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1D HMQC-TOCSY: A SELECTIVE ONE-DIMENSIONAL ANALOGUE OF HMQC-TOCSY

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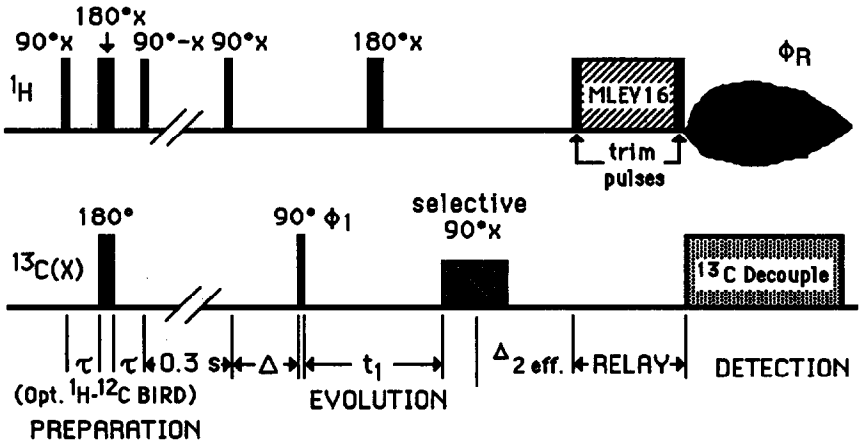
Summary: A selective, soft-pulse one dimensional analogue of the HMQC-TOCSY experiment, 1D HMQC-TOCSY, is described. An application is presented for a carbocyclic adenosine analog wherein problems of overlap between vicinally coupled protons preclude assignment by traditional methods such as COSY or relayed COSY.

We wish to report the implementation of a selective one-dimensional (1D) analogue of HMQC-TOCSY[1], which we have labeled, after the work of Kessler, Oschkinat, and Griesinger[2], one-dimensional heteronuclear multiple quantum coherence-TOCSY (1D HMQC-TOCSY). The technique may be applied using either square or Gaussian-shaped[3] selective ^{13}C-pulses with comparable success. The choice of pulse type is a function of the degree of selectivity required by the application.

Berger described a selective 1D variant of the HMQC experiment, SELINCOR, which is a technique for selectively assigning proton resonances *via* specific correlation to a known ^{13}C resonance by means of a soft pulse.[4] Since this experiment works in the reverse sense from traditional experiments, in that protons are correlated to carbons of known position, applications of SELINCOR may be limited to the solution of highly specific structural questions due to the general availability of total proton-carbon chemical shift correlations by means of 2D HMQC spectra, even at very low concentration. In contrast, hyphenated reverse detected experiments in which coherence is relayed by one means or another (HMQC-TOCSY[1], HMQC-NOESY[5,6], and HMQC-ROESY[7]) can provide vital useful structural information through the dispersal of proton connectivity information as a function of the carbon chemical shift in the F_1 frequency domain. Consequently, ^{13}C-selective 1D analogues of these hyphenated 2D experiments, in the basic form of SELINCOR, have considerable potential for the solution of a range of difficult structural problems in which only a limited portion of the total information content of the full 2D experiment is necessary.

A schematic representation of a 1D heteronuclear multiple quantum coherence (1D HMQC) relay pulse sequence is shown below[8]. In these experiments, the final refocusing delay, Δ_2,

prior to the relay step is normally optimized as a function of $1/2(^1J_{CH})$. In our experience, however, the specific optimization of this delay for the sample at hand is crucial to insure that good sensitivity is retained in the selective 1D experiment. Since the final ^{13}C pulse will need to be relatively long to permit satisfactory selectivity (6 to 20 msec, typically), calculation of this delay from the actual coupling constant ($^1J_{CH}$) is not appropriate. One-half the length of the selective pulse is effectively added to this delay[2]. The duration of the $\Delta 2$ delay can be experimentally optimized, quite simply, by recording data without using either the relay step or ^{13}C decoupling. In this form, the experiment is essentially a refocused SELINCOR experiment. After phasing the resultant satellite doublet to be antiphase, with $\Delta 2$ equal to zero, the length of $\Delta 2$ is then adjusted until a duration is found that gives the best in-phase doublet for the satellite spectrum of the selected resonant pair. Using this method we have found an optimal delay of 4.6 msec with an 8.2 msec selective 90° ^{13}C pulse. Thus, we employ a $\Delta 2$ effective of 8.7 msec.



A second critical point concerns the transmitter frequency at which the ^{13}C pulses are applied. In order to efficiently create and reconvert heteronuclear multiple quantum coherence to single quantum proton coherence, the first hard ^{13}C 90° pulse and the final soft ^{13}C 90° pulse must be applied at exactly the same frequency offset. If a BIRD pulse element is employed at the beginning of the sequence for suppression of the ^{12}C-bound protons, it may be useful to offset the ^{13}C frequency at which the BIRD pulse is applied to a point somewhere near the center of the carbon chemical shift range. If desired, broadband ^{13}C decoupling may be applied at any offset frequency as well, although we have found it beneficial to ^{13}C-decouple on-resonance with the selected ^{13}C resonance and to employ fairly low (800 Hz) decoupling power. This approach allows the option for relatively long ^1H acquisition times without concern for the poor cancellation that can result from sample heating.

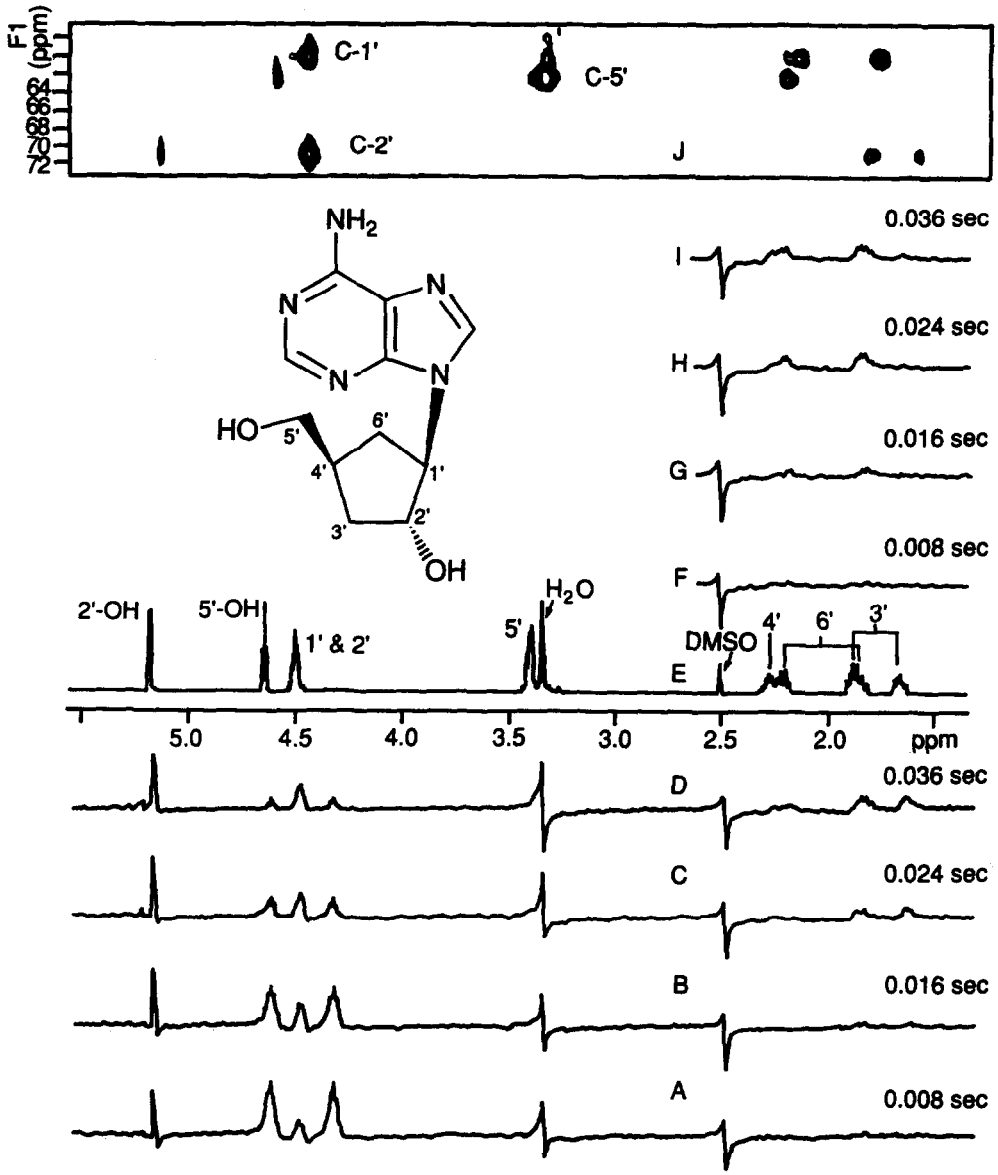


Figure 1. 1D HMQC-TOCSY data obtained for 1. Traces A-D reflect buildup of transferred magnetization from H2' to the 3' protons from the selective pulse application to C2'. The growth of the response for the vicinal coupling between the overlapped protons H1' and H2' centered at 4.5 ppm should also be noted in these traces. The one-dimensional 500 MHz reference spectrum is shown in trace E. Traces F-I reveal the buildup of magnetization at the H6' protons, transferred from H1' as a result of selective pulse application to C1'. The dispersive signals near 3.3 and 2.5 ppm are residual water and DMSOd_6. J - segment of the contour plot of the HMQC-TOCSY spectrum corresponding to the traces shown in A-I.

To illustrate the application of the 1D HMQC-TOCSY experiment, Figure 1 shows the buildup of response intensity as a function of mixing time duration for two selectively pulsed ^{13}C resonances. The model compound chosen, **1**, exhibits overlap of key proton signals, H1' and H2', even at 500 MHz, making assignments impossible.[9,10] The propagation of magnetization from H1' or H2' as a function of the carbon resonance selectively excited, C1' and C2', respectively, yields information identical to that obtained from the full two-dimensional HMQC-TOCSY spectrum.[11] It is important to note that the 1D HMQC-TOCSY experiment provides this information with a tremendous time savings relative to the two-dimensional experiment. In particular, each of the traces shown in Figure 1 represents 3 min of acquisition time as compared to approximately 2 hrs 40 min to acquire the full HMQC-TOCSY experiments with the same set of four mixing times.[11,12] Hence, the 1D HMQC-TOCSY experiment can be beneficially employed when connectivity information is needed for only a few carbon resonances rather than for every resonance in the spectrum. Selective ^{13}C bandwidth requirements could limit the usefullness of this technique for crowded spectral regions. While the savings in time for 1D HMQC-TOCSY is significant, even greater potential time savings can be realized for selective analogues of the HMQC-NOESY experiment, which suffers from extreme insensitivity. We are evaluating 1D HMQC-NOESY pulse sequences and anticipate that they will form the basis for a future report.

REFERENCES AND NOTES

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8 The pulse sequence which we employed was obtained by minor modification of the C program for HMQC-TOCSY which is provided in the "res_psglib" directory within the standard Varian VNMR NMR program. The phases were cycled as follows: Phases labelled "X" in the pulsesequence schematic were [00112233]; $\phi 1$ and the receiver phase were [02132031]; the MLEV16[13] phases were +/- X, and inverted each 8 transients; the trim pulses were effectively MLEV+1.

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10 We wish to acknowledge Dr. S. Daluge and Mr. R. McFadyen of the Division of Organic Chemistry Burroughs Wellcome Co. for the synthesis of the compound.

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12 Data were obtained at 25°C with a 20 mg/ml sample in DMSO-d_6 solution. Each trace is the result of 128 transients (3 minutes) using a Varian VXR500S spectrometer equipped with a Z*Spec ID500 probe. The hard ^{13}C 90° tip was 16 μsec and the selective ^{13}C pulse for the work shown here was an 8.2 msec square pulse. The ^{1}H 90° pulse was 13.3 μsec. The duration of the first fixed delay, Δ, was set to 3.5 msec; the duration of Δ_2 was optimized as described in the text and found to be 4.6 msec. The evolution time, t_1, was set to 2 μsec to allow both a phased presentation of the data with ample time for the 54 db power switch of the ^{13}C channel. Spin lock was achieved with an MLEV16[13] rf field with a magnitude ($\gamma H_2/2\pi$) ≈ 6344 Hz ranging in duration from 8 to 36 msec. 1 msec trim pulses flanked the MLEV period.

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