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Dynamics of electronically excited states in the eumelanin building block 5,6-dihydroxyindole

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Electronic Supplementary Information

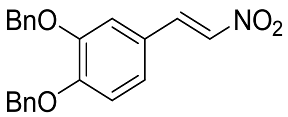
Preparation, characterization and deuteration of 5,6-dihydroxyindole

General Information

^{1}H NMR spectra were recorded on Bruker AV 300, DPX 400 and AV 400 spectrometers at 300 and 400 MHz, respectively, and referenced to residual solvent. ^{13}C NMR spectra were recorded using the same spectrometers at 75 and 100 MHz, respectively. Chemical shifts (δ in ppm) were referenced to tetramethylsilane (TMS) or to residual solvent peaks ($CDCl_3$ at δH 7.26). J values are given in Hz and s, d, dd, ddd, t, dt, q, m, br and app. abbreviations correspond to singlet, doublet, doublet of doublet, doublet of doublet of doublet, triplet, triplet of doublet, quartet, multiplet, broad and apparent, respectively. Mass spectra were obtained at the EPSRC National Mass Spectrometry Service Centre in Swansea. Infrared spectra were obtained on Perkin-Elmer Spectrum 100 FT-IR Universal ATR Sampling Accessory, deposited neat or as a chloroform solution to a diamond/ZnSe plate.

Flash column chromatography was carried out using Matrix silica gel 60 from Fisher Chemicals and TLC was performed using Merck silica gel 60 F254 pre-coated sheets and visualised by UV (254 nm) and stained by the use of aqueous acidic $KMnO_4$. Anhydrous dichloromethane (DCM) was distilled from CaH_2.

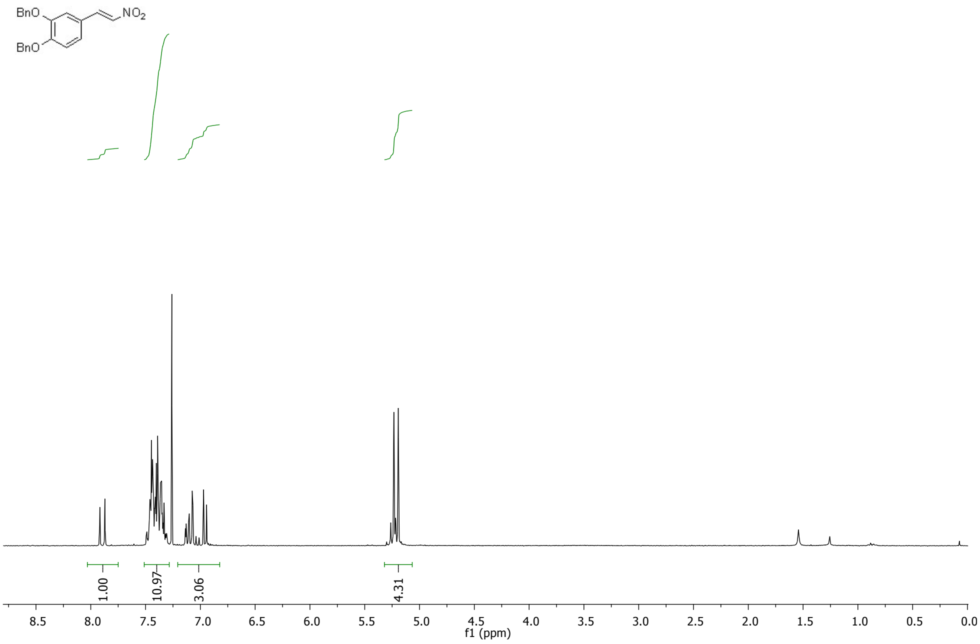
(*E*)-3,4-Dibenzyloxy-*β*-nitrostyrene[1]



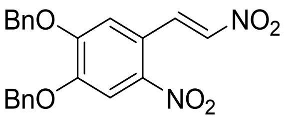
A solution of commercially available 3,4-dibenzyloxybenzaldehyde (9.63 g, 30.2 mmol, 1 eq), $MeNO_2$ (9.75 mL, 181.2 mmol, 6 eq) and NH_4OAc (9.33 g, 121.0 mmol) in AcOH (100 mL) was heated to reflux for 40 min and the dark yellow solution was cooled to room temperature. The mixture was concentrated under reduced pressure to give a yellow residue which was dissolved in DCM (100 mL).

The solution was carefully washed with sat. K_2CO_3 solution until the aqueous layer was basic. The organic layer was then washed with water (100 mL) and dried over $MgSO_4$ and concentrated to give the desired product as a bright yellow solid (9.57 g, 87 %). Crude material carried on to next step without further purification.

(300 MHz, $CDCl_3$) δ 5.20, (s, 2H), 5.23 (s, 2H), 6.96 (d, *J* = 8.3 Hz, 1H), 7.07 (d, *J* = 2.0 Hz, 1H), 7.12 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.29–7.52 (m, 11H), 7.89 (d, *J* = 13.6 Hz, 1H).
mp 116-119 °C (lit 117-118 °C)

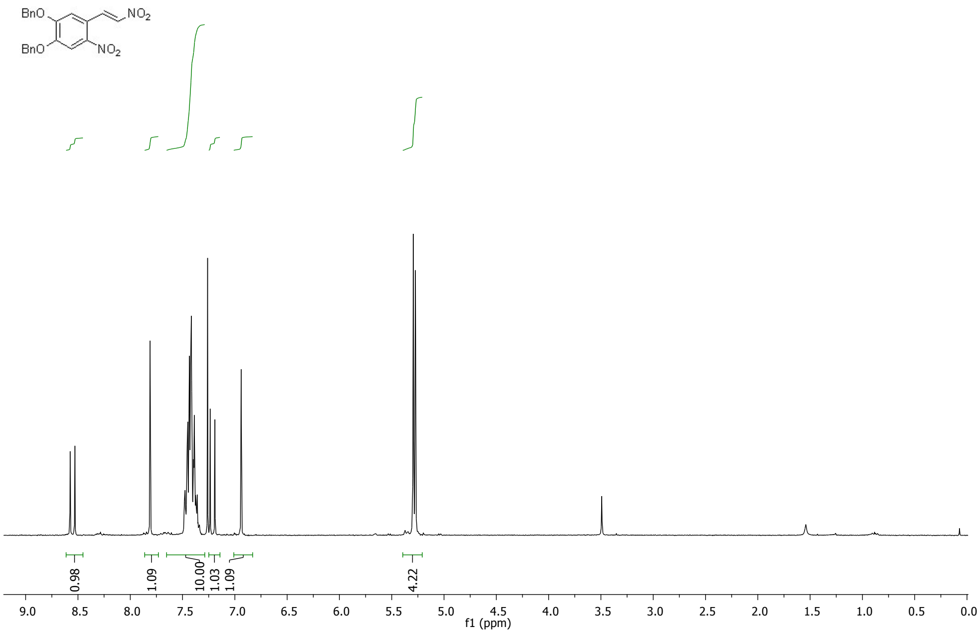


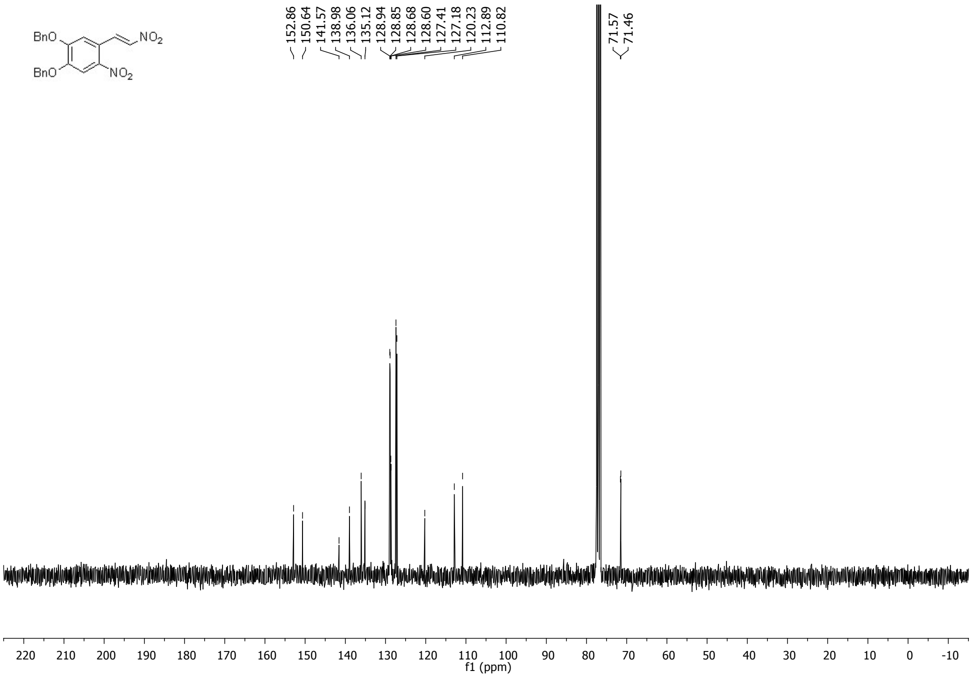
(*E*)-4,5-Dibenzyloxy-2,*β*-nitrostyrene[1]



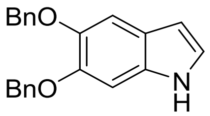
(*E*)-3,4-Dibenzyloxy-*β*-nitrostyrene (9.57 g, 26.4 mmol) was suspended in glacial acetic acid (200 mL) and HNO_3 (60 mL, 90 % fuming) was added dropwise. An increase in temperature to 30 °C was observed at which point the flask was cooled in an icebath. The mixture was allowed to reach room temperature and the addition completed. The mixture was then stirred for 3 h at room temperature and poured into ice water (400 mL). The resulting yellow precipitate was filtered off and washed with water (100 mL) and then dissolved in DCM (200 mL). The solution was washed with sat. K_2CO_3 until the aqueous layer was basic. The organic layer was dried ($MgSO_4$) and concentrated to give a bright yellow solid which was re-crystallised from DCM/MeOH to give the desired product (4.48 g, 42 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, *J* = 13.5 Hz, 1H), 7.81 (s, 1H), 7.59 – 7.31 (m, 10H), 7.21 (d, *J* = 13.5 Hz, 1H), 6.94 (s, 1H), 5.29 (s, 1H), 5.27 (s, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9, 150.6, 141.6, 138.9, 136.1, 135.1, 128.9, 128.8, 128.7, 128.6, 127.4, 127.2, 120.2, 112.9, 110.8, 71.6, 71.5.
mp 160-162 °C (lit 161-162 °C)





5,6-Dibenzyloxyindole[1]

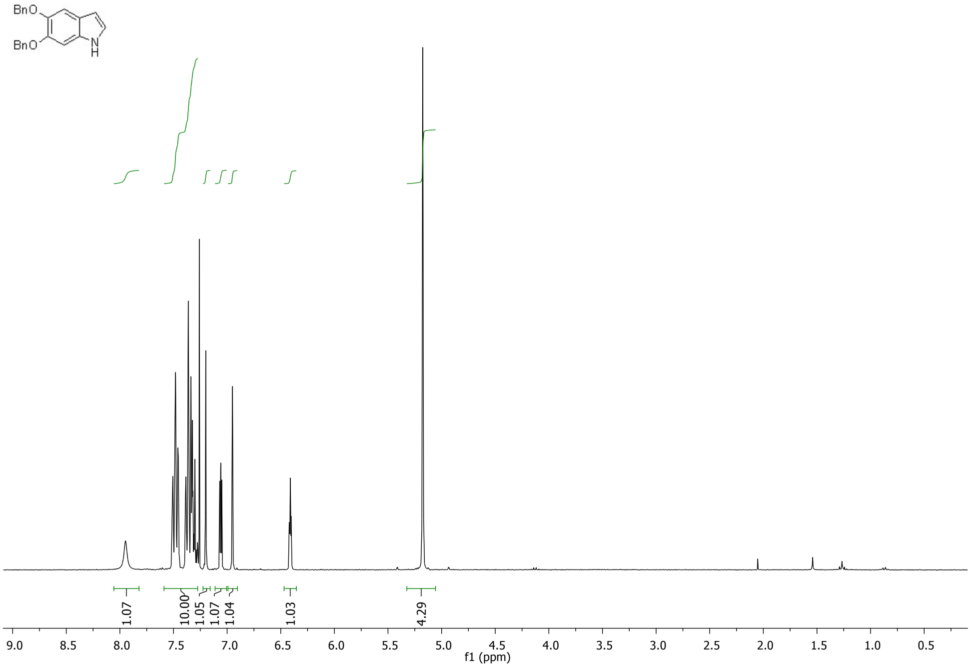


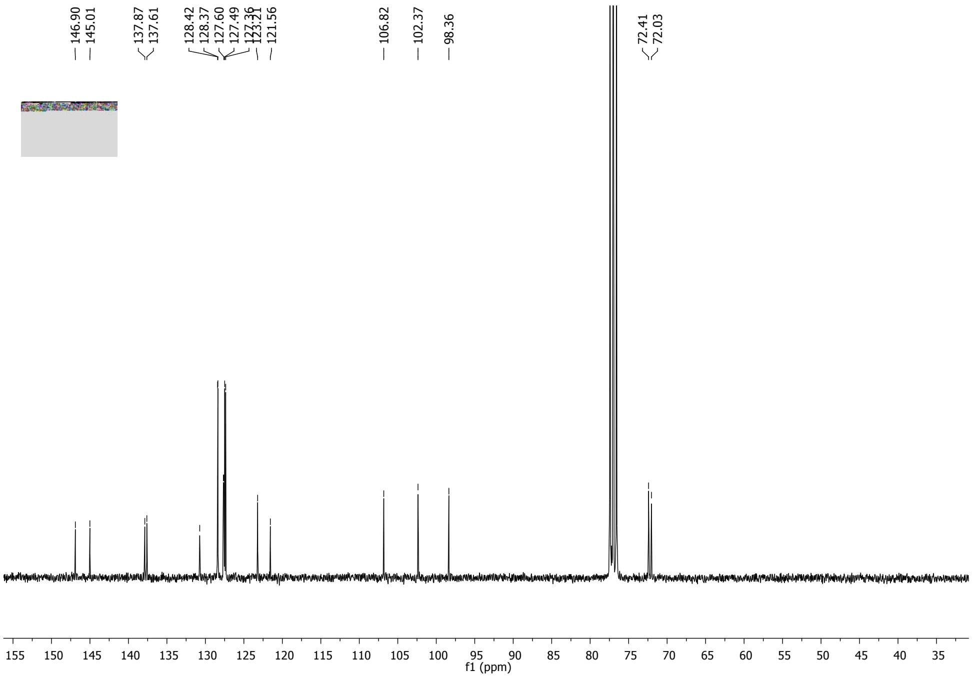
(*E*)-4,5-Dibenzyloxy-2,*β*-nitrostyrene (3.62 g, 8.89 mmol, 1 eq) was dissolved in AcOH (55 mL), cyclohexane (22 mL) and benzene (65 mL). Iron powder (9.94 g) and SiO_2 (13.38 g) were added and the green suspension was brought to reflux under nitrogen. The mixture appeared as a red solution before becoming a thick green suspension. After 30 min the mixture was cooled to room temperature and filtered through Celite with DCM: EtOAc (1:1) until the washings contained no product by TLC. The black filtrate was washed with aq K_2CO_3 until the washings were basic. The organic layer was washed with water (2 × 100 mL), dried ($MgSO_4$) and concentrated to give a black solid (3 g). The crude residue was purified by column chromatography (PE:EtOAc 3:1) to give 5,6-dibenzyloxyindole as a cream solid (1.98 g, 68%) which was stored in the freezer under nitrogen.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.55 – 7.27 (m, 10H), 7.20 (s, 1H), 7.06 (dd, *J* = 3.1, 2.5 Hz, 1H), 6.95 (d, *J* = 0.5 Hz, 1H), 6.41 (ddd, *J* = 3.0, 2.1, 0.8 Hz, 1H), 5.18 (s, 4H).

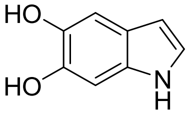
^{13}C NMR (75 MHz, $CDCl_3$) δ 146.9, 145.0, 137.9, 137.6, 130.7, 128.4, 128.4, 127.7, 127.6, 127.5, 127.4, 123.2, 121.6, 106.8, 102.4, 98.4, 72.4, 72.0.

mp 110-113 °C (lit. 113-114 °C)





5,6- Dihydroxyindole[2,3]



5% Pd/C (0.090 g) was weighed into a 100 mL flask and argon was carefully passed over the solid. EtOAc (36 mL) was added followed by 5,6-dibenzyloxyindole (0.300 g, 0.91 mmol). The flask was protected from light and the mixture was placed under a hydrogen atmosphere using a balloon. The mixture was stirred vigorously overnight. TLC showed consumption of starting material and mono-benzylated side product and the mixture was filtered through Celite and rinsed thoroughly with EtOAc with care not to let the Celite completely dry out. The filtrate was concentrated to yield a beige residue, which was purified rapidly via column chromatography (100% EtOAc). The column and test tubes were protected from light. The crude oil thus obtained crystallised to a cream-coloured solid upon trituration with hexane. 5,6- dihydroxyindole (0.136 g, 93%) was then dried under high vacuum. The compound was stored in the freezer under argon or nitrogen.

IR (cm^{-1}) v 3404(br), 3126, 3104, 3053, 1510,1496, 1470, 1376, 1335, 1279, 1174,1108;

^{1}H NMR (300 MHz, DMSO-*d*^{6}) δ 10.41 (s, 1H, NH), 8.47 (s, 1H, OH), 8.21 (s, 1H, OH), 7.04- 6.94 (m, 1H), 6.81 (s, 1H), 6.74 (s, 2H), 6.18 – 6.05 (m, 1H);

^{13}C NMR (101 MHz, DMSO-*d^6*) δ 142.5, 140.4, 130.3, 122.5, 120.2, 104.4, 100.1, 97.1;

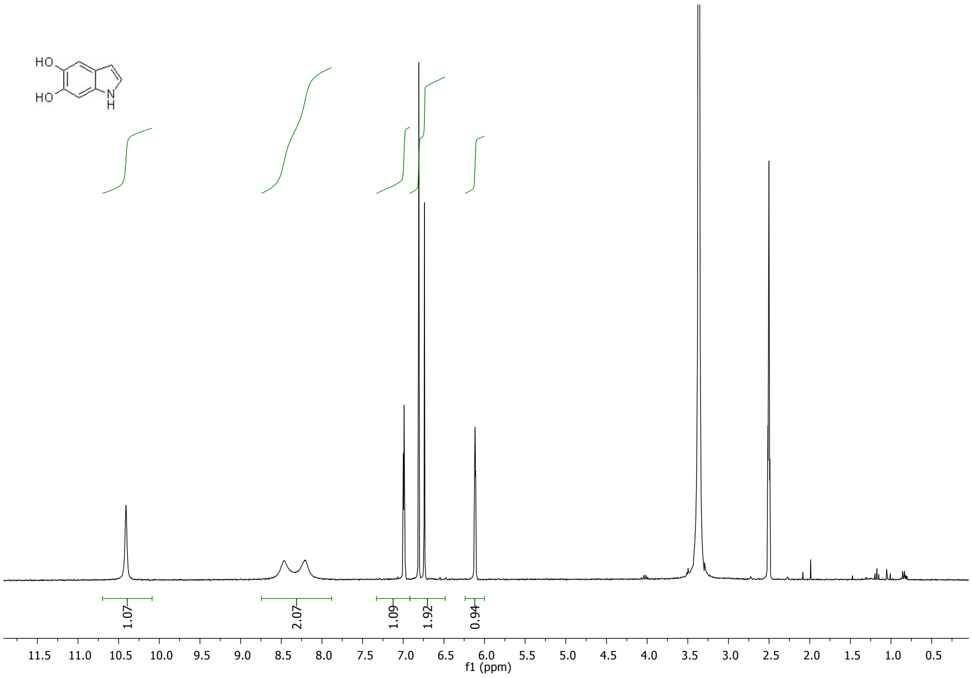
m/z (ESI$^+$) 150 (M + H$^+$, 100%);

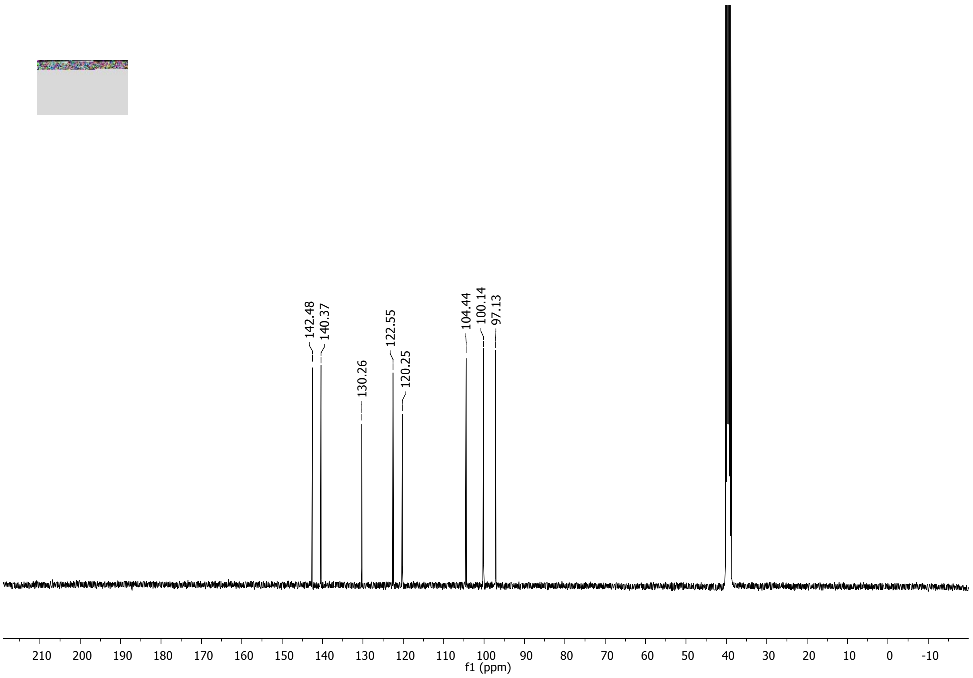
HRMS (ESI$^+$) found 150.0546, $C_8H_8O_2N$ (M + H)$^+$ requires 150.0550.

m.p. (Et_2O/Petroleum ether 40-60): 140 - 141°C dec. (lit. 144 °C, A. T. Al-Kazwini et al., *J. Phys. Chem.*,1990, *94*, 6666-6670);

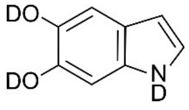
Notes: An alternative hydrogenation procedure using Pd/C and cyclohexadiene gave no conversion by TLC.

The compound is highly insoluble (requires DMSO, EtOAc) and tends to get darker in colour over time when not in the freezer, particularly in solution. It is difficult to remove residual EtOAc from samples, even after several days on high vac. 2D TLC showed no decomposition.





1*d*-Indole-5,6-diol-*d2*



5,6-dihydroxyindole (298 mg, 2 mmol) was dissolved deuterated methanol (3 ml) and stirred at room temperature for 2 hours. The sample was concentrated forming a grey solid, 1*d*-indole-5,6-diol-*d2*;

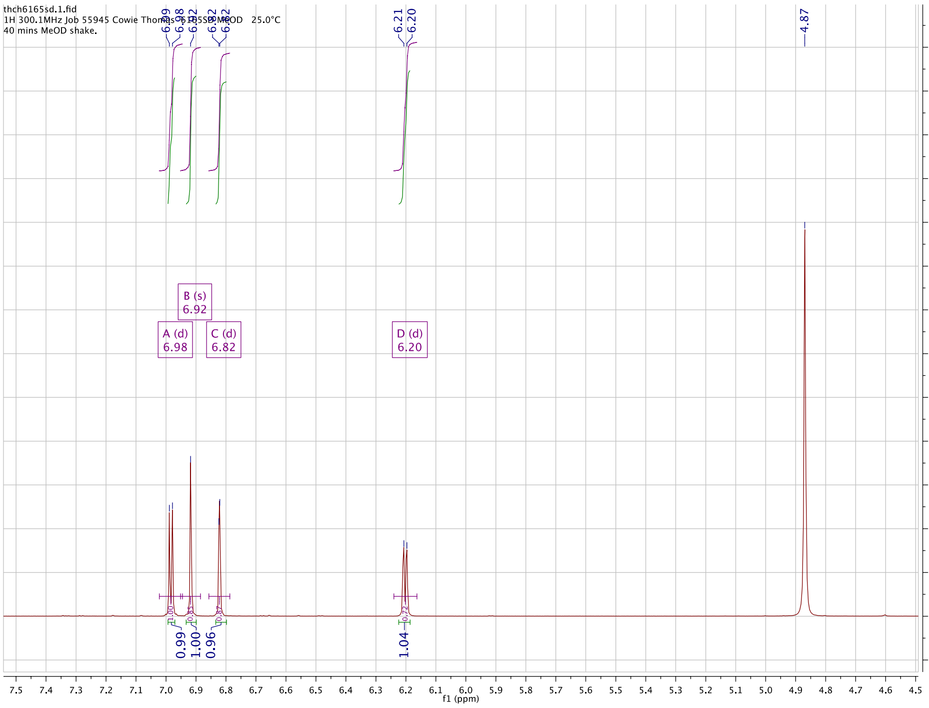
IR (cm^{-1}) v 3434br, 3121, 3045, 2554br, 1629, 1583, 1491, 1460, 1359, 1298, 1271, 1162, 1113, 1085, 949, 899.

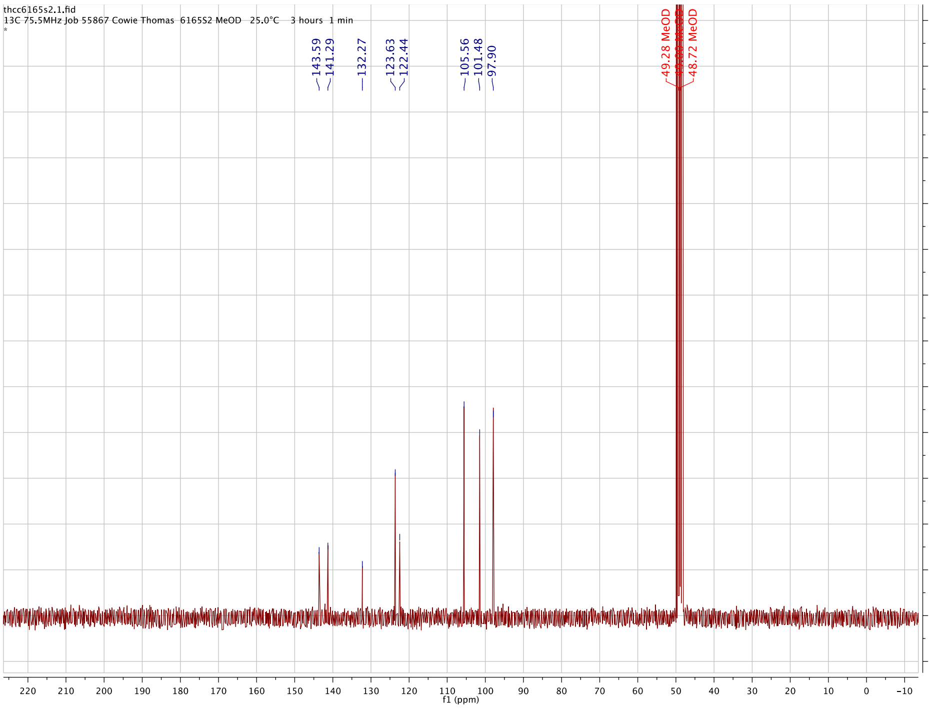
^{1}H NMR (300 MHz, *Methanol-d$_4$*) δ 6.98 (d, J = 3.1 Hz, 1H), 6.92 (s, 1H), 6.82 (s, 2H), 6.20 (d, J = 3.1 Hz, 1H);

^{13}C NMR (101 MHz, *Methanol-d$_4$*) δ 143.6, 141.3, 132.3, 123.6, 122.4, 105.6, 101.5, 97.9; m/z (ESI$^+$) 153 (M + H$^+$, 100%);

HRMS (ESI$^+$) found 153.0730, $C_8H_5D_3NO_2$ (M + H)$^+$ requires 153.0743.

mp: (*Methanol-d$_4$*) 144-146^{o}C





References:

1. S. P. H. Mee, V. Lee, J. E. Baldwin and A. Cowley, *Tetrahedron*, **2004**, *60*, 3695.
2. U. S. Pat., 1986; 4, 595, 765.
3. J. D. Benigni and R. L. Minnis, *J.Heterocycl Chem*., **1965**, *2,* 387.