(Figure 4). Similar to the H_nNF_{3-n} series (Figure 1), the force constants *increase* with *increasing* bond lengths, a trend which contradicts the relation represented by Badger's rule. In search for other examples of such an opposite bond length–force constant relationships we only found one more case, i.e., the O–F bonds in OF_2 and HOF. The bond lengthens from 1.4124 Å in OF_2[18] to 1.442 (2) Å in HOF,[19] and the force constant increases from 3.95[18] to 4.418 mdyn·Å^{-1}.[20] In other fluorine derivatives for which the bond lengths and clear-cut force constants are known, such as H_nCF_{4-n}[4] or H_nPF_{3-n},[21] the relationship shows normal behavior, i.e., the force constants decrease with increasing bond lengths.

A possible rationalization of this unusual property of the N–F bond is based on electrostatic effects.[4] Assuming that the bonding potential can be regarded as a superposition of covalent and electrostatic contributions, a repulsive coulombic term *lengthens* the bond and *increases* the force constant and vice versa, provided that the covalent part of the potential remains unchanged. This model suggests that the variation of the N–F bond lengths in the hydrogen and methylfluoroamines is predominantly due to electrostatic interactions, which vary from strongly attractive in NF_3 to repulsive in H_2NF and Me_2NF, and that the covalent contribution remains nearly constant. On the other hand, lengthening of C–F and P–F bonds can be rationalized as being partly due to electrostatic effects and partly due to lowering of the covalent bond strength. In this case, the increase of the force constant due to electrostatic effects is overcompensated by simultaneous decrease of the covalent contribution. Thus, the force constants show normal behavior, i.e., they *decrease* with *increasing* bond lengths, but this decrease is less than that predicted by Badger's rule.

Acknowledgment. We thank NATO for financial support of this cooperative research project (CRG 860785). O.D.G., R.L.K., and J.M.S. acknowledge support from the National Science Foundation (CHE-8703790) and Air Force Office of Scientific Research (87-00667). H.O. is grateful for support from the Fonds der Chemischen Industrie.

Note Added in Proof. After submission of this paper a reinvestigation of the gas-phase structure of $MeNF_2$ using electron diffraction intensities and rotational constants was initiated by K. Hagen, K. Hedberg, E. O. John, R. Kirchmeier and J. M. Shreeve. The preliminary results indicate that the N–F bond length given in Table V of this paper remains unchanged within its error limit and the N–C bond is slightly longer than the value derived from rotational constants only.

Supplementary Material Available: Total electron diffraction intensities and Q-branch rotational transitions for Me_2NF (6 pages). Ordering information is given on any current masthead page.

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Coherence Transfer in Nuclear Magnetic Resonance by Selective Homonuclear Hartmann–Hahn Correlation Spectroscopy

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Abstract: New NMR techniques are described for transferring transverse magnetization between scalar-coupled nuclei in the rotating frame. The methods involve simultaneous spin-locking of two selected spins A and X, using cosinusoidal modulation of the radio frequency (rf) carrier. If transverse magnetization of spin A is excited selectively before spin-locking, for example with a self-refocusing 270° Gaussian pulse, in-phase magnetization will be transferred from spin A to spin X, and in-phase multiplets are obtained in both one- and two-dimensional spectra. The method allows one to verify whether two particular spins are connected by a scalar coupling. Examples are shown for peptides and basic pancreatic trypsin inhibitor (BPTI). The fine structure of the multiplets yields information on couplings to further spins. In contrast to nonselective spin-lock experiments, the magnetization cannot diffuse to a manifold of coupling partners. If relayed coherence transfer is to be achieved deliberately, several spins may be locked simultaneously by imposing an additional modulation on the rf carrier.

Introduction

Much has been written[1-10] in recent years about the relative merits of conventional correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY), the latter being also known as homonuclear Hartmann–Hahn spectroscopy (HOHAHA). Both methods are intended to reveal scalar couplings or networks of couplings. While COSY multiplets only reflect direct connectivities, TOCSY multiplets also appear when two spins merely belong to the same coupling network, so the latter method may be compared to relayed magnetization-transfer experiments. Generally speaking, TOCSY experiments must be regarded as more demanding from an experimental point of view, with special requirements on radio frequency (rf) power and phase coherence. Furthermore, the interpretation may be hampered if some areas in the spectra appear "bleached" because of unfavorable conditions, notably if offset effects give rise to tilted effective fields. Difficulties may also arise from cross-relaxation effects in the rotating frame (so-called ROESY effects), so a variety of methods have been developed to separate coherent and incoherent processes in spin-locking experiments.[11,12]

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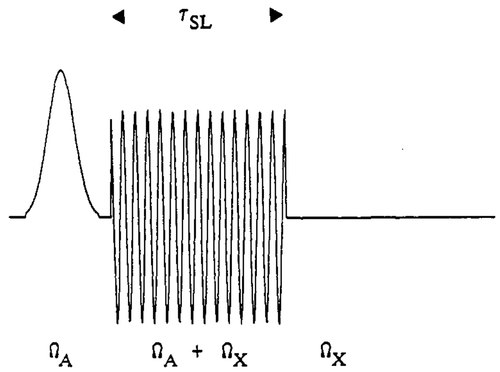

Figure 1. Pulse sequence for doubly selective HOHAHA. The initial self-refocusing 270° Gaussian pulse with a carrier frequency Ω_A excites in-phase magnetization I_x^A. During the spin-lock period τ_{SL}, the rf carrier is positioned midway between the two chemical shifts, $\omega_0 = {}^1/_2(\Omega_A + \Omega_X)$, and the audio modulation frequency is set to $\omega_a = {}^1/_2(\Omega_A - \Omega_X)$.

In recent years, we have found that it is often advantageous to use *selective* correlation experiments such as "soft" COSY and related techniques.[13-16] These methods allow one to "interrogate" the spin system by asking specific questions, such as whether spin A is coupled to spin X or whether the two coupled spins A and X have common coupling partners. Such questions may be refined, e.g. by inquiring whether particular coupling partners constitute sets of magnetically equivalent spins. Very often, questions of this type need not be answered for the entire molecule but only for some selected regions of strategic interest.

While many soft experiments, such as soft COSY, selectively inverted soft (SIS) COSY,[14,15] multiplet unravelling by selective injection of coherence (MUSIC) COSY,[17] etc., allow one to obtain quick answers to such questions, all of these techniques suffer from the same problems as the original COSY method. In particular, COSY multiplets (be they obtained by sequences involving soft or hard pulses) invariably feature signals with alternating signs ("antiphase" multiplets). Not only are these prone to mutual cancellation, but they also make it rather difficult to recognize the in-phase multiplet structures that are due to passive spins. This problem is particularly severe when many equivalent spins are present. By contrast, TOCSY multiplets are entirely in-phase, so they not only tend to be more intense but also help to identify multiplet structures due to passive couplings.

Doubly Selective HOHAHA

We have developed a new class of techniques that combine the selective features of soft COSY with the advantages of the in-phase multiplet structures that are characteristic of TOCSY or HOHAHA. Figure 1 shows a pulse sequence that is appropriate for "doubly selective" HOHAHA. The initial self-refocusing 270° Gaussian pulse[13] excites in-phase magnetization I_x^A, which is subsequently spin-locked by a modulated rf field that affects the spins A and X selectively. Before observing the in-phase magnetization that has been transferred to spin X, a purging pulse may be applied to eliminate antiphase terms that are present if the transfer is incomplete (see below). The Gaussian pulses are truncated at 2.5% and typically have a duration of 30 ms and a peak amplitude of 55 Hz, such that the phase dispersion of the components of a multiplet of about 30-Hz width does not exceed 20°. If this is not sufficient, one can use more sophisticated excitation sequences such as a G^4 Gaussian pulse cascade[18] or a so-called excitation band-selective uniform response pure phase ("E-BURP") pulse.[19-21]

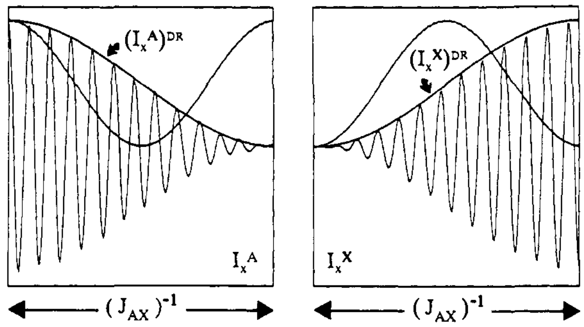

Figure 2. Simulation of doubly selective HOHAHA transfer in a two-spin AX system. The magnetization migrates from I_x^A to I_x^X. The fast oscillations refer to a frame rotating at the average chemical shift: the slowly varying envelopes with a half-period of $(J_{AX})^{-1}$ are obtained in a doubly rotating (DR) frame. For comparison, the oscillations with a period $(J_{AX})^{-1}$ indicate magnetization transfer under "normal" TOCSY conditions with nonselective spin-locking.

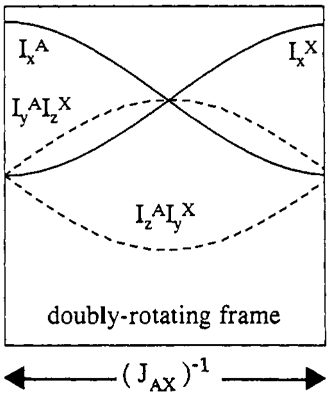

Figure 3. In-phase and antiphase magnetization of a two-spin AX system in the course of doubly selective HOHAHA transfer, viewed in a doubly rotating frame. The difference of the antiphase terms $(2I_y^A I_z^X - 2I_z^A I_y^X)$ represents zero-quantum coherence in the tilted frame.

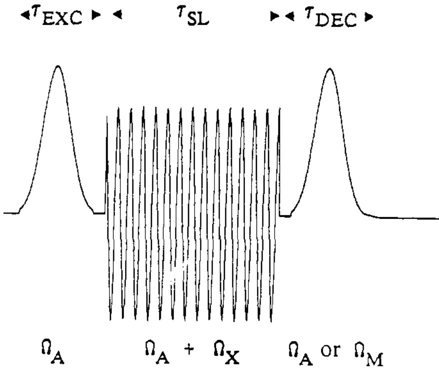

Figure 4. A 270° Gaussian purging pulse of duration τ_{DEC} with a carrier frequency Ω_A or Ω_M can be appended to the sequence of Figure 1. In a two-spin AX system, this allows one to eliminate residual antiphase terms if τ_{SL} does not exactly match the condition for optimum transfer, $\tau_{SL} = (J_{AX})^{-1}$. In larger spin systems, antiphase multiplets may be generated as explained in the text.

A spin-locking rf field that affects only two chosen spins A and X ("doubly selective" spin-locking) is implemented by modulating a square pulse with cos $(\omega_a t)$, where $\omega_a = {}^1/_2(\Omega_A - \Omega_X)$. The modulation causes the spectrum of the rf irradiation to split into two sidebands at $\omega = \omega_0 \pm \omega_a$, which coincide with the two

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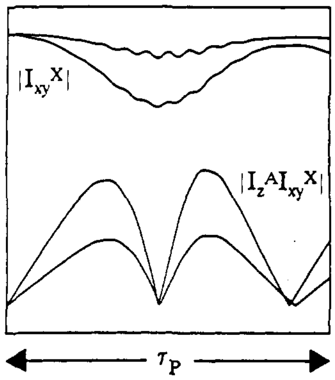

Figure 5. Evolution of initial in-phase transverse magnetization $|I_{xy}^X|$ (top) and of the antiphase term $|I_z^AI_{xy}^X|$ (below) during a 270° Gaussian purging pulse applied to spin A. Simulations are shown for coupling constants J_{AX} = 10 Hz (small excursions) and J_{AX} = 20 Hz (large excursions). Note that at the end of the pulse only a negligible amount of antiphase coherence remains.

chemical shifts Ω_A and Ω_X if the transmitter is placed midway between the A and X resonances. After the excitation pulse on spin A, a short delay (typically 500 μs) is needed to shift the transmitter from $\omega_0 = \Omega_A$ to $\omega_0 = {}^1/_2(\Omega_A + \Omega_X)$.

Figure 2 illustrates the TOCSY-type transfer of magnetization that takes place during a modulated spin-lock. If we assume that spin A was initially excited, a complete transfer to spin X is achieved after a spin-lock interval $\tau_{SL} = (J_{AX})^{-1}$. This is twice as long as the interval of $(2J_{AX})^{-1}$ required in normal homonuclear TOCSY transfer using nonselective "hard" spin-locking. The transfer involves, as an intermediate, antiphase terms of the kind $(2I_y^AI_z^X - 2I_z^AI_y^X)$, as shown in Figure 3. These have to be "purged" if we wish to monitor the buildup of in-phase coherence of spin X as a function of the spin-lock duration τ_{SL}. This can be achieved by inserting a 270° Gaussian pulse of duration τ_{DEC} applied to Ω_A just before acquisition, as in Figure 4.

Note that while the purging pulse acts on spin A and transforms the antiphase term[22] $|2I_z^AI_{xy}^X|$ into multiple-quantum coherence, the coupling partner X does not simply experience a free precession interval of duration τ_{DEC}. If this were the case, in-phase $|I_{xy}^X|$ magnetization would partly evolve into antiphase terms $|2I_z^AI_{xy}^X|$ *during* τ_{DEC}, thus reintroducing exactly the same perturbation that we sought to eliminate. The simulations in Figure 5 illustrate that some exchange between in-phase and antiphase coherences of spin X takes place while the coupling partner A is irradiated with a 270° Gaussian pulse. The important observation is that only a negligible amount of antiphase coherence is present at the end of the purging pulse. All *J* couplings to spin A appear to be refocused, so that the 270° Gaussian pulse on spin A effectively functions as a *selective decoupling* interval.

Figures 6 and 7 illustrate an application of the pulse sequence of Figure 4. The magnetization transfer in an AX spin system of a small molecule (exifone) is monitored as a function of the duration of the spin-lock period τ_{SL}. Note that, because a purging pulse has been applied to the coupling partner in this case, the doublets of both "source" and "destination" spins are pure in-phase. If the receiver reference remains set at ω_0 at the time of observation, the phase of the resulting signals will not depend on the duration of the spin-lock interval provided that τ_{SL} corresponds to a multiple of $2\pi/\omega_a$. The time scale in Figure 6 extends from 30 to 150 ms; the maximum at 100 ms corresponds to $(J_{AX})^{-1}$.

The experiment may also be carried out in two-dimensional fashion, where an evolution period t_1 with a refocusing pulse is inserted before the spin-lock period. The selective refocusing pulse, typically a G^3 Gaussian cascade,[18,24] acts on the "active" spin A and serves to refocus all couplings involving spin A, so that pure in-phase magnetization I_x^A is present at the end of the evolution interval.[25]

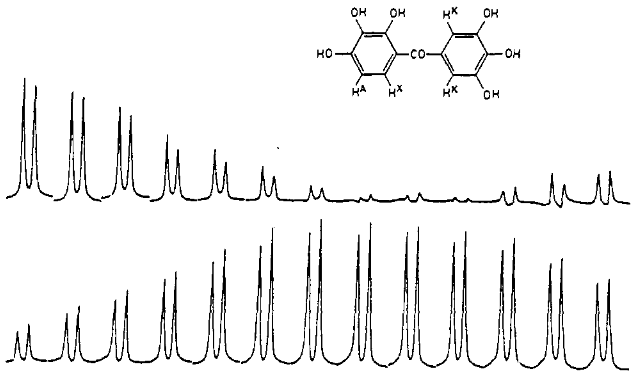

Figure 6. Experimental doublets of a two-spin system in exifone (protons H^A and H^X shown in inset) recorded with the sequence of Figure 4 as a function of the duration τ_{SL} of the spin-lock pulse varied between 30 and 150 ms. Experimental conditions: $\Delta\Omega/2\pi$ = 250 Hz, J_{AX} = 10 Hz; 270° Gaussian pulses (2.5% truncation, 30 ms, peak amplitude 55 Hz) for excitation and purging, modulated spin-lock pulse of 40-Hz amplitude (20 Hz for each sideband); spectral width 500 Hz, 1K data points, Lorentz–Gauss transformation (LB = −1.0 and GB = 0.08). The experiment was carried out on a Bruker AM-400 spectrometer equipped with a selective excitation unit.

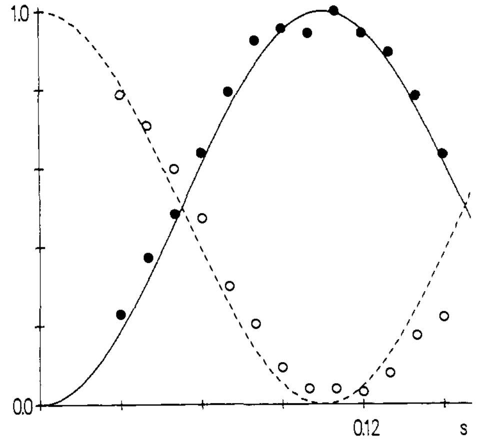

Figure 7. Theoretical curves (dotted line = I_x^A, solid line = I_x^X) and experimental values (circles) corresponding to the peak heights in Figure 6.

Mechanism of Coherence Transfer in Doubly Selective HOHAHA

The in-phase coherence transfer during a modulated spin-lock bears a closer resemblance to heteronuclear than to homonuclear Hartmann–Hahn transfer, hence the characteristic period of $(J_{AX})^{-1}$ instead of $(2J_{AX})^{-1}$ as applies to the normal homonuclear TOCSY experiment.[1] While in the latter case the complete scalar coupling Hamiltonian $\mathbf{H}_J = 2\pi J_{AX}(I_x^AI_x^X + I_y^AI_y^X + I_z^AI_z^X)$ has to be considered, one can show that in the modulated experiment only the $I_z^AI_z^X$ part of the coupling Hamiltonian is effective in the magnetization transfer. To derive an analytical expression for the transfer, we may therefore start from the following two-spin Hamiltonian in the rotating frame (assuming that the transmitter is placed midway between the chemical shifts of spins A and X):

$$\mathbf{H}(t) = 2\omega_1(\cos(\omega_a t))(I_x^A + I_x^X) + \omega_a(I_z^A - I_z^X) + 2\pi J_{AX}I_z^AI_z^X \quad (1)$$

As has been shown elsewhere,[26] it is convenient to transform the equation of motion of the density operator σ into a doubly

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[22] The amplitude of antiphase single-quantum coherences is defined[23] as $|2I_z^AI_{xy}^X| = [(2I_z^AI_x^X)^2 + (2I_z^AI_y^X)^2]^{1/2}$.

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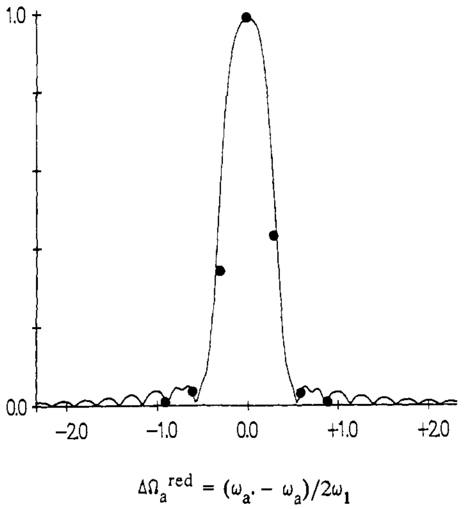

Figure 8. Theoretical curve with experimental points for magnetization transfer in the doubly selective HOHAHA experiment as a function of the reduced offset $\Delta\Omega_a^{red} = (\omega_{a'} - \omega_a)/2\omega_1$, where $\omega_a = {}^1/_2(\Omega_A - \Omega_X)$. One sideband always occurs at Ω_A, while the other varies between $\Omega_X - 3\omega_1$ and $\Omega_X + 3\omega_1$.

rotating (DR) frame, where the transformed density operator is given by

$$\sigma^{DR}(t) = U\,\sigma(t)\,U^{-1} \qquad [U = \exp[-i\omega_a t(I_z^A - I_z^X)]] \tag{2}$$

In this frame, the relevant terms in the Hamiltonian are time independent:[26]

$$\mathbf{H}^{DR} = \omega_1(I_x^A + I_x^X) + 2\pi J_{AX} I_z^A I_z^X \tag{3}$$

With an initial condition $\sigma^{DR}(0) = I_x^A$, one obtains the following time dependence for the in-phase and antiphase components of spin X in the doubly rotating frame:

$$\langle I_x^X\rangle(\tau) = {}^1\!/_2[\Delta^2\Sigma^2 + \sin^2(2\alpha)\cos(\lambda_1\tau) - \cos(\lambda_2\tau)] \tag{4}$$

$$\langle 2I_z^A I_y^X\rangle(\tau) = {}^1\!/_2[\sin(2\alpha)\sin(\lambda_1\tau) + \sin(\lambda_2\tau)] \tag{5}$$

with the coefficients

$$\lambda_1 = 2\omega_1\cos(2\alpha) - \pi J\sin(2\alpha) \tag{6}$$

$$\lambda_2 = \pi J_{AX} \tag{7}$$

$$\Sigma = \cos\alpha + \sin\alpha \tag{8}$$

$$\Delta = \cos\alpha - \sin\alpha \tag{9}$$

where $\tan(2\alpha) = -\pi J_{AX}/2\omega_1$.

The complementary antiphase term $2I_y^A I_z^X$ has the same magnitude as $2I_z^A I_y^X$ but is opposite in phase. The superposition of these two antiphase terms corresponds to zero-quantum coherence in the tilted frame. In fact, TOCSY transfer is mediated by these terms which, to first order, are insensitive to rf field inhomogeneity.[27,28] Figure 3 illustrates the time evolution of both in-phase and antiphase terms in the doubly rotating frame. Note that at the time $\tau = (J_{AX})^{-1}$ one obtains pure in-phase coherence, I_x^X.

The Hamiltonian in the doubly rotating frame, $\mathbf{H}^{DR}$, has the same appearance as the on-resonance cross-polarization Hamiltonian in a heteronuclear IS system for $\omega_{1I} = \omega_{1S}$. There is a correspondence between offset effects in heteronuclear systems and a mismatch of the modulation frequency in the modulated homonuclear experiment.[29] Figure 8 shows experimental and theoretical curves for magnetization transfer in a doubly selective HOHAHA experiment as a function of the reduced offset $\Delta\Omega_a^{red} = (\omega_{a'} - \omega_a)/2\omega_1$, where $\omega_a = {}^1/_2(\Omega_A - \Omega_X)$. The profile shows a pronounced sensitivity to mismatch of the modulation frequency, so interference with other multiplets in the same spectral range need not be feared.

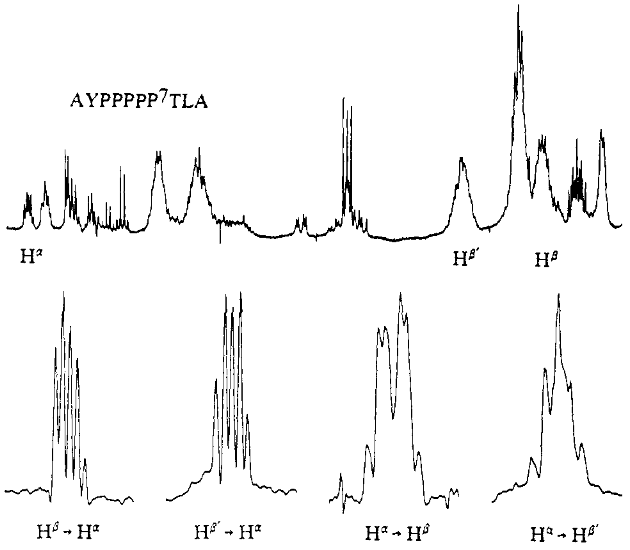

Figure 9. One-dimensional multiplets resulting from in-phase coherence transfer in doubly selective HOHAHA in the $\alpha\beta$ region of the Pro^7 residue in AYPPPPPTLA. The experimental conditions were like those for Figure 6.

Cross-Relaxation in Doubly Selective HOHAHA

In normal TOCSY experiments, cross-relaxation effects may interfere with the coherent transfer of magnetization.[11,12] Indeed, if coherences are spin-locked by a strong rf field, they become effectively degenerate in their precession frequencies. This not only leads to Hartmann–Hahn transfer but also "activates" cross-relaxation pathways.[30] By contrast, with the Hamiltonian of eq 1, the precession frequencies of the A and X spins are not degenerate. Indeed, in a frame rotating at the average chemical shift, the coherences of spins A and X rotate at the same frequency ω_a but in opposite sense. Thus we can follow Redfield's argument that the contribution of nonsecular terms to the relaxation process is negligible[30] and hence conclude that cross-relaxation does not occur in doubly selective experiments.

Doubly Selective Spin-Locking in Higher Spin Systems

If a doubly selective spin-lock is applied to a more complex spin system, the situation remains essentially unchanged. The spin-lock inhibits evolution with respect to passive couplings, so that we expect only additional in-phase splittings in the spectrum (or, if the data are recorded in 2D fashion, in the ω_2 dimension only). The transfer function (i.e. the coefficient of the desired operator term I_x^X) depends only on the "active" coupling constant, in contrast to normal TOCSY experiments, where the complete coupling network contributes to a complicated oscillatory magnetization transfer between all participating spins. The selective experiment is thus intrinsically more sensitive than conventional TOCSY.

Figures 9 and 10 show doubly selective spin-lock experiments applied to the peptide AYPPPPPTLA[31] and to basic pancreatic trypsin inhibitor (BPTI) using the sequence in Figure 1. The experiments illustrate how a quick one-dimensional experiment can help to identify two coupling partners within the crowded H$^\alpha$ and H$^\beta$ regions. No purging pulses were used in these experiments. If the coupling constant is only known approximately, we expect a slight distortion of intensities, but this is not too detrimental to the primary goal of establishing connectivities.

If a purging pulse is applied prior to detection, in the manner of Figure 4, one may unwittingly generate antiphase coherences

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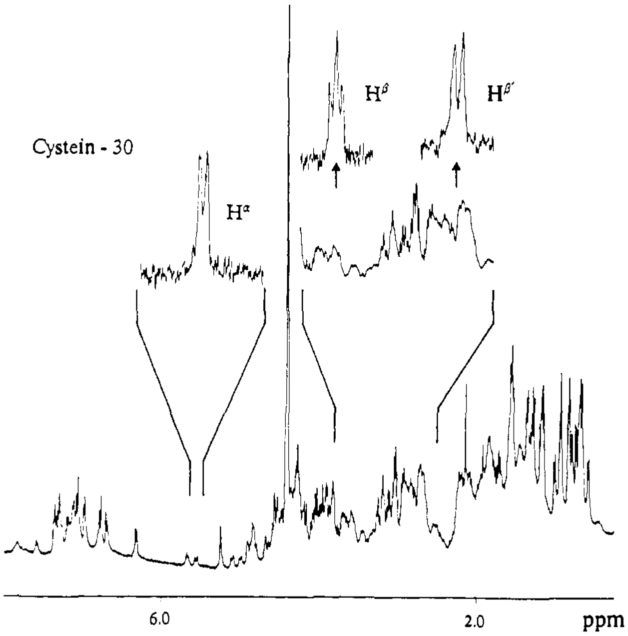

Figure 10. One-dimensional multiplets obtained by doubly selective HOHAHA in cysteine-30 of bovine pancreatic trypsin inhibitor (BPTI): (left) transfer $H^\beta \rightarrow H^\alpha$; (middle) transfer $H^\alpha \rightarrow H^\beta$; (right) transfer $H^\beta \rightarrow H^{\beta'}$. The experimental conditions were like those for Figure 6.

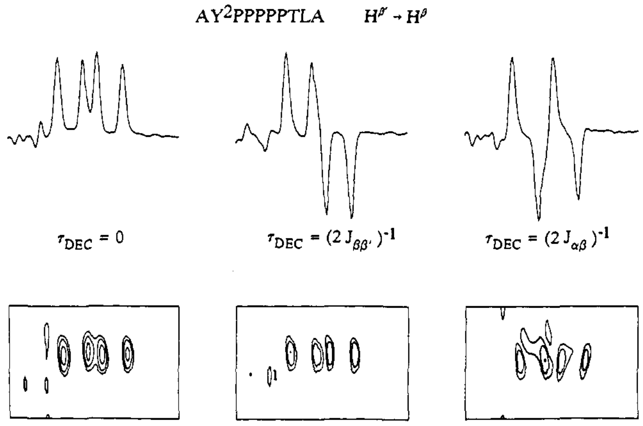

Figure 11. Two-dimensional in-phase and antiphase multiplets of the Tyr^2 residue in AYPPPPPTLA (coupling network see Figure 12), obtained by applying selective pulses prior to detection. The pulse durations were adapted to the scalar couplings involved (see text). In this case two-dimensional experiments are preferable, as the analogous 1D spectrum is perturbed by the adjacent signal of 3-(trimethylsilyl)-1-propanesulfonic acid added as internal standard.

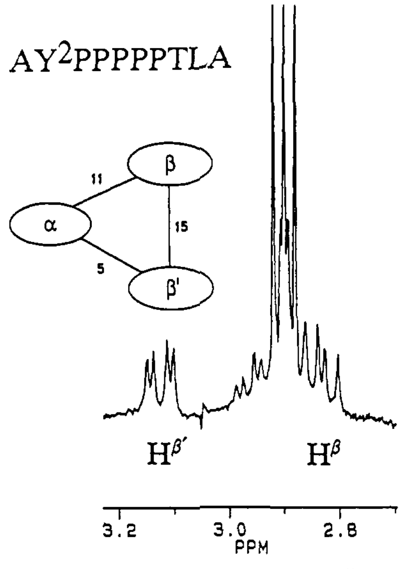

Figure 12. Coupling network of the H^α, H^β, and $H^{\beta'}$ protons in Tyr^2 of AYPPPPPTLA with the corresponding region of the 1D spectrum.

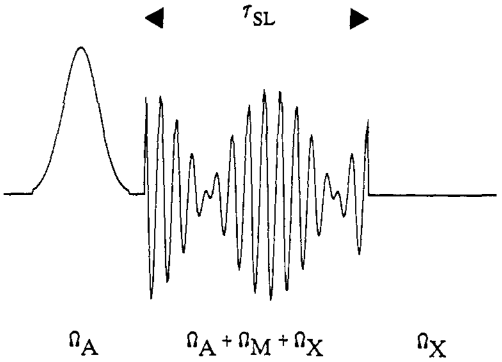

Figure 13. Pulse sequence for triply selective HOHAHA. The initial self-refocusing 270° Gaussian pulse with a carrier frequency Ω_A excites in-phase magnetization I_x^A. During the spin-lock period τ_{SL}, the rf carrier is positioned midway between the two chemical shifts, $\omega_0 = {}^1/_2(\Omega_M + \Omega_X)$, and the audio modulation frequencies are set to $\omega_a = {}^1/_2(\Omega_A - \Omega_M)$ and $\omega_b = {}^1/_2(\Omega_A - \Omega_X)$. This generates three sidebands at Ω_A, Ω_M, and Ω_X. The fourth one must be placed well outside the spectrum.

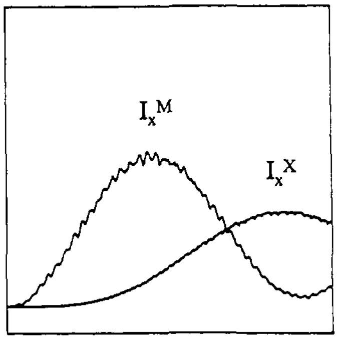

Figure 14. Simulation of a triply selective spin-lock experiment in a three-spin AMX system with $J_{AM} = 10$, $J_{MX} = 5$, and $J_{AX} = 0$ Hz. Starting from transverse magnetization of spin A, the transfer to spin M reaches a maximum at $\tau_{SL} \approx (J_{AM})^{-1}$, while X magnetization reaches a maximum determined by the coupled differential equations.

with respect to passive coupling partners. Consider a three-spin system AMX where magnetization is transferred from I_x^A to I_x^X in the doubly rotating frame. If the 270° Gaussian purging pulse acts on spin A, the couplings J_{AM} and J_{AX} may be regarded as inactive during the pulse interval τ_{DEC} (see above), while J_{MX} is effective and generates antiphase coherence $2I_z^MI_y^X$, which will be at a maximum if $\tau_{DEC} = (2J_{MX})^{-1}$. Thus the elimination of antiphase terms comprising the active spins will invariably lead to the creation of other antiphase terms involving passive coupling partners. The problem may be solved by *simultaneously* irradiating both spins A and X using a modulated purging pulse.

With a different objective in mind, one may deliberately generate a variety of different antiphase terms. Let us assume that the active coupling J_{AX} is known with sufficient accuracy, so that pure in-phase coherence I_x^X may be obtained after an interval $\tau_{SL} = (J_{AX})^{-1}$. Then pure antiphase coherence $2I_z^MI_y^X$ is obtained after a 270° Gaussian purging pulse applied to spin A with a duration of $(2J_{MX})^{-1}$. In an analogous fashion, one may obtain $2I_z^AI_y^X$ by applying a purging pulse to spin M of duration $(2J_{AX})^{-1}$. Figure 11 shows complementary in-phase and antiphase spectra obtained from the tyrosine residue of AYPPPPPTLA, which has the coupling network[31] shown in Figure 12. The two-dimensional version of the experiment was chosen because interference with an adjacent signal belonging to 3-(trimethylsilyl)-1-propanesulfonic acid (used as internal standard) is less troublesome than in the corresponding 1D experiment. The coupling constants were approximately 11 and 15 Hz, so the duration of the purging pulse

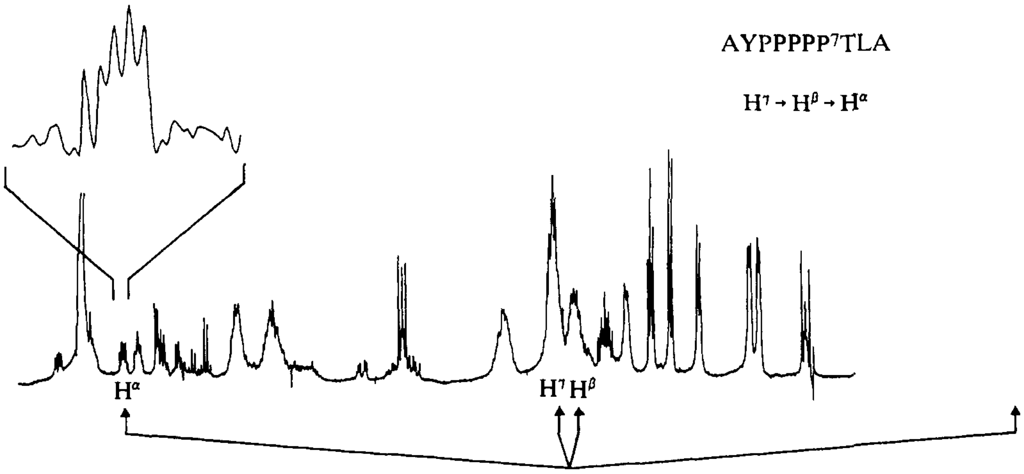

Figure 15. Experimental example for triply selective HOHAHA: $H^\gamma \rightarrow H^\beta \rightarrow H^\alpha$ transfer in Pro^7 of AYPPPPPTLA.

did not exceed 50 ms. For smaller coupling constants and correspondingly larger durations, one might resort to alternative methods of creating antiphase terms, like selective COSY.

Such complementary experiments appear useful with regard to a method proposed by Titman and Keeler,[32] which allows one to measure the coupling constants with great accuracy by comparison of in-phase and antiphase multiplets. Indeed, two such multiplets must yield identical patterns if they are convoluted with antiphase and in-phase doublets of δ functions, respectively, provided these both have the correct splitting J_{AX}.

Multiply Selective Spin-Locking

If two pairs of coupled spins are degenerate, this leads to overlapping cross-peaks in COSY-type spectra, as frequently occurs in the crowded H$^\alpha$ and H$^\beta$ regions of proteins. Connectivities to further spins may then be used to distinguish the (sub)systems involved. Relayed coherence transfer, e.g., from an H$^\gamma$ to an H$^\alpha$ via a common coupling partner H$^\beta$, allows one to unravel overlapping signals. Two approaches to relayed transfer that involve selective spin-locking may be considered. The first method would involve two sequential steps, first a transfer from H$^\gamma$ to H$^\beta$ and then one from H$^\beta$ to H$^\alpha$. This can be achieved with a succession of two doubly selective spin-lock periods. This has indeed been tried successfully. The second approach uses a multiply selective spin-lock field, which can be achieved by imposing two or more modulation frequencies on the rf carrier. For a triply selective spin-lock, we have

$$\omega_a = \tfrac{1}{2}(\Omega_A - \Omega_M) \tag{10}$$

$$\omega_b = \tfrac{1}{2}(\Omega_A - \Omega_X) \tag{11}$$

with the transmitter set at $\omega_0 = {}^1/_2(\Omega_M + \Omega_X)$. This generates three sidebands at Ω_A, Ω_M, and Ω_X. The fourth sideband (which occurs at $-\Omega_A + \Omega_M + \Omega_X$) must be placed well outside the spectrum. The appearance of a doubly modulated spin-lock pulse of this type is shown in Figure 13.

The simulation of Figure 14 illustrates the situation in an AMX spin system where $J_{AM} = 10$, $J_{MX} = 5$, and $J_{AX} = 0$ Hz. Starting from transverse magnetization of spin A, the transfer to spin M reaches a maximum at $\tau_{SL} \approx (J_{AM})^{-1}$, while X magnetization starts to build up following a behavior typical of a second-order process. Clearly, the transfer function depends on several coupling constants. Figure 15 gives an experimental example, where the connectivity between the H$^\gamma$ and H$^\alpha$ protons of the Pro^7 residue of AYPPPPPTLA is established.

Conclusions

We have proposed novel methods that allow one to transfer in-phase coherence between two or more selected spins, provided that they are connected by scalar couplings. In both one- and two-dimensional spectra, the fine structure of the resulting multiplets can be used to ascertain the interactions with further coupling partners.

There has been some dispute in recent years as to whether experiments of this type should be referred to by the acronym TOCSY (total correlation spectroscopy) or HOHAHA (homonuclear Hartmann–Hahn). So far, we have strongly tended to favor the former, which was historically the earliest designation. However, the word "total" refers to the fact that coherence can be transferred between all spins that belong to a common network and seems quite inappropriate to the selective experiments described in this paper. This is why, in the present context, we tend to favor the name of selective homonuclear Hartmann–Hahn spectroscopy.

The new doubly selective HOHAHA method can be used to verify whether a cross-peak appearing in a nonselective TOCSY spectrum at frequency coordinates $(\omega_1, \omega_2) = (\Omega_A, \Omega_X)$ is due to a scalar coupling J_{AX} or whether this cross-peak merely arises from the fact that spins A and X belong to a common coupling network. If spins A and X have a vanishing coupling $J_{AX} = 0$, no transfer can be observed with our doubly selective HOHAHA method. It is also possible to lift similar ambiguities in one-dimensional TOCSY spectra[9] that are obtained by selective excitation of one multiplet followed by nonselective spin-locking.

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[32] Titman, J.; Keeler, J. *J. Magn. Reson.* **1990**, *89*, 640.