

# Dipolar Recoupling in NOESY-Type $^1\text{H}$ – $^1\text{H}$ NMR Experiments under HRMAS Conditions

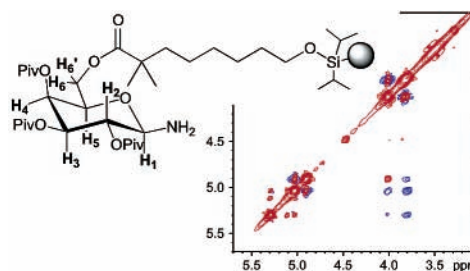
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## ABSTRACT



The concept of dipolar recoupling is introduced to  $^1\text{H}$ – $^1\text{H}$  NOESY experiments performed under HRMAS conditions. Dipole–dipole couplings are selectively recoupled during the mixing period, while MAS ensures high resolution in the spectral dimensions. Incoherent dipolar exchange is replaced by amplified coherent processes, such that time scales for polarization transfer are shortened, and dipolar double-quantum techniques become applicable. In this way, dipole–dipole couplings, as well as  $J$ -couplings, can be individually measured.

In partially immobilized systems, standard solution-state NMR experiments frequently fail because residual anisotropic interactions, mainly dipole–dipole couplings and susceptibility effects, broaden the resonance lines and hamper the spectral resolution. To overcome this problem, line narrowing by magic-angle spinning (MAS), well established in solid-state NMR, has been combined with solution-state NMR<sup>1</sup> experiments. This high-resolution (HR) MAS approach<sup>2,3</sup>

merely uses MAS to make the system appear more liquidlike. However, the anisotropies, in particular the homonuclear dipole–dipole interaction, can also be exploited by means of other solid-state NMR methods to gain information that is usually not accessible in liquids. As a first example of this new approach, the idea of dipolar recoupling is introduced to MAS–NOESY experiments. During the mixing period, the dipole–dipole couplings are then no longer averaged by MAS but selectively recoupled by radio frequency (RF) pulse sequences, while unwanted anisotropic interactions remain suppressed.<sup>4</sup> In this way, incoherent dipolar exchange is replaced by amplified coherent processes, such that (i) time scales for polarization transfer are shortened, (ii) dipolar double-quantum (DQ) filters and DQ spectroscopy become applicable, and (iii) dipole–dipole couplings can be directly measured. Moreover, isotropic ( $J$ –

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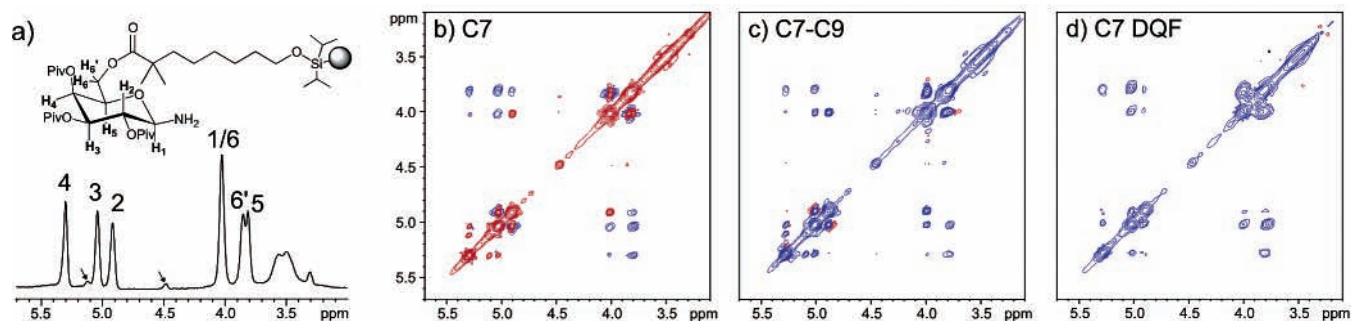
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**Figure 1.** (a) Structure,  $^1\text{H}$  MAS spectrum ( $\nu_R = 5$  kHz), and resonance assignment of the O-pivaloylated galactosylamine sample under investigation; the arrows indicate resonances of a residual amount ( $\sim 1\%$ ) of galactosyl azide that was not converted to the amine. (b)  $^1\text{H}$ – $^1\text{H}$  MAS exchange spectrum ( $\nu_R = 3571$  Hz) with dipolar recoupling during mixing ( $\text{C7}_2^1$  sequence,  $\tau_{\text{mix}} = 12$  ms). Negative contours are shown in blue and positive contours in red. (c) Difference spectrum of C7 recoupling and C9 decoupling. (d) DQ-filtered spectrum, yielding pure dipolar contacts.

driven) mixing can be realized<sup>5</sup> and distinguished from dipolar exchange.

The experiments proposed above were carried out on a sample with features similar to those typically encountered in solid-phase synthesis. This synthetic method is more and more commonly used in the chemistry of carbohydrates, which represent a class of molecules that is central in current research. They usually adopt a ring conformation, featuring short distances between the protons, which result in distinct through-space as well as through-bond contacts.

2,3,4,6-Tetra-*O*-pivaloyl- $\beta$ -D-galactosylamine has been found to be a valuable tool in several stereoselective syntheses. It has been used as a chiral auxiliary in the synthesis of alkaloids and chiral heterocycles, as well as in stereoselective Ugi and Strecker syntheses of  $\alpha$ -amino acid derivatives.<sup>6</sup> The transfer of these solution reactions to the solid phase opens up the possibility for asymmetric combinatorial chemistry, enabling the generation of stereochemically pure compound libraries. The immobilization of the O-pivaloylated galactosylamine, through an ester linkage, onto Wang resin, and its successful application in stereoselective Ugi reactions were recently reported.<sup>7</sup> The linkage between the galactosyl moiety and the resin was established at the primary, and therefore easily accessible, hydroxy group at position 6. Due to the synthetic purpose of the compound, the anchoring has to be reversible, and both the anchor and the spacer should not interfere with subsequent reactions. To render further stereoselective solid-phase reactions amenable, an alternative fluoride labile linking strategy was chosen, giving rise to the resin-bound system investigated

herein (Figure 1a). The spacer that has been found to be suitable is a sterically hindered  $\alpha,\alpha$ -branched  $\omega$ -hydroxy carboxylic acid, which resembles the pivaloyl groups, and should therefore provide a mainly uniform structure of the ester groups around the carbohydrate. The length of the spacer, which is basically a  $\text{C}_7$ -alkyl chain, is chosen such that the carbohydrate frame should show sufficient overall mobility. The linkage of the spacer to the polymeric support is achieved through formation of a silyl ether, connecting the  $\omega$ -hydroxy function of the spacer unit with silylated polystyrene, that can readily be obtained via lithiation and subsequent reaction with dichlorodisopropylsilane.<sup>8</sup> The loading of the resin was monitored by IR spectroscopy and quantified by elemental analysis to 0.414 mmol/g. An investigation using conventional solution-state NMR is not feasible, because the restriction of rapid molecular motions of the swollen sample gives rise to inhomogeneously broadened resonance lines in the spectra. The application of HRMAS provides sufficiently resolved  $^1\text{H}$  spectra (Figure 1a), although splittings arising from  $J$ -couplings are still concealed. The sample was swollen in a 4 mm HRMAS rotor using chloroform- $d_1$ . All spectra were acquired at room temperature on a Bruker DRX-700 NMR spectrometer with a  $^1\text{H}$  Larmor frequency of 700.13 MHz, using a MAS double-resonance probehead without  $^2\text{H}$  lock capabilities. The RF-fields were set to power levels corresponding to  $90^\circ$  pulses of 9.25 and 10.92  $\mu\text{s}$  (for C7 and C9 pulse sequences, respectively; see below).

For rapid molecular motions in liquids or under MAS conditions, dipole–dipole couplings are efficiently reduced and only give rise to relaxation phenomena, which are commonly evaluated on a phenomenological basis according to Macura and Ernst.<sup>9</sup> The information obtainable from cross-relaxation rates, however, is a combination of the dipole–

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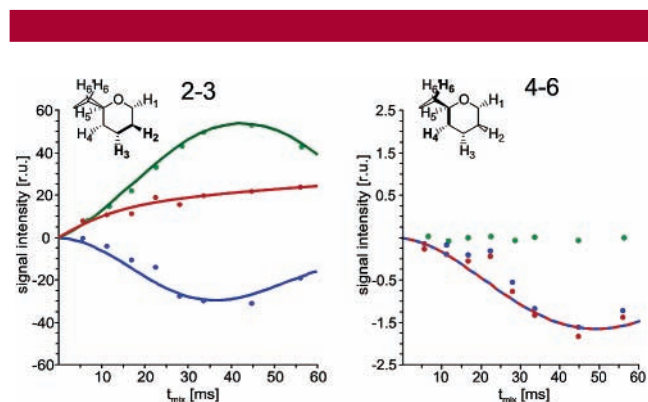
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dipole coupling,  $D_{\text{HH}}$ , and a motional correlation time,  $\tau_c$ . In the Lipari–Szabo model,<sup>10</sup> even two time scales for a fast anisotropic and a slow isotropic motion are considered in macromolecules. Thus, MAS–NOESY experiments do not provide dipole–dipole couplings directly but only in conjunction with unknown motional correlation times, and the quantification of the couplings is difficult. The samples in question for HRMAS applications, however, possess significant anisotropies and, hence, residual dipolar interactions, which should be measured directly instead of being removed by MAS. Dipolar recoupling pulse sequences such as the helical  $CN_n^v$ -types developed by Levitt and co-workers<sup>4</sup> compensate for the averaging effect of MAS. At first sight, zero-quantum (ZQ)-type recoupling might appear as the natural choice, since it resembles the character of the original homonuclear dipolar interaction. DQ-type recoupling, however, facilitates the suppression of unwanted anisotropic interactions due to its unique spin rank of two.<sup>4</sup> With regard to two-dimensional exchange experiments, the dipolar DQ-Hamiltonian retains the difference magnetization,  $I_z - S_z$ , of two coupled spins  $I$  and  $S$ , such that DQ-exchange signals are negative (off-diagonal) with respect to positive signals of uncoupled spins (on-diagonal). In particular, exchange signals arising from isotropic  $J$ -couplings are positive due to the ZQ-character of the isotropic mixing Hamiltonian. Thus, DQ-dipolar “through-space” exchange can be readily distinguished from “through-bond”  $J$ -exchange by means of the sign of the signals.

To observe exchange under dipolar recoupling, we applied the  $C7_2^1$  sequence<sup>11</sup> during the mixing period of a standard three-pulse NOESY experiment, corresponding to an experiment described by Geen et al.<sup>12</sup> The average Hamiltonian,  $H_{C7}$ , contains a recoupled dipolar DQ-part and an isotropic mixing ( $J$ -coupled) part:  $H_{C7} = H_{\text{DQ}} + H_J$ . Therefore, superpositions of negative dipolar and positive  $J$ -exchange signals are observed in the spectra (see Figure 1b). Under recoupling conditions, dipolar exchange is already observed for a mixing time of 12 ms, while  $\sim 300$  ms are needed for MAS–NOESY. Additionally, the resin signals are reduced in a similar fashion, though not as efficiently, as described by Ditty et al.<sup>13</sup> The dipolar signals can be selected either by a DQ filter (DQF) or by subtracting the  $J$ -exchange signals. The DQF is realized by dividing the recoupling pulse sequence into two blocks of equal length and performing an appropriate phase cycle.<sup>12</sup> A representative DQF spectrum is shown in Figure 1d. Alternatively, a subtraction procedure can be applied: replacing the  $C7_2^1$  pulse sequence by  $C9_3^1$  yields  $J$ -exchange spectra (not shown),<sup>5</sup> because  $C9_3^1$  removes anisotropic interactions, including dipole–dipole couplings, and provides isotropic mixing ( $H_{C9} = H_J$ ). Thus, a “ $C7$ – $C9$ ” difference spectrum represents an approximation for the action of the difference Hamiltonian  $H_{C7} - H_{C9} = H_{\text{DQ}}$ . The resulting spectrum is shown in Figure 1c. This

approach is feasible as the change from  $C7_2^1$  to  $C9_3^1$  is so small that identical effective relaxation behavior of the spins can be assumed.

Dipole–dipole,  $D_{\text{HH}}$ , and  $J$ -couplings,  $J_{\text{HH}}$ , can be determined from the off-diagonal peak intensities in  $C7$  and  $C9$  exchange spectra with varying mixing times. Ideally, the curves are of the form  $S(\tau_{\text{mix}}) \propto \sin^2(C\tau_{\text{mix}})$ , where  $C$  contains either the scaled dipole–dipole coupling<sup>4,11</sup> or the  $J$ -coupling. The  $J$ -oscillations are directly accessible from  $C9$  data, while the dipolar curve requires either a DQ-filter or the subtraction of  $C7$  and  $C9$  data. In Figure 2, the latter approach is shown



**Figure 2.** Signal intensities from  $^1\text{H}$ – $^1\text{H}$  MAS exchange spectra for two proton pairs.  $J$ - and dipole–dipole couplings are superimposed for  $C7$  recoupling (red), while  $C9$  yields pure  $J$ -oscillations (green). The difference “ $C7 - C9$ ” (blue) represents pure dipole–dipole couplings.

together with calculated curves, and the results are listed in Table 1. Motional order parameters,  $S$ , in the range of 0.5–

**Table 1.** Dipole–Dipole,  $D_{\text{HH}}$ , and  $J$ -Couplings Measured from  $C7$  and  $C9$  Exchange Spectra<sup>a</sup>

contact	$D_{\text{HH}}/2\pi$ [Hz]	$S$ [%]	$J_{\text{HH}}$ [Hz]
1–2	70	$1.7 \pm 0.2$	13
1–3	53	$0.9 \pm 0.1$	
2–3	68	$1.7 \pm 0.2$	11.5
2–4	26	$1.2 \pm 0.2$	
3–4	52	$0.6 \pm 0.1$	5
3–5	obscured		
4–5	140	$1.7 \pm 0.2$	<1
4–6	35	$0.5 \pm 0.1$	
5–6	obscured		$\sim 7$
6–6'	obscured		14

<sup>a</sup> Accuracy is approximately  $\pm 10$  Hz for  $D_{\text{HH}}$  and  $\pm 0.5$  Hz for  $J_{\text{HH}}$ . Order parameters,  $S$ , are calculated by relating experimental  $D_{\text{HH}}$  with  $D_{\text{HH}}$  calculated for a rigid molecule.

1.7% are deduced from comparing the measured  $D_{\text{HH}}$  with the  $D_{\text{HH}}$  corresponding to the proton–proton distances in the (rigid) structure. The latter was calculated for a single molecule in vacuum applying a MM2 procedure. The protons  $\text{H}_1$ – $\text{H}_2$  and  $\text{H}_2$ – $\text{H}_3$ , for example, are expected to adopt an axial–axial arrangement with a 3.1 Å distance.

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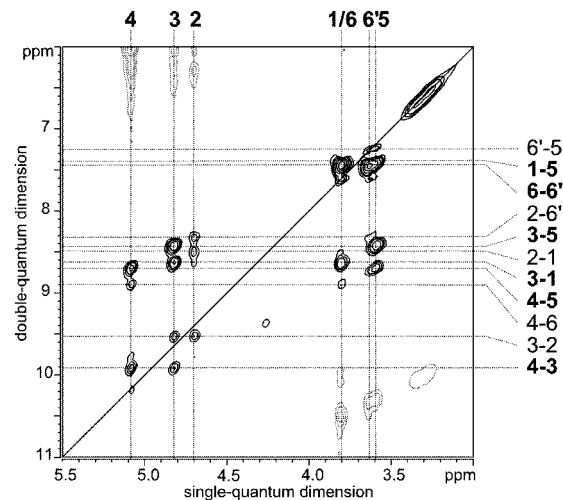
Consistently, the measured  $D_{\text{HH}}$  are identical, leading to  $S = 1.7\%$ . This indicates a high and uniform degree of mobility, as expected due to the sugar molecule being bound to the resin by a flexible linking chain. Considering the values given in Table 1, potential reasons for the observation of  $S < 1.7\%$  are (i) anisotropic molecular motions that average the couplings slightly differently or (ii) slight differences between the calculated and the actual conformation, which could arise from solvent or steric substituent effects. In particular, the low-order parameter of the 3–4 contact indicates a distortion at this ring segment, i.e., the actual  $\text{H}_3\text{--H}_4$  distance is slightly larger than calculated. Apparently, the distortion does not affect the 4–5 contact, as suggested by the value of the respective order parameter ( $S = 1.7\%$ ). For the 4–6 contact, the order parameter is low because it involves a  $\text{CH}_2$  proton (6) that is not part of the galactose ring, such that the molecular segments can move relative to each other. Table 1 also lists  $J_{\text{HH}}$  values, which have been determined from the oscillations of the intensities of C9 exchange signals (Figure 2). In principle, line splittings would yield more accurate values, but the resolution of HRMAS spectra is not sufficient. In the intensity curves, we only evaluated the initial regime, since it is dominated by the coupling of the respective spin pair, and effects of multiple couplings play a minor role. The  $J_{\text{HH}}$  values so obtained agree remarkably well with the NMR data of the solubilized sugar.<sup>14</sup>

In the experiments discussed so far, DQ coherences merely serve as transition states for dipolar exchange. Alternatively, DQ spectroscopy, which recently proved to be highly informative in solids,<sup>15</sup> can be performed by inserting the  $t_1$  dimension into the mixing period. The resulting DQ spectra contain the same information as DQF exchange spectra, but the DQ dimension allows for an unambiguous identification of peaks that would coincide on the diagonal in exchange spectra. Thus, all resonances can be readily assigned on the

(14)  $J$ -Couplings for 3–4 and 4–5 are not identical, although both pairs adopt an axial–equatorial arrangement. This suggests a slight distortion of the 3–4 segment, supporting the interpretation of the C7–C9 data.

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basis of spatial proton–proton proximities by virtue of a single dipolar  $^1\text{H}\text{--}^1\text{H}$  DQ spectrum (Figure 3).



**Figure 3.** Dipolar  $^1\text{H}\text{--}^1\text{H}$  DQ spectrum recorded under MAS ( $\nu_{\text{R}} = 3571$  Hz) applying dipolar recoupling for DQ excitation and reconversion ( $\text{C7}_2^1$  sequence,  $\tau_{\text{exc}} = \tau_{\text{rec}} = 5.6$  ms). For short  $\tau_{\text{exc/rec}} (\tau_{\text{exc/rec}} \ll 2\pi/D_{\text{HH}})$ , a semiquantitative interpretation is possible: the stronger the DQ signal, the shorter the distance between the involved nuclei.<sup>15c</sup>

These first examples demonstrate that solid-state NMR techniques can indeed be applied under HRMAS conditions to identify and directly measure dipole–dipole couplings. In this way, molecular conformations and mobilities can be locally probed, and motional order parameters can be individually determined for molecular segments. Furthermore, decoupling techniques enable the quantification of through-bond interactions, given that  $J$ -splittings cannot be observed due to insufficient spectral resolution.

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