

Note

Heteronuclear editing method for detecting NOE between chemically equivalent protons: application to polycyclic aromatic hydrocarbons

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ABSTRACT: The complete characterization of polycyclic aromatic hydrocarbons (PAHs) and tetrasubstituted ethylenes is critical to an understanding of their reactivity, for which NMR is an important tool. Chemical shifts can provide a direct measure of charge distribution and aromaticity. Unfortunately, COSY, NOESY and heteronuclear correlation cannot provide a complete assignment of the NMR spectra for some carbon-rich PAHs with symmetrical bay regions. The protons in the bay regions would yield NOE signals if they were not symmetrical. Natural substitution of ¹²C with ¹³C can be used to break the symmetry and yield these useful NOE signals. Using gradient-assisted editing, unequivocal assignments have been achieved for some previously problematic molecules. Copyright © 2000 John Wiley & Sons, Ltd.

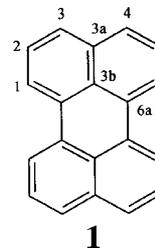
KEYWORDS: NMR; ¹H NMR; ¹³C NMR; NOE; polycyclic aromatic hydrocarbons; assignment of symmetrical polycycles

INTRODUCTION

The study of polycyclic aromatic hydrocarbons (PAHs) is of interest with respect to synthetic,^{1–3} environmental and biological chemistry. In the environment, PAHs act as pollutants produced by the combustion of fuels and other organic materials.⁴ In biology they bind to DNA via enzymatic bay region activation, causing mutations and cancer. The steric crowding of the bay region protects the attack on the DNA from detoxification increasing the mutagenic and carcinogenic activity. In synthetic chemistry, carbon-rich PAHs are an emerging class of compounds that are analogs for fullerenes, graphite and as yet undiscovered but theoretically possible allotropes of carbon.⁵

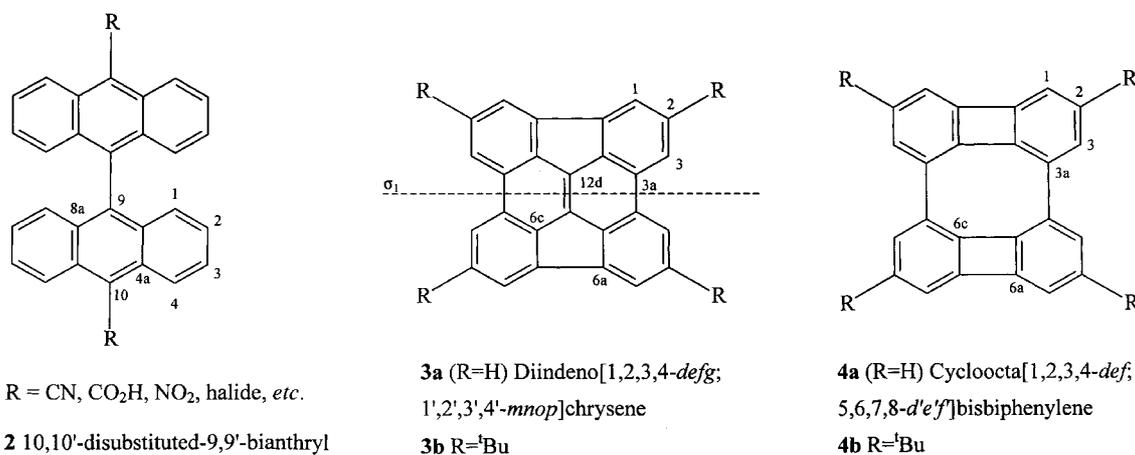
NMR spectra of these compounds are usually assigned by a combination of NOESY⁶ (or ROESY), COSY, heteronuclear single quantum coherence (HSQC) [or heteronuclear multiple quantum coherence (HMQC)] and heteronuclear multiple bond coherence (HMBC). NOESY yields correlations between neighboring rings and often the combination of COSY and NOESY is sufficient to assign the ¹H NMR spectrum. The carbon spectrum requires the use of HSQC (or HMQC) and HMBC. Where COSY and NOESY are insufficient to assign the ¹H

NMR spectrum, the HMBC experiment may complete it. An example of this is perylene (**1**) where the assignments of H-1 and H-3 are uncertain. HMBC optimized for couplings of 10 Hz usually yields three-bond correlations strongly and other correlations much more weakly in PAHs. C-4 (identical with C-3) will correlate with H-3 whereas C-1 will not correlate with H-1.



However, these techniques cannot provide a complete assignment for some carbon-rich compounds with symmetrical bays. 10,10'-Disubstituted 9,9'-bianthryl (**2**) (Scheme 1) is one such example. Its ¹H NMR spectrum contains four multiplets. However, it is impossible to differentiate between H-1 and H-4 or H-2 and H-3 by the above methods without resorting to assumptions about chemical shifts or relaxation times. Even HMBC yields correlations compatible with either assignment: H-1 correlates with C-3, C-4a and C-9 while H-4 correlates with C-2, C-9a and C-10. Much less sensitive methods such as 2D INADEQUATE⁷ are required. In many cases,

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Scheme 1

solubility or compound availability preclude the use of such an insensitive technique.

There are also cases such as diindeno[1,2,3,4-*defg*;1',2',3',4'-*mnop*]chrysene (**3a**) and cycloocta[1,2,3,4-*def*;5,6,7,8-*d'e'f'*]bisbiphenylene (**4a**) where the symmetry of the compound is such that even INADEQUATE is of no use. The HMBC correlations across the bays for H-1 and H-3 have the same pattern and no INADEQUATE correlations are observed across the symmetry plane σ_1 of the molecule. This is in addition to the extreme difficulty in preparing sufficient compound for a 2D INADEQUATE experiment and the impossibility of getting enough into solution.

Despite the above, nature supplies us with built-in ¹³C isotopic substitution, that allows NOESY ¹²C-¹H/¹³C-¹H cross peaks between otherwise identical protons to be recorded. Previous work has described HMQC-ROESY⁸ and HSQC-NOESY⁹ experiments that can yield such correlations. We have now developed a modified version of these experiments, carbon-edited NOESY, which is more sensitive and usually requires less acquisition time.

METHOD

Carbon-edited NOESY is a combination of a regular NOESY and a carbon-edited spectrum. During the evolution period (t_1), all the signals are present while only those coupled to carbon are recorded during acquisition (t_2). The resulting signals are then carbon decoupled in f_2 leaving the carbon coupling in f_1 . The result is that the carbon satellites excited in f_1 are again active in f_2 and yield a 'double diagonal' split in f_1 by the ¹H, ¹³C coupling constant (~158 Hz for PAHs). A signal arising from a proton attached to a ¹²C that has a NOESY correlation with an identical proton attached to a ¹³C will appear on the 'true' diagonal, half-way between the 'double diagonal' signals.

A number of techniques are available for carbon editing. The most common ones are experiments based on single-quantum (SQ) and multiple-quantum (MQ) coherences.^{10,11} Both methods are amenable to sensitivity improvement by refocusing and combining two in-phase proton magnetization components.¹¹ The sensitivity

improvement (SI) method that may be applied to either SQ or MQ theoretically yields a 41% improvement in sensitivity if the relaxation time is long. In practice, a yield of 35% sensitivity improvement is routinely obtainable. If the relaxation time is short (several milliseconds or less), sensitivity improvement will not work and may even cause a reduction in sensitivity.

For carbon-edited NOESY we implemented both types of carbon editing. We found the SQSI method to be preferable owing to increased sensitivity except when cross correlation artifacts overlapped the signals of interest. In these cases, there are two options: not to use sensitivity improvement or to suppress carbon-coupled signals during evolution. The latter method suppresses the double diagonal leaving only the required cross peaks. Any remaining cross relaxation artifacts are much weaker than the cross peaks.

The pulse sequence (Fig. 1) consists of a NOESY where the last pulse is replaced by a carbon editing sequence. The homospoil in the NOESY sequence is optional. It helps to reduce artifacts when there is a suspicion that transverse correlations will persist throughout the mixing time. The type of editing used is chosen based on the above considerations. Two scans per transient are required

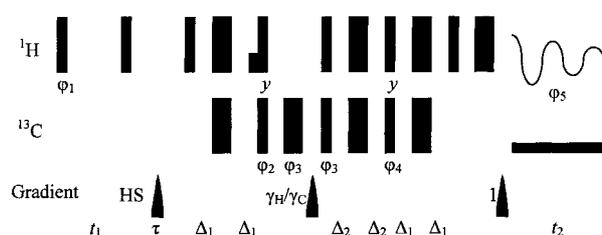
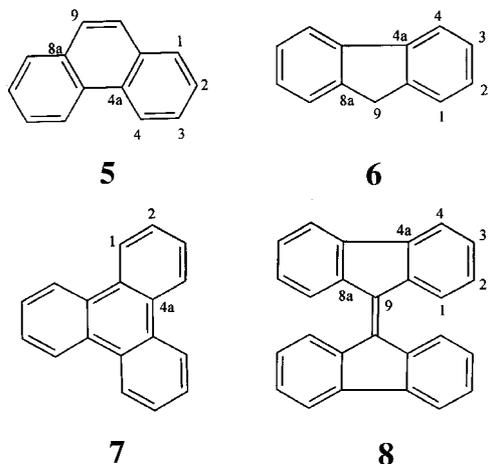


Figure 1. Pulse sequences for carbon-edited NOESY with single-quantum transfer and sensitivity improvement. Narrow rectangles are 90° pulses and wide rectangles are 180° pulses; the shorter rectangle is a spin-lock, τ = mixing time, $\Delta_1 = 1/4J(\text{C,H})$ (~3.2 ms), $\Delta_2 = 1/4J(\text{C,H})$ for CH only and $1/8J(\text{C,H})$ for all multiplicities, $\gamma_{\text{H}}/\gamma_{\text{C}} = 3.98$, HS is a homospoil (~5 G cm⁻¹ for 1 ms). $\varphi_1 = x, -x, x, -x$ (incremented by 90° each row); $\varphi_2 = x, x, -x, -x$; $\varphi_3 = x, x, x, x, -x, -x, -x, -x$; $\varphi_4 = y, y, y, y, -y, -y, -y, -y$; $\varphi_5 = x, -x, -x, x, -x, x, x, -x$; minimum phase cycle of four.

for quadrature detection in f_1 . A further two scans are advised to suppress residual signals from protons attached to ^{12}C because of incomplete suppression of the signals by the gradients.

RESULTS AND DISCUSSION

We tested the experiment with phenanthrene (**5**), fluorene (**6**) and triphenylene (**7**) because each one contains one of the symmetrical bay types found in **3**. The inter-proton distance across the bays in **5** and **7** should be 0.17 nm and in **6** 0.40 nm assuming planarity (calculated from standard bond lengths and bond angles). In reality, the distances are probably slightly different owing to possible distortions from the plane. The NOE correlations across the bays could be observed for **5** and **7** within 1 h whereas 15 h acquisition (at 400 MHz) was required to observe the correlation for **6** under the same conditions. As expected, the **6** correlation was much weaker than the **5** and **7** correlations.



Our assignment of bifluorenylidene (**8**) using HMBC was confirmed by carbon edited NOESY.

Compounds **3** and **4** defy assignment by any of the other methods listed, including 2D-INADEQUATE, owing to its symmetry combined with the isolation of its ^1H spin systems by number of bonds and symmetry. Compounds **3a** and **4a** can only be assigned directly by carbon-edited NOESY. The same problems apply to 2,5,8,11-tetrasubstituted derivatives of **3a** and **4a** such as 2,5,8,11-tetra-*tert*-butyl-**3a** (**3b**) and 2,5,8,11-tetra-*tert*-butyl-**4a** (**4b**). In **3** and **4**, the distance between H-1 and H-12 is considerably larger than that between H-3 and H-4. As a result, the NOE signal between H-3 and H-4 is expected to be nearly two orders of magnitude stronger than that between H-1 and H-12 because the NOE is proportional to the inverse sixth power of the distance. The carbon-edited NOESY spectra of **3b** and **4b** show only one correlation across a bay region that we assigned to H-3, H-4. The spectrum of $\mathbf{4b}^{2-}2\text{Li}^+$ showed two correlations, one much stronger than the other (Fig. 2). We assigned the stronger signal to H-3, H-4 and the weaker one to H-1, H-12. This assignment was then used to complete the

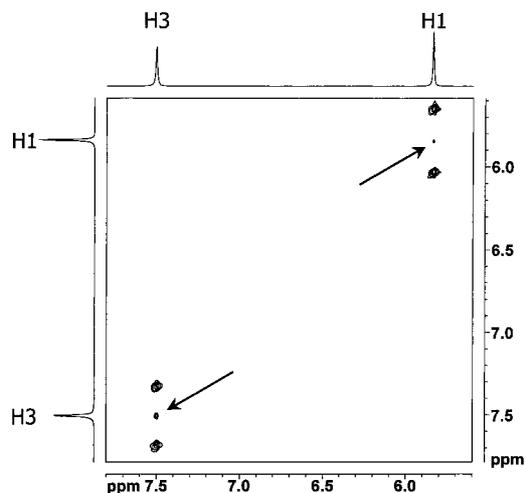
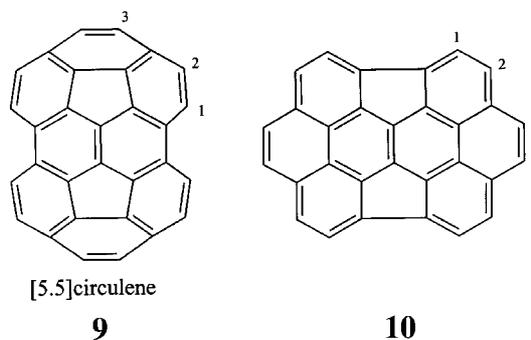


Figure 2. Carbon-edited NOESY of 2,5,8,11-Tetra-*tert*-butylcycloocta[1,2,3,4-*def*;5,6,7,8-*d'e'f'*]bisbiphenylene dianion with lithium $\mathbf{4b}^{2-}2\text{Li}^+$. The arrows point to the carbon-edited NOESY correlations.

assignment of the ^{13}C spectrum with the aid of HSQC and HMBC (see Experimental).

We expect that the carbon-edited NOESY method will also find application in distinguishing *Z*- and *E*-isomers of tetrasubstituted symmetrical ethylenes of the type $\text{RR}'\text{C}=\text{CRR}'$ including such important classes of compounds as phenylenevinylens. This is a long-standing problem in synthetic organic chemistry that at present can only be resolved by x-ray crystallography or by a stereospecific chemical modification, followed by enantiomeric excess measurement.¹²

Carbon-edited NOESY may also find an application in distinguishing large symmetrical PAHs such as [5.5]circulene¹³ (**9**) from its isomer **10**.



EXPERIMENTAL

All materials were commercial samples except for **3a**,¹⁴ **3b** and **4b**,¹⁵ which were synthesized as reported.

NMR experiments were carried out at 298.5 ± 0.5 K [except perylene (**1**) that was heated to 453 K to overcome low solubility and $\mathbf{4b}^{2-}2\text{Li}^+$ at 220 K] using a Bruker DRX-400 spectrometer equipped with a BGUII *z*-gradient. The materials (up to 20 mg depending on solubility) were dissolved in acetone- d_6 with the exception of **1** (nitrobenzene- d_5), **3b** (CD_2Cl_2), **4b** ($\text{THF}-d_8$)

and **5** (CDCl₃) and degassed under vacuum using the freeze–pump–thaw technique. The reported chemical shifts of **4b** and **4b**²⁻Li⁺ were calibrated to the downfield THF signal (δ_{H} 3.575; δ_{C} 67.394), whereas the chemical shifts of **3b** (CD₂Cl₂ solution) were referenced to internal TMS. Our data for **5**, **6** and **7** are in accord with those of earlier investigations, and for **8** the previous assignment is now corrected (verified by the standard 2D NMR experiments).^{16–18}

¹H and ¹³C assignments

2,5,8,11-Tetra-tert-butyl-diindeno[1,2,3,4-defg;1',2',3',4'-mnop]chrysene (3b). δ_{H} (CD₂Cl₂, 298.5 K) 1.44 (Me, s, 36H), 7.91 (H-1, d, *J* 0.9 Hz, 4H), 8.18 (H-3, d, *J* 0.9 Hz, 4H); δ_{C} (CD₂Cl₂, 298.5 K) 31.08 (Me), 35.25 (^tBu), 119.38 (C-1), 119.39 (C-3), 132.55 (C-3a), 136.84 (C-12d), 137.27 (C-6c), 140.38 (C-6a), 151.84 (C-2).

2,5,8,11-Tetra-tert-butylcycloocta[1,2,3,4-def;5,6,7,8-d'e'f']bisbiphenylene (4b). δ_{H} (THF-*d*₈, 298.5 K) 1.22 (Me, s, 36H), 6.49 (H-1, d, *J* 1.2 Hz, 4H), 6.77 (H-3, d, *J* 1.2 Hz, 4H); δ_{C} (THF-*d*₈, 298.5 K) 31.0 (Me), 35.8 (^tBu), 116.1 (C-1), 118.9 (C-3), 125.9 (C-3a), 147.5 (C-6c), 151.6 (C-6a), 153.4 (C-2).

2,5,8,11-Tetra-tert-butylcycloocta[1,2,3,4-def;5,6,7,8-d'e'f']bisbiphenylene dianion with lithium 4b²⁻Li⁺. Compound **4b** and lithium wire were introduced into an extended NMR tube under argon. The sample was evacuated and predried THF-*d*₈ was vacuum transferred into the tube. The tube was sealed with a flame under vacuum. δ_{H} (THF-*d*₈, 220 K) 1.40 (Me, s, 36H), 5.84 (H-1, s, 4H), 7.50 (H-3, s, 4H); δ_{C} (THF-*d*₈, 220 K) 32.3 (Me), 35.8 (^tBu), 95.1 (C-1), 99.2 (C-3a), 115.6 (C-3), 123.7 (C-6c), 130.6 (C-2), 152.5 (C-6a).

Phenanthrene (5). δ_{H} (CDCl₃, 298.5 K) 7.67 (H-2, t, *J* 7.3 Hz, 2 H), 7.72 (H-3, t, *J* 7.5 Hz, 2 H), 7.81 (H-9, s, 2 H), 7.96 (H-1, d, *J* 7.6 Hz, 2 H), 8.74 (H-4, d, *J* 8.0 Hz, 2 H); δ_{C} (CDCl₃, 298.5 K) 122.5 (C-4), 126.4 (C-2), 126.5 (C-3), 126.8 (C-9), 128.4 (C-1), 129.8 (C-4a), 131.6 (C-8a).

Fluorene (6). δ_{H} (acetone-*d*₆, 298.5 K) 3.90 (H-9, s, 2 H), 7.30 (H-2, t, *J* 7.4 Hz, 2 H), 7.37 (H-3, t, *J* 7.4 Hz, 2 H),

7.57 (H-1, d, *J* 7.4 Hz, 2 H), 7.86 (H-4, d, *J* 7.6 Hz, 2 H); δ_{C} (acetone-*d*₆, 298.5 K) 36.08 (C-9), 119.46 (C-4), 128.68 (C-1), 126.34 (C-2), 126.34 (C-3), 141.22 (C-4a), 142.88 (C-8a).

Triphenylene (7). δ_{H} (acetone-*d*₆, 298.5 K) 7.70 (H-2, AA'XX', 6 H), 8.79 (H-1, AA'XX', 6 H); δ_{C} (acetone-*d*₆, 298.5 K) 124.1 (C-1), 128.1 (C-2), 130.4 (C-4a).

Bifluorenylidene (8). δ_{H} (acetone-*d*₆, 298.5 K) 7.26 (H-2, t, *J* 7.6 Hz, 4 H), 7.37 (H-3, t, *J* 7.5 Hz, 4 H), 7.83 (H-4, d, *J* 7.2 Hz, 4 H), 8.36 (H-1, d, *J* 8.0 Hz, 4 H); δ_{C} (acetone-*d*₆, 298.5 K) 121.0 (C-4), 127.4 (C-1), 128.0 (C-2), 130.4 (C-3), 138.8 (C-8a), 141.7 (C-9), 142.2 (C-4a).

Acknowledgments

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