Inverted and Suppressed Direct Response HMQC-TOCSY Spectra - A Convenient Method of Spectral Editing

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HMQC-TOCSY spectra provide a convenient means of establishing proton-proton connectivities in congested spectra of complex aromatic heterocycles. Advantage is taken of the greater dispersion of the ^{13}C nmr spectrum to circumvent overlap which would preclude spectral interpretation through the usual COSY spectrum. A recently reported method for inverting direct responses (IDR) in HMQC-TOCSY spectra is demonstrated for [1]benzothieno[2,3-*c*]naphtho[2,1-*g*]quinoline. A modification of the IDR-HMQC-TOCSY method is also demonstrated which is capable of fully suppressing direct responses (SDR) without resorting to the timing of the onset of decoupling as in the original report of the HMQC-TOCSY experiment. SDR-HMQC-TOCSY has the further advantage of allowing the use of higher levels of digitization in F_2 than can be attained when broadband heteronuclear decoupling is employed.

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Inverse-detected 2D nmr techniques, which were reviewed recently [1], have had a profound impact on nmr spectral assignment and structure elucidation. HMQC-TOCSY [2] affords a convenient means of establishing proton-proton connectivities in highly congested spectra. Correlations are deconvoluted by sorting information with the ^{13}C resonance chemical shift of the carbon directly bound to the proton from which magnetization transfer occurs. HMQC-TOCSY data, when a series of experiments are performed, can also be utilized to indirectly establish protonated carbon-protonated carbon connectivities [1,3].

One problem inherent to the HMQC-TOCSY experiment is the necessity of identifying the direct proton-carbon pairing if longer durations of the mixing time are to be employed which can give rise to relayed responses with intensities comparable to the direct response. A method for identifying direct responses in HSQC-TOCSY spectra was recently described by Domke [4]. Quite simply, Domke demonstrated that the insertion of a delay, $\Delta = 1/2(^1J_{CH})$, followed by simultaneous 180° $^1H/^{13}C$ pulses and a second refocussing delay, Δ, afforded a spectrum in which the direct responses are inverted. Although Domke did not describe an HMQC variant of his technique it is easy to write the corresponding HMQC-based pulse sequence, IDR-HMQC-TOCSY. The IDR-HMQC-TOCSY pulse sequence is shown in Figure 1B. We have utilized this method in assigning the proton and carbon nmr spectra of [1]benzothieno[2,3-*c*]naphtho[2,1-*g*]quinoline (1) [5]. We also wish to report a simple modification of Domke's experiment which is capable of completely suppressing direct responses (SDR-HMQC-TOCSY).

IDR-HMQC-TOCSY effectively combines, in a single experiment, identification of the direct or one-bond proton-carbon correlations as inverted responses, with propagation of magnetization from the directly bound proton to neighboring protons as a function of the mixing time chosen. Relayed responses appear in the spectrum with positive intensity. We have utilized IDR-HMQC-TOCSY to establish proton-proton connectivities of **1**. To compare the IDR-and SDR- (see following discussion) modifications to conventional HMQC-TOCSY, spectra were acquired and processed under identical conditions. The HMQC-TOCSY spectrum of **1** over the F_1 range of 121-129 ppm is shown in Figure 2. The IDR-HMQC-TOCSY spectrum acquired using the pulse sequence shown in Figure 1B, $\beta = 180°$, is presented in Figure 3. Inverted direct responses are plotted in red, relayed responces are plotted in black.

Extending Domke's work a step further, we recall that heteronuclear decoupler pulses may be calibrated using a simple 90°^1H - Δ - 90°^{13}C -acquire sequence where $\Delta = 1/2(^1J_{CH})$ [6,7]. In the experiment shown in Figure 1B, we have ^1H magnetization in the xy-plane at the end of the mixing period. From a product operator formalism [8], proton magnetization can be considered the sum of two contributing terms, one arising from the proton directly bound to the ^{13}C resonance, the other for remote protons selected by the isotropic mixing

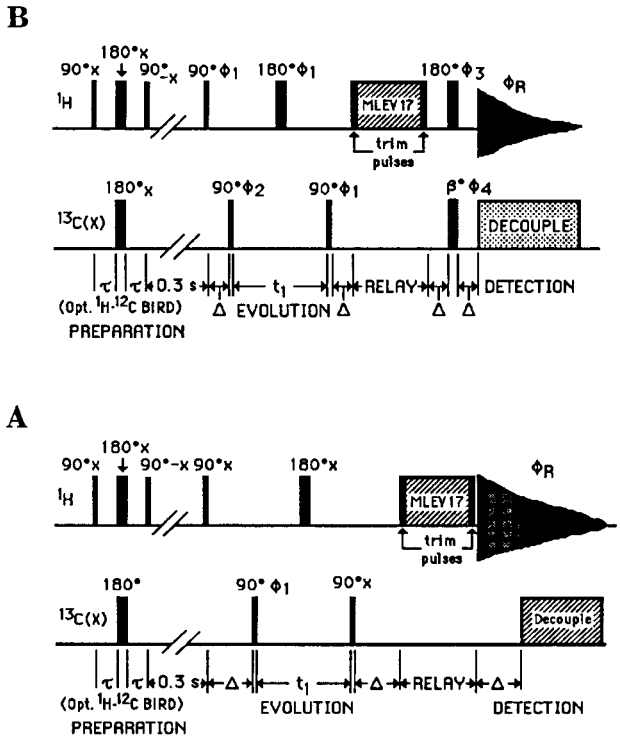
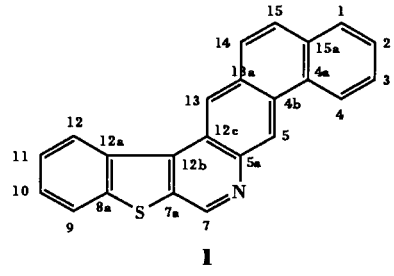


Figure 1. A) HMQC-TOCSY pulse sequence originally described by Lerner and Bax [2]. Direct responses are canceled by initiating broadband heteronuclear decoupling $\Delta = 1/2(^1J_{CH})$ after the onset of data acquisition. The portion of the FID acquired prior to beginning heteronuclear decoupling is denoted by the gray shaded area. B) IDR-HMQC-TOCSY pulse sequence to invert direct responses based on HMQC-TOCSY. The pulse sequence is patterned after the work of Domke who described the analogous HSQC-TOCSY experiment [4]. When the adjustable pulse, β is set to 180°, direct responses are inverted as in the original work of Domke. In contrast, when β = 90°, direct responses will be canceled in a fashion analogous to the procedure used to calibrate decoupler pulses [6,7]. Thus, SDR-HMQC-TOCSY is performed for β = 90°. In experiments when the direct response is to be canceled, there is no need of broadband heteronuclear decoupling during acqusiition, allowing much higher levels of digital resolution in F_2 than would otherwise be possible. Phases were cycled as follows: the relative phases of the BIRD pulse were as shown in the figure, over which was superimposed an 8 step cycle consisting of 0011 2233; ϕ_1 = 0011 2233; ϕ_2 = 0213 0213; ϕ_3 = 1122 3300; ϕ_4 = 0000 0000 2222 2222; ϕ_R = 0213 2031.

period. At the end of the mixing period, the direct component of proton magnetization is "in-phase" with respect to $^1J_{CH}$. During the first delay, Δ, which follows, the direct component of proton magnetization becomes antiphase. It was at this point, that Lerner and Bax [2] initiated broadband decoupling in their original implementation of the HMQC-TOCSY experiment (see Figure 1A). By applying a 180° ^{1}H pulse and adjusting the final carbon pulse such that β = 90°, we



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may effectively suppress direct responses. The process just described converts the observable single quantum magnetization for the direct component of proton magnetization to unobservable multiple quantum coherence. The 180° ^{1}H pulse refocuses proton chemical shift evolution after 2Δ. This idea forms the basis of the SDR-HMQC-TOCSY experiment.

An SDR-HMQC-TOCSY spectrum of **1** acquired under conditions identical to those used in the acquisition of the spectra shown in Figures 2 and 3 is presented in Figure 4. Again, direct responses would have been plotted in red if any wer observed; relayed responses are presented in black.

Since contour plots can be deceiving in terms of the threshold used to prepare the plot, it is useful to examine slices for the same carbon in all three of the TOCSY experiments performed. Traces from the various HMQC-TOCSY variants are shown in Figure 5. The proton reference spectrum of **1** is shown in trace A. The trace for the carbon resonating at 126.3 ppm from the conventional HMQC-TOCSY spectrum is shown in trace B. The corresponding trace from the IDR-HMQC-TOCSY spectrum is shown in trace C, the inverted response near 9.4 ppm obviously the direct response. Finally, the corresponding trace from the SDR-HMQC-TOCSY spectrum is shown in trace D. Only a slight vestige of the direct response remains visible, denoted by the arrow above the trace.

In conclusion, Domke [4] described a modification of the HSQC-TOCSY experiment which obviates the need to acquire a separate HMQC or HSQC spectrum to establish direct proton-carbon correlations. As shown in the present communication, the concept is equally applicable to HMQC-based pulse sequences. We have also described a further modification capable of effectively suppressing direct responses, SDR-HMQC-TOCSY. The latter method has the advantage of allowing higher levels of digitization to be employed in the F_2 frequency domain than would be possible if delayed initiation of broadband heteronuclear decoupling is used to suppress the direct response as in the original report of Lerner and Bax [2].

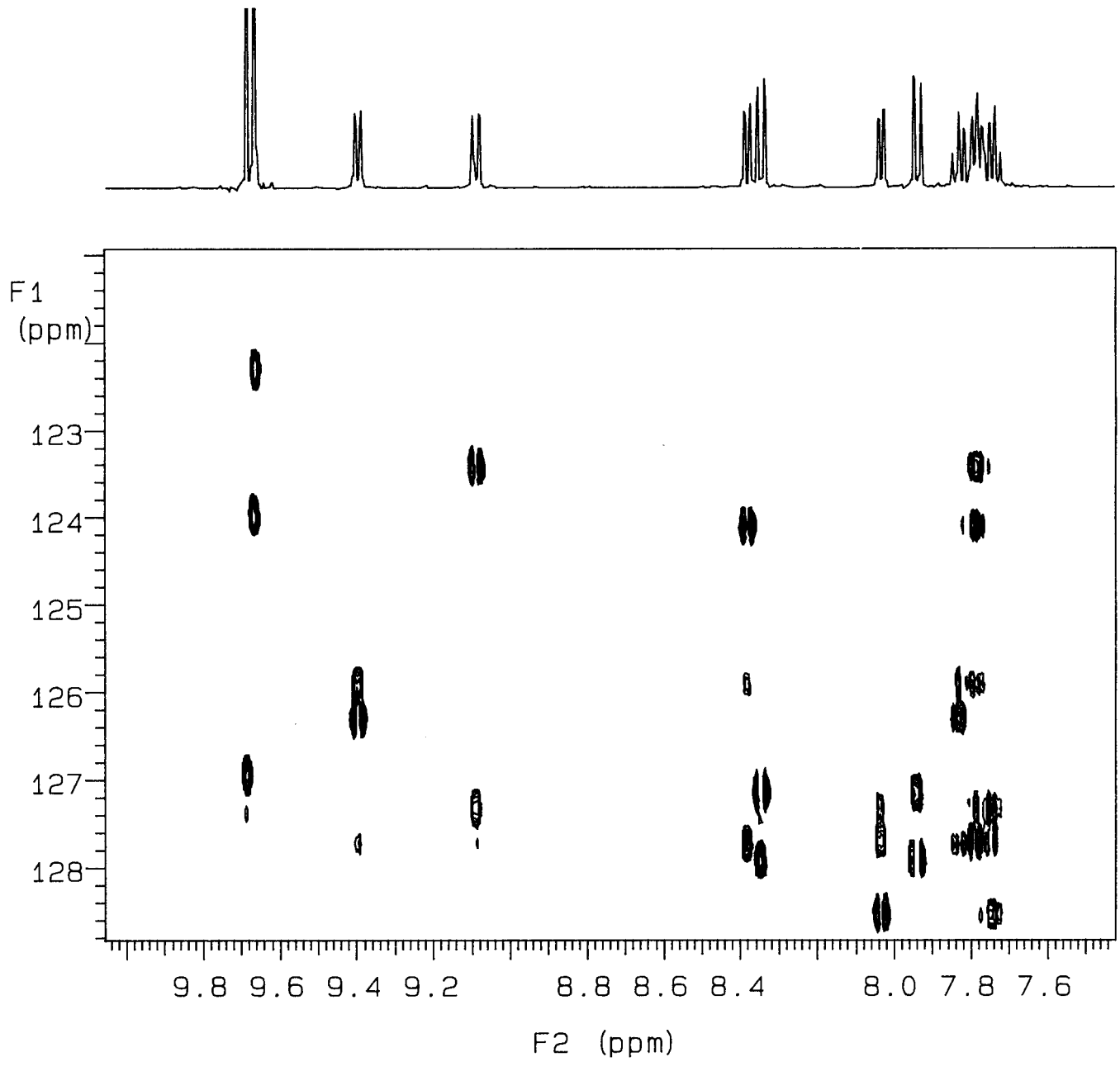

Figure 2. HMQC-TOCSY spectrum of **1** (2 mg/0.5 ml 99.96% d_6-DMSO). The data were acquired as 512 x 32 files in a phase-sensitive fashion with 64 transients/file. The mixing time was 24 msec; total accumulation time was approximately 90 minutes. Usable data were available after accumulating 8 transients/file. The spectrum was subjected to F_2 phasing and no phase correction in F_1 was performed. The response observed at $^1H \sim 9.7$ ppm, $^{13}C \sim 127$ ppm is the direct response from C7 which normally resonates at ~ 147 ppm which folded as a consequence of the F_1 spectral width chosen for the experiment.

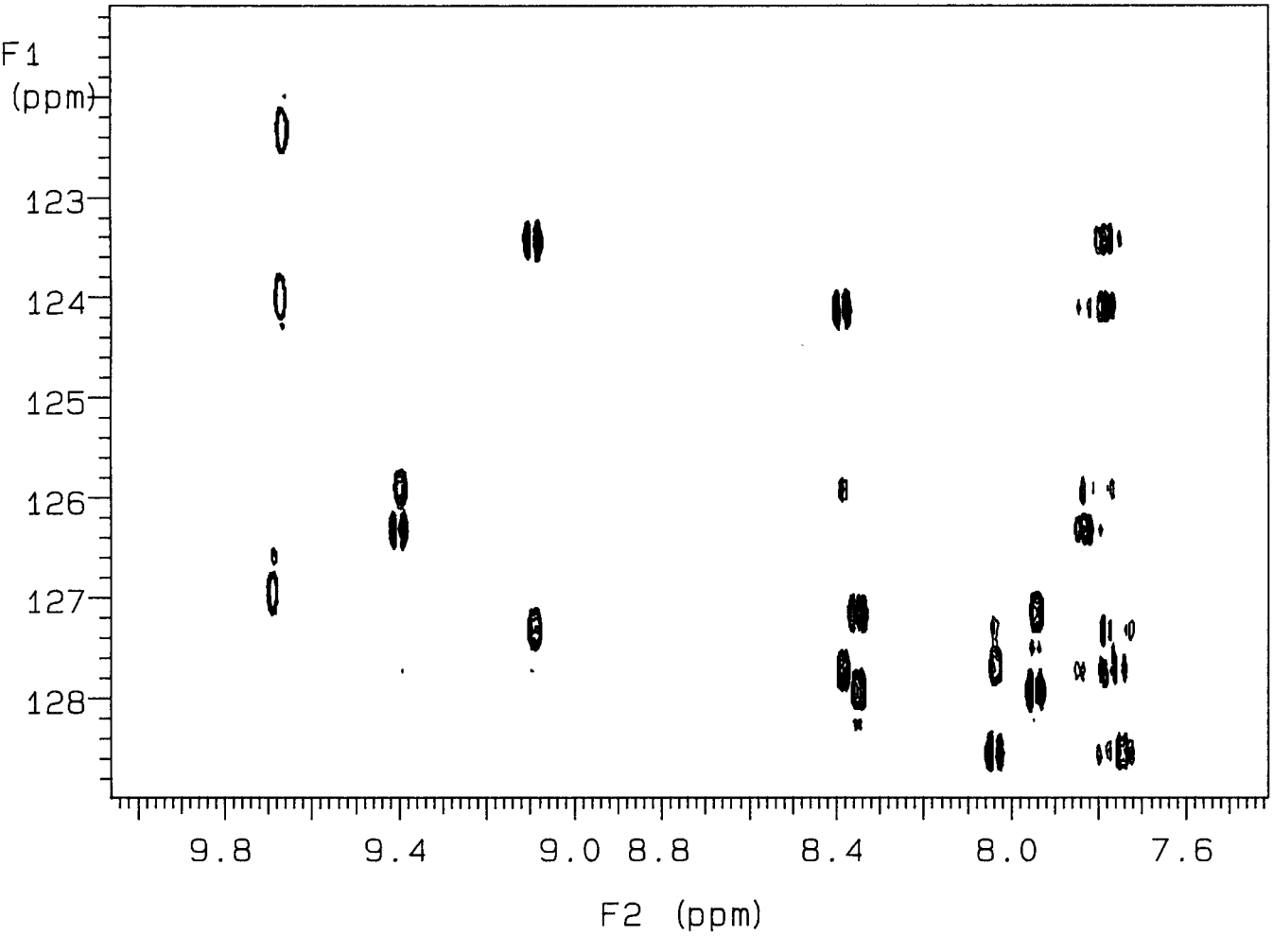

Figure 3. IDR-HMQC-TOCSY spectrum of **1**. Acquisition, processing, and presentation are identical to FIgure 2. The pulse sequence employed was that shown in Figure 1B with $\beta = 180°$. The spectrum was subjected to F_2 phasing and no phase correction in F_1 was performed. Direct responses are observed with inverted phase and are plotted in red; relayed responses have positive phase and are plotted in black. A slice comparison of the results from this experiment and the other HMQC-TOCSY experiments performed in this study is presented in Figure 5.

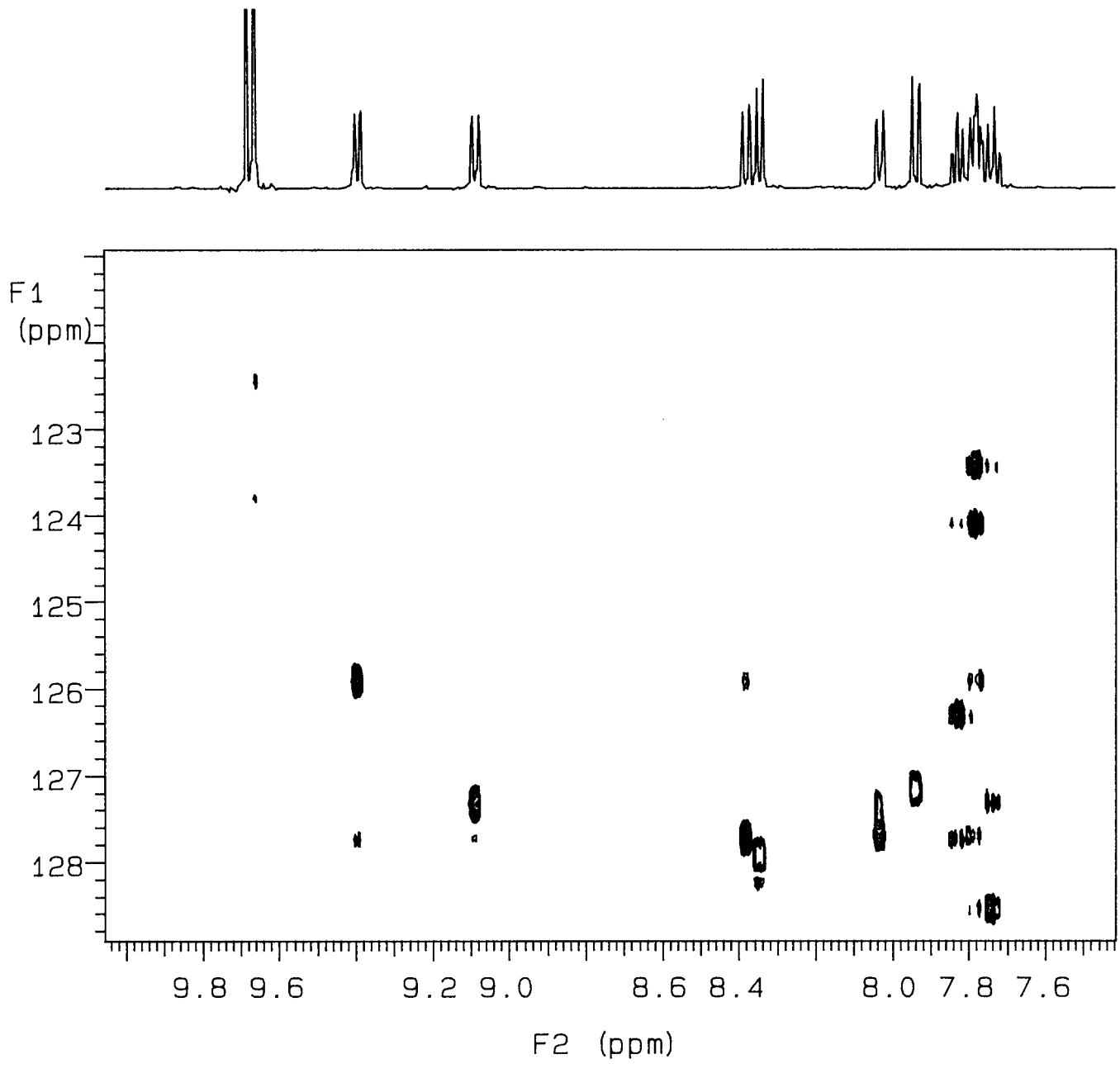

Figure 4. SDR-HMQC-TOCSY spectrum of **I**. Acquisition, processing, and presentation are identical to FIgure 2. The pulse sequence employed was that shown in Figure 1B with $\beta = 90°$. Direct responses are almost completely canceled; residual signal arising from the direct responses may appear with positive or negative intensity but, in all cases, was very weak. The spectrum was subjected to F_2 phasing and no phase correction in F_1 was performed. (For the location of the direct responses, refer to the red responses shown in Figure 3.) Relayed responses have positive phase and are plotted in black. A slice comparison of the results from this experiment and the other HMQC-TOCSY experiments performed in this study is presented in Figure 5.

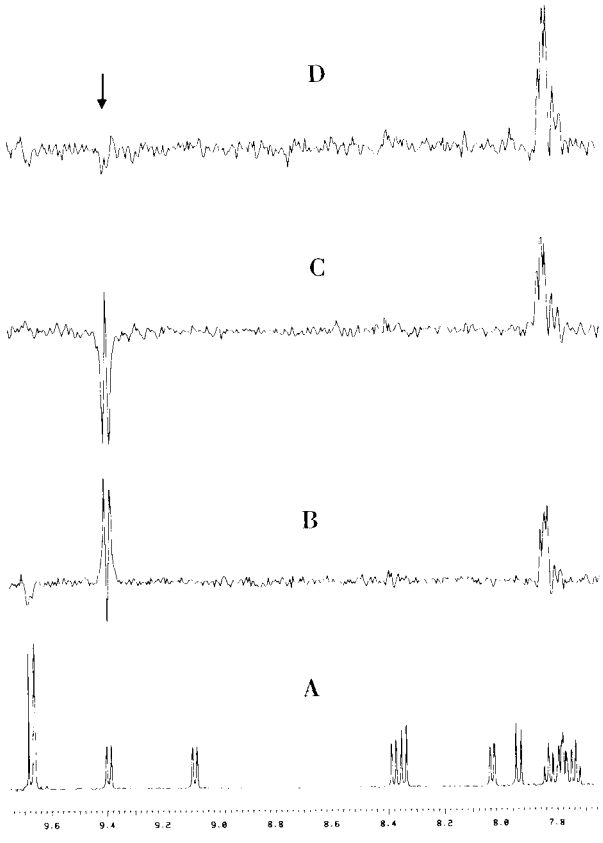

Figure 5. Comparison of the proton reference spectrum with identical slices extracted from the three HMQC-TOCSY experiments performed in this study. Trace A shows the normal high resolution proton reference spectrum. Trace B shows the slice taken at 126.3 ppm from the 24 msec HMQC-TOCSY spectrum shown in Figure 2. The direct response appears at about 9.4 ppm in this trace. Trace C shows the same slice as in B taken from the IDR-HMQC-TOCSY spectrum shown in Figure 3. The direct response is inverted in this trace and obviously identifiable. Trace D shows the corresponding slice taken from the SDR-HMQC-TOCSY spectrum presented in Figure 4. The direct response is almost completely eliminated, the residual signal denoted by the arrow above the trace.

REFERENCES AND NOTES

[1] G. E. Martin and Ronald C. Crouch, *J. Nat Prod.*, **54**, 1 (1991).

[2] L. Lerner and A. Bax, *J. Magn. Reson.*, **69**, 375 (1986).

[3] T. D. Spitzer, R. C. Crouch, G. E. Martin, M. H. M. Sharaf, P. L. Schiff, Jr., A. N. Tackie, and G. L. Boye, *J. Heterocyclic Chem.*, **28**, 2065 (1991).

[4] T. Domke, *J. Magn. Reson.*, **95**, 174 (1991).

[5] Full details of the synthesis and assignment of [1]benzothieno[2,3-*c*]naphtho[1,2-f]quinoline (**1**) and [1]benzothieno[2,3-*c*]naphtho[2,1-*g*]quinoline, also formed during the synthesis, will be reported separately.

[6] D. M. Thomas, M. R. Bendall, D. T. Pegg, D. M. Doddrell, and J. Field, *J. Magn. Reson.*, **42**, 298 (1981).

[7] A. Bax, *J. Magn. Reson.*, **52**, 76 (1983).

[8] O. W. Sørensen, G. W. Eich, M. H. Levitt, G. Bodenhausen, and R. R. Ernst, *Prog. Nucl. Magn. Reson. Spectrosc.*, **16**, 163 (1983).