

Note

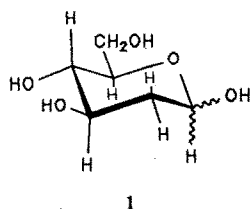
Application of gradient-selective COSY and double-quantum filtered gradient-selective COSY experiments to carbohydrates: 2-deoxy-D-arabino-hexose (“2-deoxy-D-glucose”)

T. Adrian Carpenter, Lawrence D. Colebrook, Laurance D. Hall and Gregory K. Pierens

Herchel Smith Laboratory for Medicinal Chemistry, University of Cambridge School of Clinical Medicine, University Forvie Site, Robinson Way, Cambridge, CB2 2PZ (United Kingdom)

(Received March 4th 1992; accepted in final form August 17th, 1992)

The purpose of this Note is to draw attention to an operationally simple means whereby the speed of acquisition of certain two-dimensional (2D) nuclear magnetic resonance (NMR) experiments can be enhanced by at least ten-fold, effectively reducing the measurement time from several hours to less than ten minutes. This remarkable approach is simple and robust and should revolutionize the ease with which 2D, ^1H – ^1H correlation measurements (COSY) can be used by practising carbohydrate chemists. The spectra of 2-deoxy-D-arabino-hexose (**1**) shown in Figs. 2 and 3 demonstrate the quality of the data obtainable by this method and, although a detailed quantum mechanical description is not appropriate here, a brief explanation can be given.



The 2D COSY experiment invented by Jeener¹ involves the use of two 90° radiofrequency pulses separated by an incremented delay period, t_1 (Fig. 1A). Acquisition of a data set from a single set of these pulses produces a complex,

Correspondence to: Dr. L.D. Colebrook (permanent address): Department of Chemistry and Biochemistry, Concordia University, 1455 de Maisonneuve Blvd W., Montreal, Quebec, Canada H3G 1M8.

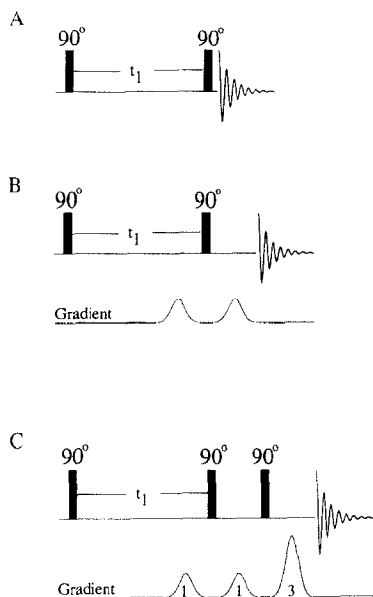


Fig. 1. (A) Pulse sequence for a normal (phase-cycled) COSY, (B) pulse sequence for gradient-selective COSY, (C) pulse sequence for double-quantum filtered gradient-selective COSY. The third gradient has three times the integrated intensity of the preceding gradients. The gradients, which are applied along the z -axis, have the same phase for n -type coherence selection.

overlapping array of peaks consisting of two diagonals and associated off-diagonal peaks, and a set of peaks corresponding to the normal spectrum at zero frequency in the second dimension.

In practice, it is possible to eliminate most of the unwanted responses by use of phase cycling², thereby reducing the COSY spectrum to the familiar display of peaks on a single diagonal, together with the off-diagonal peaks that produce the correlation between the sets of protons which share a scalar coupling. Experimentally, phase cycling involves systematically varying the transmitter and receiver phases during successive acquisitions so that the wanted signals accumulate and the unwanted signals average to zero. A major disadvantage of the phase cycling procedure is the time taken for data collection; sixteen acquisitions per increment in t_1 are required for a fully phase-cycled COSY experiment.

The number of phase cycles may be reduced to as few as four if a reduction in spectral quality can be tolerated. Insufficient phase cycling causes degradation of the spectrum by artifacts resulting from instrumental imbalances, which add during data acquisition. These are the same artifacts which are removed by phase cycling in 1D experiments.

Fortunately, the same effect can be achieved in a single acquisition^{3,4} per increment by applying pulsed magnetic field gradients, as shown in the pulse sequence (Fig. 1B). The gradient pair shown selects only the wanted single diagonal and its associated cross-peaks, so that phase cycling is unnecessary and

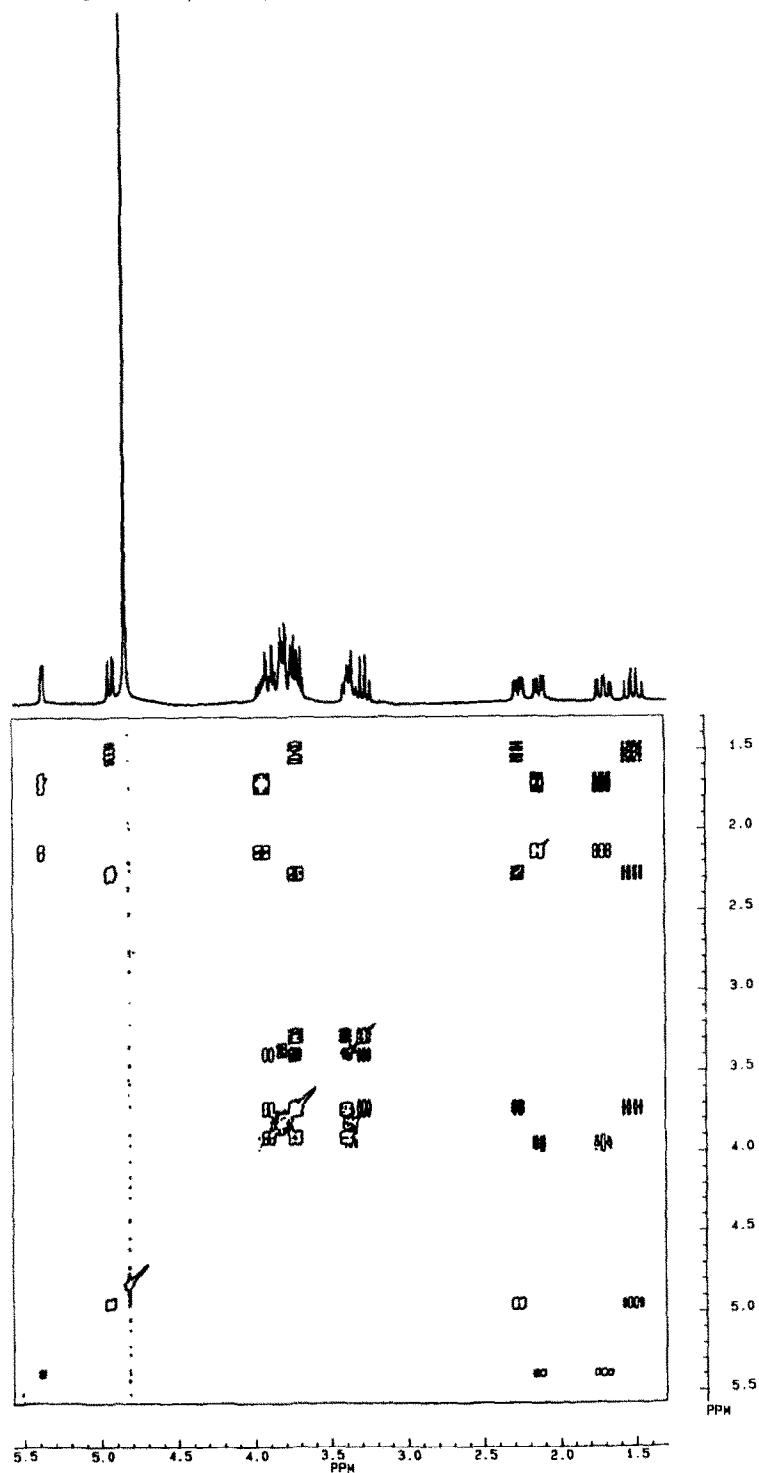


Fig. 2. Gradient-selective COSY 2D (1K×1K data block, 1.8 Hz/point digital resolution, and normal 1D 300-MHz spectra of 2-deoxy-D-arabino-hexose, 0.1 M in D₂O solution). The acquisition time of the 2D data set was 8.5 min.

the time required for the experiment is dramatically reduced. Levels of t_1 noise, which arises from instrumental instabilities and accumulates during phase cycling, are considerably reduced.

Only a minor increase in instrument time is needed to acquire a high digital resolution COSY data set, which will show more detail in the off-diagonal peaks, and a much narrower and more detailed diagonal. Use of a large data set leads to excellent definition of the components of multiplets within the cross-peaks, minimising the effects of cancellation of out-of-phase components, and reducing the

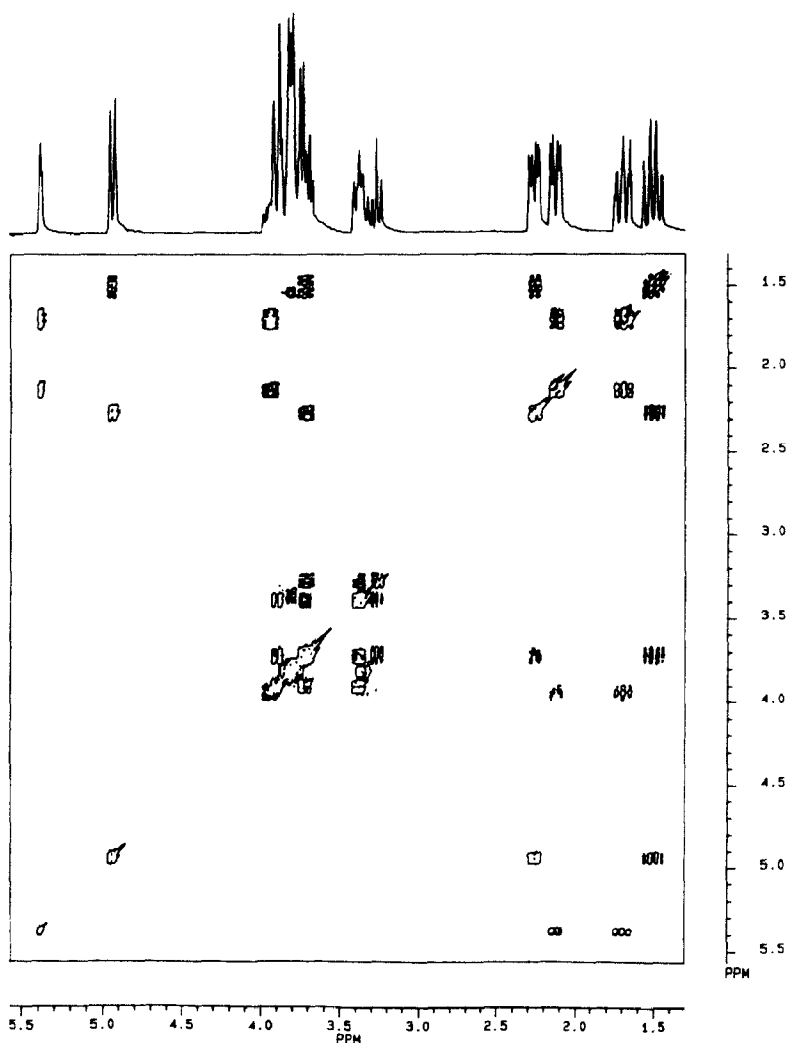


Fig. 3. Double-quantum filtered gradient-selective COSY 2D 300-MHz spectrum of 2-deoxy-D-arabino-hexose, taken under the same conditions as those of Fig. 2 except for the pulse program, with a projection of the 2D data set on the F_2 axis.

need for phase-sensitive spectra. Phase-sensitive gradient-selective experiments have been described⁴, but are unlikely to be necessary for most COSY spectra.

The effectiveness of this approach is illustrated in Fig. 2; a COSY spectrum is produced which shows only the single diagonal, with fewer artifacts than commonly appear in the corresponding phase-cycled spectrum. Despite the high intensity of the HDO signal, visible in the 1D spectrum, there is little t_1 noise on the 2D plot.

The sample of 2-deoxy-D-*arabino*-hexose contains almost equal concentrations of the α and β anomers⁵. Starting with the signals of the anomeric protons, which may be distinguished by the magnitudes of their coupling constants, it is possible to trace many of the connectivities and identify the chemical shifts of the protons involved. For the α isomer, the cross-peaks from the anomeric proton signal at δ 5.38 identify the H-2 $_{eq}$ and H-2 $_{ax}$ signals at δ 1.71 and 2.13, respectively. The cross-peaks from those signals identify the chemical shift of H-3 at δ 3.91. Connectivities from the anomeric proton of the β isomer (δ 4.94) via the H-2 protons to H-3 may be similarly established. Cross-peaks connecting chemical shifts within the multiplets centered on 3.3 and 3.8 ppm are clearly visible, and connectivities may be established, but unambiguous assignment of the chemical shifts in these regions is hampered by severe signal overlap.

It is also appropriate to draw attention to a further variant, double-quantum filtered COSY (DQCOSY)⁶. This experiment filters signals from protons with no spin coupling, so it is a useful method for eliminating or reducing the intensities of singlets. For example, this approach may be used to reduce or eliminate HDO signals from the COSY spectra of carbohydrates in D₂O solution. It may also reduce the t_1 noise associated with strong singlets. The pulsed gradient version of DQCOSY (Fig. 1C) requires only a single acquisition per increment in t_1 and uses no phase cycling, taking the same time as the gradient-selective GCOSY; however, sensitivity is reduced compared to a normal COSY. Its use is illustrated in Fig. 3, which shows complete absence of t_1 noise. The excellent suppression of the strong HDO signal at δ 4.8 is most easily seen in the projection on the F_2 axis.

CONCLUSION

In a range of studies, we have found it possible to acquire technically perfect high-resolution 2D spectra of a carbohydrate in less than 10 min of acquisition time, and practically useful spectra in still less time. We suggest that this remarkable saving of time, along with the artifact reduction achievable by use of gradients, is revolutionary in its potential. Through the use of the gradient selective method, total acquisition time in the design of a COSY-type measurement is unlikely to be a serious consideration, so digital resolution may be tailored to the requirements of the experiment².

This approach will increase the ease with which COSY spectra can be incorporated into routine structural studies and it will allow the routine use of higher resolution COSY spectra than is current practice. It will become the method of

choice for studies made at ultrahigh field (> 750 MHz), desirable to simplify the complex spectra of carbohydrates, where digital resolution problems and/or those of data acquisition times will demand optimized measurement protocols.

EXPERIMENTAL

Data were acquired at 300.13 MHz, using a Bruker MSL-300 spectrometer with a wide-bore magnet. A single frequency ^1H probe was modified by the addition of an unshielded z-gradient coil on a polyperfluoroethylene mounting and pi-section filters on each gradient line. The gradient coil was driven by a Tecron 7570 Crown power amplifier. Thirty-two step gaussian gradients of ~ 6 ms total duration and ~ 10 kHz per cm intensity (for the basic gradients) were employed. Data were collected into a 2K data block (1K real points), with 512 increments, using buffered acquisition with a recycle time of about 1 s, and the data set was zero-filled in the f_1 dimension and processed in the magnitude mode. Data sets were not symmetrized. Eight dummy scans preceded data acquisition to establish steady state conditions. 2-Deoxy-D-arabino-hexose and the internal reference, sodium 3-(trimethylsilyl)propionate-2,2,3,3- d_4 , were obtained from Aldrich Chemical Co.

ACKNOWLEDGMENT

We thank Dr. Herchel Smith for the endowment which supported this work.

REFERENCES

- 1 J. Jeener, *Ampere International Summer School, Basko Polje, Yugoslavia, 1971*.
- 2 J.K.M. Sanders and B.K. Hunter, *Modern NMR Spectroscopy. A Guide for Chemists*, Oxford University Press, Oxford, 1987; A.E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon, Oxford, 1989.
- 3 P. Barker and R. Freeman, *J. Magn. Reson.*, 64 (1985) 334–338; C.H. Sotak, D.M. Freeman, and R.E. Hurd, *ibid.*, 78 (1988) 355–361; R.E. Hurd, *ibid.*, 87 (1990) 422–428; R.E. Hurd and B.K. John, *ibid.*, 92 (1991) 658–668; I.M. Brereton, S. Crozier, J. Field, and D.M. Doddrell, *ibid.*, 93 (1991) 54–62; M. von Kienlin, C.T.W. Moonen, A. van der Toorn, and P.C.M. van Zijl, *ibid.*, 93 (1991) 423–429; B.K. John, D. Plant, S.L. Heald, and R.E. Hurd, *ibid.*, 94 (1991) 664–669; G.W. Vuister, R. Boelens, R. Kaptein, R.E. Hurd, and P.C.M. Van Zijl, *J. Am. Chem. Soc.*, 113 (1991) 9688–9690.
- 4 R.E. Hurd, B.K. John, and H.D. Plant, *J. Magn. Reson.*, 93 (1991) 666–670.
- 5 L.D. Hall and J.F. Manville, *Adv. Chem. Ser.*, 74 (1968) 228–253.
- 6 A. Wokaun and R.R. Ernst, *Chem. Phys. Lett.*, 52 (1977) 407–12; U. Piantini, O.W. Sorensen, and R.R. Ernst, *J. Am. Chem. Soc.*, 104 (1982) 6800–6801; A.J. Shaka and R. Freeman, *J. Magn. Reson.*, 51 (1983) 169–173.