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Determining hydrogen-bond strengths in the solid state by NMR: the quantitative measurement of homonuclear *J* couplings†

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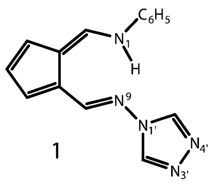
Hydrogen-bonding strengths in the solid state are quantitatively determined by the accurate measurement of ^{15}N–^{15}N *J* couplings using a straightforward 2D MAS NMR spin-echo approach.

Hydrogen bonds are one of the most important structure-directing interactions in chemistry, as is evidenced by their role in controlling self-assembly in supramolecular chemistry and in determining the secondary structure of proteins and nucleic acids. Indirect evidence for the presence of a hydrogen bond can be provided by the positions of hetero- and hydrogen atoms as determined from a diffraction study, or by the observation of characteristic IR vibration frequencies or NMR chemical shifts. Recently, *J* couplings across hydrogen bonds have been observed in solution-state NMR spectra of bio-macromolecules[1] and also smaller molecules.[2] In this way, hydrogen bonds are *directly detected*, since the presence of such a *hydrogen-bond mediated J coupling* requires a bond. In solid-state NMR, the detection of *J* couplings is a challenge, since they are usually obscured even under magic-angle spinning (MAS) by the broadenings due to anisotropic interactions. In the last few years, solid-state NMR experiments have been developed which utilize *J* couplings to establish homonuclear single-quantum[3] and double-quantum[4] as well as heteronuclear correlations.[5] Very recently, we have presented ^{15}N INADEQUATE solid-state NMR spectra of **1** which demonstrate the presence of a ^{15}N–^{15}N *J* coupling across the hydrogen bond (N1–N9);[6] we believe this to be the first observation of a hydrogen-bond mediated *J* coupling in the solid state, and hence the *first-ever direct detection* of a *solid-state hydrogen bond*. Here, the hydrogen-bond mediated ^{15}N–^{15}N *J* couplings in **1** and **2** are *accurately determined* (±0.2 Hz) by a straightforward *2D spin-echo* MAS approach. In this way, the *hydrogen-bond strengths* can be *quantified*.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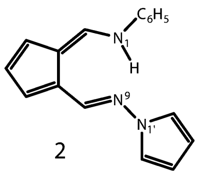
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The investigated 6-aminofulvene-1-aldimines differ in their molecular structure solely with regard to the triazole (**1**) or pyrrole (**2**) substituent; their synthesis as well as X-ray single-crystal and solution-state NMR characterisations are described in ref. 7. The molecules were fully ^{15}N-labelled. Solid-state NMR experiments were performed on a Bruker DSX 500 NMR spectrometer using a MAS frequency of 12.0 kHz. Other experimental details are available as ESI.†

The line widths observed for rigid solids in MAS experiments are typically at least 10 s of Hz—the line widths in a ^{15}N cross-polarisation (CP) MAS spectrum of **1** are 50–70 Hz.[6] Thus, only splittings due to relatively large *J* couplings can be observed. In particular, the ^{15}N–^{15}N *J* couplings in **1** and **2**—the solution-state values are listed in Table 1—are too small to give rise to an observable splitting in the ^{15}N CP MAS spectra. In NMR, the simple Carr–Purcell spin-echo (τ–π–τ) sequence achieves the refocusing of evolution due to all terms that appear as offsets, which may arise from, *e.g.*, a distribution of chemical shifts or imperfect decoupling. Importantly, in the solid state, the 'non-refocusable' line width, $\Delta' = 1/\pi T_2'$, which is defined by the time constant, T_2', measured in such a spin-echo experiment, is usually significantly less than the 'apparent' line width.[4b,9] Thus, if spin-echoes are incorporated into solid-state NMR experiments, it is possible to detect and utilize *J* couplings, *even if no splitting can be observed in the normal spectrum*. Spin echoes are important elements of the solid-state INADEQUATE[4a] and refocused INADEQUATE[4b] experiments employed in ref. 6.

We demonstrate here that rotor-synchronised 2D spin-echo solid-state NMR experiments are a straightforward method for the accurate measurement of *J* couplings, especially suitable for the case where no splitting is observable in the normal spectrum. For a ^{15}N nucleus which is *J*-coupled to a single non-equivalent neighbour, the dependence of the integrated frequency-domain signal on the spin-echo delay, τ, is given by:

$$S(\tau) = A\cos(2\pi J\tau)\exp(-2\tau/T_2') \qquad (1)$$

To extract the *J* coupling and the effective dephasing time, T_2', it is simply necessary to record a series of 1D experiments, where the spin-echo sequence follows CP from ^1H to ^{15}N, and fit the integrated intensities in the frequency-domain spectra to eqn. (1). Alternatively, it can be recognised that eqn. (1) describes a free-induction decay, where Fourier transformation (FT) with respect to 2τ yields a pair of lines with width $\Delta' = 1/\pi T_2'$ at $\pm J/2$, *i.e.*, the familiar *J*-coupled doublet. This, thus, suggests a 2D experiment, CP $- t_1/2 - \pi - t_1/2 - t_2$, which is the solid-state equivalent of homonuclear *J* spectroscopy.[10] In order to obtain an amplitude-modulated signal with respect to t_1, and hence pure absorption-mode lineshapes after FT in both dimensions, a '*z*-filter' ($\pi/2 - 2\mu s - \pi/2$)[8] can be inserted before t_2. This *z*-filtered spin-echo experiment was employed

Table 1 Parameters extracted from the fits to the spin-echo spectra

Molecule	Site	^1J/Hz	^{2h}J/Hz	T_2'/ms	$[\varepsilon^2]^a$
1	^{b}N1′	11.9 ± 0.1	—	248 ± 15	0.0019
1	^{b}N1	—	7.4 ± 0.1	149 ± 10	0.0009
1	^{c}N9	12.0 ± 0.1	7.2 ± 0.1	194 ± 15	0.0009
1	—	11.8^d	8.6^d	—	—
2	^{b}N1′	10.3 ± 0.1	—	255 ± 20	0.0026
2	^{b}N1	—	8.1 ± 0.2	90 ± 10	0.0110
2	^{c}N9	10.2 ± 0.4	8.0 ± 0.3	159 ± 25	0.0059
2	—	10.3^d	9.0^d	—	—

[a] $\varepsilon^2 = \Sigma[I_{fit}(n) - I_{exp}(n)]^2/\Sigma I_{exp}(n)^2$. [b] Fit to $A\cos(2\pi J\tau)\exp(-2\tau/T_2')$. [c] Fit to $A\cos(2\pi J\tau)\cos(2\pi J'\tau)\exp(-2\tau/T_2')$. [d] Solution-state NMR ($CDCl_3$) from (**1**) ref. 7*a* and (**2**) ref. 7*b*.

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† Electronic supplementary information (ESI) available: additional experimental details. See http://www.rsc.org/suppdata/cc/b2/b205324a/

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here, with ^{1}H TPPM decoupling[11] at a rf field of 100 kHz being applied after CP.

Fig. 1 presents plots of experimental S(τ) against τ for the N1′, N1, and N9 resonances in **1** and **2**, together with the best-fit analytical curves—see Table 1. Zero crossings are observed in all plots. A consideration of eqn. (1) reveals that such zero-crossings occur at $\tau = n/4J$, where n is an odd integer. Thus, the observation of the first zero-crossing at $\tau = 1/4J$ immediately gives a good estimate of J, *e.g.*, for N1′ and N1 in **1** zero crossings are observed at about 21 and 33 ms, corresponding to $J = 12$ and 7.5 Hz, respectively. The N9 nitrogen is J-coupled to both N1′ and N1, and there is thus an additional $\cos(2\pi J'\tau)$ modulation, and two zero-crossings corresponding to the two J couplings are observed. Comparing the plots for **1** and **2**, the two J couplings are clearly closer together in value in **2**.

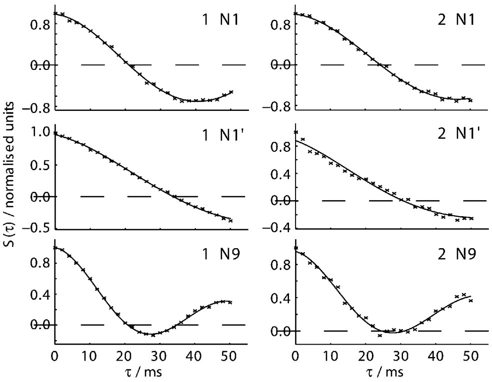

Fig. 1 Plots of $S(\tau)$ against τ for the N1, N1′, and N9 resonances in **1**and **2**, together with the best-fit analytical curves (see Table 1).

A 2D spin-echo spectrum of **1** is shown in Fig. 2 together with extracted rows for the four distinct ^{15}N resonances. For the equivalent N3′ and N4′ nitrogens, there is no observable J coupling, and a single resonance at zero frequency is observed—in a plot of $S(\tau)$ against τ (not shown) no zero crossing is observed. For N1, N1′, and N9, the refocused line widths, Δ', are sufficiently small to allow the familiar J coupling patterns to be identified—for N9, the two resonances at $\pm\{^1J(\text{N9,N1}')$—$^{2h}J(\text{N1}\cdots\text{H}\cdots\text{N9})\}$ are not resolved. It is to be noted that the J couplings and effective dephasing times could be equally determined from a fit of these frequency-domain spectra.

Considering both experimental values obtained from solution-state NMR of RNA and DNA fragments and quantum-chemical calculations, $^{2h}J(\text{N}i\cdots\text{H}\cdots\text{N}j)$ increases with decreasing N$i\cdots$Nj and increasing Ni–H (Ni is the donor) distances, and is thus a measure of *hydrogen bond strength*.[12] Comparing the $^{2h}J(\text{N1}\cdots\text{H}\cdots\text{N9})$ couplings for the two molecules given in Table 1, a smaller value is found for **1** in both the solid- and solution-state, indicating a weaker hydrogen-bond. This conclusion is supported by the ^{1}H chemical shifts of the hydrogen-bonded proton (determined by ^{1}H MAS (30 kHz) NMR, spectra not shown) being 11.5 and 11.9 ppm in **1** and **2**, respectively. The N1$\cdots$N9 distances given by the X-ray single-crystal structures of 2.84 Å in **1** and 2.83 Å in **2** are identical within the experimental error,[7a,7b] *i.e.*, the difference is most likely manifested in a harder-to-detect difference in the proton position. Table 1 also compares the solid- and solution-state J couplings: while the solid- and solution-state $^1J(\text{N9,N1}')$ values are very similar, the solid-state $^{2h}J(\text{N1}\cdots\text{H}\cdots\text{N9})$ values are about 1 Hz smaller than those measured in solution for both **1** and **2**. It, thus, appears that the hydrogen bonds are weaker in the solid state than in solution.

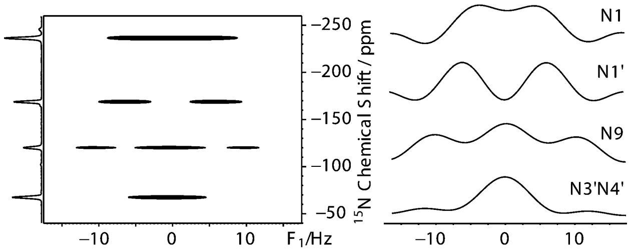

Fig. 2 A ^{15}N 2D MAS spin-echo (with z-filter) spectrum of **1**, together with extracted rows corresponding to the different ^{15}N resonances.

Our approach is, of course, not restricted to ^{15}N NMR; as will be described elsewhere, ^{13}C experiments carried out on fully-labelled amino acids and partially-labelled cellulose allow, in addition to the determination of the J couplings, the assignment of the resonances and the determination of the degree of enrichment, respectively, while ^{31}P experiments have been used to characterise P–O–P bonds. We note that ^{31}P–^{31}P J couplings have been extracted from absolute-value 2D spectra obtained using the spin-echo sequence without the z-filter.[13] We have also very recently used the heteronuclear equivalent of these spin-echo experiments to measure ^{13}C–^{1}H J couplings in surface species.[14]

To conclude, we have shown that a 2D spin-echo MAS solid-state NMR experiment allows the accurate measurement of small J couplings, the splittings due to which are lost under the residual line broadening in a CP MAS experiment. In this way, hydrogen-bond mediated ^{15}N–^{15}N J couplings, which provide direct evidence for the existence of hydrogen bonds and are a direct measure of hydrogen-bond strength, have been measured for **1** and **2**. Many related applications to, *e.g.*, supramolecular systems and fully-labelled biomolecules can be envisaged.

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