Supplementary Information

Curcumin-based molecular probes for fluorescence imaging of fungi

Joana R. M. Ferreira,[1] Mariana Alves,[2] Bárbara Sousa,[2] Sandra I. Vieira,[2] Artur M. S. Silva,[1] Samuel Guieu,[1,3] Ângela Cunha,[4] Raquel Nunes da Silva[1,2]

[1]LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, Aveiro, Portugal
[2]iBiMED, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
[3]CICECO Aveiro-Institute of Materials, University of Aveiro, Aveiro, Portugal
[4]CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal

Synthesis

General

Melting points were measured in a BÜCHI Melting point B-540 apparatus fitted with a microscope and are uncorrected. NMR spectra were recorded with Bruker DRX 300 and 500 spectrometers (300 and 500 for ^{1}H, 75 and 126 for ^{13}C and 282 MHz for ^{19}F), in $CDCl_3$ as solvent, if not stated otherwise. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz; internal standard was residual peak of the solvent. Unequivocal ^{13}C assignments were made with the aid of 2D gHSQC and gHMBC (delays for one-bond and long-range *J*C/H couplings were optimised for 145 and 7 Hz, respectively) experiments. High resolute mass spectra analyses (HRMS-ESI) were performed on a microTOF (focus) mass spectrometer. Ions were generated using an ApolloII (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage between 100 and 150 V applied to the capillary. Absorption spectra were collected with UV-2501PC, emission and excitation spectra were collected with JASCO Spectrofluorometer FP-8300.

Preparative thin-layer chromatography was performed with Macherey-Nagel G/ UV 254. Column chromatography was performed with ACROS Organics silica gel 60A (0.030-0.200 mm). All other chemicals and solvents used were obtained from commercial sources and were either used as received or dried by standard procedures.

General procedure for the synthesis of derivatives **3a-f**

Acetylacetone (1 equiv., 1 mmol, 0.105 mL) and $BF_3{\cdot}OEt_2$ (1.5 equiv., 1.5 mmol, 0.185 mL) were mixed in toluene (4 mL) at 65ºC during 30 minutes. To the mixture at 65ºC, the appropriate benzaldehyde (2 equiv., 2 mmol) or benzaldehydes (1 equiv. each, 1 mmol each) and $B(OEt)_3$ (2 equiv., 2 mmol, 0.340 mL) were added. Then, $nBuNH_2$ (0.2 equiv., 0.2 mmol, 0.020 mL) was added and the mixture was stirred at 65ºC for 5 to 21 hours. Water was added and the organic layer was extracted with CH_2Cl_2 (3 x 15 mL), collected, and dried over anhydrous sodium sulfate. Finally, the solution was concentrated to dryness. The product was precipitated from CH_2Cl_2 and hexane and dried in air or purified by silica gel thin-layer chromatography using CH_2Cl_2 as eluent.

2,2-difluoro-4,6-dimethyl-2*H*-1λ^3,3,2λ^4-dioxaborinine (**1**)[1]

Acetylacetone (1 equiv., 1 mmol) and $BF_3{\cdot}OEt_2$ (1.5 equiv., 1.5 mmol) were mixed in toluene (5 mL) at 65ºC. After stirring for 30 minutes, water was added and the organic layer was extracted with CH_2Cl_2 (3 x 15 mL), collected, and dried over anhydrous sodium sulfate. Finally, the solution was concentrated to dryness to afford the compound **1** as an off-white solid.

Using the procedure described in section 2.2.2.i., 1 mmol of acetylacetone and 1.5 mmol of $BF_3{\cdot}OEt_2$ were mixed for 30 minutes at 65 ºC and a yellow solution was obtained. Water was added (15 mL) and the product was extracted with 3 x 15 mL of CH_2Cl_2 and dried over anhydrous sodium sulfate. The pure product was obtained as an off-white solid (0.162 g). Yield: quantitative; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.00 (s, 1H), 2.25 (s, 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -134.51 (^{10}B - 0.2 ^{19}F), -134.57 (^{11}B - 0.8 ^{19}F).

(*E*)-4-(2-(2,2-difluoro-6-methyl-2*H*-1λ^3,3,2λ^4-dioxaborinin-4-yl)vinyl)-*N*,*N*-dimethylaniline (**2a**)

Acetylacetone (1 equiv., 1 mmol, 0.105 mL) and $BF_3{\cdot}OEt_2$ (1.5 equiv., 1.5 mmol, 0.185 mL) were mixed in toluene (4 mL) at 65ºC during 30 minutes. To the mixture at 65ºC and while stirring, the appropriate benzaldehyde (1 equiv., 1 mmol) and $B(OEt)_3$ (2 equiv., 2 mmol, 0.340 mL) were added. Then, $nBuNH_2$ (0.2 equiv., 0.2 mmol, 0.020 mL) was added and the mixture was stirred at 65ºC for 5 hours. Water was added and the organic layer was extracted with CH_2Cl_2 (3 x 15 mL), collected, and dried over anhydrous sodium sulfate. Finally, the solution was concentrated to dryness. The product was precipitated from CH_2Cl_2 and hexane and dried in air or purified by silica gel thin-layer chromatography using CH_2Cl_2 as eluent.

Using the procedure described in section 2.2.2.ii., *p*-(dimethylamino)benzaldehyde (1 equiv., 1 mmol, 0.149 g) was added. After purification by silica gel thin-layer chromatography with CH_2Cl_2/Hex (1:1) and CH_2Cl_2, the product **2a** was obtained as a red solid (0.0187g). Yield: 10%; mp: 181-184ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 15.3 Hz, 1H), 7.52 (d, *J* = 9.0 Hz, 2H), 6.69 (d, *J* = 9.0 Hz, 2H), 6.38 (d, *J* = 15.3 Hz, 1H), 5.87 (s, 1H), 3.10 (s, 6H, NMe_2), 2.27 (s, 3H, Me); ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.27 (CO), 180.83 (CO), 153.36, 150.22, 132.23, 121.84, 113.32, 112.05, 100.57, 40.28 (NMe_2), 24.09 (Me); ^{19}F NMR (282 MHz, $CDCl_3$) δ -137.09 (^{10}B - 0.2 ^{19}F), -137.15 (^{11}B - 0.8 ^{19}F); ESI/HRMS m/z: $[M + Na]^+$ Calcd for $C_{14}H_{16}O_2NBF_2Na$ 302.1140; Found 302.1128.

4,6-bis((*E*)-4-bromostyryl)-2,2-difluoro-2*H*-1λ^3,3,2λ^4-dioxaborinine (**3a**)[2]

Using the procedure described in section 2.2.2.iii., *p*-bromobenzaldehyde (2 equiv., 2 mmol, 0.185 g) was added. After column purification with CH_2Cl_2/Hex (1:1) and CH_2Cl_2, the product **3a** was obtained as an orange solid (0.320g). Yield: 66%; mp > 289 ºC (degradation); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 15.6 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 4H), 7.49 (d, *J* = 8.5 Hz, 4H), 6.73 (d, *J* = 15.6 Hz, 2H), 6.10 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.21 (CO), 146.38, 132.81, 132.62, 130.43, 126.71 (CBr), 121.00, 102.54; ^{19}F NMR (282 MHz, Acetone) δ -135.47 (^{10}B - 0.2 ^{19}F), -135.53 (^{11}B - 0.8 ^{19}F).

2,2-difluoro-4,6-bis((*E*)-4-methoxystyryl)-2*H*-1λ^3,3,2λ^4-dioxaborinine (**3b**)[2]

Using the procedure described in section 2.2.2.iii., *p*-methoxybenzaldehyde (2 equiv., 2 mmol, 0.242 mL) was added. The product **3b** was obtained by crystallization from CH_2Cl_2 and hexane as purple crystals (0.172g). Yield: 45%; mp: 242-245ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, *J* = 15.5 Hz, 2H), 7.57 (d, *J* = 8.8 Hz, 4H), 6.94 (d, *J* = 8.8 Hz, 4H), 6.58 (d, *J* = 15.5 Hz, 2H), 6.01 (s, 1H), 3.87 (s, 6H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.48 (CO), 162.80, 146.90, 131.21, 126.99, 118.07, 114.76, 102.06, 55.55 (OMe); ^{19}F NMR (282 MHz, $CDCl_3$) δ -137.38 (^{10}B - 0.2 ^{19}F), -137.44 (^{11}B - 0.8 ^{19}F); ESI/HRMS m/z: $[M - F]^+$ Calcd. for $C_{21}H_{19}O_4BF$ 365.1360; Found 365.1349.

4,4'-((1*E*,1'*E*)-(2,2-difluoro-2*H*-1λ^3,3,2λ^4-dioxaborinine-4,6-diyl)bis(ethene-2,1-diyl))bis(*N*,*N*-dimethylaniline) (**3c**)[2]

Using the procedure described in section 2.2.2.iii., *p*-(N,N-dimethylamino)benzaldehyde (2 equiv., 2 mmol, 0.300 g) was added. The product **3c** was obtained by precipitation from CH_2Cl_2 and hexane as a black solid (0.115g). Yield: 28%; mp: 312-315ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 15.3 Hz, 2H), 7.51 (d, *J* = 8.9 Hz, 4H), 6.68 (d, *J* = 8.9 Hz, 4H), 6.46 (d, *J* = 15.3 Hz, 2H), 5.89 (s, 1H), 3.08 (s, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.87 (CO), 152.66, 146.84, 131.40, 122.37, 114.96, 111.90, 100.979, 40.15 (NMe_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ -138.77 (^{10}B – 0.2 ^{19}F), -138.84 (^{11}B – 0.8 ^{19}F); ESI/HRMS m/z: $[M - F]^+$ Calcd. for $C_{23}H_{25}O_2N_2BF$ 391.1993; Found 391.1982.

4-((*E*)-4-bromostyryl)-2,2-difluoro-6-((*E*)-4-methoxystyryl)-2*H*-1λ^3,3,2λ^4-dioxaborinine (**3d**)

Using the procedure described in the section 2.2.2.iii., *p*-bromobenzaldehyde (1 equiv., 1 mmol, 0.185 g) and *p*-methoxybenzaldehyde (1 equiv., 1 mmol, 0.121 mL) were added. After column purification with CH_2Cl_2 and silica gel thin-layer chromatography with CH_2Cl_2/Hex (1:1) two main products were obtained: derivative **3b** (0.085 g, 22% yield) and derivative **3d**. **3d** was obtained as a red solid (0.0463g). Yield: 11%; mp: 242-245ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, *J* = 15.5 Hz, 1H), 7.93 (d, *J* = 15.7 Hz, 1H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.56 (d, *J* = 7.3 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 15.7 Hz, 1H), 6.60 (d, *J* = 15.5 Hz, 1H), 6.05 (s, 1H), 3.88 (s, 3H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.91 (CO), 178.54 (CO), 163.18, 148.26, 144.96, 133.04, 132.49, 131.53, 130.23, 126.78, 126.17, 121.31, 117.76, 114.86, 102.18, 55.59 (OMe); ^{19}F NMR (282 MHz, $CDCl_3$) δ -136.89 (^{10}B – 0.2 ^{19}F), -136.95 (^{11}B – 0.8 ^{19}F); ESI/HRMS m/z: $[M + Na]^+$ Calcd. for $C_{20}H_{16}O_3BrBF_2Na$ 455.0242; Found 455.0232.

4-((*E*)-2-(6-((*E*)-4-bromostyryl)-2,2-difluoro-2*H*-1,3λ^3,2λ^4-dioxaborinin-4-yl)vinyl)-*N*,*N*-dimethylaniline (**3e**)

Using the procedure described in section 2.2.2.iii., *p*-bromobenzaldehyde (1 equiv., 1 mmol, 0.185 g) and *p*-(N,N-dimethylamino)benzaldehyde (1 equiv., 1 mmol, 0.149 g) were added. After purification by silica gel chromatography with CH_2Cl_2/Hex(1:1)/5%

MeOH, product **3e** was obtained as a green solid (0.009 g) Yield: 2%; mp: 271-274ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 15.1 Hz, 1H), 7.87 (d, *J* = 15.6 Hz, 1H), 7.56 – 7.52 (m, 4H), 7.45 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 9.0 Hz, 2H), 6.66 (d, *J* = 15.6 Hz, 1H), 6.48 (d, *J* = 15.1 Hz, 1H), 5.97 (s, 1H), 3.12 (s, 6H, NMe_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.64 (CO), 175.88 (CO), 153.38, 149.94, 143.02, 133.47, 132.35, 129.98, 125.46, 121.94, 121.77, 113.90, 111.98, 101.96, 40.17 (NMe_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ -138.02 (^{10}B - 0.2 ^{19}F), -138.08 (^{11}B - 0.8 ^{19}F); ESI/HRMS m/z: $[M - F]^+$ Calcd. for $C_{21}H_{19}O_2NBrBF$ 426.0676; Found 426.0667.

4-((*E*)-2-(2,2-difluoro-6-((*E*)-4-methoxystyryl)-2*H*-1,3λ^3,2λ^4-dioxaborinin-4-yl)vinyl)-*N*,*N*-dimethylaniline (**3f**)

Using the procedure described in section 2.2.2.iii., *p*-methoxybenzaldehyde (1 equiv., 1 mmol, 0.121 mL) and *p*-(N,N-dimethylamino)benzaldehyde (1 equiv., 1 mmol, 0.149 g) were added. After purification by silica gel chromatography with Toluene/5% MeOH product **3f** was obtained as a green solid (0.036 g). Yield: 9%; mp: 255-258 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 15.2 Hz, 1H), 7.94 (d, *J* = 15.5 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.6 Hz, 2H), 6.56 (d, *J* = 15.5 Hz, 1H), 6.47 (d, *J* = 15.2 Hz, 1H), 5.94 (s, 1H), 3.87 (s, 3H, OMe), 3.10 (s, 6H, NMe_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.65 (CO), 177.16 (CO), 162.31, 153.05, 148.64, 145.00, 131.92, 130.80, 127.34, 122.07, 118.55, 114.65, 114.33, 111.93, 101.38, 55.51 (OMe), 40.15 (NMe_2); ^{19}F NMR (282 MHz, $CDCl_3$) δ -138.26 (^{10}B - 0.2 ^{19}F), -138.32 (^{11}B - 0.8 ^{19}F); ESI/HRMS m/z: $[M - F]^+$ Calcd. for $C_{22}H_{22}O_3NBF$ 378.1677; Found 378.1670.

Synthesis of 4,4'-((1*E*,1'*E*)-(2,2-difluoro-2*H*-1λ^3,3,2λ^4-dioxaborinine-4,6-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (**4**)[2]

Using a similar procedure as described in section 2.2.2.i to obtain the intermediary BF_2–complex, the commercial curcumin 95% (1 equiv., 2.7 mmol, 0.100 mg) was dissolved in toluene (5 mL) and $BF_3{\cdot}OEt_2$ (1,5 equiv., 1.5 mmol, 0.05 mL) was added and the solution was stirred for 1 hour at 65ºC. Product **4**, after precipitation with CH_2Cl_2 and hexane, was obtained by purification by silica gel chromatography using EtOAc as an eluent as a red solid (0.017g). Yield: 15%; mp > 216 ºC (degradation); ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.65 (s, 2H, OH), 7.95 (d, *J* = 15.6 Hz, 2H), 7.48 (d, *J* = 2.0 Hz, 2H), 7.37 (dd, *J* = 8.3, 2.0 Hz, 2H), 6.97-6.90 (m, 4H), 6.36 (s, 1H), 3.94 (s, 6H, OMe); ^{13}C NMR (75 MHz, Acetone) δ 179.70 (CO), 148.10, 146.73, 126.73, 124.86, 118.19, 115.67, 111.69, 101.09, 55.47 (OMe); ^{19}F NMR (282 MHz, Acetone) δ -137.53 (^{10}B – 0.2 ^{19}F), -137.59(^{11}B – 0.8 ^{19}F).

NMR spectra

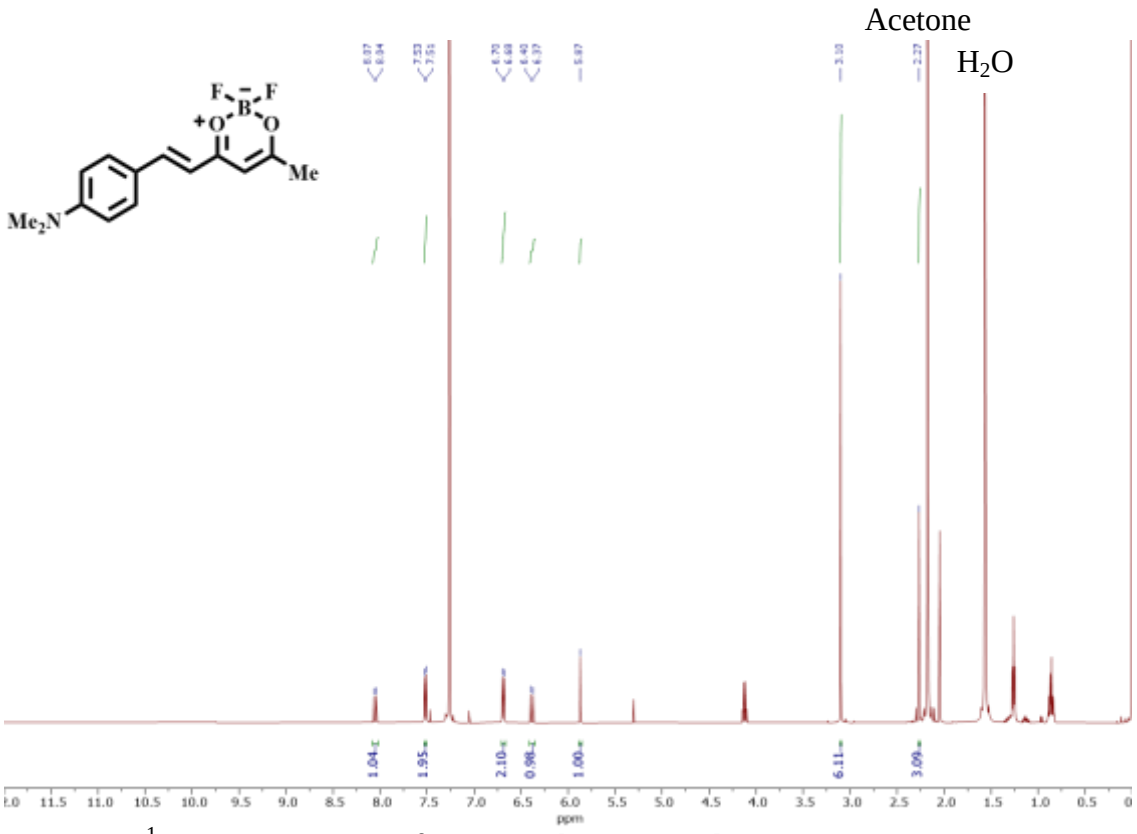

Figure S1. ^{1}H-NMR spectrum of compound **2a** in $CDCl_3$.

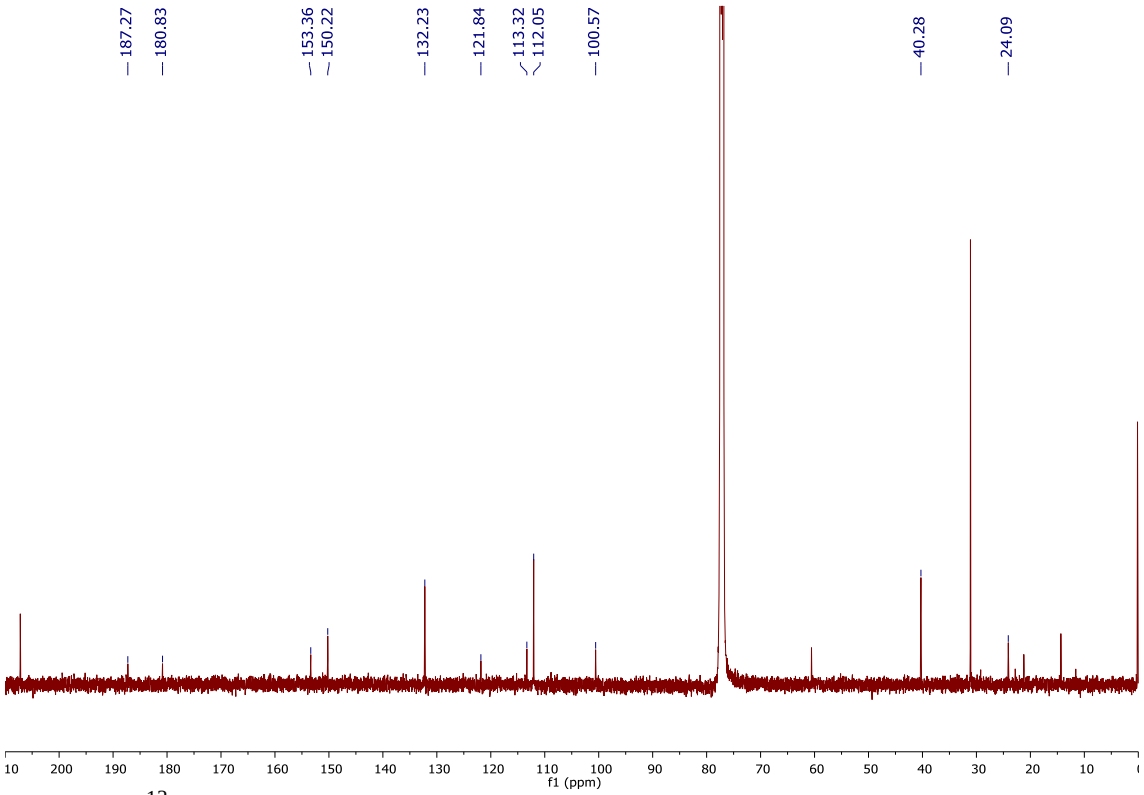

Figure S2. ^{13}C-NMR spectrum of compound **2a** in $CDCl_3$.

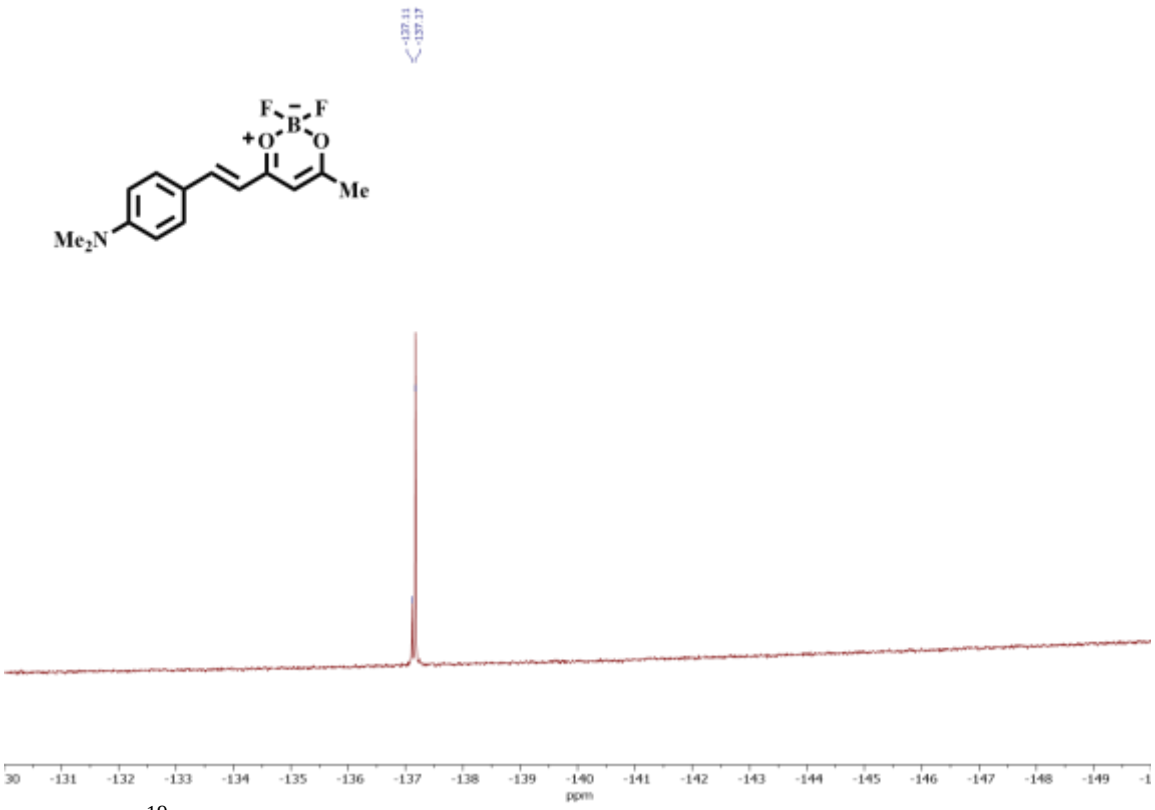

Figure S3. ^{19}F-NMR spectrum of compound **2a** in $CDCl_3$.

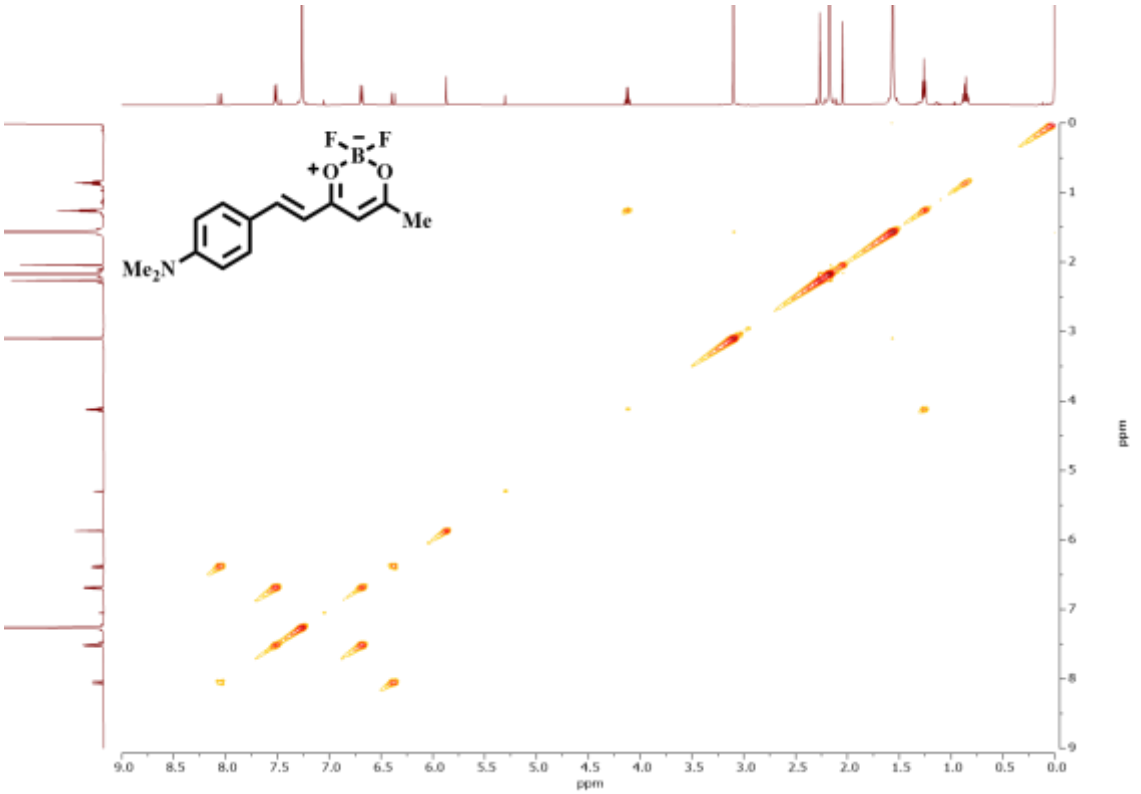

Figure S4. COSY-NMR spectrum of compound **2a** in $CDCl_3$.

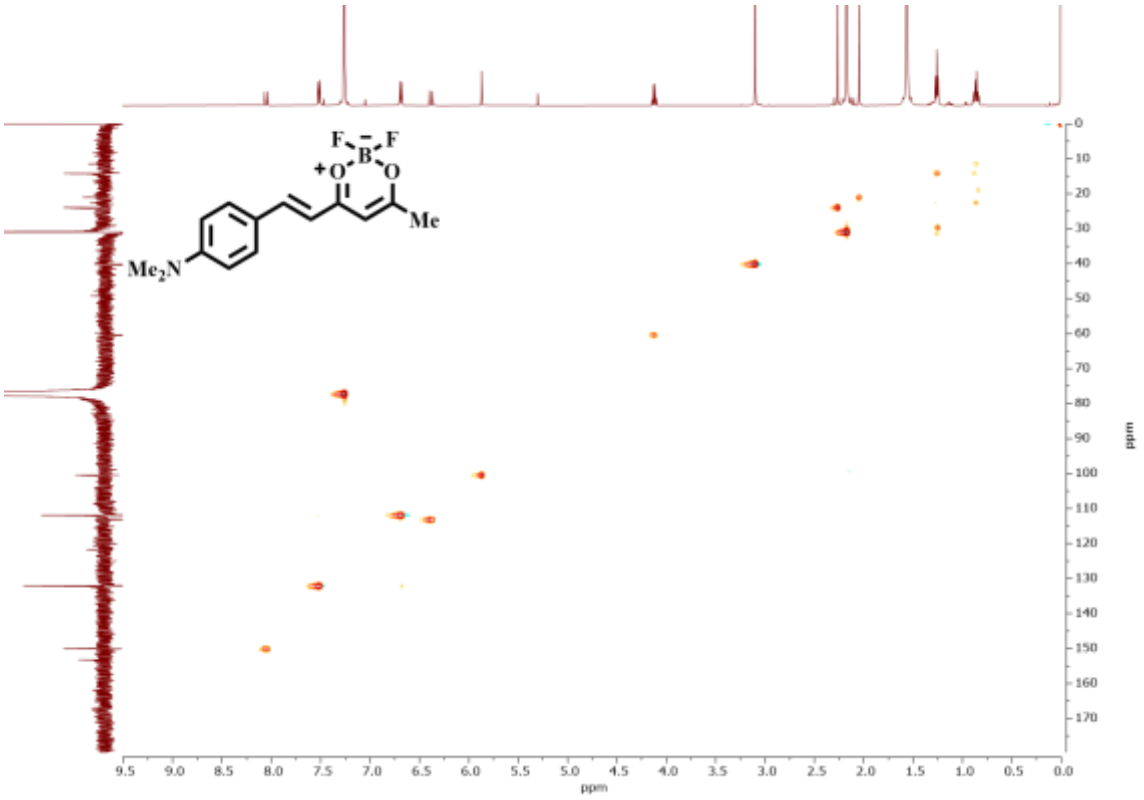

Figure S5. HSQC-NMR spectrum of compound **2a** in $CDCl_3$.

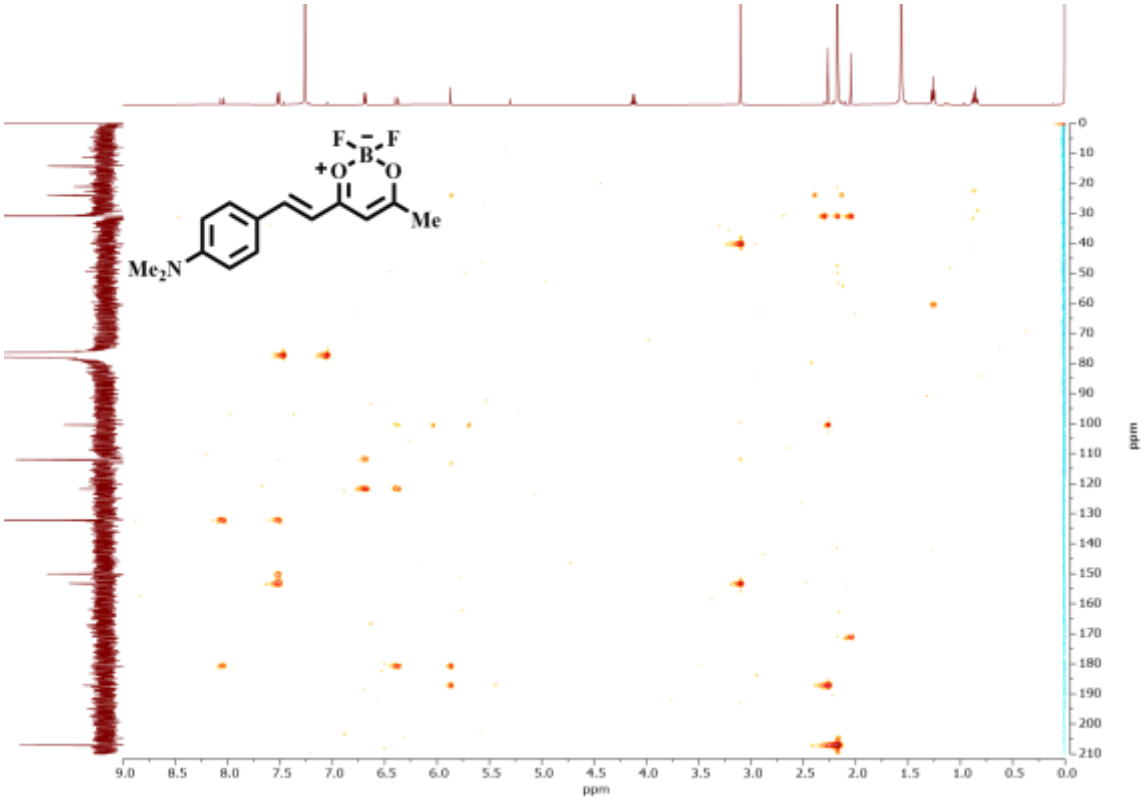

Figure S6. HMBC-NMR spectrum of compound **2a** in $CDCl_3$.

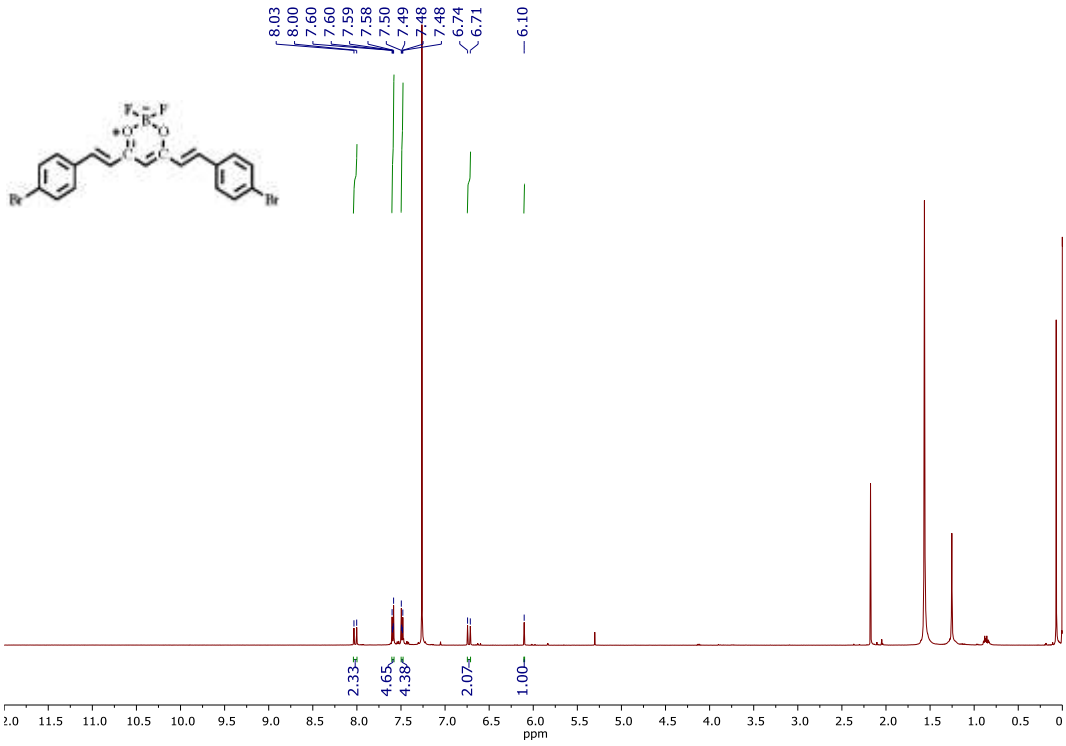

Figure S7. ^{1}H-NMR spectrum of compound **3a** in $CDCl_3$.

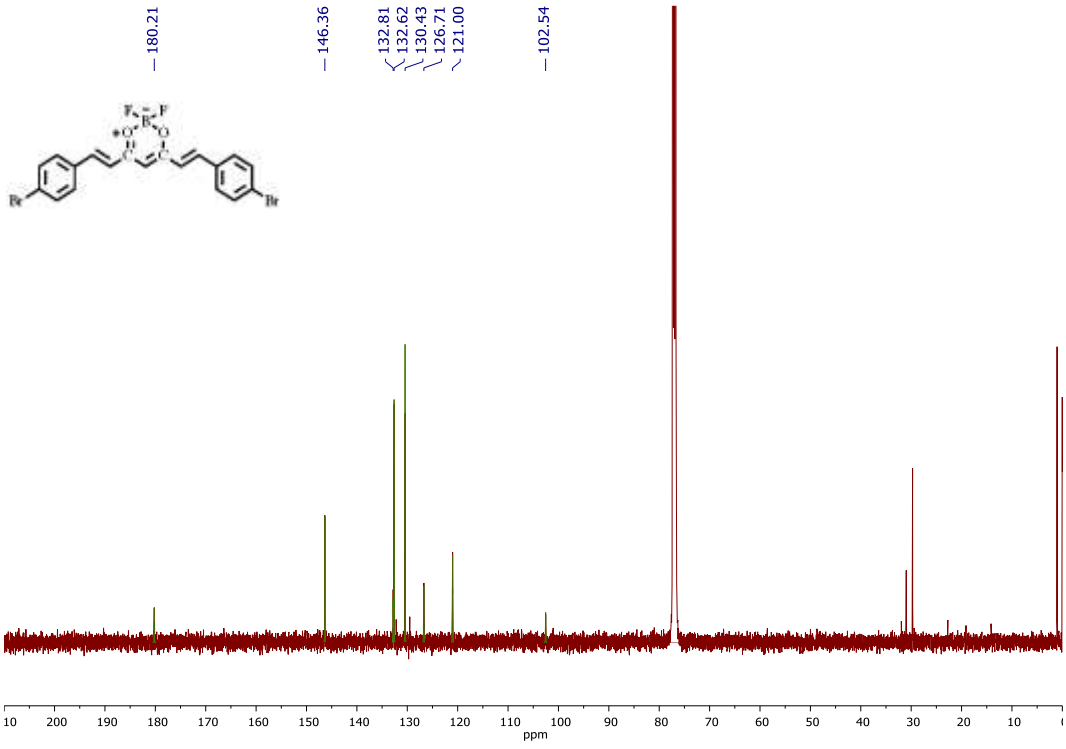

Figure S8. ^{13}C-NMR spectrum of compound **3a** in $CDCl_3$.

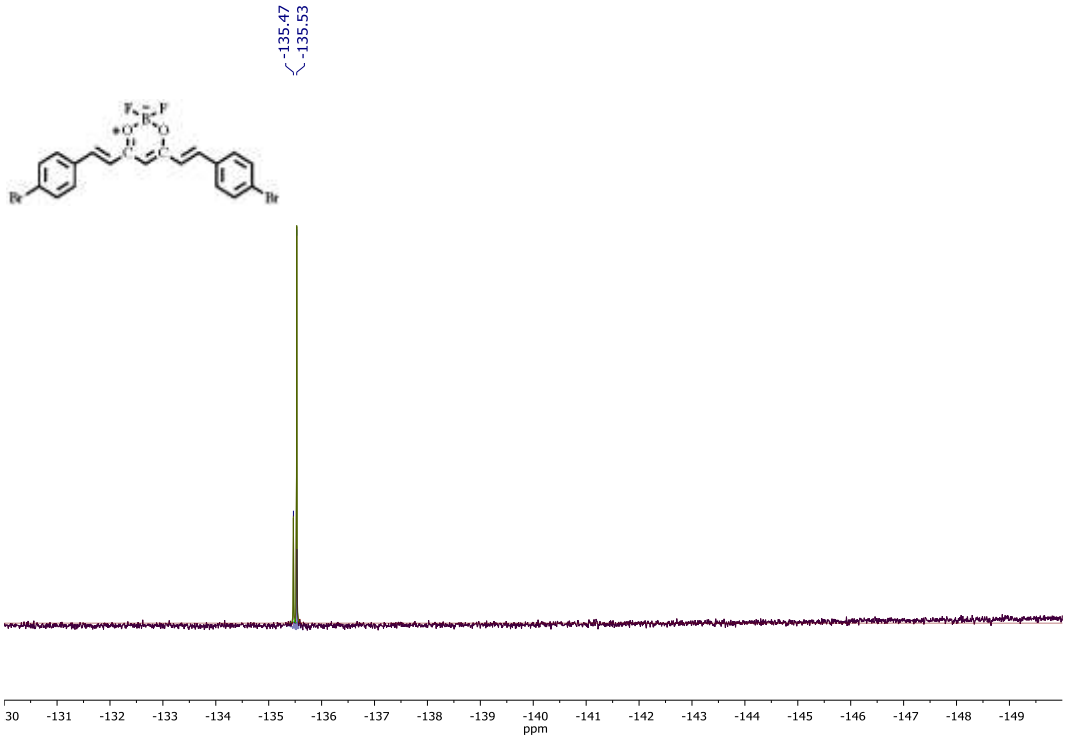

Figure S9. ^{19}F-NMR spectrum of compound **3a** in $CDCl_3$.

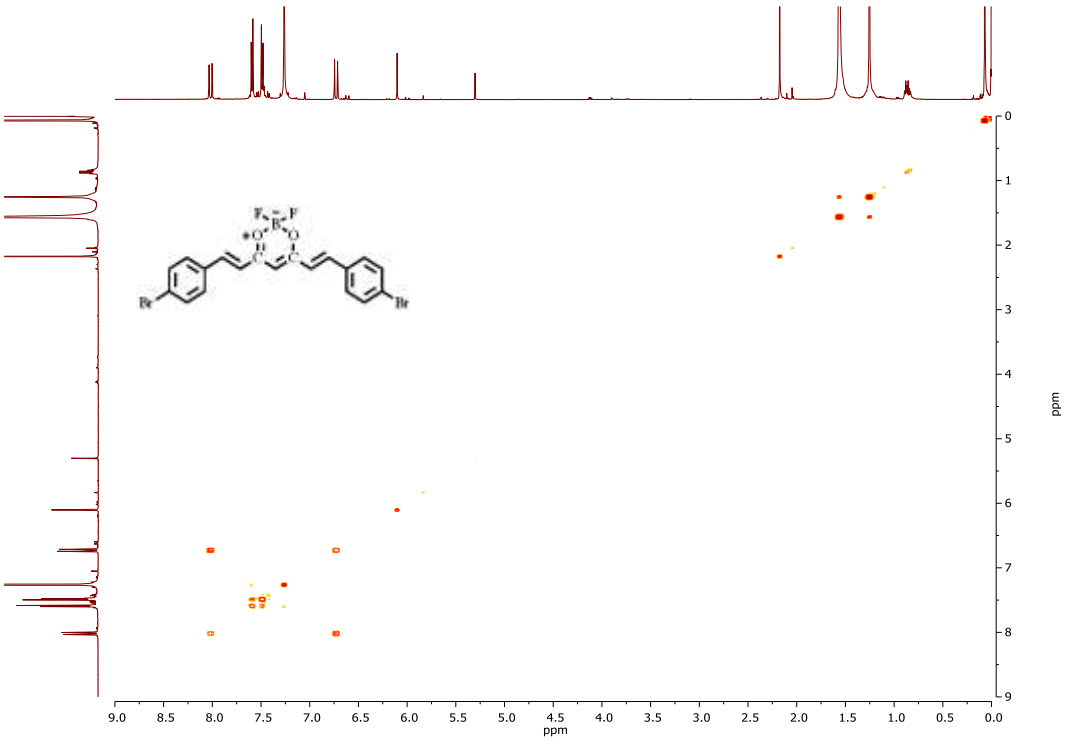

Figure S10. COSY-NMR spectrum of compound **3a** in $CDCl_3$.

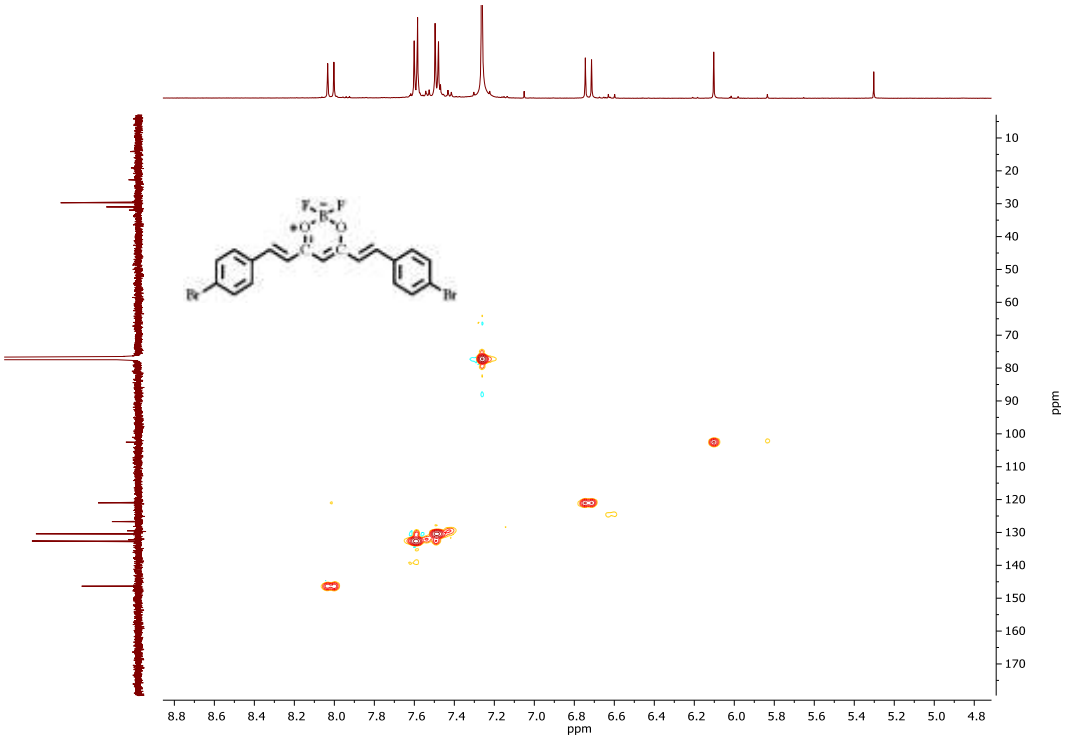

Figure S11. HSQC-NMR spectrum of compound **3a** in $CDCl_3$.

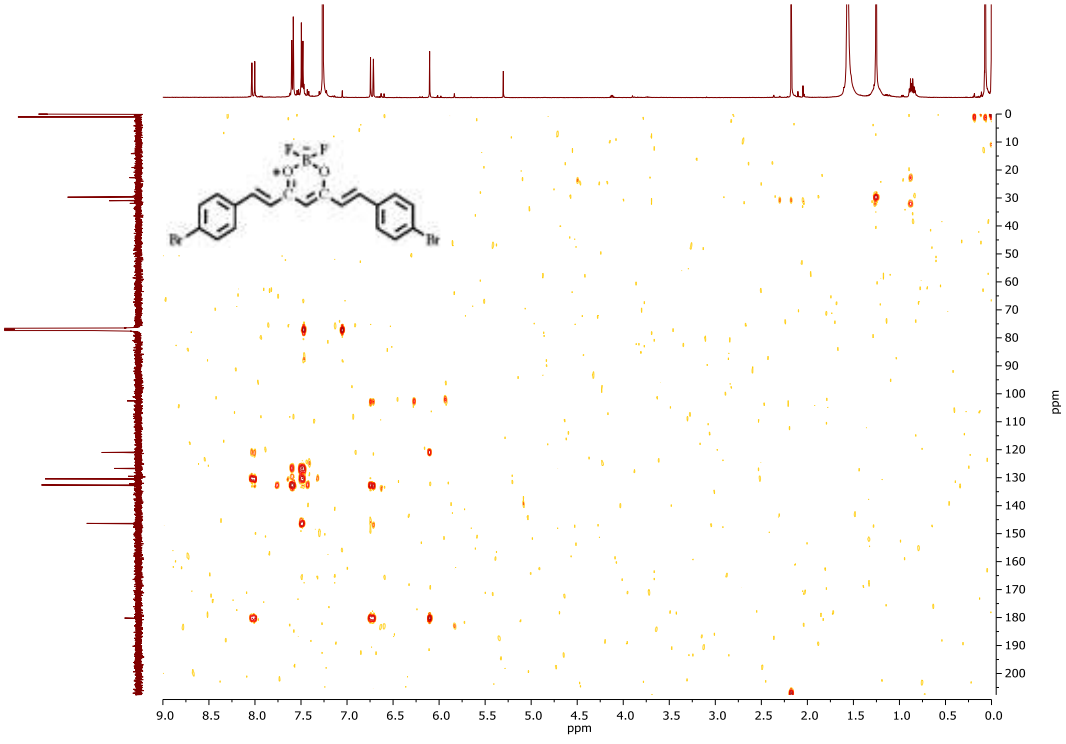

Figure S12. HMBC-NMR spectrum of compound **3a** in $CDCl_3$.

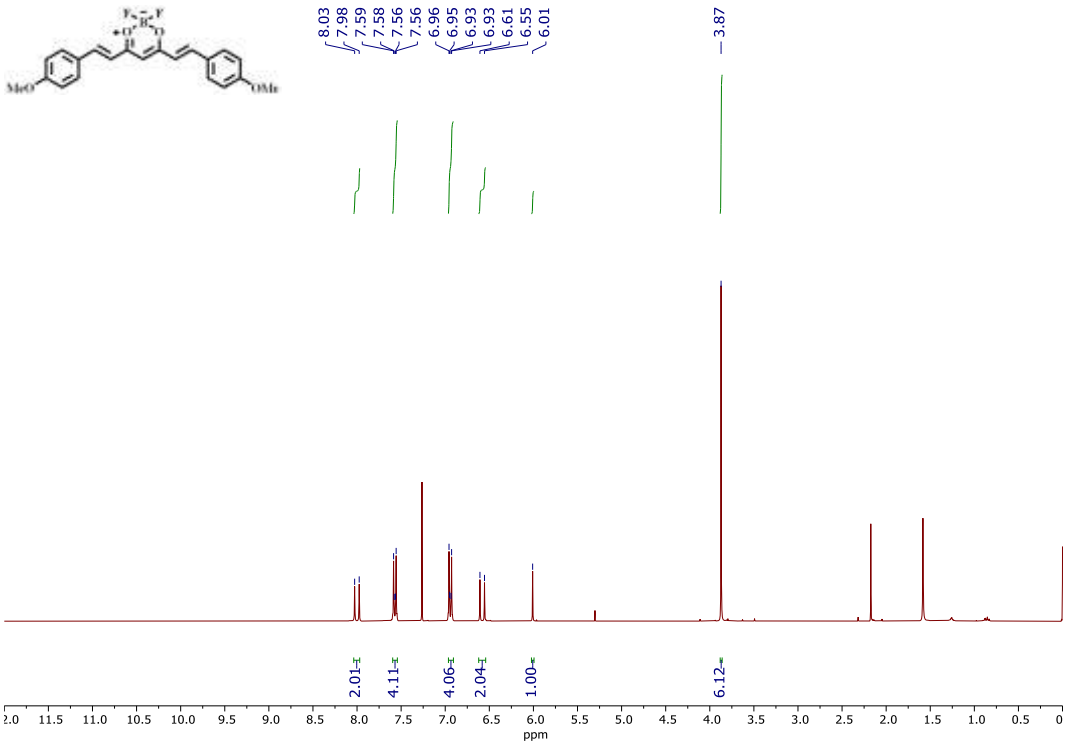

Figure S13. ^{1}H-NMR spectrum of compound **3b** in $CDCl_3$.

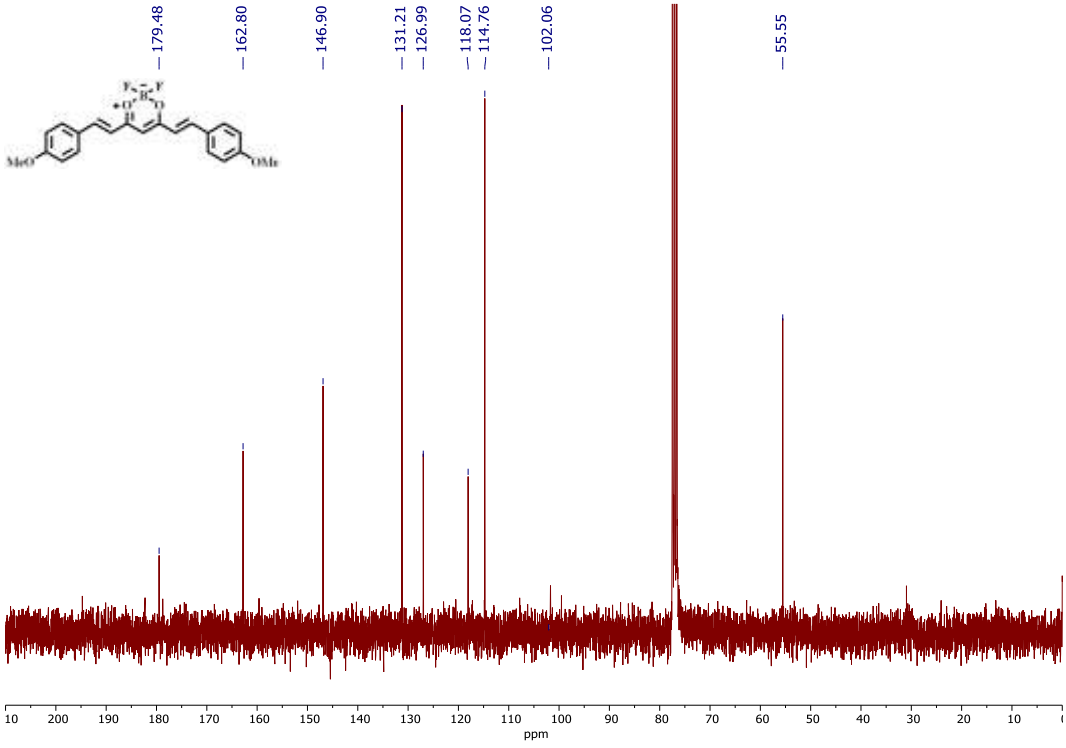

Figure S14. ^{13}C-NMR spectrum of compound **3b** in $CDCl_3$.

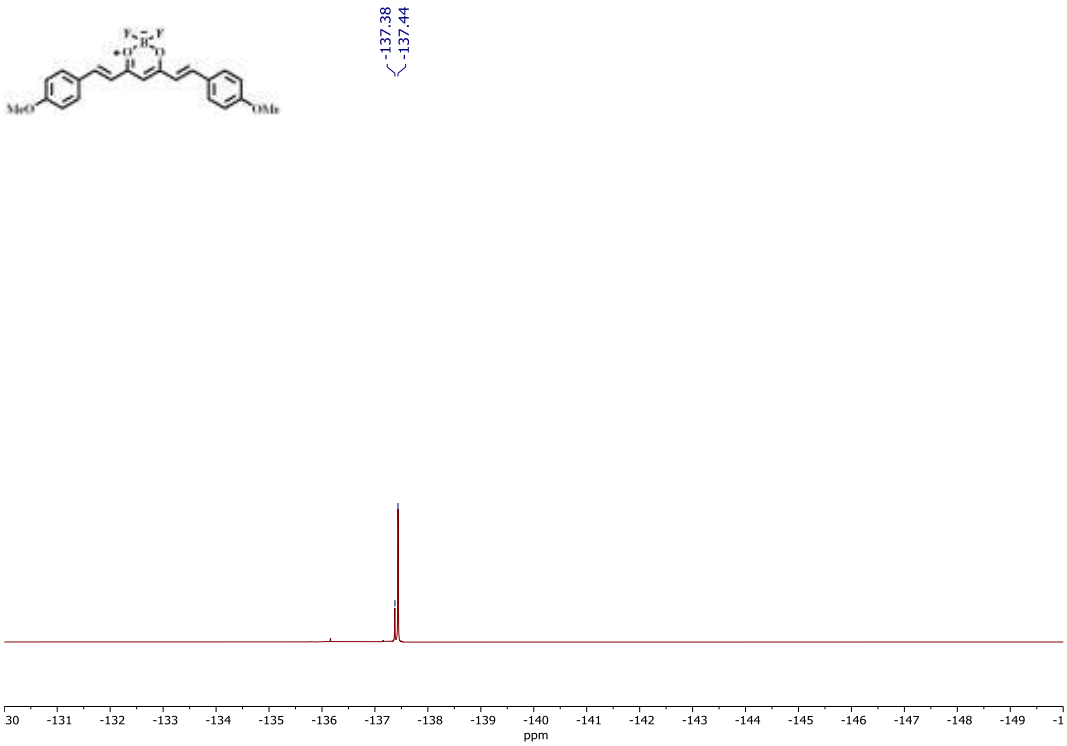

Figure S15. ^{19}F-NMR spectrum of compound **3b** in $CDCl_3$.

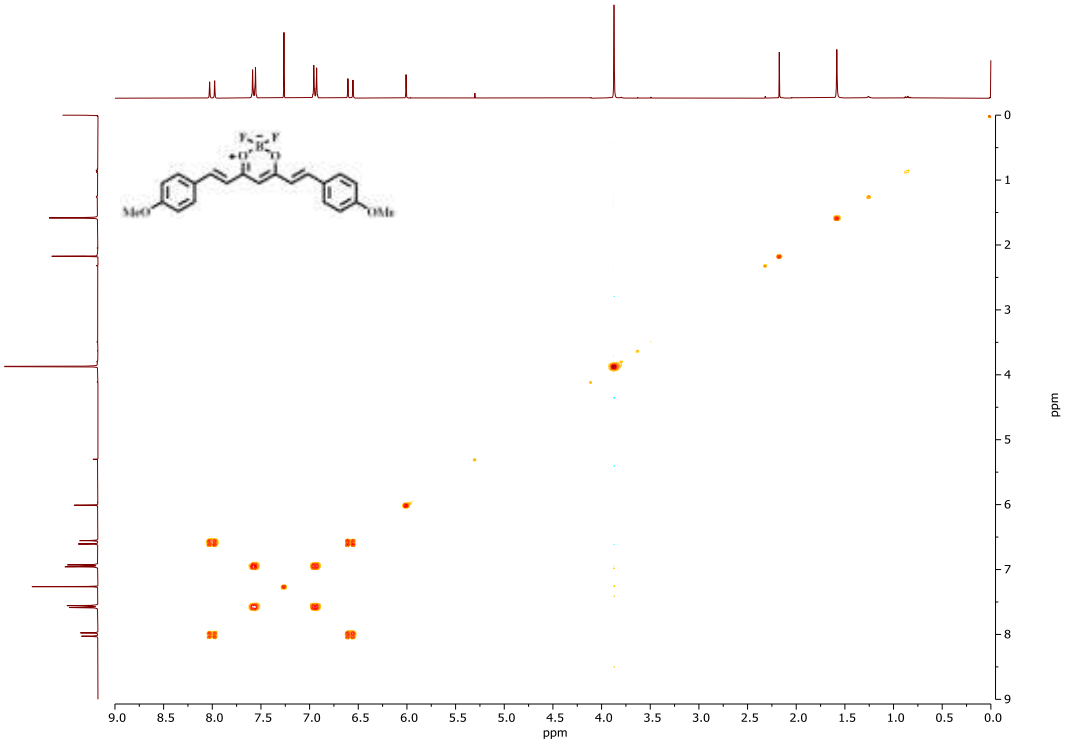

Figure S16. COSY-NMR spectrum of compound **3b** in $CDCl_3$.

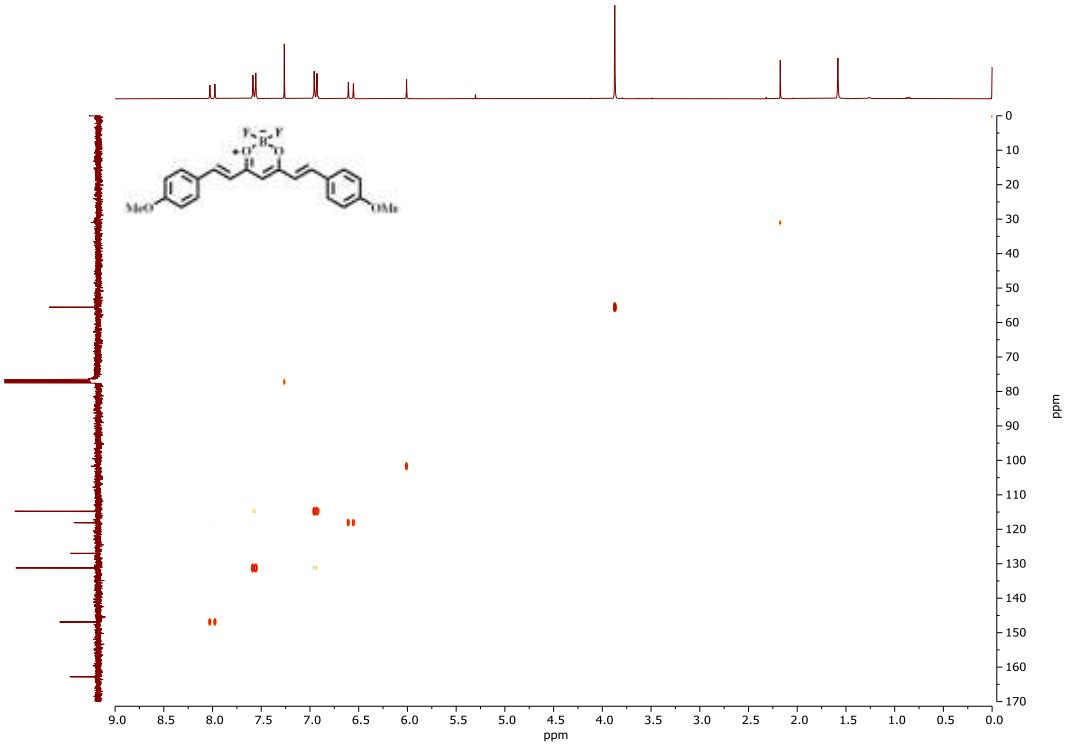

Figure S17. HSQC-NMR spectrum of compound **3b** in $CDCl_3$.

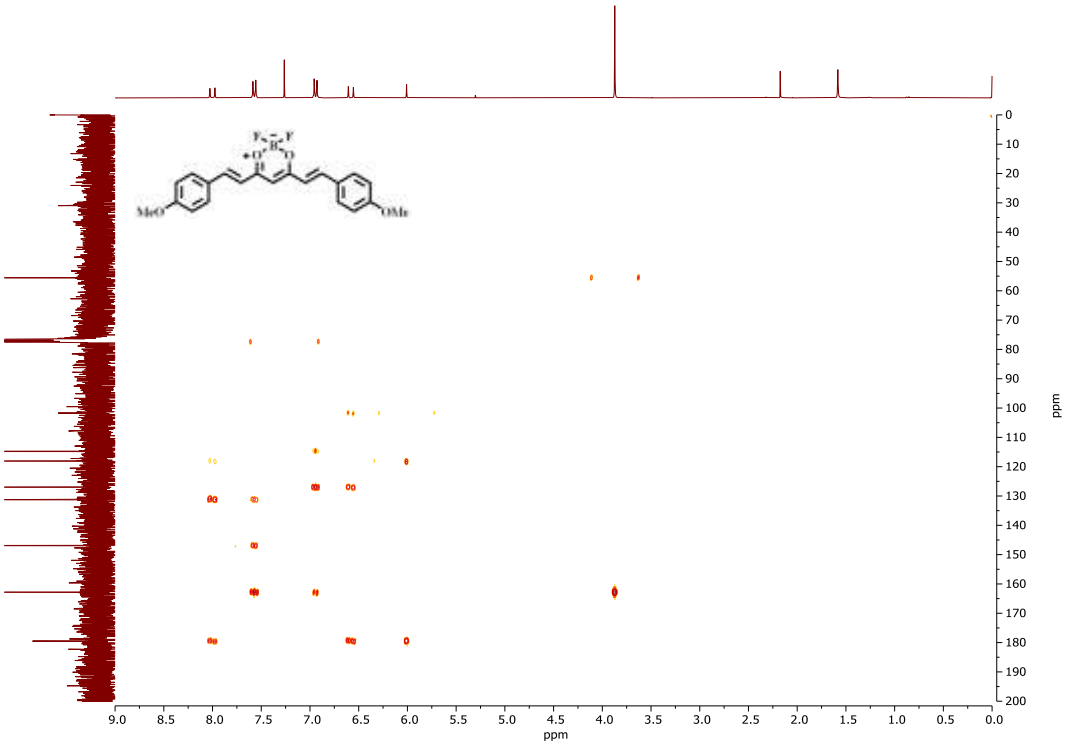

Figure S18. HMBC-NMR spectrum of compound **3b** in $CDCl_3$.

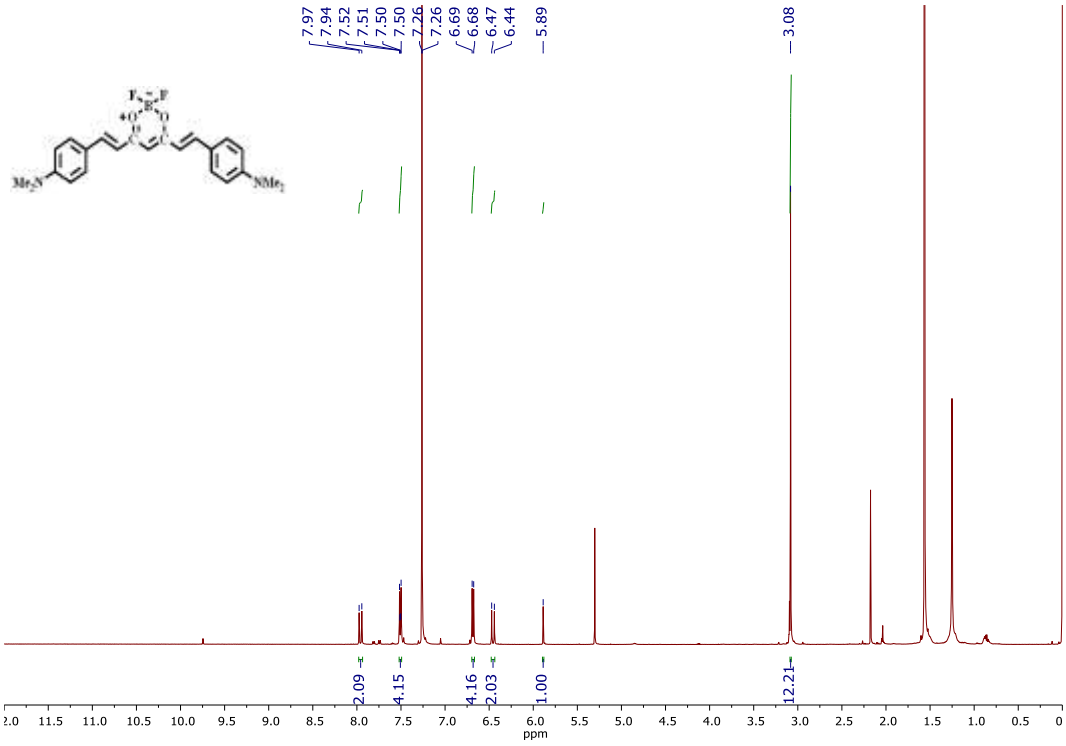

Figure S19. ^{1}H-NMR spectrum of compound **3c** in $CDCl_3$.

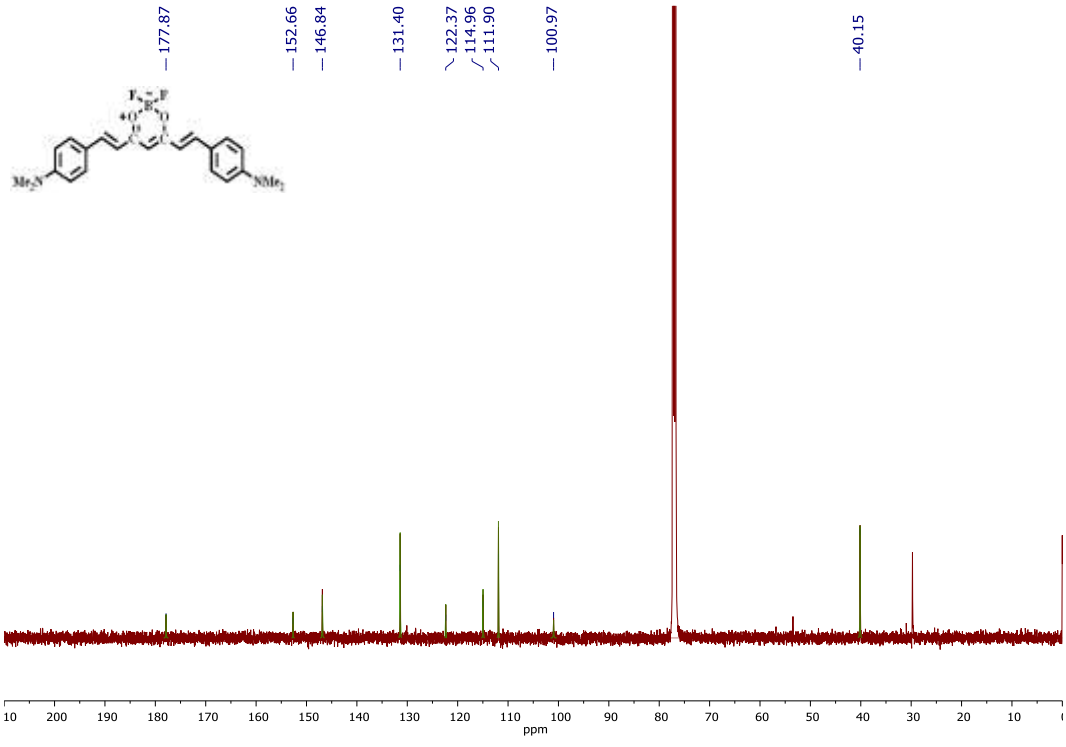

Figure S20. ^{13}C-NMR spectrum of compound **3c** in $CDCl_3$.

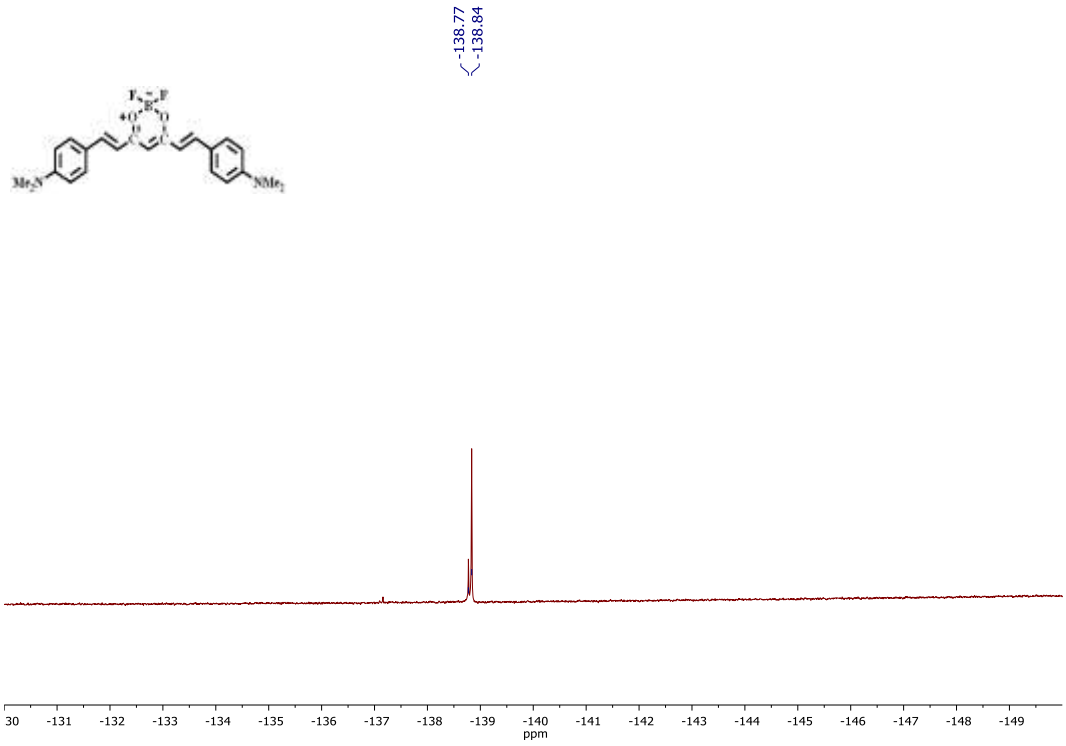

Figure S21. ^{19}F-NMR spectrum of compound **3c** in $CDCl_3$.

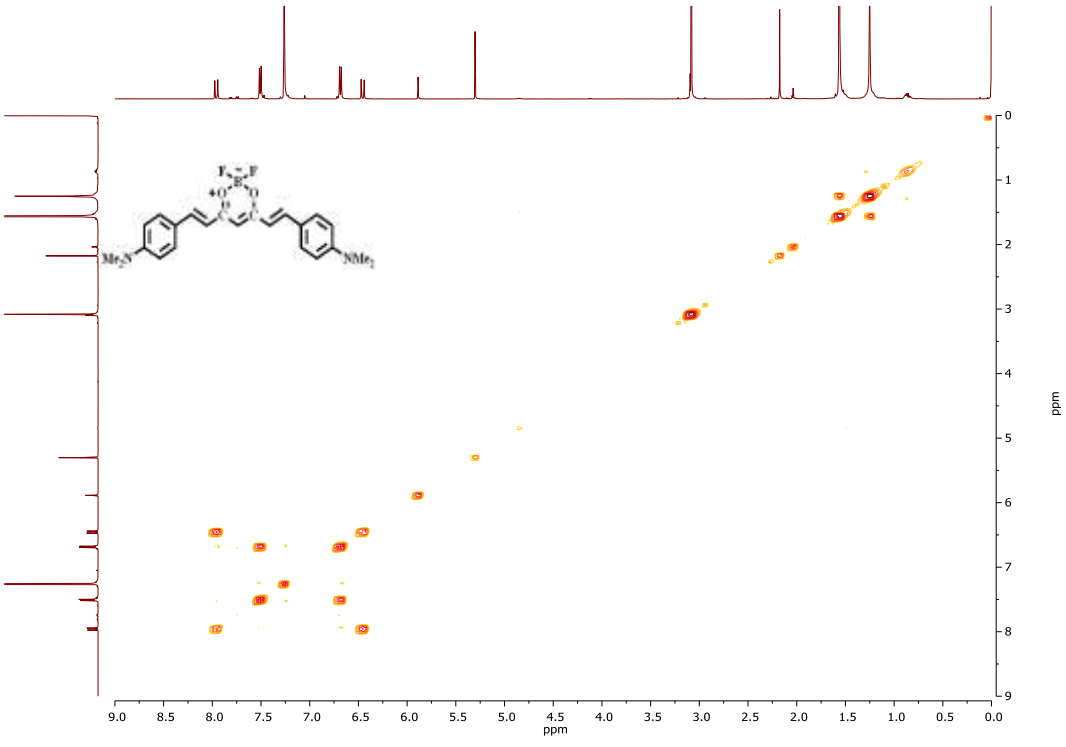

Figure S22. COSY-NMR spectrum of compound **3c** in $CDCl_3$.

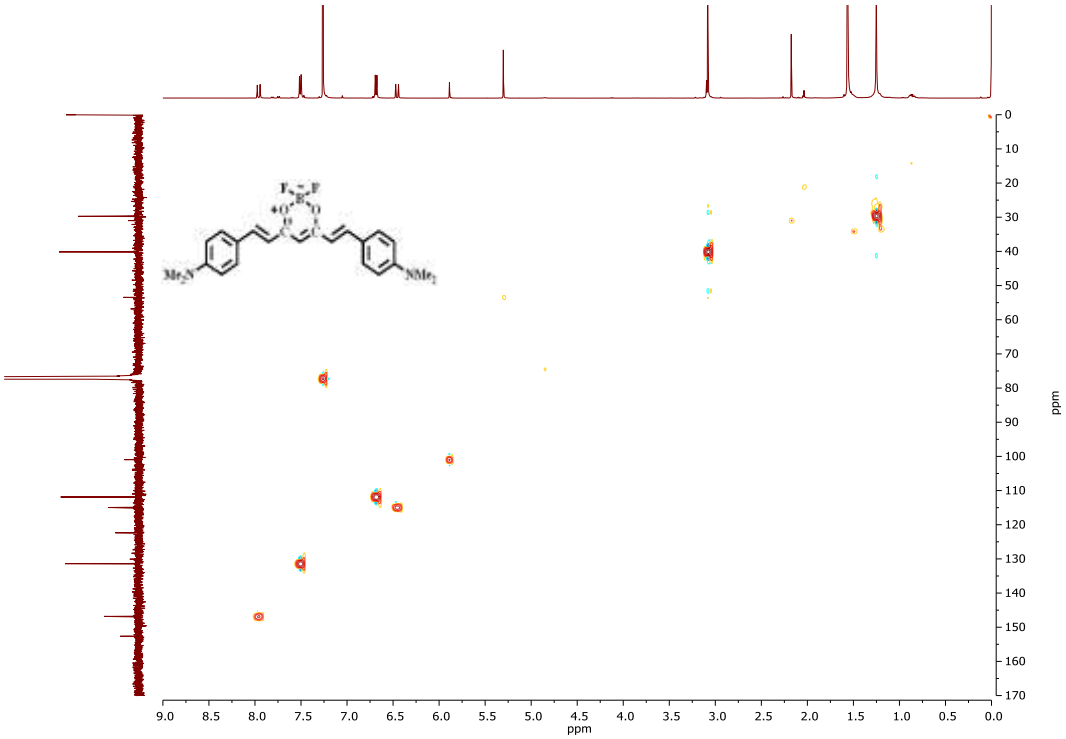

Figure S23. HSQC-NMR spectrum of compound **3c** in $CDCl_3$.

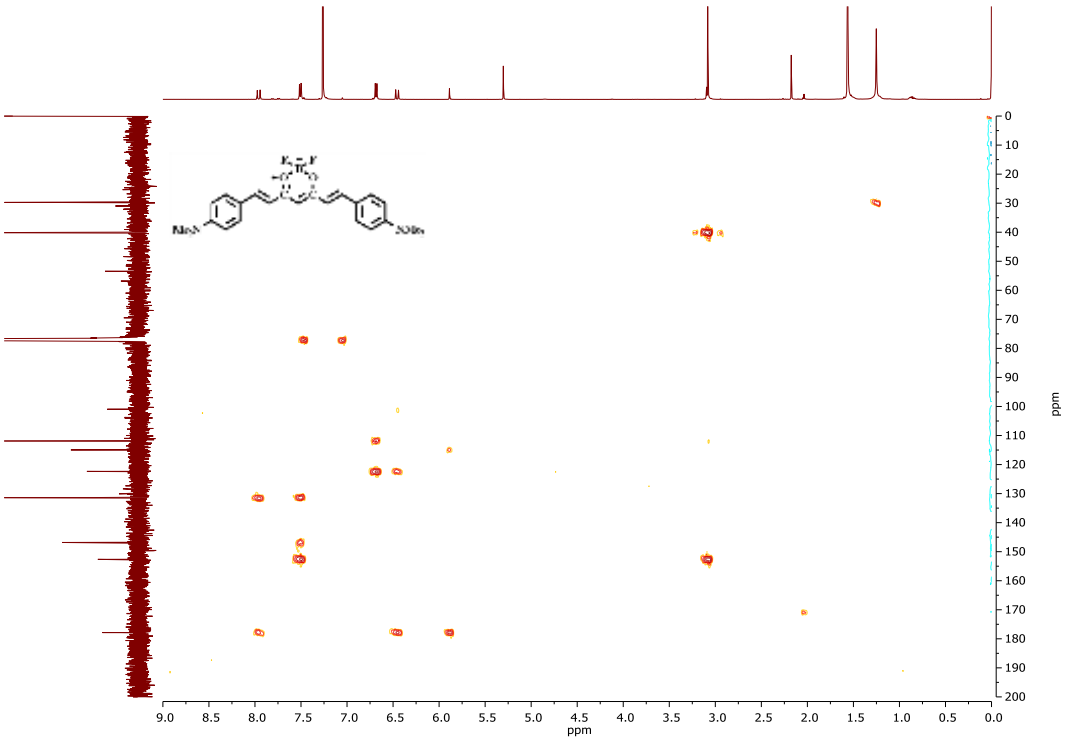

Figure S24. HMBC-NMR spectrum of compound **3c** in $CDCl_3$.

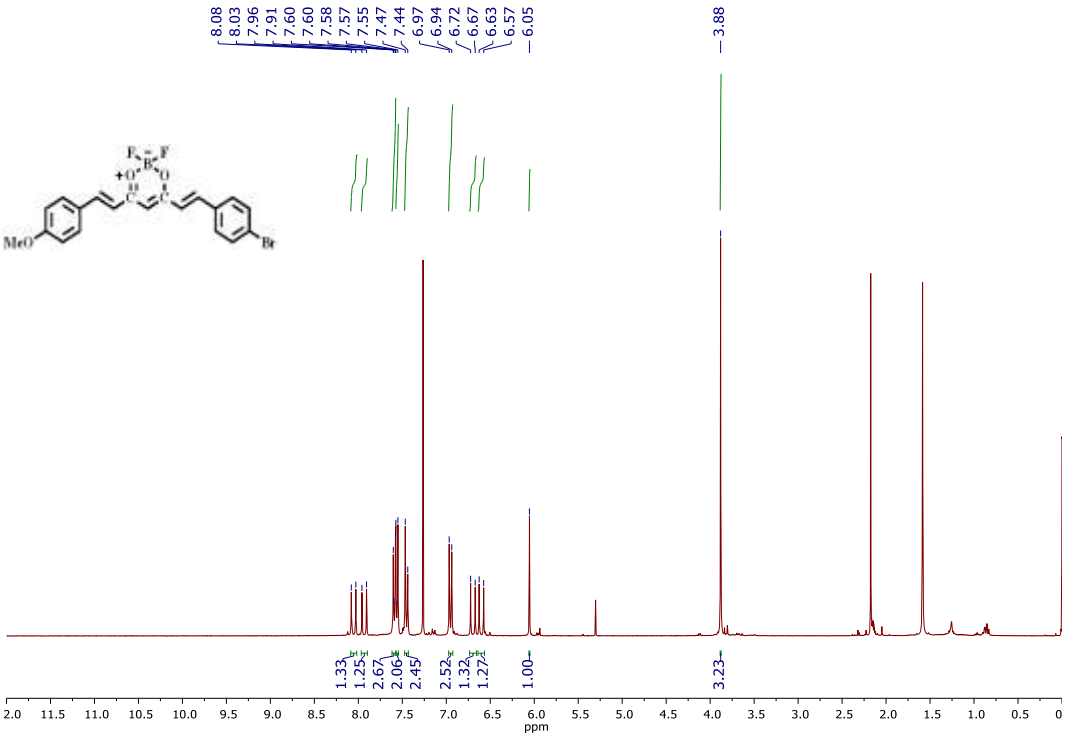

Figure S25. ^{1}H-NMR spectrum of compound **3d** in $CDCl_3$.

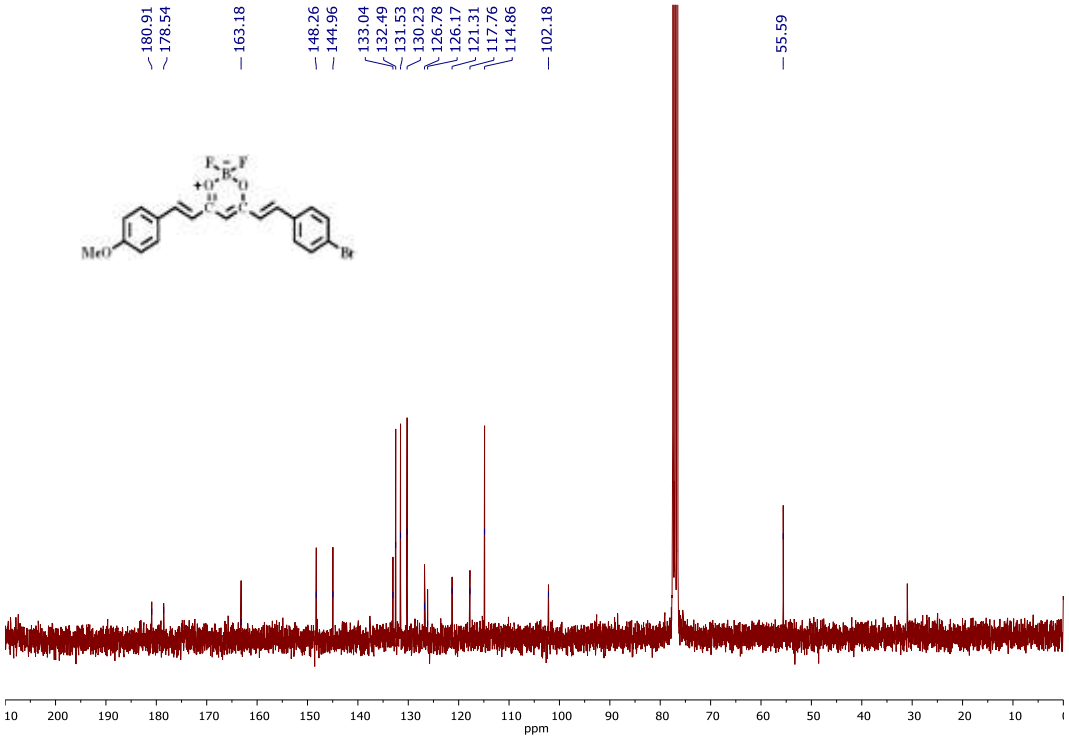

Figure S26. ^{13}C-NMR spectrum of compound **3d** in $CDCl_3$.

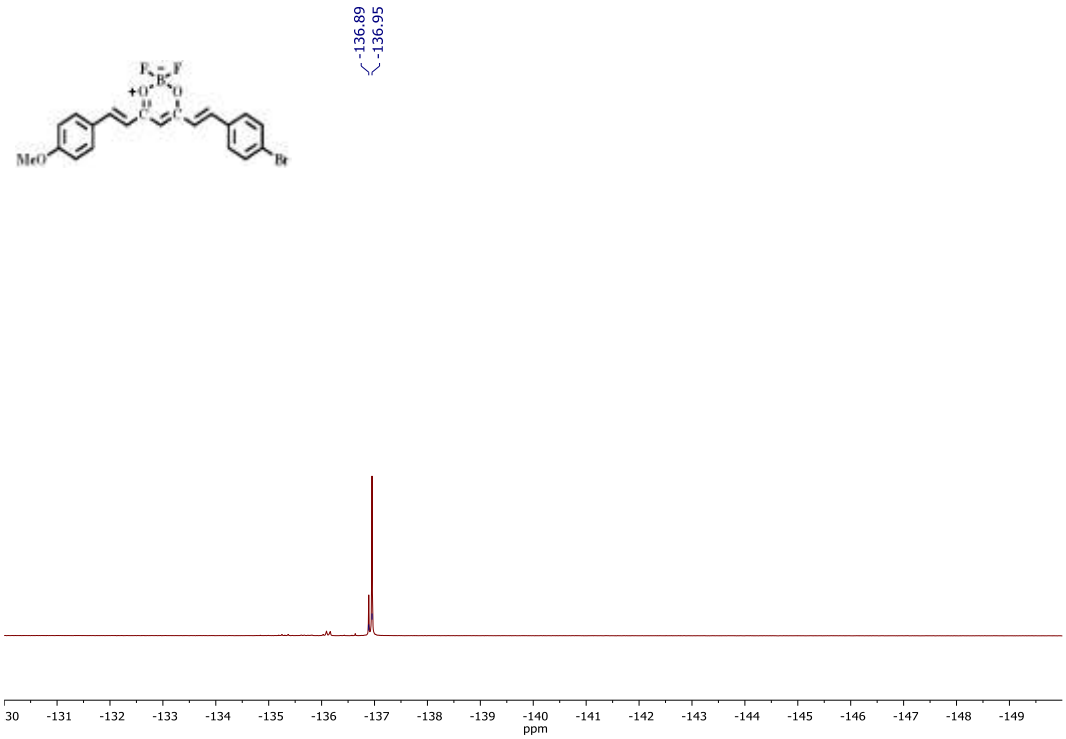

Figure S27. ^{19}F-NMR spectrum of compound **3d** in $CDCl_3$.

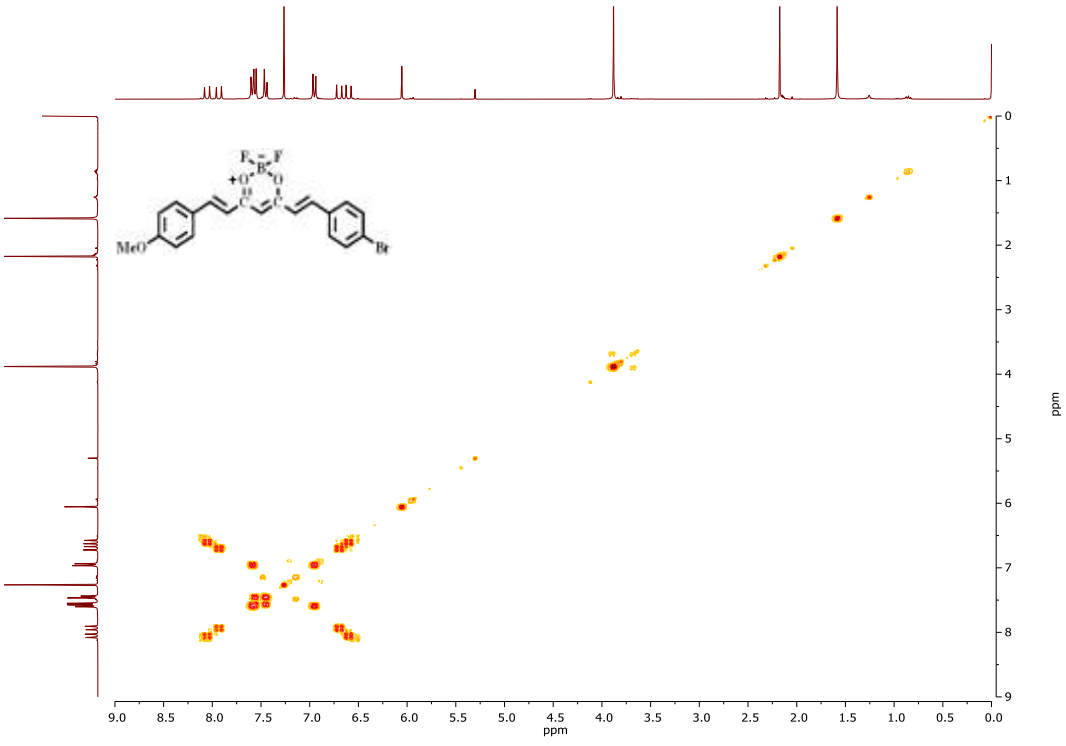

Figure S28. COSY-NMR spectrum of compound **3d** in $CDCl_3$.

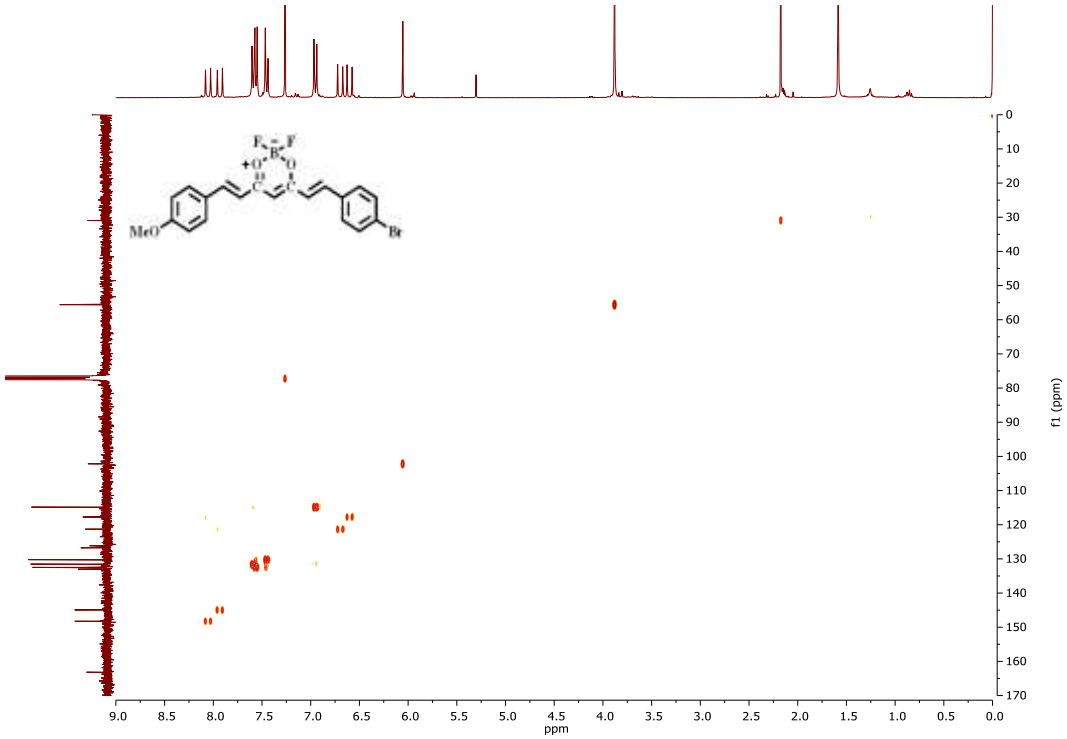

Figure S29. HSQC-NMR spectrum of compound **3d** in $CDCl_3$.

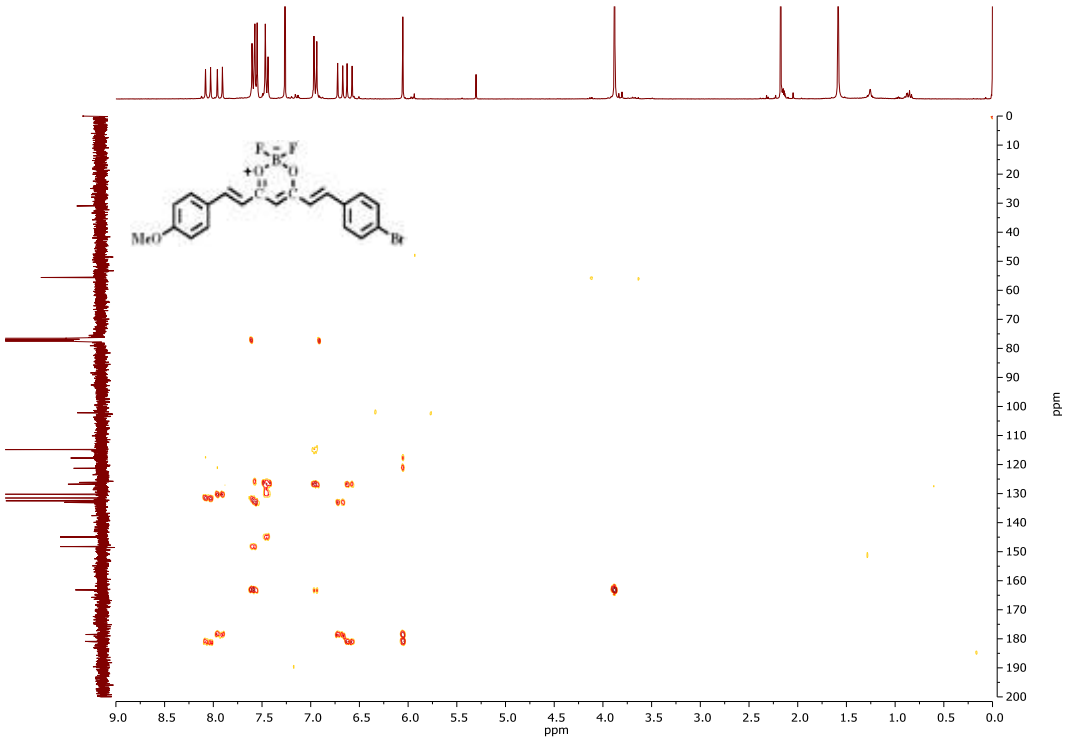

Figure S30. HMBC-NMR spectrum of compound **3d** in $CDCl_3$.

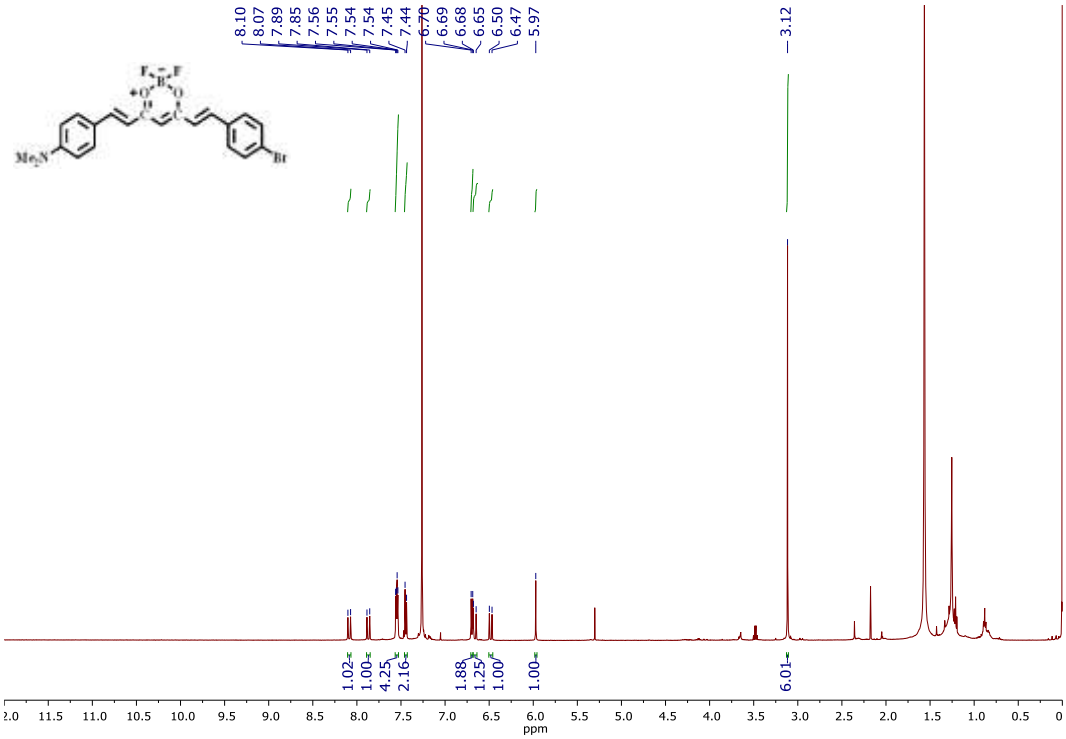

Figure S31. ^{1}H-NMR spectrum of compound **3e** in $CDCl_3$.

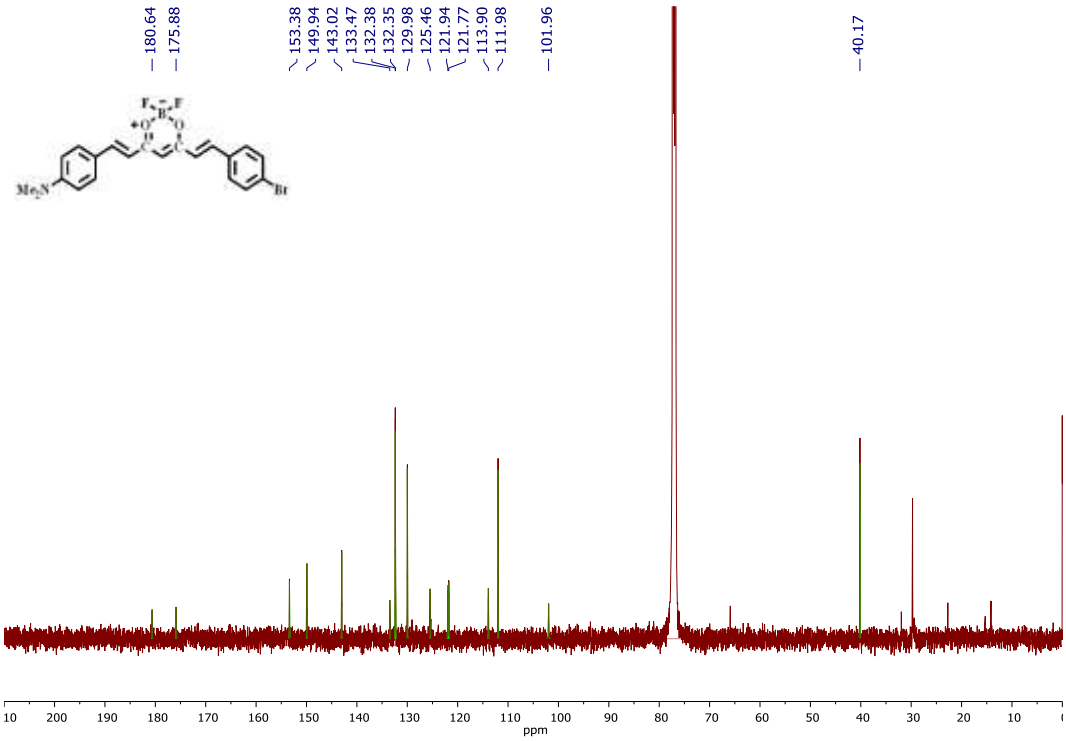

Figure S32. ^{13}C-NMR spectrum of compound **3e** in $CDCl_3$.

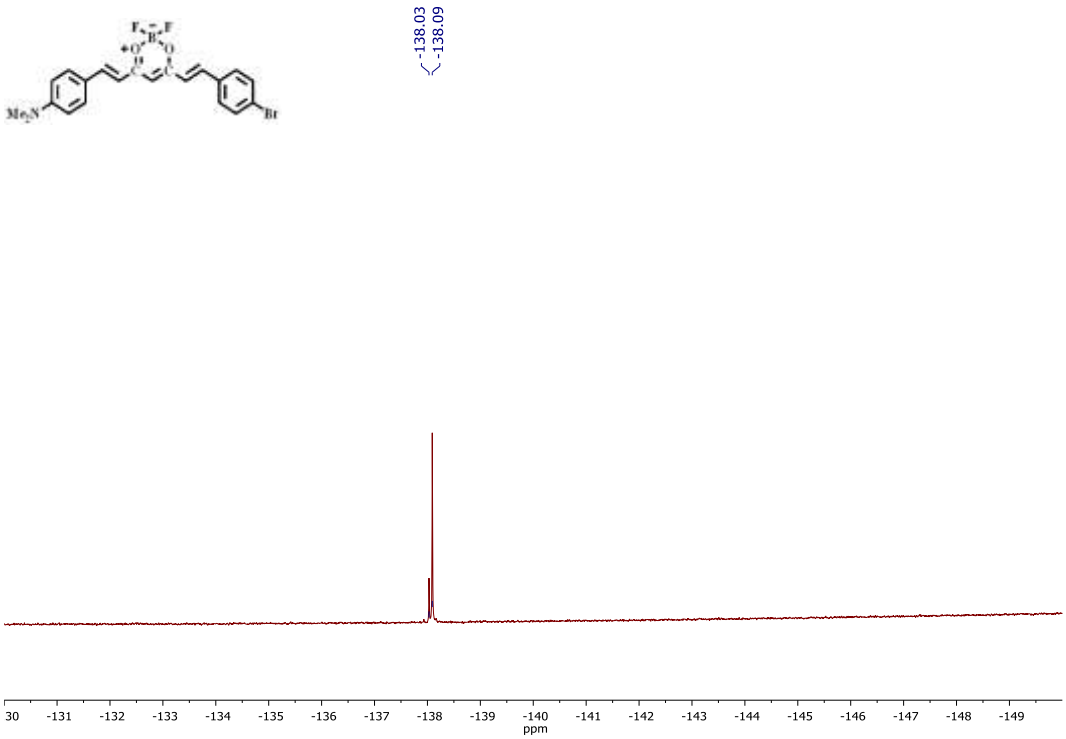

Figure S33. ^{19}F-NMR spectrum of compound **3e** in $CDCl_3$.

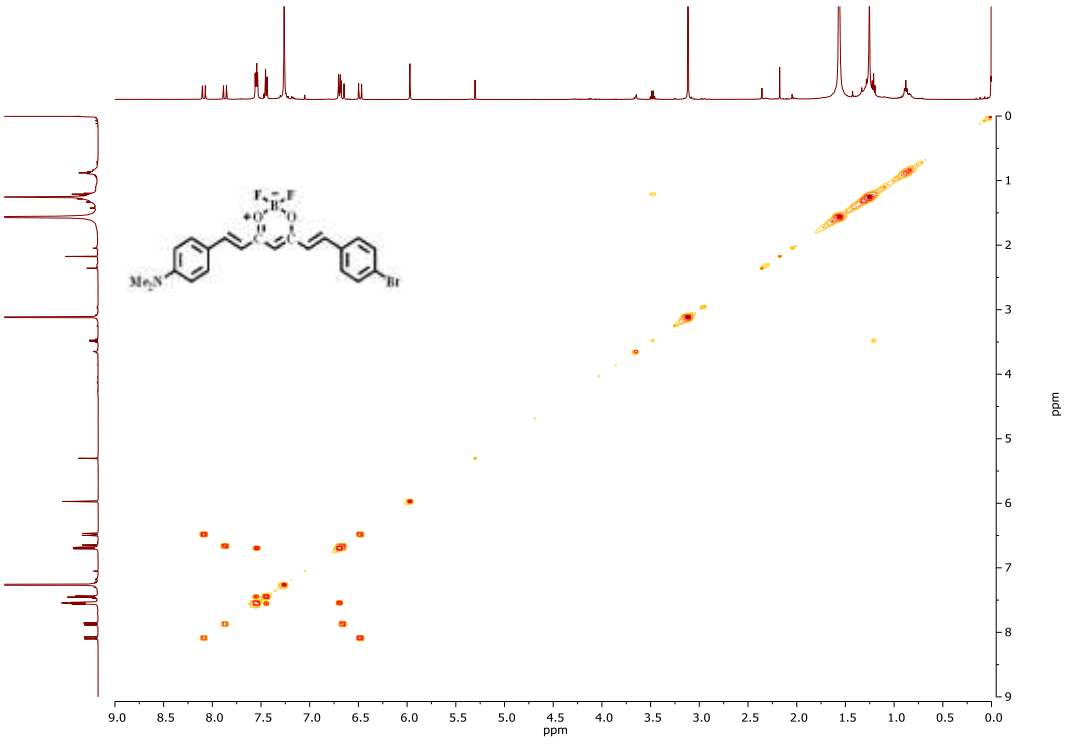

Figure S34. COSY-NMR spectrum of compound **3e** in $CDCl_3$.

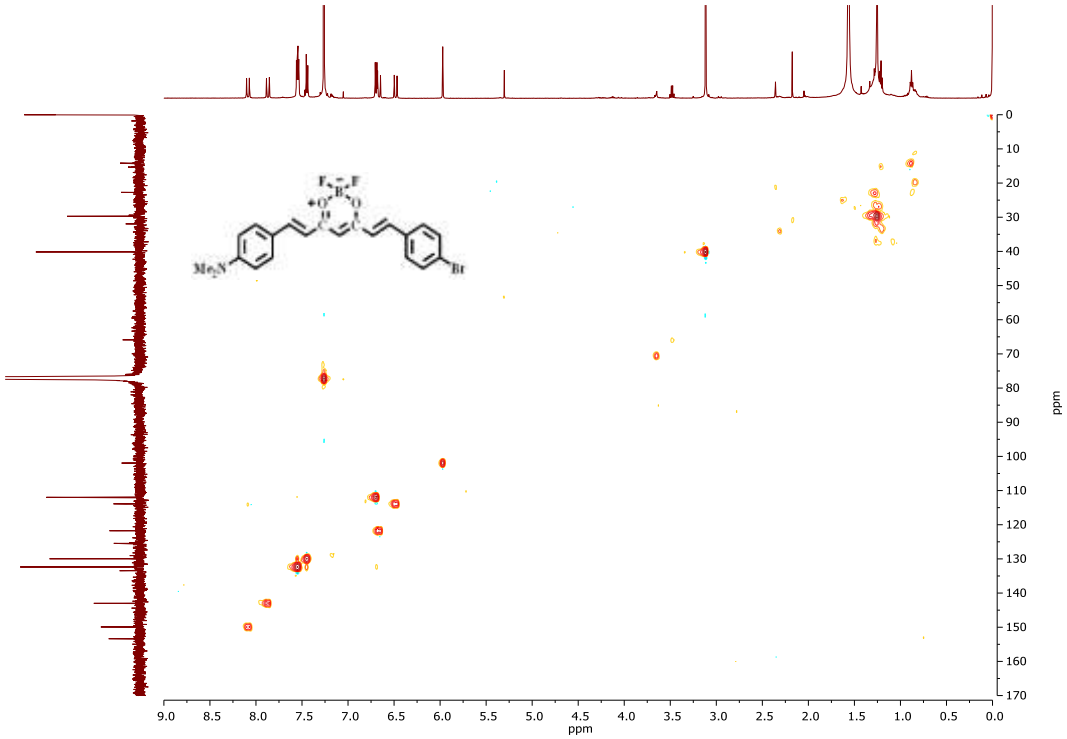

Figure S35. HSQC-NMR spectrum of compound **3e** in $CDCl_3$.

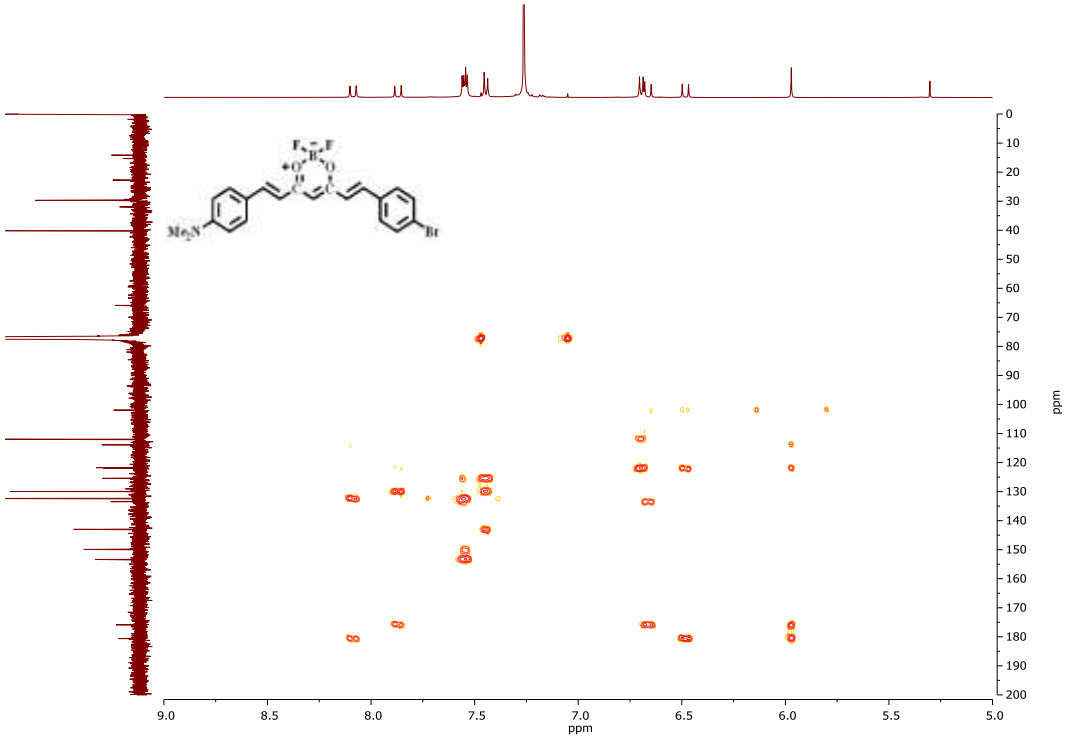

Figure S36. HMBC-NMR spectrum of compound **3e** in $CDCl_3$.

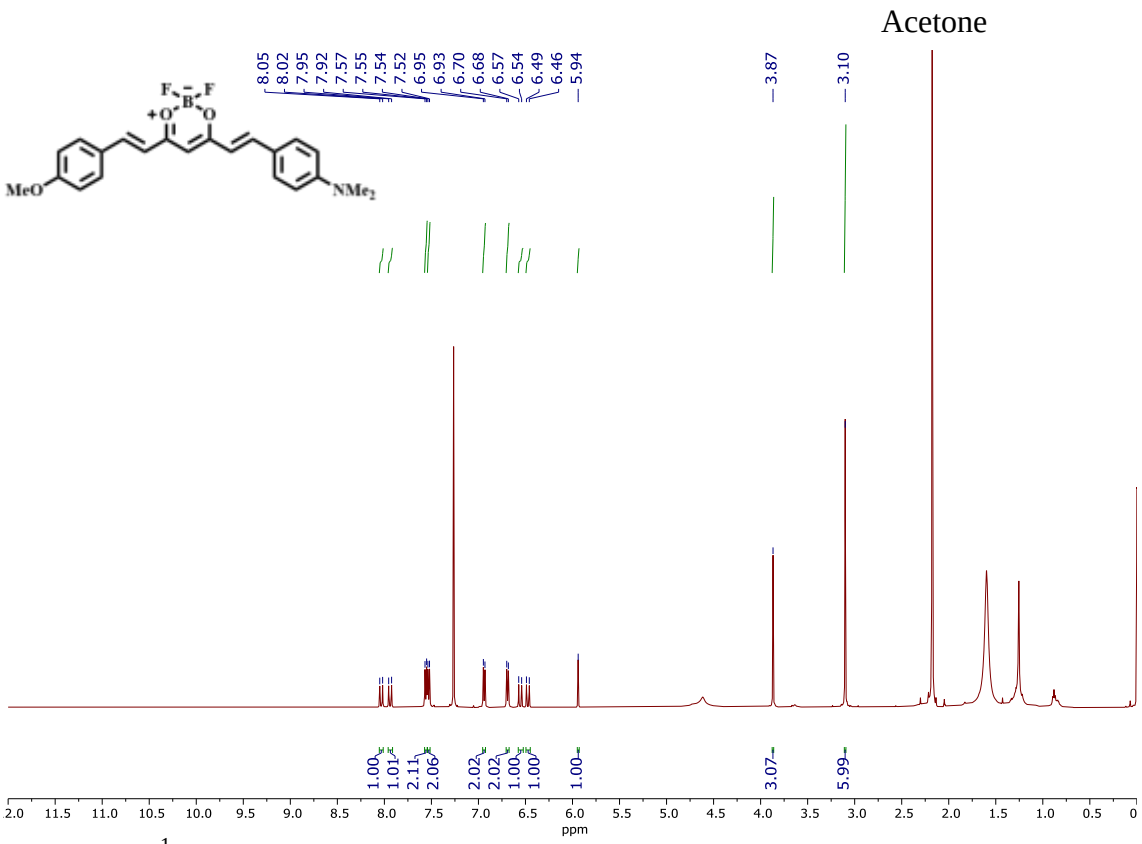

Figure S37. ^{1}H-NMR spectrum of compound **3f** in $CDCl_3$.

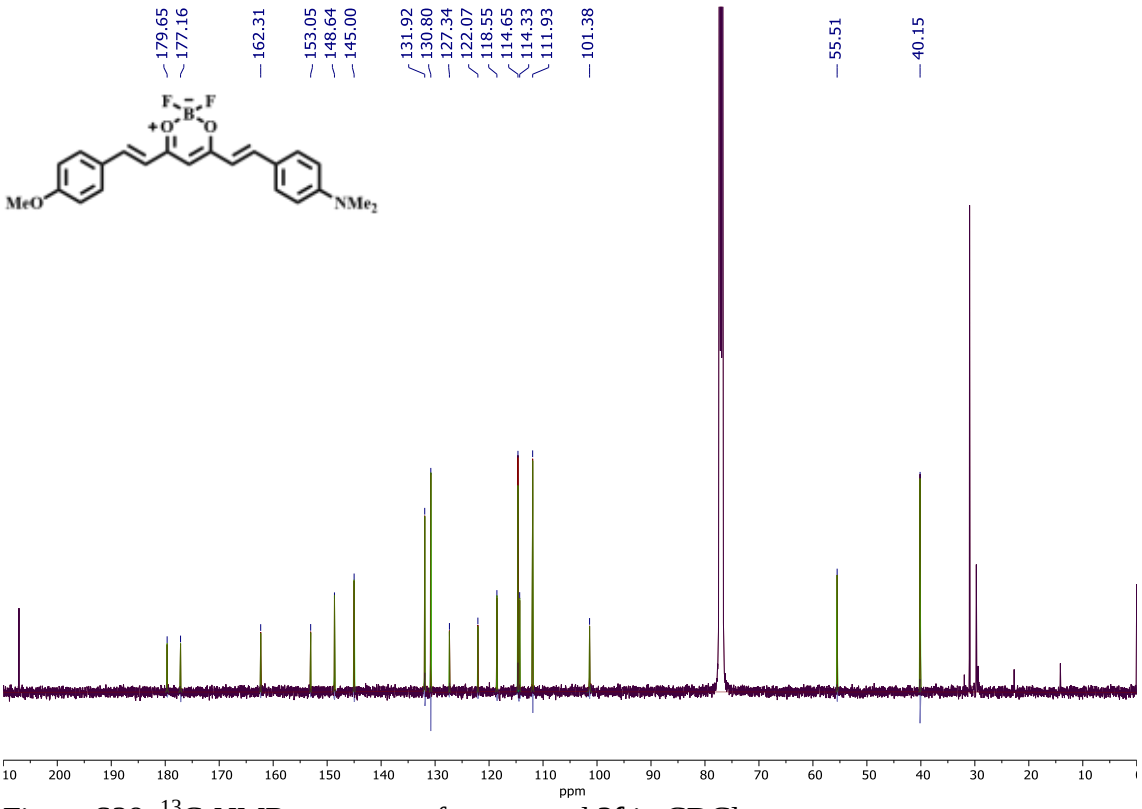

Figure S38. ^{13}C-NMR spectrum of compound **3f** in $CDCl_3$.

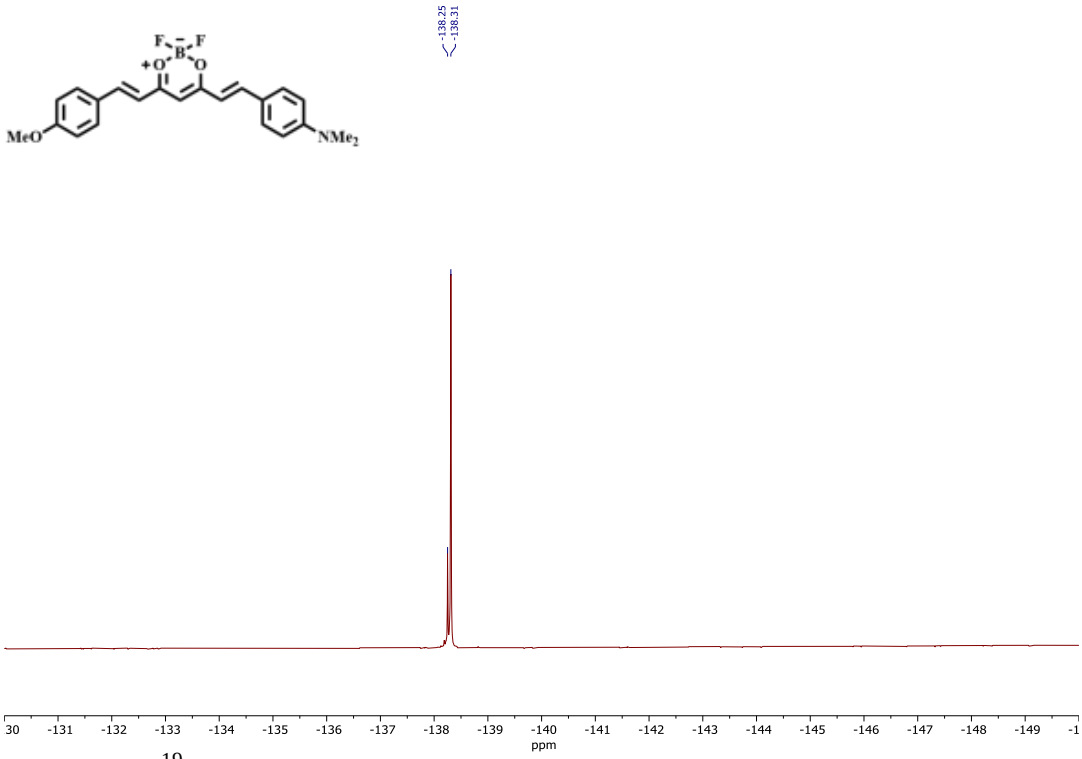

Figure S39. ^{19}F-NMR spectrum of compound **3f** in $CDCl_3$.

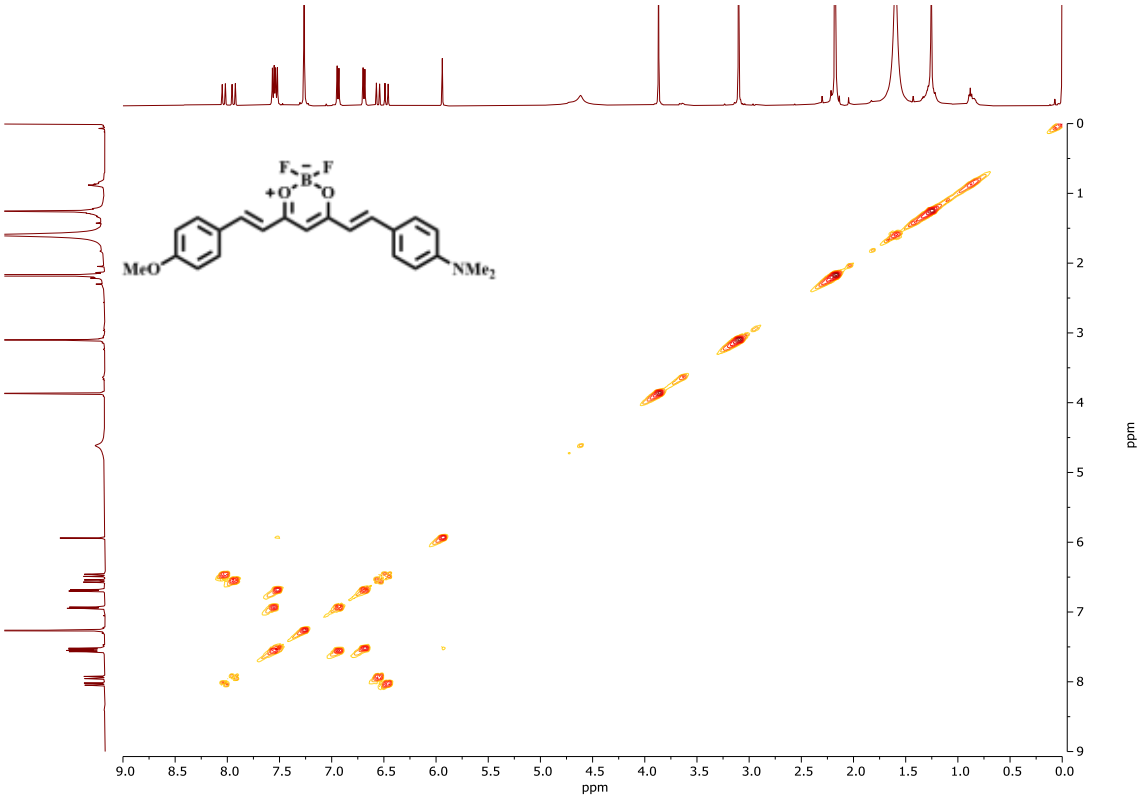

Figure S40. COSY-NMR spectrum of compound **3f** in $CDCl_3$.

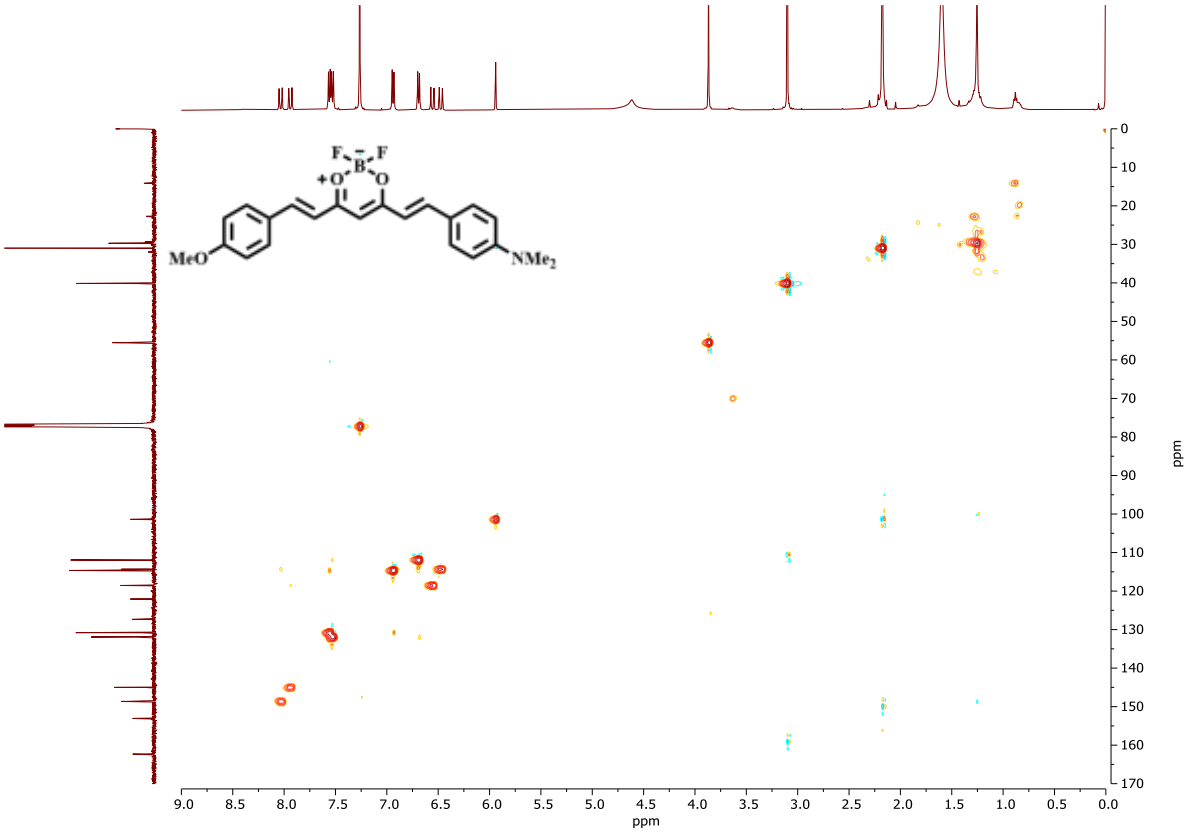

Figure S41. HSQC-NMR spectrum of compound **3f** in $CDCl_3$.

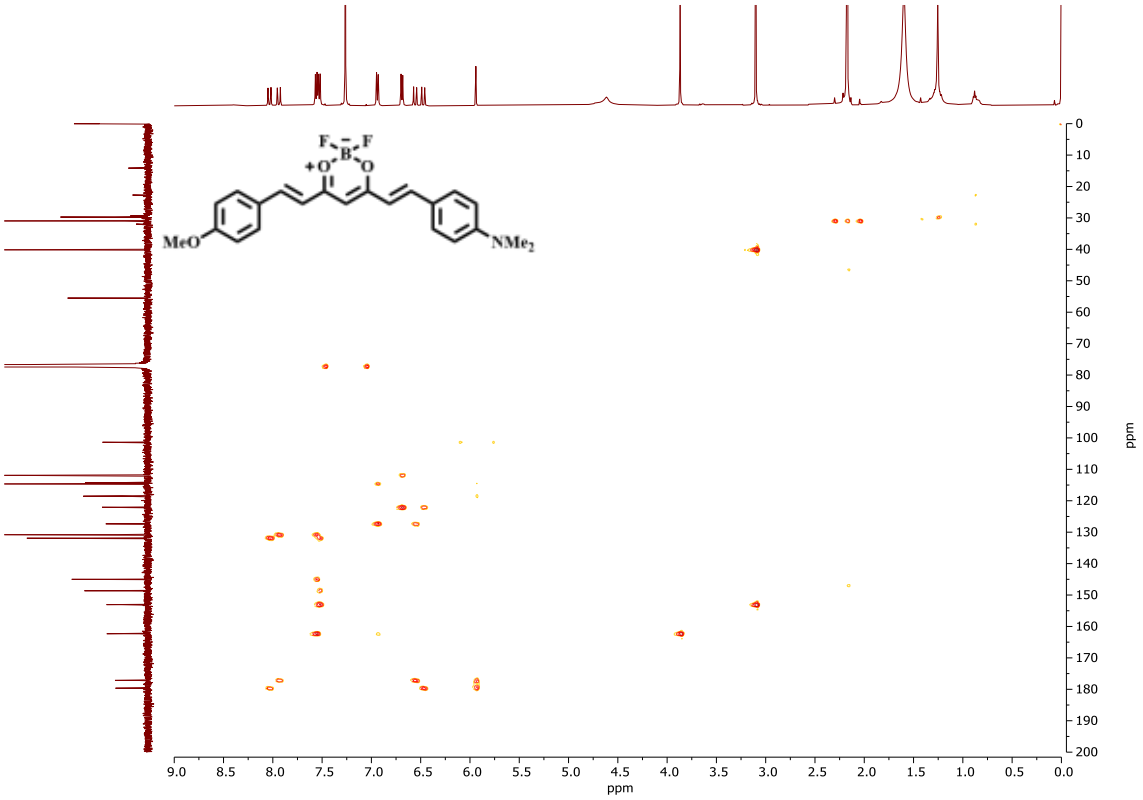

Figure S42. HMBC-NMR spectrum of compound **3f** in $CDCl_3$.

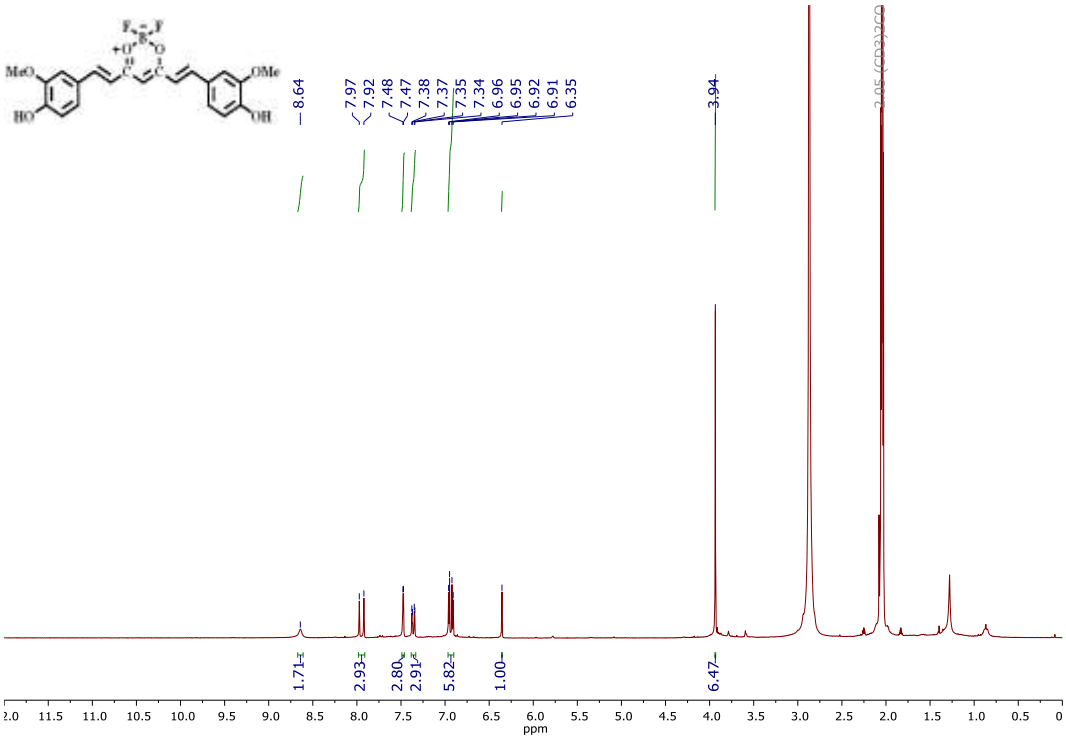

Figure S43. ^{1}H-NMR spectrum of compound **4** in Acetone-d_6.

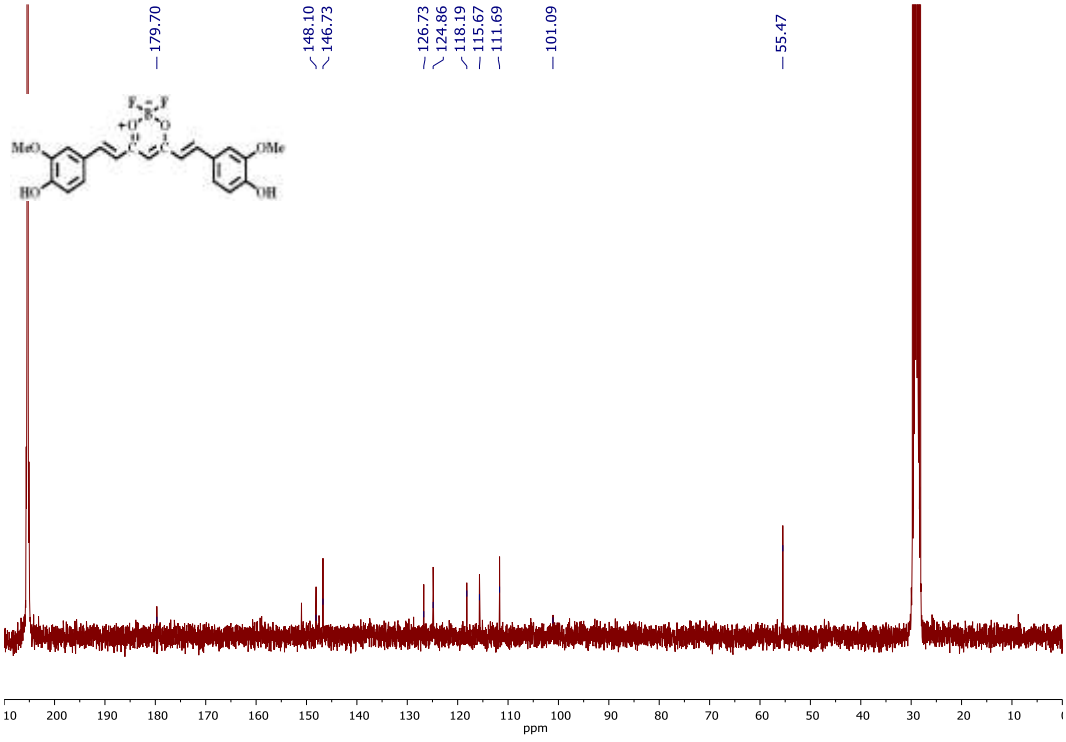

Figure S44. ^{13}C-NMR spectrum of compound **4** in Acetone-d_6.

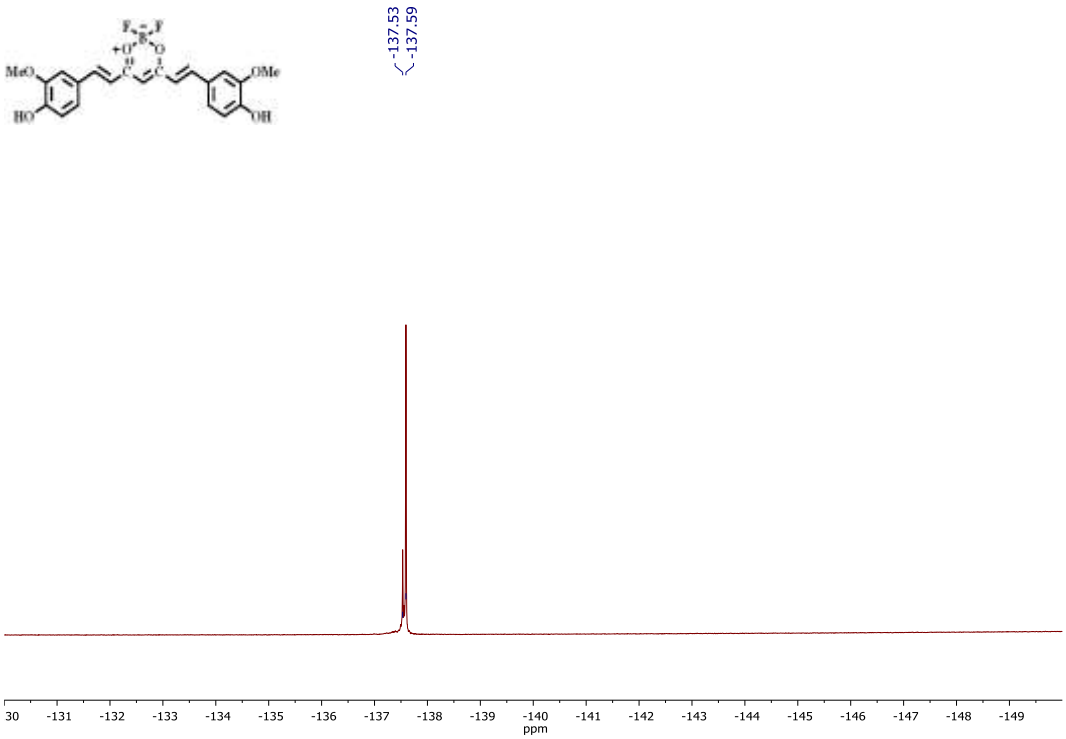

Figure S45. ^{19}F-NMR spectrum of compound **4**in Acetone-d_6.

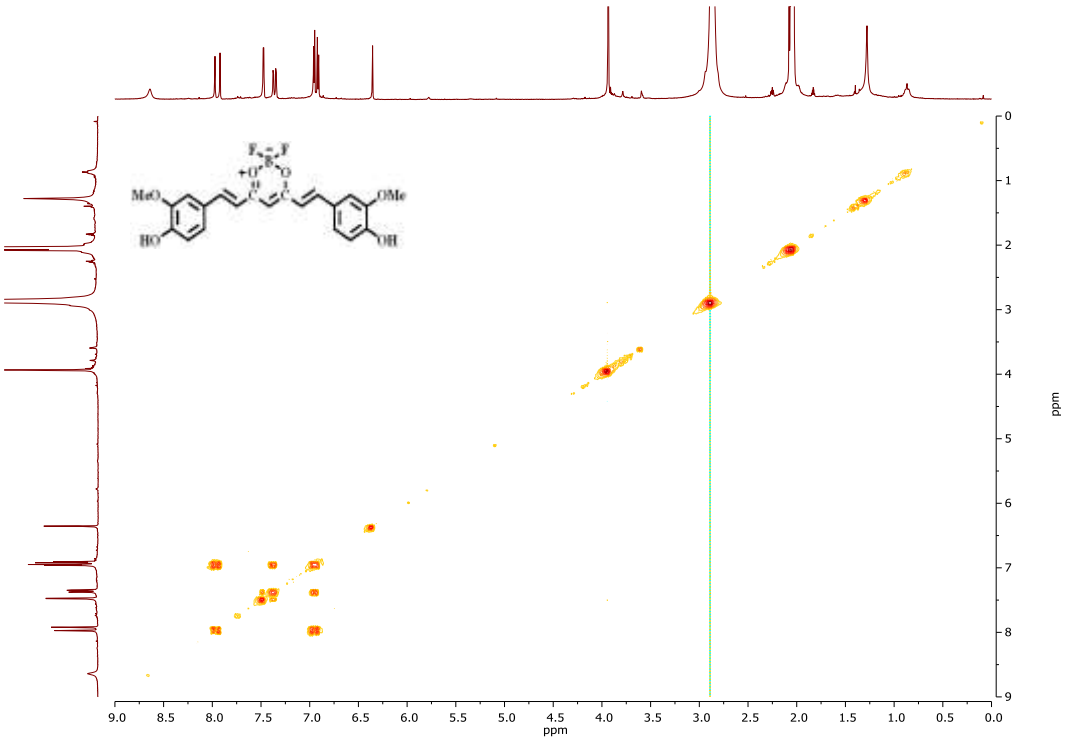

Figure S46. COSY-NMR spectrum of compound **4** in Acetone-d_6.

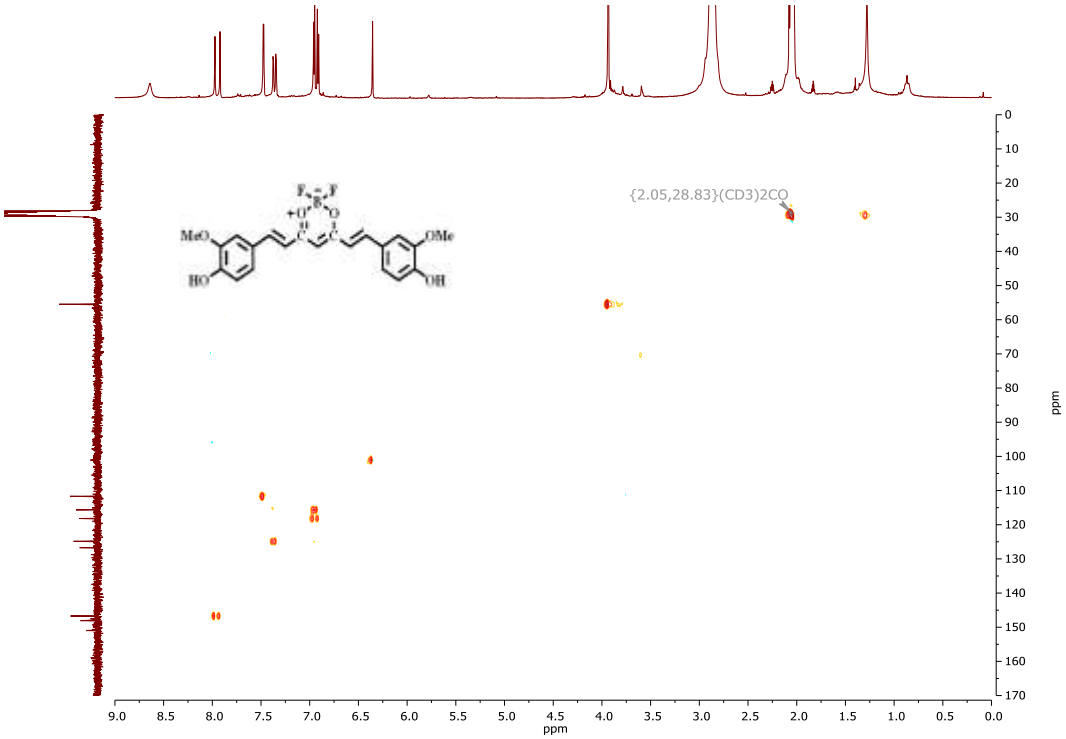

Figure S47. HSQC-NMR spectrum of compound **4** in Acetone-d_6.

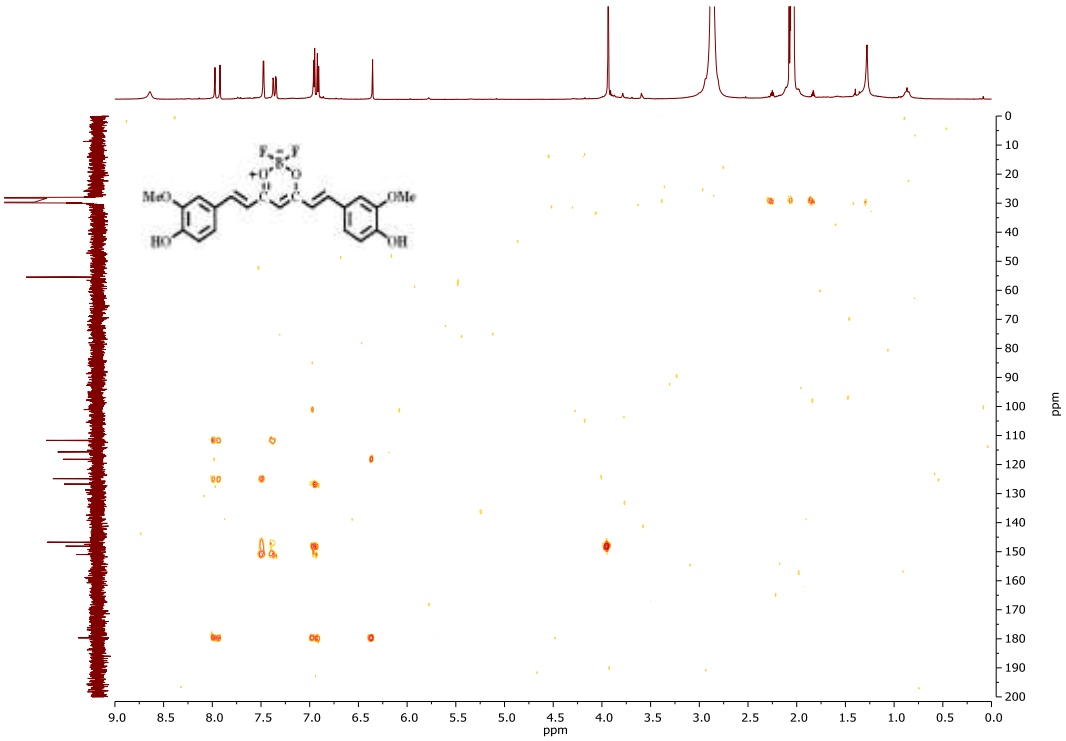

Figure S48. HMBC-NMR spectrum of compound **4** in Acetone-d_6.

Crystal structures

Single-crystals of compounds **3b** (purple) and free **ligand** (orange) were manually selected from the crystallization vial. A suitable single-crystal was mounted on a glass fiber with the help of silicon grease. Data were collected at 150(2) K on a Bruker X8 Kappa APEX II charge-coupled device (CCD) area-detector diffractometer (Mo K_a graphite-monochromated radiation, λ = 0.71073 Å) controlled by the APEX2 software package,[1] and equipped with an Oxford Cryosystems Series 700 cryostream monitored remotely using the software interface Cryopad.[2] Images were processed using the software package SAINT+,[3] and data were corrected for absorption by the multi-scan semi-empirical method implemented in SADABS.[4] The structure was solved using the direct methods algorithm implemented in SHELXS-97,[5,6] which allowed the immediate location of the majority of the atoms. All remaining non-hydrogen atoms were located from difference Fourier maps calculated from successive full-matrix least squares refinement cycles on F^2 using SHELXL-97.[5,7] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters.

Hydrogen atoms bound to carbon were located at their idealized positions using appropriate *HFIX* instructions in SHELXL (*43* for the aromatic and vinylic, *23* for the $-CH_2-$ moieties and *13* for the chiral tertiary carbon atoms) and included in subsequent refinement cycles in riding-motion approximation with isotropic thermal displacements parameters (U_{iso}) fixed at 1.2 times U_{eq} of the atom to which they are attached.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 2161374-2161375. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 2EZ, U.K. Fax: (+44) 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

(1) APEX2, *Data Collection Software Version 2.1-RC13, Bruker AXS, Delft, The Netherlands* **2006**.
(2) Cryopad, *Remote monitoring and control, Version 1.451, Oxford Cryosystems, Oxford, United Kingdom* **2006**.
(3) SAINT$^+$, *Data Integration Engine v. 7.23a* © 1997-2005, *Bruker AXS, Madison, Wisconsin, USA*.
(4) G. M. Sheldrick, *SADABS v.2.01, Bruker/Siemens Area Detector Absorption Correction Program* **1998**, *Bruker AXS, Madison, Wisconsin, USA*.
(5) G. M. Sheldrick, *Acta Cryst. A*, **2008**, *64*, 112-122.
(6) G. M. Sheldrick, *SHELXS-97, Program for Crystal Structure Solution, University of Göttingen* **1997**.
(7) G. M. Sheldrick, *SHELXL-97, Program for Crystal Structure Refinement, University of Göttingen* **1997**.

Absorption and emission spectra

The absorption, excitation and emission spectra of compounds **2a**, **3a-f**, **4** and curcumin were recorded at different concentrations (between $1x10^{-7}$ M and $3x10^{-5}$ M) in tetrahydrofuran, ensuring that the Beer-Lambert law is followed and that the emission is a non-saturated curve. The standard used for quantum yield calculation of compounds **2a**, **3a-b**, **3d**, and **4** (in THF) was compound **4** which presents a quantum yield of 0.62 in dichloromethane,[3] and for compounds **3c**, **3e** and **3f** (in THF) was compound **3c** in DCM which presents a quantum yield of 0.47.[4]

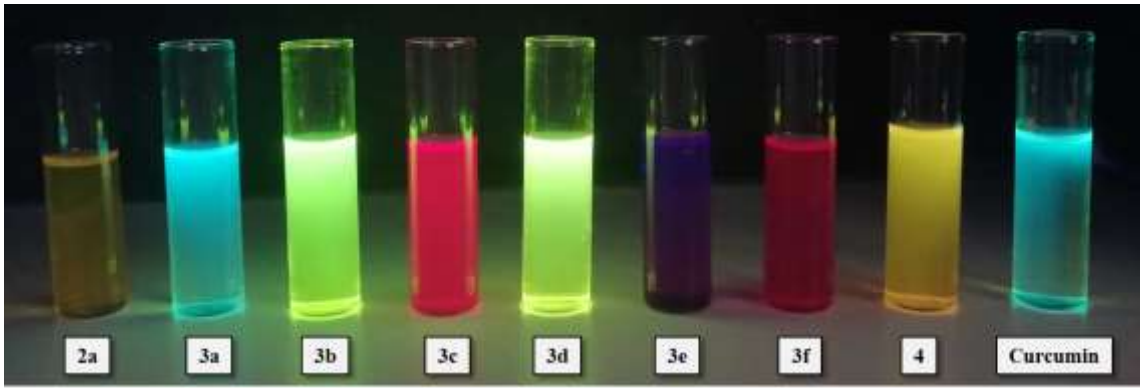

Figure S49. Solutions of the different compounds in THF under a UV light (365nm).

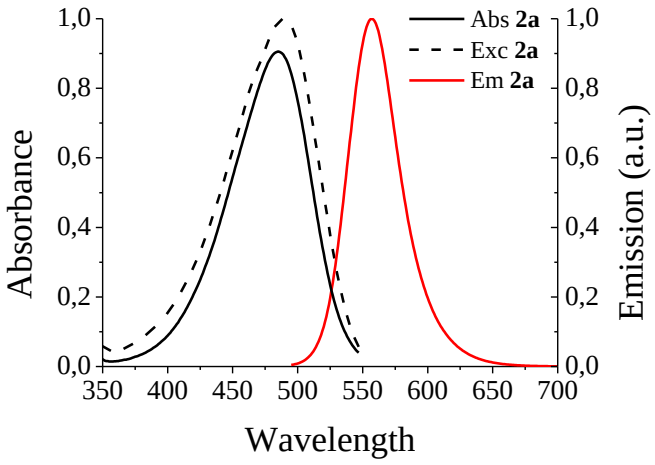

Figure S50. Absorption, excitation and emission spectra of compound **2a** in THF.

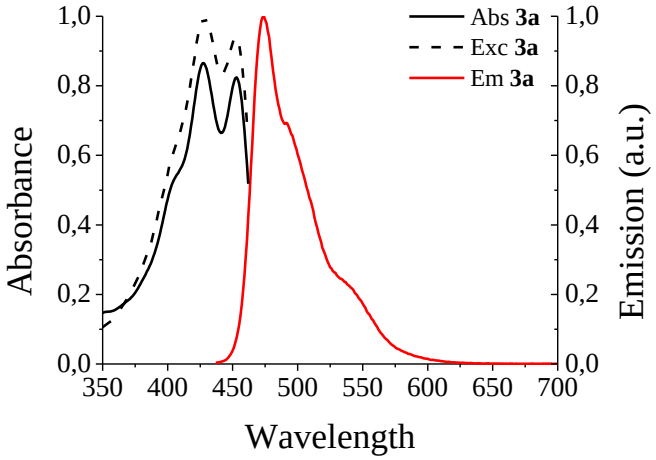

Figure S51. Absorption, excitation and emission spectra of compound **3a** in THF.

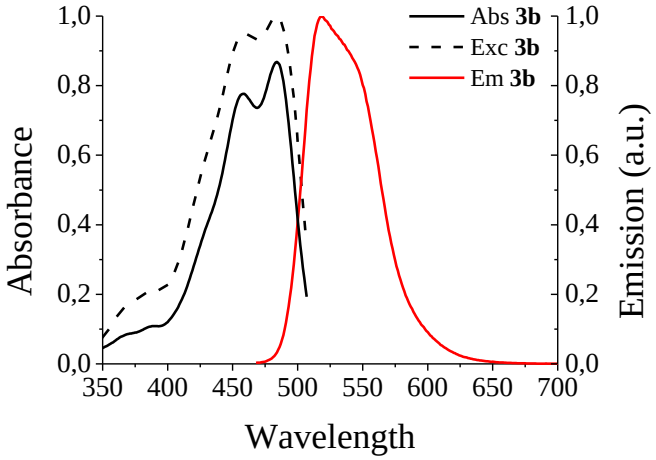

Figure S52. Absorption, excitation and emission spectra of compound **3b** in THF.

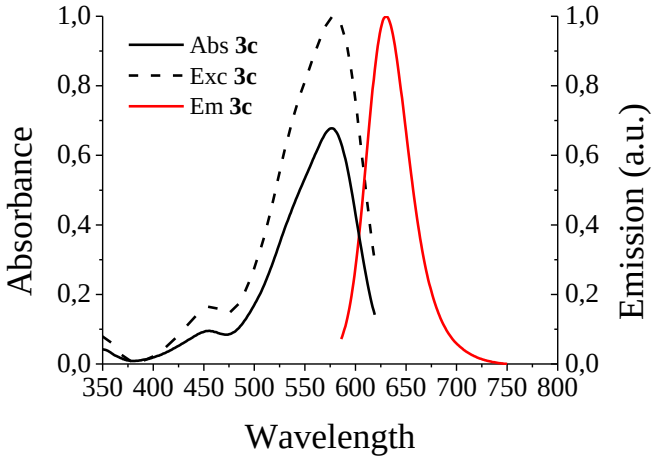

Figure S53. Absorption, excitation and emission spectra of compound **3c** in THF.

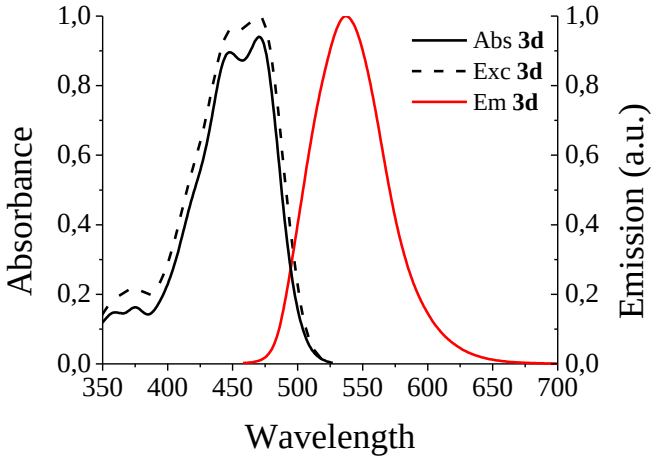

Figure S54. Absorption, excitation and emission spectra of compound **3d** in THF.

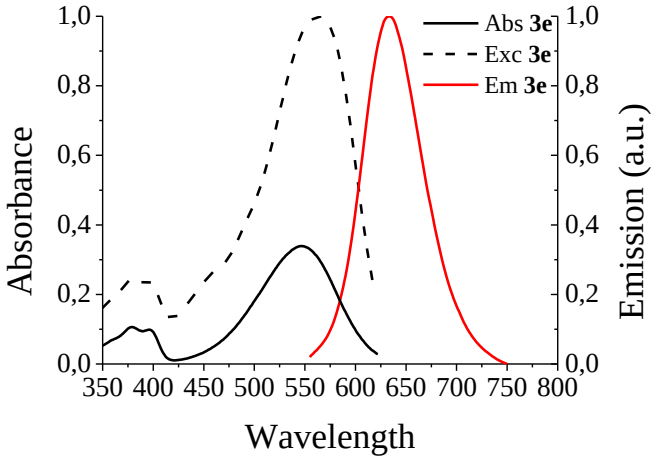

Figure S55. Absorption, excitation and emission spectra of compound **3e** in THF.

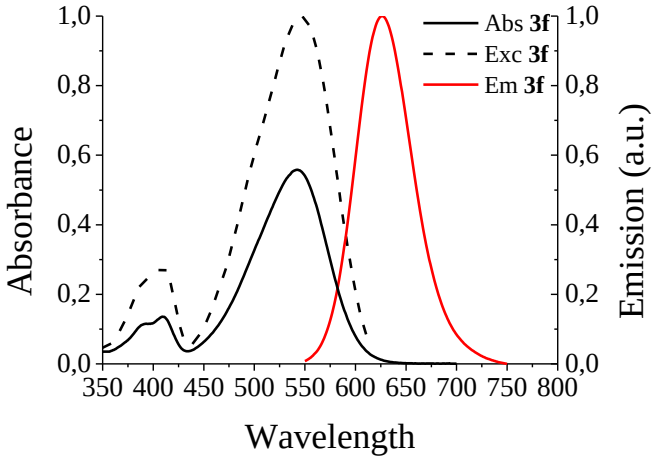

Figure S56. Absorption, excitation and emission spectra of compound **3f** in THF.

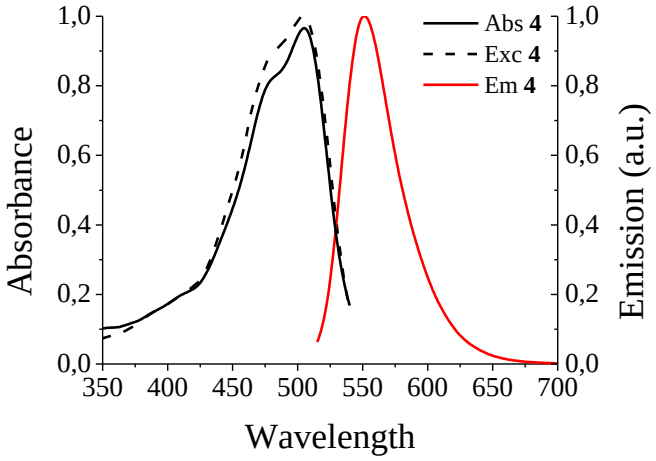

Figure S57. Absorption, excitation and emission spectra of compound **4** in THF.

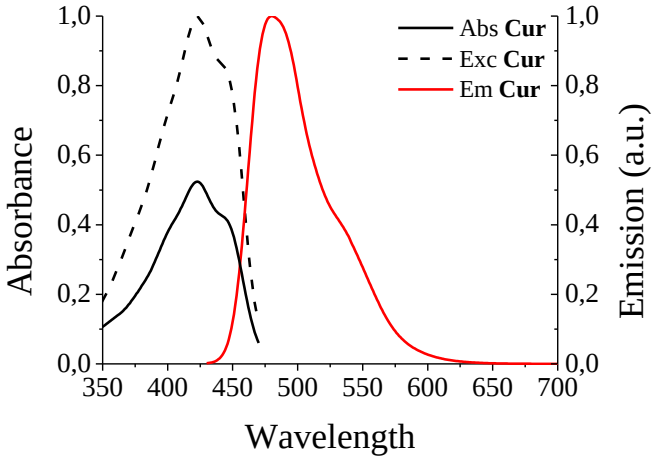

Figure S58. Absorption, excitation and emission spectra of curcumin in THF.

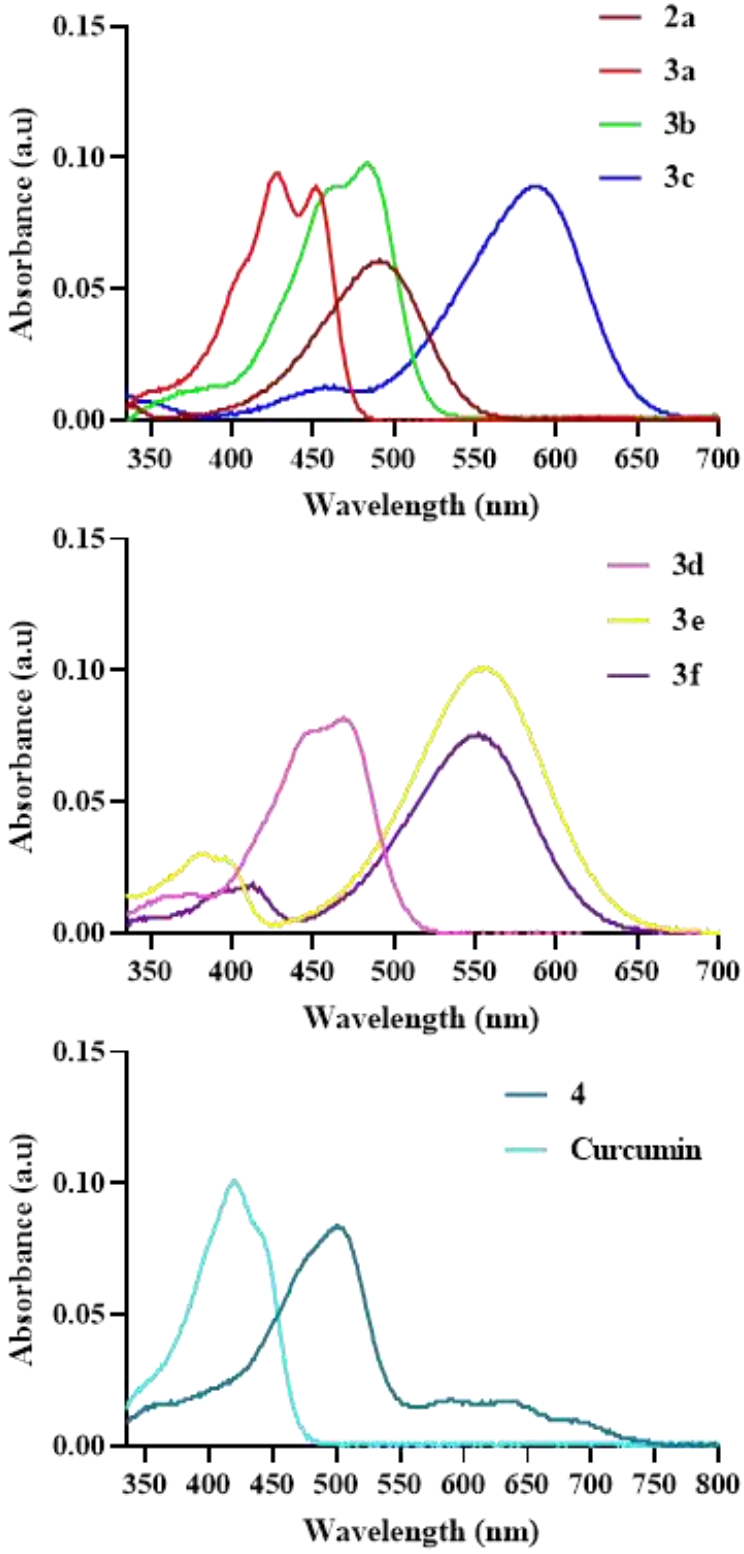

Figure S59. Absorption spectra of the dyes in acetone.

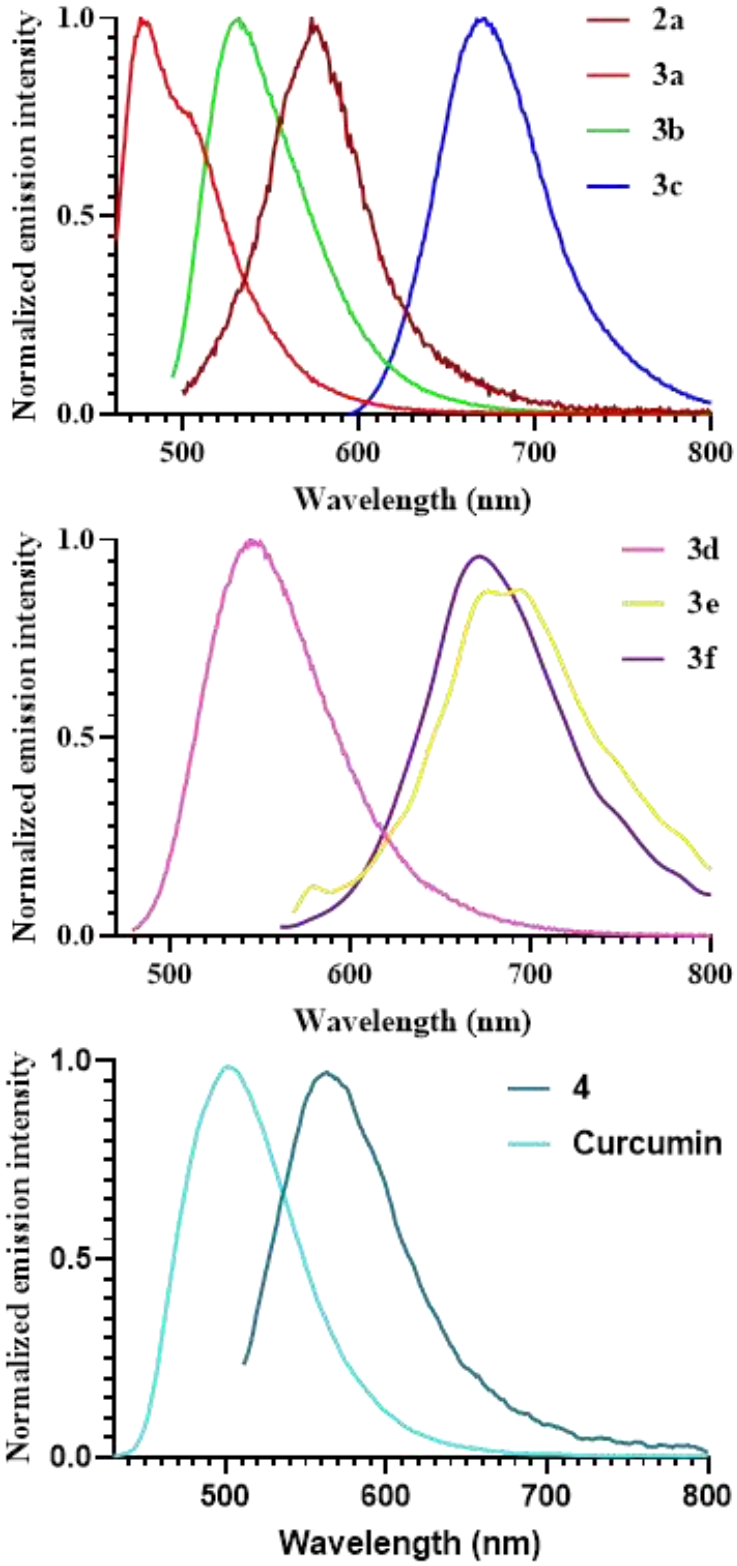

Figure S60. Emission spectra of the dyes in acetone.

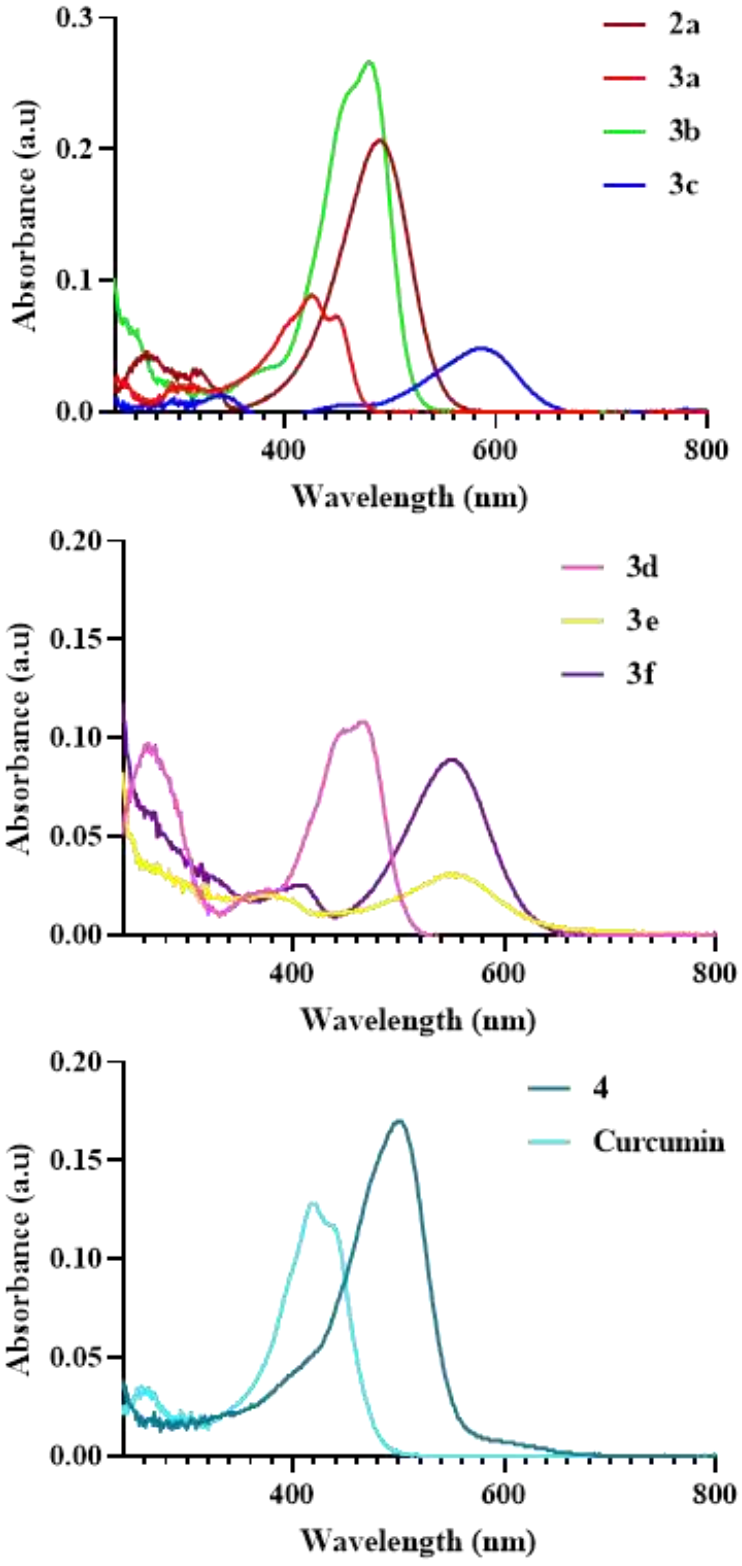

Figure S61. Absorption spectra of the dyes in methanol.

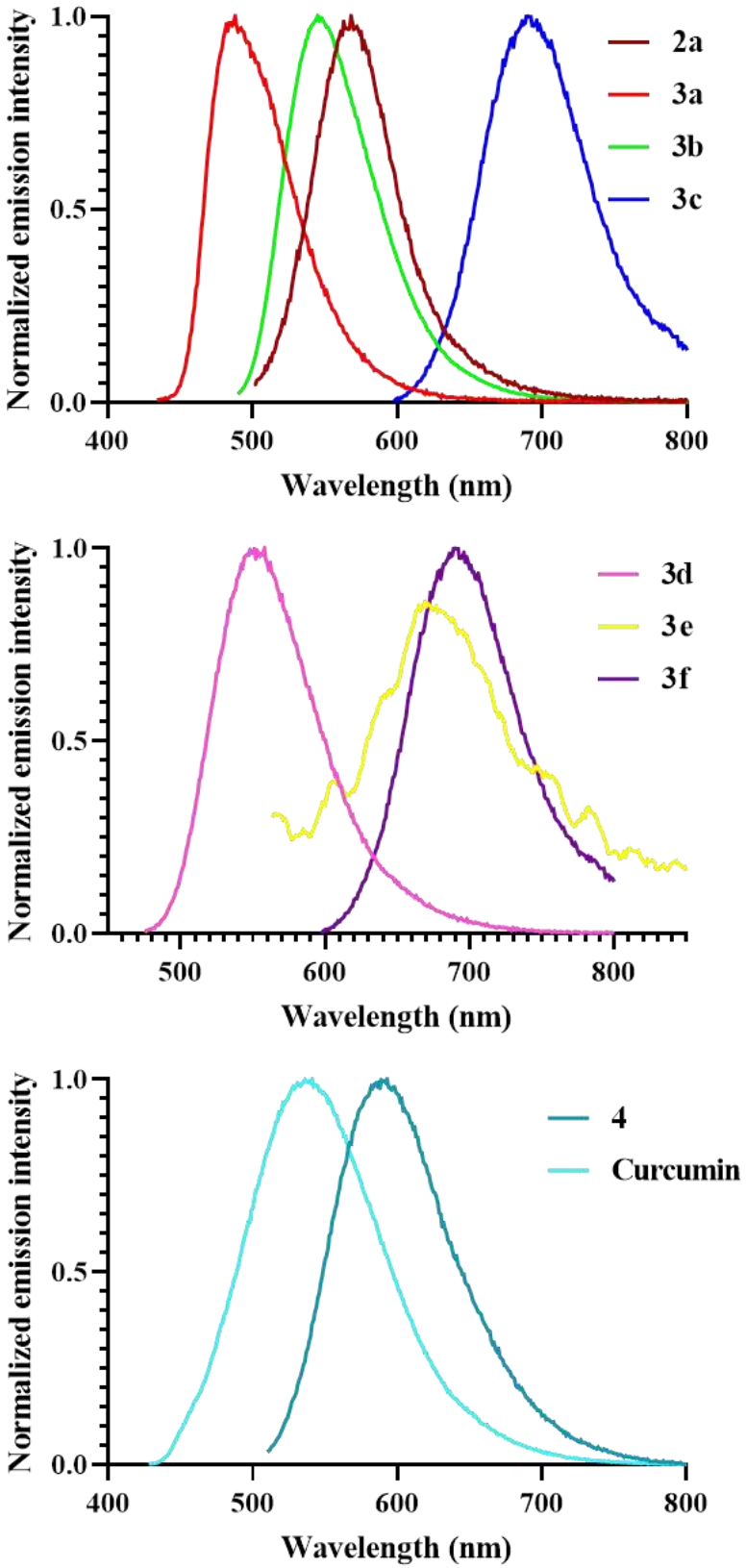

Figure S62. Emission spectra of the dyes in methanol.

Biological evaluation

Fusarium oxysporum cytotoxicity

Cytotoxicity assay was performed using a double layer medium technique, where the fluorophores (**2a**, **3a-f**, **4**, and **Curcumin**) dissolved in acetone, were incorporated in Potato Dextrose Agar (PDA) soft medium (5 g/L of agar) ensuring a fluorophore final concentration of 100 μM. PDA soft medium was subsequently poured in a thin layer onto a PDA solid medium (15 g/L of agar). In the culture plate, two perpendicular lines were drawn and a plug of *F. oxysporum* mycelium was inoculated in the intersection of these two lines (mycelium plug of 6 mm). Two types of controls were performed: a control where no acetone was incorporated in the PDA soft medium and a control where the same percentage of acetone was incorporated in PDA soft medium to understand the effect of acetone in the cell growth. All the cultures were incubated for 7 days, at 25 ºC, in the dark, and during the incubation, the mean diameter of mycelial growth was daily measured. Three replicates for three biological replicates were made for each fluorophore and controls.

Statistical Analysis

Significant differences in mycelial growth between different experimental conditions were assessed by One-Way ANOVA with the software Graphpad Prism 8.4.2. with a 95% significance threshold. Normality of variances was checked by the Shapiro-Wilk test.

Fluorescence microscopy of *Fusarium oxysporum* hyphae

The fungi were grown on PDA. A solution of the pretended dye (50 μL of fluorophore in acetone and 50 μL of PBS, for a total of 100 μL of solution at a total concentration of 100 μM) was added in the well, and the fungal hyphae were incubated in the dark for 6 hours at 25ºC. The fluorophore solution was then removed carefully (to try not to remove spores and hyphae that possibly could be in solution) with a pipette. The fungi was scratched from the medium with an inoculation loop and transferred to 1mL of distilled water. This aqueous solution was frozen at -80°C and hyphal material was lyophilized (Telstar e o LYOQUEST- 80% Plus Eco) overnight. For observation, fresh mounts of lyophilized material were prepared: the lyophilized fungus was rehydrated in one drop of distilled water on the slide and a coverslip was placed on top. The margins of the coverslip were sealed with transparent polish.
Hyphae were visualized by confocal microscopy [Zeiss LSM 880 with Airyscan confocal microscope (Zeiss, Germany); 100x/0.3 objective]. To determine intracellular fluorophore incorporation, hyphae were observed using common excitation wavelengths similar to the absorbance wavelengths determined for each fluorophore in tetrahydrofuran (405 nm for **3a** and **Curcumin**; 488 nm for **2a**, **3b**, **3d**; 514 nm for **4**; 561 nm for **3c**, **3e**, **3f**), and the emission was recorded in an interval corresponding to the emission maximum of each fluorophore (503-654 nm for **2a**; 443-592 nm for **3a**; 497-654 nm for **3b**; 571-731 nm for **3c**; 503-654 nm for **3d**; 582-735 nm for **3e**; 582-735 nm for **3f**; 518-678 nm for **4**; 438-600 nm for **Curcumin**). Control hyphae, without

fluorophore, were observed using the same excitation wavelength to account for normal cellular autofluorescence.

The results of the fluorescent microscopy images were analysed using FIJI.[5]

Bibliography

[1] A. G. Mirochnik, B. V. Bukvetskii, E. V. Gukhman, P. A. Zhikhareva, V. E. Karasev, *Russ. J. Gen. Chem.* **72**, 737–740 (2002).
[2] K. Liu, J. Chen, J. Chojnacki, S. Zhang, *Tet. Lett.* **54**, 2070–2073 (2013).
[3] A. Chaicham, S. Kulchat, G. Tumcharern, T. Tuntulani, B. Tomapatanaget, *Tetrahedron* **66**, 6217–6223 (2010).
[4] G. Bai, C. Yu, C. Cheng, E. Hao, Y. Wei, X. Mu, L. Jiao, *Org. Biomol. Chem.* **12**, 1618–1626 (2014).
[5] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nature Methods* **9**, 676–682 (2012).