A Gaussian broadening was applied to the truncated signal before the t_1 Fourier transformation. The lowest contour corresponds to 12% of the maximum signal. Traces extracted for DQ frequencies of -357 and -289 ppm are shown in (b) and (c), respectively.

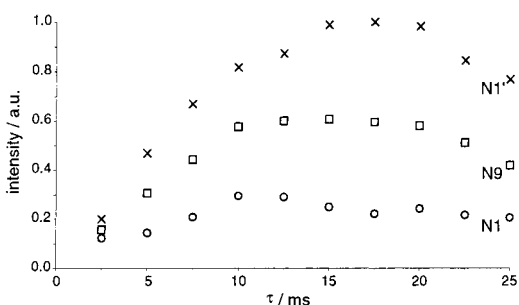


Figure 3. The build-up of signal intensity in one-dimensional ^{15}N INADEQUATE spectra of **1** as a function of the J coupling evolution period, τ ,⁵ for N9 (squares), N1' (crosses), and N1 (circles). All spectra were recorded under the same experimental conditions as those of Figure 1b.

with the case of the corresponding dipolar-coupling mediated DQ MAS experiment, in which peaks between equivalent spins are observed.¹⁵ Figures 1b and 1c show one-dimensional ($t_1 = 0$) (b) INADEQUATE⁵ and (c) refocused INADEQUATE⁷ spectra of **1**. In the latter case, the addition of a second spin-echo leads to the observation of in-phase as opposed to anti-phase line shapes. The better sensitivity of the refocused INADEQUATE experiment is evident. The absence of signal above the noise level at the position of the N3', N4' resonance in both Figures 1b and 1c proves that it is exclusively the through-bond J coupling which mediates the creation of DQC in these experiments, i.e., the through-space dipolar coupling (which is 490 Hz for the N3'–N4' pair) does not lead to any coherence transfer processes.

Significant peak intensity is observed for the N1 nitrogen in both Figures 1b and 1c. This can only be explained by a J coupling to a different nitrogen. The J -coupled partner of N1 can be identified by means of the two-dimensional INADEQUATE experiment in Figure 2a. In such a spectrum, signals are observed at the DQ frequency (vertical dimension) corresponding to the sum of the SQ frequencies (horizontal dimension) of the two J -coupled nuclei. The extracted trace in Figure 2b for a DQ frequency of -357 ppm demonstrates that the N1 nitrogen (-236.4 ppm) is J -coupled across the hydrogen bond to the N9 nitrogen (-119.9 ppm). As expected, the N9 nitrogen is also J coupled to its directly bonded neighbor, N1' (-168.9 ppm), as is evident from the trace extracted for a DQ frequency of -289 ppm (Figure 2c).

Figure 3 shows the build-up of INADEQUATE signal intensity for the three nitrogens, obtained from a series of spectra of the type in Figure 1b recorded with different τ values. For each of the three nuclei in the three-spin J -coupled system, the intensity depends

on the two J couplings,¹⁶ as well as the relaxation rates and initial intensities (after CP). As will be described elsewhere, the two J couplings can be determined straightforwardly from the observed zero-crossings in a two-dimensional spin-echo experiment: A preliminary analysis for **1** yields $^1J(\text{N9}, \text{N1}') = 12 \pm 1$ Hz and $^2J(\text{N1}' \cdots \text{H} \cdots \text{N9}) = 8 \pm 1$ Hz.

To conclude, we have observed signal intensity in ^{15}N INADEQUATE experiments for the N1–N9 pair in **1**, which prove that the two nuclei are J coupled across a hydrogen bond. We believe this to be the first observation in the solid state of a hydrogen-bond mediated J coupling, and, hence, the first-ever direct detection of a solid-state hydrogen bond, i.e., we do not infer the presence of a bond from either the position of the proton (X-ray) or from the ^1H chemical shift, but rather directly from the presence of an interaction that requires a bond. The future observation of a heteronuclear ^{15}N – ^1H hydrogen-bond mediated J coupling can also be envisaged.

Solid-state NMR is already well-suited to the investigation of hydrogen bonding; for example, the ^1H chemical shift and the ^2H quadrupolar coupling constant are sensitive indicators of hydrogen-bonding strength,¹⁷ while the measurement of dipolar couplings allows the quantitative determination of hydrogen-bond distances.¹⁸ The ability to measure hydrogen-bond mediated J couplings provides a further probe of hydrogen bonding in solids. Numerous applications to, e.g., supramolecular systems and membrane proteins can be envisaged.

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