Two-Dimensional Relayed Coherence Transfer Experiments as a Means of Subgrouping Protonated Carbon Resonances of Complex Polynuclear Heteroaromatics on the Basis of Vicinal Proton-Proton Connectivities

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Application of autocorrelated homonuclear (COSY) and proton-carbon chemical shift correlation two-dimensional nmr experiments to the problem of assigning the ^{1}H- and ^{13}C-nmr spectra of phenanthro[4,3-*a*]dibenzothiophene fails despite their considerable utility in the assignment of the spectra of the smaller phenanthro[*b*]thiophenes. The failure of these techniques in the case of phenanthro[4,3-*a*]dibenzothiophene is predominantly due to the inability of the COSY spectrum to provide the means of subgrouping the proton resonances into their component spin subsystems. Two-dimensional relayed coherence transfer nmr experiments which first established coherence between vicinally coupled protons which is then transferred to the carbon spins which are ultimately observed circumvent these problems. The application of two-dimensional relayed coherence transfer to phenanthro[4,3-*a*]dibenzothiophene is described, the technique leading to the subgrouping of all five of the proton spin subsystems and thus providing a means of beginning the total assignment.

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Two-dimensional nmr spectroscopy has progressed substantially from the first suggestion of the experiment by Jeener in 1971 [1], these techniques now allowing the detailed study of molecules whose nmr spectra would have been totally intractable by conventional techniques only a few years ago. In particular, our interest in two-dimensional nmr experiments centers around their application in the assignment of the highly congested proton and carbon

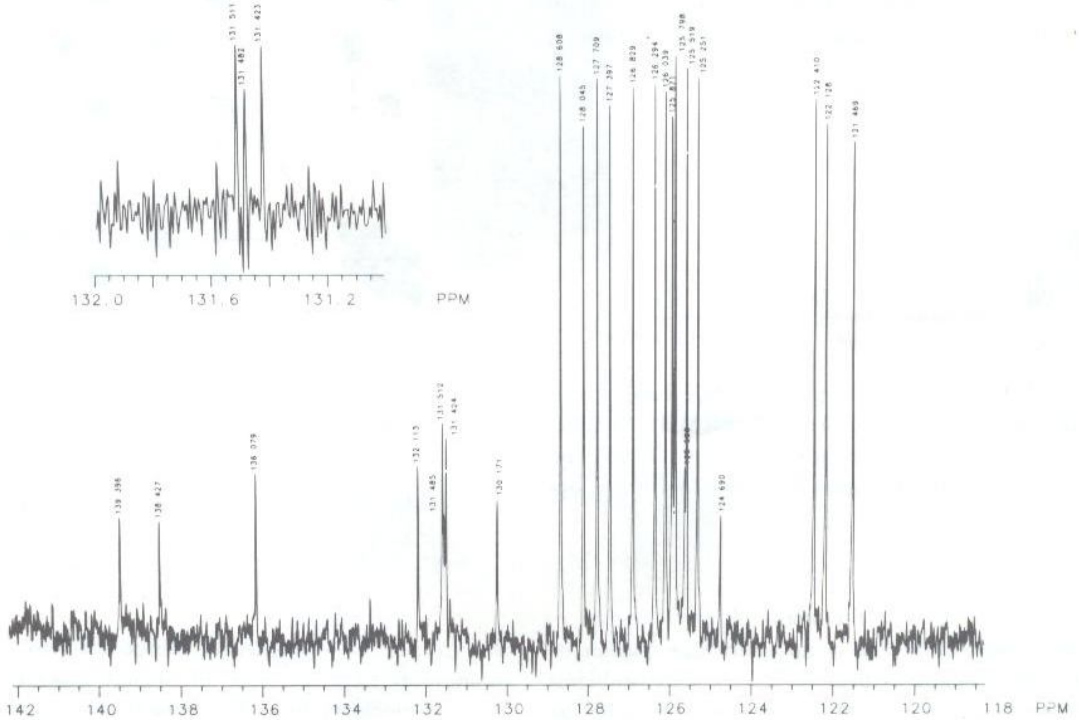
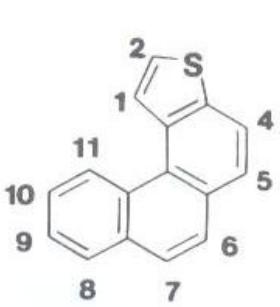


Figure 1. ^{1}H-Decoupled ^{13}C-nmr spectrum of phenanthro[4,3-*a*]dibenzothiophene recorded in deuteriochloroform at 75.45 MHz at ambient temperature.

nmr spectra of polynuclear aromatic thiophene systems. Thus, we have previously reported studies on phenanthro-[1,2-*b*]thiophene [2], phenanthro[3,4-*b*]thiophene (**1**) [3] and most recently benzo[2,3]phenanthro[4,5-*bcd*]thiophene [4]. Although there are numerous interesting features associated with the study of these potential sulfur contaminants of coal, perhaps one of the more interesting features associated with these compounds was the pronounced helical character associated with phenanthro[3,4-*b*]thiophene (**1**) [3]. In pursuing this observation further, we were interested in examining other related systems with a high probability of being helically distorted. Unfortunately, however, the necessary additional rings which must be fused to a nucleus such as **1** to produce a more helically distorted system also add significantly to the complexity of the proton and carbon nmr spectra of the molecule. In particular, such fusions add to the numbers of AB proton spin systems, thus making interpretation increasingly more difficult. An example of such a system is found in the case of phenanthro[4,3-*a*]dibenzothiophene (**2**) [5] which forms the focal point of the application of a new two-dimensional nmr technique described in this report. As will be noted from the ^{1}H-decoupled ^{13}C-nmr spectrum of **2** shown in Figure 1, the fourteen protonated carbon resonances are contained in a spectral region only 8 ppm wide with eleven of the resonances clustered in only a 3 ppm wide portion of that region. The proton spectrum of **2** at 300 MHz is similarly complex, nine of the fourteen protons contained in a region only ~0.2 ppm wide (Figure 2). Thus, the detailed nmr study of such a system is expected to be difficult from the outset, even with the availability of two-dimensional nmr experiments. The complement of experiments which may be brought to bear on assignment problems such as those represented by **2** has been expanded by the work of Bolton [6,7] and others [8-11] through the pioneering development of two-dimensional relayed coherence transfer (RCT2D). We would like to illustrate the application of this powerful new experiment in heterocyclic chemistry through its ability to sub-group protonated carbon resonances *via* vicinal proton-proton connectivities.



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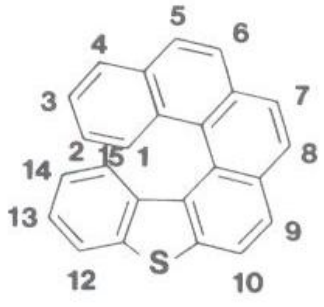

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The complexity of the assignment problem encountered with phenanthro[4,3-*a*]dibenzothiophene (**2**) becomes more obvious when the autocorrelated two-dimensional proton (COSY) nmr spectrum [2-4,12-14] and the normal two-dimensional proton-carbon chemical shift correlation spectra [2,3,15-17] are examined (Figures 3 and 4 respectively).

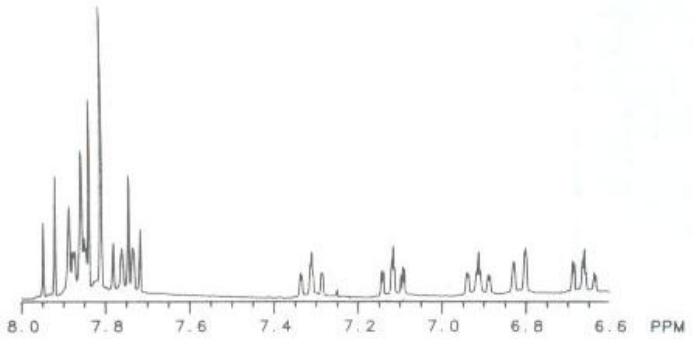

Figure 2. ^{1}H-nmr spectrum of phenanthro[4,3-*a*]dibenzothiophene in deuteriochloroform at 300.068 MHz. Chemical shifts are reported in ppm downfield from TMS referenced externally from the residual protiochloroform signal.

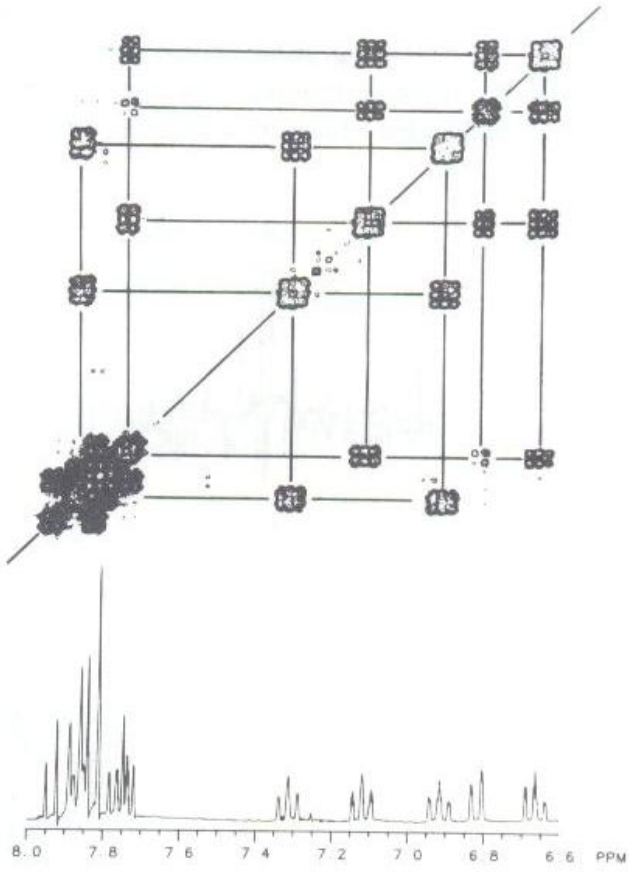

Figure 3. Two-dimensional autocorrelated (COSY) homonuclear nmr spectrum of phenanthro[4,3-*a*]thiophene in deuteriochloroform. The data is presented as a twelve level contour plot comprising 256 × 256 real data points, the data symmetrized [27] prior to plotting.

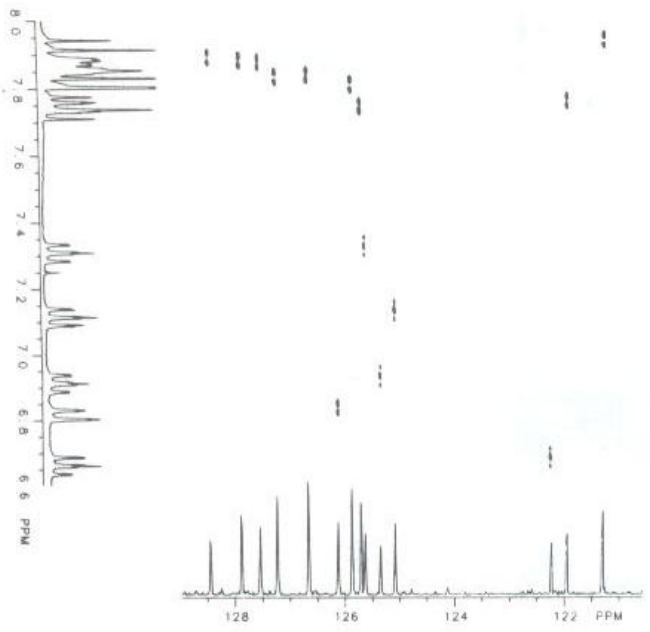

Figure 4. Two-dimensional proton-carbon chemical shift correlation nmr spectrum of phenanthro[4,3-*a*]dibenzothiophene in deuteriochloroform. The data is presented as a six level contour plot consisting of 256 × 512 real data points.

Cursory examination of the structure of **2** readily leads to the realization that the proton spectrum will be comprised of five essentially independent spin systems. The protons of rings A and F will be four spin systems which, on the basis of our previous studies [2-4], would be expected to be either ABMX or ABXY systems, these connectivities illustrated in the contour plot of the COSY spectrum shown in Figure 3. Components of both four spin systems are contained in the complex multiplet observed between 7.7 and 7.95 ppm thus making the location of the correct carbons to which these resonances correspond in the two-dimensional proton-carbon chemical shift correlation spectrum rather difficult. Within the three AB systems, the problem of correlating the proton resonances with one another and also with the corresponding directly attached carbons is even worse, clearly underscoring the need for a reliable and sensitive means of obtaining such correlations. This need is filled in large part by the two-dimensional relayed coherence transfer (RCT2D) experiment [6-11] which despite some shortcomings, is of considerable utility.

The pulse sequence for the RCT2D experiment [10] is shown in Figure 5; functions of the various components of the sequence are described by Bax [9]. The plot of the full RCT2D nmr spectrum is shown in Figure 6 and an expansion of the highly congested downfield region from 125 to 128 ppm is shown in Figure 7. The experiment was performed on an 80 mg sample of **2** dissolved in 0.4 ml of deuteriochloroform in a 5 mm sample tube. The spectrum was

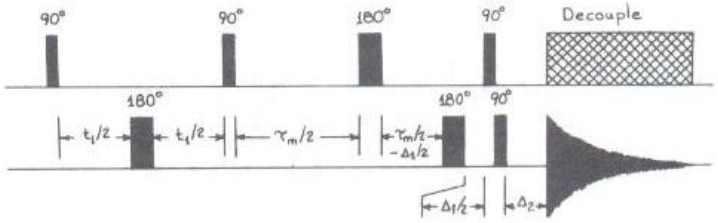

Figure 5. Pulse sequence for two-dimensional relayed coherence transfer nmr. Details of the experiment are given in the experimental section.

run in a 5 mm dual tuned $^1H/^{13}C$ probe on a Nicolet NT-300 wide bore spectrometer. Phase cycling of the first 90° and 180° proton pulses and the second 180°, and 90° carbon pulses along with the receiver reference phase was employed to provide the equivalent of quadrature detection in both frequency domains [18]. The spectrum shown in Figure 6 is a six level contour plot [19] of the final data matrix (F_2F_1) which consisted of 512 × 256 real data points. Total data accumulation time for the experiment was approximately 36 hours (216 increments of the evolution period out of 256 actually performed).

Analysis of the data provided by the RCT2D experiment requires a knowledge of the proton-carbon connectivities obtained by the prior acquisition of a two-dimensional proton-carbon chemical shift correlation experiment. Proton-proton connectivities derived from the COSY experiment, although a useful adjunct, are not mandatory. The resonances furthest upfield (in the carbon spectrum) comprise two of the resonances of one of the four spin systems and one member of one of three AB spin systems. Connectivities for both of these spin systems are shown in Figure 6. On the basis of the assigned ^{13}C-nmr spectrum of phenanthro[3,4-*b*]thiophene (**1**) [3], it is possible to assign the resonance at δ = 121.47 as C10, the RCT2D spectrum (Figure 6) readily providing the location and identity of the C9 resonance as the remaining member of the AB system at δ = 126.83. The connectivities of the remaining four spin ABXY system and those for the two remaining AB spin systems are shown in Figure 7. Because of the helical nature of **2** expected at the outset of this study and supported by the significant upfield shifts of some of the resonances, only tentative assignments could be made on the basis of chemical shift or structural arguments which we have chosen to avoid, preferring instead to make these assignments unequivocally after the completion of a two-dimensional double quantum coherence experiment [3, 20-26].

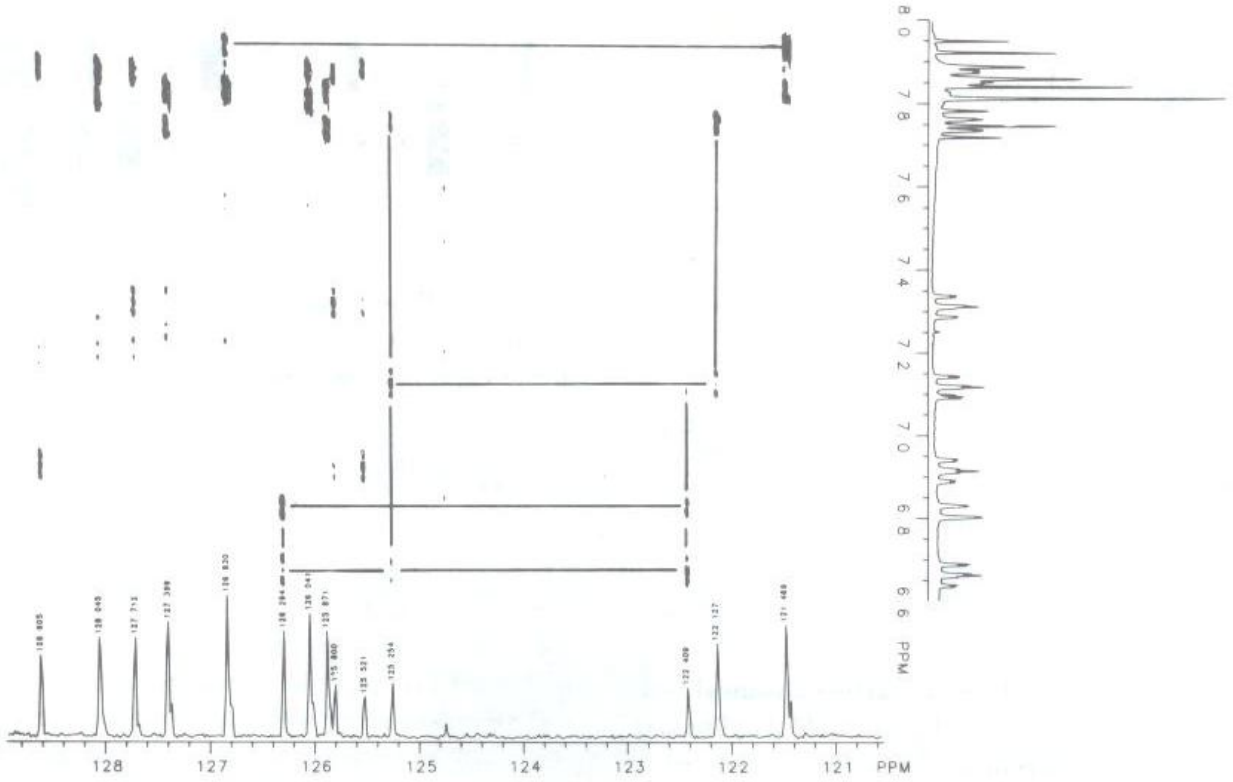

Figure 6. Two-dimensional relayed coherence transfer (RCT2D) nmr spectrum of phenanthro[4,3-*a*]dibenzothiophene in deuteriochloroform. The data is presented in the form of a six level contour plot consisting of 256 × 512 real points. Connectivities for one of the four spin systems and for one of the two spin systems are shown.

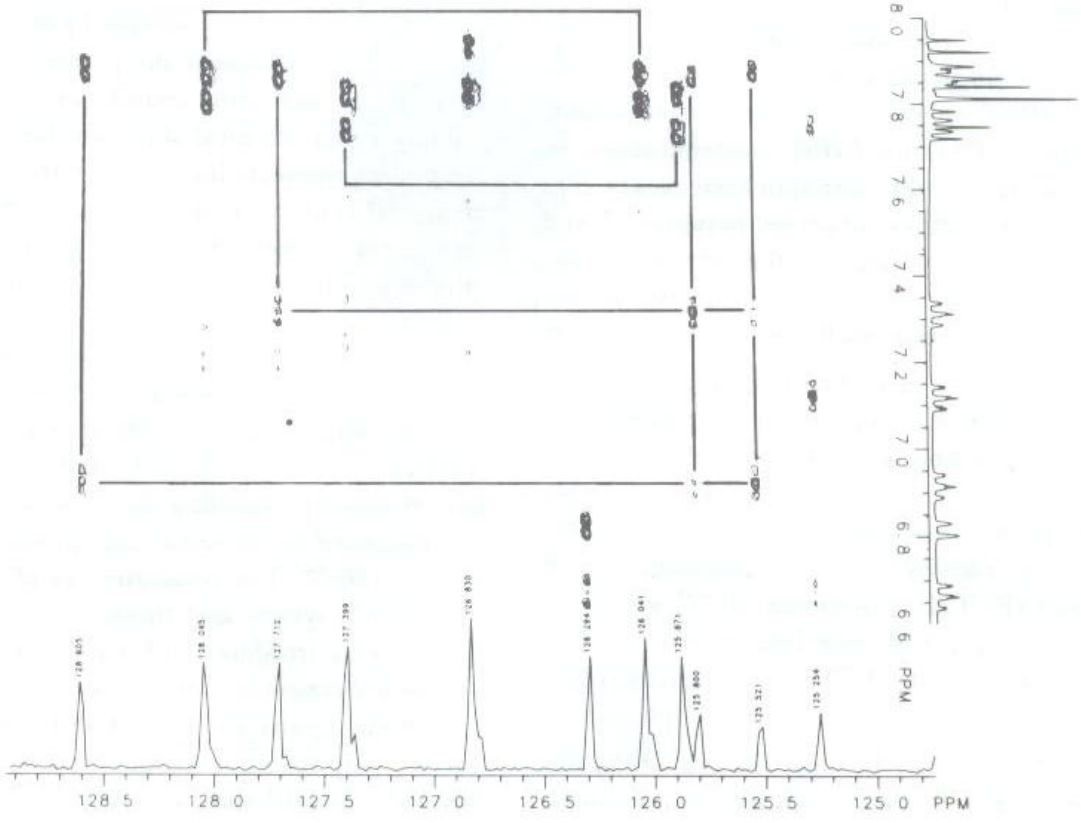

Figure 7. Expansion of the two-dimensional relayed coherence transfer (RCT2D) nmr spectrum of phenanthro[4,3-*a*]-dibenzothiophene shown in Figure 6. Connectivities for the remaining four spin systems and for the two remaining two spin systems are shown.

In conclusion, the RCT2D experiment, as has been shown, very importantly establishes proton-carbon connectivities of the spin systems in the molecule based upon the vicinal proton-proton coupling networks. The importance of this experiment when it is extended to systems of unknown structure is obvious. Furthermore, the experiment is more sensitive than the two-dimensional double quantum coherence experiments and also, will not suffer from breaks in the connectivity network when the spectrum contains a mixture of carbon-carbon AB and AX pairings [27]. Final work toward the completion of the spectral assignments of **2** and those of the benzo[*b*]phenanthro[4,3-*d*]thiophene system which is intermediate in complexity between **1** and **2** is at present under way in these laboratories and will be the subject of a forthcoming report.

EXPERIMENTAL

A sample of benzo[*c*]phenanthro[4,3-*d*]benzo[*b*]thiophene (**2**) of analytical purity (80 mg) employed in this study was obtained using the synthetic method previously described by Castle and co-workers [5]. The sample was prepared by dissolving the material in 0.5 ml of deuteriochloroform in a 5 mm nmr tube which was subsequently degassed with zero grade argon for a period of 20 minutes. All spectra were taken at ambient temperature and were internally referenced to either the residual protiochloroform resonance or to the center line of the deuteriochloroform multiplet. Pulse widths were as follows: 90° proton observe pulse = 12 μsec; 90° proton pulse from the decoupler coils = 37 μsec; 90° ^{13}C observe pulse = 19.5 μsec and a 180° carbon pulse = 37 μsec. The COSY spectrum was taken using the pulse sequence described by Freeman and co-workers with quadrature phase cycling [14], the raw data consisting of 256 × 512 point blocks. The total data accumulation time was 26 minutes and the data was processed using sinusoidal multiplication in both Fourier transforms followed by symmetrization [28] to provide the twelve level contour plot shown in Figure 3. The CSCM experiment was performed using the pulse sequence of Freeman and Morris [16] modified with phase cycling [17] to provide quadrature detection in both frequency domains. The spectrum was accumulated in 6 hours and consisted of 256 × 1K point blocks processed to provide the 256 × 512 point data matrix shown as a contour plot in Figure 4. To improve sensitivity, the coherence transfer echo was detected [29]. The RCT2D experiment was performed using the pulse sequence shown in Figure 5 which is an adaption of that reported by Kessler and co-workers [10]. The intervals in the pulse sequence were set as follows: $t_1/2$ = proton dwell time/2; $\tau_m/2$ = D4 + D5 where D4 = $1/10^3J_{HH}$ ($^3J_{HH}$ taken as 7 Hz) and D5 = $1/4\ ^1J_{CH}$ ($^1J_{CH}$ taken as 165 Hz); the second 180° ^{13}C pulse was placed $\Delta_1/2$ before the 90° proton pulse from the decoupler coils where $\Delta_1/2$ = D5; the magnetization was brought back into phase using the Δ_2 delay prior to when the decoupler was gated on, this delay set = $1/3\ ^1J_{CH}$. An interpulse delay of 1 sec was uniformly employed for all three two-dimensional experiments described in this work.

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