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Homonuclear Broadband-Decoupled NMR Spectra

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The ^{1}H resonance assignment of complex molecules, which is a prerequisite for their structure determination, is often hampered by spectral overlap. If proton spectra could be homonuclear broadband decoupled, meaning that each multiplet is replaced by a singlet, the evaluation of chemical shifts would be facilitated. A number of different experiments have been described in the literature which achieve proton decoupling in the indirectly detected dimension in two-dimensional spectra, like time-reversal sequences (*1*), pseudo-echo weighting (*2*) and constant-time (*3, 4*) experiments. The latter two methods severely distort the relative intensities of the decoupled signals. Moreover, in all of these experiments, the homonuclear decoupling is a result of the processing procedure, and they are therefore not suitable for continuous decoupling, which would be necessary for applications like the measurement of transverse in-phase relaxation times (*5*). Several methods of semiselective homonuclear decoupling are presently known where a whole region is decoupled during an evolution period (*6, 7*) or even during acquisition (*8*). The most promising solution at the moment for broadband-decoupled proton spectra, where the decoupling is accomplished by a processing scheme, is the extraction of chemical shifts from two-dimensional reflected *J* spectra (*9*). Another way of extracting homonuclear broadband-decoupled proton spectra is the ω_2 projection of phase-sensitive *J*-resolved spectra which can be obtained by time reversal of NMR signals by linear prediction (*10*).

We suggest a completely different route to homonuclear broadband-decoupled spectra, which is based on the use of selective pulses and weak gradient fields. During the application of a magnetic field gradient, different parts of the sample experience different magnetic fields which lead to a location-dependent frequency shift across the sample volume.

Thereby, the induced frequency shift $\Delta\nu$ (in hertz) of any given signal over a length of s (centimeters) is $\Delta\nu = \gamma G \times s$, where γ is the gyromagnetic constant of the observed nucleus and G is the gradient strength in gauss/centimeter. Thus, to excite a range of 10 ppm (at 8.46 T) using a sample length of 1 cm, a gradient strength of 0.9 G/cm is necessary. Because of the gradient-induced shifts of the frequencies, a selective pulse leads to an excitation over the whole spectrum, provided a sufficient gradient strength is used. However, in different parts of the sample, different signals are excited; i.e., this is a spatially resolved experiment.

In the zeroth-order average Hamiltonian approximation, decoupling of a distinct signal at time τ can be achieved by inverting all of its scalar coupling partners at time $\tau/2$. This idea has already been used in the so-called BIRD approach (*12*), which can be used, for instance, to decouple carbon nuclei from protons. However, in our experiment, the signals which have been selectively excited during a gradient can be homonuclear decoupled by inverting all spins except those which are on-resonance. This can be done by combining a hard 180° pulse and a soft 180° pulse during a gradient field of the same strength. Because of the gradient, different signals are selectively decoupled at different positions across the sample volume. The combination of a hard and a soft 180° pulse for the inversion of all off-resonance spins has also been used by Hwang and Shaka (*12*).

To obtain one-dimensional homonuclear broadband-decoupled ^{1}H spectra, we record several spectra in a two-dimensional fashion (Fig. 1a), where the delay between excitation and detection is incremented stepwise. In the middle of this delay, the mentioned combination of hard and soft 180° pulses refocuses the evolution of scalar coupling. As at the beginning of the detection period, the effects of coupling are completely refocused; the first data points of each FID must be consecutively arranged to a new FID. This can be done by excising the first data points of each FID and pasting them together to form a new FID. The number of these points is given by

$$n = \frac{\mathrm{IN}}{\mathrm{DW}},$$

where IN is the time increment and DW is the dwell time. The size of the resulting FID is $n \cdot$ NE. As the proton–proton coupling constants are quite small (usually less than 20 Hz), it suffices to use increments of several milliseconds. The number of experiments NE usually does not exceed 32. This new FID can then be processed and transformed by ordinary procedures. It must, however, be noted that, if the scalar coupling constants are larger, the increments must be set to

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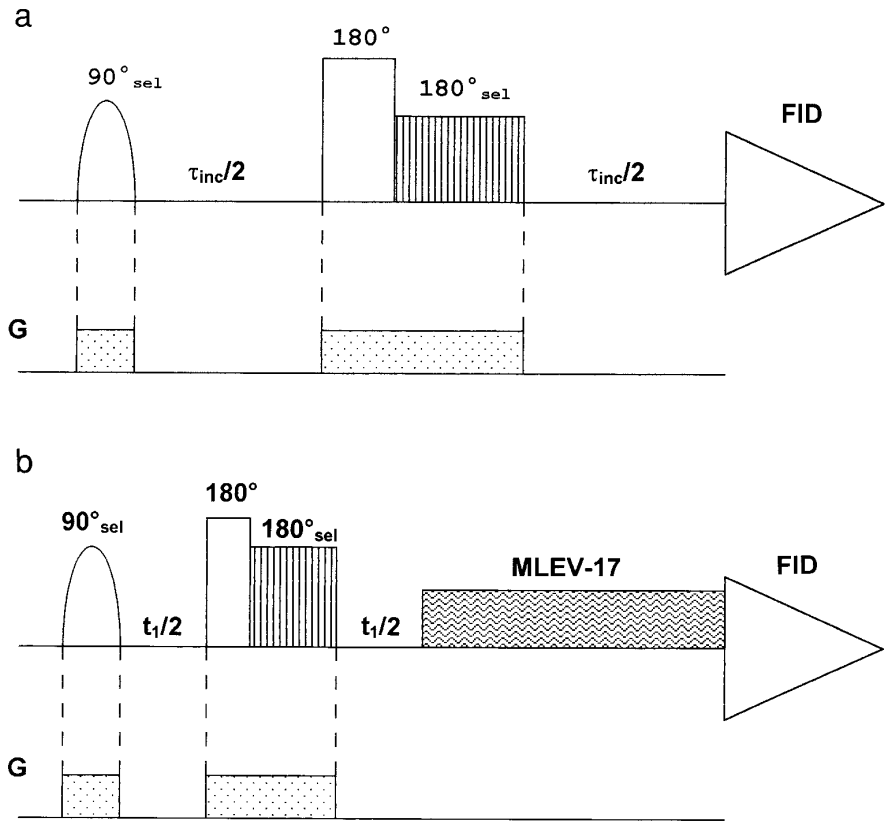

FIG. 1. Pulse sequences of (a) the method, which was used to obtain one-dimensional proton-decoupled proton spectra, and (b) the ω_1-decoupled TOCSY experiment. The delay τ_{inc} was incremented by several milliseconds for every experiment, as outlined in the text. The phases of the selective 90° pulse and the receiver were $x, -x, -x, x, y, -y, -y, y$, and both 180° pulses were cycled according to $x, x, -x, -x$. The gradients were used as shown on the line denoted G. Their amplitude was mostly less than 1 G/cm. The duration of the gradients is the same as the duration of the selective pulses. For the calculation of the 1D spectrum with the pulse sequence of (a), the first points of all the FIDs were put together into a new FID, which can then be processed and transformed like an ordinary FID.

smaller values in order to avoid sidebands in the processed spectra as a modulation in the FID arises earlier.

The described procedure leads to the same result as an ω_1 projection of the corresponding two-dimensional spectrum without, however, the inherent drawback of a long measuring time. As the attenuation of signal intensity due to scalar coupling is given by $[1 - \cos(\pi Jt)]$, an intensity reduction by just 3% after 8 ms of detection is observed for a coupling constant of 10 Hz.

The advantage of recording an homonuclear broadband-decoupled spectrum is best seen when applied to more complex systems. As an example, the decoupled ^{1}H spectrum of sucrose in DMSO is shown in Fig. 2 together with an ordinary ^{1}H spectrum. The simplification of the decoupled spectrum allows the measurement of the chemical shifts of many signals which are overlapped in the ordinary spectrum. Signals as close as 0.01 ppm, such as the two hydroxy resonances at 4.8 ppm, can be separated. Problems arise if two signals are strongly coupled, as the selective pulse cannot invert a signal, which is too close to the originally excited one. In this case, rather small signals appear in the spectrum, for instance, for the proton at position 3 of the fructose residue (at 3.87 ppm), which is only 0.1 ppm away from the proton at position 4 (at 3.77 ppm).

In 3-heptanone (not shown), the two resonances at 2.46 ppm can be detected unambiguously, although they are separated by only 0.02 ppm. The resolution is thereby also enhanced by the fact that only a limited volume of the sample is measured for each frequency, which thus reduces field inhomogenities. As a result of the location-dependent excitation, the sensitivity of the proposed method is quite low. The sensitivity loss compared to a nonselectively excited spectrum can be estimated by calculating the portion of the sample where any given signal is excited by the selective pulse. Since the gradient-induced shift is γG Hz/cm, the selective pulse with an excitation bandwidth of $\Delta\omega$ Hz leads to an excitation of $(\Delta\omega/\gamma G)100\%$, if a detected sample volume of 1 cm is assumed. If, for example, a pulse with an excitation bandwidth of 50 Hz is used for the selective excitation, and the gradient field strength is 0.5 G/cm, the sensitivity of the proposed experiment is 2.4% of the nonselective experiment. Another way of implementing this broadband decoupling is to record ω_1-proton-decoupled two-dimensional proton–proton correlations, like TOCSY (*13, 14*) spectra.

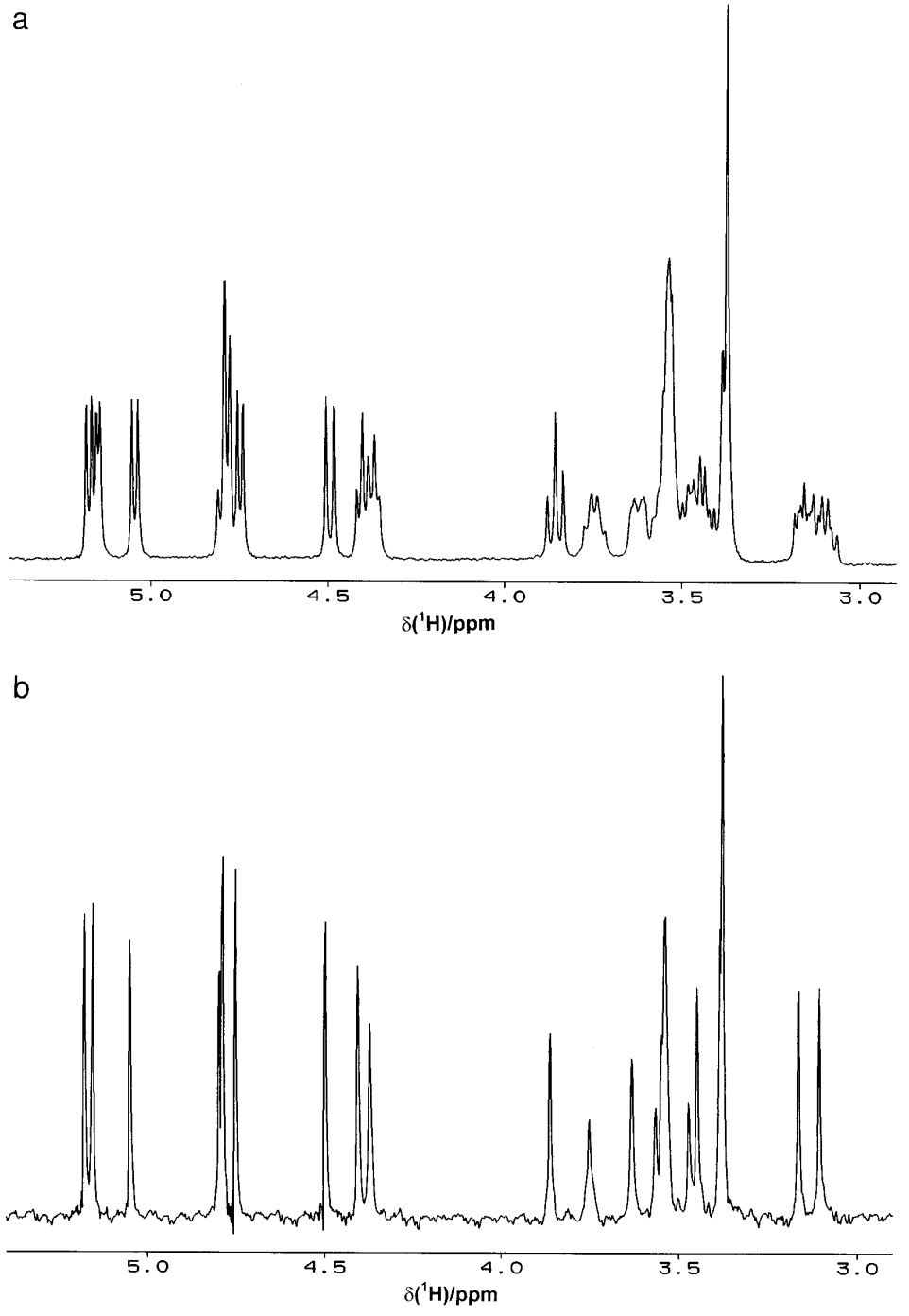

FIG. 2. (a) A 360 MHz ^{1}H NMR spectrum of sucrose in DMSO. Sixteen scans of 1024 complex data points were accumulated. (b) Spectrum of the same compound, obtained by the pulse sequence of Fig. 1a; 64 scans were accumulated for each of the 64 experiments; the time increment was 8 ms and the dwell time 250 μs. The first 32 complex data points of each FID were combined into a new FID consisting of 1024 complex data points, which after Fourier transformation led to the shown spectrum.

The pulse sequence for this experiment is shown in Fig. 1b. The first 90° pulse in the standard TOCSY experiment is interchanged by a selective 90° pulse during a gradient, and in the middle of the evolution delay t_1, a hard and a soft 180° pulse are applied during a gradient field of the same strength, which leads to the refocusing of the evolution of the scalar coupling. The better resolution in f_1 compared to an ordinary TOCSY spectrum (Fig. 3) is obvious. Another application could be the nonselective measurement of transverse in-phase relaxation times $T_2^{\text{in-phase}}$. We think that in

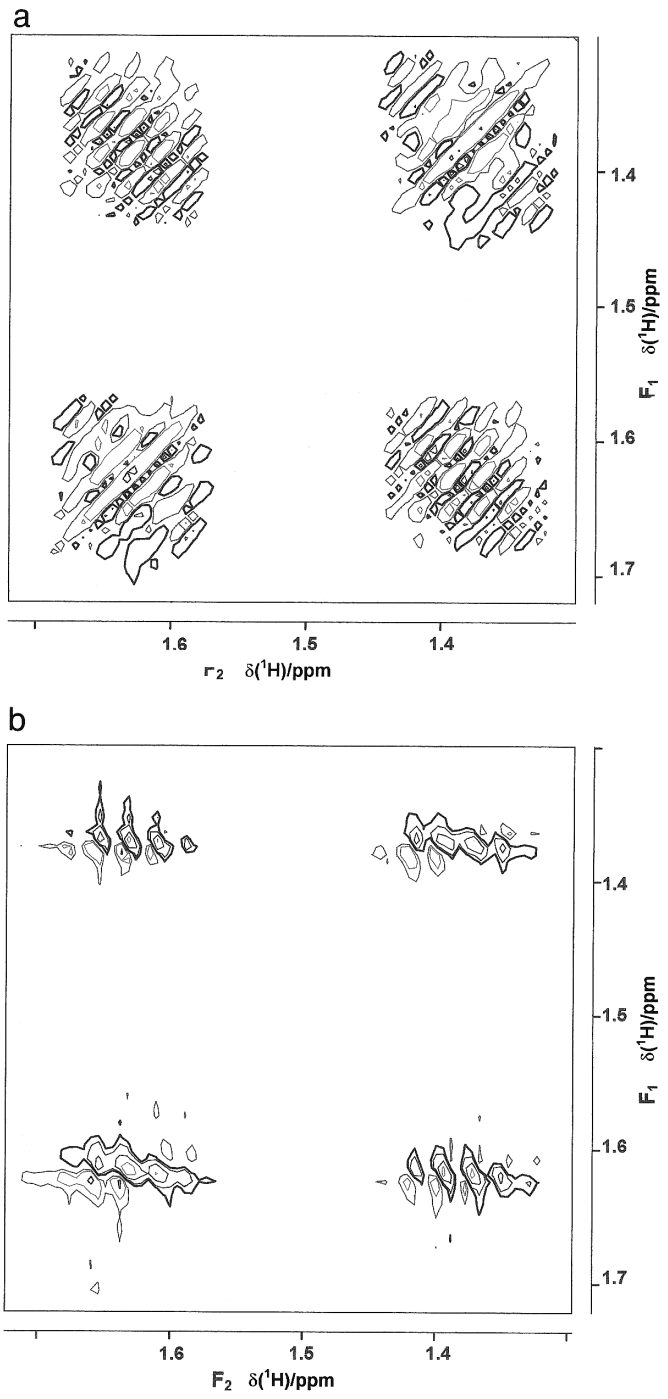

FIG. 3. Part of the 360 MHz (a) TOCSY and (b) ω_1-decoupled TOCSY spectra of 3-heptanone, showing the region of the methylene protons H-5 and H-6. Both spectra consisted of 512 experiments, each of which had a size of 1024 data points. After zero filling, the whole spectra, with a spectral width of 1000 Hz, consisted of 1024 × 1024 Bruker sequential data points. The number of scans per t_1 increment was 4 for the TOCSY spectrum (a), and 32 scans were acquired for each experiment of the ω_1-decoupled TOCSY spectrum (b).

cases where the inherently low sensitivity of this proposed scheme, of physical homonuclear broadband decoupling, is not a limiting factor, the broadband-decoupled spectra will lead to an enormous simplification of proton spectra.

All experiments were carried out on a Bruker AM 360 MHz NMR spectrometer equipped with a selective excitation unit (SEU) for the generation of amplitude-modulated shaped radiofrequency pulses and an ARX Z-gradient accessory. The decoupling experiments were carried out on solutions of 1% 3-heptanone in 99% $CDCl_3$ and 50 mg sucrose in DMSO-d_6. We used a 270° GAUSS pulse (*15*) for the selective excitation and a 180° DANTE pulse train (*16*) for the selective inversion. The phase of the selective 180° pulse must be the same as that of the hard 180° pulse or shifted by 180°. Due to phase differences between soft and hard pulses, we resorted to a DANTE pulse instead of a shaped 180° pulse. The gradients were rectangular, to assure the same frequency shift over the whole excitation and inversion period. In contrast to the other high-resolution techniques, where gradients affect the signal intensities by diffusion, here diffusion can just lead to a lower degree of decoupling for spins that changed the position between the selective excitation and the application of the selective 180° pulse.

The data processing needed for the 1D spectra was carried out by a Felix macro, which can be obtained from the authors.

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