

**The First 5,6-Dihydroxyindole Tetramer by Oxidation of 5,5',6,6'-Tetrahydroxy-2,4'-
biindolyl and an Unexpected Issue of Positional Reactivity En Route to Eumelanin-Related
Polymers.**

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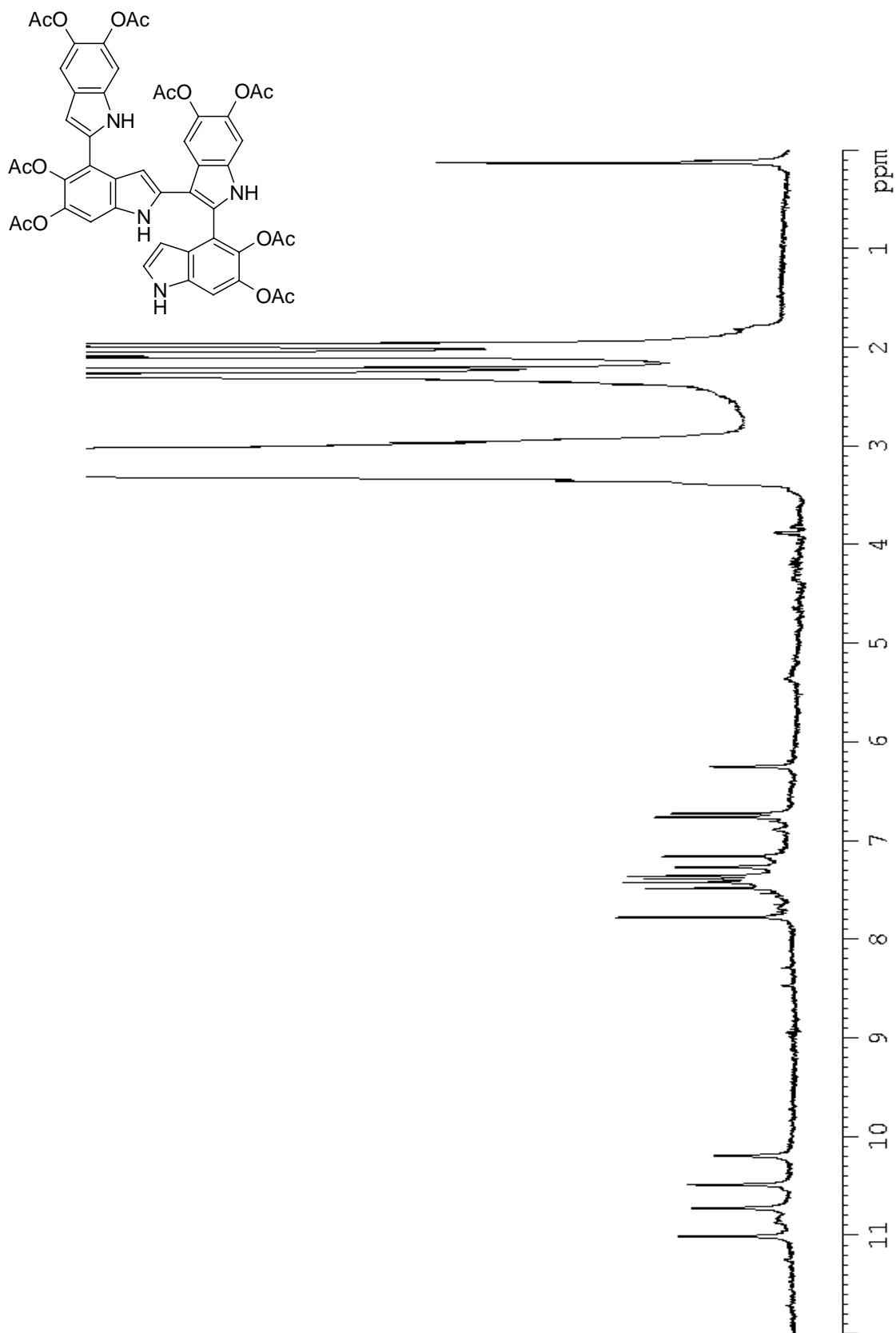
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Experimental Procedures

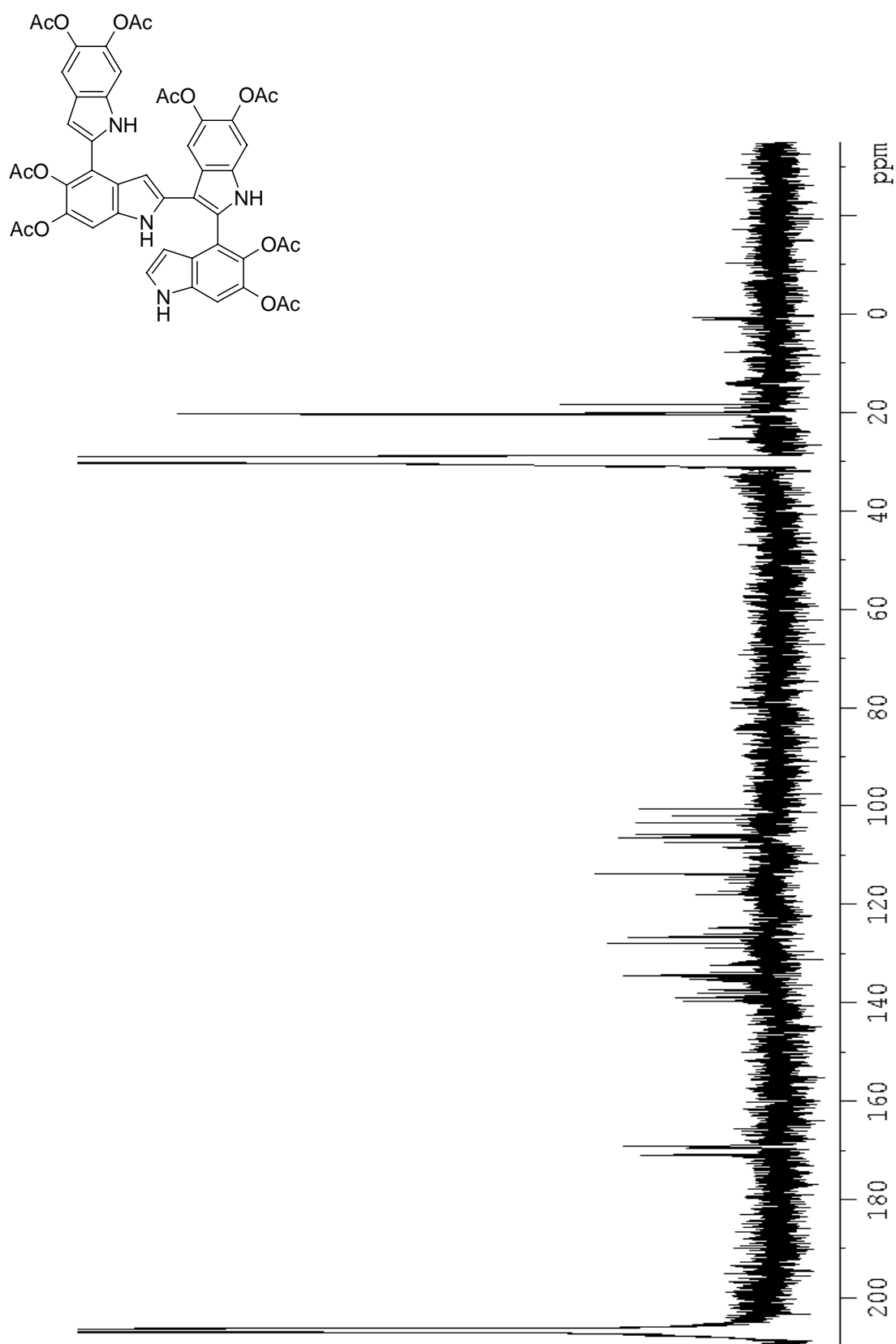
General Experimental Methods. Dimer **2** as tetraacetyl derivative was prepared as previously reported.⁹ All other reagents were purchased from commercial sources and used as obtained. UV spectra were obtained on a diode array spectrophotometer. ESI+/MS(HR) spectra were obtained in 1% formic acid-methanol 1:1 v/v. ¹H NMR spectra were recorded at 400 MHz, ¹³C NMR spectra at 100 MHz. ¹H, ¹H COSY, ¹H, ¹³C HMBC, ¹H, ¹³C HSQC and ROESY experiments were run at 400 MHz on an instrument equipped with a 5 mm ¹H/broadband gradient probe with inverse geometry using standard pulse programs. Analytical and preparative TLC were carried out on silica gel plates (0.25 and 0.50 mm, respectively). CHCl₃: ethyl acetate 65:35 was used as the eluant.

Oxidation of 2. Isolation of acetylated tetramer 7. A solution of the tetraacetyl derivative of **2** (45 mg, 0.096 mmol) in methanol (3 mL) was taken under a flux of argon and treated with NaOH 10 mM (9 mL) that had prior been purged with argon. After 10 min 0.1 M HEPPSO buffer (pH 7.4) (45 mL) containing ZnSO₄ × 7H₂O (27 mg, 1 molar equivalent) was added followed by peroxidase (450 pyrogallol units) and 30% hydrogen peroxide (45 μL). The mixture was taken under vigorous stirring and after about 30 s the reaction was stopped by addition of 0.2 M sodium dithionite in water (10 mL). The mixture was then acidified to pH 4 by addition of 3 M HCl and extracted with ethyl acetate (3 × 30 mL); the combined organic layers were dried over sodium sulphate and taken to dryness, and the residue was acetylated with acetic anhydride/pyridine 95:5 v/v. The residues obtained from three reaction runs as described above were combined and fractionated by preparative TLC to give acetylated **7** (*R_f* = 0.14, 3 mg, 2% yield, > 90% purity estimated by ¹H NMR analysis). HR ESI+/ MS: found *m/z* 927.2369 [M+H]⁺, calcd for C₄₈H₃₉N₄O₁₆ 927.2361; found *m/z* 949.2173 [M+Na]⁺, calcd for C₄₈H₃₈NaN₄O₁₆ 949.2180. UV (CH₃OH): λ_{max} 214, 313, 339 (sh) nm.

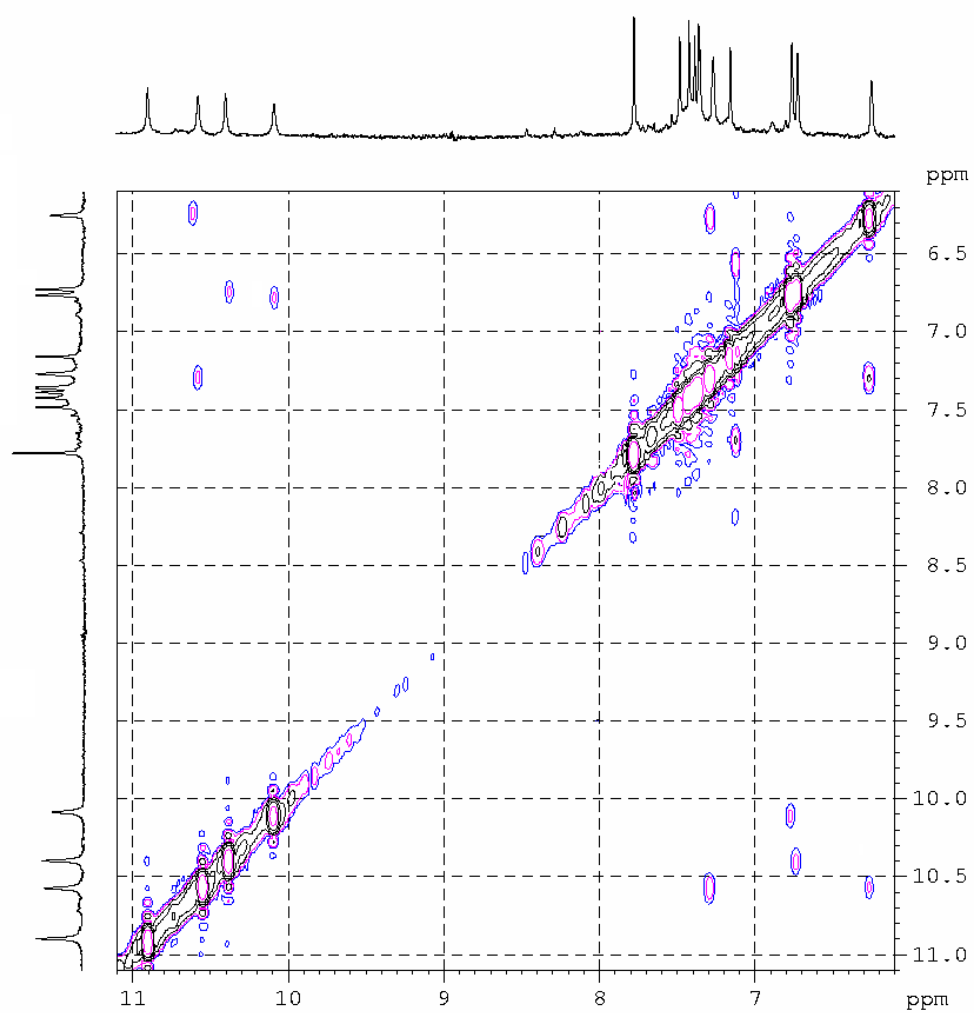
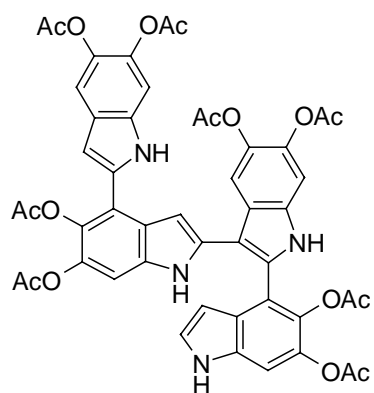
^1H NMR spectrum of acetylated **7**



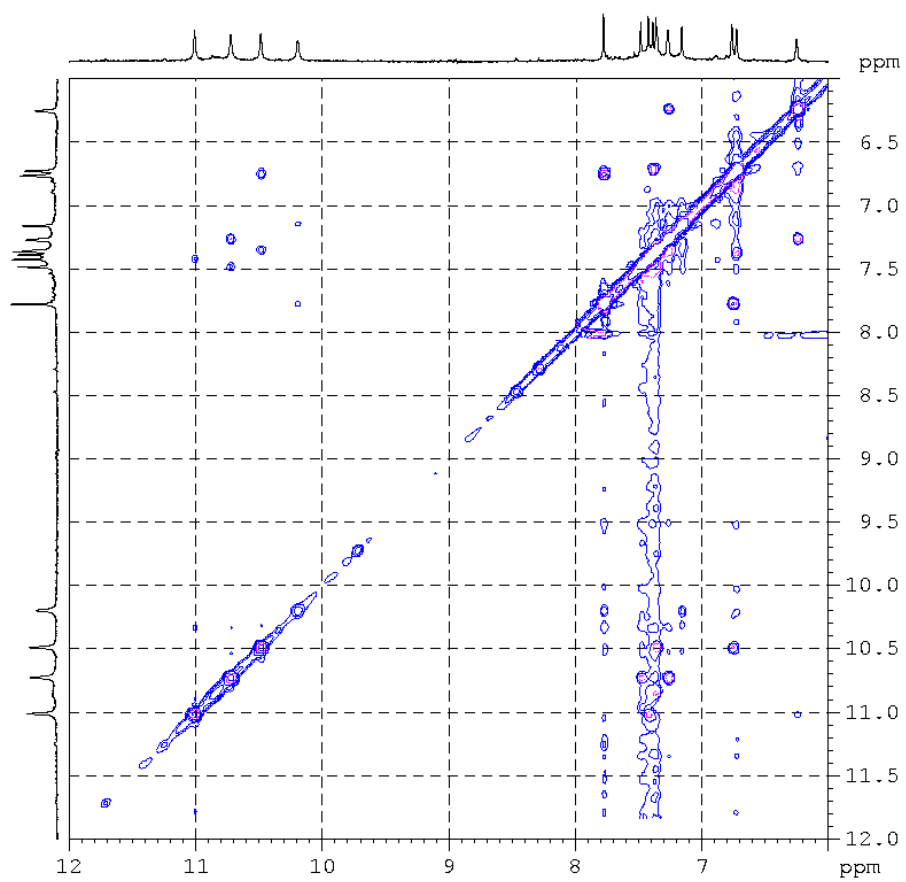
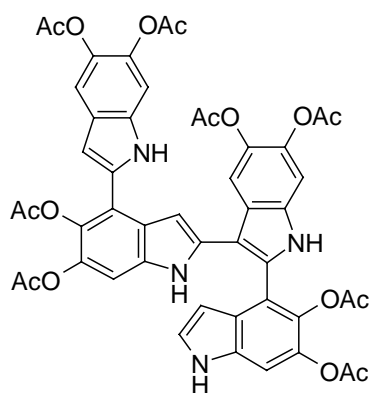
^{13}C NMR spectrum of acetylated **7**



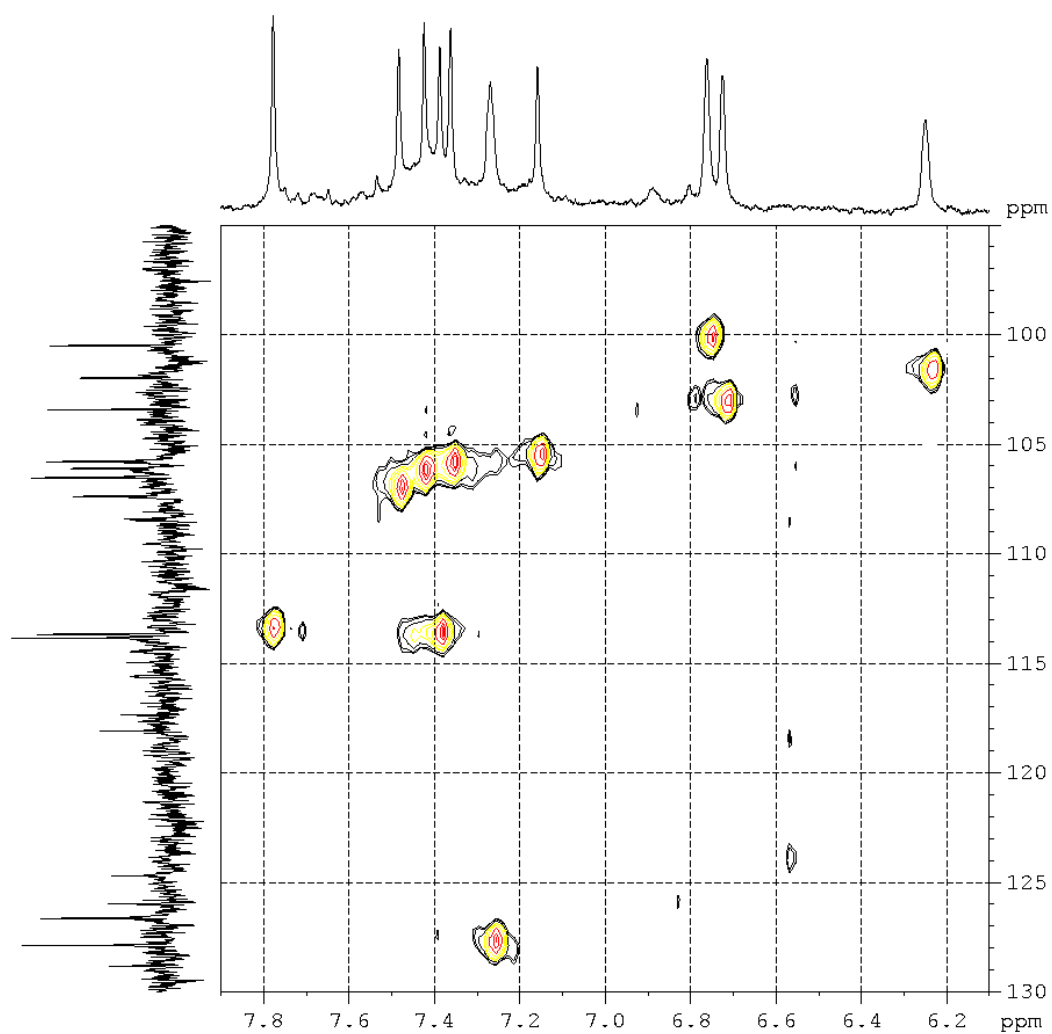
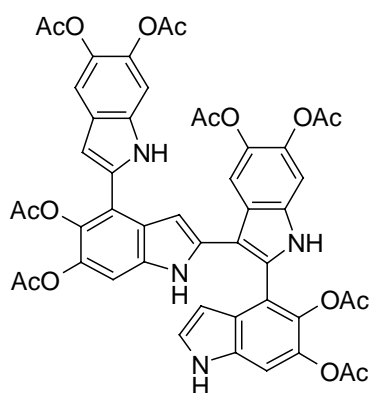
^1H , ^1H COSY spectrum of acetylated **7**



ROESY spectrum of acetylated **7**



^1H , ^{13}C HSQC spectrum of acetylated **7**



^1H , ^{13}C HMBC spectrum of acetylated **7**

