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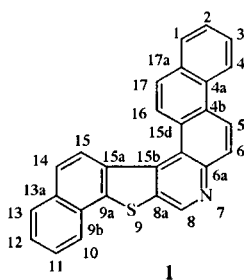
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Received January 10, 2000**Dedicated to the Memory of Professor Raymond N. Castle**

The assignment of the NMR spectra of the polynuclear heteroaromatic naphtho[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-c]quinoline is reported. The analysis was based on the homonuclear ROESY, heteronuclear direct GHSQC, IDR-GHSQC-TOCSY, and long-range GHMBC experiments. The complete ^1H and ^{13}C shift assignments are reported.

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The assignment of the proton and carbon NMR resonances of the polynuclear heteroaromatic naphtho[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-c]quinoline (**1**) provided a significant challenge due to spectral overlap in both frequency domains. In general, assignment strategies for molecules such as **1** begin by focusing on the establishment of the proton-proton connectivity network.



These correlations are subsequently expanded to provide concordance between the protons and carbons to which they are directly bound. Once the heteronuclear spin systems have been established, they must be correctly oriented relative to one another, and linked *via* the quaternary carbons and/or heteroatoms to assemble the larger structural fragments. In the context of assigning the NMR spectra of a synthetically prepared molecule, this task generally amounts to correctly attributing and orienting the various spin systems to the intended target molecular skeleton. For example, in the present case there are three two-spin and two four-spin systems comprising the naphthalene- and phenanthrene-based portions of the molecule. Locating these five different spin systems in the

correct position and orientation within the skeletal framework of the molecule is an integral facet of assigning the spectra. However, in the case of an unknown molecule, such as a natural product, the problem is somewhat more challenging in that the skeletal framework of the molecule must be simultaneously deduced during the spectral assignment process.

In the present case, one of the two four-spin systems must first be associated with the naphthalene-derived portion of the molecule, after which it must be oriented within the skeletal framework relative to the correct two-spin system. For the phenanthrene-derived portion of the molecule, the four-spin system must be correctly oriented relative to the two different two-spin systems. The required orientation process can be accomplished *via* through space correlations using *nOe* (nuclear Overhauser effect) or *rOe* (rotating Overhauser effect) connectivities, or *via* common long-range heteronuclear couplings to the intervening quaternary carbon resonances. Both experiments are viable for this task, with the latter required in assigning the quaternary carbon resonances.

Conventionally, we sought first to establish the proton-proton connectivity network from a homonuclear experiment since these are, by far, more sensitive. However, the extensive overlap of the five proton resonances near 7.8 ppm (even at 600 MHz, see the ^1H reference spectrum plotted above Figure 1) precluded the successful utilization of homonuclear COSY (CORrelated SpectroscopY) or TOCSY (TOTal CORrelated SpectroscopY) experiments. The problem was further exacerbated by the additional overlap of the pairs of resonances at 8.52 and 8.07 ppm.

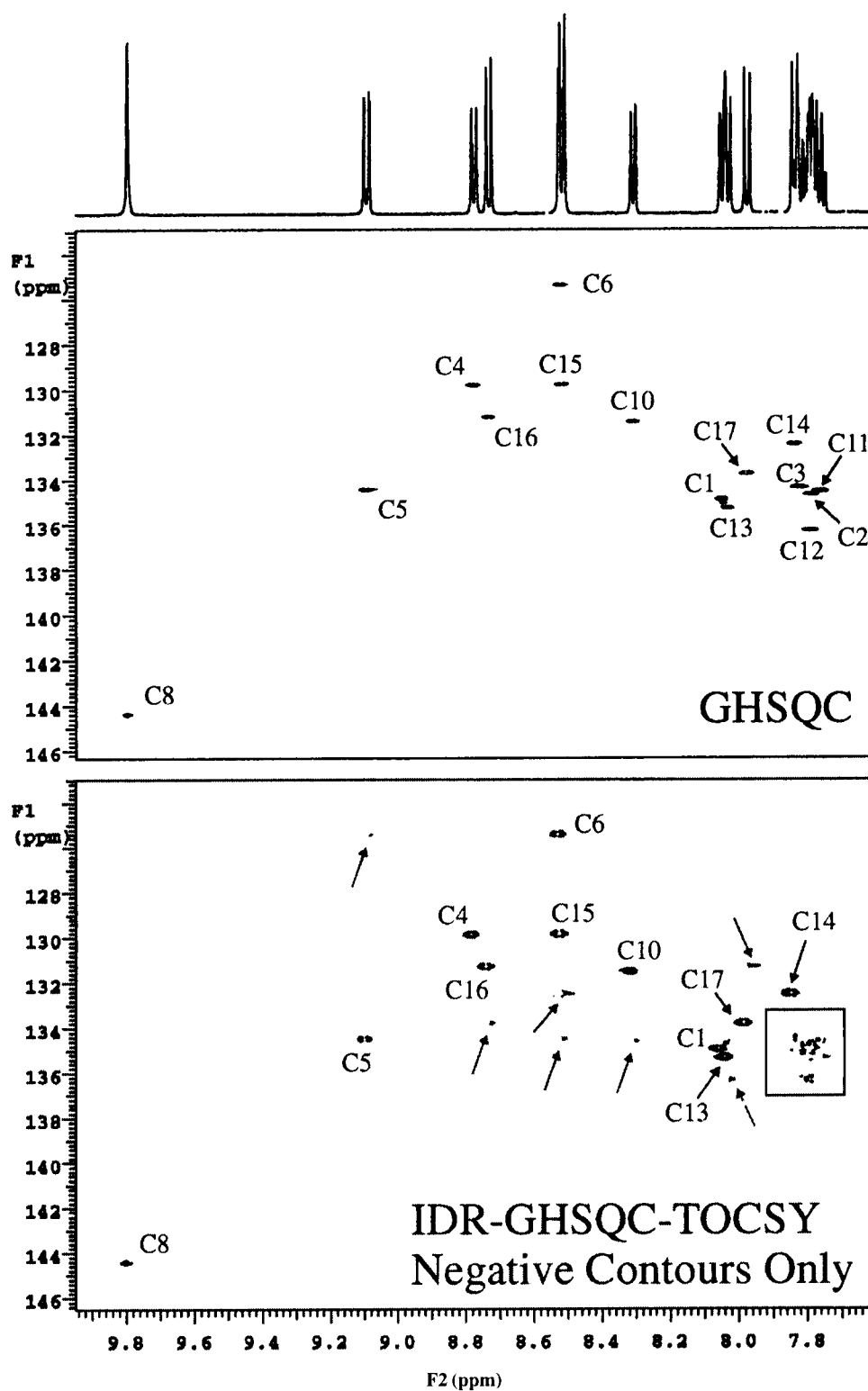


Figure 1. Direct ^1H - ^{13}C correlations for **1**. Top panel: conventional GHSQC. Bottom panel: negative, direct responses from the IDR-GHSQC-TOCSY spectra, with a mixing time of 24 msec. The tremendous overlap in both frequencies for both experiments is obvious, making necessary the acquisition of 160 files, affording just under 30 Hz per point resolution prior to linear prediction. The forward prediction of 480 points (three times the number of increments) afforded 7 Hz per point resolution, and allowed the separation of the congested areas. The boxed area in the bottom panel is more difficult to interpret. Phase irregularities (denoted with red arrows) of the responses require care to be exercised when interpreting the direct responses in the IDR-GHSQC-TOCSY in lieu of the conventional GHSQC.

Each site of overlap provided a source of potential ambiguity and discontinuity of the proton-proton connectivity network during the spectral assignment. Furthermore, the inability to correctly assign the central spins of a four-spin system can lead to the potential misassignment of the quaternary carbon resonances from the long-range heteronuclear data, since these resonances are normally assigned from correlations to the central proton resonances of four-spin systems.

An alternative approach when dealing with highly congested spectra involves the use of either the IDR-GHMQC-TOCSY or IDR-GHSQC-TOCSY experiments [1,2]. These are inverse-detected 2D NMR experiments that first establish the direct correlation between a proton and its directly bound carbon either by a multiple (GHMQC-, or Gradient Heteronuclear Multiple Quantum Coherence) [3] or single quantum (GHSQC-, or Gradient Heteronuclear Single Quantum Coherence) coherence [4]. After magnetization is refocused, it can be propagated *via* vicinal scalar coupling pathways during an isotropic

mixing period analogous to that in a TOCSY experiment. In this fashion, vicinally coupled protons are associated, and the ensemble is sorted by the chemical shift of the directly bound carbon. The GHSQC- portion of the experiment [5-9] establishes the direct ^1H - ^{13}C correlations first, followed by the establishment of the vicinal connectivity network in the -TOCSY portion of the experiment. The overall difference relative to conventional homonuclear experiments, obviously, is that the proton-proton connectivity information in the second frequency domain is sorted as a function of the carbon chemical shift. Since the spectral dispersion of carbon is generally much greater than that of proton, the likelihood of avoiding overlaps and thereby establishing the needed connectivities is correspondingly much higher in the hyphenated heteronuclear experiment than its simple homonuclear counterpart.

The IDR- (Inverted Direct Response) portion of this experiment inverts the phase of the direct responses [10], simplifying interpretation. Plotting only the negative

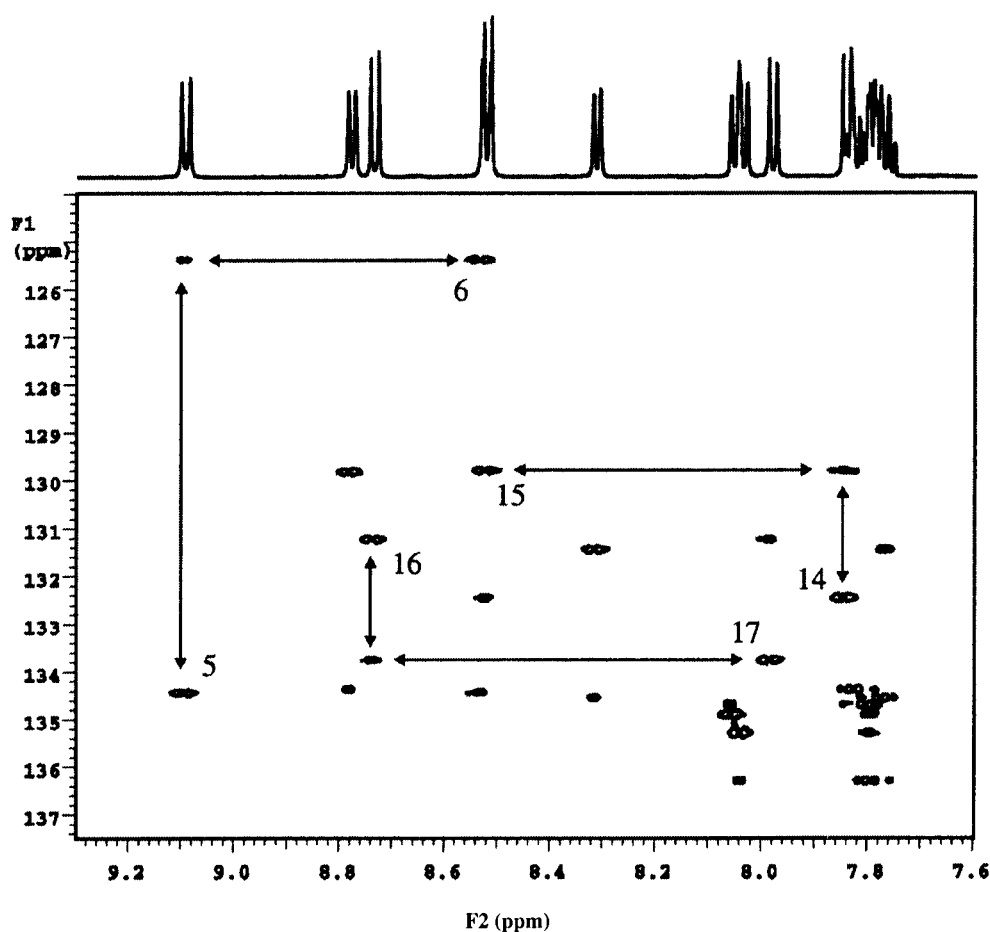


Figure 2. IDR-GHSQC-TOCSY spectrum of 1. The IDR- portion inverts the direct responses, shown in red, from the cross-correlations, shown in black. The three two-spin systems were readily identifiable, and have been labeled. Tracing from the red direct response through the black correlation and to the adjacent direct response, the vicinal proton neighbor can be identified. The opposite correlation is also observed, but was not annotated in the contour plot.

phase, direct responses nominally affords the chemical shifts of the directly bound proton-carbon pairs as shown in Figure 1. Inverted direct responses clearly identify the majority of the protonated carbons in the molecule. The boxed region of the spectrum was problematic. Care must also be taken not to misinterpret phase irregularities of some of the relayed responses as direct correlation response artifacts, denoted by red arrows in Figure 1. The conventional GHSQC data are also included in Figure 1 as a comparison for the IDR-GHSQC-TOCSY data, however, these data were not acquired prior to characterization. The negative, direct responses facilitate the interpretation of the full IDR-GHSQC-TOCSY data. The congestion in F_2 at ~ 7.8 ppm, with a total of five protons resonating in a region of the ^1H spectrum less than 0.1 ppm in width, is

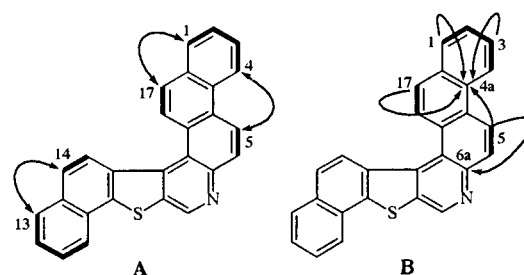


Figure 3. Key correlations necessary for the placement of the spin systems onto the molecular skeleton. ROESY correlations (A) easily distinguished the assignments of the naphthalene system with the correlation of H13 to H14. The phenanthrene system, however, needed both the ROESY (A) and GHMBC (B) correlations to correctly orient the spin systems to the molecule.

Table 1

^1H and ^{13}C Chemical Shift Assignments of **1**, in CDCl_3 , with a Drop of TFA. The Observed Long-range Couplings from the 10 Hz Optimized GHMBC are Given as the Positional Proton Correlated to the listed carbon. Weak Long-range Correlations in the GHMBC Spectrum are Denoted by (w).

Position	^1H	^{13}C	Long-Range GHMBC
1	8.05	134.9	3, 4a, 17, 17a(w)
2	7.79	134.7	4, 17a
3	7.83	134.4	1, 4(w), 4a
4	8.78	129.8	2, 4b, 17a
4a	--	135.6	
4b	--	136.7	
5	9.09	134.4	4a, 6a, 4b(w), 15c(w), 15d
6	8.52	125.4	4b, 15c, 15d(w)
6a	--	141.8	
8	9.80	144.4	6a, 8a, 15b, 15c(w)
8a	--	139.0	
9a	--	153.4	
9b	--	134.3	
10	8.31	131.4	9a, 9b(w), 12, 13a
11	7.76	134.5	9b, 13, 13a(w)
12	7.79	136.3	10, 13(w), 13a
13	8.04	135.3	9b, 11, 13a(w), 14
13a	--	139.5	
14	7.84	132.5	9a(w), 9b, 13, 13a, 15a
15	8.52	129.8	9a, 9b(w), 13a, 15a(w), 15b
15a	--	137.6	
15b	--	149.0	
15c	--	129.1	
15d	--	131.8	
16	8.74	131.2	4(w), 4a(w), 4b, 15c, 15d(w),
17	7.98	133.8	1, 4a, 4b(w), 15d, 17a
17a	--	139.1	

partially removed as three of these five closely spaced resonances are well resolved in the second frequency domain. Responses for the closely spaced resonance pairs at 8.06 and 8.52 ppm are also reasonably resolved in F_1 .

Interpreting the entire IDR-GHSQC-TOCSY spectrum, Figure 2, identified all of the proton-proton connectivity networks. The direct responses are again plotted in red. The correlation for H8/C8 was not included in the contour plot, as it is an isolated resonance and thus exhibited no

relayed correlations. The three two-spin systems were easily identified, and are annotated in the contour plot. Sorting the remaining correlations afforded the proton/carbon pairs of the two four-spin systems as 8.05/134.9, 7.79/134.7, 7.83/134.4, 8.78/129.8, and 8.31/131.4, 7.76/134.5, 7.79/136.3, 8.04/135.3.

Orienting the heteronuclear spin systems extracted from the IDR-GHSQC-TOCSY experiment pair-wise on the molecular skeleton was easily accomplished *via* the

concerted analysis of the homonuclear ROESY (Rotating Overhauser Enhanced Spectroscopy) and a 10 Hz optimized long-range GHMBC (Gradient Heteronuclear Multiple Bond Correlation) experiment. As shown by Figure 3, only a few key ROESY through space and GHMBC long-range through bond correlations were necessary to orient the spin systems relative to the molecular skeleton. Other than the expected ROESY correlations within individual spin systems, key correlations were observed from the terminal proton of one of the four-spin systems resonating at 8.05 ppm to a resonance at 7.98 ppm contained in a two-spin system. The corresponding opposite terminal proton resonance of the same four-spin system at 8.78 ppm was correlated *via* a ROESY response to a resonance at 9.09 ppm in a second two-spin system. These two key ROESY correlations connected the three spin systems adjacent. The appropriate four-spin and two two-spin systems can only fit into the phenanthrene nucleus of the molecular skeleton, identifying the spin systems as H1-H4, H5-H6, and H16-H17.

The ROESY correlations did not, however, orient the three associated spin systems relative to the molecular framework. Fortunately, there was a single carbon that exhibited long-range GHMBC correlations (Figure 3B) to four proton resonances in the three spin systems. The carbon resonance at 135.6 ppm exhibited key three-bond correlations to proton resonances at 8.05, 7.83, 9.09, and 7.98 ppm. This carbon could only be C4a, and identified the proton resonances as H1, H3, H5, and H17, respectively, and appropriately oriented the systems to the phenanthrene nucleus.

Rotating all three spin systems 180 degrees and maintaining the same skeletal framework does not result in an equivalent set of correlation possibilities. After rotation, the key ROESY correlations (H1-H17 and H4-H5) would remain the same, and H5 and H17 would still long-range correlate *via* 3-bonds to C4a. H1 and H3, however, would have the less probable 2- and 4- bond correlations, respectively, to C4a. Additionally, H5 exhibited a correlation to C6a at 141.8 ppm, which is a unique chemical shift representative of a carbon adjacent to a pyridine nitrogen. The H5/C6a correlation irrefutably oriented the three spin systems in the molecular framework of **1**.

Of the remaining two spin systems for the naphthalene nucleus, only a single ROESY correlation (Figure 3a) was needed to orient the spin systems relative to one another and to the molecular skeleton. A correlation was observed from the terminal proton resonance of the four-spin system at 8.04 ppm to the two-spin resonance at 7.84 ppm, identifying the protons as H13 and H14. The assignments for the remaining ^1H and ^{13}C resonances are listed in Table 1.

EXPERIMENTAL

NMR data were acquired at natural abundance on a Varian INOVA 600 MHz, three channel NMR spectrometer with 28 channel Oxford shims, operating at a ^1H observation frequency of 599.75 MHz, equipped with a Nalorac Z•SPEC MIDTG-600-3 (micro inverse-detected triple resonance gradient) probe. Naphtho[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-c]quinoline (**1**) was synthesized by standard photocyclization methods. Details of the synthesis and of the title compound and related congeners will be reported separately. The sample was prepared in a dry box under argon gas, dissolved in 150 μL CDCl_3 (99.996 %, Isotec), and transferred to a Wilmad 3mm NMR tube. The sample was sparingly soluble in deuteriochloroform, which was counteracted by the addition of one drop of TFA to the 300 μL ampule of deuterated solvent prior to sample prep.

Assignments were accomplished *via* homonuclear ROESY, IDR-GHSQC-TOCSY, GHSQC, and GHMBC experiments. The IDR-GHSQC-TOCSY was acquired with a mixing time of 24 msec, the ROESY with a mixing time of 300 msec, and the GHMBC was optimized for an assumed 10 Hz long-range coupling. Standard pulse sequences from the vendor-supplied pulse sequence library were employed without modification. The 90° pulses were calibrated using standard methods, and were as follows: 7.30 μs at 48 dB (63 max) for ^1H , and 13.0 μs at 59 dB (63 max) for ^{13}C .

REFERENCES AND NOTES

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