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# In-Phase Double Selective Excitation of Coupled Spin Systems Using Excitation Sculpting

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**An experiment that excites exclusively coupled nuclei pairs is presented. It involves the biselective defocusing and refocusing of coupled transverse magnetization under double pulsed field gradient spin echo conditions. Application to the extraction of subspectra from crowded COSY spectra is presented, as well as a doubly selective version of the homonuclear *J*-resolved experiment.** © 1998 Academic Press

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The effect produced by simultaneous soft Gaussian pulses to a pair of scalar coupled spins depends on the relative strengths of the coupling interaction and of the excitation field. The deviation from the effect produced by hard pulses was observed for inversion pulses (*1*) and later given the name TSETSE (twin spin effect) for excitation pulses (*2*). General analytic solutions of the underlying spin physics equations were reported (*3*). A practical application of TSETSE is the spectral ''fishing out'' of pairs of coupled proton signals from crowded spectral regions. Heteronuclear TSETSE was applied to the measurement of long-range heteronuclear coupling constants (*4*). A related scheme named TSETSE-2 (*5*) combines spin pinging (*6*) and Gauss-shaped (*7*) refocusing pulses instead of E-BURP pulses (*8*). Whatever the scheme is, the magnetization of the selected nuclei appears with an antiphase pattern that reveals the mutual coupling constant. This leads to its accurate measurement through the *J*-doubling technique (*9, 10*). The present communication shows how the TSETSE-2 experiment can be improved and extended in order to build a coupled nuclei pair selective, pure in-phase excitation sequence. Applications to 1D TOCSY (*11*) and 2D TOCSY–COSY (*12*) are presented.

The TSETSE-2 experiment (Fig. 1a) relies on spin pinging for the elimination of signals from unselectively excited spins that are not subjected to selective refocusing. The discrimination between the spin pair of interest and other spins is achieved by a double quantum filter (*13*). The spin pinging procedure can be replaced with profit by excitation sculpting (*14*), resulting in the gradient-enhanced doubly selective spin echo sequence, as shown in Fig. 1b. The advantages are the elimination of spin pinging subtraction artifacts, the reduction of phase cycle length, a neat inversion profile, and a simplified control of the phase of the produced signals. If an unknown phase difference exists between hard and soft pulse channels, the magnetization produced by the sequence in Fig. 1a ends up with an undefined position in the transverse plane, making it difficult to manipulate it subsequently. The double spin echo sequence takes advantage of inherent refocusing properties to alleviate this problem. The desired selectivity may be incompatible with the overall sequence length, causing unacceptable signal loss by relaxation. Then, a single spin echo can be used, keeping in mind that the phase of a soft refocusing pulse is defined at its middle point.

The optimum duration $\Delta$ of coupling action can be adjusted by recording an array of spectra according to pulse sequence 1b in which $\delta$ is systematically incremented. The correct $\Delta$ value produces a maximum of antiphase magnetization and should be about $(2n + 1)/2J$ (*5*). An incorrect $\Delta$ value causes a signal loss without phase distortion. The pulse sequence in Fig. 1c incorporates a second biselective double spin echo period, leading to multiplets completely in-phase, as in refocused homonuclear INEPT (*15*). Quadruple multiselective spin echoes were already used in the ''exside'' experiment, designed for the measurement of heteronuclear long-range coupling constants (*16*). Experimental results for gradient-enhanced antiphase and in-phase double selective excitation of coupled spins are presented in Fig. 2. The test compound is the peracetylated derivative of melezitose **1,** a trisaccharide present in honey and sweet exudates of many plants. The signals of the methyl groups are highly rejected.

The pulse sequence in Fig. 1b was used with $\delta$ regularly incremented, like for a 2D experiment. It produces the spectrum in Fig. 3 after a 2D Fourier transformation, tilting and symmetrization. This is a biselective version of a fil-

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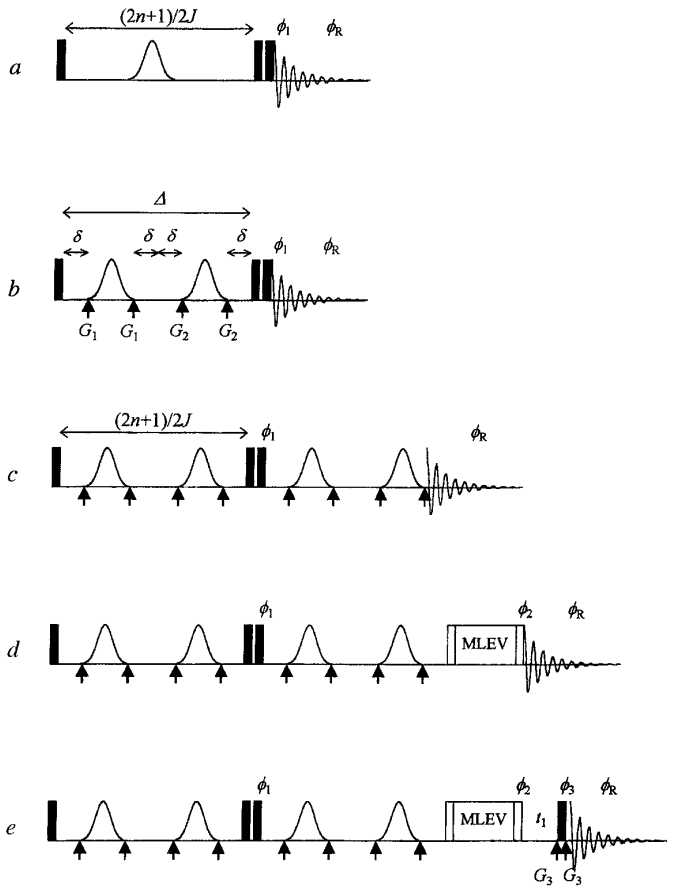


**FIG. 1.** Thin vertical bars are 90°$_x$ hard pulses, unless otherwise specified. Arrows symbolize gradient pulses. (a) The TSETSE-2 pulse sequence with double quantum filtering according to Ref. (*5*). (b) DPFGSE-enhanced version of (a). $\phi_1 = x, -y, -x, y$ and $\phi_R = x, y, -x, -y,$. (c) In-phase excitation of coupled nuclei pairs. The second double echo sequence is identical to the first one. (d) Biselective 1D TOCSY. $\phi_1 = x, -y, -x, y$, and $\phi_2 = x, y, -x, -y, -x, -y, x, y$, and $\phi_R = = x, y, -x, -y$. Phases of MLEV pulses follow the variations of $\phi_2$. (e) Biselective 2D TOCSY–COSY sequence. Phases are as in (d) with $\phi_3 = x, -x$.

tered homonuclear *J*-resolved spectrum, which could have been produced by the SERF experiment (*17*). The H-4/H-5 pair in the Glc residue is excited and only its coupling constant appears along $F_1$. The passive coupling constants (if any) are visible along $F_2$. The introduction of a recent filtering scheme could lead to narrower 2D peaks and therefore to accurate measurements of coupling constants (*18*).

The selection of a single frequency in a spectrum requires a well-separated multiplet. The biselective scheme presented here requires only a cross peak of a COSY spectrum to be well separated, as far as the parameters of interest are a pair of frequencies and the coupling constant of the corresponding nuclei. Finding an isolated 2D cross peak is in principle easier than finding an isolated 1D peak due to the spreading of information onto a surface instead of a line. The production of purely in-phase magnetization allows the use of the building block in Fig. 1c to be incorporated as an excitation sequence in other experiments. Excitation sculpting has been already reported as a useful tool in the construction of singly and doubly selective 1D NMR experiments (*19*). The pulse sequence in Fig. 1d produces the ''COSY cross-peak selective'' version of the TOCSY experiment, which formally extracts a 1D trace from a 3D COSY–TOCSY experiment. The selected in-phase magnetization is spread along the spin systems by means of an MLEV-17 isotropic mixing sequence (*20*). Starting it with antiphase magnetization would lead to signal cancellation. The double selectivity advantage apart, this pulse sequence involves the initial magnetization of two nuclei, resulting in a theoretical improvement of the signal-to-noise ratio by a

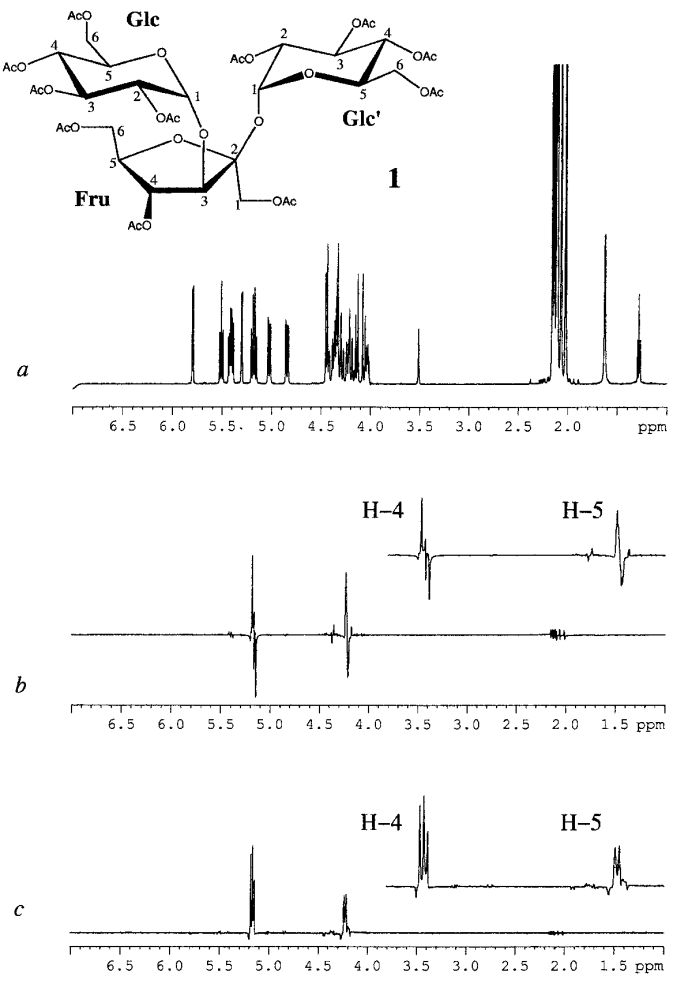


**FIG. 2.** (a) The 1D spectrum of peracetylated melezitose **1.** (b) DPFGSE-enhanced TSETSE-2 spectrum of **1,** showing antiphase patterns for H-4 and H-5 in Glc. Gradient strength $G_1$ and $G_2$ are 38 and 10 G cm$^{-1}$, respectively. Signal-to-noise ratio is optimum with $\Delta = 162.4$ ms. (c) is as (b) but with selective refocusing. The second double echo sequence is identical to the first one. The number of scans is 32, 8, and 8 for spectra (a), (b), and (c), respectively.

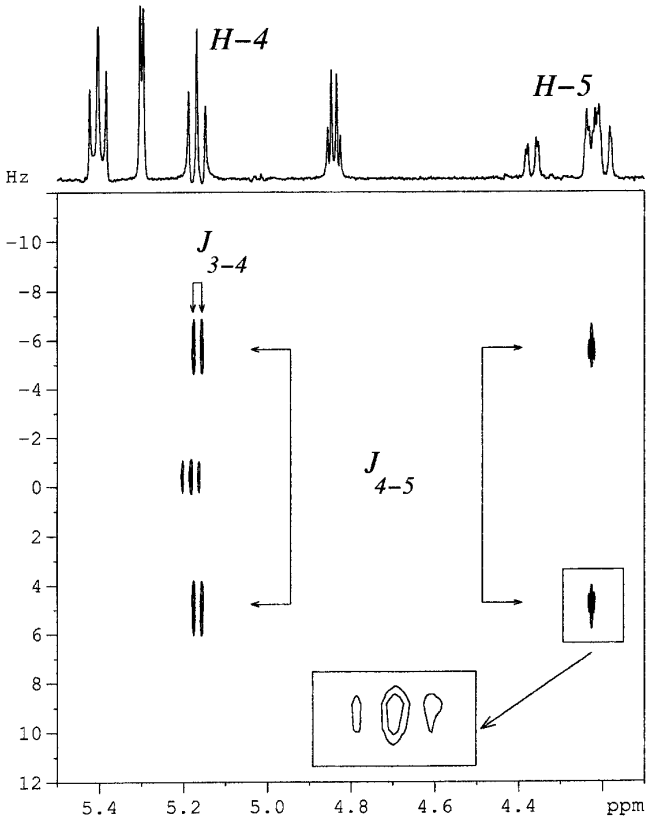


**FIG. 3.** The biselective *J*-resolved spectrum of the H-4/H-5 protons pair in **1** (8 scans, 64 experiments). The delay δ is incremented in steps of 5 ms. The top trace is the biselective 1D TOCSY spectrum (32 scans) of the Glc unit. An attenuated signal from H-4′ is visible at $F_1 = 0$.

factor of 2 over the usual 1D TOCSY (*11*) pulse sequence. The improvement is effective only if the transverse magnetization does not vanish by relaxation during the defocusing–refocusing process.

A further extension of the pulse sequence in Fig. 1d consists in building a COSY experiment that displays all coupling relationships between the nuclei within a doubly selected spin system. Formally, the pulse sequence in Fig. 1e extracts a plane from a 4D COSY–TOCSY–COSY experiment. From the COSY spectrum of **1** (Fig. 4a) such a plane is presented in Fig. 4b, using the H-4/H-5 cross peak as a starting point. The 1D spectrum at the top of Fig. 4b is the corresponding 1D-TOCSY spectrum recorded by means of the pulse sequence in Fig. 1d.

All spectra were recorded on a Bruker DRX 500 instrument, fitted with an inverse mode, *z*-axis gradient probe. The sample is made of 25 mg of **1** in 0.7 ml of $CDCl_3$ (37 mM). The shape of Gaussian biselective pulses is produced using the standard Bruker shape tool utility program. They last 50 ms and are truncated at the 1% level, and no absolute phase control is required when using a double echo technique. Gradient pulses are sine-bell shaped, their duration is 1 ms, and they are followed by a 100-μs recovery delay.

The pulse sequences presented here are practical developments of TSETSE, a phenomenon that was initially considered as a nuisance, making it difficult, for example, to use template excitation (*21*) in proton NMR. The presented experiments are easy to set up once a selective refocusing Gaussian pulse has been properly calibrated. They should

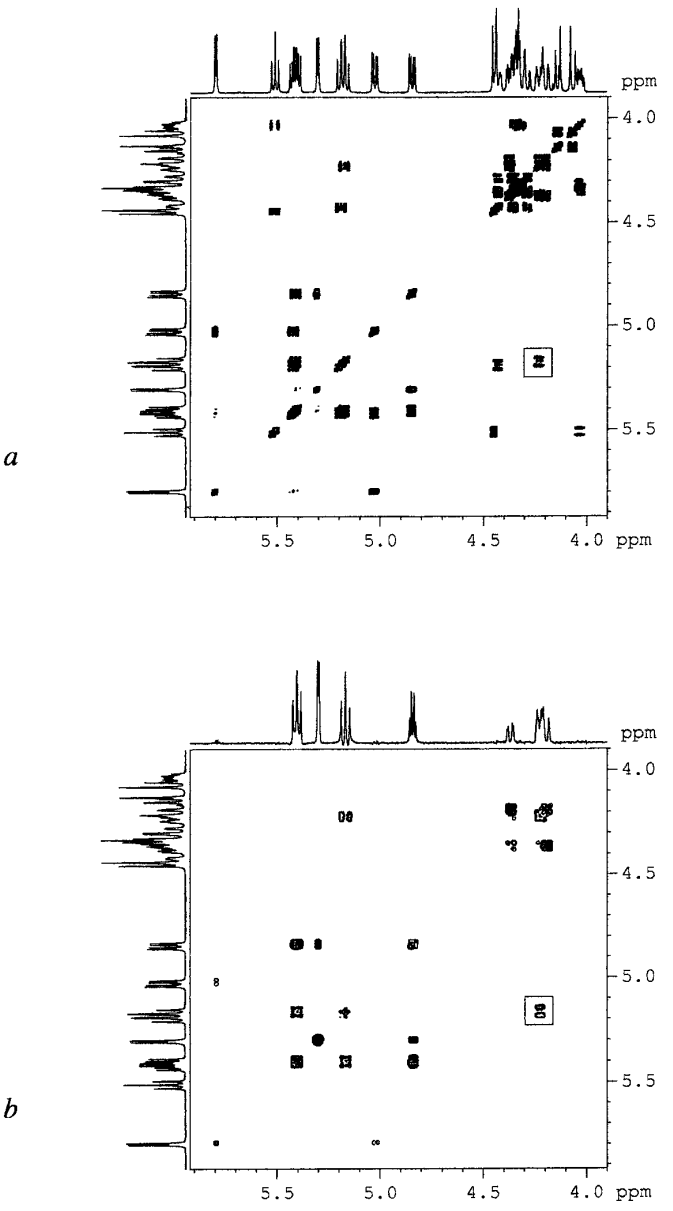


**FIG. 4.** (a) The gradient-enhanced N-type magnitude-mode COSY spectrum of **1.** The box encloses the correlation H-4/H-5 from which spectrum (b) originates. (b) The N-type magnitude-mode COSY spectrum (16 scans, 256 experiments) of the Glc spin system in **1.** Isotropic mixing and trim pulses last 188 ms ($\gamma B_1 = 8.6$ kHz) and 2.5 ms, respectively. Gradient strength $G_3$ is 7 G $cm^{-1}$. The top trace is the biselective 1D TOCSY spectrum of the Glc unit.

find applications in the field of the structural analysis of complex molecules, as carbohydrates or secondary metabolites.

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