Total Assignment of the ^1H and ^{13}C NMR Spectra of Benzo[*f*][1]benzothieno[2,3-*c*]quinoline by Inverse-Detected Two-Dimensional NMR Techniques

John P. Shockcor* [a], Ronald C. Crouch [b] and Gary E. Martin [b]

Divisions of Pharmacokinetics & Drug Metabolism [a]
and Organic Chemistry [b], Wellcome Research Laboratories,
Research Triangle Park, NC 27709

Abdallah Cherif

Department of Medicinal Chemistry, College of Pharmacy, University of Houston,
Houston, TX 77204-5515

Jiann-Kuan Luo and Raymond N. Castle

Department of Chemistry, University of South Florida,
Tampa, FL 33620-5250

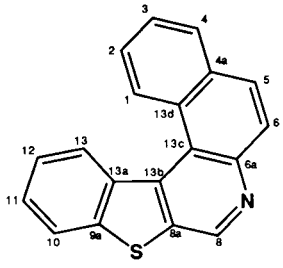
Received February 11, 1991

The proton and carbon nmr spectra of benzo[*f*][1]benzothieno[2,3-*c*]quinoline have been totally assigned using a combination of 2D nmr methods including concerted use of HMQC (heteronuclear multiple quantum correlation) and HMBC (heteronuclear multiple bond correlation) experiments.

J. Heterocyclic Chem., **28**, 2035 (1991).

Introduction.

The total nmr spectral assignment of polynuclear aromatic and heteroaromatic compounds of unknown or known structure can be a very difficult task. We have used benzo[*f*][1]benzothieno[2,3-*c*]quinoline (**1**) to demonstrate the usefulness of inverse-detected two-dimensional nmr experiments in the assignment process of these types of compounds. One of the major advantages of the inverse-detected experiments is the increased sensitivity they provide. This is critical when sample size is limited or solubility poor. In the case of **1**, we had neither of these problems. However, by using inverse-detection methods, we were able to collect all the data necessary for the total assignment of the spectra, which unequivocally confirm the structure, in less than four hours. The rate limiting step in the process now becomes the interpretation of the data, which requires more time than acquiring it when the study is not sample limited.



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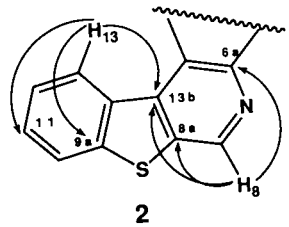
Discussion.

The ^1H nmr spectrum of **1**, although well resolved, shows two four spin-systems which cannot be unambiguously assigned from The COSY [1] spectrum (Figures 1 and 2). The ^{13}C nmr spectrum (Figure 3) also shows fairly good resolution, but here again, unequivocal assignment of the spectrum is not possible by inspection. We chose, as a rapid solution to this assignment problem, the concerted use of HMQC [2] and HMBC [3] which are in routine use in these laboratories. Using standard parameter sets, and a sample concentration of 4 mg/ml, both the HMQC and HMBC spectra were obtained (Figures 4 and 5). With the direct correlations established from the HMQC spectrum and the vicinal ^1H-^1H connectivities, obtained from the COSY spectrum, the solution of the assignment problem was afforded by correlations observed in the HMBC spectrum.

The singlet assigned to H8 at 9.57 ppm, on the basis of the lack of coupling and chemical shift, was the obvious place to begin the assignment. This singlet has a direct correlation to the carbon at 145.27 ppm as seen in the HMQC spectrum. Long-range couplings to this singlet were observed at 144.79 ppm, 135.42 ppm, and 134.21 ppm in the HMBC spectrum. From the structure of **1**, H8 should correlate to quaternary carbons C6a, C8a, and C13b. The weak correlation response at 135.42 ppm is as-



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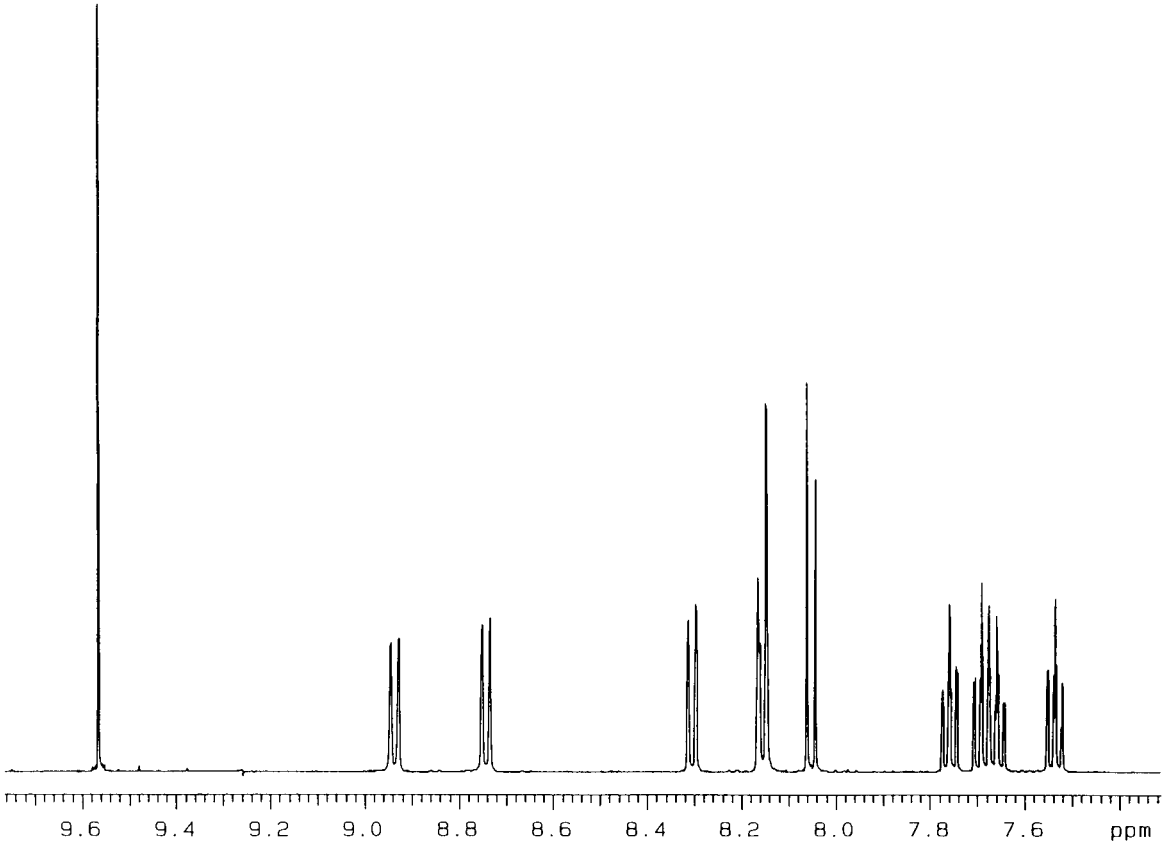


Figure 1

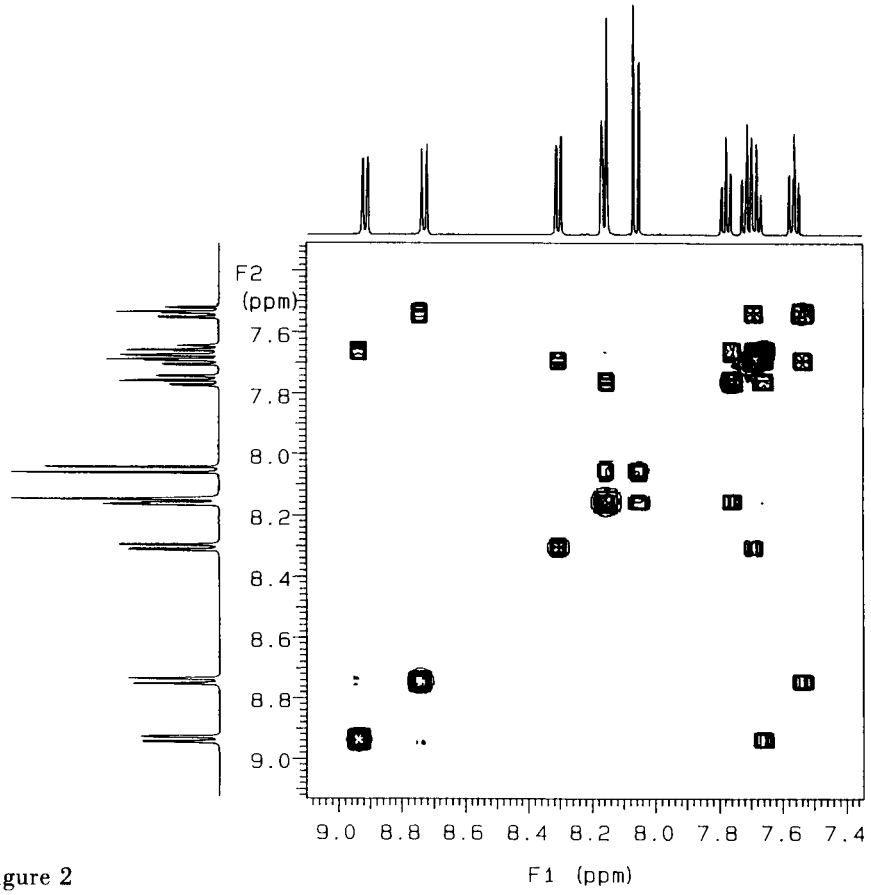


Figure 2

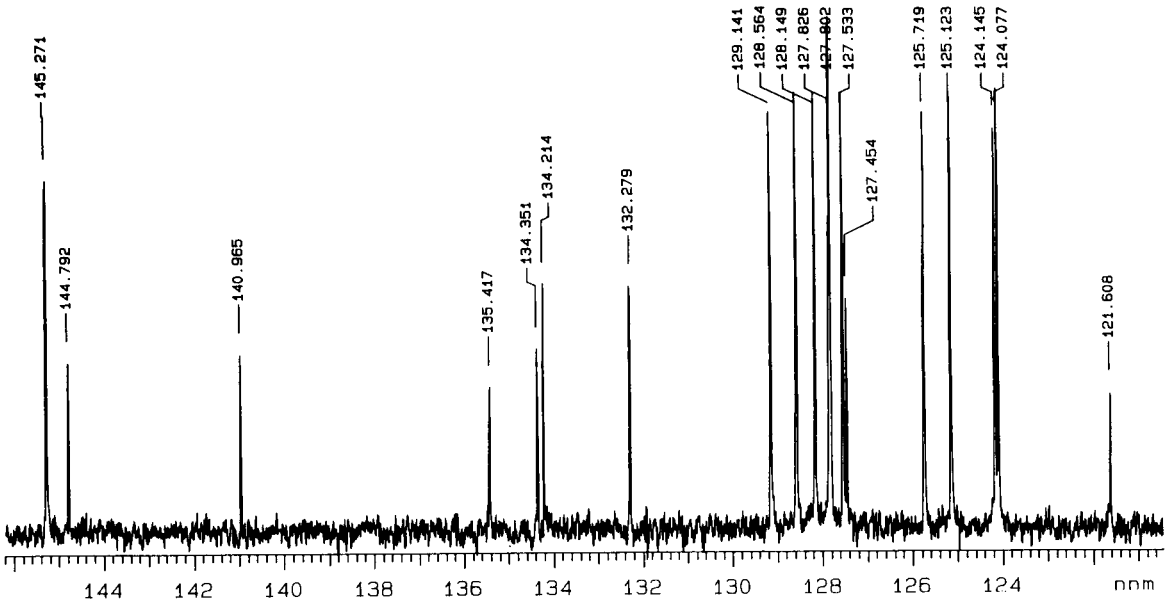


Figure 3

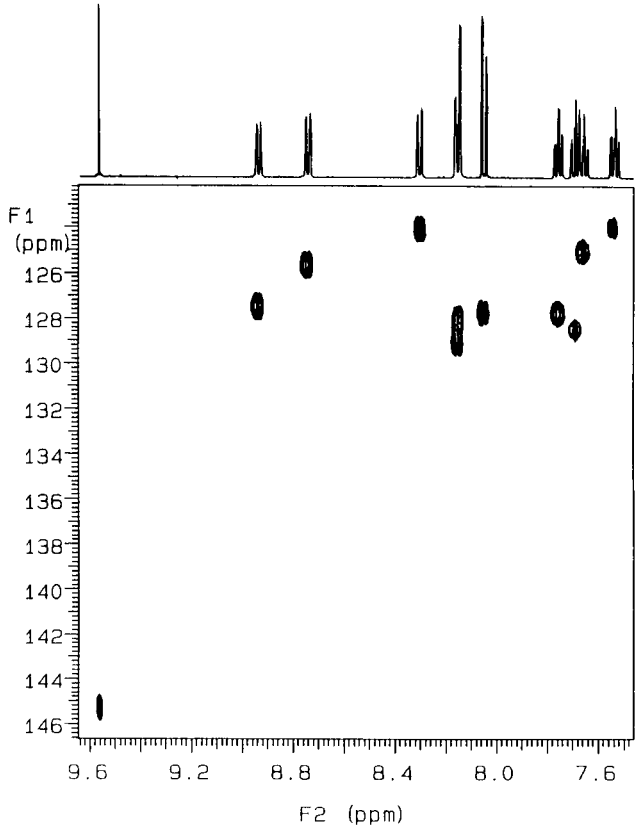


Figure 4

signed to C8a. As one would expect from structure **1**, this carbon has no other correlations to it. The correlation at 144.79 ppm is assigned to C6a on the basis of chemical shift. This leaves the correlation at 134.21 ppm, which is assigned to C13b. These correlation pathways are shown in **2**.

Table I

^{1}H and ^{13}C Chemical Shifts in ppm Relative to TMS

Position	^{1}H Shift	^{13}C Shift
1	8.94	127.53
2	7.66	125.12
3	7.76	127.83
4	8.16	128.15
4a	–	132.28
5	8.15	129.14
6	8.05	127.80
6a	–	144.79
8	9.57	145.27
8a	–	135.42
9a	–	140.97
10	8.31	124.15
11	7.69	128.56
12	7.54	124.08
13	8.74	125.72
13a	–	134.35
13b	–	134.21
13c	–	121.61
13d	–	127.45

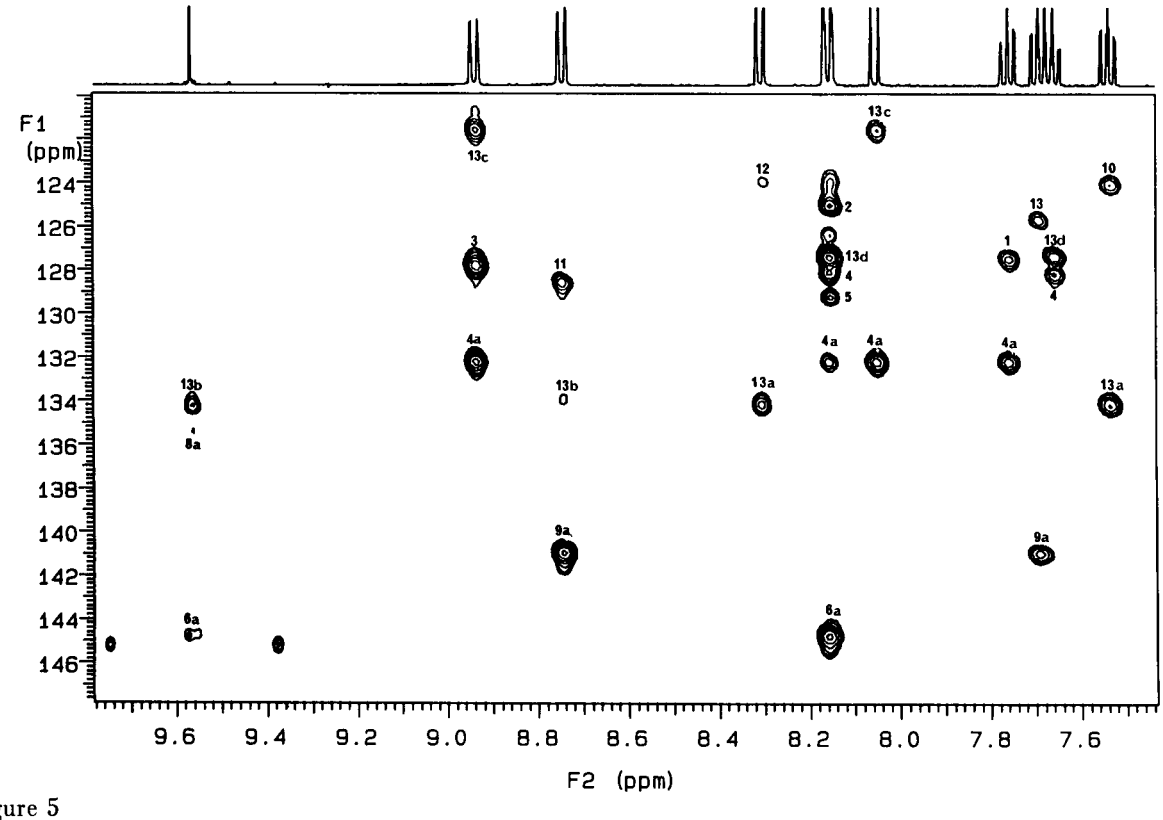


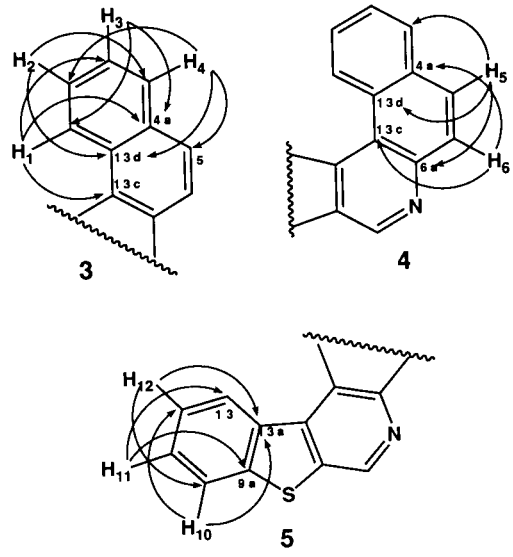
Figure 5

From **2** one can see tha C13b provides a link between the isolated singlet, H8, and the four spin-system H10, H11, H12, and H13. The quaternary C13b should show only two correlations: one to H8, as already assigned; the other to H13. The latter correlation is observed to the doublet at 8.74 ppm in the HMBC spectrum. The other correlations to H13 are to C11 at 128.56 ppm, and C9a at 140.97 ppm, as shown in **2**. At this point one can complete the assignment of the ^{1}H nmr spectrum using the data from the COSY spectrum by assuming that H1, as the other bay region proton, is the doublet resonating at 8.94 ppm.

Table II
Coupling Constants (J_{HH}) in Hz

J_{HH}	Hz
1,2	8.3
1,3	1.2
1,4	0.5
2,3	6.9
2,4	1.5
3,4	8.0
5,6	8.9
10,11	8.1
10,12	1.2
10,13	0.6
11,12	7.0
11,13	1.2
12,13	8.3

With the proton spectrum assigned, the remaining quaternary carbons can be easily assigned. For example, the doublet at 8.94 ppm, assigned as H1, shows direct correlation in the HMQC spectrum to C1 at 127.53 ppm, with long-range correlation in the HMBC spectrum to C13c at 121.61 ppm, C3 at 127.83 ppm, and C4a at 132.28 ppm. The remainder of the quaternary carbons were assigned in similar fashion. These correlation pathways are shown in **3**, **4**, and **5** and the correlations are labeled on the HMBC



spectrum (Figure 5). The ^1H and ^{13}C nmr chemical shifts and J_{HH} coupling constants are presented in Tables I and II, respectively.

Conclusions.

With a now nominal amount of time and effort, it is possible to completely assign complex nmr spectra using HMQC and HMBC. These techniques should not be viewed as methods applicable only when sample concentration is low. The reduction in time, and the excellent quality of the data obtainable from these experiments, makes them valuable routine tools for many structural problems.

EXPERIMENTAL

The ^1H nmr spectrum of **1** was obtained in DMSO-d_6 at 25° on a Varian VXR-500S spectrometer operating at a frequency of 499.984 MHz. The data were collected over a 1478 Hz spectral width using 32K data points (10.829 sec acquisition time) and zero-filled to 64K. The rf pulse employed was 8.3 μsec (60°). The relaxation delay was 0.2 sec. The ^{13}C nmr spectrum was obtained in DMSO-d_6 at 25° on a Varian VXR-300S spectrometer operating at a frequency of 75.426 MHz. The data were collected over a 3020 Hz spectral width using 8K data points (1.356 sec acquisition time) and zero-filled to 16K. The rf pulse employed was 7.0 μsec (55°). The relaxation delay was 1.0 sec. The spectrum was proton-decoupled using WALTZ modulation with a field strength (γH_2) of 3000 Hz.

The COSY spectrum was obtained using the standard COSY pulse sequence [1]. The spectrum was acquired as 512 x 256 data points, and was zero-filled and subjected to sinusoidal multiplication prior to both Fourier transforms to afford a final 1024 x 1024 point data matrix. The data were symmetrized prior to plotting. The spectral width was 1478 Hz in F_1 and F_2 with an acquisition time of 0.173 sec. The ^1H 90° pulse width was 13.3 μsec. A 1.0 sec interpulse delay was employed.

The HMQC spectrum was obtained using the pulse sequence described by Bax and Subramanian [2]. The refocusing delay was optimized to 160 Hz (3.1 msec). The "null" delay following the BIRD pulse was 300 msec. The spectrum was acquired using the TPPI method for phase-sensitive acquisition as 512 x 256 data points, and was zero-filled and subjected to apodization with a Gaussian function prior to both Fourier transforms to afford a 1024 x 1024 point data matrix. Spectral widths were 3070 Hz in F_1 (^{13}C) and 1478 Hz in F_2 (^1H). The ^1H 90° degree pulse width was 13.3 μsec. The ^{13}C 90° pulse width was 11.0 μsec. A 0.9 sec interpulse delay was employed. WALTZ ^{13}C decoupling was employed during acquisition with a field strength (γH_2) of 3000 Hz. Total experiment time was 0.6 hours.

The HMBC spectrum was obtained using the pulse sequence described by Bax and Summers [3]. The low-pass J-filter portion (Δ_1) of the experiment was optimized for an average one-bond heteronuclear coupling of 160 Hz (3.1 msec). The long-range delay (Δ_2) utilized to excite the heteronuclear multiple quantum coherence was optimized for 10 Hz (50 msec). The spectrum was acquired as 512 x 256 data points, was zero-filled and subjected to apodization with a Gaussian function prior to both Fourier transforms to afford the 1024 x 1024 point final data matrix. Spectral widths were 10,056 Hz in F_1 (^{13}C) and 1478 Hz in F_2 (^1H). The ^1H 90° pulse width was 13.3 μsec. The ^{13}C 90° pulse width was 11.0 μsec. A 1.6 sec interpulse delay was employed. Total experiment time was 1.6 hours.

REFERENCES AND NOTES

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