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Benzo[*b*]phenanthro[4,3-*d*]thiophene: Spectral Assignment by Two-Dimensional NMR Methods and Tertiary Structure Determination

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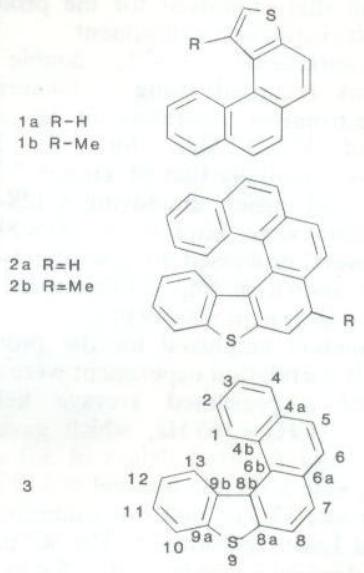
Two-dimensional heteronuclear chemical shift correlation, heteronuclear relayed coherence transfer and autocorrelated $^{13}C—^{13}C$ double quantum coherence have been used to assign the proton and ^{13}C NMR spectra of benzo[*b*]phenanthro[4,3-*d*]thiophene. The highly complementary nature of the heteronuclear relay and carbon–carbon double quantum spectra conveniently provides the means of avoiding breaks in the carbon–carbon connectivity network assembled by the latter, which may arise when strongly AB rather than AX carbon–carbon pairings are encountered. Nuclear Overhauser difference spectra have shown the title compound to possess a tertiary helical structure similar to that of phenanthro[3,4-*b*]thiophene; the average separation between the H-1 and H-13 atoms of the title compound was found to be 2.06 ± 0.04 Å.

KEY WORDS Heteronuclear relayed coherence transfer Carbon–carbon double quantum coherence Two-dimensional NMR 2D NMR Polynuclear aromatics

INTRODUCTION

Heterocyclic analogs of polynuclear aromatic systems, by virtue of the many similar chemical environments possible in such molecular structures, possess ^{1}H and ^{13}C NMR spectra which may be difficult to assign unambiguously. These difficulties are largely alleviated, however, by the application of two-dimensional NMR techniques which have been successfully utilized in the total assignment of phenanthro[1,2-*b*]thiophene,[1] phenanthro[3,4-*b*]thiophene (**1a**)[2] and its 1-methyl analog **1b**,[3] phenanthro[4,3-*a*]dibenzothiophene (**2a**)[4,5] and its 9-methyl analog **2b**.[6] Assignment strategies employing heteronuclear proton–carbon chemical shift correlation[7,8] in concert with heteronuclear relayed coherence transfer[9–12] have made it possible to map the vicinal proton–proton connectivity networks even in the case of ^{1}H NMR spectra which are far too congested to allow the successful utilization of the COSY experiment, such as in **2a** and **2b**.[4–6] Finally, orientation of the heteronuclear spin systems relative to one another may be accomplished either by



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^{13}C–^{13}C double quantum coherence[13–19] as in the case of **1**[2] and **2a**,[4] or via long-range heteronuclear chemical shift correlation (two to four bonds). Such was the case in a study of **2b**[6] where the quaternary carbons were also assigned in the process. Since the total assignment and tertiary solution structure of **1**[2] and the assignments of **2a** and **2b**[3–6] and the crystal structure of **2b**[20] have been determined, we now report the total assignment and tertiary solution structure of benzo[*b*]phenanthro[4,3-*d*]thiophene (**3**), the intermediary homolog of this series.

EXPERIMENTAL

All experiments reported, with the exception of the high-resolution ^1H NMR spectrum plotted as a reference below the heteronuclear relay spectrum, were recorded using a 0.75 M solution of **3** in $CDCl_3$. The high-resolution proton spectrum plotted along the F_1 axis of the heteronuclear relayed coherence transfer spectrum shown in Fig. 1 was acquired at 300 MHz using a 0.025 M degassed solution in $CDCl_3$. All experiments were performed at 290 K. The proton–carbon chemical shift correlation and heteronuclear relayed coherence transfer experiments were recorded using a Nicolet NT-300 wide-bore spectrometer operating at observation frequencies of 300.068 and 75.459 MHz for ^1H and ^{13}C, respectively. The proton–carbon chemical shift correlation experiment employed the pulse sequence and the 16-step phase cycle of Bax and Morris;[8] the coherence transfer echo was acquired.[21] The data were acquired as $128 \times 1K$ complex points in a period of 6 h and were zero filled during processing to afford a final matrix consisting of $256 \times 1K$ real points. The heteronuclear relayed coherence transfer experiment was recorded using the pulse sequence of Bax[12] with a 32-step phase cycle, the details of which have been reported previously.[4] Data accumulation took 14 h and the processing was identical with that employed for the proton–carbon chemical shift correlation experiment.

The autocorrelated ^{13}C–^{13}C double quantum spectrum was recorded using a General Electric GN-500 spectrometer operating at an observation frequency of 125.762 MHz for ^{13}C. The pulse sequence was essentially that of Turner,[15,16] modified with a 135° read pulse[17] employing a 128-step phase cycle. The data were acquired as $30 \times 8K$ complex points and were processed to give the $64 \times 4K$ real point matrix shown in Fig. 2. The total acquisition time for the experiment was 19 h.

The parameters employed for the proton–carbon chemical shift correlation experiment were selected on the basis of a presumed average heteronuclear coupling of $^1J(CH) = 165$ Hz, which gave durations from the Δ_1 and Δ_2 fixed delays of 3.0 and 2.0 ms, respectively, with a recycle interval of 1 s. The 90° ^{13}C pulse length was 17.5 μs and was calibrated using the procedure of Lawn and Jones.[22] The 90° proton pulse from the decoupler coils was 25.4 μs and was determined from $1/4(\gamma H_2/2\pi)$. For the heteronuclear relayed coherence transfer experiment, the Δ_1 and Δ_2 delays were set as in the proton–carbon chemical shift correlation experiment, except that the duration of the Δ_1 delay was halved because of the 180° ^{13}C pulse applied midway through this interval. The mixing interval ($\tau_m/2$) was set to $1/10\ ^3J(HH) = 14.3$ ms for an assumed vicinal proton–proton coupling constant of 7 Hz. The experiment was recycled using a 1 s interpulse delay. The 90° pulse length on the GN-500 spectrometer used to perform the carbon–carbon double quantum experiment was 17.5 μs.[22] Finally, the autocorrelated ^{13}C–^{13}C double quantum employed a delay of $1/4J(CC) = 5.0$ ms based on an assumed ^{13}C–^{13}C coupling of 50 Hz, a 135° read pulse[17] and an interpulse delay of 3 s.

The nuclear Overhauser effect (NOE) data (300 MHz) were collected as an interleaved data set (4 acq./irradiation frequency × 100) employing a reference irradiation 600 Hz downfield of the lowest field resonance and irradiations at both the H-1 and H-13 resonances. The irradiation was performed for a period of 7 s with an interpulse delay of 7 s. Difference spectra were computed using a 3 Hz exponential apodization prior to subtraction. Data were collected in triplicate, the calculated interproton distance reported as the average of the six independent measurements of the NOE value observed between H-1 and H-13.

RESULTS AND DISCUSSION

Structurally, **3** would be expected to have a tertiary helical structure similar to that of **1a**,[2] **1b**[3] and **2b**.[20] The ^1H NMR spectrum consists of two ABMX and two AB spin systems, with H-1 and H-13 projecting into a bay region and consequently shifted downfield. One-bond proton–carbon chemical shift correlations were established using a proton–carbon chemical shift correlation experiment[7,8] providing the identities of the direct responses labeled on the heteronuclear relayed coherence transfer spectrum shown in Fig. 1. Carbon–hydrogen pairings of the two ABMX systems (H-1–H-4 and H-10–H-13) and one of the AB pairs (H-7–H-8) are readily established from the heteronuclear relayed coherence transfer experiment, despite the fact that the H-7 resonance is heavily overlapped by the H-5–H-6 AB system.[3–6,10–12] The remaining AB spin system consisting of the H-5–H-6 pair is less readily pinpointed in Fig. 1. Specifically, the H-5–H-6 AB pair is very strongly non-first order, so much so that the relay responses are obscured by the direct responses.

Whereas the heteronuclear spin systems are readily delineated using the heteronuclear relayed coherence transfer experiment, ambiguity in the assignments still exists since the spin systems cannot be readily distinguished from one another, nor, in the case of the AB pairs, can they be oriented relative to the other spin systems. Orientation of the spin systems relative to one another may be unequivocally established using either long-range heteronuclear chemical shift correlation[6] or the carbon–carbon double quantum experiment.[2,5] However, the carbon–carbon double quantum experiment also has inherent limitations. For

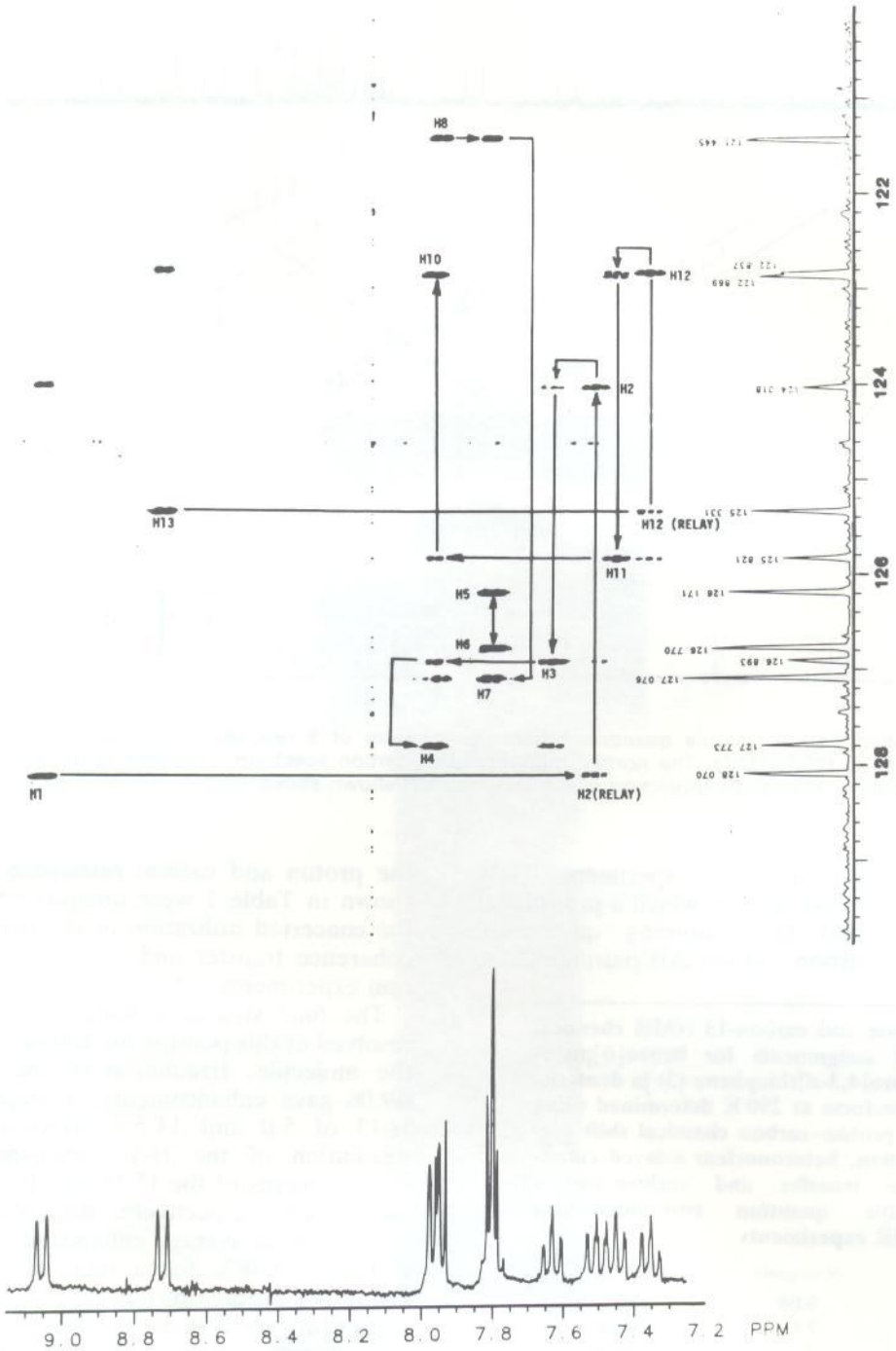

Figure 1. Contour plot of the heteronuclear relayed coherence transfer NMR spectrum of benzo[*b*]phenanthro[4,3-*d*]thiophene (**3**) recorded in deuteriochloroform at 290 K at ^{1}H and ^{13}C observation frequencies of 300.068 and 75.459 MHz, respectively. A normal high-resolution proton reference spectrum is plotted along the F_1 axis and a projected carbon spectrum is plotted along the F_2 axis. Direct proton–carbon correlations were established by the prior acquisition of a conventional proton carbon heteronuclear chemical shift correlation spectrum.

example, in the case of **3**, the carbon–carbon double quantum spectrum shown in Fig. 2 fails to establish the C-5–C-6 connectivity (δ126.13 and 126.77) because of the AB character of this pair even at 125 MHz. Importantly, the heteronuclear relayed coherence transfer and carbon–carbon double quantum experiments are mutually complementary in nature. Although the protonated carbons may have carbon–carbon AB character, thereby leading to a potential break in the connectivity in the carbon–carbon double quantum spectrum, it will generally be possible to establish this link through the heteronuclear relayed coherence transfer spectrum as in the case of the C-5–H-5/C-6–H-6 system described above. Conversely, the inability of the heteronuclear relayed coherence transfer experiment to orient the spin systems relative to one another and to assign the quaternary carbon resonances is circumvented by the

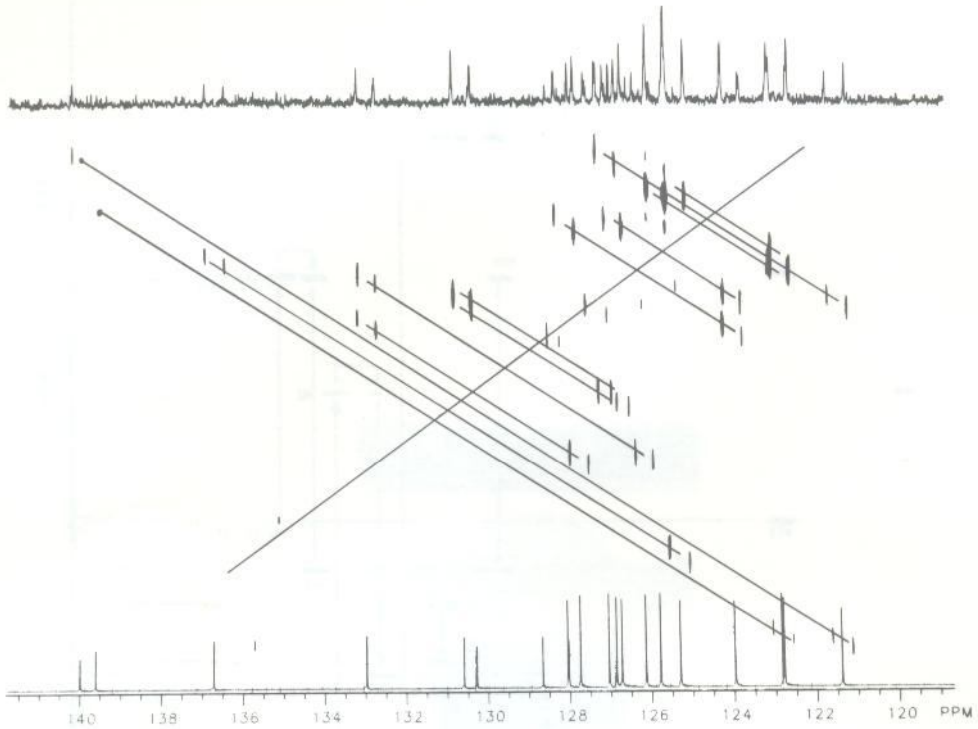

Figure 2. Autocorrelated $^{13}C-^{13}C$ double quantum coherence spectrum of **3** recorded in deuteriochloroform at 290 K and an observation frequency of 125.762 MHz. The normal high-resolution carbon spectrum is plotted below the contour plot and the $^{13}C-^{13}C$ coupled spectrum obtained by projection of the data matrix is shown above.

carbon–carbon double quantum experiment. Only very rarely would the case arise in which a protonated carbon resonance and the adjoining quaternary carbon consist of a carbon–carbon AB pairing. Thus, the proton and carbon resonance assignments for **3** shown in Table 1 were unequivocally established via the concerted utilization of the heteronuclear relayed coherence transfer and carbon–carbon double quantum experiments.

Table 1. Proton and carbon-13 NMR chemical shift assignments for benzo[*b*]phenanthro[4,3-*d*]thiophene (3) in deuteriochloroform at 290 K determined using the proton–carbon chemical shift correlation, heteronuclear relayed coherence transfer and carbon–carbon double quantum two-dimensional NMR experiments

Position	$\delta(^1H)$ (ppm)	$\delta(^{13}C)$ (ppm)
1	9.06	128.07
2	7.51	123.97
3	7.63	126.86
4	7.97	127.74
4a	—	132.94
4b	—	128.65
5	7.80	126.13
6	7.77	126.77
6a	—	130.57
6b	—	128.02
7	7.79	127.08
8	7.94	121.45
8a	—	139.96
8b	—	130.26
9a	—	139.58
9b	—	136.68
10	7.97	122.83
11	7.45	125.82
12	7.36	122.79
13	8.73	125.33

The final structural feature of **3** remaining to be resolved at this point is the tertiary helical structure of the molecule. Irradiation of the H-1 resonance at δ9.06 gave enhancements, as expected, at H-2 and H-13 of 5.0 and 14.5%, respectively. Conversely, irradiation of the H-13 resonance at δ8.73 gave enhancements of the H-12 and H-1 resonances of 5.3 and 13.4%, respectively. Repetitions of the experiment gave an average enhancement between H-1 and H-13 of 13.48% for a total of six determinations. Using the average distance (2.42 Å) between the bay region protons and their vicinal neighbors determined crystallographically for **2b**[20] and hexahelicene[23] as a model for the H-1–H-2 and H-13–H-12 distances, the calculated average H-1····H-13 distance was 2.06 ± 0.04 Å, which is slightly greater than the 1.99 Å determined using the same technique for **1a**.[2] Based on the congruence between the solution (2.10 Å) and crystallographically determined separation (2.12 Å) of the bay region substituents of **1b**,[3] it is probable that the distance computed between H-1 and H-13 for **3** is reasonably accurate. Interestingly, the separation between the bay region protons of **3** is very considerably smaller than that of **2b** at 3.18 Å.[20] Further work will be necessary to address the question of the sudden increase in the separation between the bay region protons in going from **1** or **3**, which are essentially the same, to **2b**, in which the distance increases by approximately 1.2 Å.

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