

Elimination of Refocusing Pulses in NMR Experiments

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Received June 13, 1984

A large number of one- and two-dimensional NMR experiments employ 180° refocusing pulses with the main purpose of obtaining absorption-mode spectra. Imperfections in the flip angle of the refocusing pulse will generally lead to degradation of the sensitivity of the experiment and may give rise to unwanted resonances in 2D spectra. Those problems can sometimes be significantly alleviated by proper use of the composite pulse concept (1-4). However, we will demonstrate that those problems can be completely avoided by omitting the refocusing pulses altogether.

As a first example, consider the refocused INEPT experiment (5-8). Its sequence is sketched in Fig. 1a. A pair of 180° pulses applied simultaneously to the ^1H and ^{13}C nuclei at the midpoint of the Δ_2 period removes the effect of chemical shift during this period and average evolution during this period is only due to the heteronuclear scalar coupling, J . If, for a methine group, the delay Δ_2 is set to $1/2J$, the two ^{13}C doublet components will be in phase at the end of this period and will be aligned along the x axis (7, 8). If broadband proton decoupling is started at this time, an absorption mode proton-decoupled ^{13}C spectrum can be recorded. We propose to omit this pair of refocusing pulses in the INEPT experiment and to start data acquisition immediately after the 90° ^{13}C detection pulse (Fig. 1b). Broadband proton decoupling is switched on a time, Δ_2 , later. This means that during the first 2-3 ms (for ^{13}C) of the data acquisition the signal is in the proton-coupled mode, whereas during the rest of the free induction decay the signal is in the proton-decoupled mode. The envelope amplitude of this signal shows a strong similarity with the time domain signal to which a convolution difference filter (9) has been applied, and consequently, a dip in the baseline will occur after Fourier transformation. For the normal case, where the decay constant T_2^* of the ^1H -decoupled ^{13}C signal is much larger than the reciprocal of the coupling constant, J^{-1} , the depth, ϵ , in the baseline is calculated to be

$$\epsilon = (1 - 2/\pi)/2J \quad [1]$$

whereas the intensity I of the resonance involved equals T_2^* . For a typical case with a nominal line width of 1 Hz ($T_2^* = 1/\pi$) and a 150 Hz coupling constant, the dip in the baseline is only 0.4% of the peak height and does not cause any serious

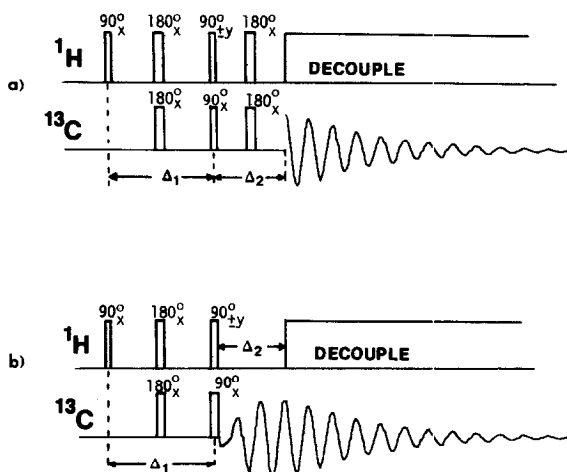


FIG. 1. (a) Pulse scheme of the refocused INEPT experiment. (b) Scheme of the modified experiment where data acquisition is started immediately after the 90° ^{13}C pulse and proton decoupling is switched on a time Δ_2 thereafter.

problems. However, one should note that the total integrated intensity of the spectrum, according to the Fourier theory, has to be zero.

As an example, results for the aldotrionic acid derivative sketched at the top of Fig. 2 are presented. Figure 2a shows the regular NOE-enhanced FID spectrum of the protonated ^{13}C nuclei, obtained from 200 scans. Figure 2b shows the INEPT spectrum obtained with omission of the 180° (^1H , ^{13}C) pulse pair and Fig. 2c shows the result of the optimized refocused INEPT experiment. The signal intensity is slightly ($\sim 10\%$) higher without the 180° pulses, but a baseline distortion is visible. This distortion is much larger than calculated on the basis of Eq. [1], because each of the ^{13}C resonances causes a dip in the baseline with a width of several hundred hertz. Clearly, those dips overlap and reinforce each other.

Another experiment where refocusing pulses can be omitted is the APT sequence (10). This experiment is widely used for multiplicity determination of ^{13}C sites and its sequence is sketched in Fig. 3a. Figure 3b shows our modified sequence, in which the 180° ^{13}C refocusing pulse has been omitted and data acquisition is started immediately after the 90° ^{13}C pulse. The functional dependence of the observed resonance intensity on the length of time delay Δ is unchanged with the original version of the experiment. However, as the duration of Δ in this type of experiment is typically much longer than in the INEPT sequence, the baseline distortion will be worse, but will usually not obscure any of the information desired. Because only one ^{13}C pulse per experiment is applied in our modification, a reduced flip angle can be used for this pulse, allowing a higher experiment repetition rate and consequently higher sensitivity (11).

Figure 4a shows the conventional NOE-enhanced high-field ^{13}C spectrum of the sugar sketched in the inset of Fig. 2. Figure 4b shows the result obtained with the nonrefocused APT experiment for a Δ value of 6.5 ms. Baseline distortion is clearly visible, but the methylene (up) and methine (down) sites can easily be distinguished.

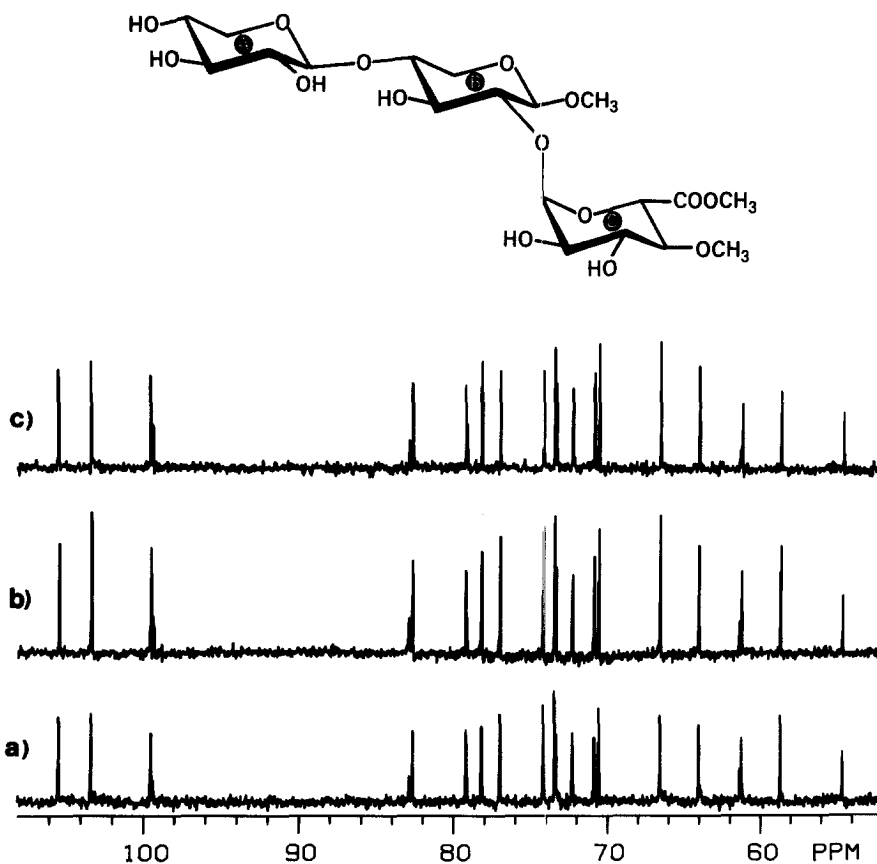


FIG. 2. Regular NOE-enhanced FID spectrum of the aldotronic acid derivative shown at the top. (b) INEPT spectrum obtained with the sequence of Fig. 1b. (c) INEPT spectrum obtained with the regular refocused INEPT experiment (Fig. 1a). Each spectrum is the result of 200 scans. Ring C is partly (30%) demethylated at the 6 position.

One might argue that starting data acquisition directly after the observe pulse and switching on the proton decoupling a time, Δ , later produces results very similar to starting data acquisition at time Δ and right shifting the data by a number of data points that would have been acquired during this time (which is identical to applying a strong linearly frequency-dependent phase correction to the final spectrum). However, this approach gives rise to a rather strong undesirable baseline roll (8).

The ideas of right shifting the data points and omission of refocusing pulses can be combined in order to obtain a two-dimensional absorption-mode heteronuclear chemical-shift correlation spectrum. The theoretical basis for the 2D heteronuclear chemical-shift correlation experiment has been discussed in detail in a number of papers (12-17) and will not be repeated here. The regular absorption-mode pulse scheme employs two 180° (^1H , ^{13}C) pulse pairs, applied at the midpoints of the Δ_1

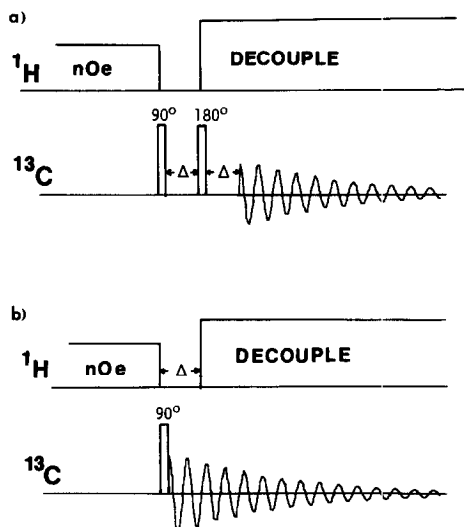


FIG. 3. (a) Pulse sequence of the regular APT sequence. (b) Pulse sequence of the modified APT sequence, where data acquisition is started immediately after the ^{13}C observe pulse. To optimize sensitivity the flip angle of the ^{13}C pulse may be chosen smaller than 90° .

and Δ_2 intervals (Fig. 5a). The pulse pair in the center of Δ_1 , can be omitted if the data points are right-shifted by $\Delta_1/\Delta t_1$ data points, prior to Fourier transformation with respect to t_1 . As discussed above for the INEPT experiment, the pulse pair in the center of the Δ_2 interval can be omitted if data acquisition is started immediately after the ^{13}C detection pulse (Fig. 5b). The experiment has to be performed in the

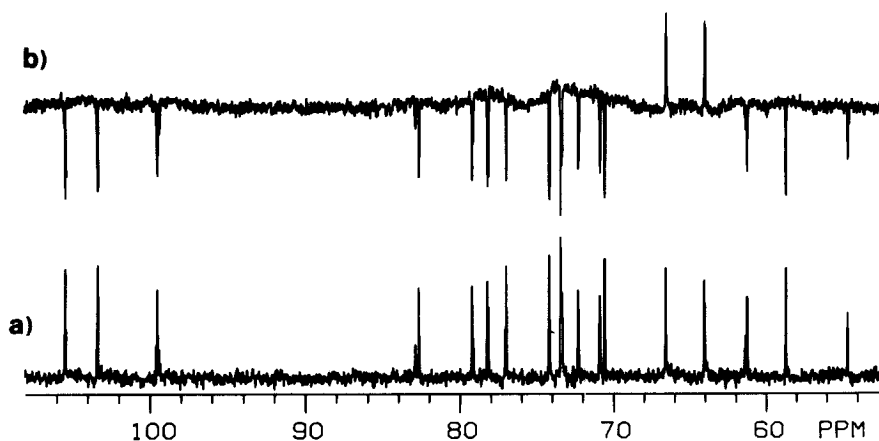


FIG. 4. (a) Regular NOE-enhanced FID spectrum of the aldotronic acid derivative sketched in the inset of Fig. 2. (b) Modified APT spectrum obtained with the pulse sequence of Fig. 3b. Each spectrum is the result of 200 accumulations.

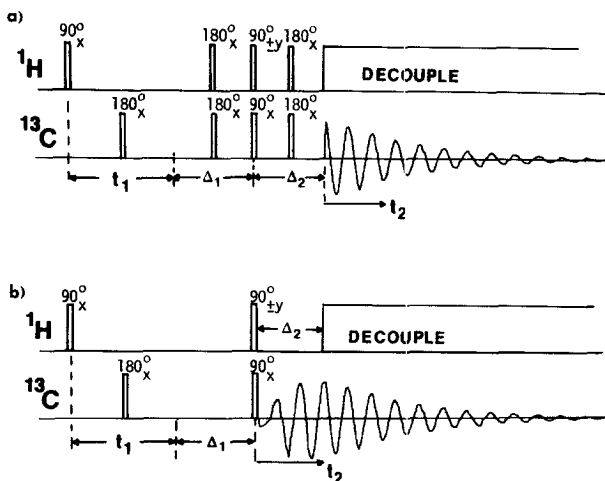


FIG. 5. (a) Pulse scheme of the regular absorption-mode heteronuclear chemical-shift correlation experiment. (b) Sequence of the modified heteronuclear chemical-shift correlation experiment. The phase of the second 90° ^1H pulse is alternated along the $\pm y$ axis in consecutive scans and data are correspondingly added and subtracted to memory. For further experimental details, see text.

amplitude-modulated mode, with the decoupler placed at either the low or the high field side of the ^1H spectrum. The decoupler can be positioned in the center of the proton spectrum if additional (TPPI) phase shifting of the decoupler pulses is used (18–20). For the simplest case where the decoupler frequency is placed at one side of the ^1H spectrum, more decoupler power is needed to decouple effectively the ^1H spectrum. However, with the introduction of the new composite pulse decoupling techniques (21), this usually does not present any practical problems. More data storage space by a factor of two is needed in the amplitude-modulated version compared with the phase-modulated experiment (16) in order to obtain the same digital resolution, but it can be shown (22) that the amplitude-modulated version yields an improvement in sensitivity by $\sqrt{2}$ over the phase-modulated experiment.

Figure 6 shows the 2D absorption-mode heteronuclear chemical-shift correlation spectrum, obtained from a 128×1024 data matrix on a Nicolet NT-270 spectrometer. Gaussian line broadening is used in both dimensions to avoid truncation and zero filling by a factor of two is used in both dimensions, resulting in a 128×1024 data matrix for the displayed absorption part of the spectrum. Along the sides of the 2D spectrum the projections onto the F_2 and F_1 axes are shown, displaying the absorption-mode lineshapes. The resolution in the F_1 dimension is limited by the digital resolution (compare with conventional ^1H spectrum recorded on the same sample and shown above the F_1 projection) and could be improved by using a longer acquisition time in the t_1 dimension.

The idea of omitting refocusing pulses and starting proton decoupling and data acquisition at different times is not limited to the examples presented above, but applies to a large number of heteronuclear 1D and 2D pulse schemes. Sequences with fewer pulses tend to be less sensitive to practical instrumental problems and

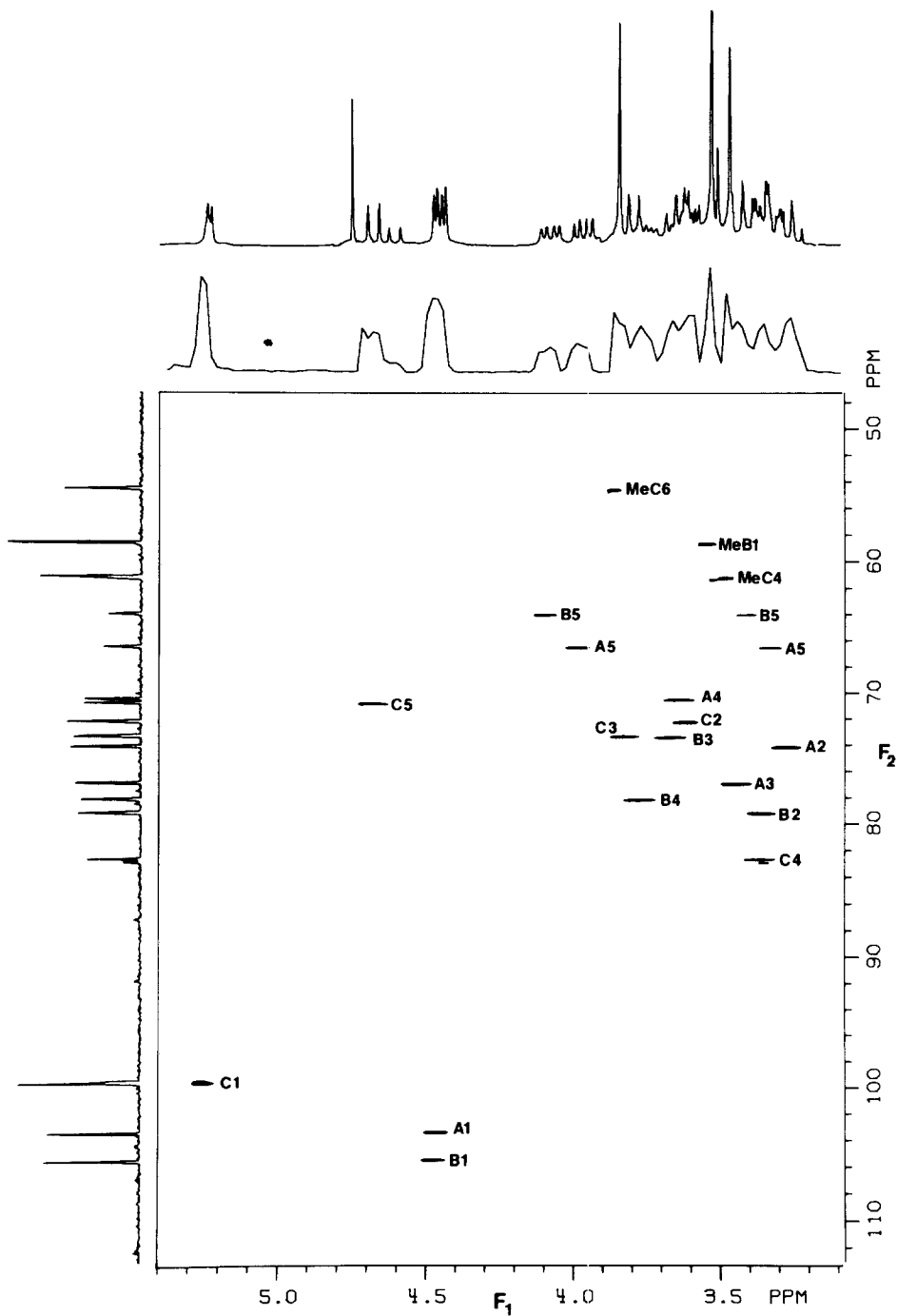


FIG. 6. Two-dimensional absorption-mode heteronuclear chemical-shift correlation spectrum of the sugar sketched in the inset of Fig. 2, obtained with the sequence of Fig. 5b. The spectrum results from a 128×1024 data matrix and 80 accumulations were performed for each t_1 value (total measuring time 4 h). Along both axes the projections of the 2D spectrum on these axes are shown. Along the F_1 axes the conventional ^1H spectrum (high resolution) is also displayed.

will generally be less susceptible to possible misadjustments of experimental parameters and may therefore produce more reliable results.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Geoffrey Bodenhausen and Dr. Malcolm Levitt for sending preprints of Refs. (20) and (22), and thank Mr. Rolf Tschudin for continuous technical support.

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JOURNAL OF MAGNETIC RESONANCE

Published monthly (semi-monthly in February, June, and October) by Academic Press, Inc., 111 Fifth Avenue, New York, NY 10003. Number of issues published annually: 13. Editor: Dr. Wallace Brey, Department of Chemistry, University of Florida, Gainesville, FL 32611.

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