Double Quantum Filtered Zero Quantum Coherence With Broadband Homonuclear F_1 Decoupling

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A pulse sequence is described which combines double quantum filtration and broadband homonuclear F_1 decoupling in a two-dimensional zero quantum (ZQCOSY) nmr experiment.

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Proton zero quantum coherence two-dimensional nmr spectroscopy (ZQC2D) is presently an infrequently utilized and quite probably an underappreciated technique. Early efforts in the area were hampered by difficulties in the excitation of zero quantum transitions (ZQTs). Simple excitation sequences such as that shown in Eq 1, although capable of exciting ZQTs, do so with a strong dependence on the scalar coupling constant and chemical shift [1,2].

$$90°_x - \tau - 90°_x \qquad \text{Eq 1}$$

Matters were exacerbated by the early misperception that the sequence used to excite double quantum transitions (DQTs), shown in Eq 2, was incapable of efficiently exciting ZQTs. This prompted Müller [3] to propose an alternative excitation scheme which is shown in Eq 3.

$$90°_x - \tau - 180°_y - \tau - 90°_x \qquad \text{Eq 2}$$

$$90°_x - \tau - 180°_y - \tau - 45°_y \qquad \text{Eq 3}$$

Both Braunschweiler, Bodenhausen and Ernst [4] and Hall and Norwood [5,6] have since, however, demonstrated that the sequence shown in Eq 2 is capable of exciting ZQTs for all but A_nX_n type spin systems. In contrast, the excitation scheme proposed by Müller shown in Eq 3 is capable of exciting A_nX_n type spin systems albeit at the expense of all other zero quantum coherences (ZQCs).

With improved excitation schemes available, the major remaining obstacle to the widespread use of ZQC2D has been the intense $F_1 = 0$ Hz axial artifact responses which arise due to longitudinal relaxation during the evolution period. Unfortunately, axial responses cannot be removed by phase cycling. Farmer, Ramachandran and Brown [7] observed that axial artifacts, which arise from single quantum coherences (SQCs), cannot be converted into DQC by the application of a single pulse. In contrast, the evolved ZQC can be interchanged with DQC by a single pulse. Hence, Farmer and co-workers [7] proposed a double quantum filtered zero quantum nmr experiment which largely eliminates the axial artifact responses.

In an extension of the work of Farmer and co-workers [7], Cavanagh and Keeler [8] have examined multiplet effects in double quantum filtered zero quantum spectra. Rather surprisingly, the work of both Farmer and coworkers [7] and that of Cavanagh and Keeler [8] employed the chemical shift dependent excitation scheme shown in Eq 1. Recently [9] we have reported a double quantum filtered zero quantum pulse sequence which incorporates the excitation sequence shown in Eq 2. A comparison of projections through the F_1 frequency domain of two spectra, one recorded using the conventional Müller [3] experiment which has served as the basis for all of our previous work in the area of ZQC2D [10-14], the other using the excitaton scheme shown in Eq 2 combined with a double quantum filter showed the latter to give superior response intensity with near total suppression of $F_1 = 0$ Hz artifact responses.

Although work by Hall and Norwood [5,6,15] has incorporated a varibly positioned 180° pulse in the evolution period to provide broadband F_1 decoupled ZQC2D spectra analogous to those pioneered with the F_1 decoupled COSY spectrum of Bax and Freeman [16], there still remained the problem of axial artifacts with the Hall and Norwood experiments. In our first efforts at ameliorating the problem of axial artifacts, we recently have communicated [17] the incorporation of broadband F_1 decoupling in the

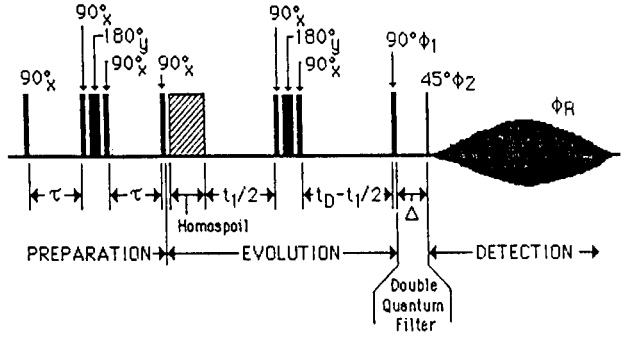


Figure 1. Modified proton quantum pulse sequence using chemical shift independent excitation and designed to provide broadband F_1 decoupling and elimination of axial artifacts through double quantum filtering prior to detection. Phases are cycled according to Table 1.

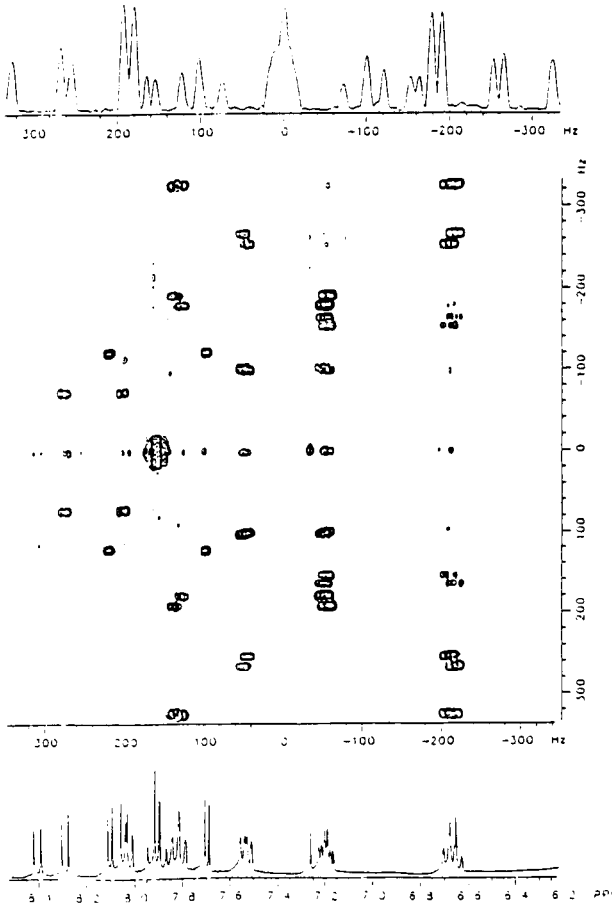


Figure 2. Proton zero quantum spectrum of phenanthro[3',4':3,4]phenanthro[2,1-*b*]thiophene (**1**) recorded using the pulse sequence shown in Figure 1. These data were acquired as 128 x 512 complex points and were processed using sinusoidal multiplication prior to both Fourier transformations with zero filling prior to the second to afford a final data matrix consisting of 512 x 256 points. Fixed delays during evolution, τ, were optimized for 7 Hz as a function of $\frac{1}{4}(J_{HH})$ giving a duration of 35.7 mseconds. Total duration of the evolution period (t_D) was fixed at 183 mseconds.

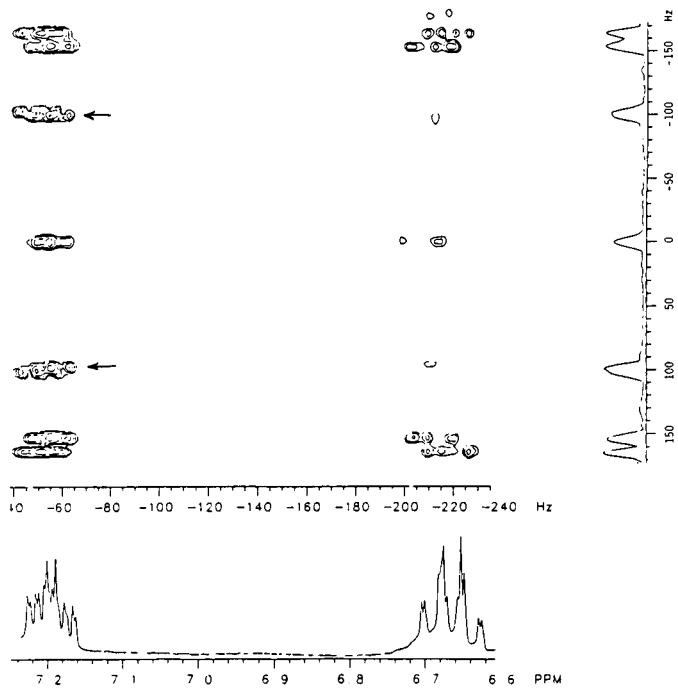


Figure 3. Expansion of the region of the zero quantum spectrum shown in Figure 2 containing responses correlation H11-H12 and H15-H16. The projection through F_1 for this region demonstrates the efficiency of the double quantum filter (compare Figure 4); the intensity of the axial response at F_1 = 0 Hz is relatively minimal. It should also be noted that the zero quantum or K-splittings in the F_1 frequency domain [8] have been collapsed to singlets (responses at ±164 and 154 in F_1). The significance of this observation is clarified by comparing the F_1 projection of Figure 3 with the corresponding projection of Figure 4. Finally, the responses denoted by arrows located at ±101 Hz arise from correlations to protons resonating further downfield (see Figure 2).

double quantum filtered zero quantum experiment of Farmer, Ramachandran and Brown [7]. We now wish to communicate the results obtained with a modified ZQC2D pulse sequence shown in Figure 1 which incorporates the more efficient excitation scheme shown in Eq 2 using a composite 180°$_y$ pulse, broadband F_1 decoupling using a composite 180° pulse and finally double quantum filtering.

The 7 Hz optimized zero quantum spectrum of phenanthro[3',4':3,4]phenanthro[2,1-*b*]thiophene (**1**) is shown in Figure 2. It will be noted that there is generally excellent response intensity and that responses appear as singlets in the F_1 frequency domain. The significance of the latter portion of the preceding statement is dramatically illustrated by the responses correlating H11-H12 and H15-H16 shown in the expansion presented in Figure 3. Here,

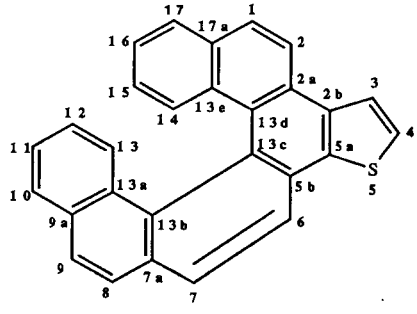
Table 1

Phase Cycling Scheme for the Modified Zero Quantum Pulse Sequence Shown in Figure 1 [a]

Acquisition	ϕ1	ϕ2	ϕR
1	x	x	x
2	x	y	-y
3	x	-x	-x
4	x	-y	y
5	y	x	-x
6	y	y	y
7	y	-x	x
8	y	-y	-y
9	-x	x	x
10	-x	y	-y
11	-x	-x	-x
12	-x	-y	y
13	-y	x	-x
14	-y	y	y
15	-y	-x	x
16	-y	-y	-y

[a] The Nicolet pulse sequence listing may be obtained from the authors upon request.

although the differences in chemical shifts range from only 0.011 to 0.02 ppm, the responses in F_1 are clearly defined and the correlation easily made. In contrast, an expanded segment of the spectrum of **1** recorded using the Müller [3] pulse sequence which was described in our



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earlier work [11] shows a substantially more complex pattern of responses. Based upon the analysis of Cavanagh and Keeler [8] the zero quantum responses of the interior

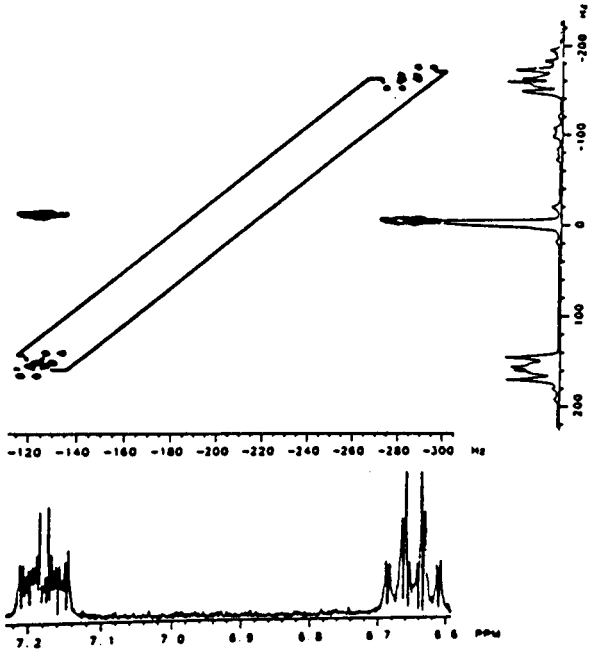

Figure 4. Expansion of the proton zero quantum spectrum of **1** recorded using the pulse sequence proposed by Müller, as reported in a previous communication [11]. Whereas correlations can be drawn between well defined singlet responses in F_1 in the spectrum shown in Figure 3, responses in this case exhibit "K-triplet" structures in F_1 which are substantially overlapped thereby making interpretation much more difficult. It is also worth noting the intensity of the $F_1 = 0$ Hz axial artifact response in this spectrum which is substantially more intense than the desired zero quantum responses. Overall, compared with the results shown in Figure 3 obtained using the new pulse sequence described in this work, the benefits of broadband F_1 decoupling and double quantum filtration are obvious.

spins of a four spin system will exhibit zero quantum splittings, also referred to as K-splittings [8,17], which are triplet-like in character. The overlap of the pairs of K-triplets is shown in the F_1 projection which is also plotted in Figure 4. Obviously, the interpretation of the coupling pathways is complicated by the multiplet structure in F_1 in this case. In contrast, each of the K-triplets is reduced to a singlet (± 164 and 154 Hz in F_1) as shown both in the contour plot and the F_1 projection in Figure 3, with a commensurate large improvement in the ease of interpretation. Furthermore, it should also be noted that there is essentially no $F_1 = 0$ Hz axial artifact response in Figure 3 whereas the axial response is substantially more intense than the desired zero quantum responses obtained using the Müller sequence as shown in Figure 4.

A further point worthy of comment is the use of the composite $180°_y$ pulse located in the evolution period in the pulse sequence shown in Figure 1. Hall and Norwood [6] mention the use of this pulse in the acquisition of zero and double quantum spectrum of dehydrotestosterone only briefly in the experimental section of the paper, suggesting that the composite pulse minimizes coherence transfer artifacts which tend to arise due to miscalibration of the 180° pulse. A preliminary density matrix calculation suggests, however, that their statement may be an oversimplification of the effects of the composite pulse cluster. Assuming a density matrix for an AX spin system containing purely zero quantum coherence immediately prior to the $90°_x$ pulse of the composite pulse cluster we find that following pre- and post multiplication of the matrix by the appropriate rotation operators that zero quantum coherence is converted to real double quantum coherence terms and imaginary zero and single quantum terms. The matrix is then inverted by the $180°_y$ pulse and finally reconverted to zero quantum coherence by the final $90°_x$ pulse of the cluster. This amounts, in effect, to a second double quantum filtration of the evolving zero quantum coherence which may account for the superior results obtained in this study and that of Hall and Norwood [6]. A rigorous analysis of pulse sequence shown in Figure 1, examining in particular the effects of the composite pulse on evolving zero quantum coherence, has been undertaken and will be reported in a subsequent paper on the topic.

In conclusion, the modified ZQC2D pulse sequence shown in Figure 1 affords zero quantum spectra which are substantially simplified and virtually free of undesirable axial artifacts. We hope that the sequence shown in Figure 1 or a variant thereof will facilitate increased usage of zero quantum nmr for structure elucidation and assignment applications. We are currently exploring further modifications of the pulse sequence shown in Figure 1 as well as applications of zero quantum which will serve as the basis for forthcoming reports.

EXPERIMENTAL

All spectra were recorded using a Nicolet NT-300 controlled by a Model 293-C pulse programmer and operating at an observation frequency of 300.068 MHz. Data were acquired using the pulse sequence shown in Figure 1 and the phase cycling scheme shown in Table 1 as 128 x 512 complex points and were processed using sinusoidal multiplication prior to both Fourier transformations with zero filling prior to the second to afford a final data matrix consisting of 512 x 512 real points. The spectral width in F_2 was +/-349 Hz. Fixed delays during evolution were optimized as a function of $\frac{1}{4}(J_{HH})$ which for 7 Hz gave a delay of 35.7 mseconds. The total duration of the evolution period, t_d, was fixed at 183 mseconds. Longer evolution times required for higher levels of digitization in F_1 were observed to give a consequent diminution of response intensity. The interpulse delay was set to 2 seconds and a total of four dummy scans were acquired and discarded prior to actual data accumulation.

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REFERENCES AND NOTES

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[1] W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).

[2] G. Pouzard, S. Sukumar, and L. Hall, *J. Am. Chem. Soc.*, **103**, 4209 (1981).

[3] L. Müller, *J. Magn. Reson.*, **59**, 326 (1984).

[4] L. Braunschweiler, G. Bodenhausen and R. R. Ernst, *Mol. Phys.*, **48**, 535 (1983).

[5] L. D. Hall and T. J. Norwood, *J. Magn. Reson.*, **69**, 391 (1986).

[6] L. D. Hall and T. J. Norwood, *J. Magn. Reson.*, **76**, 241 (1988).

[7] B. T. Farmer, II, R. Ramachandran, and L. R. Brown, *J. Magn. Reson.*, **73**, 534 (1987).

[8] J. Cavanagh and J. Keeler, *J. Magn. Reson.*, **77**, 612 (1988).

[9] L. R. Soltero and G. E. Martin, *Magn. Reson. Chem.*, submitted (1988).

[10] A. S. Zektzer and G. E. Martin, *J. Nat. Prod.*, **50**, 455 (1987).

[11] A. S. Zektzer, G. E. Martin, and R. N. Castle, *J. Heterocyclic Chem.*, **24**, 879 (1987).

[12] A. S. Zektzer, L. D. Sims, R. N. Castle, and G. E. Martin, *Magn. Reson. Chem.*, **26**, 287 (1988).

[13] D. E. Barnekow, J. H. Cardellina, II, A. S. Zektzer, and G. E. Martin, *J. Am. Chem. Soc.*, in press (1989).

[14] M. J. Musmar, S. R. Khan, A. S. Zektzer, G. E. Martin, and K. Smith, *J. Heterocyclic Chem.*, in press (1989).

[15] L. D. Hall and T. J. Norwood, *J. Magn. Reson.*, **69**, 397 (1986).

[16] A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).

[17] L. R. Soltero, M. D. Johnston, Jr., R. N. Castle, and G. E. Martin, *J. Magn. Reson.*, **81**, 406 (1989).