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# J-ONLY-TOCSY: Efficient suppression of RDC-induced transfer in homonuclear TOCSY experiments using JESTER-1-derived multiple pulse sequences

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**Abstract**

The main purpose of homonuclear Hartmann–Hahn or TOCSY experiments is the assignment of spin systems based on efficient coherence transfer via scalar couplings. In partially aligned samples, however, magnetization is also transferred via residual dipolar couplings (RDCs) and therefore through space correlations can be observed in COSY and TOCSY experiments that make the unambiguous assignment of covalently bound spins impossible. In this article, we show that the JESTER-1 multiple pulse sequence, originally designed for broadband heteronuclear isotropic Hartmann–Hahn transfer, efficiently suppresses the homonuclear dipolar coupling Hamiltonian. This suppression can be enhanced even further by variation of the supercycling scheme. The application of the resulting element in homonuclear TOCSY periods results in coherence transfer via *J*-couplings only. As a consequence, the assignment of scalar coupled spin systems is also possible in partially aligned samples. The bandwidth of coherence transfer for the JESTER-1-derived sequences is comparable to existing TOCSY multiple pulse sequences. Results are demonstrated in theory and experiment.


*Keywords:* TOCSY; RDCs; Partial alignment; Hartmann–Hahn transfer; Isotropic mixing

## 1. Introduction

Homonuclear Hartmann–Hahn or TOCSY experiments are among the most important techniques in liquid state NMR-spectroscopy [1,2]. The majority of their applications concerns the assignment of scalar coupled spin systems, for which the resulting isotropic mixing Hamiltonian leads to very efficient coherence transfer [3–7]. The introduction of alignment media with sufficiently low alignment strength for aqueous [8–14], polar organic [15–18], and apolar organic solutions [19–23], however, increased the complexity and flexibility of Hartmann–Hahn experiments. As was shown in the so-called DCOSY experiment [24], TOCSY transfer via residual dipolar couplings (RDCs) can be used to connect protons through space over quite long distances.

The coherence transfer via RDCs and combinations of RDCs and scalar couplings is well understood [25–27]. In contrast to purely scalar coupled spins with an isotropic coupling Hamiltonian, the symmetry of the interaction is reduced to an axially symmetric or cylindrical Hamiltonian if dipolar couplings are present. As a consequence, coherence transfer can result in either positive or negative cross peaks, depending on the orientation of the initial magnetization with respect to the principal axis of the effective Hamiltonian of a given multiple pulse sequence [28,29].

Among the multitude of existing isotropic mixing sequences (see e.g. [2] for an overview) the properties change significantly with respect to coherence transfer through dipolar couplings. Most importantly, the offset dependence of the transfer efficiency of existing multiple pulse sequences differs for scalar and dipolar couplings

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[30] and the effective dipolar coupling constant is significantly scaled for the different sequences [28,31].

In this article, we will show theoretically and experimentally that the JESTER-1 multiple pulse sequence optimized for heteronuclear isotropic Hartmann–Hahn (HIHAHA) [2] transfer readily fulfills the conditions for effectively removing the (homonuclear) dipolar interaction from the average Hamiltonian active during mixing. The suppression of dipolar coupling evolution can be increased even further using supercycles of the XY-type [32,33]. Based on this main building block, we propose the J-ONLY-TOCSY as a pulse sequence which allows the acquisition of TOCSY spectra on oriented samples that only contain correlations via scalar couplings. As a consequence the assignment of scalar coupled spin systems is made possible independent of the presence of RDCs.

## 2. Theory

Consider a homonuclear spin system with two spin-$\frac{1}{2}$ nuclei, termed $I_1$ and $I_2$. Then the cylindrical coupling Hamiltonian in an anisotropic medium is given by [26,27]

$$\mathcal{H}_{cyl} = \mathcal{H}_J + \mathcal{H}_D, \tag{1}$$

where the isotropic coupling Hamiltonian is described by

$$\mathcal{H}_J = 2\pi J\{I_{1x}I_{2x} + I_{1y}I_{2y} + I_{1z}I_{2z}\} = 2\pi J\mathbf{I}_1\mathbf{J}\mathbf{I}_2 \tag{2}$$

with

$$\mathbf{J} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \tag{3}$$

and the anisotropic dipolar component with the definition following [30] is of the form

$$\mathcal{H}_D = 2\pi D\{I_{1x}I_{2x} + I_{1y}I_{2y} - 2I_{1z}I_{2z}\} = 2\pi D\mathbf{I}_1\mathbf{D}\mathbf{I}_2. \tag{4}$$

with

$$\mathbf{D} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -2 \end{pmatrix}. \tag{5}$$

In both cases the coupling tensors **J** and **D** are real and diagonal matrices which can be treated separately since all appearing product operators commute as long as chemical shift contributions are neglected. The orientational component of the scalar interaction **J** is proportional to the identity matrix, independent of the choice of reference system. In a toggling frame defined by a non-selective rf-irradiation, the isotropic scalar coupling tensor **J** therefore is constant, whereas the anisotropic coupling tensor **D** is modulated by the irradiation of pulses. Since $\mathrm{Tr}(\mathbf{D}) = 0$, the dipolar interactions can be averaged to zero. If the pulse sequence is cyclic (with cycle time $\tau_c$), i.e. if the orientation of the toggling frame is identical to the orientation of the rotating frame at the beginning and at the end of the pulse sequence (which is for example the case for all TOCSY-sequences, as they have an effective flip angle that corresponds to multiples of 360°), the effect of a pulse sequence can be described by the average coupling terms [2,34,35]

$$\overline{\mathcal{H}_{\mathrm{cyl}}} = \mathcal{H}_J + \overline{\mathcal{H}}_D \tag{6}$$

with the corresponding average dipolar coupling Hamiltonian

$$\overline{\mathcal{H}_D} = 2\pi D\mathbf{I}_1\overline{\mathbf{D}}\mathbf{I}_2. \tag{7}$$

As long as rf-pulses dominate all other interactions, the average Hamiltonian can be derived straightforwardly in the toggling frame [2] where the isotropic and dipolar interactions are modulated as a function of time. The integration over all orientations adopted by the tensor $\mathbf{D}'(t)$ during the pulse sequence divided by the "residence times" at the corresponding orientations yields the average coupling tensor $\overline{\mathbf{D}}$,

$$\overline{\mathbf{D}} = \frac{1}{\tau_c}\int_0^{\tau_c} \mathbf{D}'(t)\mathrm{d}t. \tag{8}$$

The time-dependent coupling tensor $\mathbf{D}'$ is again a real $3\times 3$ matrix. As shown in [2,30], its elements $d_{\alpha\beta}$ ($\alpha,\beta = x, y, z$) can be calculated via three-dimensional rotation matrices according to

$$\mathbf{D}' = \begin{pmatrix} d_{xx}(t) & d_{xy}(t) & d_{xz}(t) \\ d_{yx}(t) & d_{yy}(t) & d_{yz}(t) \\ d_{zx}(t) & d_{zy}(t) & d_{zz}(t) \end{pmatrix} \tag{9}$$

with

$$d_{\alpha\beta}(t) = a^1_{x\alpha}(t)a^2_{x\beta}(t) + a^1_{y\alpha}(t)a^2_{y\beta}(t) - 2a^1_{z\alpha}(t)a^2_{z\beta}(t), \tag{10}$$

where the coefficients $a^i_{\alpha\beta}(t)$ ($\alpha,\beta = x, y, z$; $i = 1,2$ for the two spins $I_1$ and $I_2$) denote the elements of real, three-dimensional rotation matrices and are a function of the flip angles, phases, and tilt angles of the effective rotation at a given time $t$ [30,36].

A first approximation of the transfer properties can easily be achieved in this framework assuming two on-resonant spins. In Fig. 1 the time evolution of the tensor elements $d_{\alpha\beta}$ for the JESTER-1 pulse sequence [2,37] is given. Here, the shortest possible supercycle ($RR$, with $R$ being the basic multiple pulse sequence element $90^\circ_x\,270^\circ_y\,450^\circ_x$) is chosen which aligns the toggling frame with the rotating frame at the beginning and at the end of the pulse sequence. As can be seen in Fig. 1, all tensor elements average to zero apart from $d_{xy}$ and $d_{yx}$. This result remains valid for more sophisticated MLEV-type supercycles [38] like MLEV-4, MLEV-8, MLEV-16, or MLEV-32 and the average dipolar coupling tensor has the form

$$\overline{\mathbf{D}}^{\mathrm{MLEV}}_{\mathrm{JESTER\text{-}1}} = \begin{pmatrix} 0 & \frac{1}{3\pi} & 0 \\ \frac{1}{3\pi} & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}. \tag{11}$$

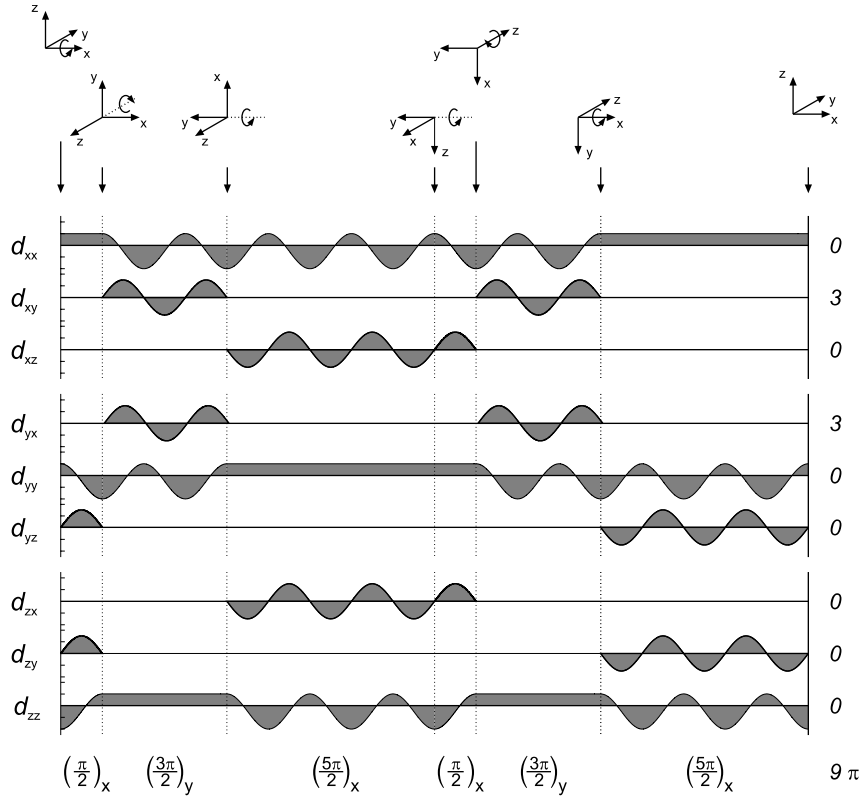



Fig. 1. Evolution for the tensor elements $d_{\alpha\beta}$ of the dipolar coupling tensor $\overline{\mathbf{D}}$ as defined in Eqs. (8)–(10). The tensor elements evolve under the basic JESTER-1 multiple pulse sequence element $R = 90^\circ_x\, 270^\circ_y\, 450^\circ_x$ extended by an $RR$ supercycle. At the top of the plot rotations of the toggling frame as a result of the applied pulses are drawn. The integrals over the supercycle for each tensor element are shown at the very right.

In this case, the diagonal elements are zero and the effective dipolar coupling Hamiltonian is reduced to the double quantum term $\mathcal{H}_D^{\mathrm{MLEV}} = a_{\mathrm{DQ}}\{I_{1x}I_{2y} + I_{1y}I_{2x}\}$ with $a_{\mathrm{DQ}} = (2/3)D$ and vanishing $a_{\mathrm{ZQ}}$ which, according to [2]

$$T_z^{12} = \frac{1}{2}\{\cos(a_{\mathrm{DQ}}t) - \cos(a_{\mathrm{ZQ}}t)\} \tag{12}$$

leads to negative coherence transfer between two isolated spins. The double quantum transfer is very weak, resulting in only 2.75% transferred inphase magnetization compared to ideal transfer at the mixing time of $1/(2D)$. The suppression of dipolar coupling evolution is already very good, but it can be suppressed even further if supercycles of the XY-type [32,33] (XY-8 or higher) are used for the expansion of the JESTER-1 basic sequence, as has previously been explored for the creation of clean TOCSY transfer [39]. In this case the dipolar coupling tensor for two on-resonant spins fully averages to zero

$$\overline{\mathbf{D}}^{\mathrm{XY8}}_{\mathrm{JESTER}-1} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}, \tag{13}$$

i.e. the dipolar interaction is completely suppressed allowing only transfer via scalar couplings.

A more detailed view on the transfer amplitudes including off-resonance effects can be achieved by quantum mechanical simulations of a two-spin system including chemical shift offsets in the overall Hamiltonian. The transfer amplitudes for inphase transfer via scalar and dipolar couplings for four different pulse sequences (DIPSI-2 [40], MOCCA-XY16 [31,41], JESTER-1-MLEV16, and JESTER-1-XY16) with the initial magnetization oriented along $z$ have been calculated according to

$$T_z^{12}(\tau) = \frac{\mathrm{Tr}\{I_{2z}^{\dagger}U(\tau)I_{1z}U^{\dagger}(\tau)\}}{\mathrm{Tr}\{I_{2z}^{\dagger}I_{2z}\}}, \tag{14}$$

using the simulation program SIMONE [42] (Fig. 2). In the definition of the transfer amplitude $I_{1z}$ and $I_{2z}$ represent the initial and the target state, respectively, and $U(\tau)$ the propagator

$$U(\tau) = \exp(-\mathrm{i}\mathcal{H}_{\mathrm{eff}}\tau), \tag{15}$$

with the overall effective Hamiltonian $\mathcal{H}_{\mathrm{eff}}$ of the corresponding pulse sequences. From these simulations it is apparent that all tested pulse sequences show comparable transfer properties for scalar coupled spins, but very different properties for purely dipolar coupled spins. While the MOCCA-XY16 multiple pulse sequence shows strong zero quantum-based positive transfer amplitudes for dipolar coupled spins, the DIPSI-2 sequence results in negative amplitudes. The JESTER-1 sequence with MLEV-16 supercycle yields dipolar transfer amplitudes generally below 3%. The JESTER-1 sequence with XY16-expansion,

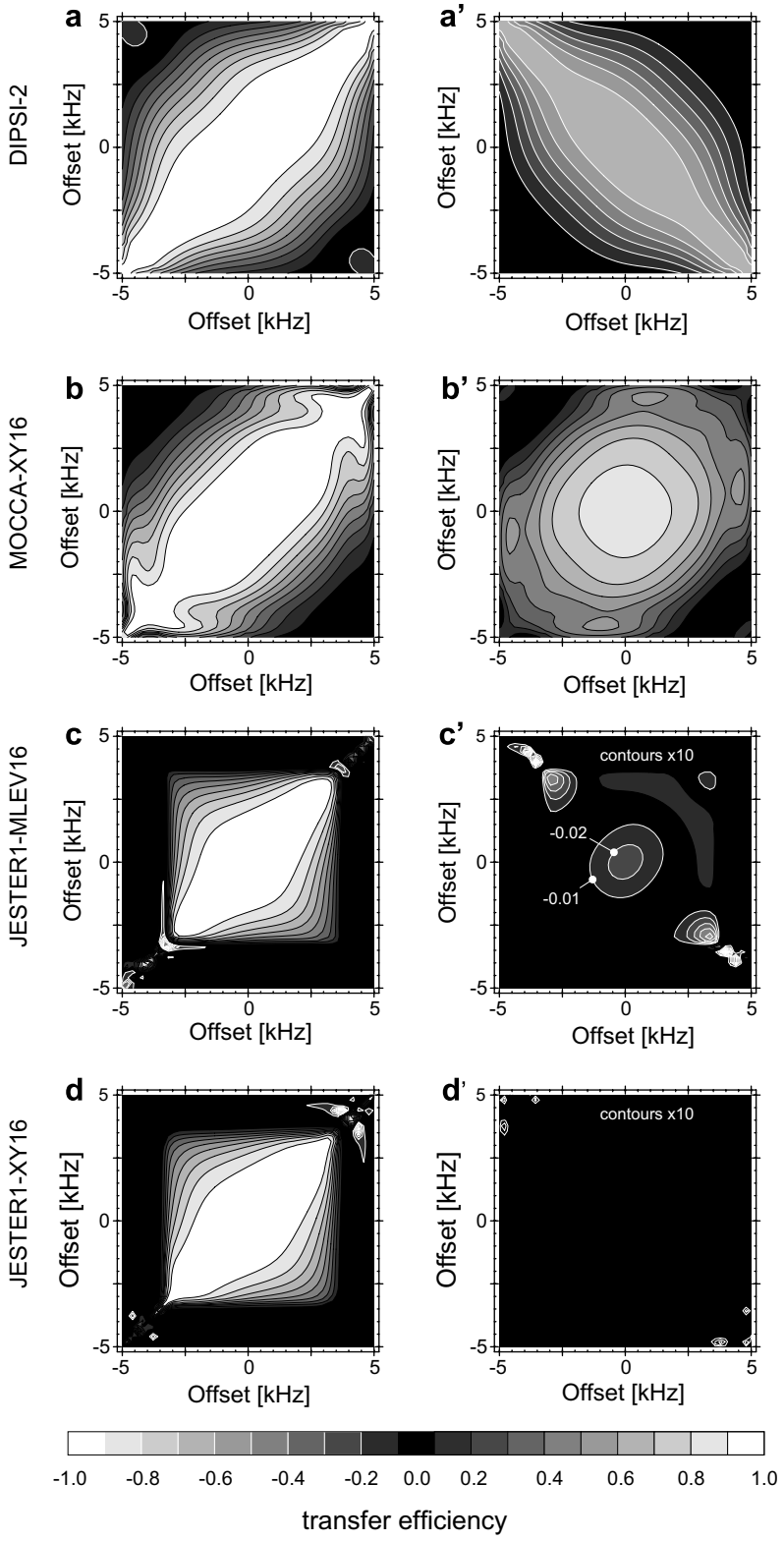



Fig. 2. Simulated offset profiles of the transfer amplitudes via scalar and dipolar couplings for various TOCSY multiple pulse sequences: DIPSI-2 (a, a′), MOCCA-XY16 (b, b′), JESTER-1 with MLEV-16 expansion (c, c′), and JESTER-1 with XY16 expansion (d, d′). In all cases the transfer between two coupled spins was analyzed with $I_{1z}$ as the initial spin state and $I_{2z}$ as the target state according to Eq. (14). On the left hand side offset profiles for the transfer via scalar couplings are shown (a, b, c, d), while the right hand side (a′, b′, c′, d′) represents the corresponding dipolar transfer profiles. Coupling constants of $J = D = 10$ Hz and a mixing time of 50 ms were used for the calculations. Maximum rf-amplitudes have been 12.5 kHz for the MOCCA-XY16 sequence (with an inter pulse delay of 88 μs) and 6.25 kHz for all other sequences. Transfer amplitudes were simulated with the program SIMONE [42]. No $B_1$-field inhomogeneity was considered.

finally, fully suppresses dipolar coupling contributions over the whole bandwidth calculated.

## 3. Experimental

To verify the findings in practice, homonuclear $^1H$–$^1H$-TOCSY experiments have been recorded for the cyclic undecapeptide Cyclosporin A (CspA) in isotropic and partially oriented samples. For measurements in isotropic solution 6.6 mg CspA have been dissolved in 600 μL $CDCl_3$. The aligned sample was prepared using a cross-linked poly(dimethylsiloxane) gel (PDMS, diameter = 3.0 mm, cross-linked with 200 kGy of accelerated electrons) which was equilibrated in $CDCl_3$ (1 mL) in an NMR-tube for several days. After one week the sample showed a constant $CDCl_3$ quadrupolar deuterium splitting of $\Delta\nu_Q = 33$ Hz. This results in $^1H$–$^1H$ dipolar couplings between −35 and 39 Hz for geminal protons in $CH_2$ moieties when assuming the alignment tensor determined in reference [43] with a linear scaling factor. Supernatant solvent was removed and CspA dissolved in $CDCl_3$ was added to a final concentration of approximately 34 mM in the gel. The sample could be analyzed after 2 days of incubation.

Using the pulse sequence shown in Fig. 3, four experiments with the multiple pulse sequences used in Fig. 2 and a conventional ROESY for comparison (pulse sequence not shown) were recorded for the aligned CsA-sample. For all TOCSY-sequences the magnetization for inphase-transfer was positioned along $z$ before the mixing period. The differences for the various sequences can best be seen in the $N$-methyl to aliphatic region shown in Fig. 4. The DIPSI-2 sequence produces positive as well as negative cross-peaks depending on whether scalar or dipolar couplings dominate the transfer. In the region where transfer is only expected via dipolar transfer (2.6–3.7 ppm of the indirect dimension), all cross peaks are negative and signals expected from scalar coupling transfer, as e.g. peaks along 3.83 ppm, are significantly reduced (Fig. 4a).

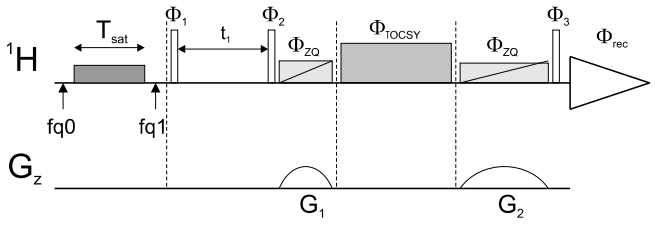



Fig. 3. The basic pulse sequence for the acquired TOCSY spectra. Presaturation of unwanted signals originating from the PDMS/$CDCl_3$-gel as alignment medium [23,43] was achieved by frequency jumps forth and back (fq0, fq1) and cw-irradiation at the corresponding resonance. Zero-quantum suppression was achieved by the combination of adiabatic CHIRP pulses [46,47] (with durations of 30 ms before and 50 ms after the TOCSY mixing period) and simultaneously applied gradients as described in [44,45]. The gradient strengths of the corresponding gradients $G_1$ and $G_2$ were adjusted by the procedure described in [44]. Pulse phases have been: $\Phi_1 = x, -x$, $\Phi_2 = 4(x), 4(-x)$, $\Phi_3 = 2(x), 2(-x)$, $\Phi_{ZQ} = x$, and $\Phi_{rec} = x, 2(-x), x, -x, 2(x), -x$. Quadrature detection following the TPPI scheme was achieved by cycling the pulse phase $\Phi_1$ accordingly ($x$, $y$, $-x$, $-y$).

The MOCCA-XY16 sequence, instead, results in positive cross-peaks throughout the spectrum (Fig. 4b). Its cross peaks are generally most intense because of the very efficient transfer via dipolar couplings [31] and its favorable relaxation properties [41]. A distinction between scalar and dipolar-mediated cross peaks is generally not possible in this case. The TOCSY spectra with the two JESTER-1-derived mixing periods are shown in Fig. 4c and d with generally positive cross peaks. As expected from the simulations in Fig. 2 practically no transfer is visible in the region from 2.6 to 3.7 ppm, clearly demonstrating the suppression of coherence transfer via dipolar couplings. Residual negative cross peaks in the selected region for the JESTER-1 sequence with MLEV16-expansion are very weak (note the factor 10 used for selected slices in Fig. 4). Since none of the multiple pulse sequences are compensated for cross-relaxation transfers, cross peaks for both JESTER-1 variants as well as DIPSI-2 and MOCCA-XY16 experience contributions from nuclear Overhauser enhancement in the rotating frame (ROE). The corresponding ROESY spectrum is shown in Fig. 4e for comparison. Residual cross peak intensities in the slices for the JESTER-1 sequences in 4 that are not explained by scalar coupling transfer or ROE contributions can be attributed to $t_1$-noise.

The applicability of the J-ONLY-TOCSY approach is demonstrated in Fig. 5, where the JESTER-1 TOCSY spectrum acquired on the aligned CsA sample (Fig. 5a) is compared with a conventional DIPSI-2 TOCSY recorded on an isotropic sample of CsA dissolved in $CDCl_3$ (Fig. 5b). Although cross peak intensities vary slightly because of variations in the multiplet patterns due to dipolar couplings being present in the aligned sample, the appearance of the two spectra is basically identical and the assignment of scalar coupled spin systems in the partially oriented sample is possible.

As has been shown already in [50], solute molecules can experience significant changes in resonance frequencies when dissolved in an alignment medium. To demonstrate the usefulness of the J-ONLY-TOCSY approach on a practical example, we prepared a strychnine sample dissolved in a stretched polystyrene (PS)/$CDCl_3$ gel following the procedure described earlier [22,50]. The resulting spectra are shown in Fig. 6: by just looking at the 1D spectra and assuming only a uniform shift of all resonances due to PS acting as a kind of aromatic cosolvent, one would probably make the wrong guess in assigning the resonances for protons 11 and 14. The multiplet width of 11 is unexpectedly reduced by dipolar couplings while signal 14 appears broadened and the order of the resonance frequencies for the two protons has changed. A similar difficulty arises for the assignment of resonances 20 and 11′, which partially overlap in the aligned spectrum. However, using the J-ONLY-TOCSY with the JESTER-XY16 multiple pulse sequence, the assignment is easily achieved by following the scalar coupling network in comparison with the corresponding experiment applied to the unaligned sample.

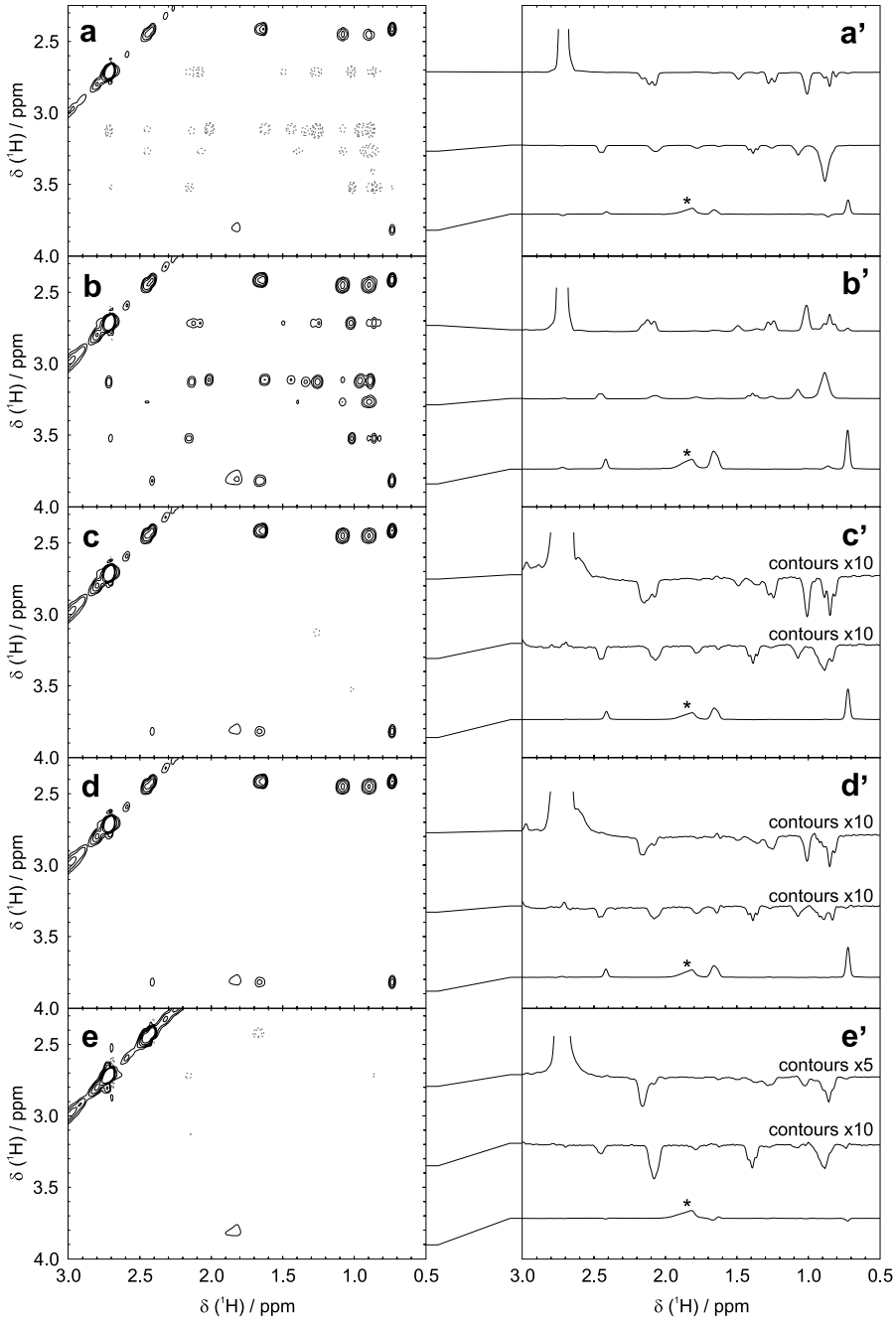



Fig. 4. Enlargement of the *N*-methyl-to-aliphatic region of TOCSY and ROESY spectra acquired on Cyclosporin A partially aligned in a stretched PDMS/$CDCl_3$-gel using the basic pulse sequence as described in Fig. 3. DIPSI-2 (a), MOCCA XY16 (b), JESTER-1 with MLEV-16 expansion (c), and JESTER-1 with XY16 expansion (d), as well as a cw spin lock for ROESY-transfer (e) were applied during the mixing period. Field strengths for the TOCSY periods were chosen according to the maximum rf-amplitudes given in the caption of Fig. 2. ROESY spin locking was achieved by cw irradiation with a rf-amplitude of 6.25 kHz. Mixing times were 82.88 ms for DIPSI-2, 81.92 ms for MOCCA XY16, 80.64 ms for JESTER-1 with MLEV16 and JESTER-1 XY16 expansion, and 80 ms for the ROESY, respectively. For better visualization example traces (two traces with purely dipolar transfer and one trace with expected transfer via scalar couplings) are shown on the right with scaling by a factor 5 or 10 whenever annotated. Signals marked with an asterisk originate from chemical exchange with residual water.

The experiments were recorded on a Bruker 600 MHz DMX spectrometer equipped with a triple resonance probe head with actively shielded *z*-gradients. The DIPSI-2 and the JESTER-1 multiple pulse sequences were both applied with rf-amplitudes of 6.25 kHz while 180° pulses in the MOCCA-XY16 sequence were applied with an rf-amplitude of 12.5 kHz resulting in an average introduced rf-power equivalent to continuous wave irradiation of constant rf-amplitude of 6.99 kHz. Spin-locking in the ROESY [48,49] experiments was achieved via a cw spin-lock with a rf-power of 6.25 kHz (see caption of Fig. 4 for details). In all TOCSY and ROESY experiments the mixing time was set to 80 ms.

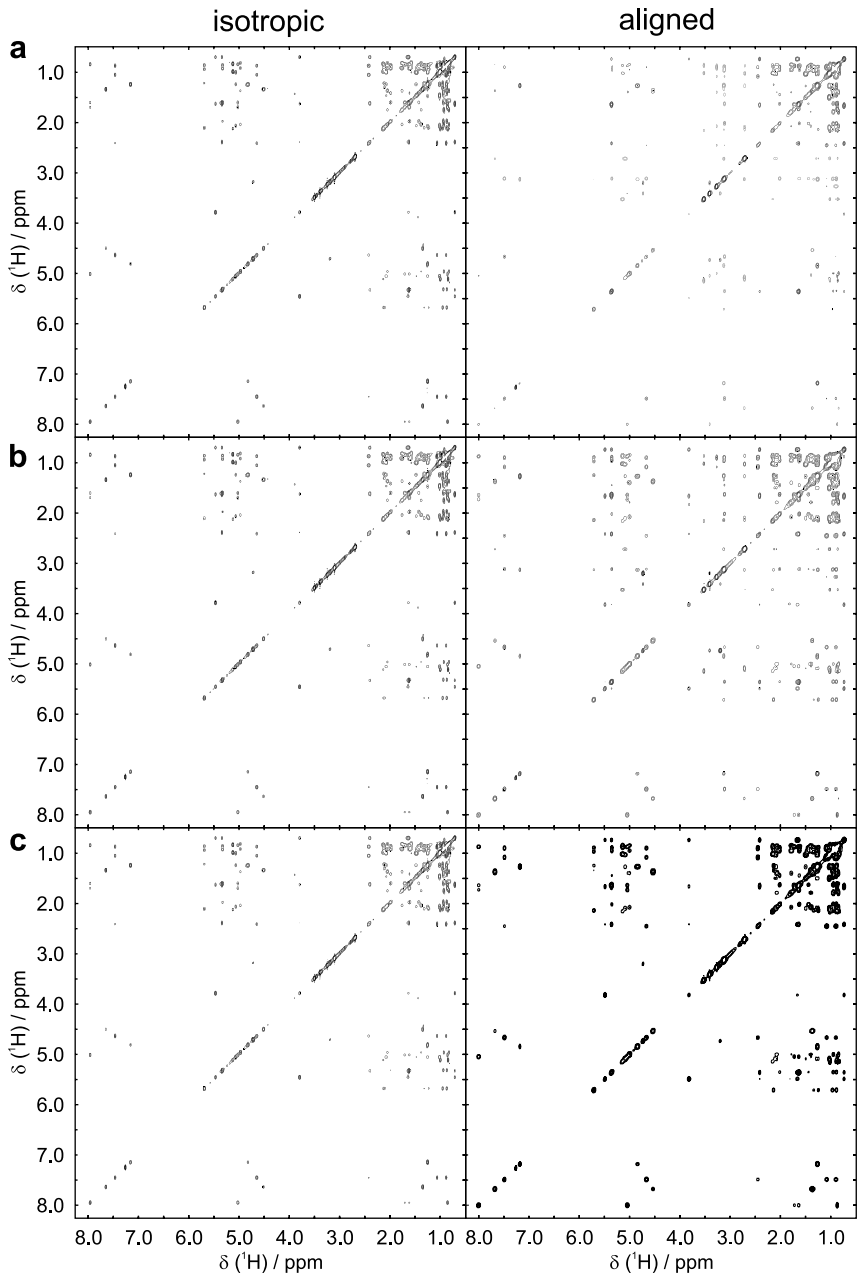
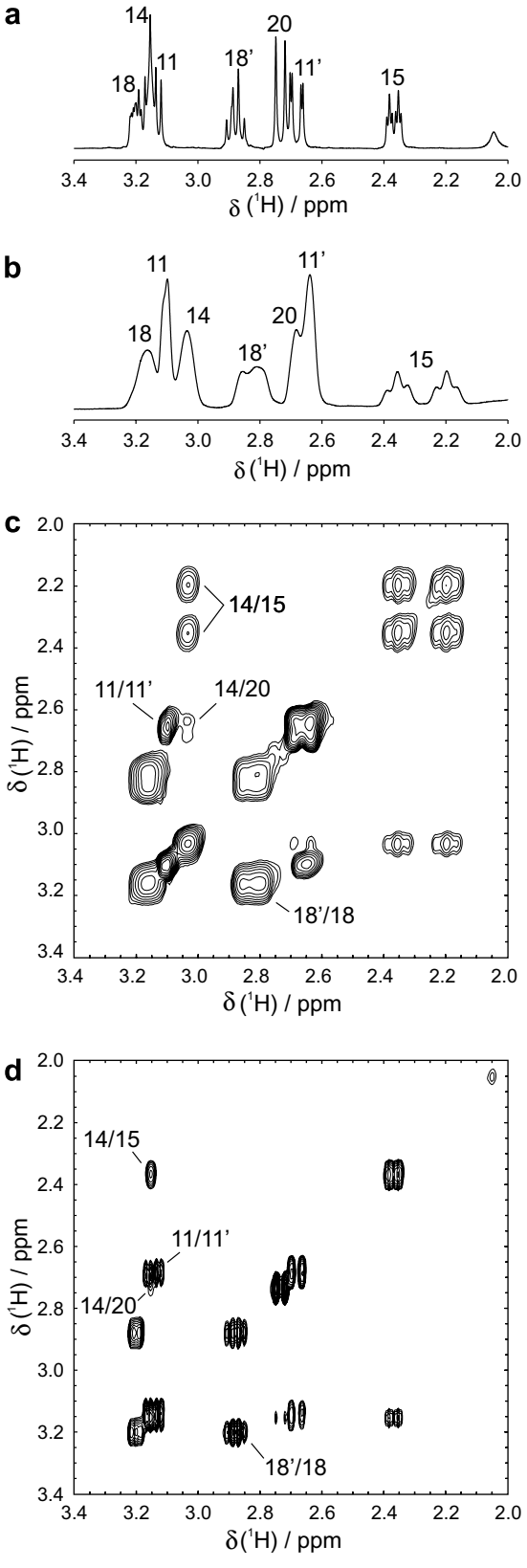



Fig. 5. Experimental TOCSY spectra of isotropic Cyclosporin A (left) and Cyclosporin A aligned in a stretched PDMS/$CDCl_3$-gel (right). Spectra were acquired using the DIPSI-2 (top), MOCCA-XY16 (middle) and JESTER-XY16 (bottom) multiple pulse sequences. TOCSY mixing sequences were applied with an rf-amplitude of 6.25 kHz for DIPSI-2 and JESTER-XY16 and 12.5 kHz for MOCCA-XY16 with delays $\Delta/d = 2.2$ [31]. Mixing times are as described in Fig. 2. While the TOCSY-spectra using DIPSI-2 and MOCCA-XY16 are very different in the isotropic and aligned cases, they are virtually identical for JESTER-XY16.

## 4. Discussion

Solute molecules dissolved in a liquid crystalline or gel-based alignment medium as well as paramagnetically tagged molecules will experience chemical shift changes due to the medium acting as a cosolvent [50], residual chemical shift anisotropy, or pseudocontact shifts [51]. In such cases, it can be necessary to reestablish the assignment of the molecule of interest (see Fig. 6). But the means for the assignment process are limited: unambiguous connectivity information via covalent bonds, usually obtained by conventional HMBC-type, COSY-type or TOCSY-type experiments, cannot be used since coherence transfer also occurs through space via residual dipolar couplings. Heteronuclear correlations via scalar and/or residual dipolar couplings can generally not be distinguished by any pulse sequence, since they have coupling Hamiltonians of identical form. The same situation applies for homonuclear correlation experiments that are based on weakly coupled spins like COSY-type experiments. Only in the strong

coupling limit the scalar and dipolar interactions differ significantly and a general distinction of their contribution is possible. In this article, we have developed a homonuclear TOCSY-experiment, which we call J-ONLY-TOCSY, that fully suppresses dipolar contributions during its mixing period.




Isotropic mixing conditions, as present in homonuclear TOCSY-experiments in liquid state samples, typically result in positive transfer amplitudes [1,6,7]. Exceptions are only known for heteronuclear spin systems containing a spin 1 nucleus [52] and for homonuclear spin systems consisting of five or more spins 1/2 [53]. In planar coupled two-spin systems the transfer amplitude is also generally positive [54–56], but three or more planar coupled spins are known to undergo negative transfer under certain conditions [57]. The situation in dipolar coupled spin systems is fundamentally different, as TOCSY-type multiple pulse sequences can give rise to ZQ-based positive transfers as well as DQ-based negative transfers between any two coupled spins (see Eq. (12) and [25–27,30]). The derivation of the vanishing average Hamiltonian for the JESTER-1 sequence with XY16-expansion is given in the theory section, but a simplified view to look at it would be that the sequence produces equal amounts of ZQ- as well as DQ-based transfer therefore leading to zero net transfer.

JESTER-1 as a simple multiple pulse sequence initially designed for heteronuclear isotropic Hartmann–Hahn (HIHAHA) transfer with a relatively large bandwidth [2,37] expectedly removes all diagonal elements in the average homonuclear dipolar interaction tensor $\overline{\mathbf{D}}$ (see Eq. (11)) since HIHAHA sequences obey the condition of an isotropically distributed principal interaction axis. As has been shown in the theory section, off-diagonal elements further disappear by the expansion in XY-type supercycles [32,33]. Although not thoroughly tested, this construction principle can most likely be applied to the majority of HIHAHA multiple pulse sequence for the use in J-ONLY-TOCSY experiments. Most HIHAHA sequences published so far, however, have significantly smaller bandwidths compared to JESTER-1 (see e.g. [2,58,59]).

The bandwidth along the antidiagonal of JESTER-1 with XY16-expansion for its use in J-ONLY-TOCSY experiments is approximately 0.9 $B_1$, which is on the same order as for conventional TOCSY sequences like MLEV-16 [38], DIPSI-2 [40], or MOCCA-XY16 [31,41]. However, other multiple pulse sequences with improved bandwidths might be constructed out of HIHAHA sequences or

Fig. 6. Reassignment of strychnine aligned in a PS/$CDCl_3$ gel using the JESTER-XY16 J-ONLY-TOCSY. (a) Region of the 1D spectrum of strychnine dissolved in $CDCl_3$ with the assignment corresponding to [50], (b) the same region of the 1D spectrum of strychnine in a stretched PS/$CDCl_3$ gel with a quadrupolar deuterium splitting of $\Delta\nu_Q = 121$ Hz and the assignment derived from the J-ONLY-TOCSY. (c) Region of the 2D J-ONLY-TOCSY used for reassigning the significantly shifted resonance frequencies, and (d) the same spectral region of the JESTER-XY16 J-ONLY-TOCSY acquired on the isotropic sample for comparison. By simply comparing the 1D spectra, resonances 11 and 14 would probably be misassigned and the assignment of resonances 20 and 11′ would be highly questionable. With the J-ONLY-TOCSY assignment via the homonuclear *J*-coupling network is straightforward. The J-ONLY-TOCSY experiments were acquired using the pulse sequence shown in Fig. 3 with a JESTER-XY16 mixing time of 72.6 ms.

optimized using techniques like optimal control of spin dynamics, which in the last few years has been applied successfully to a number of problems concerning pulse and pulse sequence design [60–67].

For a number of multiple pulse sequences like e.g. DIPSI-2, polarization transfer via purely dipolar couplings in isolated two-spin systems results in negative cross peaks, which, in principle, can easily be distinguished from transfer via scalar couplings. However, one should always be aware in this case that the transfer is a *combination* of scalar and dipolar couplings [27] and the identification of covalently bound spin systems is not unambiguously possible. This is especially the case for large spin systems with very complex transfer functions and also applies to multiple pulse sequences designed for clean TOCSY transfer through RDCs [39]. Only the J-ONLY-TOCSY presented in this article fully suppresses the dipolar interaction Hamiltonian and allows the unambiguous assignment of scalar coupled spin systems in partially aligned samples.

The usual limitations of zero order average Hamiltonian theory apply for the suppression of dipolar interactions, i.e. RDCs must be considerably smaller than the inverse basic cycle time. Since the JESTER-1-XY16 sequence has a minimum expansion in XY8 in order to fully suppress the dipolar interactions, the basic cycle time corresponds to the time needed for a 6480° pulse, i.e. at an rf-amplitude of 7 kHz all residual dipolar couplings should be significantly smaller than ≈400 Hz. The multiple pulse sequences presented in this article therefore are fully applicable in combination with most of the recently developed alignment media. Applied with correspondingly increased rf-amplitudes, they might well be applicable also in solid state experiments.

The JESTER-1-based multiple pulse sequences are not clean-TOCSY sequences in the sense that they do not suppress transfer via transverse or longitudinal dipolar relaxation in the spin diffusion limit [68–70]. Such sequences require magnetization to be oriented twice as long along $z$ as in the transverse $xy$-plane to compensate negative ROE and positive NOE-relaxation contributions for large molecules in the spin diffusion limit. Especially for small to medium-sized molecules, however, the ROE-contribution will be very weak and corresponding negative cross peaks of low intensity can easily be distinguished from positive TOCSY peaks via scalar couplings.

## 5. Conclusion

In summary, we introduced the so-called J-ONLY-TOCSY experiment, which suppresses transfer via residual dipolar couplings and therefore allows the unambiguous identification of scalar coupled spin system in partially aligned samples. The key step of the experiment is the use of JESTER-1-derived multiple pulse sequences that eliminate the effective dipolar coupling Hamiltonian while retaining good transfer properties via scalar couplings.

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