JOURNAL OF MAGNETIC RESONANCE **125,** 145–148 (1997)
ARTICLE NO. MN961069

Quick Recording of Pure Absorption 2D TOCSY, ROESY, and NOESY Spectra Using Pulsed Field Gradients

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Received August 9, 1996; revised November 12, 1996

With the advent in 1990 of pulsed-field-gradient (PFG) techniques for coherence selection in high-resolution NMR experiments, the concept of routine in chemical and biochemical applications has changed. Many new, highly reliable NMR experiments use PFGs instead of the conventional phase cycle procedure to select the desired coherence-transfer pathway (CTP) (*1, 2*). The main advantages of the gradient-selection method are: (i) its greater speed, particularly for multidimensional determinations, resulting in much shorter spectrometer time requirements; and (ii) the much higher quality of the resulting spectra, always devoid of subtraction errors, thus allowing a much easier analysis of NMR parameters. Both advantages are due to the effective suppression by the gradients of unwanted magnetizations arising from undesired CTPs. Outstanding examples are the perfect suppression of $^{1}H–^{12}C$ (or $^{1}H–^{14}N$) magnetization effortlessly achieved in inverse experiments, or the frequency-independent suppression of the water signal from aqueous solutions. Another interesting advantage of PFG selection is that quadrature detection can be readily obtained in the indirect dimensions of *n*D experiments using a single scan per increment (*3*).

However, some precautions must be taken into account when designing novel PFG-based NMR methods aimed at obtaining spectra with maximum sensitivity and with signals in pure absorption lineshape (*4*). Thus, a PFG inserted into the variable evolution period in a multidimensional experiment selects only one of the two desired CTPs (P- or N-type selection) in each scan. Therefore, the resulting spectra are usually presented in magnitude mode, thus overcoming the undesirable mixed lineshapes of P or N singly selected signals (*5–7*). In order to obtain phase-sensitive spectra, pulse sequences must be modified so that no PFGs are applied during the variable evolution period. This option generally results in a decrease of the signal intensity by a factor of 2, in comparison with the phase-cycled analogous experiments. However, for samples in which concentration is not a limiting factor, excellent results are usually obtained with these phase-sensitive PFG procedures (*8, 9*).

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Here we show several alternatives to obtain phase-sensitive homonuclear 2D spectra in very short times with no phase cycling. Thus, phase-sensitive 2D TOCSY and 2D ROESY spectra can be obtained with a single scan per t_1 increment (Fig. 1) using an approach similar to that described previously for 2D NOESY spectra (*10*).

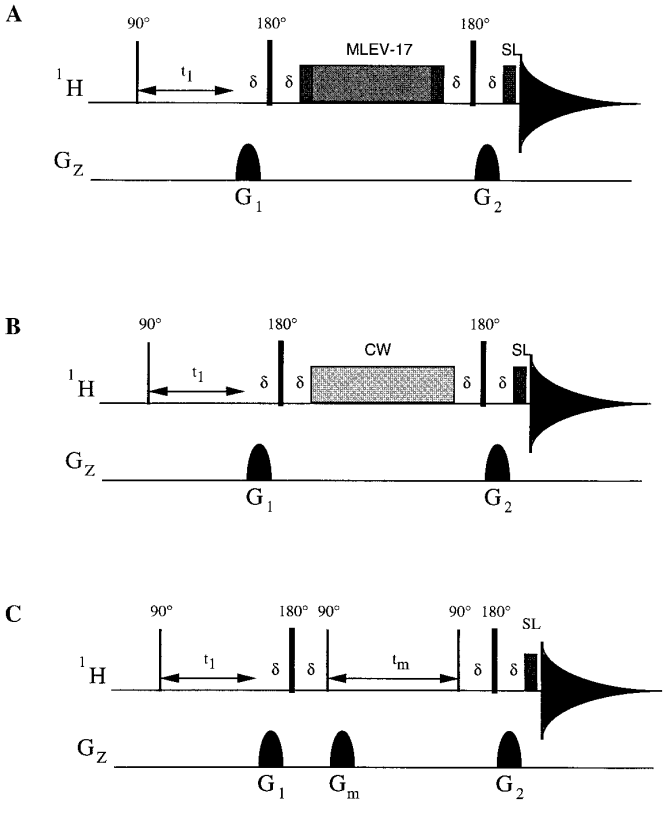

FIG. 1. Pulse sequences to obtain single-scan (a) ge-2D TOCSY, (b) ge-2D ROESY, and (c) ge-2D NOESY (*10*) spectra with pure-absorption lineshapes. Hard 90° and 180° pulses are indicated by vertical narrow and wide black bars. Short, strong spin-lock periods (SL) (1 ms at $\gamma B_1 = 7.8$ kHz) are applied just prior to acquisition. Pulsed field gradients of duration δ are also indicated by shaded shapes on the line G_Z. The PFG recovery time was 100 μs. In all cases, $G_1 = G_2$ and G_m acts as a spoil. All pulses are applied from the x axis unless otherwise indicated. To obtain pure-absorption lineshapes, it was necessary to cycle the first 90° proton pulse preceding the t_1 period in the TPPI manner.

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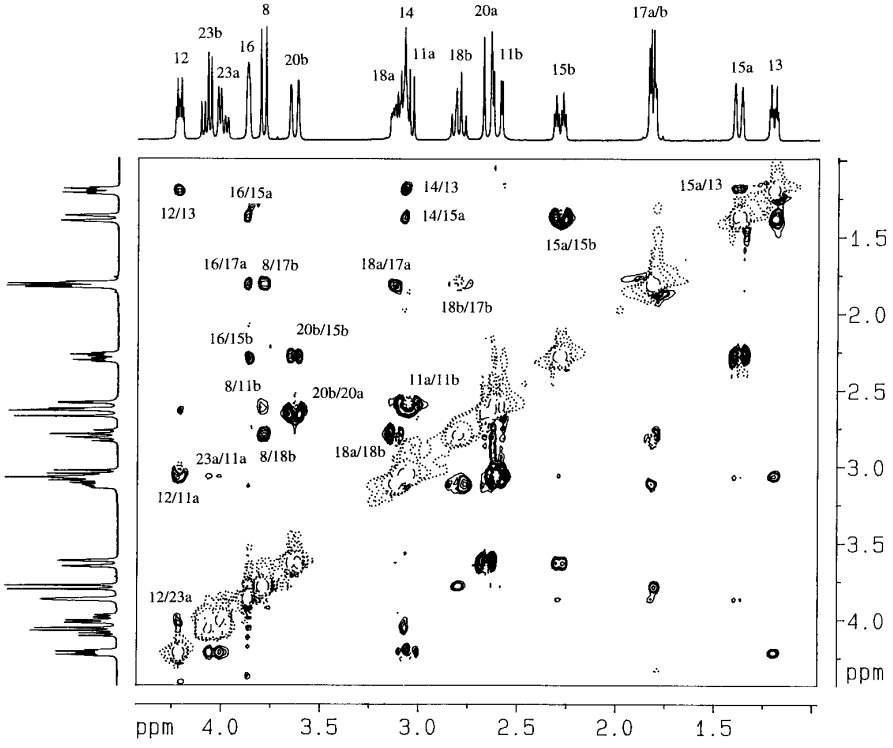
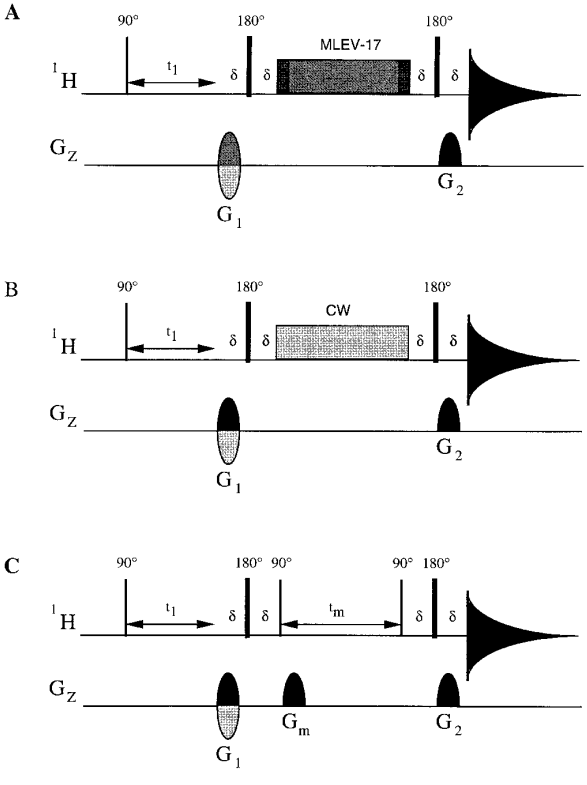


FIG. 2. Aliphatic part of the phase-sensitive ge-2D ROESY spectrum of **1** acquired with the pulse sequence of Fig. 1b. Negative ROE peaks are shown as continuous lines and positive diagonal peaks as dotted lines. A single scan was recorded for each of the 256 t_1 values giving a total experimental time of 6 min 50 s. The relaxation delay was 1 s and the mixing was achieved by an on-resonance low-power pulse ($\gamma B_1 = 2.6$ kHz) applied along the *y* axis during 500 ms. The length of all gradients was 1 ms and their strength around 5 G/cm. The data were multiplied with a cosine window function in both dimensions prior to Fourier transformation. All NMR data were obtained in a Bruker ARX400 spectrometer using an inverse broadband probehead incorporating a Z-gradient spoil.



In our experiments, we use basically the same pulse train as for the analogous phase-cycled experiments, with the addition of a pair of PFGs, inserted before and after the mixing period in order to select a specific CTP. Offset-dependent phase errors, due to evolution during the gradient length, are suppressed by replacing each gradient by a spin echo consisting of δ—180°—G, where δ is the length of the gradient G. This approach is used to record the N-type or echo data by choosing a G_1:G_2 gradient ratio of 1:1, and by incrementing the phase of the initial proton pulse in the TPPI manner (*11*). In all these and subsequent experiments, the proper choice of gradient strengths is also important in order to minimize extra sensitivity losses due to lateral diffusion present during the mixing time. Finally, an intense, short spin-locking field is applied along the *x* axis prior to acquisition to effectively remove the strong dispersive *y* components of the acquired signal. In this way, pure-phase 2D spectra

FIG. 3. Pulse sequences to obtain (a) ge-2D TOCSY, (b) ge-2D ROESY, and (c) ge-2D NOESY spectra with pure-absorption lineshapes. Two different data sets which correspond to the N-type and P-type data are recorded for all the experiments by reversing the sign of G_1 (the ratio of G_1:G_2 is 1:1 and −1:1, respectively) and then properly processed. All other experimental details are as described in the legend to Fig. 2.

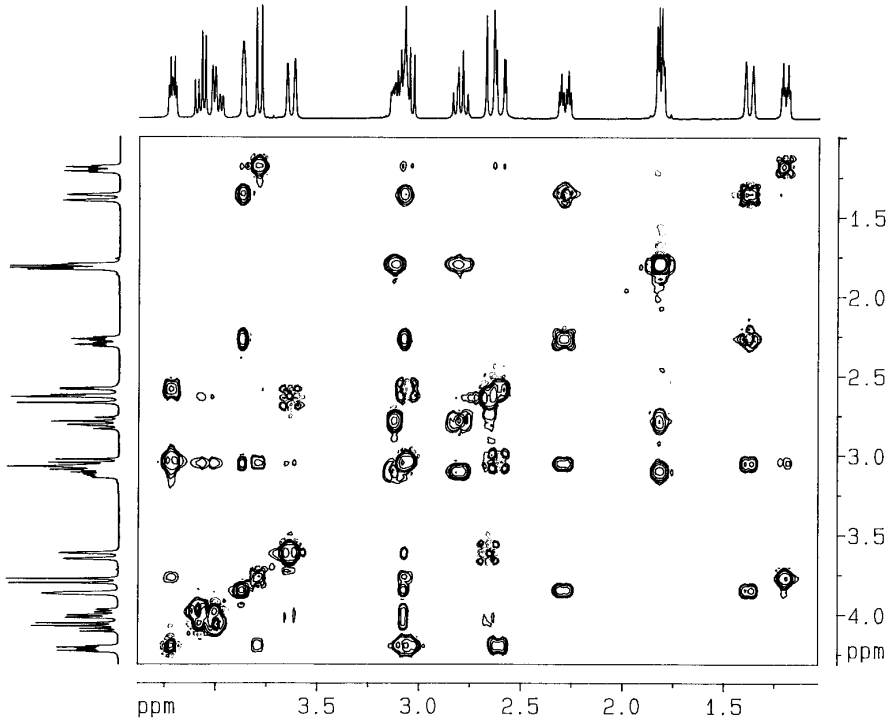


FIG. 4. Aliphatic part of the phase-sensitive 2D TOCSY spectrum of **1** acquired with the scheme of Fig. 3a. The mixing was achieved with a 60 ms MLEV-17 pulse train (γB_1 = 7.8 kHz) flanked by two 2.5 ms trim pulses. The strengths of the two sine-shaped PFGs were about 10:10 G/cm for one data set and −10:10 for the other data set. A single scan was recorded for each of the 128 t_1 points, giving a total acquisition time of 5 min.

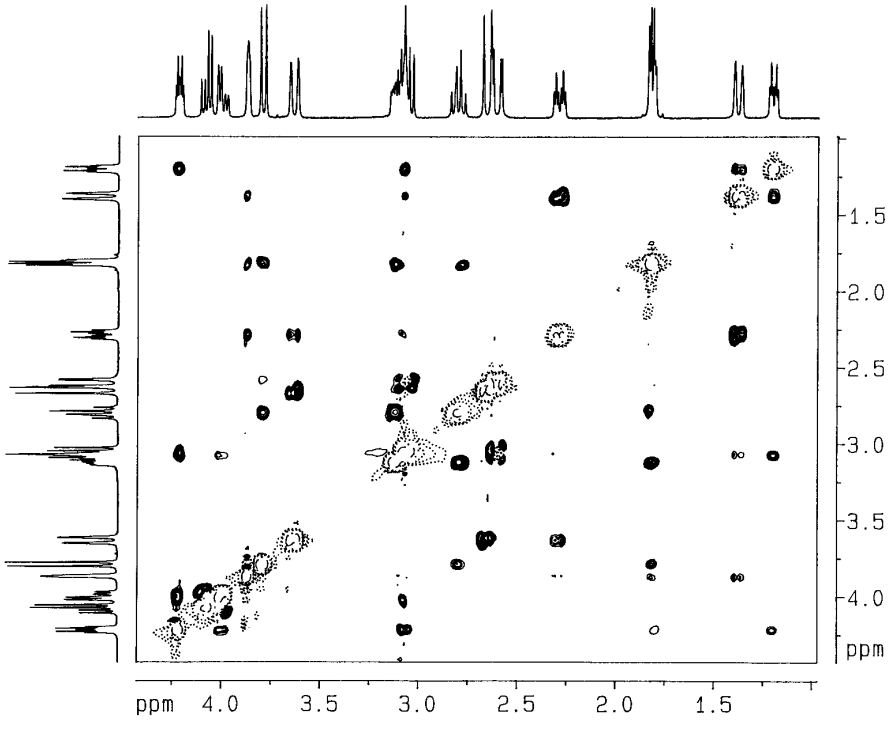
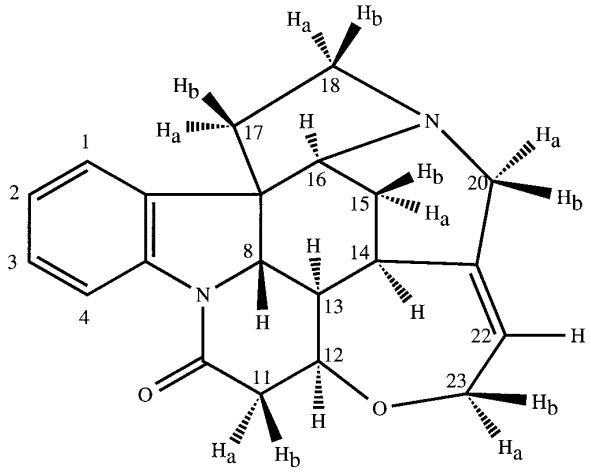


FIG. 5. Aliphatic part of the phase-sensitive 2D NOESY spectrum of **1** acquired with the scheme of Fig. 3c. The mixing time was 500 ms and the strengths of the three sine-shaped PFG were about 10, 7, and 10 G/cm for one data set and −10, 7, and 10 for the other data set. A single scan was recorded for each of the 128 t_1 points, giving a total acquisition time of 6 min 50 s.

can be obtained in a few minutes, as shown for the 2D ROESY spectrum (Fig. 2) of a 0.2 *M* solution of strychnine



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in $CDCl_3$. Although this approach produces a sensitivity loss by a factor of two in comparison with the analogous phase-cycled experiment, it can be very useful when the desired signal can be detected in a single scan.

Improved approaches to frequency-discriminated phase-sensitive spectra use the echo-antiecho methodology, in which both P-type and N-type data are recorded and stored in separate blocks for each t_1 value. This is achieved by inverting the gradient strength of one specific PFG and requires proper combination during data processing to yield an absorption-mode spectrum (*12–14*). Thus, amplitude-modulated cosine and sine data are obtained by combining the phase-modulated P-type and N-type data and further processed as complex data by using the States method of frequency discrimination (*15*). This data-processing procedure, available as a black box in the conventional software packages supplied by the manufacturers, results in the construction of *x* and *y* components by means of sums and differences of the original echo and antiecho data. Figure 3 shows the basic schemes for gradient-enhanced phase-sensitive 2D TOCSY, ROESY, and NOESY experiments with the P/N approach.

Figure 4 shows as an example the phase-sensitive 2D TOCSY spectrum of **1** obtained in only five minutes. Although distinguishing between positive and negative cross peaks is not critical for 2D TOCSY spectra of medium-sized molecules, the resolution of the phase-sensitive spectrum is much higher than that of the corresponding magnitude-mode spectrum, obtained as described in Ref. (*5*), and therefore *J*-coupled spin subsystems can be more easily studied in the phase-sensitive case.

Phase-sensitive data are, however, mandatory in 2D NOESY (and 2D ROESY) experiments because negative NOE (and ROE) cross peaks must be distinguished from undesired positive cross peaks arising from chemical exchange or *J* contributions. For instance, all negative NOE cross peaks are clearly observed in the NOESY spectrum of **1** (Fig. 5) acquired in less than 7 minutes using the pulse sequence of Fig. 3c. Similar results can be obtained from the ROESY spectrum acquired with the pulse sequence of Fig. 3b.

In summary, several of the most common and useful 2D homonuclear correlation spectra, such as TOCSY, ROESY, and NOESY, can be obtained in just a few minutes and in pure absorption lineshapes, provided the amount of sample is not limiting. These PFG methods should, therefore, be suitable for routine NMR protocols. Although a theoretical signal-to-noise loss is unavoidable, in comparison with techniques employing phase-cycling selection (TPPI or hypercomplex methods) and recorded in the same amount of measuring time, these PFG methodologies should be clearly preferred (except in cases of very low sample concentration) because the desired signal is obtained with a single scan per t_1 value.

ACKNOWLEDGMENTS

Financial support for this research was provided by DGICYT (Project PB95-0636) and is gratefully acknowledged. We also thank the Servei de Ressonància Magnètica Nuclear, UAB, for allocating instrument time to this project.

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