

Supporting Information

Supramolecular Self-assembly Mediated Aggregation-Induced Emission of Fluorene Derived Cyano Stilbenes: Multifunctional Probes for Live Cell-Imaging

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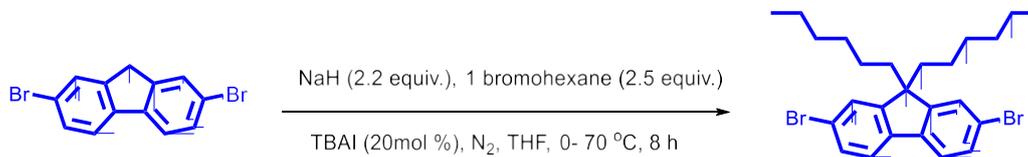
1. General remarks Materials and methods

The chemicals and reagents used in this study were obtained from Sigma Aldrich (India), Alfa Aesar (India), or S.D. Fine Chemicals Ltd (India) and were used without further purification. All the solvents used in the synthesis as well as for optical spectroscopic studies were dried thoroughly using the reported procedures. Reagents such as 9H-fluorene, 1-bromohexane, *n*-butyllithium solution 2.5 M in hexanes, tributyl borate, 1,3-Propanediol, phosphoryl chloride, *N,N*-Dimethylformamide anhydrous, 4-dimethylamino benzaldehyde, 4-(Dimethylamino)cinnamaldehyde, 4-bromotoluene, *N*-bromosuccinimide, triphenylamine, 4,4'-Bis(diethylamino)benzophenone, triethylamine, sodium hydride, potassium cyanide, potassium carbonate, tetrakis (triphenylphosphine) palladium(0) Pd(PPh₃)₄ were purchased from Aldrich and used without further purification. Toluene was dried over sodium and diphenylketone. DMF was dried over phosphorus pentoxide. The other chemicals and reagents were used as received without further purification. All the reactions were carried out in oven-dried glassware. The progress of reactions was monitored by Thin Layer Chromatography (TLC) while purification of crude compounds was done by column chromatography using neutral alumina (Brokmann activity-1). NMR spectra were recorded at 500 MHz on Bruker-400 MHz at Bruker-400 MHz. Chemical shifts are reported in δ (ppm) relative to TMS (¹H) or CDCl₃ (¹³C) as internal standards. Integrals are in accordance with assignments; coupling constants are given in Hz. All ¹³C spectra reported are proton-decoupled. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). FTIR spectra were recorded on a Perkin-Elmer RX-IFT-IR and absorbencies are reported in cm⁻¹. Mass spectra were obtained with Agilent 1100 MS series and AXIMA CFR MALDITOF (Compact) mass spectrometers. XRD patterns were obtained on an Empyrean X-ray diffraction instrument, and the samples of the as-synthesized crystals, ground powders and fumed samples on glass slides were determined at room temperature. Yields refer to quantities obtained after chromatography. UV spectra were measured on a Milton Roy spectronic 3000 Array spectrophotometer. Photoluminescence (PL) was recorded on a Perkin-Elmer LS 55 spectrofluorometer with a slit-width of 1 nm and 2 nm. Quantum yields (ϕ) of fluorescence were obtained using quinine sulfate (0.545 in 1 N H₂SO₄) as a reference standard. Thermo gravimetric analysis (TGA) was carried on a TA TGA Q5000 under nitrogen at a heating rate of 10 °C/min. The ground-state geometries were optimized using the density functional (DFT) with B3LYP hybrid functional

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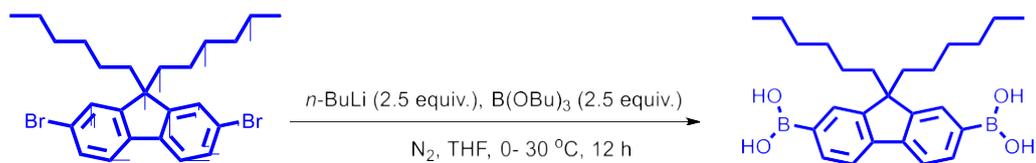
at the basis set level of 6-31G*. All the calculations were performed using Gaussian 03 package.

2. Experimental procedure

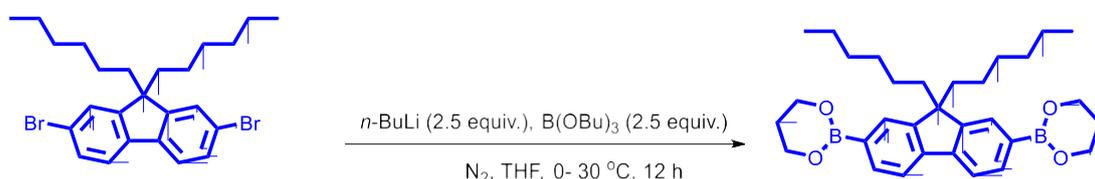


General/Typical Procedure: General Procedure Followed for the di-alkylation of fluorene 2,7-dibromofluorene (5.0g, 15.4 mmol) was dissolved in dried THF solution (30 ml) and a catalytic amount of tetra-butyl-ammonium iodide (0.046 g, 20 mol %) were added to a flask. Then, 2.2 eq. Sodium hydride (60 %) (1.86 g, 46.2 mmol) was added into the THF solution at 0 °C and then flask was degassed three times by applying freeze-thaw cycles refluxed for 5 hours. BrCH₂CH₂CH₂CH₂CH₂CH₃ (6.112 g, 37 mmol) in was added dropwise via syringe (degassed) and the mixture allowed to heated at 70 °C to stir for continuously 8 hours, after which the reaction was allowed to cool for rt. The THF was evaporated and then the remaining solid was dissolved in hexanes with passed over an alumina plug using 500 ml of hexanes which was then evaporated to dryness and poured into crushed ice and extracted with chloroform (2 × 100 ml). The combined organic solutions were washed with saturated NaCl solution (2 × 100 ml) and distilled water (1 × 100 ml). The solvent was removed under vacuum, and the crude was purified via column chromatography over a small pad of silica gel with 10 % ethyl acetate in hexane as the eluent to give the desired alkylated oil product was dried and further purified by recrystallization twice in Hexane. The synthesized 2, 7-dibromo- 9,9-dihexyl-9H-fluorene was thoroughly characterized by ¹H NMR, ¹³C NMR, IR and ESI- MASS.

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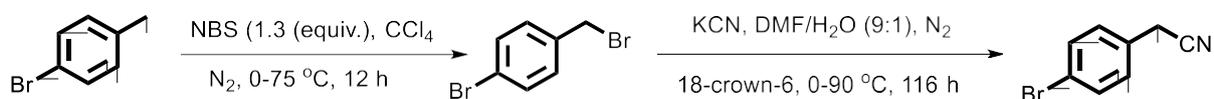
9,9-Dihexyl-2,7-diboronic acid. To a solution of 2,7-dibromo-9,9-dihexylfluorene (9.84 g, 20 mmol) in dry THF (100 ml), *n*-BuLi (2.5 M in *n*-hexane, 18 ml, 45 mmol) was added dropwise under N₂ atmosphere at -78 °C within 20 min. After further stirring for 1 h at this temperature, freshly distilled B(OBu)₃ (13 ml, 48 mmol) was added. The cooling bath was removed and stirring was continued for 12 h at room temperature. The reaction mixture was hydrolyzed with 1 M HCl solution (150 ml) and extracted with ether (3 X 70 ml). The organic layer was dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. The product was obtained. White solid, yield (7.68 gm, 18.2 mmol, 91%). The synthesized 9,9-Dihexyl-2,7-diboronic acid was thoroughly characterized by ¹H NMR, ¹³C NMR, IR and ESI-MASS. M.P. 179-180 °C



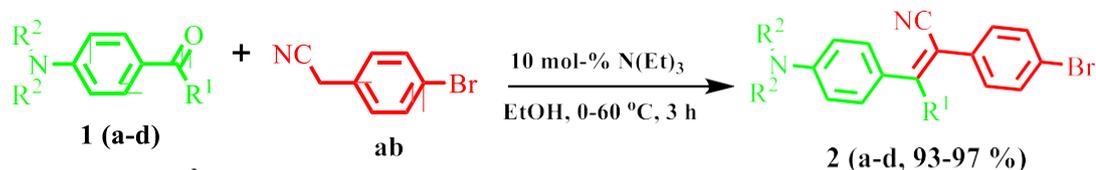
2,2'-(9,9-dihexyl-9H-fluorene-2,7-diyl)bis(1,3,2-dioxaborinane)³. To a solution of 9,9-dihexyl-2,7-diboronic acid (6.31 gm, 15 mmol) in toluene (50 ml) was added 1,3-propanediol (2.04 gm, 33 mmol). The reaction mixture was refluxed at 130 °C for 24 h under nitrogen. The mixture was poured into water and extracted with ethyl acetate (3 X 30 ml) and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure and the crude product was recrystallized from hexane to afford the title compound. White solid, yield (5.72 gm, 76%). The synthesized 2,2'-(9,9-dihexyl-9H-fluorene-2,7-diyl)bis(1,3,2-dioxaborinane)

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was thoroughly characterized by ^1H NMR, ^{13}C NMR, IR and ESI-MASS. M. P. 121-122°C (lit. 123-124°C).



Accordingly, a suspension of bromotoluene (1.5 g, 8.42 mmol) and *N*-bromo succinimide (1.65 g, 9.27 mmol) in 50 ml of carbon tetrachloride was heated to reflux for overnight. The resulting suspension was dissolved in CH_2Cl_2 and washed successively with 100 ml each of saturated aqueous sodium bicarbonate, water and brine. The organic layer was separated and dried over sodium sulphate anhydrous. Removal of solvent by rotary evaporation gave 2.0 g of yellow oil containing *p*-bromobenzyl bromide. The mixture was not separated for next step. To a stirred solution of crude *p*-bromobenzyl bromide (2.0 g, 7.81 mmol) in 10 ml DMF/H₂O (9:1) was added KCN (1.52 g, 23.44 mmol) and 18-crown-6 (2.06 g, 7.81 mmol). The reaction mixture was stirred vigorously at 90 °C for 16 h. After the completion of reaction, mixture was cooled, quenched with 20 ml water and extracted with ethyl acetate (2×100 ml). The organic layers were washed with 10 ml of water and dried over anhydrous sodium sulphate. The solvent was removed by evaporation and resulting crude material was purified by column chromatography on silica gel using petroleum ether: EtOAc (10:1) in a 50% isolated yield over three steps (1 g, 4.95 mmol) as off-white solid.

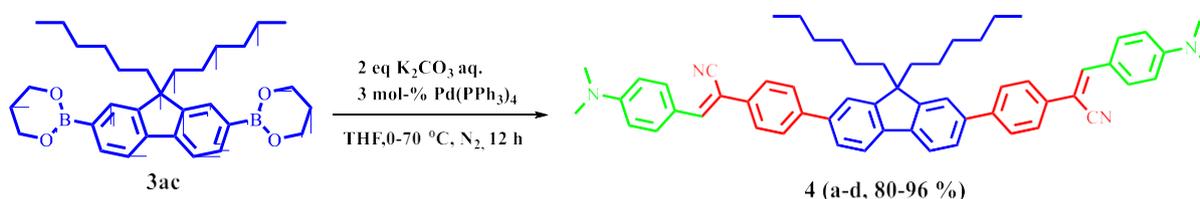


$\text{R}^1 = \text{H}, N,N\text{-diethylaniline}$; $\text{R}^2 = \text{Methyl, Ethyl, Phenyl}$.

All α cyanostilbene compounds were synthesized by Knoevenagel condensation depicted in Scheme 1. Typically, a solution of the aromatic aldehyde donor (Ar-CHO, 1.0 mmol) and active methylene acceptor (AM, 1.0 mmol) in absolute EtOH (10 ml) was treated portion

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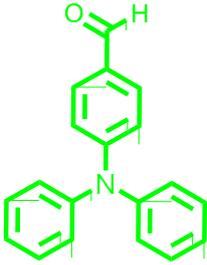
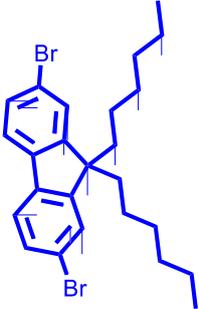
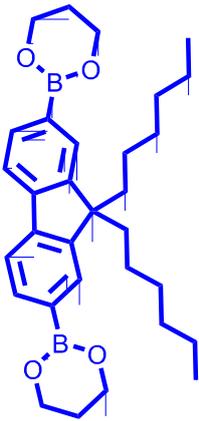
wise with triethylamine (0.1 mmol) and stirred at 60 °C for 2-3 h. After cooling to 0 °C, the precipitate was filtered and washed with chilled EtOH. The dried crude product was purified by column chromatography on silica gel using chloroform as an eluent afforded a pure solid.



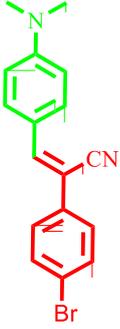
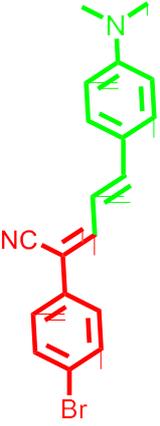
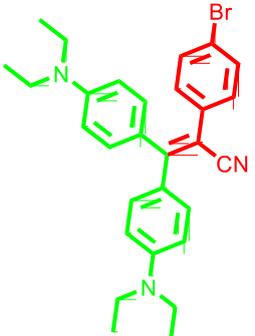
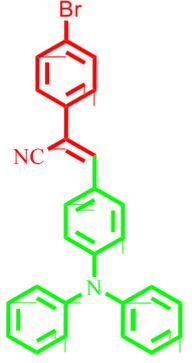
2,2'-((9,9-dihexyl-9H-fluorene-2,7-diyl)bis(4,1-phenylene))bis(3-(phenyl(or)benzophenyl acrylonitrile). Degassed THF (5 ml) and 2 M aqueous K_2CO_3 (4 ml) were added to the solution of compound **3ac** (0.24 g, 0.50 mmol), compound **2a-d** (0.18 g, 0.25 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (15 mg, 0.013 mmol) in 8 ml THF under nitrogen atmosphere. After being refluxed at 110 °C for 12 h, the mixture was poured into water (50 ml) and the organic layer was separated. The aqueous layer was extracted with chloroform (3×20 ml) and the combined organic layers were dried over anhydrous Na_2SO_4 . The organic solvent was evaporated under reduced pressure and the crude product was purified by silica column chromatography eluting with petroleum ether/ CH_2Cl_2 (v:v, 2:1) to afford compound **4a-4d** as a solid. The synthetic routes for **4a-4d** were shown in Scheme 1. The 2,7-dibromo-9H-fluorene, 4-(Diphenylamino)benzaldehyde were prepared according to the reported methods. The target molecules **4a-4d** showed good solubility in common organic solvents, such as THF, DCM, chloroform, DMF and DMSO.

3. Spectroscopic data of new compound.

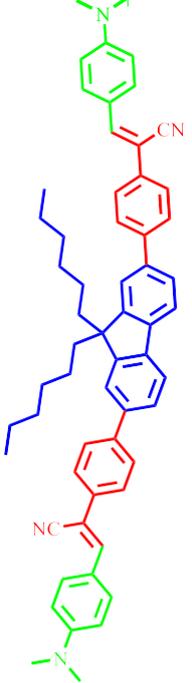
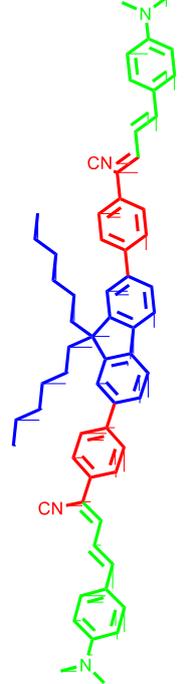
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 <p>The structure shows a central benzamide group where the nitrogen atom is substituted with two phenyl rings. The benzamide ring has a formyl group (-CHO) at the para position relative to the nitrogen.</p>	<p>Nature: Pale Yellow Powder, M.p: 130-132 °C, ^1H NMR (400 MHz, CDCl_3) δ 9.79 (s, 1H), 7.66 (d, J = 8.8 Hz, 2H), 7.32 (dd, J = 8.4, 7.4 Hz, 4H), 7.16 (d, J = 7.3 Hz, 6H), 7.01 (d, J = 8.7 Hz, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 190.45, 153.40, 146.19, 131.35, 129.78, 126.35, 125.16, 119.39, 77.44, 77.13, 76.81.</p>
 <p>The structure shows a benzene ring with a cyano group (-CN) at the 1-position and a bromine atom (-Br) at the 4-position.</p>	<p>Nature: White Crystal, M.p: 48-51 °C, Rf (3 % ; Ethyl Acetate in Hexane): 0.79, ^1H NMR (400 MHz, CDCl_3) δ 7.42 (dd, J = 8.8, 2.8 Hz, 3H), 7.18 – 7.12 (m, 3H), 3.66 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 132.18, 129.75, 129.26, 121.99, 117.71, 23.13.</p>
 <p>The structure shows a ferrocene core with two bromine atoms at the 1 and 1' positions. Each cyclopentadienyl ring is substituted with a 4-phenylbutyl group at the 2 and 2' positions.</p>	<p>Nature: White Powder, M.p: 68 – 71 °C. Rf (30% EtOAc-Hexane): 0.43, ^1H NMR (400 MHz, CDCl_3) 7.28 – 7.23 (m, 6H), 3.06 (s, 3H), 2.98 (s, 3H), 2.16 – 1.97 (m, 2H), 1.45 – 1.33 (m, 2H), 1.27 (d, J = 9.6 Hz, 11H), 1.11 (dd, J = 16.9, 10.0 Hz, 2H), 0.88 (t, J = 6.7 Hz, 3H), 0.76 (t, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 152.58, 139.09, 130.18, 126.21, 121.51, 121.13, 55.71, 40.21, 31.46, 29.59, 23.67, 22.58, 14.00.</p>
 <p>The structure shows a ferrocene core with two 2,4,6-tris(methylamino)phenyl groups at the 1 and 1' positions. Each phenyl ring has three methylamino groups (-NHMe) at the 2, 4, and 6 positions.</p>	<p>Nature: White Powder, M.p: 122-125 °C, Rf (3% Ethyl Acetate in -Hexane): 0.63, ^1H NMR (400 MHz, CDCl_3) δ 8.51 – 7.48 (m, 6H), 4.19 (t, J = 5.3 Hz, 8H), 2.07 (dd, J = 10.5, 5.2 Hz, 4H), 1.98 (dd, J = 9.9, 6.4 Hz, 4H), 1.03 (ddd, J = 23.6, 11.6, 6.5 Hz, 12H), 0.74 (t, J = 7.1 Hz, 6H), 0.53 (dd, J = 14.8, 7.4 Hz, 4H). ^{13}C NMR (101 MHz, CDCl_3) δ 150.31, 143.55, 132.36, 127.88, 119.18, 77.38, 77.06, 76.74, 62.03, 54.90, 40.41, 31.58, 29.82, 27.47, 23.74, 22.67, 14.04.</p>

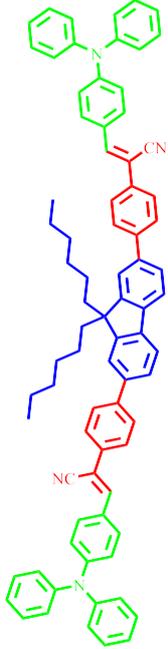
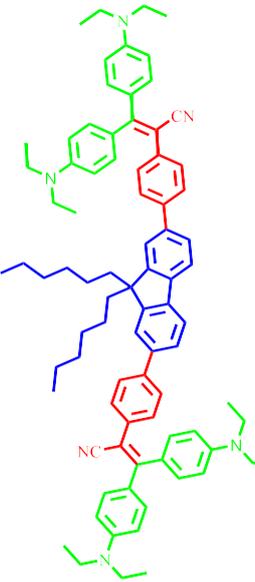
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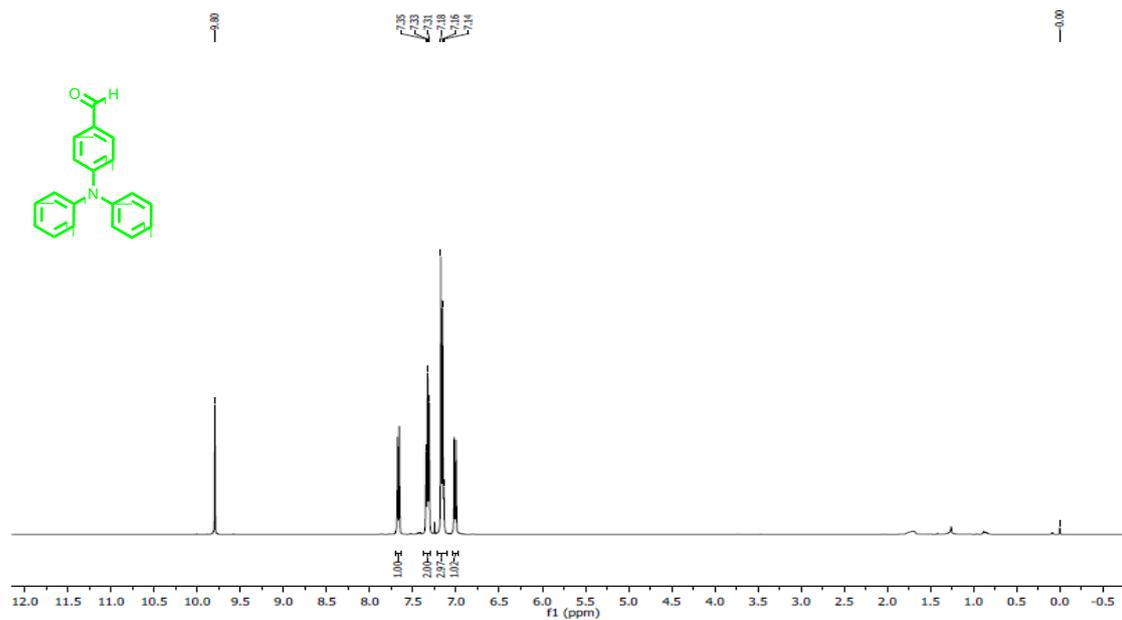
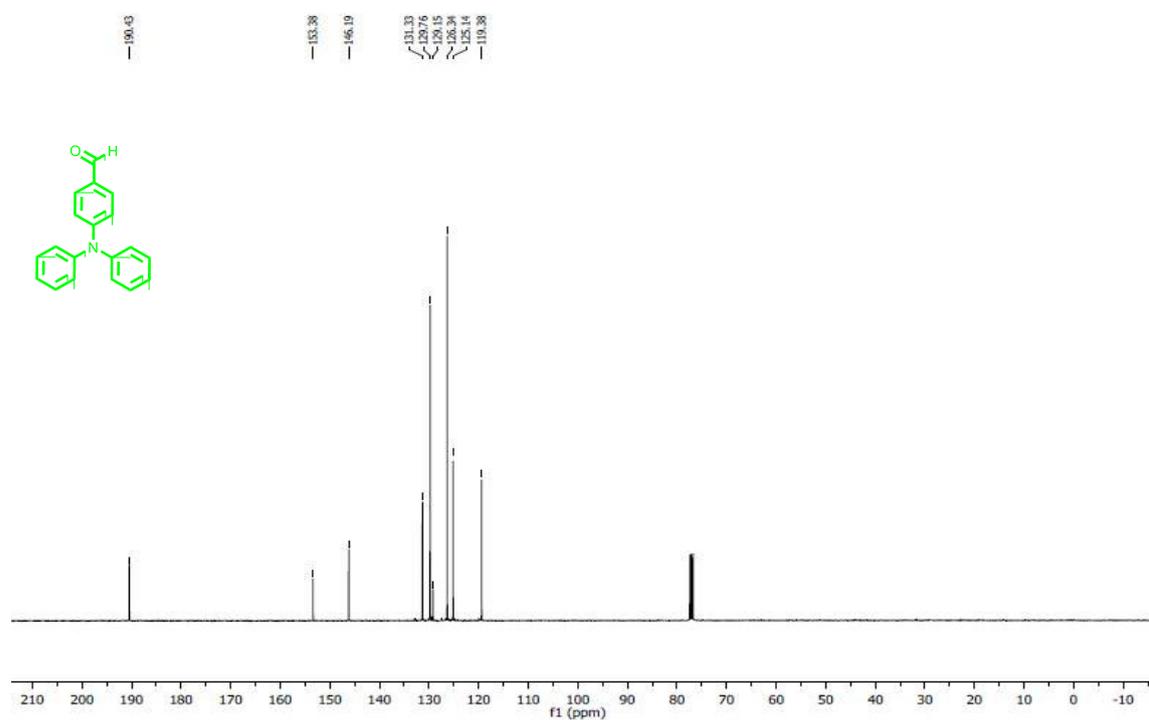
	<p>Nature: Yellow solid, M.p: 180-182 °C, ^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 8.9 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.36 (s, 1H), 6.70 (d, J = 9.0 Hz, 1H), 3.05 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 151.9, 142.8, 134.6, 132.0, 131.5, 126.9, 121.8, 121.3, 119.1, 111.6, 103.2, 40.0.</p>
	<p>Nature: Dark Yellow solid, M.p: 190-192 °C, R_f (3% Ethyl Acetate in Hexane): 0.75, ^1H NMR (400 MHz, CDCl_3) δ 7.40 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.8 Hz, 3H), 7.26 (d, J = 11.1 Hz, 1H), 7.06 (dd, J = 15.1, 11.3 Hz, 1H), 6.85 (d, J = 15.1 Hz, 1H), 6.57 (d, J = 8.8 Hz, 2H), 2.93 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 151.4, 143.3, 143.0, 133.0, 132.1, 129.4, 126.7, 123.6, 122.2, 120.4, 117.4, 112.0, 107.9, 40.2.</p>
	<p>Nature: Yellow Powder, M.p: 116-118 °C, R_f (30 % ; Ethyl Acetate in Hexane): 0.86, ^1H NMR (400 MHz, CDCl_3); δ 7.25 (dd, J = 15.3, 8.7 Hz, 2H), 7.10 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 6.55 (d, J = 8.9 Hz, 1H), 6.35 (d, J = 8.9 Hz, 1H), 3.32 (q, J = 7.0 Hz, 2H), 3.25 (q, J = 7.0 Hz, 2H), 1.12 (t, J = 7.0 Hz, 3H), 1.07 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3); δ 159.5, 149.2, 148.5, 136.2, 133.4, 132.5, 131.4, 131.2, 126.9, 125.2, 122.4, 120.5, 110.4, 110.4, 101.7, 44.4, 44.3, 12.9, 12.6.</p>
	<p>Nature: Yellow Powder, M.p: 118-120 °C, R_f (3 % ; Ethyl Acetate in Hexane): 0.87, ^1H NMR (400 MHz, CDCl_3) δ 7.64 (d, J = 8.7 Hz, 1H), 7.38 (q, J = 8.7 Hz, 3H), 7.24 – 7.07 (m, 5H), 7.06 – 6.81 (m, 10H). ^{13}C NMR (101 MHz, CDCl_3) δ 149.1, 145.4, 140.9, 133.0, 131.0, 129.7, 128.5, 128.5,</p>

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	128.1, 126.1, 124.7, 123.5, 123.1, 119.6, 105.2.
	<p>Nature: Yellow powder, M.p: 309-311 °C, Rf (30% EtOAc-Hexane): 0.83, FTIR (CH₂Cl₂) ν_{\max}: 3286, 2960, 2933, 2873, 1714, 1596, 1556, 1460, 1376, 1263, 1156, 1059, 961, 826, 758, cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.89 (t, <i>J</i> = 8.5 Hz, 1H), 7.79 (d, <i>J</i> = 7.6 Hz, 1H), 7.77 – 7.59 (m, 10H), 7.55 (td, <i>J</i> = 7.3, 1.3 Hz, 4H), 7.46 (ddd, <i>J</i> = 8.4, 5.2, 2.2 Hz, 7H), 7.28 – 7.23 (m, 1H), 3.06 (s, 3H), 2.98 (s, 3H), 2.16 – 1.97 (m, 2H), 1.45 – 1.33 (m, 2H), 1.27 (d, <i>J</i> = 9.6 Hz, 14H), 1.11 (dd, <i>J</i> = 16.9, 10.0 Hz, 2H), 0.88 (t, <i>J</i> = 6.7 Hz, 3H), 0.76 (t, <i>J</i> = 6.6 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 151.9, 151.7, 132.9, 132.2, 132.1, 132.0, 132.0, 131.8, 131.4, 129.4, 128.6, 128.5, 127.5, 125.8, 125.2, 121.3, 120.2, 116.0, 114.1, 111.7, 111.6, 111.3, 55.4, 40.1, 40.0, 33.8, 31.9, 29.7, 22.7, 22.6, 14.1, 14.0. MALDI-TOF Mass: Calcd. for C₆₃H₆₆N₄ Exact Mass: 826.17; Found 826.027 (M⁺).</p>
	<p>Nature: Dark Orange Powder, M.p: 283-285 °C, Rf (30% EtOAc-Hexane): 0.53, FTIR (CH₂Cl₂) ν_{\max}: 3168, 2990, 2853, 1800, 1556, 1460, 1376, 1156, 961, 826 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.40 (d, <i>J</i> = 8.7 Hz, 2H), 7.33 (d, <i>J</i> = 8.8 Hz, 3H), 7.26 (d, <i>J</i> = 11.1 Hz, 1H), 7.06 (dd, <i>J</i> = 15.1, 11.3 Hz, 1H), 6.85 (d, <i>J</i> = 15.1 Hz, 1H), 6.57 (d, <i>J</i> = 8.8 Hz, 2H), 2.93 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 151.4, 143.3, 143.0, 133.0, 132.1, 129.4, 126.7, 123.6, 122.2, 120.4, 117.4, 112.0, 107.9, 40.2. MALDI-TOF Mass: Calcd. for C₆₃H₆₆N₄ Exact Mass: 878.5287; Found 878.785 (M+1).</p>

Supporting Information

	<p>Nature: Yellowish-green, Powder, M.p: 250-252 °C, Rf (30% EtOAc-Hexane): 0.63, FTIR (CH₂Cl₂) ν_{\max}: 3280, 2960, 2933, 2873, 1910, 1556, 1460, 1263, 1156, 1005, 858 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.70 (dd, J = 15.4, 5.7 Hz, 1H), 7.67 – 7.61 (m, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.43 (d, J = 29.7 Hz, 1H), 7.19 (dt, J = 14.6, 6.6 Hz, 2H), 7.11 – 6.93 (m, 3H), 2.08 – 1.87 (m, 1H), 1.31 (d, J = 17.0 Hz, 1H), 1.24 – 1.09 (m, 2H), 0.98 (s, 3H), 0.83 – 0.72 (m, 1H), 0.66 (d, J = 6.8 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 150.9, 149.0, 145.6, 140.7, 140.2, 139.4, 138.0, 132.8, 130.2, 129.7, 128.6, 128.5, 126.6, 125.5, 125.1, 125.0, 124.7, 123.4, 120.3, 119.9, 119.2, 117.7, 106.3, 54.4, 39.4, 30.4, 28.7, 28.6, 21.5, 13.0. MALDI-TOF Mass: Calcd. for C₇₉H₇₀N₄ Exact Mass: 1074.5600; Found 1074.982 (M⁺).</p>
	<p>Nature: Yellow Powder, M.p: 400-402 °C, Rf (40% EtOAc-Hexane): 0.53. FTIR (CH₂Cl₂) ν_{\max}: 3096, 2958, 2873, 1919, 1596, 1460, 1263, 1156, 961, 626 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.74 (t, J = 9.5 Hz, 2H), 7.70 – 7.63 (m, 4H), 7.54 (dt, J = 6.7, 4.6 Hz, 4H), 7.43 (ddd, J = 22.8, 13.7, 5.7 Hz, 7H), 6.96 (d, J = 8.8 Hz, 1H), 6.65 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 8.9 Hz, 1H), 3.52 – 3.28 (m, 7H), 2.02 (dd, J = 8.1, 5.8 Hz, 1H), 1.92 – 1.45 (m, 1H), 1.34 – 0.93 (m, 17H), 0.92 – 0.81 (m, 1H), 0.74 (t, J = 6.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 151.7, 150.3, 149.1, 148.4, 140.1, 139.6, 139.4, 133.5, 133.0, 132.5, 132.2, 132.1, 132.0, 132.0, 130.0, 128.6, 128.5, 126.8, 125.6, 122.7, 121.1, 120.0, 110.5, 110.4, 110.0, 55.3, 44.5, 44.3, 40.5, 34.7, 34.5, 29.7, 26.9, 22.6, 14.0, 12.7, 12.6. MALDI-TOF Mass: Calcd. for C₈₃H₉₆N₆ Exact Mass: 1176.7696; Found 1177.114 (M+1).</p>

4.0 Scanned copy of spectra (^1H , ^{13}C NMR, DEPT-135, FTIR, HRMS and ESI-mass)Figure 4.1. ^1H NMR spectrum of compound **1c**Figure 4. 2. ^{13}C NMR spectrum of compound **1c**

Supporting Information

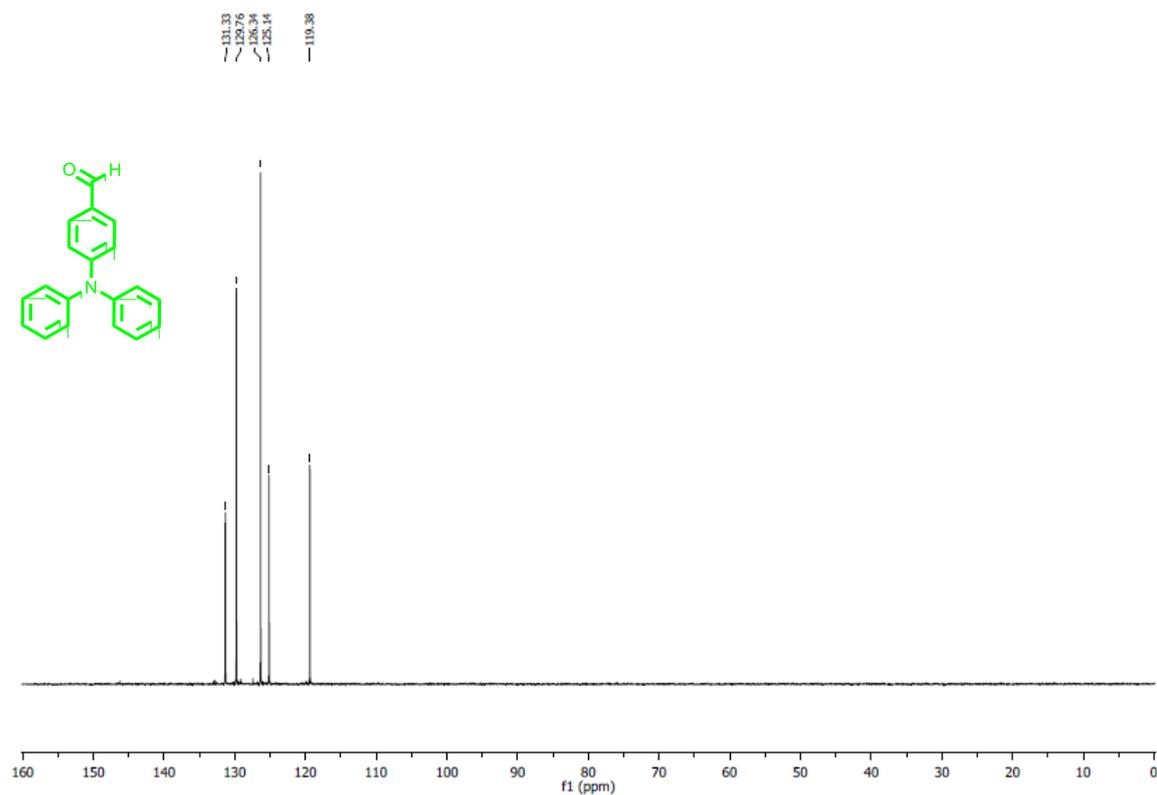


Figure 4. 3. DEPT-135 NMR spectrum of compound **1c**

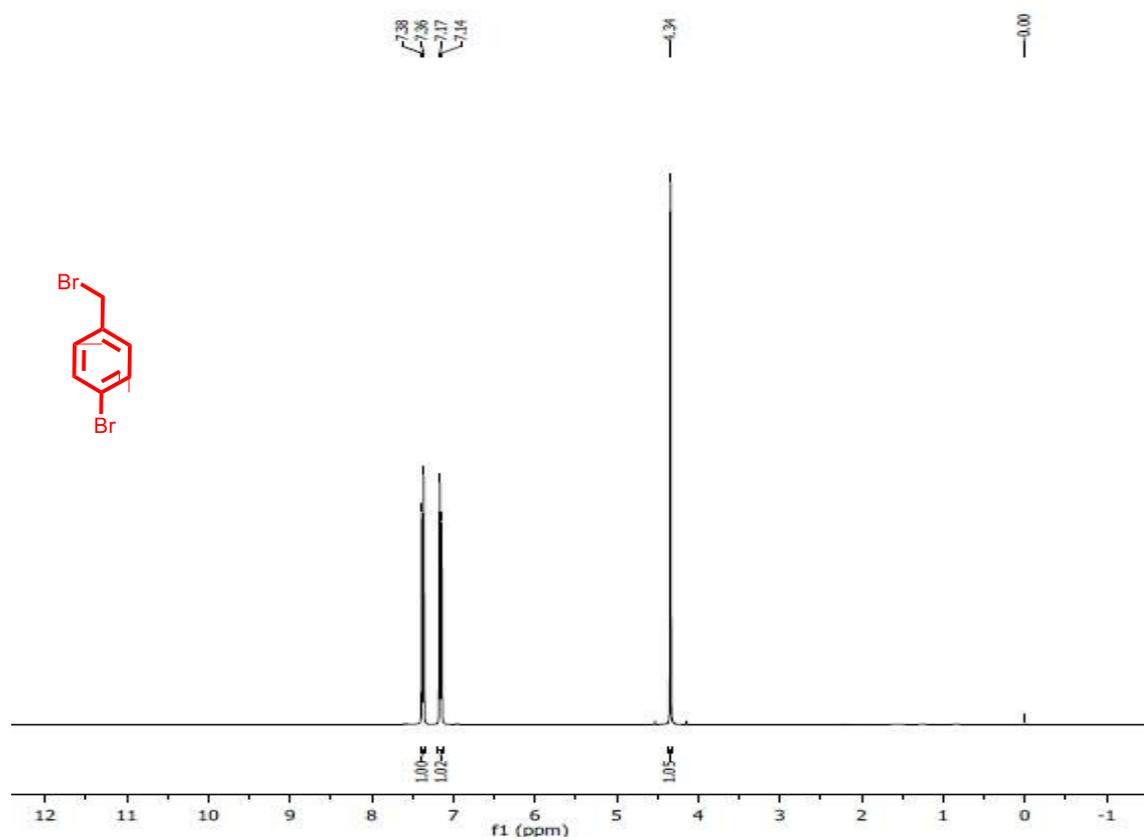


Figure 4. 4. ¹H NMR spectrum of compound **a**

Supporting Information

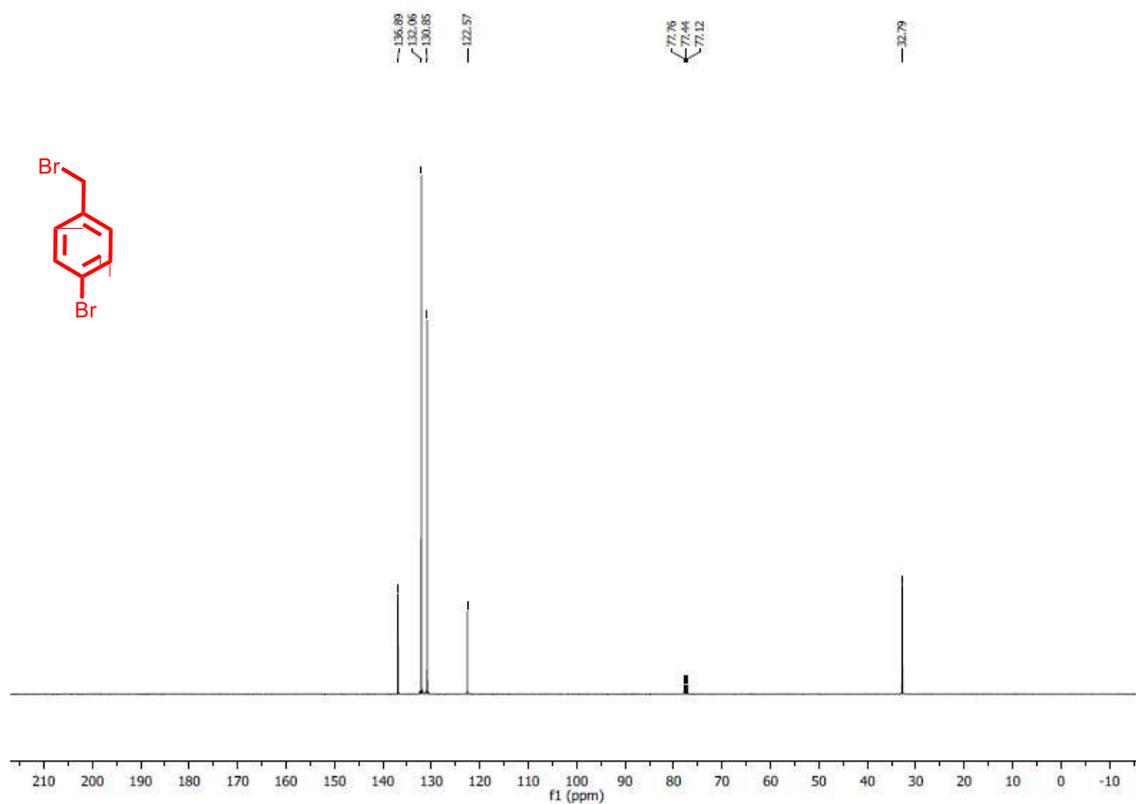


Figure 4.5. ^{13}C NMR spectrum of compound **a**

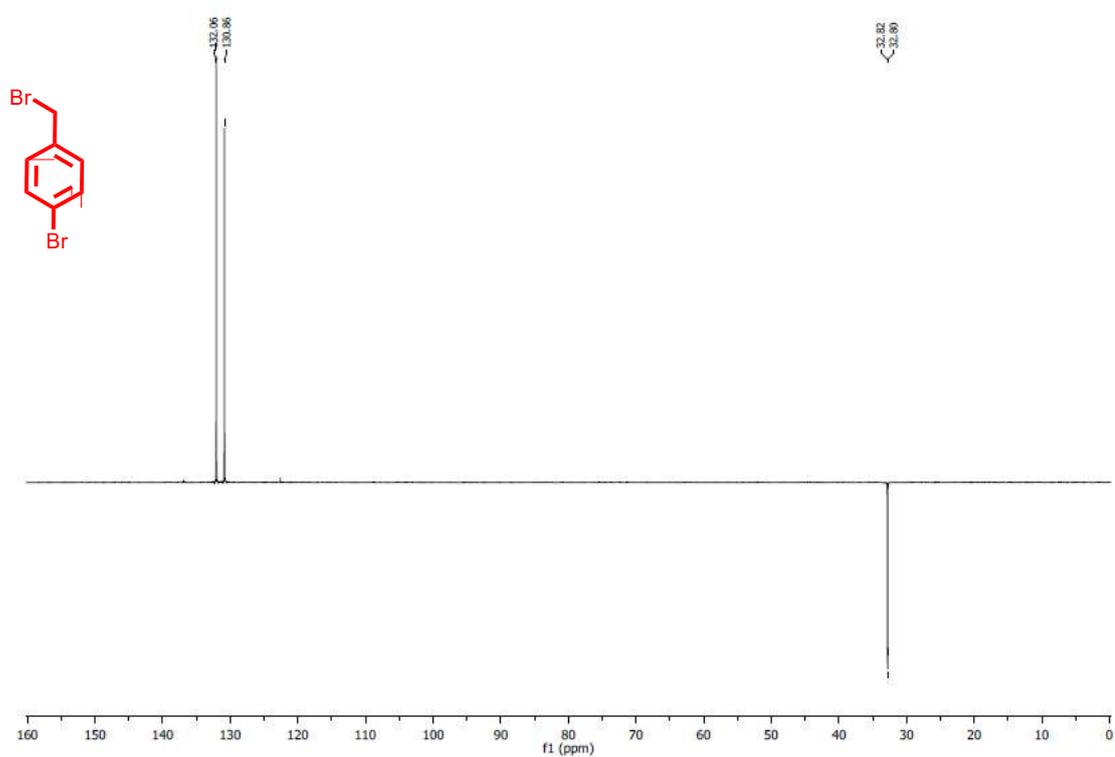


Figure 4. 6. DEPT-135 NMR spectrum of compound **a**

Supporting Information

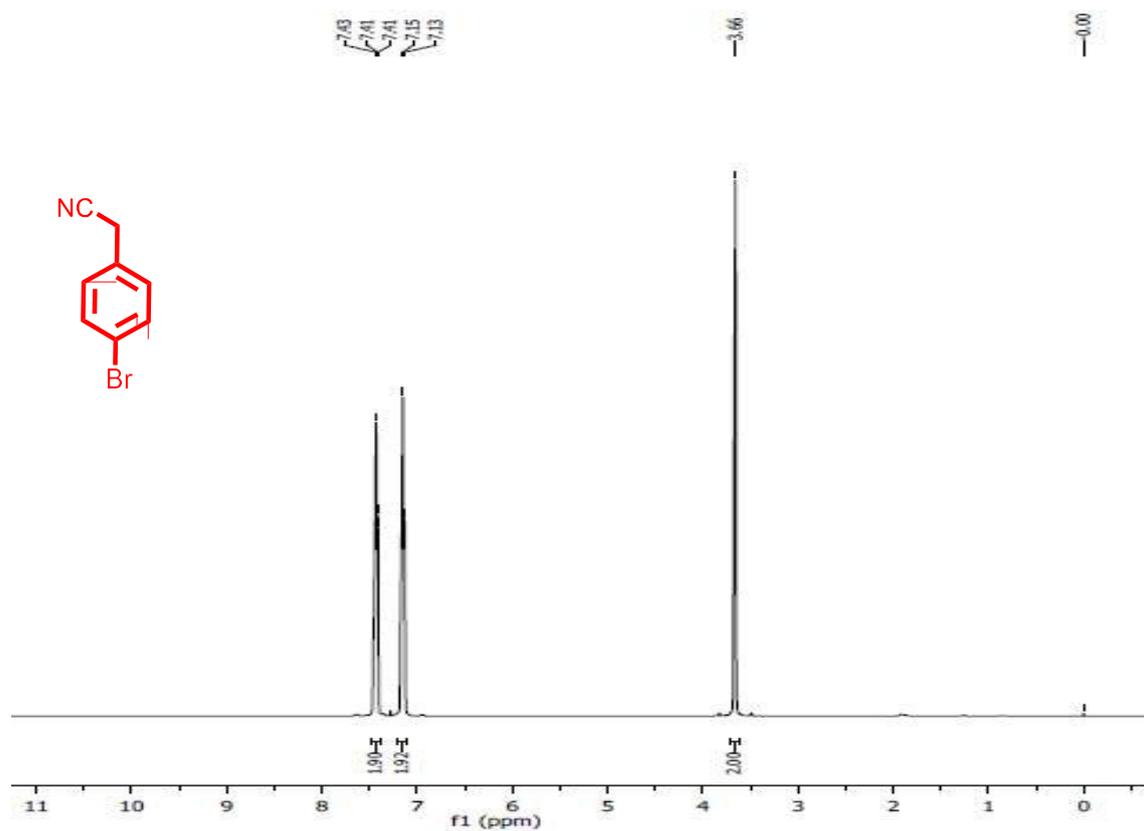


Figure 4. 7. ¹H NMR spectrum of compound **ab**

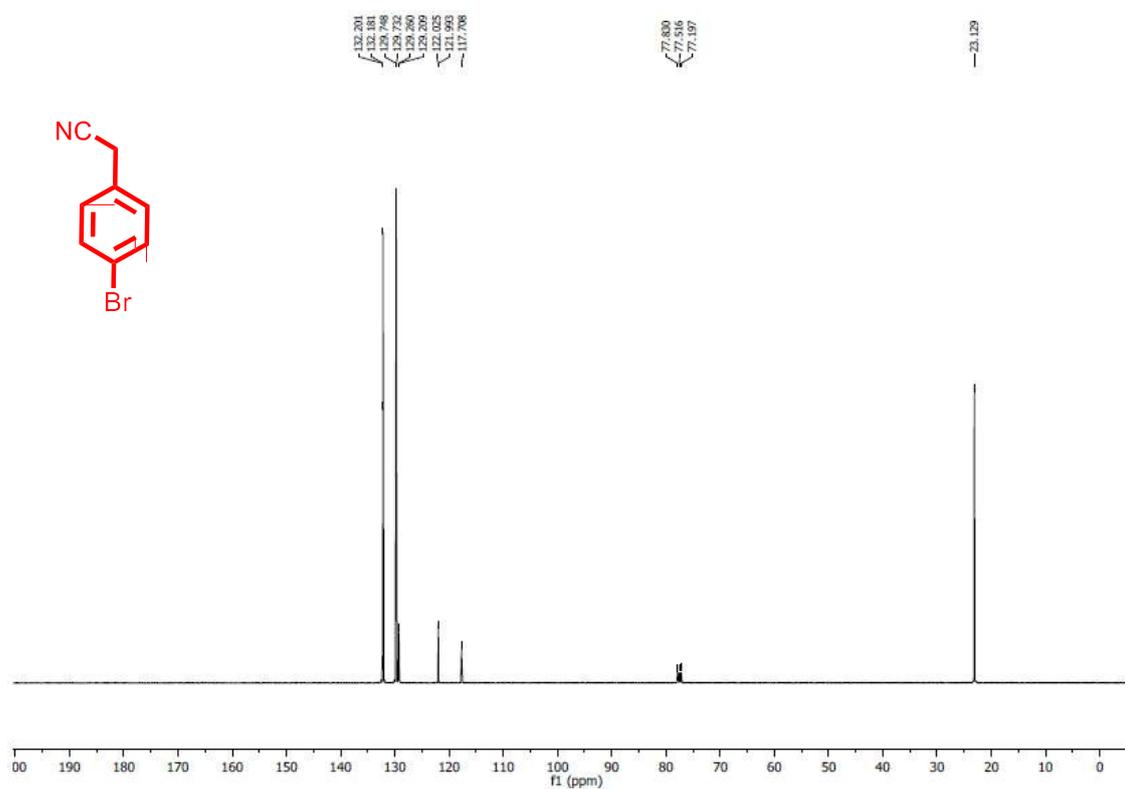


Figure 4. 8. ¹³C NMR spectrum of compound **ab**

Supporting Information

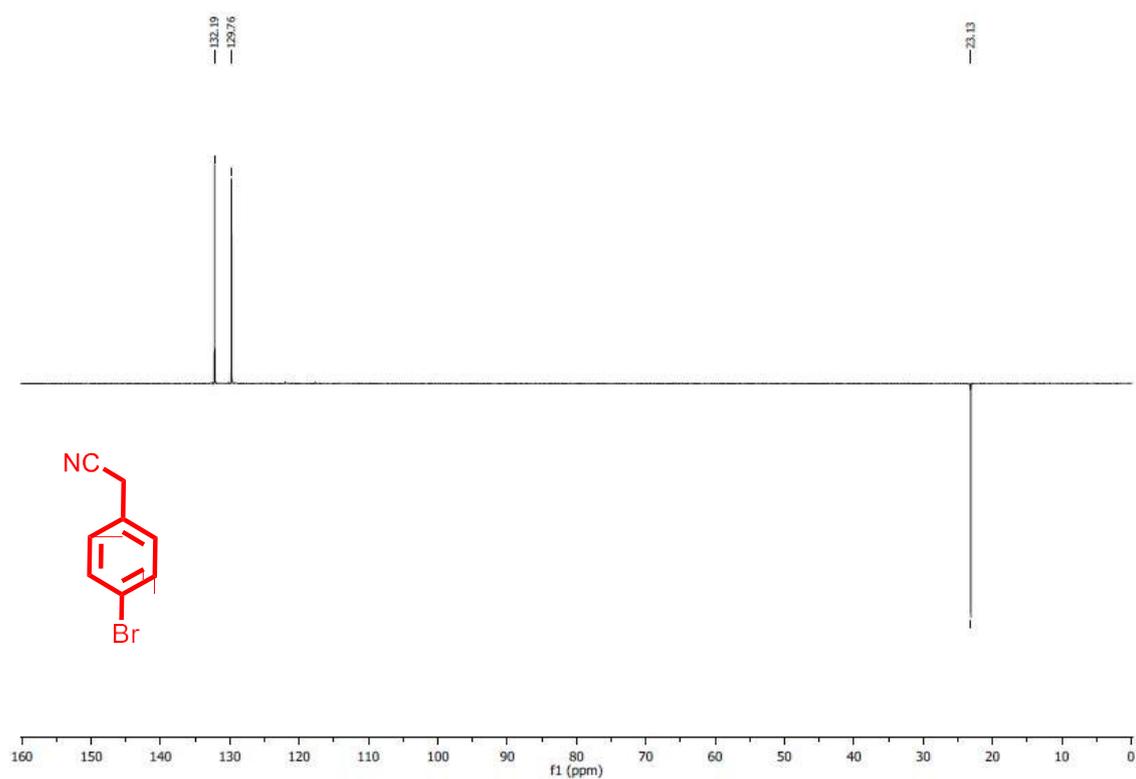


Figure 4. 9. DEPT-135 NMR spectrum of compound **ab**

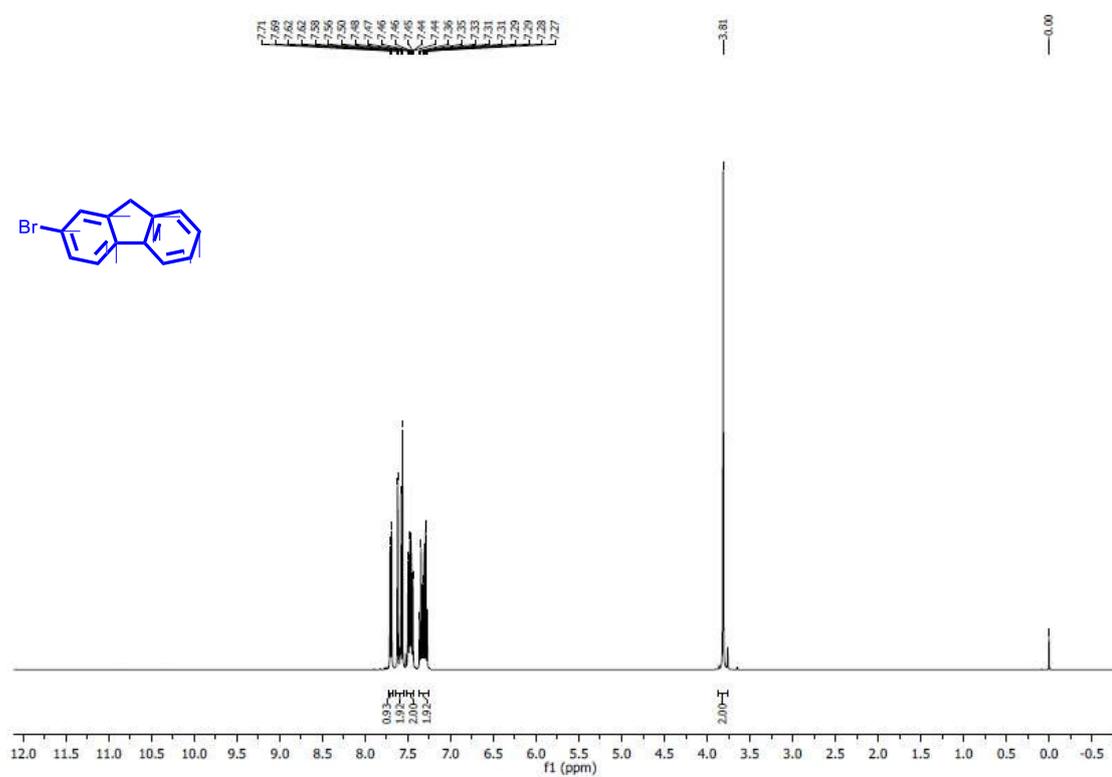


Figure 4. 10. ¹H NMR spectrum of compound **3a**

Supporting Information

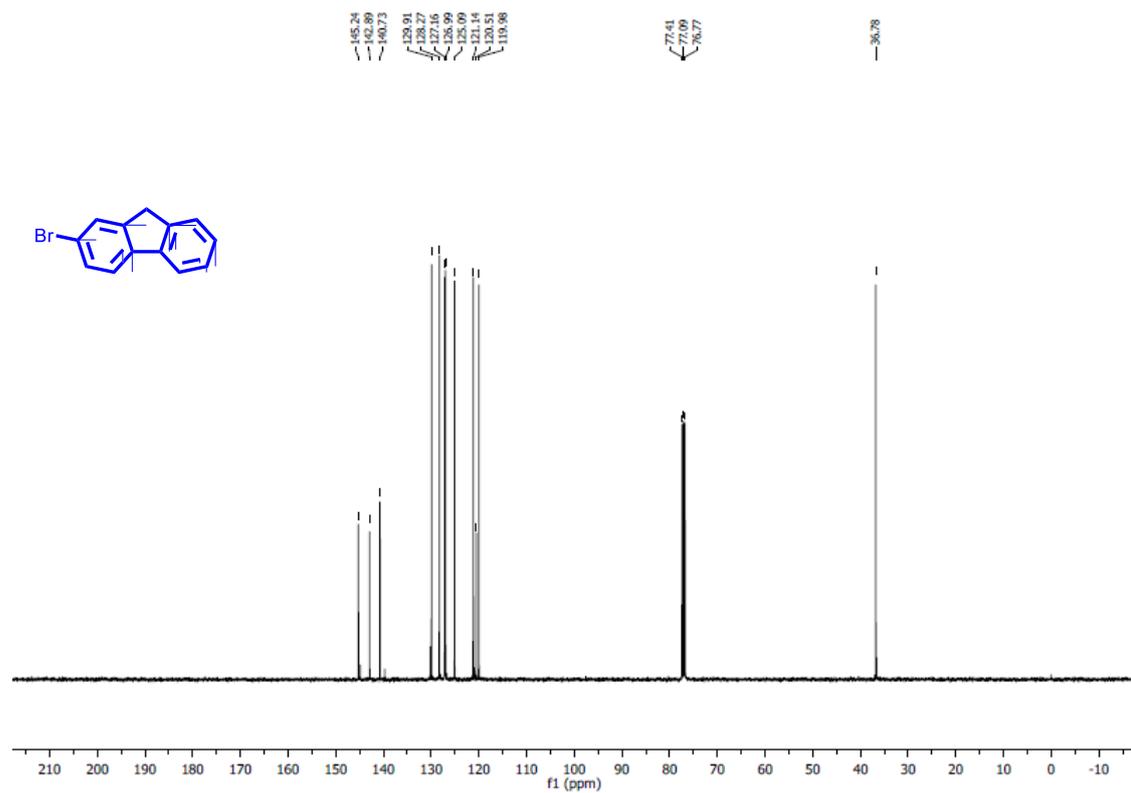


Figure 4. 11. ^{13}C NMR spectrum of compound **3a**

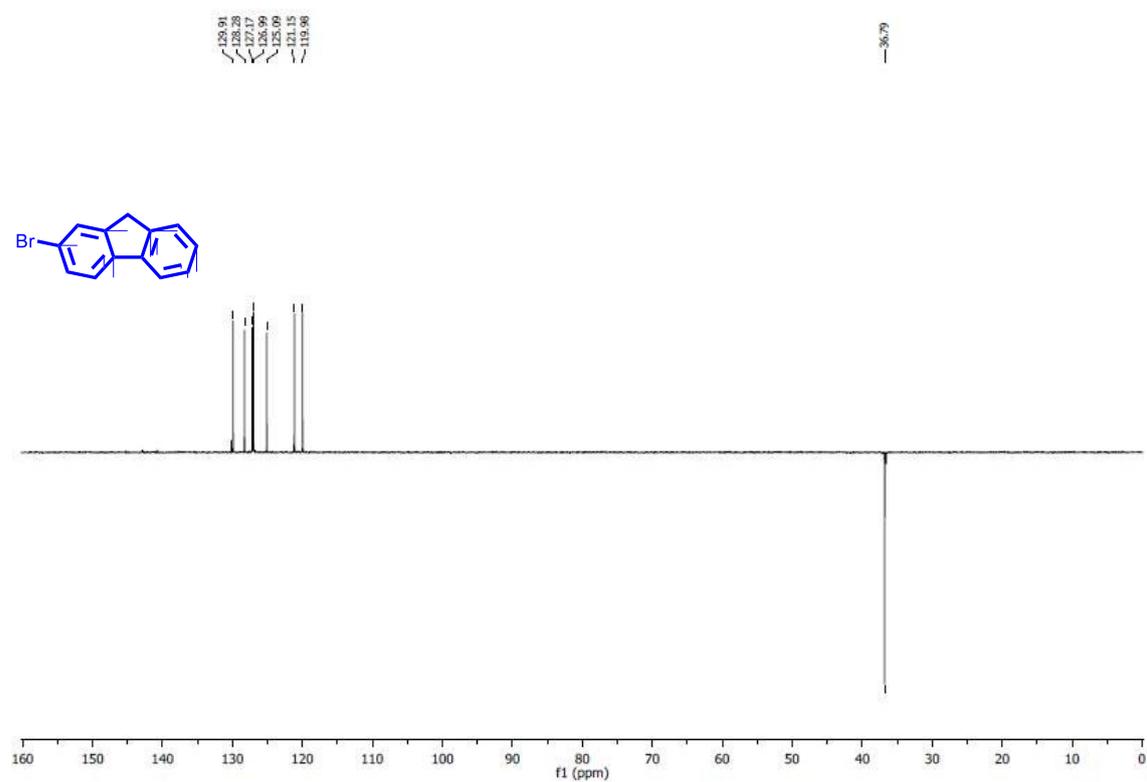


Figure 4. 12. DEPT-135 NMR spectrum of compound **3a**

Supporting Information

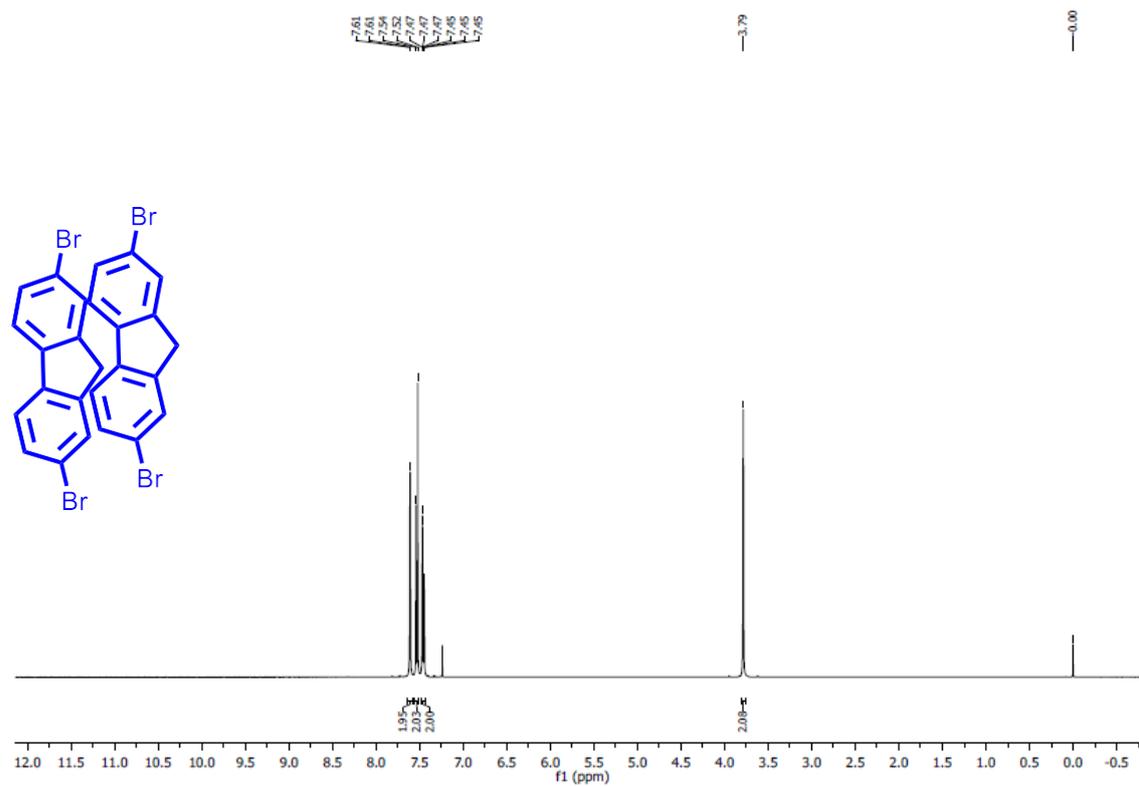


Figure 4. 13. ¹H NMR spectrum of compound **3b**

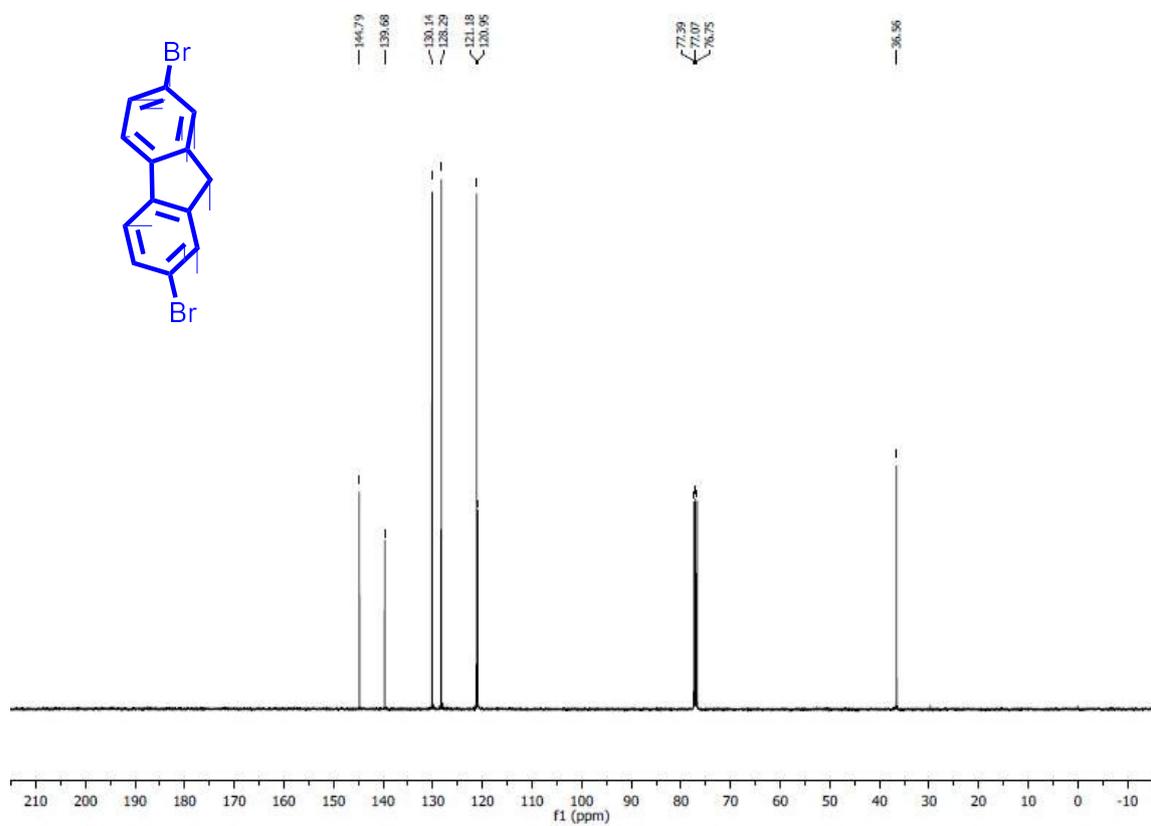


Figure 4. 14. ¹³C NMR spectrum of compound **3b**

Supporting Information

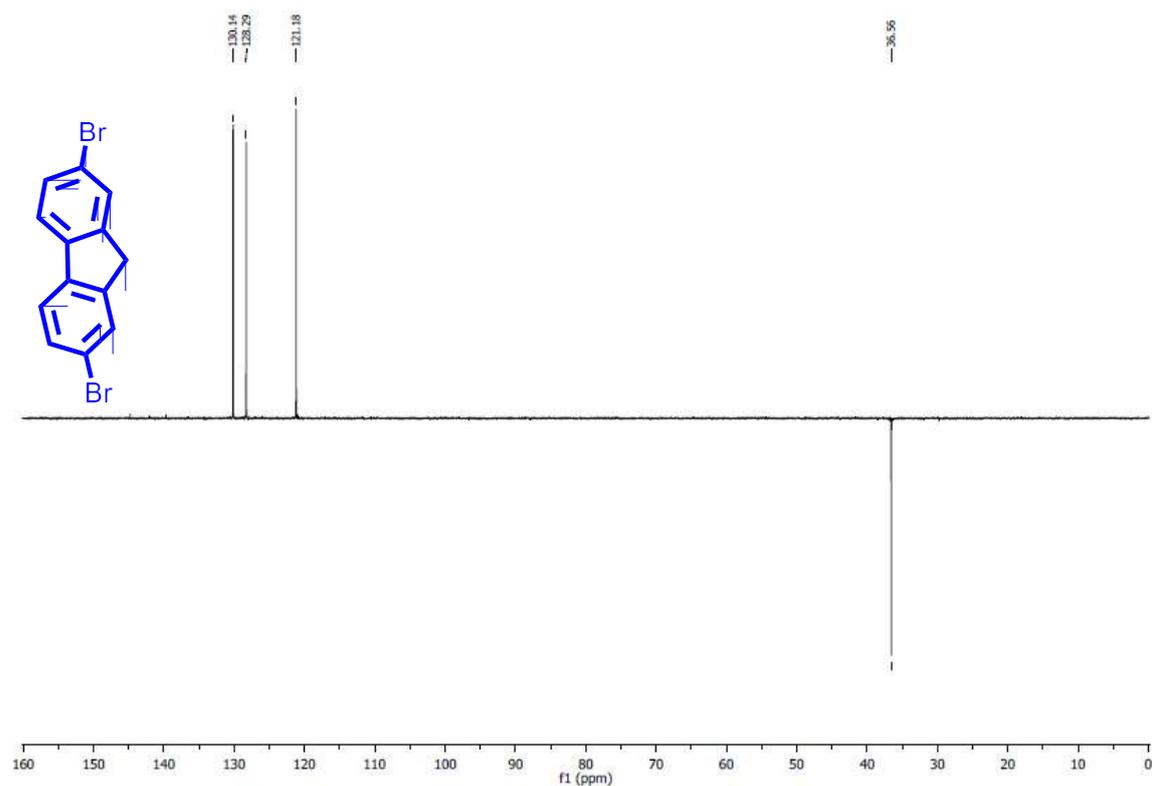


Figure 4. 15. DEPT-135 NMR spectrum of compound **3b**

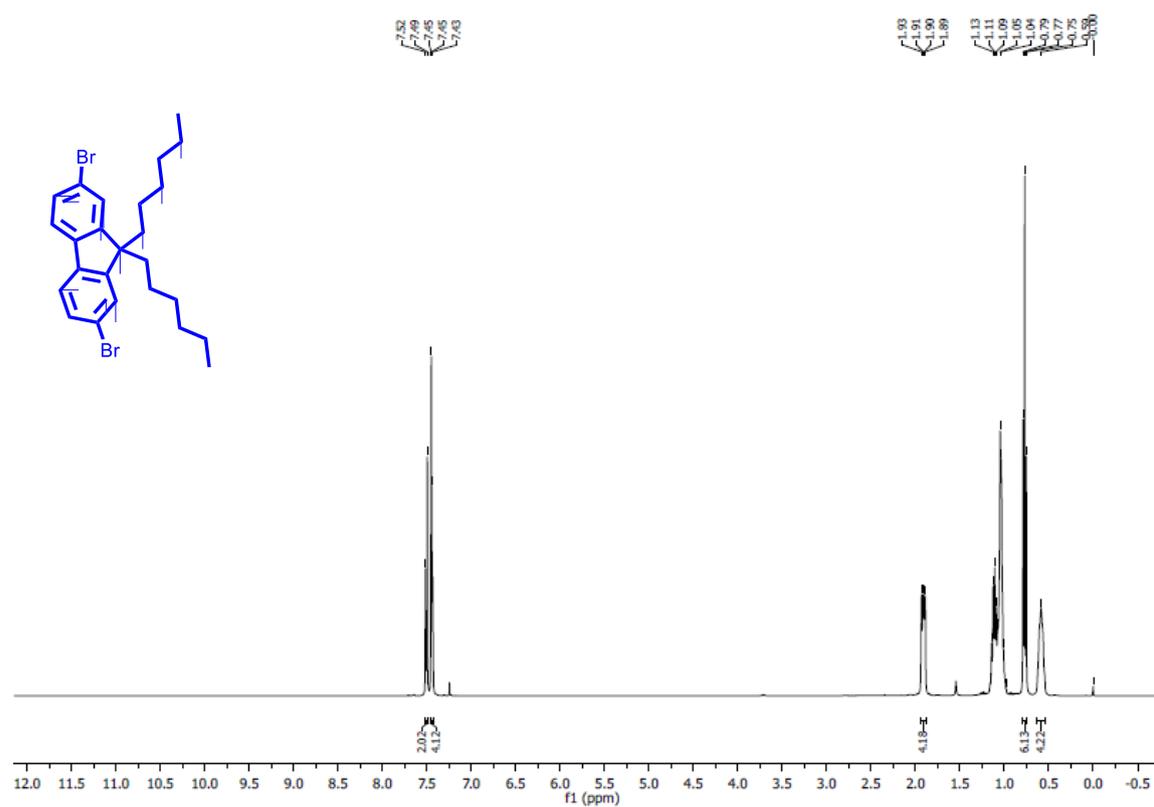


Figure 4. 16. ¹H NMR spectrum of compound **3ba**

Supporting Information

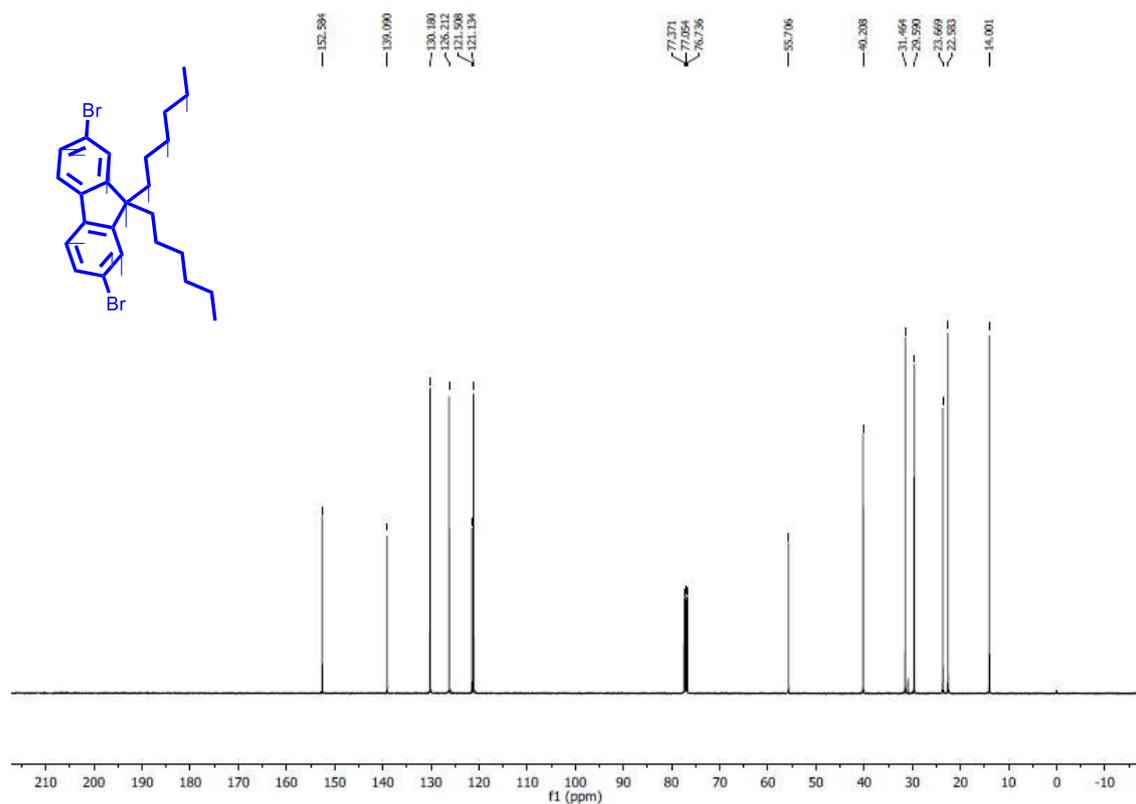


Figure 4. 17. ¹³C NMR spectrum of compound **3ba**

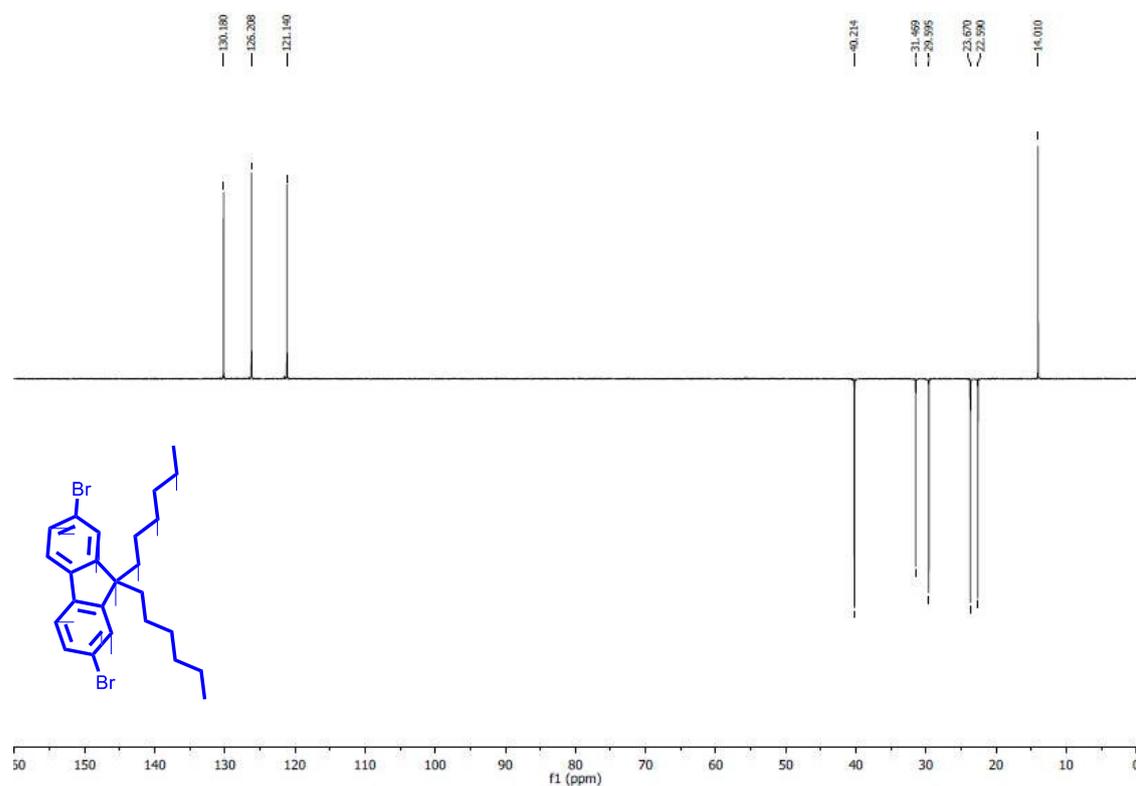


Figure 4. 18. DEPT-135 NMR spectrum of compound **3ba**

Supporting Information

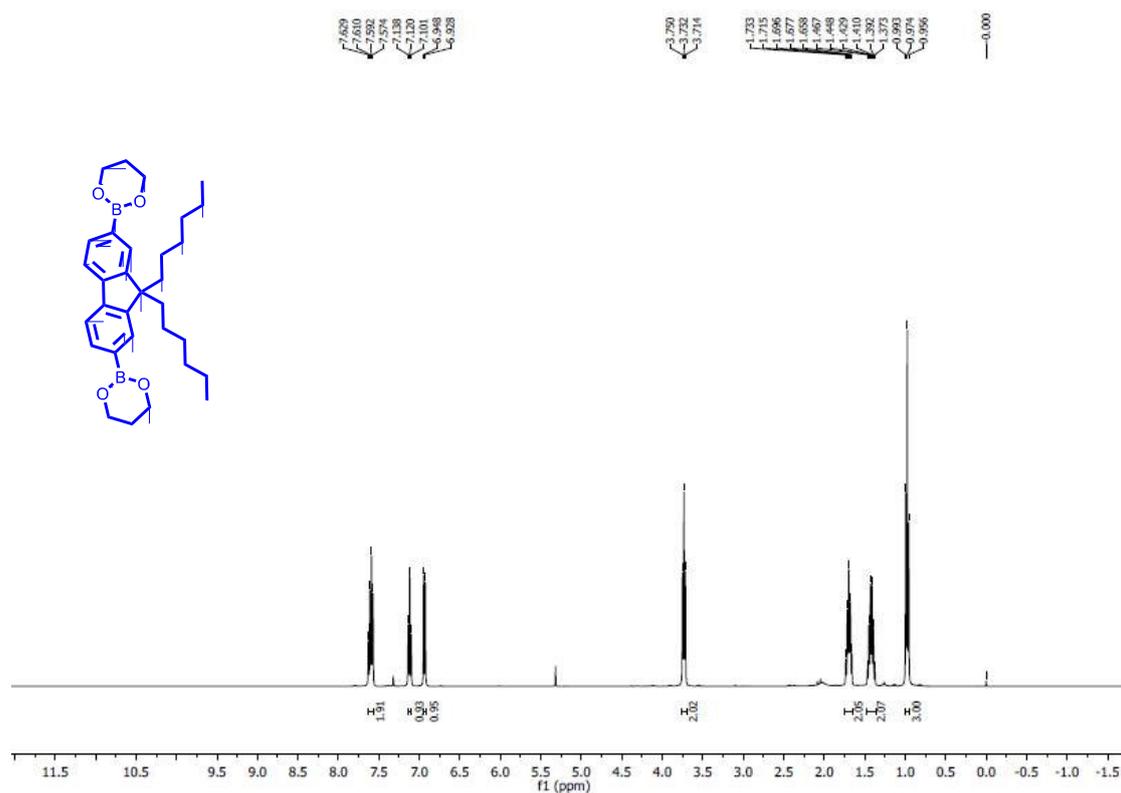


Figure 4. 19. ¹H NMR spectrum of compound **3bc**

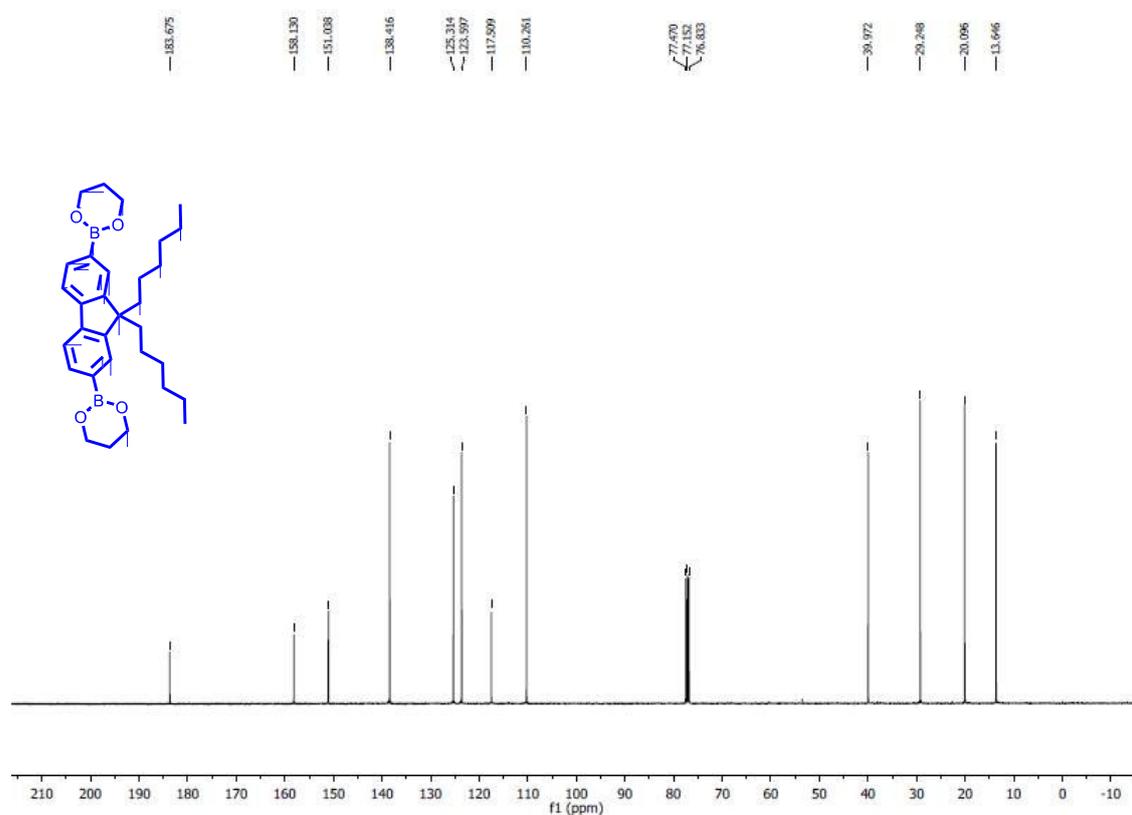


Figure 4. 20. ¹³C NMR spectrum of compound **3bc**

Supporting Information

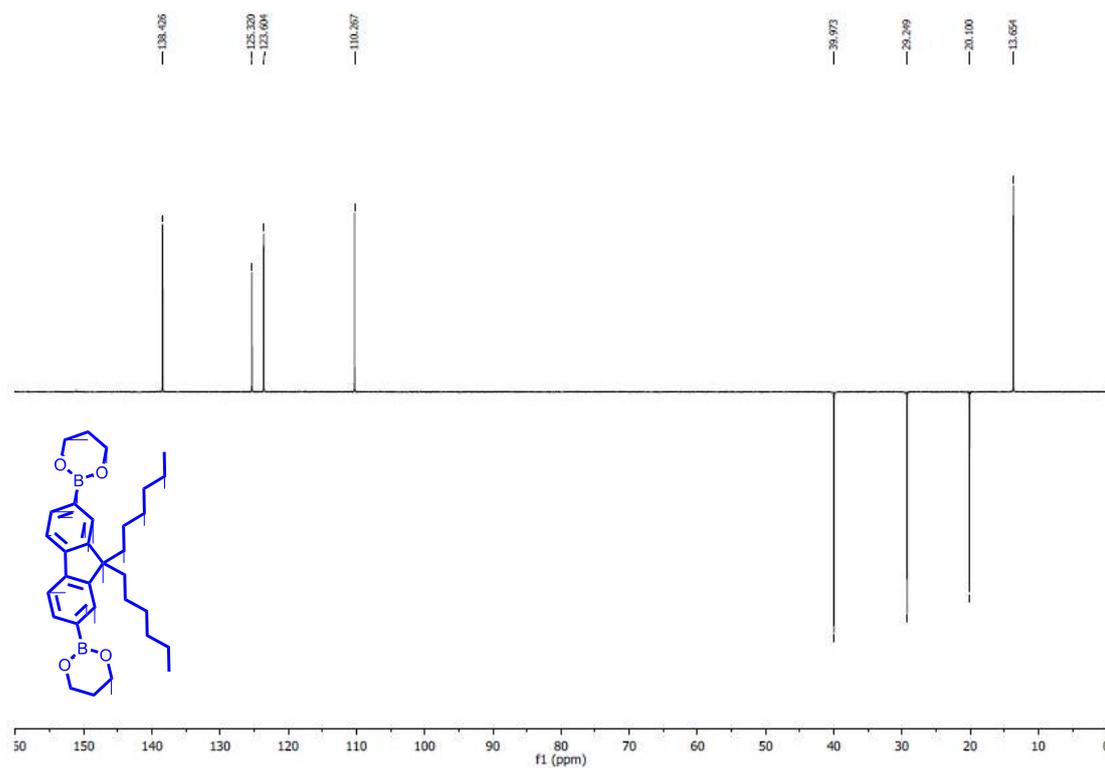


Figure 4. 21. DEPT-135 NMR spectrum of compound **3bc**

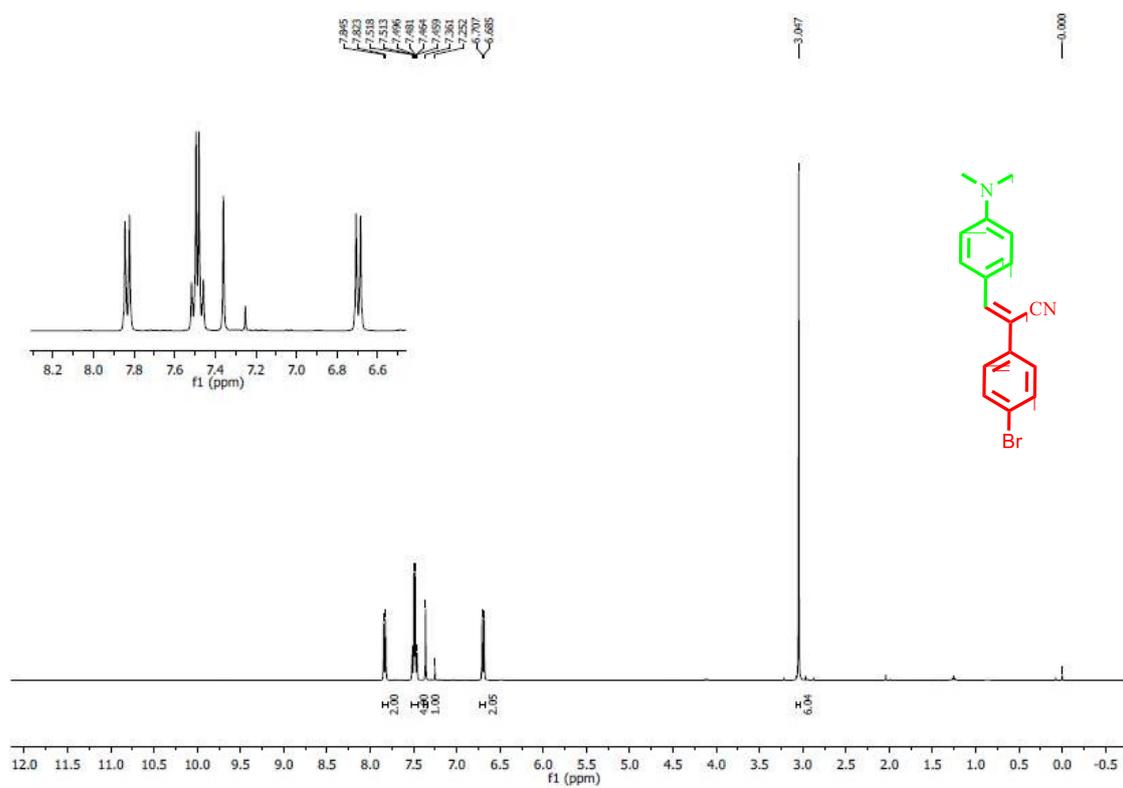


Figure 4. 22. ¹H NMR spectrum of compound **2a**

Supporting Information

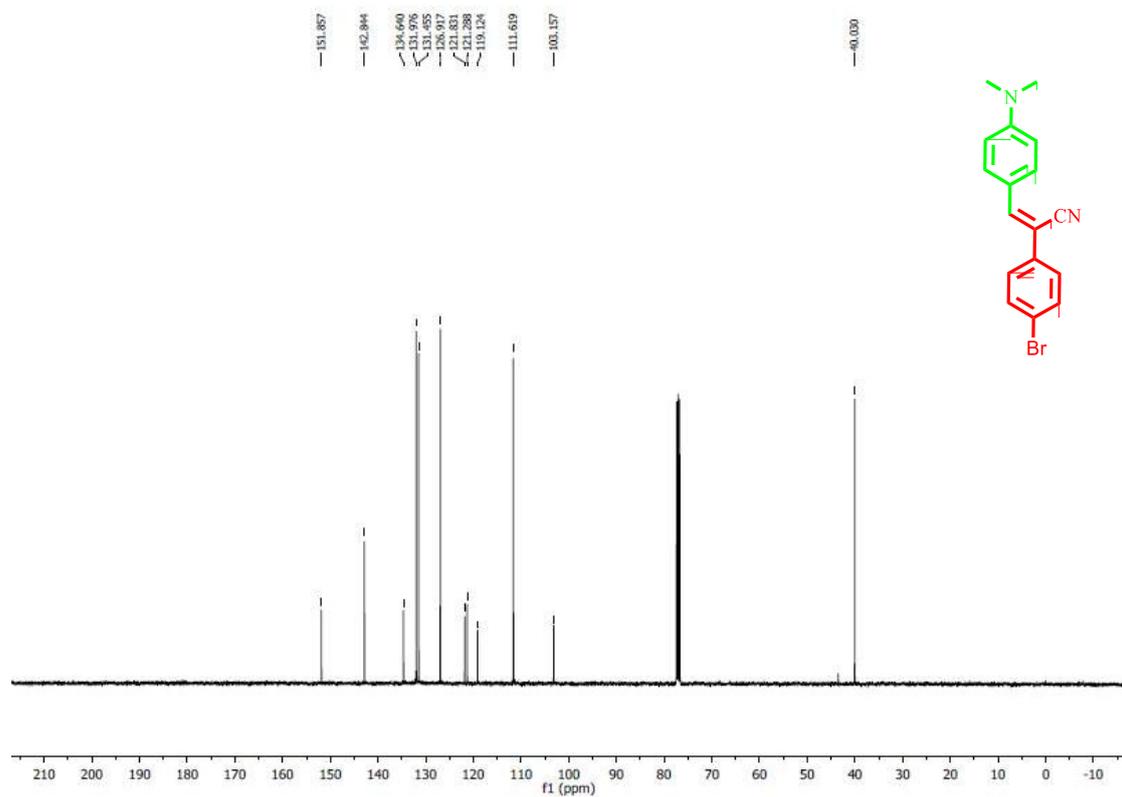


Figure 4. 23. ¹³C NMR spectrum of compound **2a**

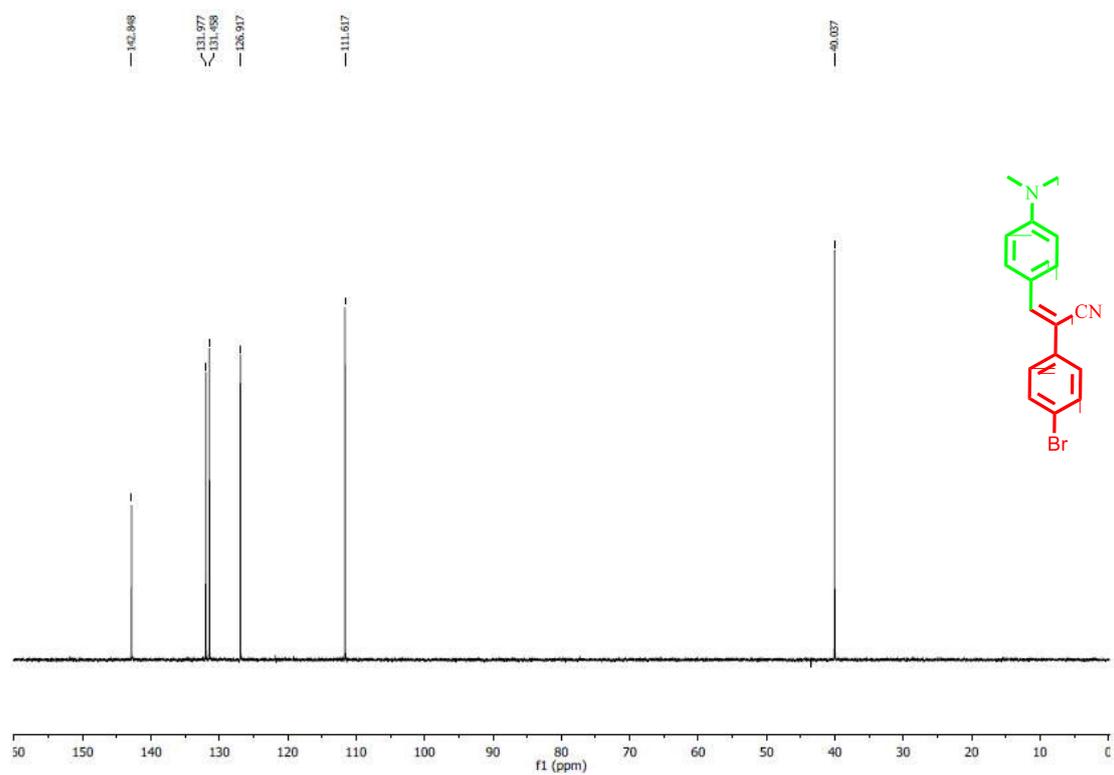


Figure 4. 24. DEPT-135 NMR spectrum of compound **2a**

Supporting Information

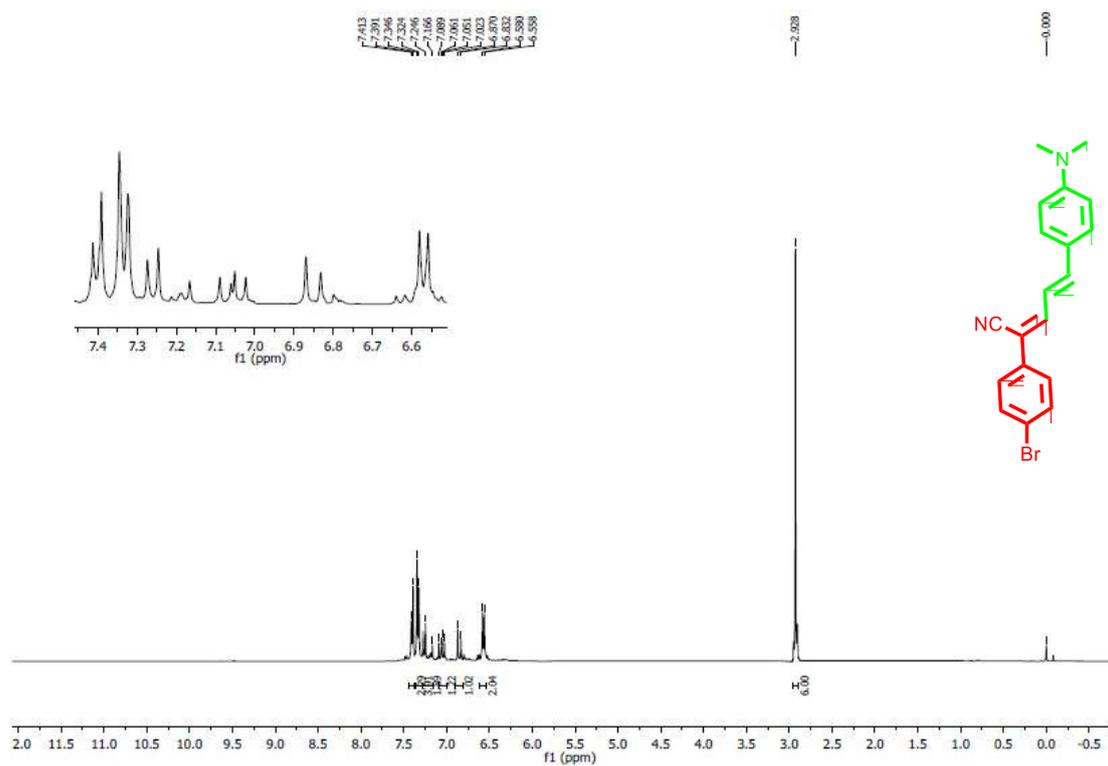


Figure 4. 25. ¹H NMR spectrum of compound **2b**

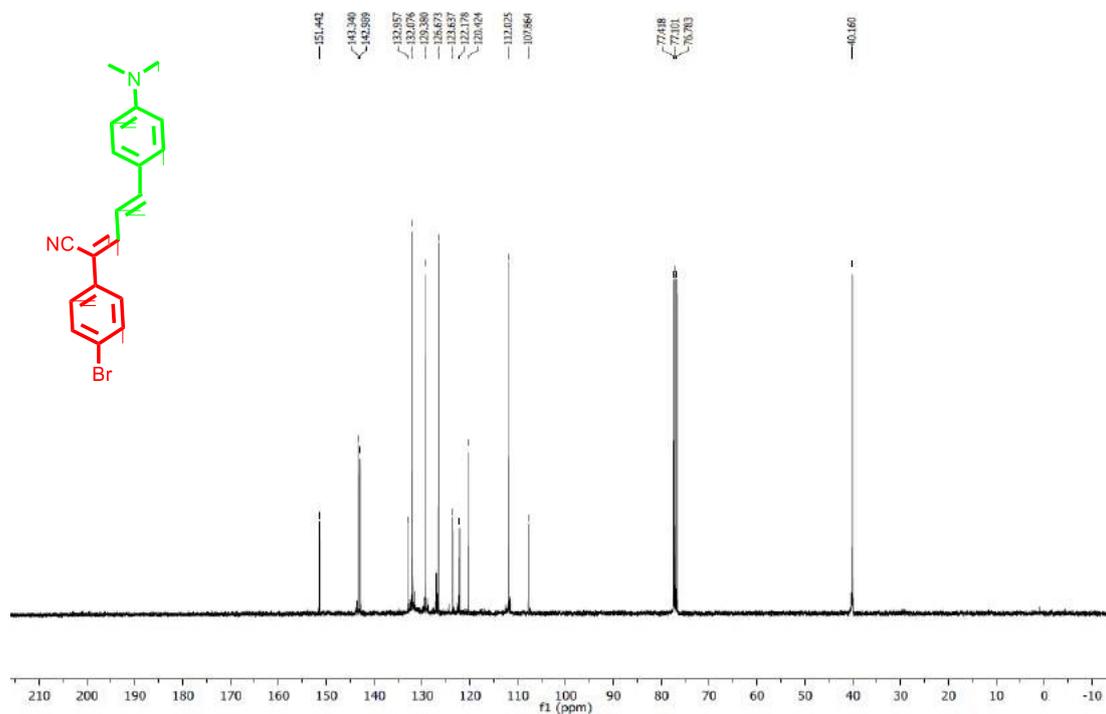


Figure 4. 26. ¹³C NMR spectrum of compound **2b**

Supporting Information

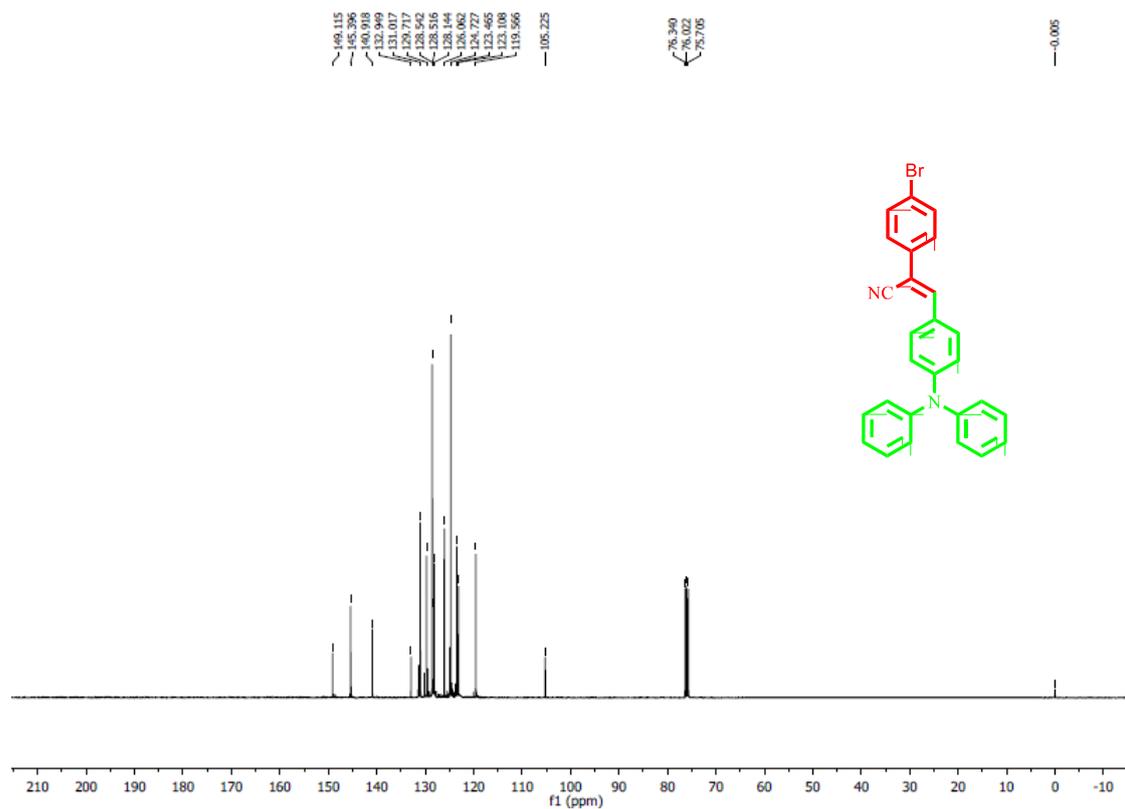


Figure 4. 29. DEPT-135 NMR spectrum of compound **2c**

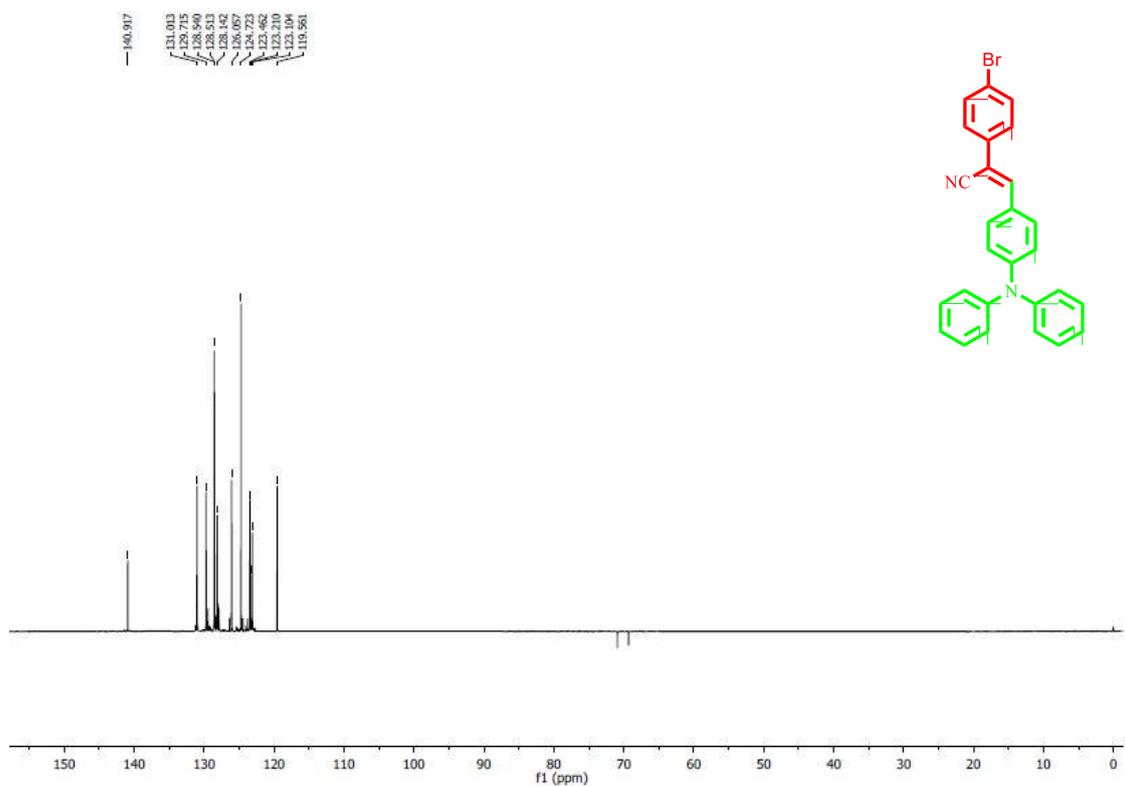


Figure 4. 30. DEPT-135 NMR spectrum of compound **2c**

Supporting Information

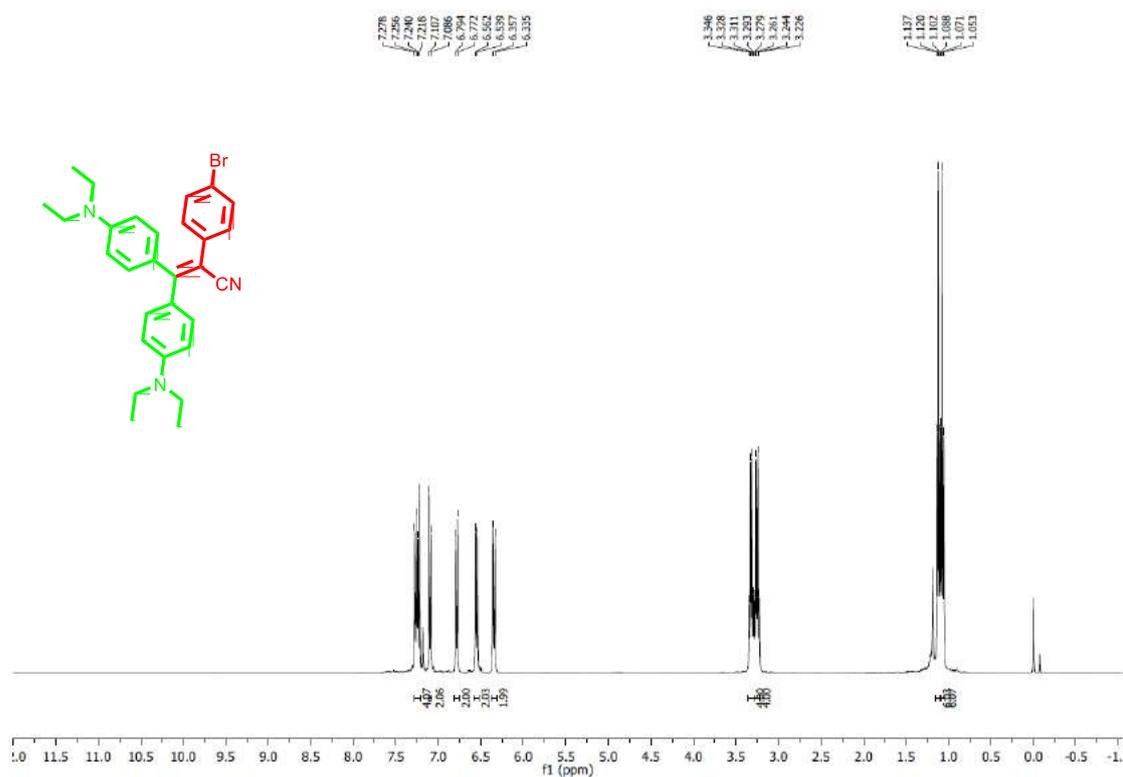


Figure 4. 31. ¹H NMR spectrum of compound 2d

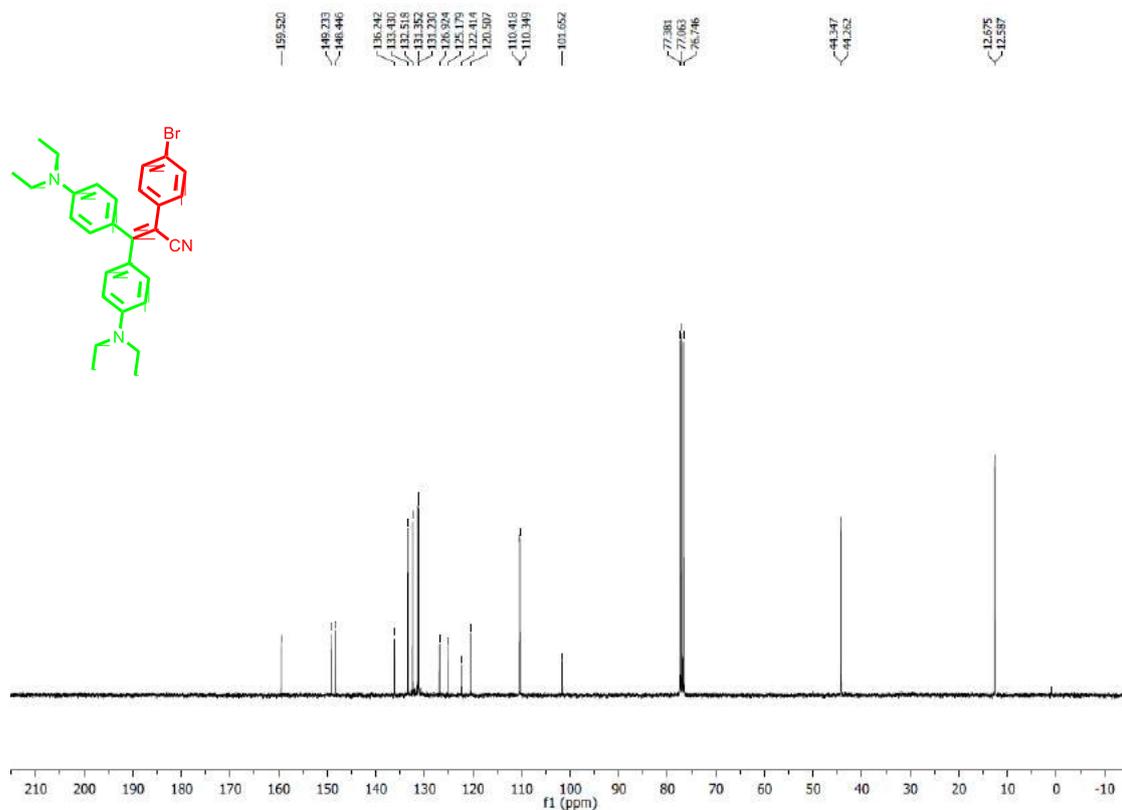


Figure 4. 32. ¹³C NMR spectrum of compound 2d

Supporting Information

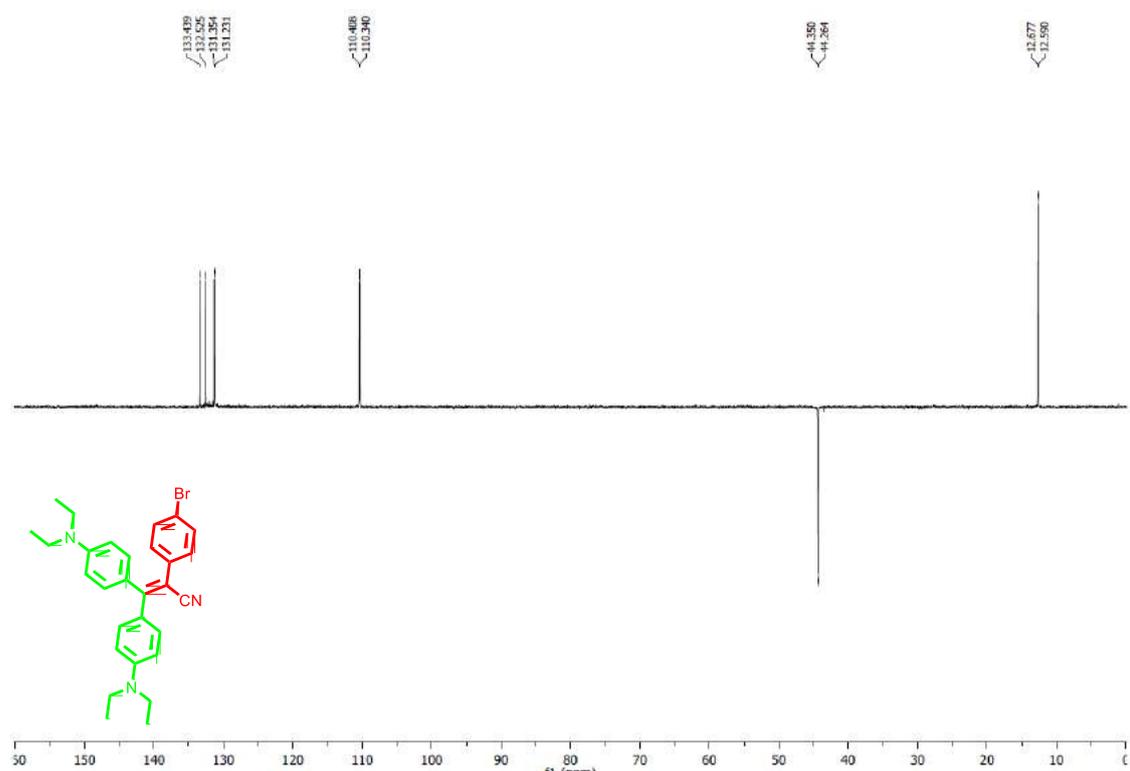


Figure 4. 33. DEPT-135 NMR spectrum of compound **2d**

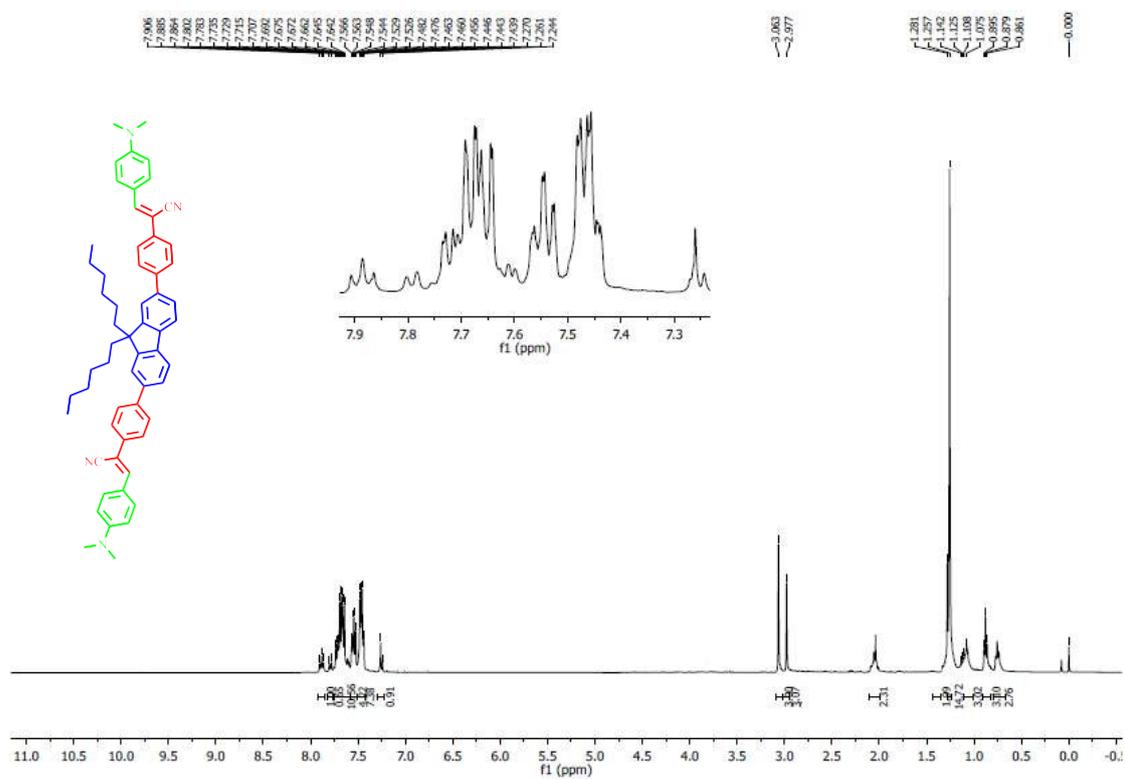


Figure 4. 34. DEPT-135 NMR spectrum of compound **4a**

Supporting Information

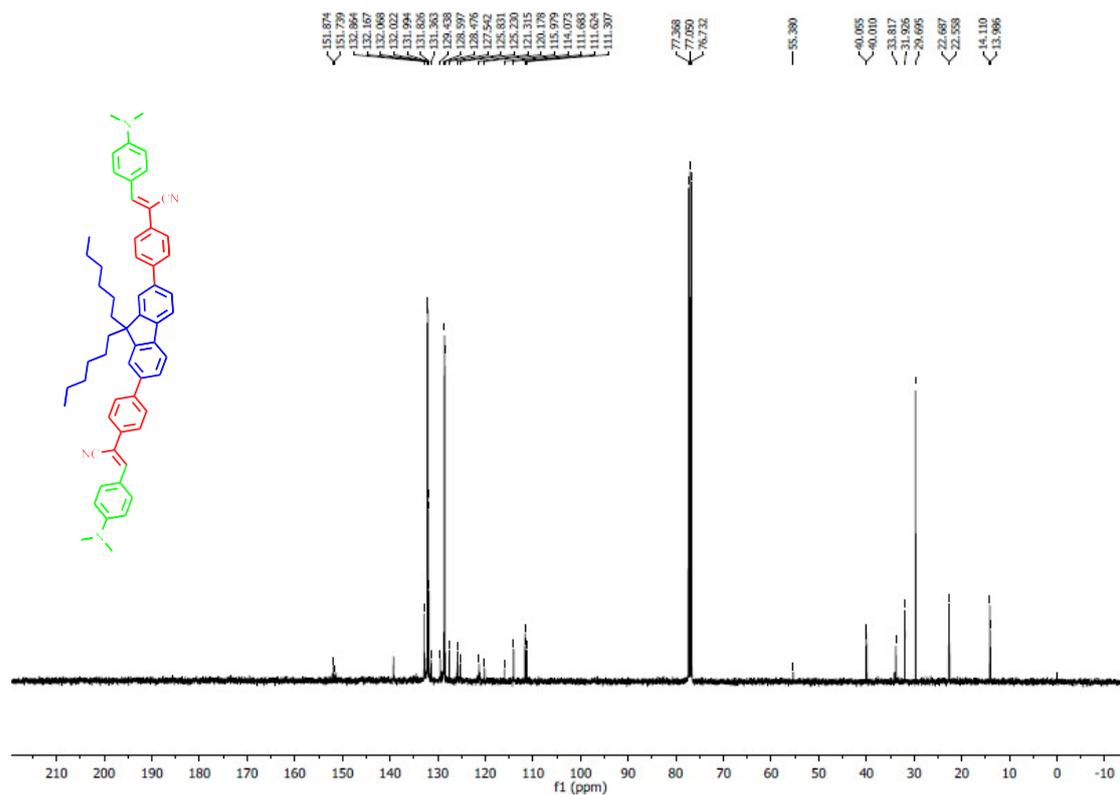
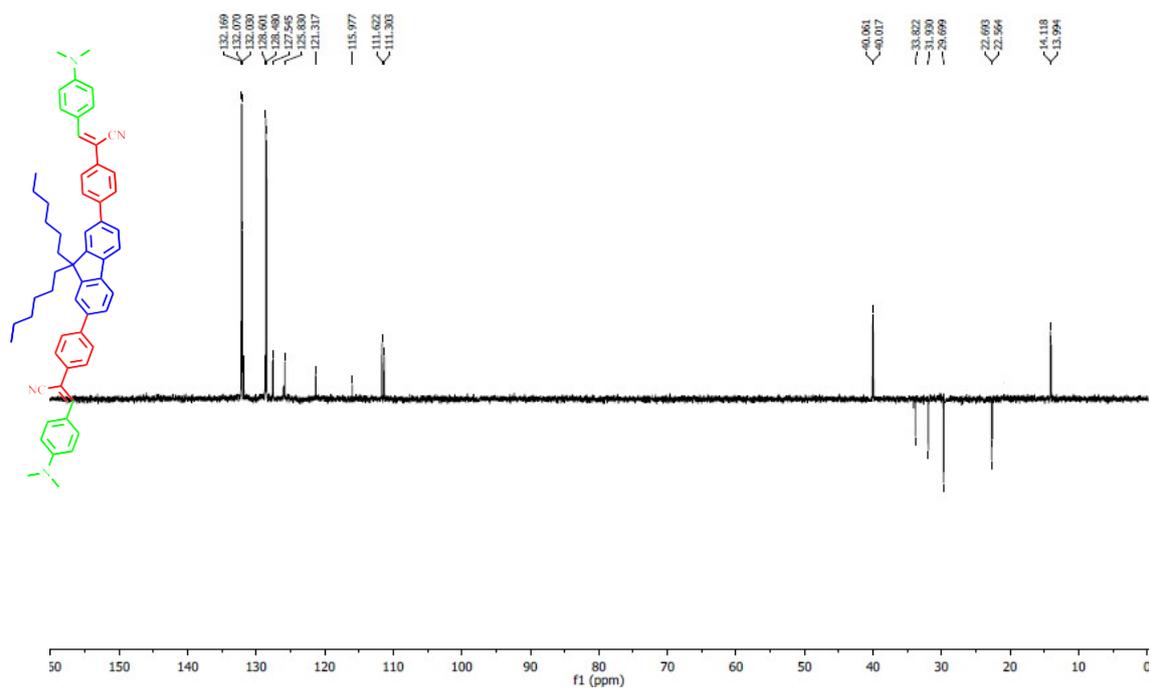


Figure 4. 35. DEPT-135 NMR spectrum of compound 4a



Supporting Information

Figure 4. 36. DEPT-135 NMR spectrum of compound **4a**

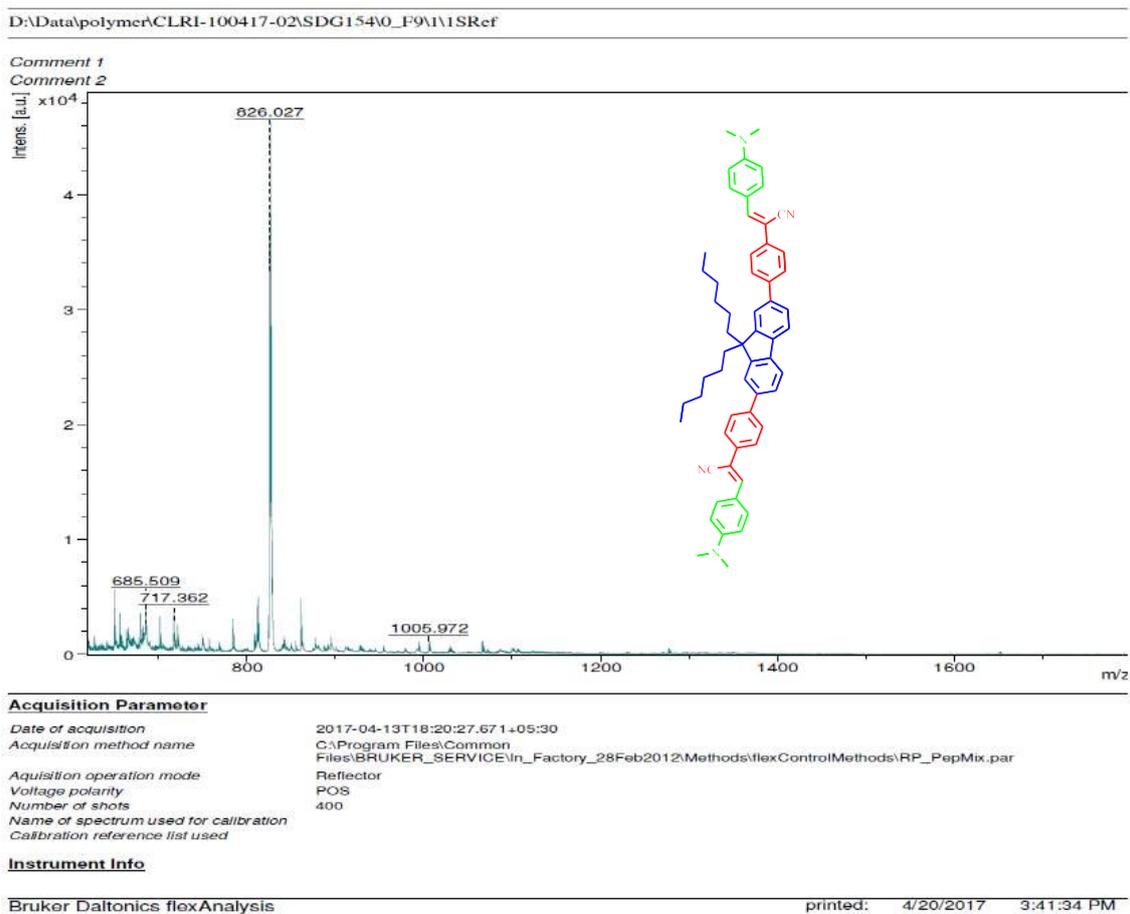
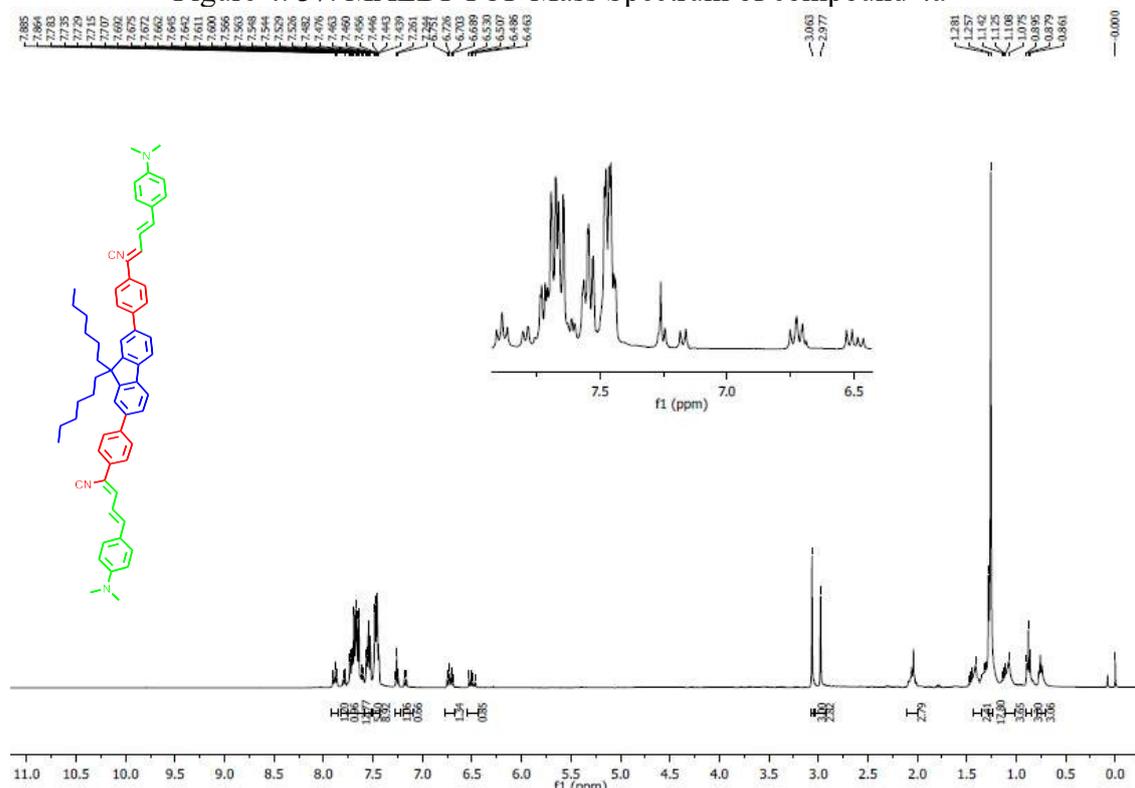
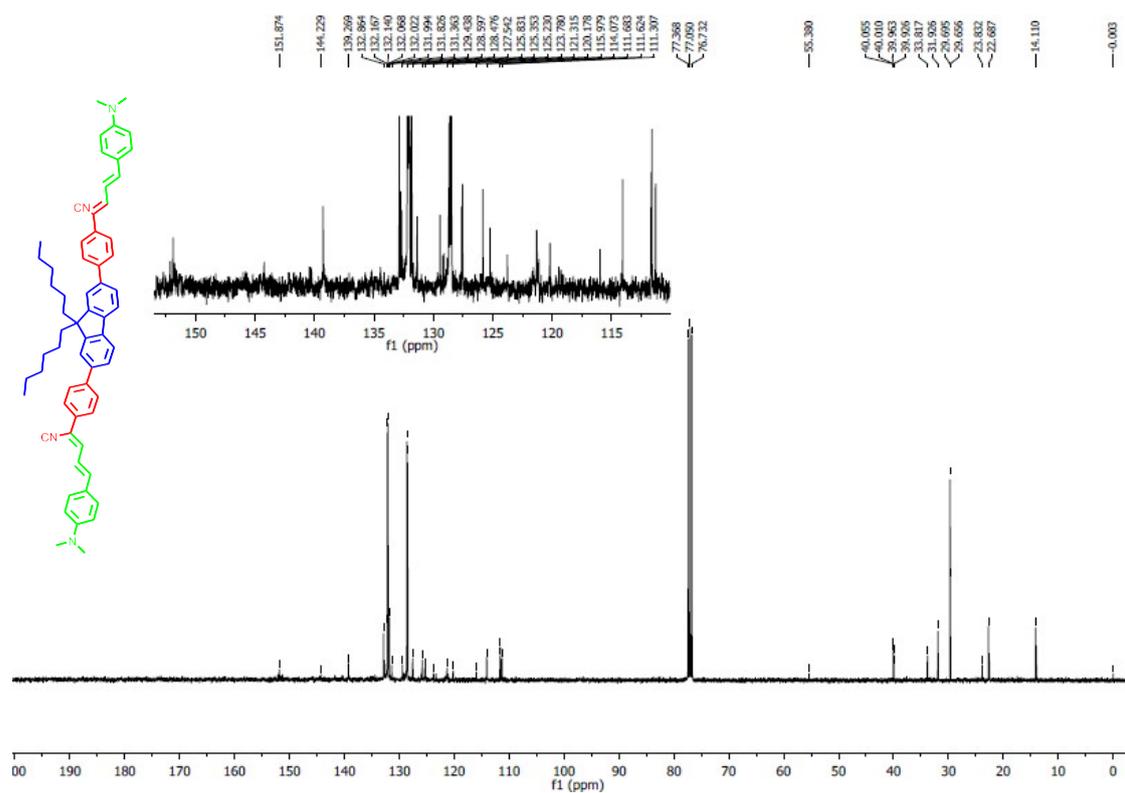


Figure 4. 37. MALDI-TOF Mass Spectrum of compound **4a**



Supporting Information

Figure 4. 38. $^1\text{H-NMR}$ spectrum of compound **4b**



Supporting Information

Figure 4. 40. DEPT-135 NMR spectrum of compound **4b**

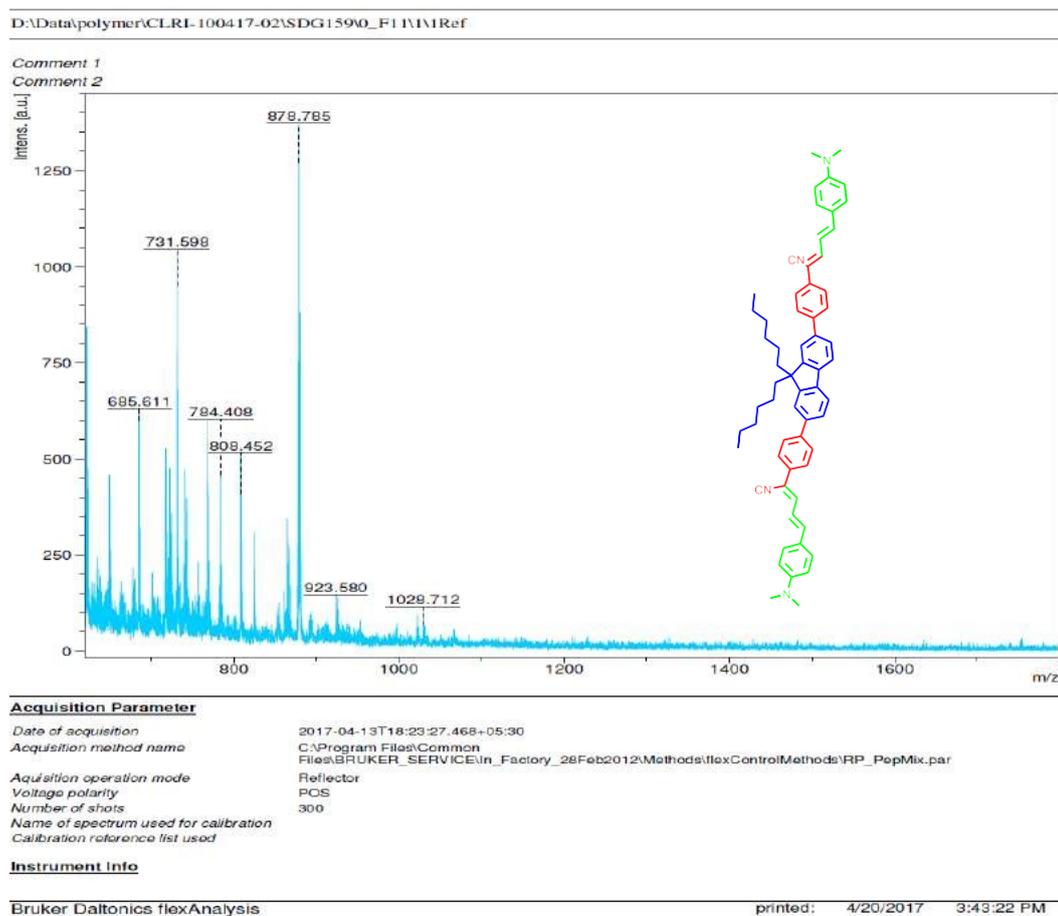


Figure 4. 41. MALDI-TOF Mass Spectrum of compound **4b**

Supporting Information

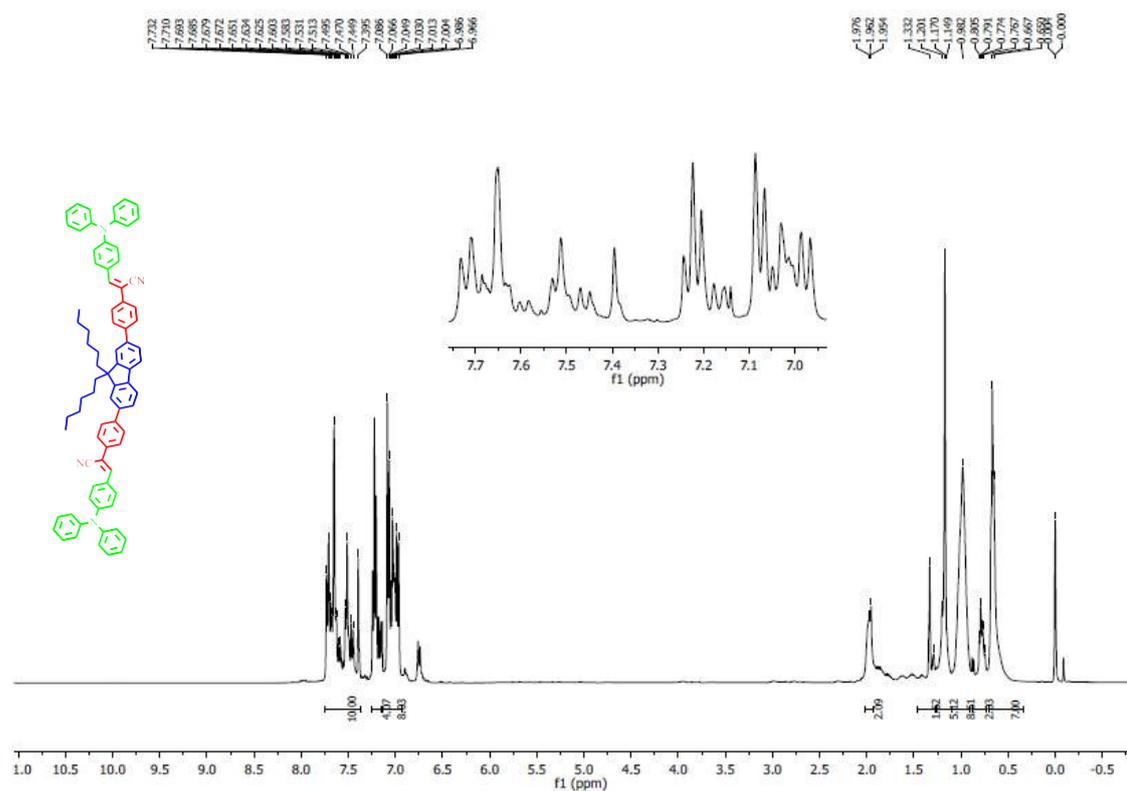


Figure 4. 42. ^1H NMR spectrum of compound **4c**

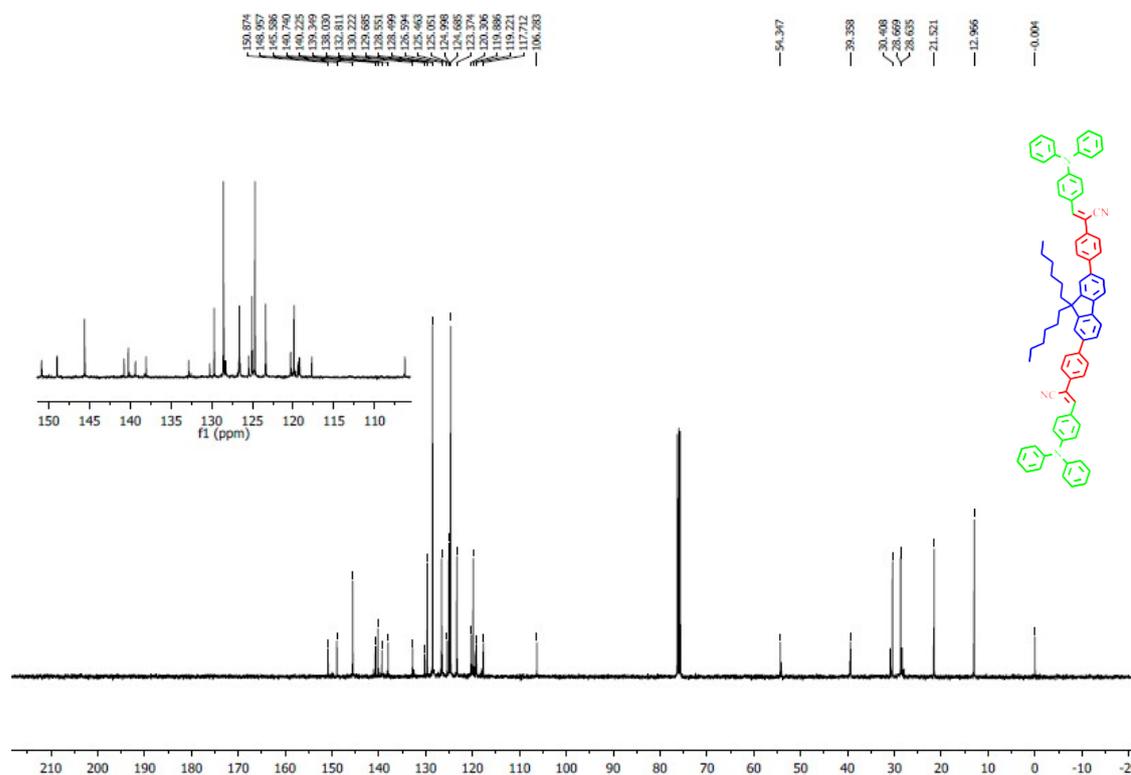


Figure 4. 43. ^{13}C NMR spectrum of compound **4c**

Supporting Information

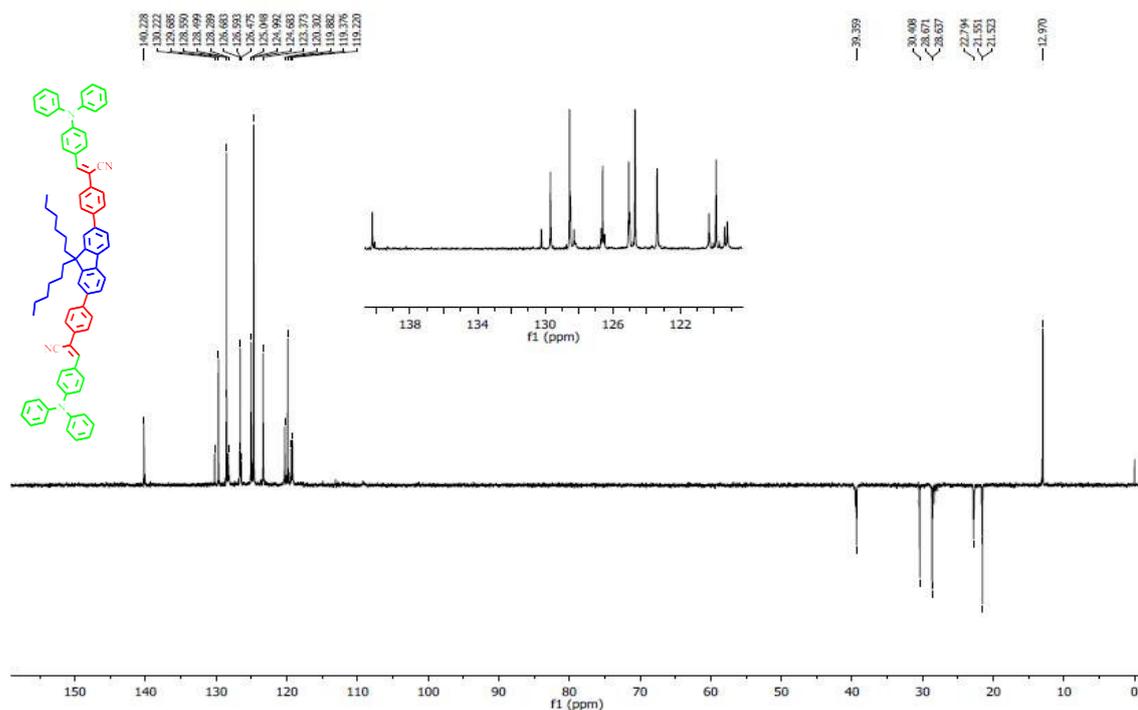
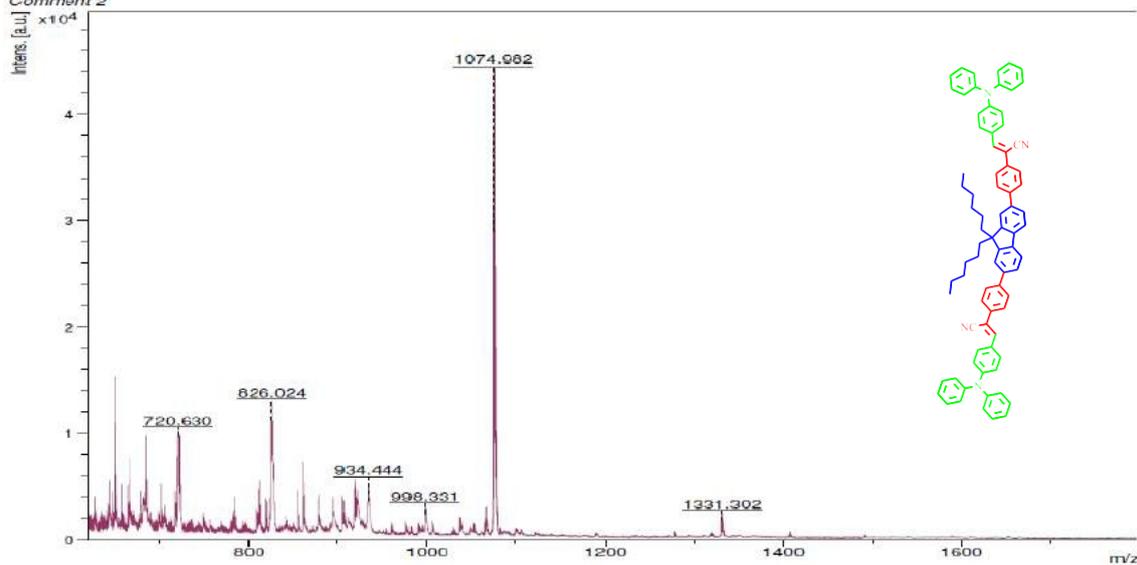


Figure 4. 44. DEPT-135 spectrum of compound 4c

D:\Data\polymer\CLRI-100417-02\SDG158\0_F10\1\1SRef

Comment 1

Comment 2



Acquisition Parameter

Date of acquisition: 2017-04-13T18:21:44.875+05:30
 Acquisition method name: C:\Program Files\Common Files\BRUKER_SERVICE\in_Factory_28Feb2012\Methods\flexControlMethods\RP_PepMix.par
 Acquisition operation mode: Reflector
 Voltage polarity: POS
 Number of shots: 1000
 Name of spectrum used for calibration:
 Calibration reference list used:

Instrument Info

Bruker Daltonics flexAnalysis

printed: 4/20/2017 3:42:22 PM

Figure 4. 45. MALDI-TOF Mass Spectrum of compound 4c

Supporting Information

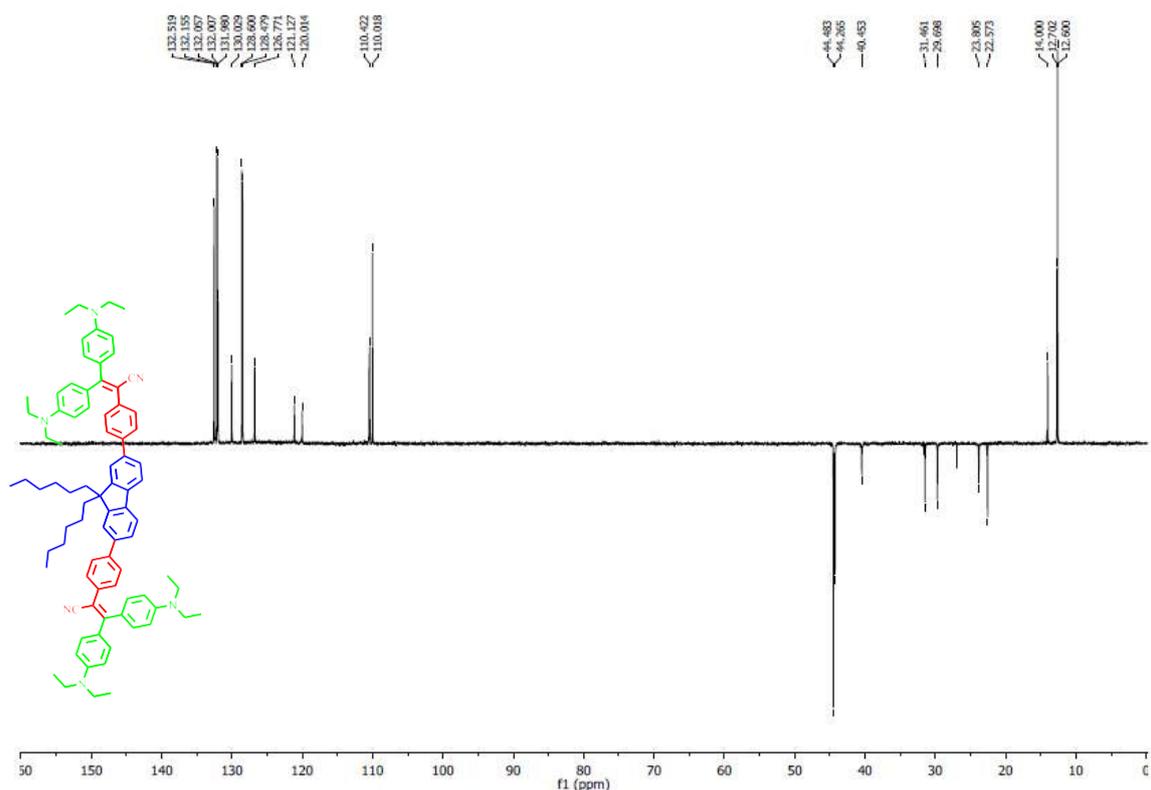


Figure 4. 48. DEPT-135 spectrum of compound 4d

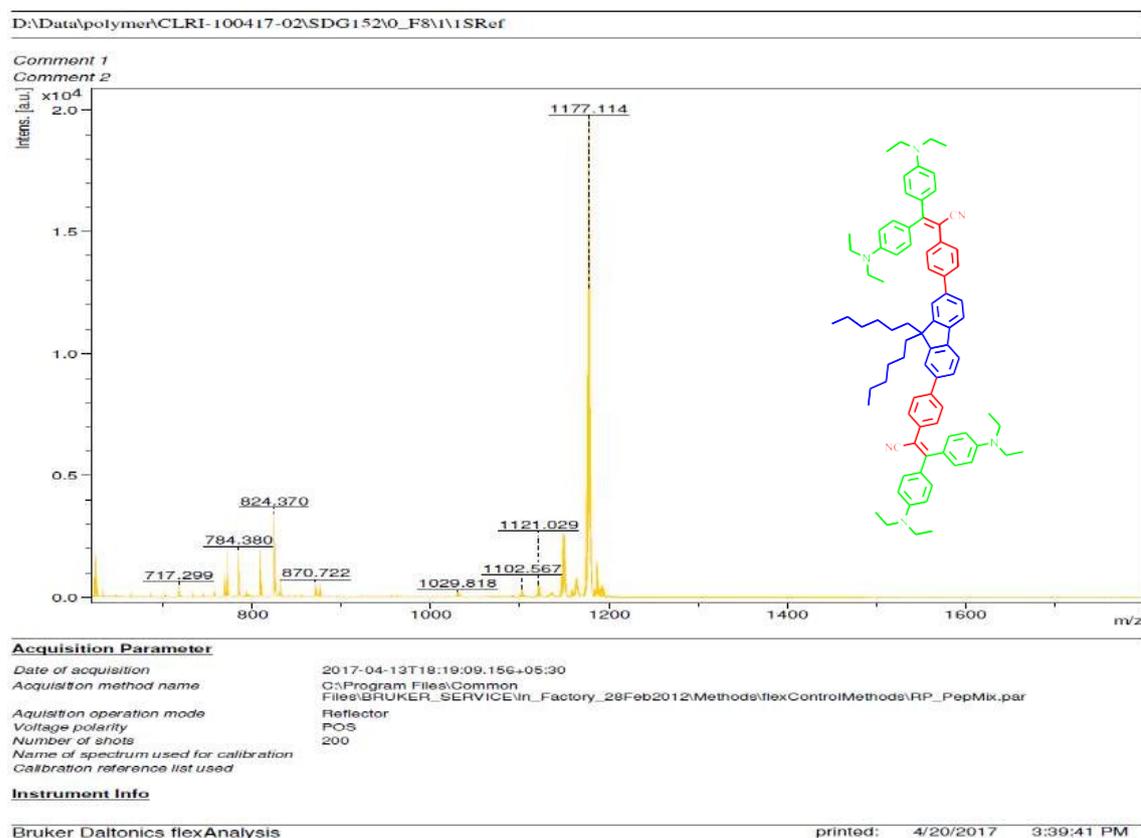
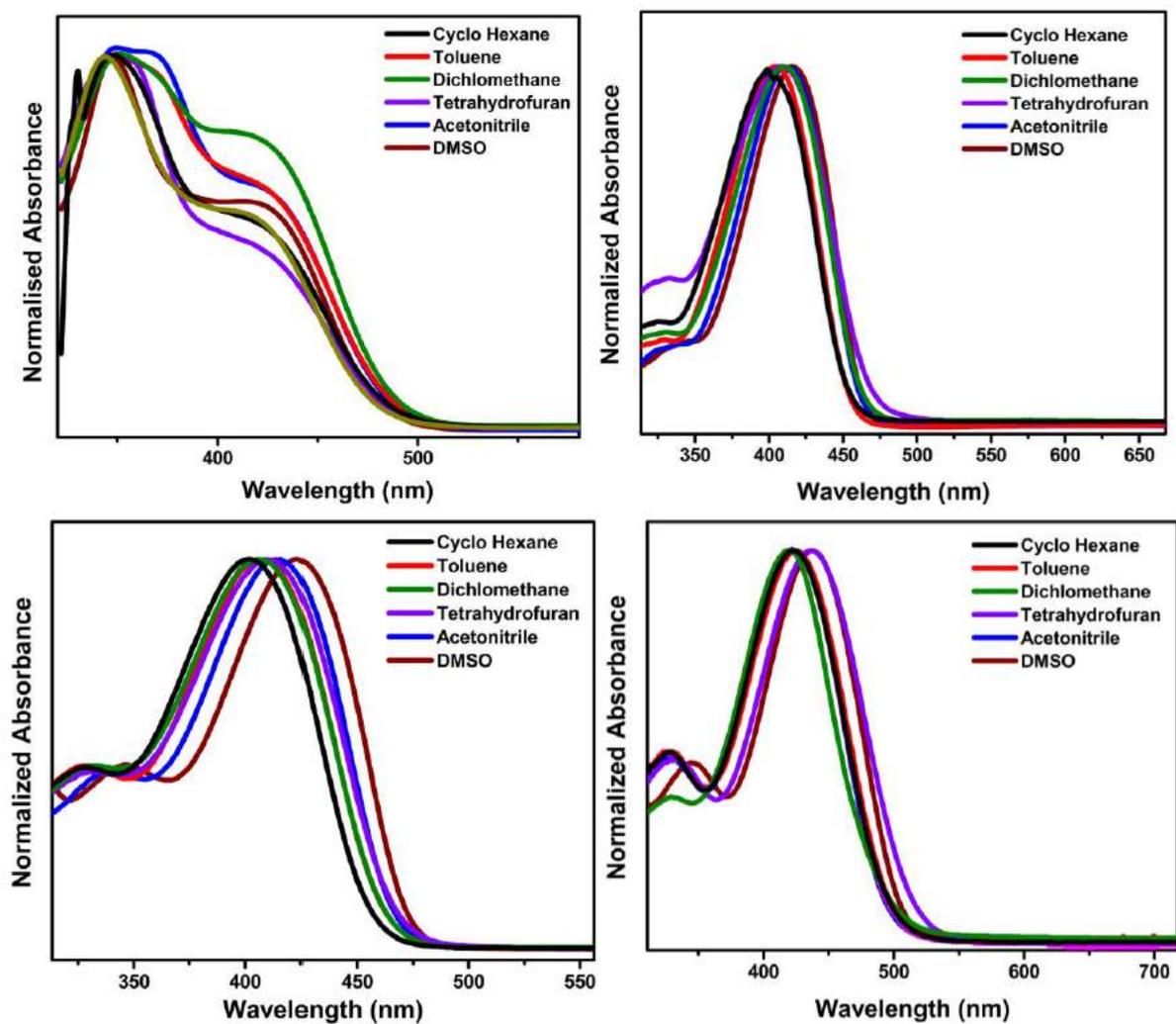


Figure 4. 49. MALDI-TOF Mass Spectrum of compound 4d

5.0 Absorption and emission spectra of stilbenes (4a-4d) in homogeneous solvents

Fig 5. 1: Normalised Absorption spectra of stilbenes (**4a-4d**) in different polarity of solvents(10 μ M)

Supporting Information

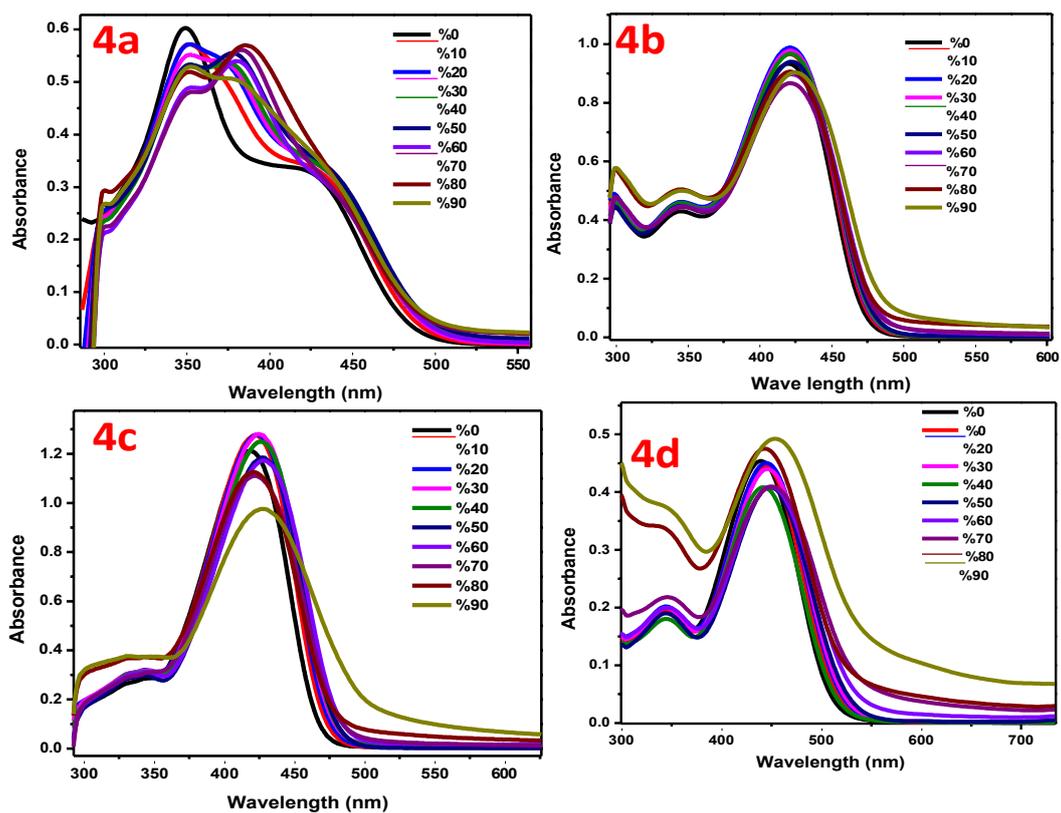
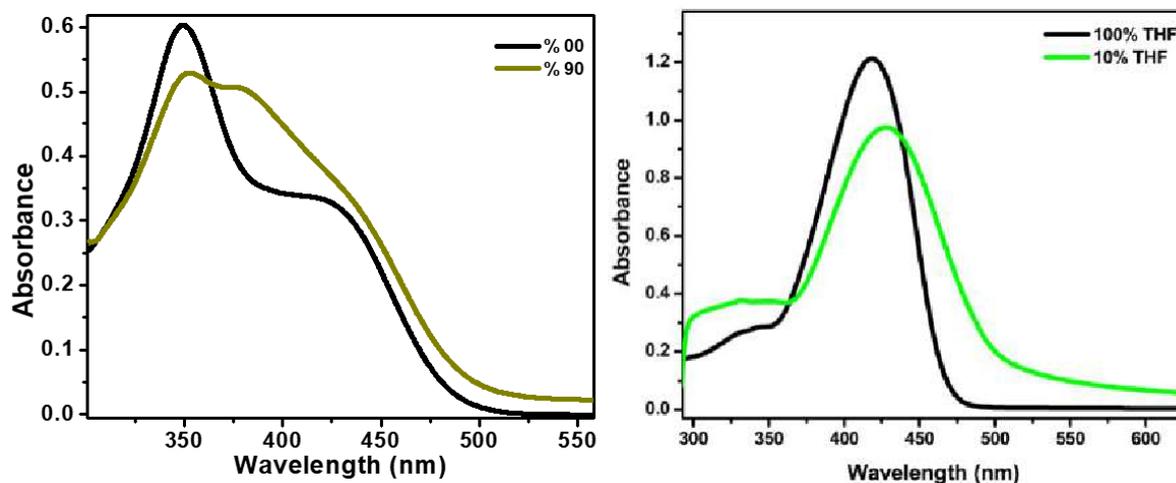


Fig 5. 2: Absorption spectra of (10 μ M) spectra of **4a-4d** in THF/water mixtures with various water fractions from top to bottom, f w = 0 to 90%



Supporting Information

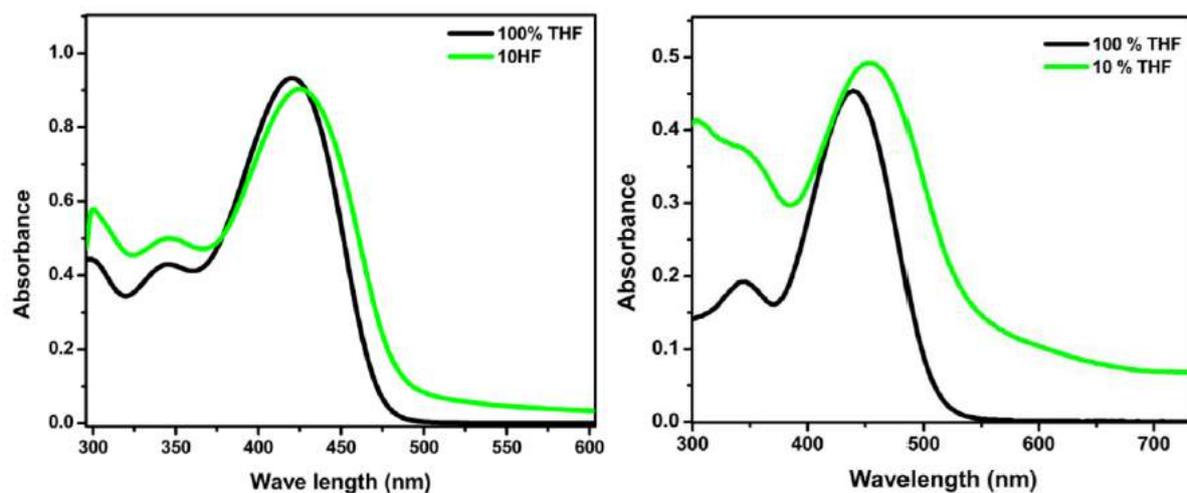


Fig 5. 3: Absorption spectra of (10 μ M) spectra of **4a-4d** in THF/water mixtures with various water fractions, $f_w = 0$ and 90 from top.

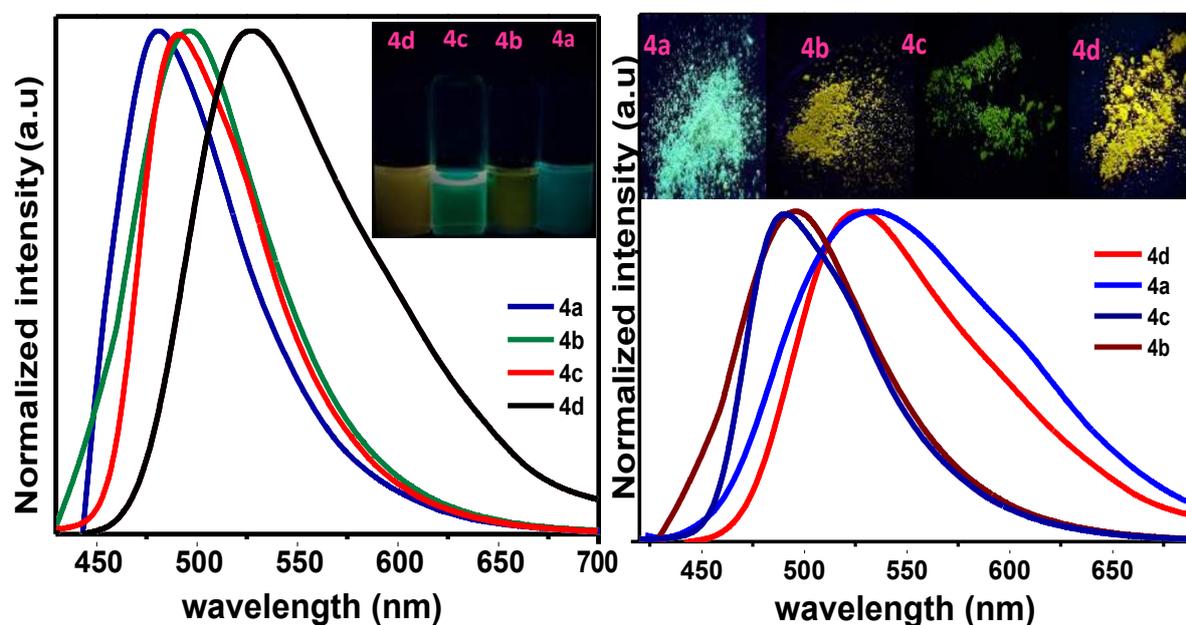


Fig 5. 4: Photoluminescence (PL) normalized spectra of (10 μ M) in THF solvent (left) and solid state emission (right) of **4a-4d**. Inset shows the visual fluorescence change in THF solvent of **4a-4d**. The photos were taken under a handheld UV (365 nm) lamp as soon as the substances were added.

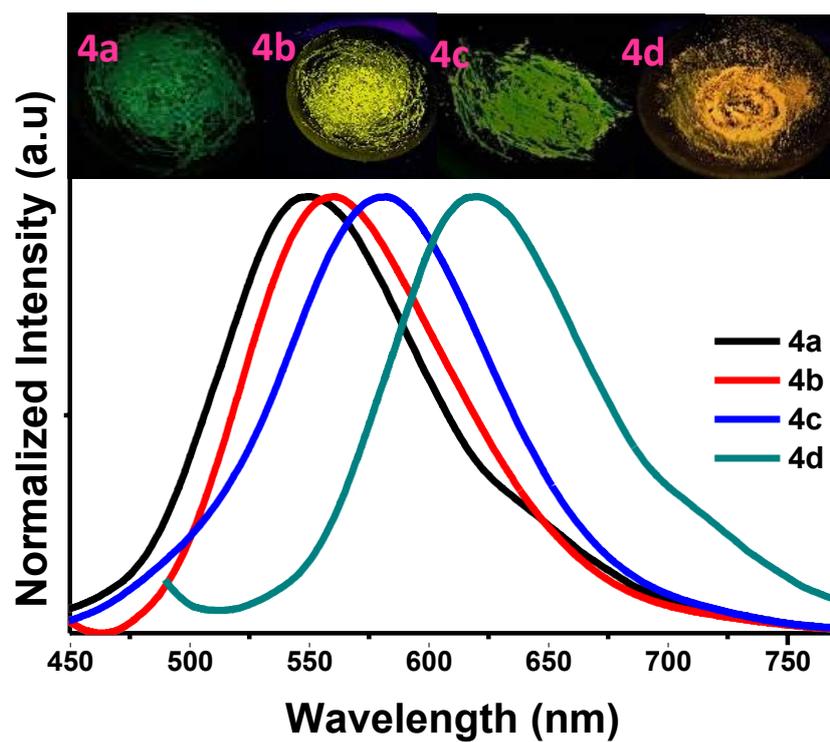


Fig 5. 5: Photoluminescence (PL) normalized spectra of **4a-4d** in ground processes and inset shows the visual fluorescence change of **4a-4d**. The photos were taken under a handheld UV (365 nm) lamp as soon as the substances were added.

Supporting Information

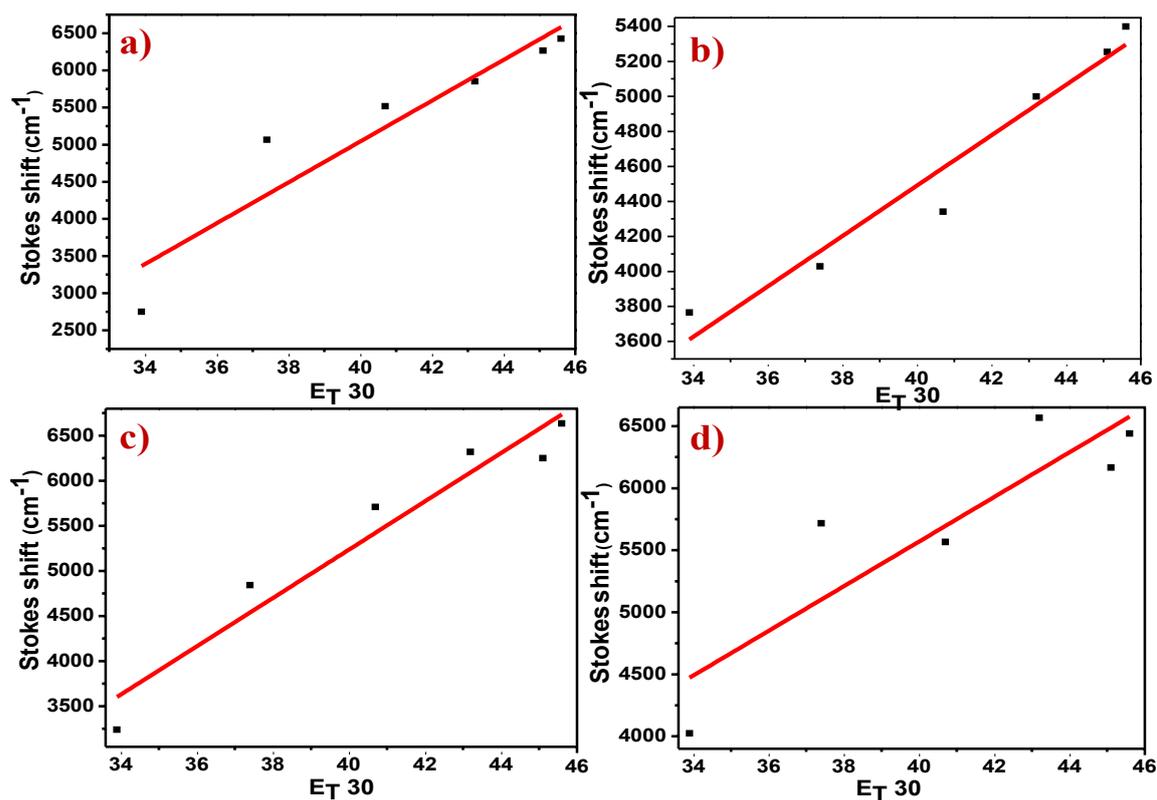


Table. S2. Photo physical spectral studies of 10 mM **4a-4d** samples different solvents (excitation wavelength is 390 nm)

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Quantum yields ^a
Cyclohexane	423	495	72	0.52
Toluene	424	517	93	0.48
DCM	419	528	109	0.46
THF	416	534	118	0.12
ACN	412	542	130	0.08
DMSO	437	550	113	0.078
Pure water^b	426	559	133	0.015
Buffer^{b, c}	422	561	136	0.012

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Quantum yields ^a
Cyclohexane	400	485	85	0.62
Toluene	407	496	89	0.55

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DCM	407	510	103	0.57
THF	405	520	115	0.17
ACN	406	525	119	0.05
DMSO	423	540	117	0.051
Pure water^b	419	552	133	0.011
Buffer^{b, c}	420	554	134	0.015

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Quantum yields ^a
Cyclohexane	406	490	84	0.59
Toluene	408	505	97	0.50
DCM	407	530	123	0.53
THF	404	550	146	0.20
ACN	400	556	156	0.11
DMSO	405	570	165	0.081
Pure water^b	401	576	175	0.042
Buffer^{b, c}	402	581	179	0.030

	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Quantum yields ^a
Cyclohexane	402	485	83	0.59
Toluene	403	505	102	0.47
DCM	407	530	123	0.49
THF	409	560	151	0.18
ACN	405	572	167	0.10
DMSO	418	590	172	0.075
Pure water^b	412	596	184	0.024
Buffer^{b, c}	415	592	177	0.035

- a) Quantum yields are calculated using Coumarine 153 (EtOH, $\Phi_F = 0.38$) solution as reference and using the following formula $\Phi = \Phi_F \times (I/I_R) \times (A_R/A) \times (\eta^2/\eta_R^2)$ where Φ = quantum yield, I = intensity of emission, A = absorbance at $\lambda_{ex} = 400$ nm, η = refractive index of solvents; The quantum yields of compounds are determined in a different solvent system and the standard error is equal to the standard deviation of three independent measurements.
- b) Compounds are not soluble in pure water and buffer solution, so we added minimum amount organic solvent (DMSO) was used.
- c) HEPES buffer was used and maintained the pH = 7.4

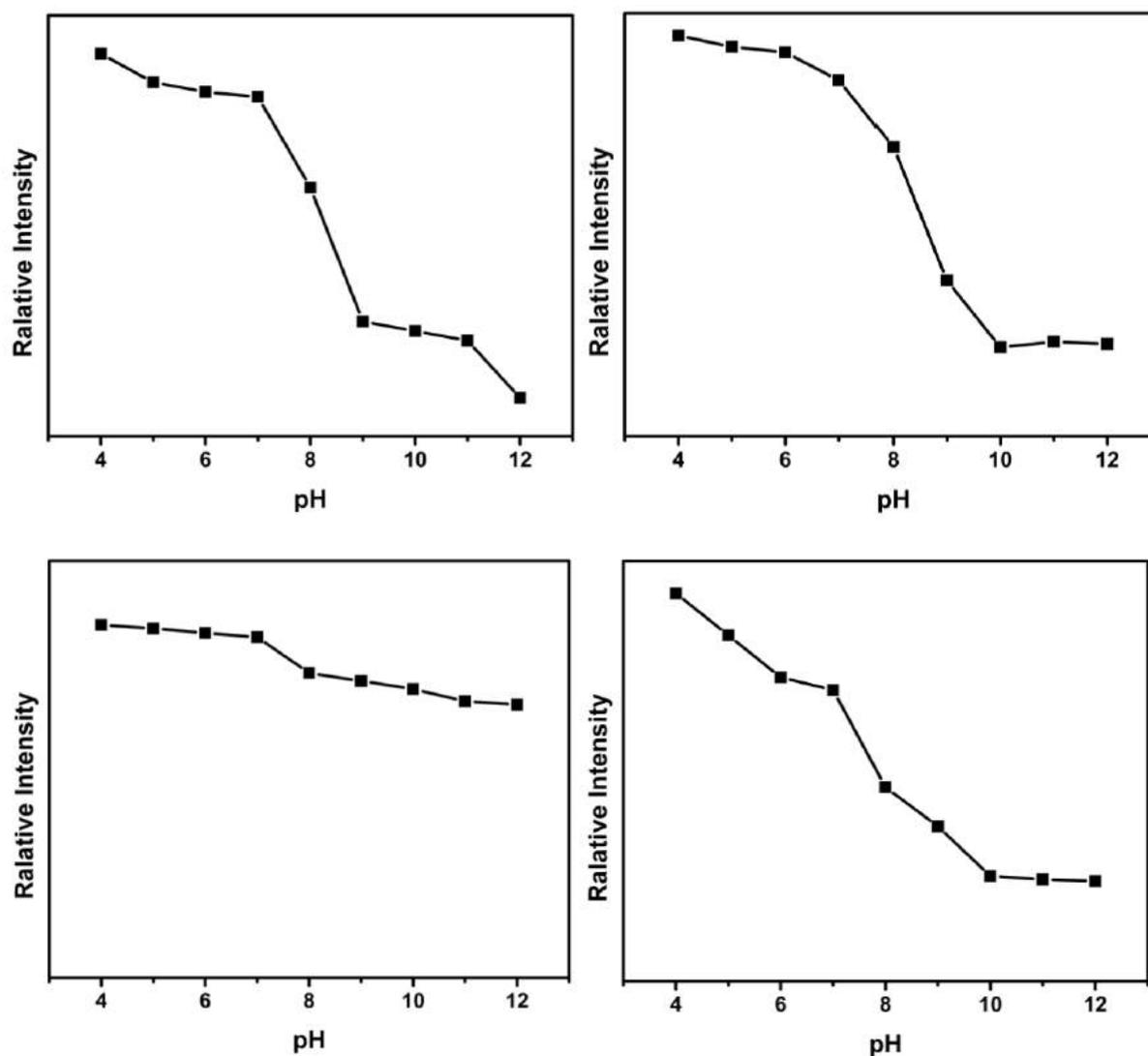


Figure 5.7. The fluorescence spectra of compounds **4a** (top left), **4b** (top right), **4c** (bottom left), **4d** (bottom right) with different pHs.

6. Dynamic light scattering (DLS) study

Size (nm)	Intensity	Volume	Number
0.6	0.0	22.0	24.4
1.0	2.1	47.0	49.4
1.7	1.5	29.0	25.6
2.9	0.0	3.0	0.6
5.1	0.0	0.0	0.0
8.8	0.0	0.0	0.0
15.3	0.0	0.0	0.0
26.4	0.0	0.0	0.0
45.8	6.3	0.0	0.0
79.4	1.9	0.0	0.0
137.7	0.0	0.0	0.0
238.6	1.0	0.0	0.0
413.5	52.2	0.0	0.0
716.7	35.0	0.0	0.0
1242.2	0.0	0.0	0.0
2153.1	0.0	0.0	0.0
3731.7	0.0	0.0	0.0
6457.7	0.0	0.0	0.0
11209.7	0.0	0.0	0.0
19428.6	0.0	0.0	0.0
33673.6	0.0	0.0	0.0
58362.8	0.0	0.0	0.0
101154.0	0.0	0.0	0.0
175319.3	0.0	0.0	0.0

Peak Analysis by intensity			
Peak	Area	Mean	Width
1	3.6	1.3	1.3
2	8.2	53.7	33.9
3	88.2	532.1	526.1

Peak Analysis by volume			
Peak	Area	Mean	Width
1	100.0	1.1	1.3

Peak Analysis by number			
Peak	Area	Mean	Width
1	100.0	1.1	1.2

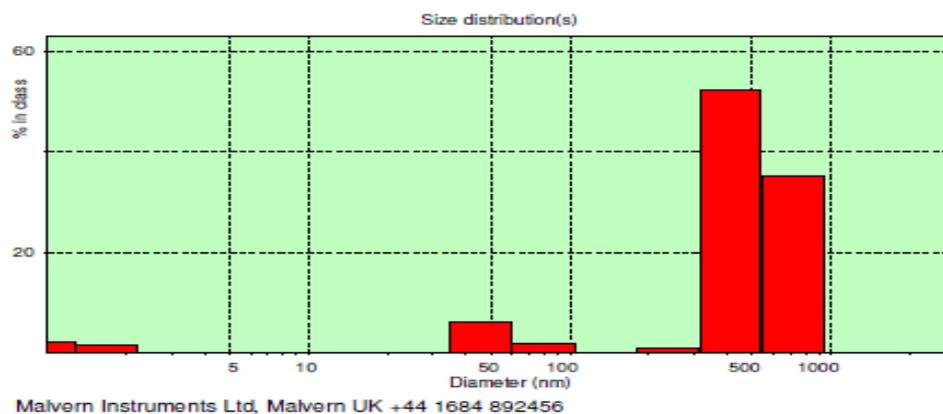


Fig. 6.1 Particle size distribution of BTPEPBI-NP50 in water studied via laser light scattering. Inset: HR-TEM image of BTPEPBI-NP50.

7.0 Thermogravimetric analysis (TGA)

The thermal properties of all compounds are investigated by thermogravimetric analysis (TGA). Excellent thermal stability is highly profound for the optoelectronic applications for organic molecular conjugates. Thermal stability is also one of the important factors for the practical application of organic electronic materials. The thermal properties of **4a-4d** were measured by TGA in N₂ flow with a heating rate of 10 °C min⁻¹. To check the thermal stability of fluorine-based α -cyano stilbene **4a-4d**, TGA was performed (Figure 1). The thermal decomposition temperatures (Td) corresponding to 5% weight loss under nitrogen atmosphere at 150 °C - 210 °C under nitrogen and are 311 °C, 283 °C, 252 °C, and 402 °C for **4a**, **4b**, **4c**, and **4d**, respectively and that indicating these molecules possessed moderate thermal stability for the application of organic devices and other application. The glass transition temperature of **4a-4d** shows in the range of 243-366°C. The thermal stability of the fluorine-based α -cyanostilbene **4a-4d** follows the order **4d** > **4a** > **4b** > **4c**. This reveals that the thermal stability of the fluorine-based α - cyanostilbene **4a-4d** decreases with the

bulkiness of substituent on α -cyanostilbene unit. This finding indicates the molecules having good thermal and morphological stability and the thermal curves were showed in figures 8.1.

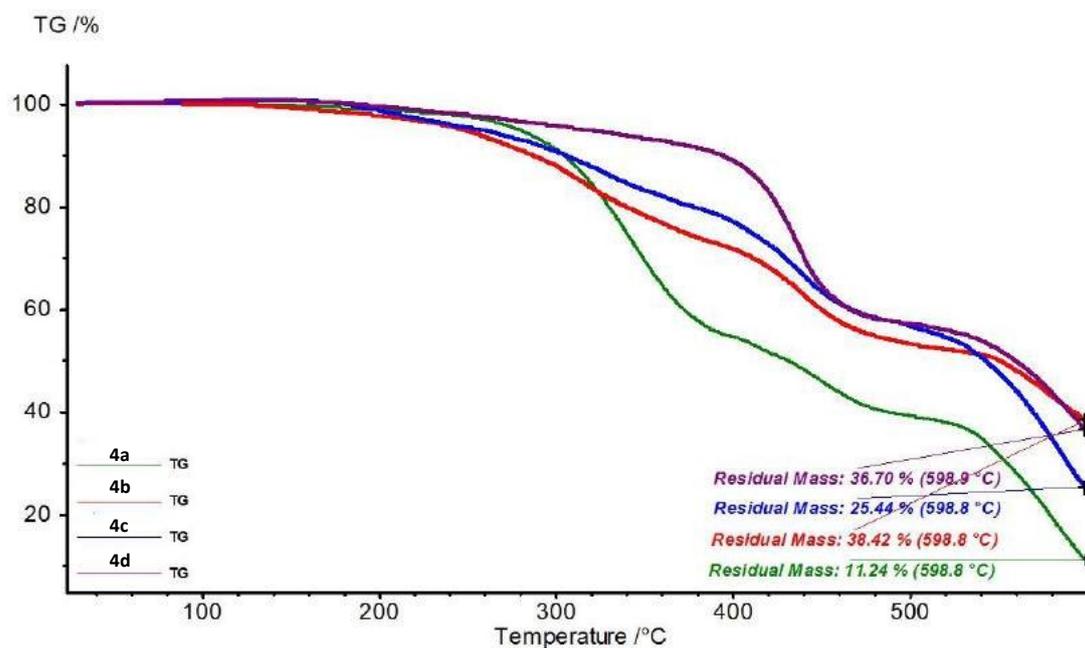


Fig. 7.1. Thermogravimetric analysis (TGA) of synthesised organic compounds: (a) compound 4b (red) compound 4d (violet), compound 4c (blue) compound 4a (green).

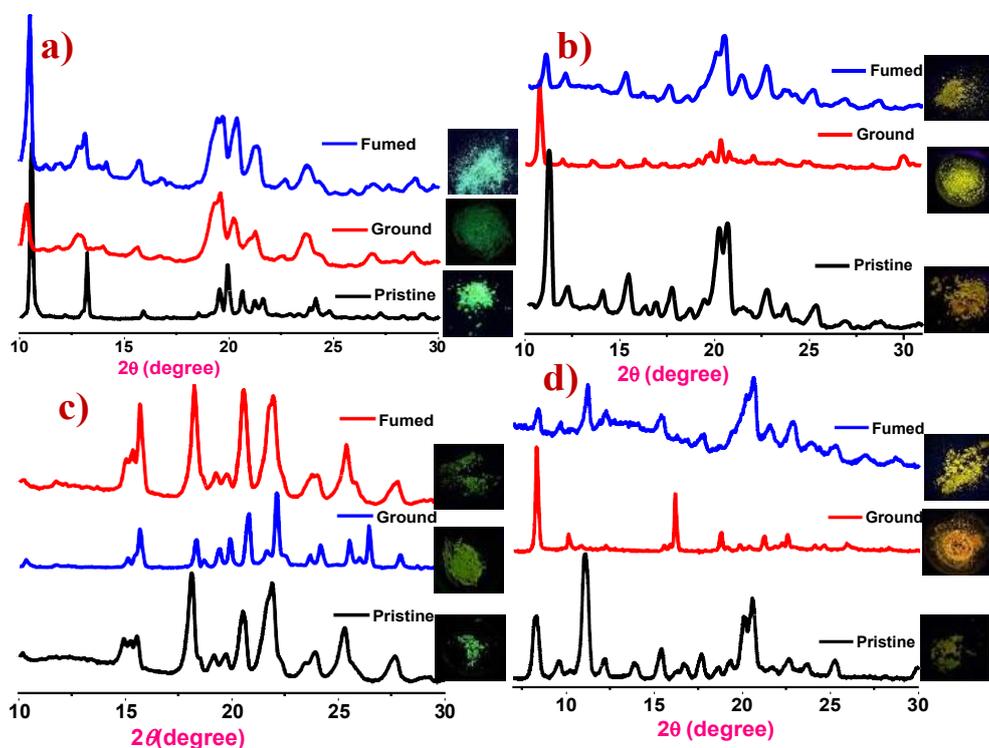


Fig. 7.2 PXRD patterns of 4a-4d Pristine (back line), Ground (red line), and Fumed (blue line)

8. Application for Live Cancer Cell Imaging

Supporting Information

Human Lung Carcinoma cells (A549) cells was procured from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in DMEM medium containing penicillin, streptomycin and amphotericin B (100 U mL^{-1}) and 10% heat-inactivated FBS and cells were maintained in a humidified incubator at $37 \text{ }^\circ\text{C}$ with 5% CO_2 for the application of Live Cancer Cell Imaging. For live cancer cell imaging, among the all, **4d** was investigated using A549 (lung cancer cells), due to its high quantum yield under aggregates state. Compound **4d** ($10 \text{ } \mu\text{M}$) was added to the tested cell cultures and incubated at $37 \text{ }^\circ\text{C}$ for 30 min. Later, the cells were stained with nuclear staining dye 4',6-diamidino-2-phenylindole (DAPI) ($2 \text{ } \mu\text{M}$) for another 20 min. To our delight, **4d** effectively entered the cell membranes of the tested cancer cells, labeled with an intense FR luminescence when compared to the fluorescence images of the cells before and after the treatment with **4d** (Figure 8.1). This showed the potential of compound **4d** in intracellular imaging in the tested cancer cells. It is noteworthy that during the cell imaging experiment, the tested cells were healthy and unharmed and displayed an adherent morphology. From this study, we conclude that compound **4d** could be used for live cancer cell imaging (Supporting Information).

8.1 Cell Viability Assay

We studied the cytotoxicity of the synthesized compound **4d**, which is an important factor for cellimaging applications (Figure 6). The MTS assay method was applied to test the cytotoxicity of compound **4d** by adding $100\text{-}200 \text{ } \mu\text{M}$ for 24 h of the compound in the cancer cell culture. The observed cytotoxicity is very nominal for compound **4d** against A549 cells, because of the excellent biocompatibility, non-toxic to the cells up to $200 \text{ } \mu\text{M}$ of the synthesized compound **4d** could be utilized as a fluorescent bioprobe in live cancer cell imaging.

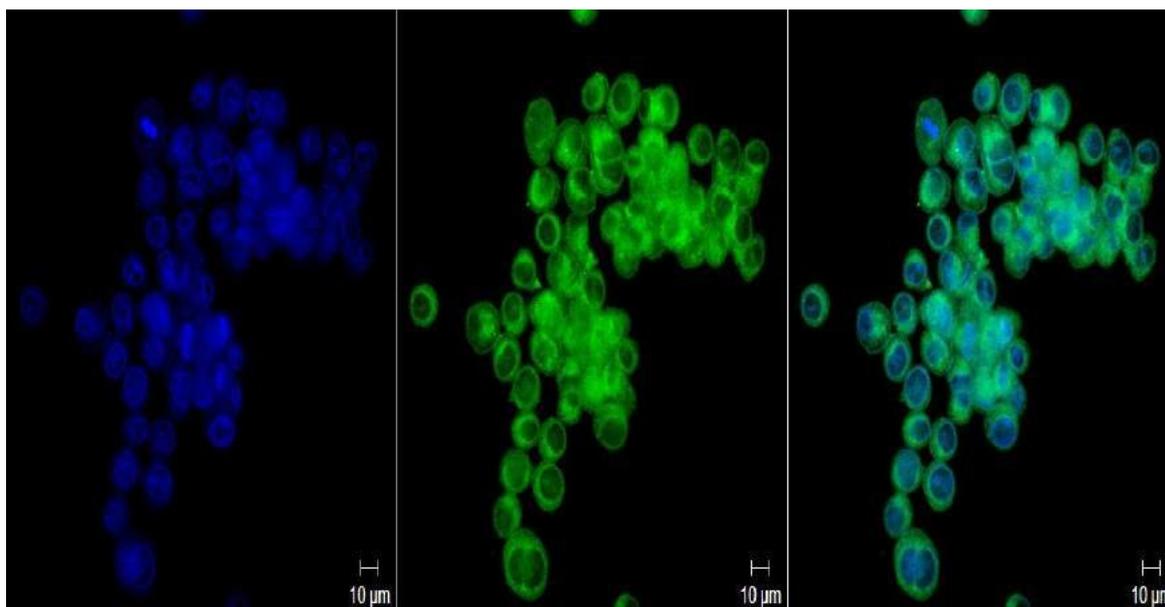


Fig. 8.1 Live cell imaging of compound **4d** in A549 cell. (A) Row 1 (a1–a3): A549 cells treated with 25 μM X for 30 min. DAPI stained blue fluorescence images; column 2 (a2 and b2): green fluorescence images; column 3 (a3 and b3): merging of the blue and green fluorescence images. Scale bar = 5 μm . Excitation and emission wavelength: 405 nm and 410–460 nm for blue fluorescence images; 488 nm and 490–561 nm for green fluorescence images.

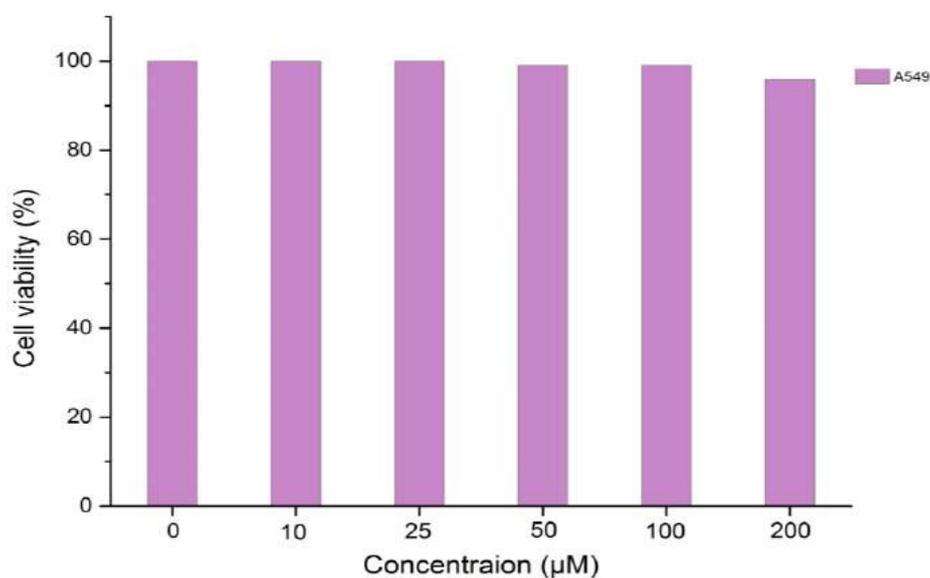


Fig. 8. 2. Cell viability of A549 cells upon treatment with DAPI stained at different concentrations in dark at a power density of 0.10 W cm^{-2} for 4 min and further incubation in fresh medium for 24 h.

Supporting Information

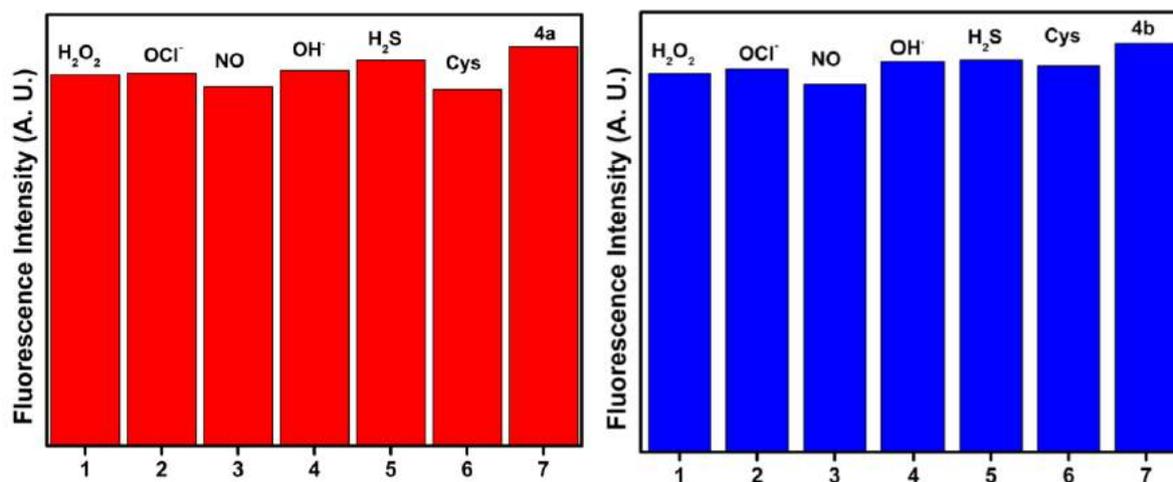


Figure 8.3. Comparison of ROS fluorescence intensity with **4a** (left) and **4b** (right). The observations clearly showed that in the presence of ROS, the fluorescence of **4a** or **4b** altered only slightly, demonstrating that the compounds are stable in the presence of ROS.

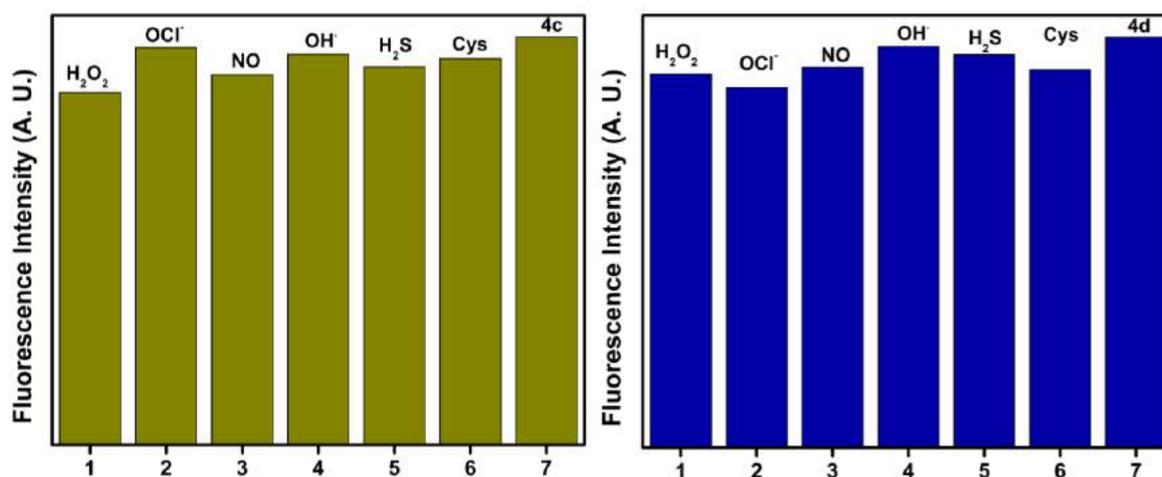


Fig. 8. 4. Comparison of ROS fluorescence intensity with **4c** (left) and **4d** (right). The observations clearly showed that in the presence of ROS, the fluorescence of **4c** or **4d** altered only slightly, demonstrating that the compounds are stable in the presence of ROS.

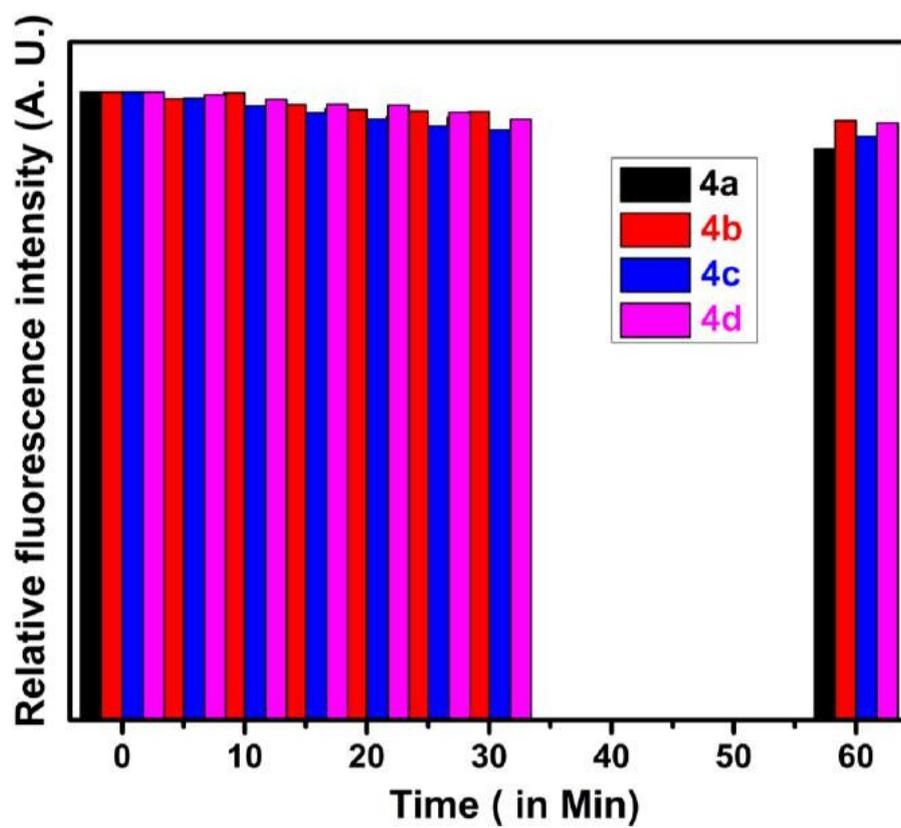


Fig. 8. 5. Photo stability of **4a**, **4b**, **4c** and **4d**.