

Spin state selectivity and heteronuclear Hartmann–Hahn transfer

Burkhard Luy*

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, Garching D-85747, Germany

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Abstract

Spin state selectivity can be obtained if inphase and antiphase magnetization can be transformed into each other. Heteronuclear Hartmann–Hahn transfer is usually used for the transfer of inphase magnetization. For a two spin system, however, a building block can be constructed that transfers inphase into antiphase magnetization and vice versa. The article gives a detailed description of the building block as well as experiments with elements similar to spin state selective coherence transfer (S^3CT) and spin state selective excitation (S^3E). The possibility of ‘sensitivity enhancement’ is pointed out and an application in the double selective measurement of coupling constants using a combination of CW-cross-polarization and the S^3CT -type approach is demonstrated.

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Keywords: Hartmann–Hahn transfer; HEHAHA; Spin state selectivity; CW-cross-polarization

1. Introduction

Spin state selective pulse sequences have found widespread use in the last years. Applications are mainly focused in the measurement of coupling constants [1–9] and as the main element in transverse relaxation optimized spectroscopy (TROSY) experiments [10–14]. The main idea is the clever combination of inphase and antiphase signals to reduce the multiplet pattern of a cross-peak, which can be easily achieved by conventional pulse–delay experiments. However, for greater flexibility it is desirable to have alternative transfer elements for use in spin state selective experiments. Heteronuclear Hartmann–Hahn transfer for example has several advantages over conventional pulse–delay experiments: computer-optimized modern Hartmann–Hahn sequences are usually well compensated for B_1 -field inhomogeneity. This is especially useful for modern cryogenic probeheads where significant losses due to uncompensated hard pulses limit the application of more complex pulse sequences. In the large number of multiple pulse sequences (see, for example [15]) many special designs can be found like the selective PLUSH-TACSY [16] or the kin-HEHAHA

with different selectivities on the two participating nuclei [17] or the double selective CW-cross-polarization approach [18,19] that open many possibilities in the development of new pulse sequences. Hartmann–Hahn sequences are also known to refocus effectively exchange broadened resonances [20] and allow efficient transfer of magnetization comparable to CPMG sequences in this case. In conventional experiments, HEHAHA is used to transfer inphase magnetization. In this article we show an easy way to convert inphase into antiphase magnetization and vice versa in an effective two spin system. With this transfer element, all basic HEHAHA building blocks are given for the design of spin state selective experiments. Elements corresponding to spin state selective coherence transfer and spin state selective excitation are given. In addition, an application for highly selective and sign-sensitive measurement of coupling constants using double selective CW-cross-polarization is shown.

2. Theory

The effective Hamiltonian of a HEHAHA experiment with the sequence irradiated along the x -axis is given by the planar mixing Hamiltonian

* Fax: +49-89-289-13210.

E-mail address: Burkhard.Luy@ch.tum.de.

$$H_P^x = 2\pi J^{\text{eff}}(I_y S_y + I_z S_z) \quad (1)$$

with the effective coupling constant $J^{\text{eff}} \leq J/2$. For the initial operator I_x , the transfer function under this mixing condition is given by [21–23]

$$I_x \rightarrow I_x \cos^2(\pi J^{\text{eff}} \tau) + S_x \sin^2(\pi J^{\text{eff}} \tau) + \frac{1}{2}(2I_y S_z - 2I_z S_y) \sin(2\pi J^{\text{eff}} \tau). \quad (2)$$

This coherence transfer function implies the well-known result that maximum inphase transfer is achieved after $\tau = \frac{1}{2}J^{\text{eff}}$. It also can be seen that after $\tau = \frac{1}{4}J^{\text{eff}}$ the antiphase term is of same magnitude as the inphase magnetization, leading to the density operator

$$I_x \xrightarrow{\frac{1}{4J^{\text{eff}}}} \frac{1}{2}[I_x + S_x + 2I_y S_z - 2I_z S_y]. \quad (3)$$

On the other hand, evolution of antiphase magnetization $2I_y S_z$ under identical mixing conditions is given by

$$2I_y S_z \rightarrow 2I_y S_z \cos^2(\pi J^{\text{eff}} \tau) + 2I_z S_y \sin^2(\pi J^{\text{eff}} \tau) + \frac{1}{2}(S_x - I_x) \sin(2\pi J^{\text{eff}} \tau) \quad (4)$$

with the result that after $\tau = \frac{1}{2}J^{\text{eff}}$ $2I_y S_z$ is transformed into $2I_z S_y$ and at a mixing time of $\tau = \frac{1}{4}J^{\text{eff}}$, we find a density operator of the form

$$2I_y S_z \xrightarrow{\frac{1}{4J^{\text{eff}}}} \frac{1}{2}[2I_y S_z + 2I_z S_y + S_x - I_x]. \quad (5)$$

Comparing Eqs. (3) and (5) it is obvious that a single $180^\circ_y(I)$ pulse will convert the density operators into each other. Therefore, inphase I_x magnetization can easily be transformed into antiphase $2I_z S_y$ after $\tau = \frac{1}{2}J^{\text{eff}}$.

A generalization of this approach leads to the pulse sequence element shown in Fig. 1 for which all possible permutations of 180° pulses for initial operators I_x and $2I_y S_z$ are summarized in Table 1. An experimental verification of these transfers is given in Fig. 2 for inphase magnetization as initial spin density ρ_a . Having in hand a HEHAHA building block that is able to convert inphase into antiphase and vice versa allows the design of spin state selective experiments.

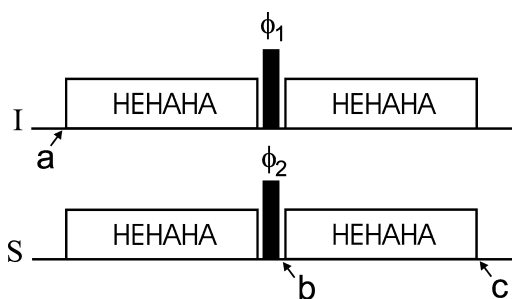


Fig. 1. Building block for the interconversion of inphase and antiphase magnetization using heteronuclear Hartmann–Hahn transfer. The positions a, b, and c correspond to the density operators described in Table 1 after application of 180° pulses with phases ϕ_1 and ϕ_2 . The HEHAHA transfer periods are $1/(4J^{\text{eff}}) \leq 1/(2J)$ each.

3. Experimental

The pulse sequence element shown in Fig. 1 can be used for the design of spin state selective experiments. A basic version of such an experiment can be seen in Fig. 3A: the preparation period is used to create inphase S_x magnetization on the heteronucleus as in most HEHAHA experiments. The back transfer after t_1 evolution, however, is used to generate the four different transfers $I_z S_x \rightarrow I_x S_z$, $S_x \rightarrow I_x$, $S_x \rightarrow I_y S_z$, and $I_z S_x \rightarrow I_y$ in sequential experiments. The four different pathways lead

Table 1
Transfer properties for the building block of Fig.1 for different phases of 180° pulses and initial operators I_x and $2I_y S_z$

ρ_a	ϕ_1	ϕ_2	ρ_b	ρ_c
I_x	—	—	$\frac{1}{2}[I_x + S_x + 2I_y S_z - 2I_z S_y]$	S_x
	x	—	$\frac{1}{2}[I_x + S_x - 2I_y S_z + 2I_z S_y]$	I_x
	—	x	$\frac{1}{2}[I_x + S_x - 2I_y S_z + 2I_z S_y]$	I_x
	y	—	$\frac{1}{2}[-I_x + S_x + 2I_y S_z + 2I_z S_y]$	$2I_z S_y$
	—	y	$\frac{1}{2}[I_x - S_x - 2I_y S_z - 2I_z S_y]$	$-2I_z S_y$
	x	x	$\frac{1}{2}[I_x + S_x + 2I_y S_z - 2I_z S_y]$	S_x
	y	y	$\frac{1}{2}[-I_x - S_x - 2I_y S_z + 2I_z S_y]$	$-S_x$
	x	y	$\frac{1}{2}[I_x - S_x + 2I_y S_z + 2I_z S_y]$	$2I_y S_z$
$2I_y S_z$	—	—	$\frac{1}{2}[-I_x + S_x + 2I_y S_z + 2I_z S_y]$	$2I_z S_y$
	x	—	$\frac{1}{2}[-I_x + S_x - 2I_y S_z - 2I_z S_y]$	$-2I_y S_z$
	—	x	$\frac{1}{2}[-I_x + S_x - 2I_y S_z - 2I_z S_y]$	$-2I_y S_z$
	y	—	$\frac{1}{2}[I_x + S_x + 2I_y S_z - 2I_z S_y]$	S_x
	—	y	$\frac{1}{2}[-I_x - S_x - 2I_y S_z + 2I_z S_y]$	$-S_x$
	x	x	$\frac{1}{2}[-I_x + S_x + 2I_y S_z + 2I_z S_y]$	$2I_z S_y$
	y	y	$\frac{1}{2}[I_x - S_x - 2I_y S_z - 2I_z S_y]$	$-2I_z S_y$
	x	y	$\frac{1}{2}[-I_x - S_x + 2I_y S_z - 2I_z S_y]$	$-I_x$
y	x	$\frac{1}{2}[I_x + S_x - 2I_y S_z + 2I_z S_y]$	I_x	

Density operators ρ_a , ρ_b , and ρ_c are present at positions a, b, and c in Fig. 1.

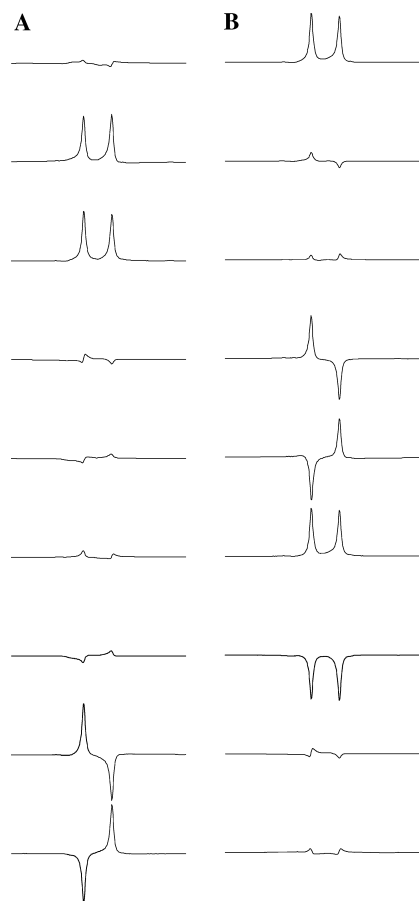


Fig. 2. Experimentally determined transfer properties of the building block described in Fig. 1 for the transfer starting with inphase I_x magnetization. The spectra are determined on the ^1H - ^{19}F two spin system of diethylfluoro malonate. Starting with inphase magnetization on protons the resulting spectra for protons (A) and fluorine (B) are shown with the rows corresponding to the first nine rows in Table 1. Dispersive antiphase signals are phase corrected by 90° .

to four different multiplet patterns after two-dimensional Fourier transformation that can be added up to allow any spin state selective multiplet in two dimensions. The method is demonstrated on ^{13}C -labeled formate in Fig. 4. Since the experiment of Fig. 3A provides spin state selection only during the coherence transfer period after t_1 evolution, it corresponds to spin state selective coherence transfer (S^3CT) as introduced in [3]. This approach is also used in most TROSY-type experiments to select for the least relaxing multiplet component [10–13].

Another way of implementing spin state selectivity is, of course, spin state selective excitation (S^3E), where the α - and β - selective density operators $S_x I^\alpha$ and $S_x I^\beta$, respectively, are produced in the preparation period and afterwards transferred in a spin state conserving way. In the original experiments, S^3E is achieved by the excitation of $\frac{1}{2}S_x \pm S_x I_z = S_x I^{\alpha/\beta}$ in every scan and subsequent subtraction of unwanted magnetization in a phase cycling scheme [1]. The S^3E -type experiment with

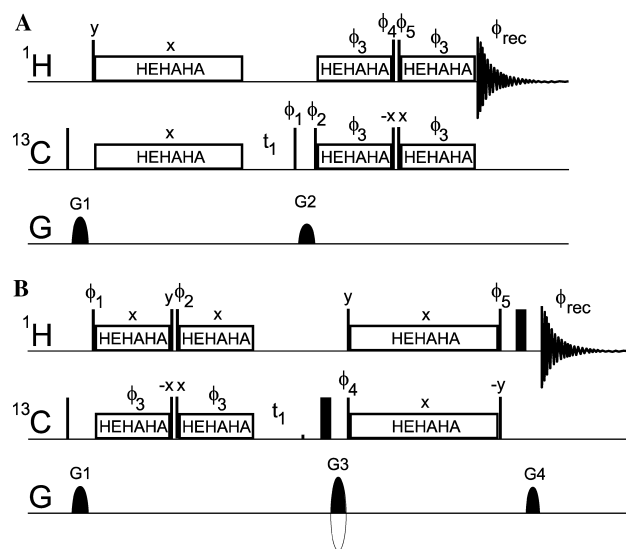


Fig. 3. Basic spin state selective HEHAHA experiments. (A) After conventional HEHAHA transfer of inphase magnetization spin state selectivity is achieved in four different ways of the back transfer, similar to the S^3CT approach. Phases independent of the back transfer are $\phi_1 = y, -y$, $\phi_2 = y, y, -y, -y$. Four different experiments with phases A: $\phi_3 = y$; $\phi_4 = x$; $\phi_5 = -x$; $\phi_{rec} = y, -y, -y, y$; B: $\phi_3 = x$; $\phi_4 = x$; $\phi_5 = -x$; $\phi_{rec} = x, -x, -x, x$; C: $\phi_3 = x$; $\phi_4 = y$; $\phi_5 = y$; $\phi_{rec} = y, -y, -y, y$; D: $\phi_3 = y$; $\phi_4 = x$; $\phi_5 = x$; $\phi_{rec} = x, -x, -x, x$; result in multiplet pattern as shown in Figs. 4A–D. (B) HEHAHA experiment similar to the S^3E approach with conventional planar mixing for the back transfer and coherence order selective transfer using the echo/antiecho method. Four different experiments with multiplet patterns corresponding to Figs. 5A–D can be recorded. A: $\phi_2 = y$; $\phi_3 = y$; $\phi_5 = -y$; B: $\phi_2 = -y$; $\phi_3 = x$; $\phi_5 = -y$; C: $\phi_2 = y$; $\phi_3 = y$; $\phi_5 = y$; D: $\phi_2 = -y$; $\phi_3 = x$; $\phi_5 = -y$. Additional phases $\phi_1 = y, -y$, and $\phi_{rec} = x, -x$ are independent of the chosen experiment, ϕ_4 is cycled with the echo (y) and antiecho ($-y$) increments in order to achieve phase-sensitive spectra. Gradients G3 and G4 need to satisfy the ratio 80:20.1 for ^{13}C as the heteronucleus for coherence order selection. All other gradients are of arbitrary strength.

HEHAHA transfer steps shown in Fig. 3B uses a different approach by exciting S_x and $S_x I_z$ in two different experiments that can be added or subtracted to obtain the desired multiplet component. The transfer back to the I nucleus is achieved easily by HEHAHA_x transfer with flanking 90°_y pulses, since its inherent planar mixing Hamiltonian is well known to conserve the spin state and allow the transfers $S^\pm I^{\alpha/\beta} \rightarrow S^{\alpha/\beta} I^\pm$ [24,25]. The spin state conserving transfer results already in reduced multiplet patterns as verified experimentally in Fig. 5. By changing the phase ϕ_5 in the experiment of Fig. 3B either the diagonal (Figs. 5C and D) or anti-diagonal (Figs. 5A and B) multiplet components are obtained with inphase (Figs. 5A and C) or antiphase pattern (Figs. 5B and D), respectively. Again, pure spin state selective signals can be derived by addition or subtraction of the corresponding spectra (Figs. 5E–H). It should be noted that the experiment shown in Fig. 3B uses coherence order selection with echo/antiecho acquisition. The resulting ‘sensitivity enhancement’ is

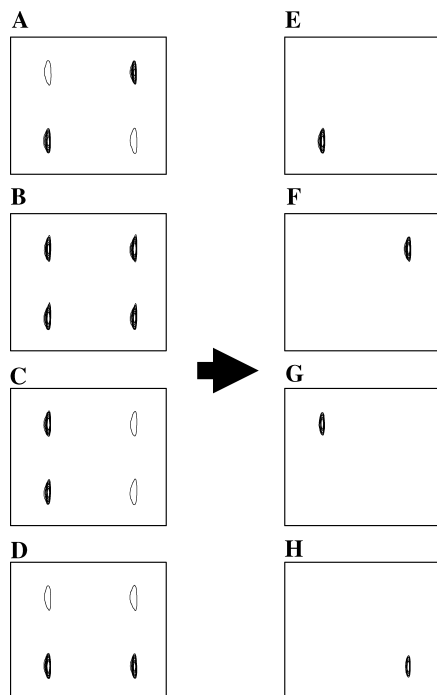


Fig. 4. Experimental multiplet patterns acquired on the ^1H - ^{13}C two spin system of ^{13}C -labeled formate. As HEHAHA sequence a MLEV-16 expanded 180° pulse is used [30] with 3104 Hz amplitude, corresponding to a cycle time of $2.58\text{ ms} = 1/(2J)$ for the two spin system with $J = 194\text{ Hz}$. Four experiments as described in Fig. 3A are recorded in (A)–(D). Positive signals are shown with the full set of contour levels while negative signals are represented only by one contour. The experiments can be used to construct pure spin state selective signals by adding/subtracting the spectra: (E) $A + B + C + D$; (F) $A + B - C - D$; (G) $-A + B + C - D$; and (H) $-A + B - C + D$.

included inherently in the planar mixing conditions of the back transfer produced by the HEHAHA sequence [26–29].

As an application the combination of the S^3E -E.COSY principle [1,2] with the double selective transfer properties of CW-cross-polarization [18,19] shall be demonstrated. CW-cross-polarization is well suited for the spin state selectivity approach described above since an effective two spin system is automatically achieved in most cases by the high bandwidth selectivity of the transfer. An experiment for the sign-sensitive measurement of long range J_{NH} coupling constants is shown in Fig. 6A and the corresponding approach for J_{HNH} coupling constants in Fig. 6B: proton magnetization of a selected amide group is transferred to the ^{15}N nucleus and flipped along the z -axis, where a spinlock/gradient combination [31] effectively dephases all undesired coherences. The transfer back to proton magnetization is done with the building block described in Fig. 1. In a first experiment, the coherence is transferred to inphase I_y magnetization and in a second experiment to antiphase $2I_x S_z$ magnetization. With a period of any kind of homonuclear mixing with

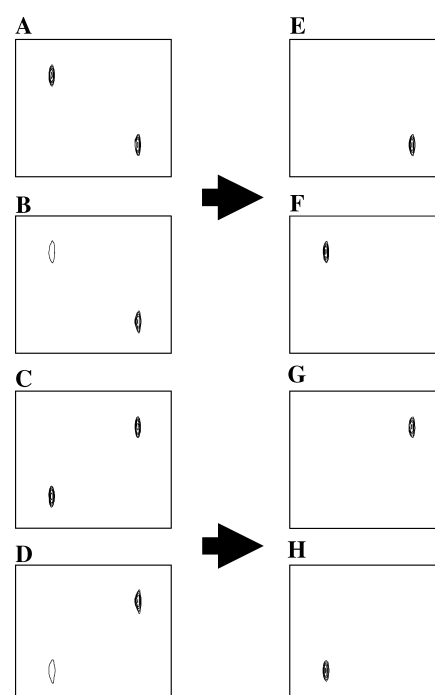


Fig. 5. Experimental multiplet patterns acquired on the ^1H - ^{13}C two spin system of ^{13}C -labeled formate. As HEHAHA sequence a MLEV-16 expanded 180° pulse is used [30] with 3104 Hz amplitude, corresponding to a cycle time of $2.58\text{ ms} = 1/(2J)$ for the two spin system with $J = 194\text{ Hz}$. Four experiments as described in Fig. 3B are recorded in (A)–(D). Positive signals are shown with the full set of contour levels while negative signals are represented only by one contour. The experiments can be used to construct pure spin state selective signals by adding/subtracting two of the spectra: (E) $A + B$; (F) $A - B$; (G) $C + D$; (H) $C - D$.

NOESY, COSY, or TOCSY-type transfer magnetization is transferred from the amide protons to surrounding spins. Addition and subtraction of the antiphase experiment from the inphase experiment results in α - and β -subspectra, respectively, of the protons with respect to their J_{NH} coupling constant. The measurement of the J_{HNH} coupling constants is achieved in the same way by simply adding a ^1H - ^{15}N -planar mixing transfer step (Fig. 6B) as described in [1].

The method is demonstrated on the uniformly ^{15}N - ^{13}C -labeled cyclic pentapeptide D-Pro-Ala-Ala-Ala-Ala (PA_4). The Ala_5 amide resonance is used for the selective transfer and a 45 ms TOCSY period was applied as homonuclear mixing. Inphase and antiphase spectra of the Ala_5 spin system are shown in Figs. 7B and C, respectively. It turns out that the intensity of the antiphase amide signal is only 69% of the intensity of the inphase peak. The origin of this difference can probably be found in the non-ideal mixing Hamiltonian of the CW-cross-polarization with amplitude $\sqrt{3}/4 J_{\text{NH}}$. However, by simply scaling the two spectra to identical amplitudes of the amide signal, the α - and β -subspectra could be obtained as described before. The resulting $J_{\text{NH}\alpha}$ and

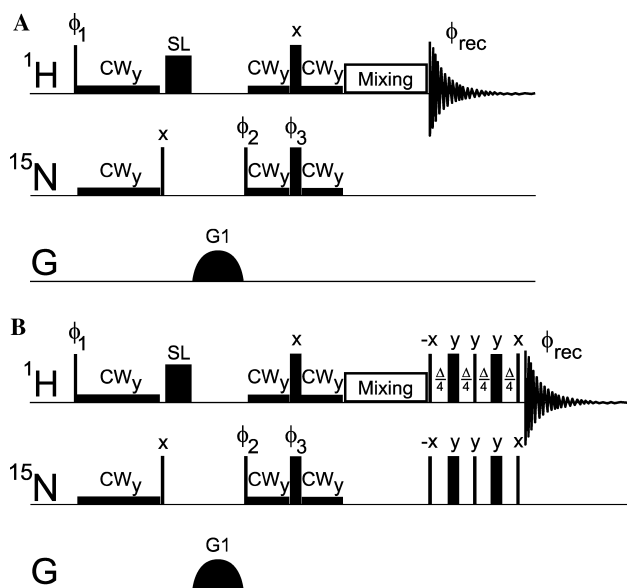


Fig. 6. Sequences for the double selective measurement of J_{NH} and J_{HNH} coupling constants using the building block of Fig. 1 with CW-cross-polarization as HEHAHA-transfer element. The experiments are a combination of the IPAP approach [5] and the S^3E -E.COSY principle [1]. Phases are $\phi_1 = x, x, -x, -x$ and $\phi_2 = x, -x$ in all experiments. For the detection of inphase magnetization the phase settings are $\phi_3 = x$; $\phi_{rec} = -y, y, y, -y$, and for antiphase detection the phase settings are $\phi_3 = x$; $\phi_{rec} = x, -x, -x, x$ for antiphase detection. After the HEHAHA period magnetization is distributed via a homonuclear mixing period. In (B), the detection of homonuclear $J_{H^N H}$ coupling constants is achieved via an additional spin state conserving planar transfer step [2,24].

$J_{NH\beta}$ coupling constants are given in Figs. 7D and E together with the α - and β -components of the $H\alpha$ and $H\beta$ signals. The corresponding $J_{H^N H\alpha}$ and $J_{H^N H\beta}$ coupling constants obtained by the experiments described in Fig. 6B are shown in Figs. 7F and G.

4. Discussion

As demonstrated theoretically and experimentally in the previous sections, spin state selectivity and TROSY spectroscopy can be achieved with HEHAHA-based transfer elements. With these results a variety of applications seems to be possible considering the large number of Hartmann–Hahn multiple pulse sequences designed for different purposes (for a review see, e.g. [15]).

HEHAHA sequences produce a constant effective field over a certain offset range, which allows efficient transfer of exchange broadened signals as long as the inverse exchange rate is on the order of the duration of a basic cycle of the corresponding multiple pulse sequence [20]. In contrast to conventional hard pulse and delay sequences, HEHAHA multiple pulse sequences are usually well compensated for B_1 -field inhomogeneity. This can lead to significantly improved transfer

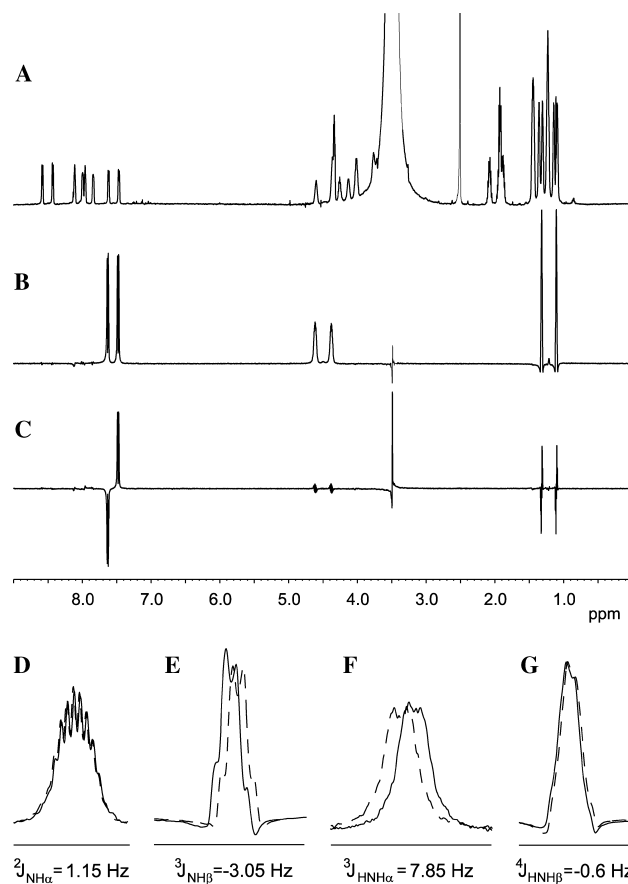


Fig. 7. Application of the double selective experiments of Fig. 6 to Ala_5 of the cyclic pentapeptide PA_4 . (A) 1D of the ^{15}N - ^{13}C -labeled PA_4 . (B,C) Inphase and antiphase spectra of the experiment shown in Fig. 6A using CW-cross-polarization with 39 Hz amplitude and a DIPSI-2 [32] mixing period of 50 ms for homonuclear transfer. After scaling the amide signal of the two spectra to identical intensity, sign-sensitive measurement of J_{NH} coupling constants (D,E) is possible. Using the identical scaling for inphase and antiphase spectra of the experiment shown in Fig. 6B leads to the corresponding $J_{H^N H}$ coupling constants (F,G).

efficiencies especially on modern cryogenic probehead systems where signal loss due to B_1 -field inhomogeneity is quite strong.

The use of HEHAHA transfer elements in spin state selective experiments, of course, also has a number of limitations. The theory derived here in previous sections is only strictly valid in effective two spin systems. An additional 1H - 1H -coupling active during the HEHAHA transfer period, for example, will lead to an effective PIP coupling topology with far more complicated transfer properties [33,34]. Also the rare case of an additional planar coupled spin will lead to strongly modified results [35,36]. Hence, the right choice of HEHAHA multiple pulse sequence will be crucial for many applications. A second limitation inherent to all Hartmann–Hahn sequences is the occurrence of zero-quantum artifacts and the sensitivity to stronger miscalibrations. Unfortunately, heteronuclear Hartmann–Hahn sequences for

compensation of miscalibration equivalent to adiabatic isotropic mixing sequences in the homonuclear case do not yet exist, but the suppression of ZQ-artifacts seems to be possible now with the recently introduced method of applying an adiabatic inversion pulse and a gradient simultaneously [37]. Inphase to inphase and antiphase to antiphase transfer efficiencies are given by the term $\sin^2(\pi J^{\text{eff}} \tau)$ (see Eqs. (2) and (4)). In contrast, inphase to antiphase and antiphase to inphase transfer is limited by the smallest operator present in the spin density ρ_b (see Fig. 1 and Table 1), leading to a transfer efficiency of $2 \sin^2(\pi J^{\text{eff}} \tau/2) \sin^2(\pi J^{\text{eff}} \tau)$ for effective couplings smaller than the optimal coupling J_0^{eff} and $2 \cos^2(\pi J^{\text{eff}} \tau/2) \sin^2(\pi J^{\text{eff}} \tau)$ for $J^{\text{eff}} > J_0^{\text{eff}}$. With the substitution $J^{\text{eff}} = J_0^{\text{eff}} + \Delta J^{\text{eff}}$ we can therefore express the ratio of transfer efficiencies of the two transfer pathways with respect to misadjusted transfer periods by $|1 - 2 \sin^2(\pi \Delta J^{\text{eff}} \tau/2 + (\pi/4))|$. This implies that spin state selective experiments introduced in this article are rather sensitive to J -mismatch. It should also be noted that not all HEHAHA multiple pulse sequences create an ideal planar mixing Hamiltonian since they are usually only optimized for inphase transfer. Also the effective coupling J^{eff} might differ significantly from the ideal value $J/2$ and B_1 -field inhomogeneity can have considerable impact on sequences that are not well compensated for it. In the case of CW-cross-polarization, for example, antiphase magnetization was significantly less produced compared to inphase magnetization (Figs. 7B and C).

In general, spin state selectivity via HEHAHA transfer is not limited to J -coupled spins but can also be applied to dipolar spin pairs since the effective heteronuclear Hamiltonian in both cases is identical [33]. The approach is also applicable to isotropic mixing conditions as produced by heteronuclear isotropic Hartmann–Hahn (HIHAHA) sequences and homonuclear Hartmann–Hahn (HOHAHA/TOCSY) experiments, for which the transfer into inphase and antiphase magnetization was already used in the SIAM-TACSY approach [38,39]. HOHAHA experiments applied to dipolar coupled spins, however, do not produce isotropic mixing conditions [40–42] and the spin state selective approach can only be applied along the principle axis of the transfer element [43,33].

The generation of antiphase out of inphase magnetization is not only limited to spin state selective experiments. The application of HEHAHA transfer elements in zero- and double-quantum experiments like the ZQ/DQ-TROSY [44,45], for example, seems to be a further promising field of applications.

5. Conclusion

It could be shown that in an effective two spin system transfer of inphase into antiphase magnetization and

vice versa is possible using HEHAHA transfer steps. The approach was used to create S^3CT and S^3E -type experiments and a doubly selective way of accurate and sign-sensitive measuring J_{NH} and $J_{H^N H}$ coupling constants using CW-cross-polarization as the heteronuclear Hartmann–Hahn sequence has been demonstrated. The approach should be applicable to experiments where transfer via exchange broadened signals, significant losses due to B_1 -field inhomogeneity or the need of tailored transfer bandwidths pose significant problems to conventional pulse–delay experiments.

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