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EASY IMPLEMENTATION OF HOMONUCLEAR 1D CORRELATION NMR TECHNIQUES. APPLICATION TO OLIGOSACCHARIDES.

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ABSTRACT: A combination of selective excitation by DANTE pulse train and chemical shift selective filtering (CSSF) allows one to record clean 1D COSY, RELAY and NOE spectra.

After the seminal papers of Bauer et al.[1] and Kessler et al.[2] on the transformation of homonuclear two-dimensional NMR techniques into their 1D analogues, conventional decoupling experiments became obsolete.

In most of these straightforward techniques the selective frequency labelling period is followed by a nonselective mixing period. The crucial aspect of such experiments is that the first selective pulse (or pulse cluster) should be phase coherent with the forthcoming pulse sequence events.

In this article we show how to easily meet these conditions with old fashioned spectrometers and compare the results to some recent methods.

The new method presented here is a simple combination of the DANTE selective excitation method[3] and chemical shift selective filters[4]. The rationale behind this approach is that the DANTE pulse train uses the same high power transmitter with some attenuation as for other pulse steps. Hence, phase coherence is automatically accomplished. On the other hand, unsuitable frequency characteristics of DANTE (side bands etc.) can be compensated for by chemical shift selective filtering (CSSF). CSSF is a special "filter" using constructive interference of selected signals by combination of free induction decays.

^{1}H-NMR assignment problems of oligosaccharides can be nicely solved by recent 2D[5] or even 3D[6] methods such as COSY[7], delayed COSY[8], RELAY[9], TOCSY[10], NOESY[11] (ROESY)[12] and their combinations. However, these techniques are rather time consuming, and poor digitization of signals in the 2nd or 3rd dimension may lead to loss of information, e.g., one can't determine important coupling constants with reasonable accuracy, which can lead to false stereochemical conclusions.

One-dimensional analogues of the aforementioned techniques have proven to be very economical for studying oligosaccharide structure[13]. Very loosely said, 1D correlation techniques are worth-while to try for solving problems of medium complexity. Each interrupted spin network should contain at least one separate multiplet, a condition which can often be met for oligosaccharides. Overlapping (but not coinciding) multiplets can also often be selectively excited. Digital resolution is no more restricted, e.g. ± 0.1 Hz accuracy is available as for routine spectra. Spectrometer time is substantially reduced even at higher magnetic fields. Disc storage capacity will not be exhausted.

In the following we demonstrate the concerted use of simple 1D COSY, multiple-RELAY, and NOESY. The problem is of medium complexity at 200 MHz, as the ^{1}H-NMR spectrum (Fig. 1) of the simple disaccharide hepta-O-acetyl-α-cellobiosyl azide[14] (1) contains both separated and overlapping multiplets, as well as some strong couplings.

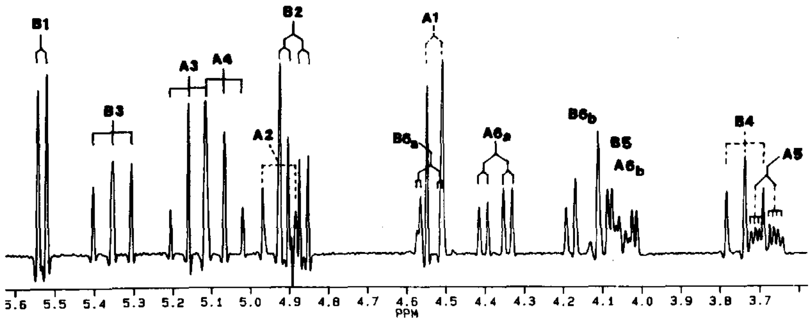
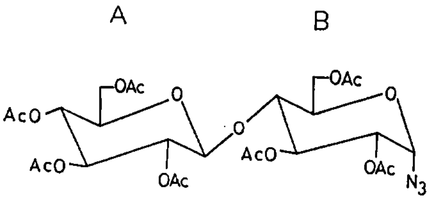


Figure 1. 200 MHz ^{1}H-NMR survey spectrum of the disaccharide (1). Assignments are given with a A (1 → 4) B shortened notation.



(1)

In Fig. 2, a simple COSY-60 experiment with optimized phase cycle[15] is shown in magnitude representation. An overnight experiment was run using 256 incremented delays. Transformation in a 1 MW data table afforded ca. 1 Hz digital resolution, which was sufficient to assign most of the cross-peaks. It is worthy of note that the unresolved $^4J_{B1,B3}$ coupling also yielded a cross-peak.

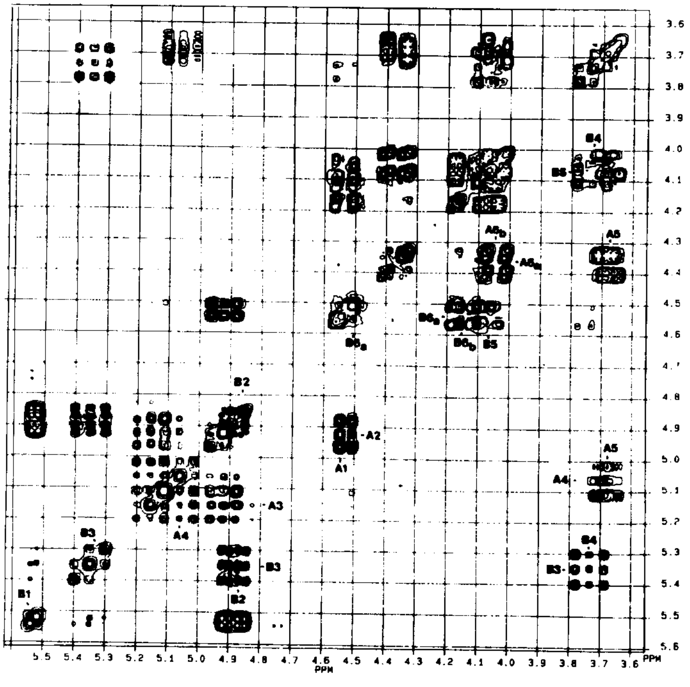


Figure 2. A COSY-60 magnitude spectrum of (1).

As a demonstration of the combined DANTE + CSSF technique and for corroboration of the assignments we ran some 1D experiments. The pulse sequence of the 1D COSY is shown in Fig. 3. A selective DANTE pulse train consists of a series of small flip angle θ_x pulses interrupted by long intervals, τ , to decrease the average power of the transmitter. The overall flip angle $\Psi = n\theta_x$ is determined by the number of pulses n. $\Psi = 90 + l \cdot 360$ ($l = 0,1,2, \ldots$) and may have different values for selective 90 degree excitation. A typical value for n is n = 100. This means that the duration of the θ_x pulse is $\tau_\theta = \tau 90/n = \tau 90/100$, assuming a calibrated hard pulse of τ_{90}. Usually, a 10-20 dB fixed attenuator should be used at the transmitter output yielding sufficient time resolution for τ_θ (typically 1-2 μs), but supporting non-selective excitation of the spectral region of interest.

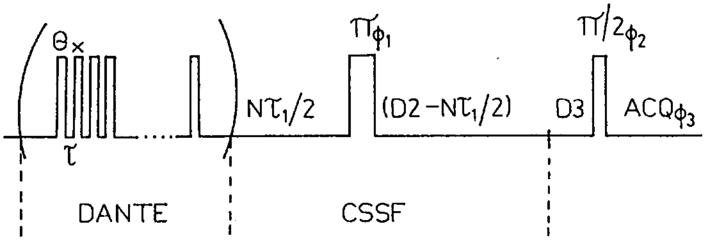


Figure 3. 1D-COSY sequence using DANTE + CSSF. Phases are cycled as follows: Φ_1 = 00002222 Φ_2 = 0123 Φ_3 = 0

The selectivity of the DANTE train is determined by its overall duration, e.g. $\Delta\nu_{DANTE} = 1/n(\tau + \tau_\theta)$. τ is typically three orders of magnitude longer than the short pulses (τ = 0.5-2 ms), hence $\Delta\nu$ = 5-20 Hz, which may be sufficient for multiplet selective perturbation. Unsuitable modulation sidebands of DANTE appear at ca. $\nu_k = \pm k/\tau$ ($k = 1,2, \ldots$) frequencies away from transmitter offset.

The chemical shift selective filter works like a constant evolution time 2D experiment, where a wandering inversion pulse causes chemical shift evolution only. For the incremented delays, however, FID-s are simply coadded, instead of using a new dimension. The number of incremented delays, N, with the duration τ_1 of one increment determines the transmission bandwidth of the filter at the frequency of irradiated spin

$$\Delta\nu_{CSSF} = 1/(N + 1)(\tau_1).$$

It should be noted that "modulation sidebands" are also disturbing at ν_t frequencies away from the selected frequency,

$$\nu_t = \pm k/\tau_1 \quad (k = 1,2,3, \ldots),$$

with the same bandwidth. Coincidence of DANTE sidebands and bypass signals of CSSF can be avoided - if necessary - by setting the incremented delay τ_1 as follows:

$$1/\tau \neq 1/\tau_1.$$

The incremented delay period may include a fixed interval D3 to give a (D2 + D3) interval. For an AX spin system with J_{AX} coupling, a DANTE/2 + D2 + D3 $\approx 1/2J_{AX}$ (2p + 1) (p = 0,1,2, ...) setting is optimal for maximal transfer of magnetization, where D2 = $N\tau_1/2$, DANTE = $= n\,(\tau + \tau_\theta)$.

For complicated spin networks it is recommended that one uses a shorter evolution period in order to avoid interference with passive spins.

In practice it is useful to set the transmitter offset frequency to the same value of the selected spin for both the DANTE and the CSSF sequence. Then the frequency may be switched to the center of the spectrum for the non-selective mixing period to accomplish quadrature detection.

Figure 4 shows a 1D COSY spectrum obtained by selective DANTE excitation of B_1 (top) and B_3 (bottom) protons. Pure phase multiplets are ob-

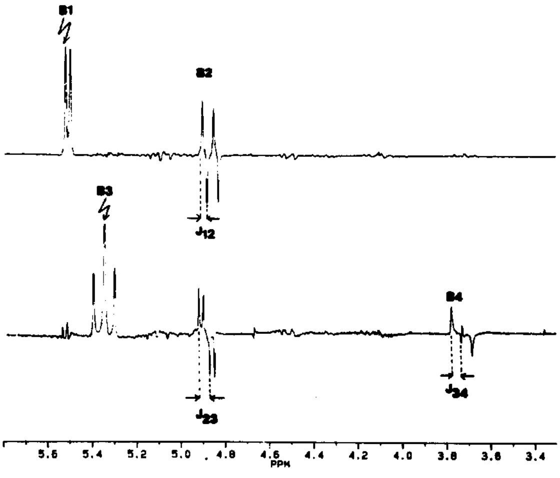


Figure 4. 1D-COSY spectrum of (1) when B1 (top) or B3 (bottom) protons are irradiated. The experimental setup is discussed in the text.

tained with splitting antiphase with respect to the active coupling. The central line of B_4 is reduced considerably due to antiphase signals. In this particular case the DANTE train contained 100 x 1 μs pulses for selective 90 degree excitation (21 dB attenuation of 50 W pulse power and τ = 1.3 ms was set). The number of incremented delay periods N of CSSF N = 16 with τ_1 = 2.598 ms yielded D2 = 20.78 ms and D3 = 15 ms was also inserted.

In practice, the finite length of the DANTE train should be taken into account, so that its half duration DANTE/2 = (100 x 1.3)/2 ms = 65 ms contributes to the effective evolution period.

The effective evolution period τ_{eff} = DANTE/2 + D2 + D3 = 100.78 ms may be close to optimal for ca. 5 Hz couplings, which could be reduced to τ_{eff} = 48 ms (using τ = 0.6 ms, τ_1 = 2 ms, and D3 = 2 ms) for couplings of ca. 10 Hz. The total number of transients was 16 x 8 = 128 in both cases. In Fig. 5 a selective 1D-COSY experiment is also shown, however with only 16 transients. This experiment with selective DANTE-Z pulse[16] offers higher selectivity, so the CSSF may be discarded. In this sequence a selective DANTE-Z inversion pulse is followed by a non-selective 90 degree pulse instead of a selective 90 degree pulse. The DANTE-Z train contained 200 x 1 μs pulses interrupted by 1 ms delays, and the phase of every fourth pin pulse was inverted. The 1/2 J delay period was 50 ms. Comparison of Figures 4 and 5 shows better selectivity for the combined technique, but a higher sensitivity for the simpler DANTE-Z COSY.

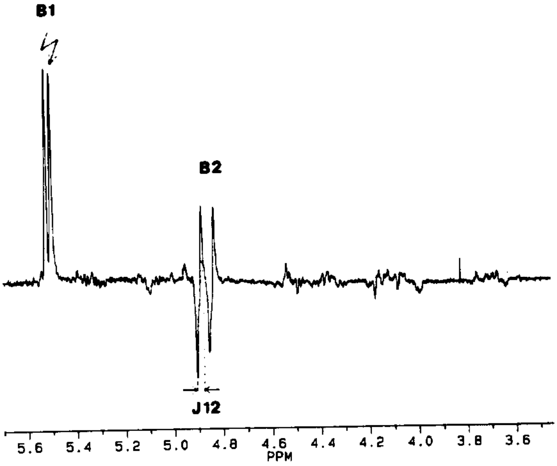


Figure 5. 1D-COSY using DANTE-Z type excitation without CSSF.

Nowadays, some promising 1D correlation techniques (DICE = direct correlation and ICE = indirect correlation/RELAY) have appeared[17] which seem to be more sensitive than the former methods, and may use an incoherent decoupler instead of the DANTE excitation. Contrary to regular 1D COSY experiments, the mixing pulse is now semiselective, and difference experiments are created using a hard pulse reference COSY experiment. The lower trace in Figure 6 shows the DICE type direct correlation (16 transients), while the upper trace presents a one step relay (ICE) in 32 scans. Both spectra are represented in magnitude mode, or large phase distortions appear.

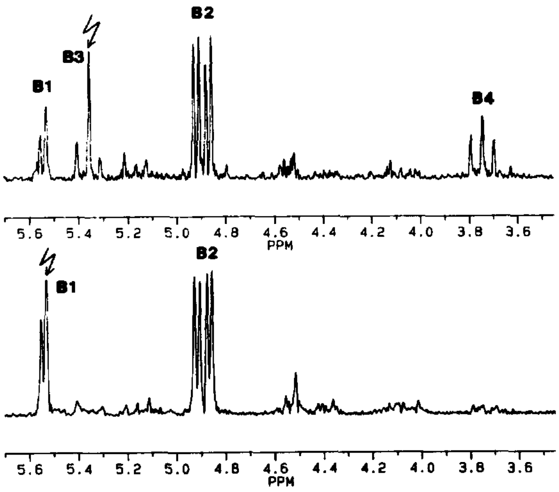


Figure 6. Direct (DICE, bottom) and indirect (ICE, top) correlations in magnitude representation.

A DANTE + CSSF combination can also be successfully used for relayed 1D correlation experiments (Figure 7.). The appearance of single, double and triple relay peaks in phase sensitive representation immediately gives the assignment of the "B" glucopyranosyl ring. Experimental parameters are similar to those of Fig. 4, except that the D2' relay period was set to shorter than the average 1/2 J period to avoid loss due to relaxation (D2' = 50 ms).

For completeness, a 1D NOESY spectrum of compound (1) demonstrates the efficiency of the DANTE + CSSF method (Fig. 9) using the sequence of Fig. 10. (Note the very clean spectrum.) As has been noted,[2] this way of NOE measurement has distinct advantages over the conventional steady-

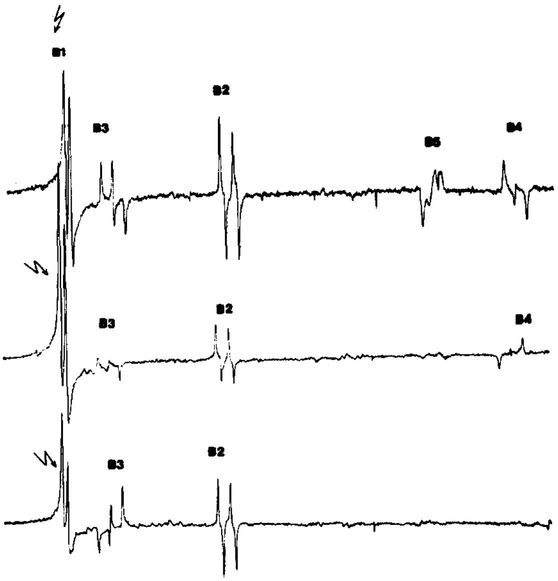


Figure 7. Single (bottom), double and triple (top) RELAY experiments using DANTE + CSSF technique (pulse sequence as shown in Fig. 8).

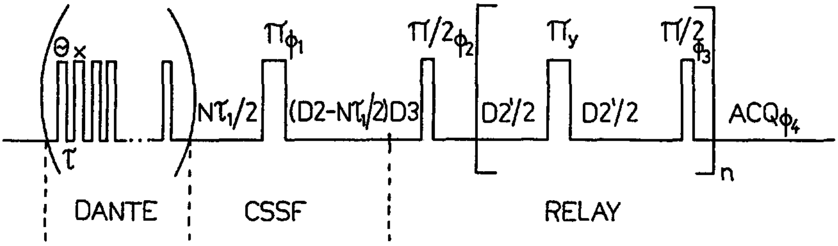


Figure 8. 1D-multiple relay pulse sequences. Phases are cycled as:

ϕ_1 = 00002222 ϕ_2 = 0123 ϕ_3 = 0000111122223333 ϕ_4 = 02

-state[18], transient NOE[19] or truncated driven (TOE)[20] methods, as in those cases selective saturation or inversion of a ^1H multiplet is mandatory, but experimental setup strongly influences the results. In this 1D NOESY measurement the in-phase magnetization of the selected spin was allowed to build up by setting an appropriate D3' delay so that DANTE/2 + D2 +

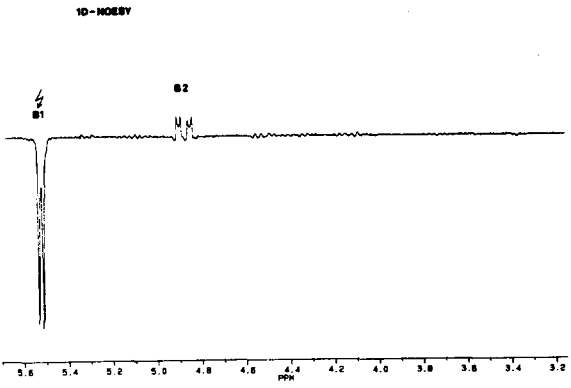


Figure 9. 1D NOESY experiment using pulse sequence of Fig. 10. B1 proton is excited by DANTE pulse train.

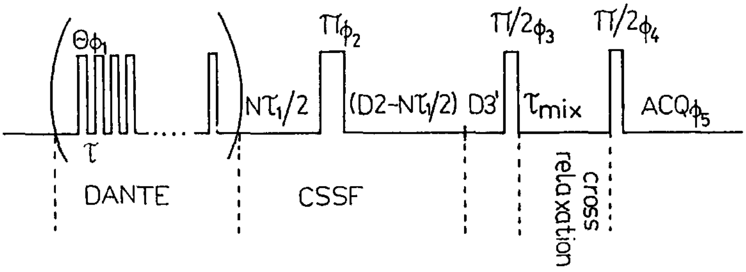


Figure 10. 1D NOESY pulse sequence using DANTE + CSSF combination. Phases are cycled as ϕ_1 = 0000222222220000 ϕ_2 = 11113333 ϕ_3 = 0000000022222222 ϕ_4 = 0123 ϕ_5 = 01232301

+ D3' = k/J (k = 1,2, ...). In 1D NOESY τ_{eff} and τ_{mix} intervals are well separated, hence the initial build-up rate of the NOE-s can be followed at very short mixing times, which is essential for determining cross-relaxation rates and internuclear distances. (For Fig. 4, τ_{eff} = 200 msec and τ_{mix} = 0.1 sec have been applied.)

Conclusion

It has been demonstrated that a great variety of 1D analogues of 2D correlation techniques can be easily implemented on existing, old fashioned spectrometers. This approach may save measuring time and yield new (or more accurate) NMR data.

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