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# Supporting Information

## *meso*-Substituted BODIPY Fluorescent Probes for Cellular Bio-imaging and Anticancer Activity

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**Instrumentation and Materials**

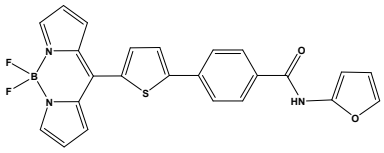
$^{1}H$ NMR (300 MHz) and $^{13}C$ NMR (500 MHz) spectra were recorded on a Bruker-Avance spectrometer. All the NMR spectra were recorded in $CDCl_3$ with TMS as internal reference. Hitachi UV-2910-spectrophotometer was used for UV studies. Florescence studies, quantum yield measurements were done with Agilent Technologies Cary Eclipse Fluorescence spectrophotometer. Mass spectra were recorded on Micromass Quarttro micro API Mass spectrometer. Unless otherwise noted, materials were used as obtained without further purification.

**General Procedure for Suzuki cross-coupling:**

**Bodipy (1a):**

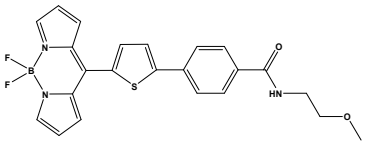
A degassed solution of THF: $H_2O$ (9:1) was added to mixture of **1** (40mg, 0.1133mmol), 4-(2-methoxyethylaminocarbonyl) benzene boronicacid pinacolester (41.5 mg 0.136 mmol), $K_3PO_4$ (0.34mmol, 72.2mg) and $Pd_2(dba)_3$ (0.0068mmol, 6.2mg), $P(t\text{-}Bu)_3.HBF_4$ (0.0136 mmol, 3.95mg) under an inert atmosphere. The reaction mixture was heated to $70^0$C. Monitoring the reaction by TLC indicated the completion of reaction in 90min. Purification by $SiO_2$-gel Column chromatography afforded red solid: 28.7 mg, yield 56%.The same protocol has been employed for all the analogues from **1a** to **1h**.

**Bodipy (1a):**



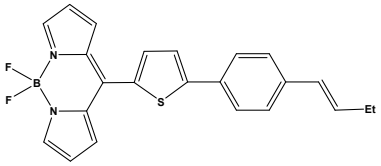

$^{1}H$ NMR (300 MHz, $CDCl_3$):δ 7.946 (s,2H), δ 7.88(d,2H,J= 8.309), δ 7.757(d,2H,J=8.309), δ 7.59(d,1H, J=3.77), δ 7.548 (d,1H,J=3.77), δ 7.364 (d,2H,J=4.532), δ 6.612 (d,2H,J=3.77), δ 3.69 (m,2H), δ 3.59 (m,2H), δ 3.42 (s,3H). $^{13}C$ NMR (500MHz, $CDCl_3$): δ 166.65, δ 149.44, δ 143.85, δ 138.88, δ 135.733, δ 134.56, δ 134.34, δ 134.04, δ 131.21, δ 128.89, δ 127.99, δ 127.05, δ 125.22, δ 118.575, δ 115.37, δ 71.087, δ 58.854, δ 39.82. MS-ESI: 452

**Bodipy (1b):**



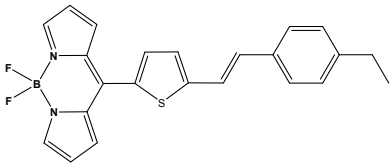
$^{13}$C NMR (500MHz, $CDCl_3$): δ 166.344, δ 150.854, δ 149.266, δ 143.878, δ 142.405, δ 138.823, δ 135.921, δ 134.628, δ 134.281, δ 134.043, δ 131.191, δ 128.96, δ 128.013, δ 126.063, δ 125.247, δ 118.568, δ 115.397, δ 110.566, δ 107.923. Yield:61% MS-ESI: 460

**Bodipy (1c):**



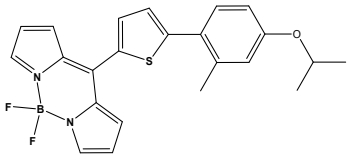
$^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.916 (s,2H), δ 7.523 (d,1H,J=3.77), δ 7.458 (d,2H,J=7.931), δ 7.365 (d,2H,J=3.21), δ 7.24 (m,5H, including with merged $CDCl_3$ peak), δ 7.127 (s,1H,J=16.05), δ 6.59 (m,2H), δ 2.69 (q,2H,J=7.554), δ 1.26 (t,3H,J=7.5)**.** $^{13}$C NMR (500MHz, $CDCl_3$): δ 150.143, δ 145.20, δ 143.18, δ 139.344, δ 134.486, δ 133.919, δ 133.490, δ 132.755, δ 131.912, δ 128.428, δ 126.799, δ 119.607, δ 118.262, δ 29.678, δ 28.711, δ 15.425. Yield:63% MS-ESI: 405

**Bodipy (1d):**



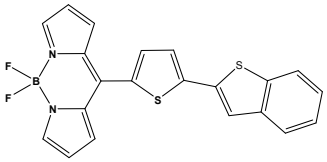
$^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.917 (s,2H), δ 7.520 (d,1H,J=3.509), δ 7.453(d,2H,J=7.782), δ 7.365 (d,2H,J=3.052), δ 7.237 (m,5H, including with merged $CDCl_3$ peak), δ 7.121 (d,1H,J=16.021), δ 6.593 (m,2H), δ 2.686 (q,2H,J=7.629), δ 1.261 (t,3H,J=7.78)**.** $^{13}$C NMR (500MHz, $CDCl_3$): δ 150.157, δ 145.222, δ 143.210, δ 139.361, δ 134.477, δ 133.967, δ 133.530, δ 132.786, δ 131.948, δ 130.986, δ 128.434, δ 126,816, δ 119.63, δ 118.28, δ 118.25, δ 28.72, δ 15.403**.** Yield:69%

**Bodipy (1e):**



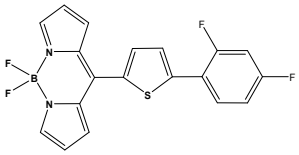
$^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.922 (s,2H), δ 7.582 (d,1H,J=3.777), δ 7.390 (m,3H), δ 7.198 (d,1H,J=3.777), δ 6.852 (d,1H,J=2.266), δ 6.811 (m,1H), δ 6.599 (m,2H), δ 4.613 (m,1H), δ 2.490 (s,3H), δ 1.385 (d,6H,J=6.043)**.** $^{13}$C NMR (500MHz, $CDCl_3$): δ 158.37, δ 150.99, δ 143.17, δ 137.57, δ 133.95, δ 133.76, δ 133.534, δ 124.792, δ 131.541, δ 131.236, δ 127.272, δ 118.35, δ 118.255, δ 113.164, δ 69.85, δ 22.013, δ 21.548. Yield 62% MS-ESI: 423

**Bodipy (1f):**



$^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.94 (s,2H), δ 7.815 (m,2H), δ 7.55 (m,2H), δ 7.4 (m,5H), δ 6.61 (s,2H),**.** $^{13}$C NMR (500MHz, $CDCl_3$): δ 143.91, δ 143.76, δ 140.14, δ 139.6, δ 138.7, δ 134.16, δ 134.04, δ 131.08, δ 130.85, δ 125.88, δ 125.44, δ 25.09, δ 123.97, δ 122.27, δ 121.14, δ 118.51. Yield 51%

**Bodipy (1g)**:



$^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.941 (s, 2H), δ 7.687 (m, 1H), δ 7.590 (d,1H,J=3.815), δ 7.566 (d,1H,J=3.815), δ 7.354 (d,2H,J=4.272), δ 7.001 (m,2H), δ 6.602 (m,2H)**.** $^{13}$C NMR (500MHz, $CDCl_3$): δ 163.89 δ 161.89, δ 160.31, δ 158.38, δ 143.8 δ 143.66, δ 138.94, δ 134.41, δ 134.08, δ 133.7, δ 131.27, δ 129.7, δ 127, δ 112.42, δ 112.3, δ 112.2**.** Yield 91% MS-ESI: 386

**Biological Studies:** The mouse melanoma cells (B16 F10), human ovarian carcinoma cells (SKOV3) and human breast cancer cells (MCF-7) were purchased from American Type Culture Collection (Manassas, VA). ECV 304 cells were a kind donation from Dr. V. Shah, Gastroenterology and Hepatology Department, Mayo Clinic, Rochester, MN, USA. HUVECs and different components of EBM complete media were obtained from Lonza. Dulbecco's

modified eagle medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), Hank's balanced salt solution (HBSS), fetal bovine serum (FBS), penicillin/streptomycin, propidium iodide, Hoechst 33258, 2′,7′-dichlorofluorescein diacetate (DCFDA), BCIP/NBT premixed and MTT reagent were purchased from Sigma-Aldrich, USA. Anti-Caspase 3 and Anti-GAPDH were purchased from Thermo Scientific Pierce and Santa Cruz Biotechnology respectively.

**Sample preparation for cell culture:** The stock solutions of compounds **1** and **1a-1g** were prepared in sterile DMSO with a concentration of 10 mM. The freshly prepared stock solutions of each sample were used for cell culture experiments.

**Cell culture experimentation:** B16 F10, SKOV3, MCF-7 and ECV-304 cells were cultured in DMEM complete media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, while HUVECs were maintained in EBM complete media containing 5% FBS at 37 °C humidified incubator with 5% $CO_2$. After 70% confluency, the cells were seeded into 96 well plate ($10^4$ cells/well) and 24 well plate ($3x10^4$ cells/well) for cytotoxicity assays and fluorescence microscopy studies respectively.

**Fluorescence microscopy:** B16 F10 cells were seeded in a 24 well cell culture plate at $3x10^4$ cells/mL for 24 h at 37 °C humidified incubator with 5% $CO_2$ in complete DMEM media. Next day, the cells were treated with **1** and **1a-1g** at different concentration (5-20 μM) for 24 h. In another set of experiment, the cells were also incubated with selective compounds **1**, **1c**, **1d**, **1e**, **1f** and **1g** at different concentration (5-20 μM) for 6 h. After desired incubation time period, the cells were washed carefully for several times to remove those compounds from the cell surface. The cells were then incubated with 2.5 μg/mL Hoechst 33258 for 30 min for nuclear staining and washed again with DPBS for several times. The fluorescence images of B16 F10 cells treated with different compounds in HBSS were observed by using a fluorescence microscope Nikon Eclipse: TE 2000-E Japan[1] with λex = 518 nm and λem = 605 nm (red fluorescence).

**MTT assay:** The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay is a well-known test to measure the activity of enzymes which can reduce MTT to purple coloured formazan dyes.[2-5] In brief, $10^4$ of B16 F10, SKOV3, MCF-7, ECV-304 and HUVEC cells were seeded in each well of a 96-well tissue culture plate with 100 μL of complete media at 37 °C humidified incubator with 5% $CO_2$ for 24 h. After that, the media was replaced with 100 μL of fresh media in each well and the cells were incubated with different compounds **1** and **1a-1g** at different concentrations (0.1-20 μM) for another 24 h for non-cancerous cell line (ECV 304 and

HUVEC) and 48 h for cancerous cell line (B16 F10, SKOV3 and MCF-7). Then, 1 mL of MTT stock solution (5 mg/mL) was diluted to 10 mL using media and 100 µL of that MTT solution was added to each well of 96 well plate by just replacing the old media and the plate was incubated for about 4 h at 37 °C. After that, the media in each well was replaced by 100 µL 1:1 DMSO-Methanol mixture (v/v) such that the purple formazan product can be solubilised. Then, the plate was shaken well on a shaker for homogeneous mixture of the solution. Finally, the absorbance of the solution in each well of the 96 well plate was measured by using a multimode reader (Biotek Synergy) at 570 nm.

**Cell cycle assay:** Cell cycle assay was performed according to our published literature.[3, 6] In brief, B16 F10 cells ($2x10^6$ cells/mL) were seeded in 60 mm dishes for 24 h. Next day the cells were incubated with compounds **1c** and **1d** for 24 h. After that the cells were carefully washed in DPBS, fixed in 70% ethanol and kept at -20°C for 48 h. Then, the cells were resuspended in ethanol, washed with cold DPBS and stained with propidium iodide solution containing RnaseA and TritonX. Quantification for the DNA content in untreated control cells and cells treated with **1c** and **1d** was carried out by using a FACScan flow cytometer (BD Bioscience).

**Determination of intracellular ROS ($H_2O_2$):** B16 F10 cells ($2\times10^4$ cells/mL) were seeded in 24 well plate for 24 h and next day the cells were incubated with **1c** (20 µM) and **1d** (20 µM) for another 24 h. After that, the cells were thoroughly washed with DPBS and incubated with 30 µM 2′,7′-dichlorofluorescein diacetate (DCFDA) for 30 min. The cells were again washed with DPBS for several times and incubated with 2.5 µg/mL Hoechst 33258 for another 30 min. The cells were then thoroughly washed with DPBS before acquiring the fluorescence images. The green fluorescence ($\lambda_{Em}$ = 525 nm), suggesting the presence of ROS ($H_2O_2$), was observed by using a fluorescence microscope (Nikon Eclipse: TE 2000-E Japan) with a 20x microscopic objective after excitation at $\lambda_{Ex}$ = 488 nm.

**Western blot analysis:** B16 F10 cells ($2 \times 10^5$ cells/2 mL) were seeded in 60 mm dishes for 24 h and the cells were then incubated with **1c** (20 µM) and **1d** (20 µM) for 48 h. The cells were lysed and all the proteins were extracted using RIPA (Radio immune precipitation assay) buffer with 1% protease inhibitor cocktail. The cell lysate was then centrifuged at $10^4$ rpm for 10 min at 4ºC and the supernatant was collected for western blot analysis. Bradford assay was employed to estimate the protein concentration in cell lysate and 50 µg protein samples were loaded in 15% sodium dodecyl sulfate poly acrylamide gel. The proteins were blotted on PVDF membrane after

electrophoretic separation. The membranes were then blocked in 5% BSA for 4 h and washed thrice in TBST (10 mM Tris, pH 7.5, 150mM NaCl, 0.05% Tween-20) solution. After that the membranes were incubated with Caspase 3 and GAPDH primary antibodies in TBST for 2 h at room temperature. Then membranes were further washed thrice with TBST and incubated in goat anti-rabbit IgG-ALP conjugate secondary antibody in TBST for 1 h at room temperature. BCIP/NBT solution was used to develop the blot under dark condition.

**References:**


1. N. R. Chereddy, S. Krishnan, A. K. Barui, C. R. Patra, J. R. Vaidya and S. Thennarasu, *RSC Advance,* 2014, **4**, 24324–24327.
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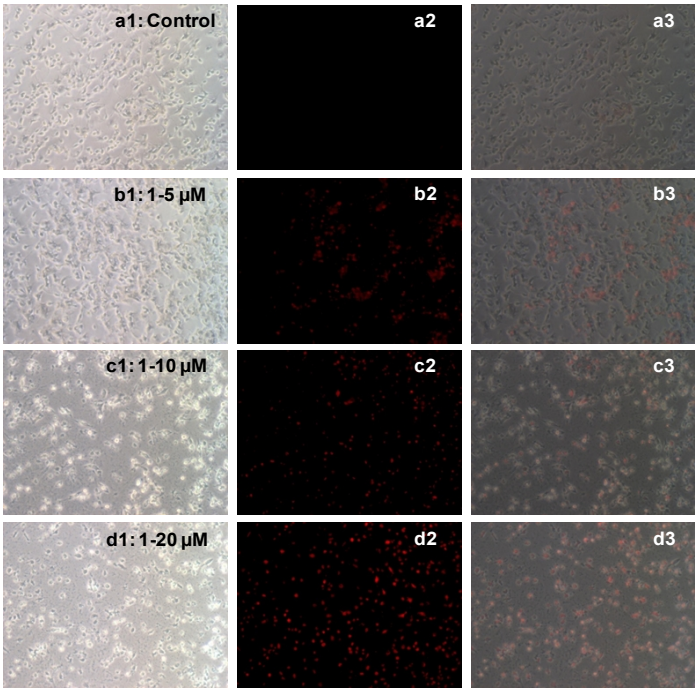
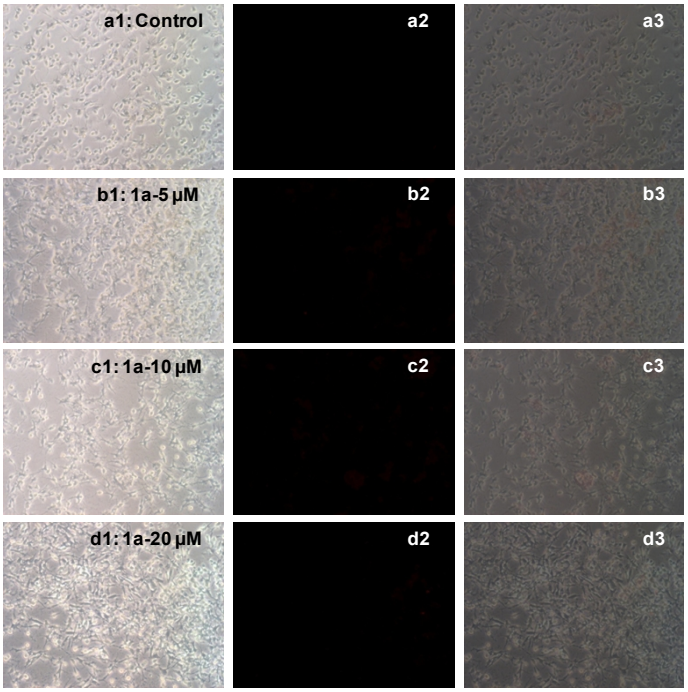
# Fluorescence Microscopic Images

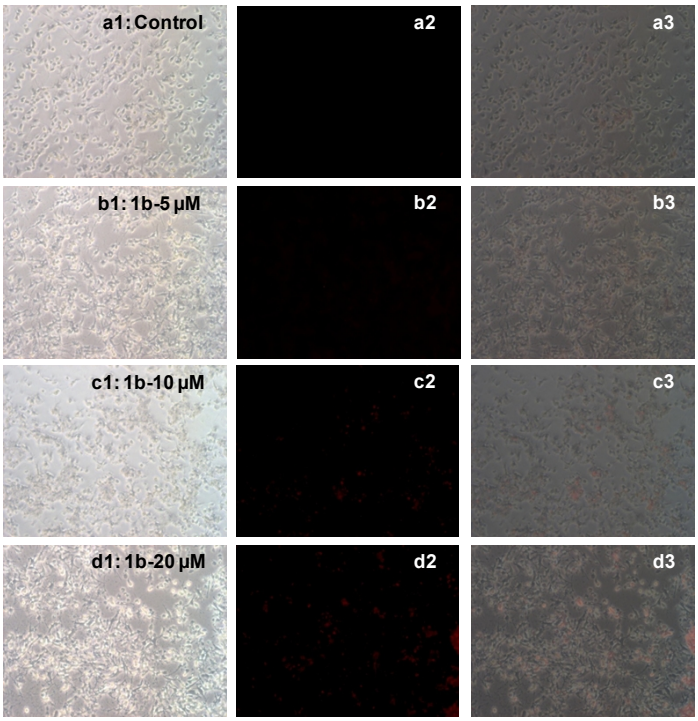
SI-Fig.1




SI-Fig.2

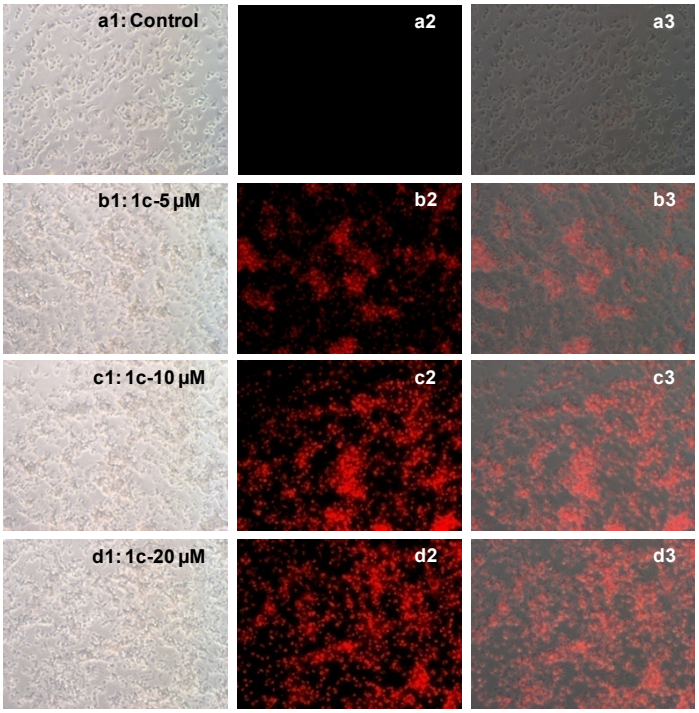


SI-Fig.3



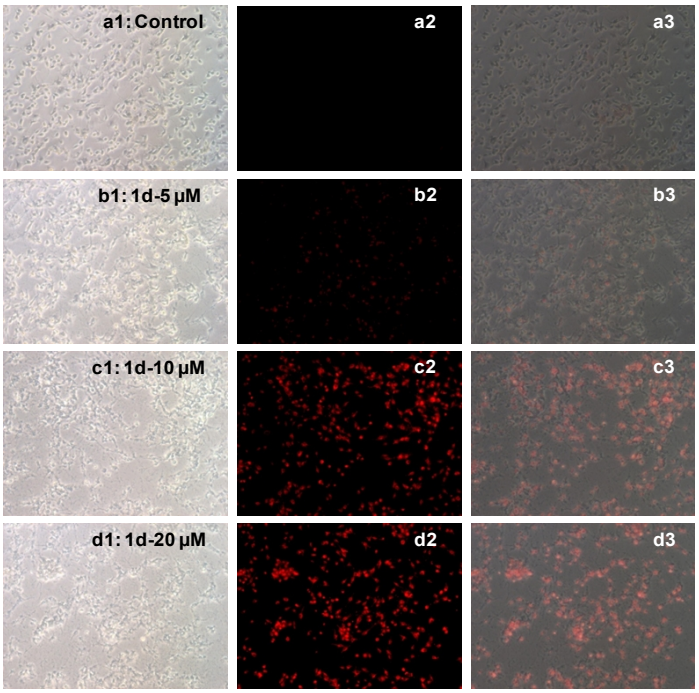

**SI-Fig.3:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): untreated control cells; Row 2 (**b1-b3**): cells treated with 5 µM **1b**; Row 3 (**c1-c3**): Cells treated with 10 µM **1b** and Row 4 (**d1-d3**): cells treated with 20 µM **1b**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1b** in B16 F10 cells is 24 h.

SI-Fig.4

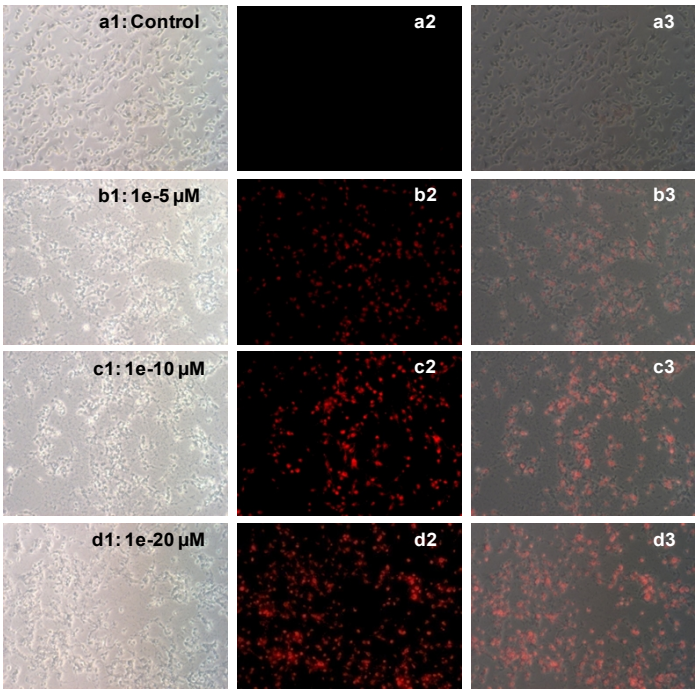



**SI-Fig.4:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): untreated control cells; Row 2 (**b1-b3**): cells treated with 5 µM **1c**; Row 3 (**c1-c3**): Cells treated with 10 µM **1c** and Row 4 (**d1-d3**): cells treated with 20 µM **1c**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1c** in B16 F10 cells is 24 h.

SI-Fig.5

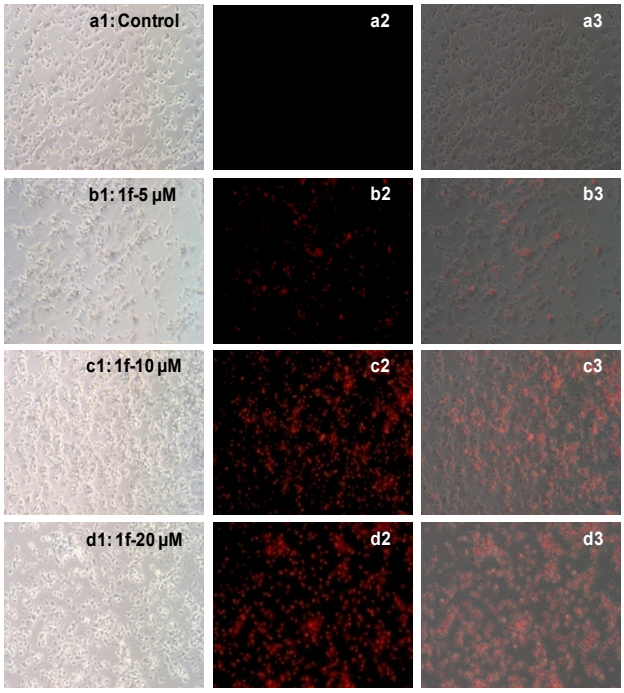



SI-Fig.6



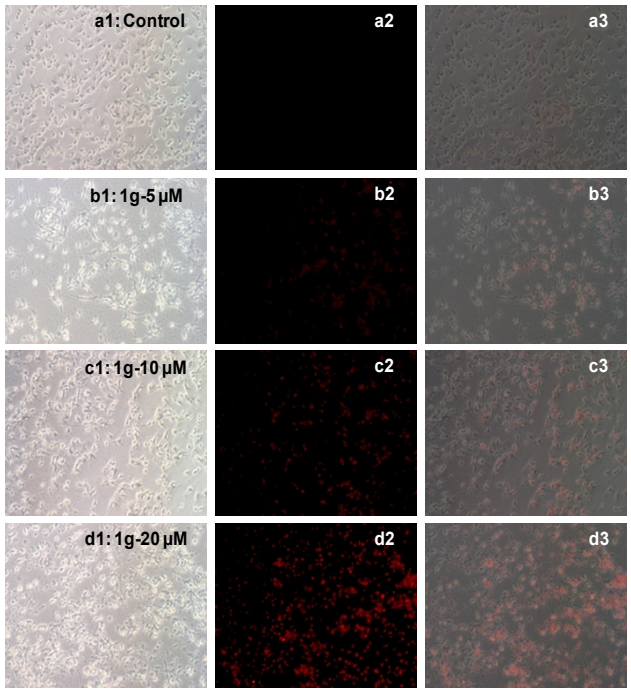

**SI-Fig.6:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): untreated control cells; Row 2 (**b1-b3**): cells treated with 5 µM **1e**; Row 3 (**c1-c3**): Cells treated with 10 µM **1e** and Row 4 (**d1-d3**): cells treated with 20 µM **1e**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1e** in B16 F10 cells is 24 h.

SI-Fig.7



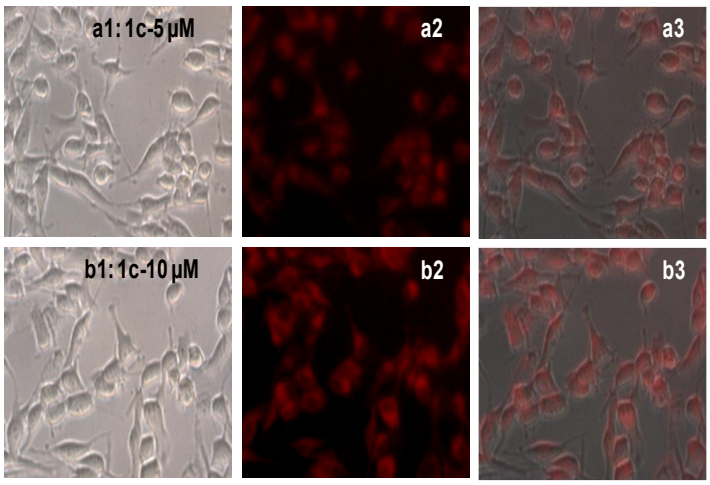

**SI-Fig.7:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): untreated control cells; Row 2 (**b1-b3**): cells treated with 5 µM **1f**; Row 3 (**c1-c3**): Cells treated with 10 µM **1f** and Row 4 (**d1-d3**): cells treated with 20 µM **1f**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1f** in B16 F10 cells is 24 h.

SI-Fig.8



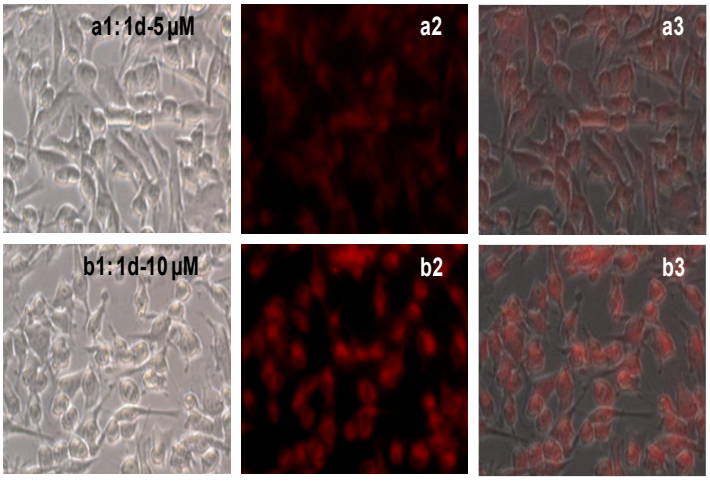

**SI-Fig.8:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): untreated control cells; Row 2 (**b1-b3**): cells treated with 5 µM **1g**; Row 3 (**c1-c3**): Cells treated with 10 µM **1g** and Row 4 (**d1-d3**): cells treated with 20 µM **1g**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1g** in B16 F10 cells is 24 h

SI-Fig.9



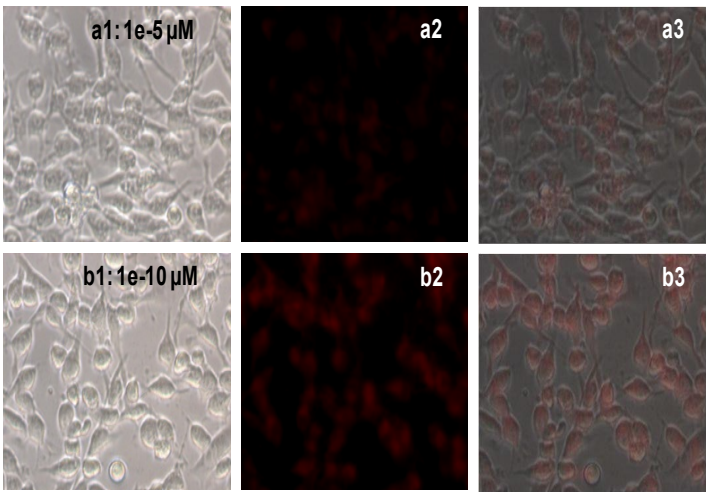

**SI-Fig.9:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): cells treated with 5 µM **1c**; Row 2 (**b1-b3**): cells treated with 10 µM **1c**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1c** in B16 F10 cells is 6 h.

SI-Fig.10



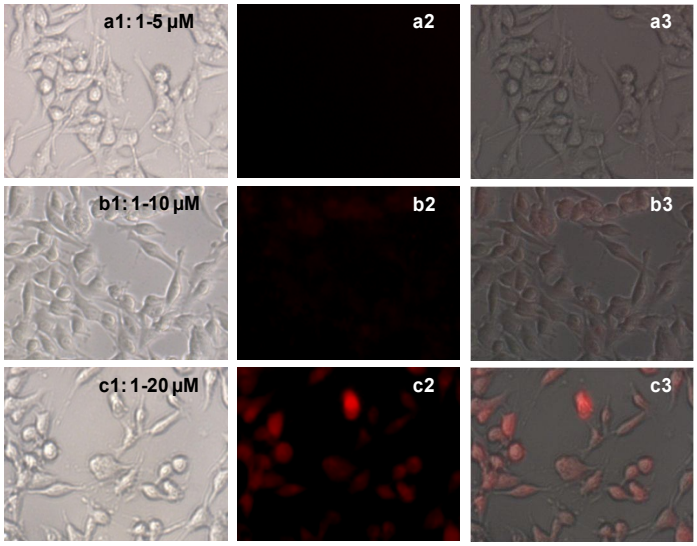

**SI-Fig.10:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): cells treated with 5 µM **1d**; Row 2 (**b1-b3**): cells treated with 10 µM **1d**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1d** in B16 F10 cells is 6 h.

SI-Fig.11



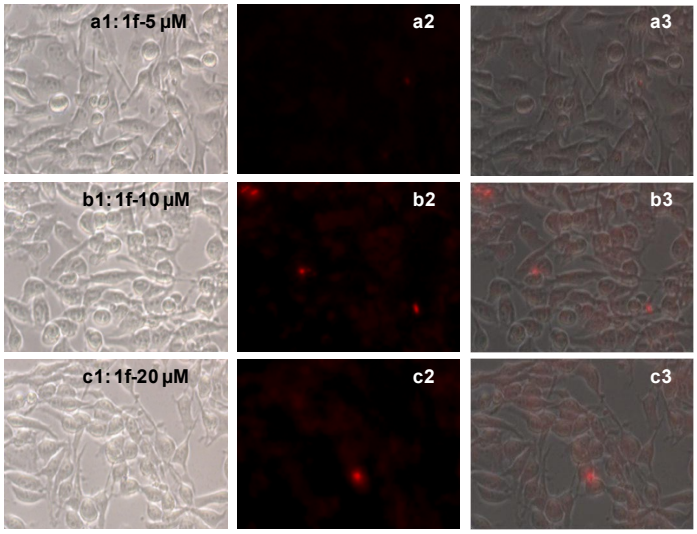

**SI-Fig.11:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): cells treated with 5 µM **1e**; Row 2 (**b1-b3**): cells treated with 10 µM **1e**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1e** in B16 F10 cells is 6 h.

SI-Fig.12

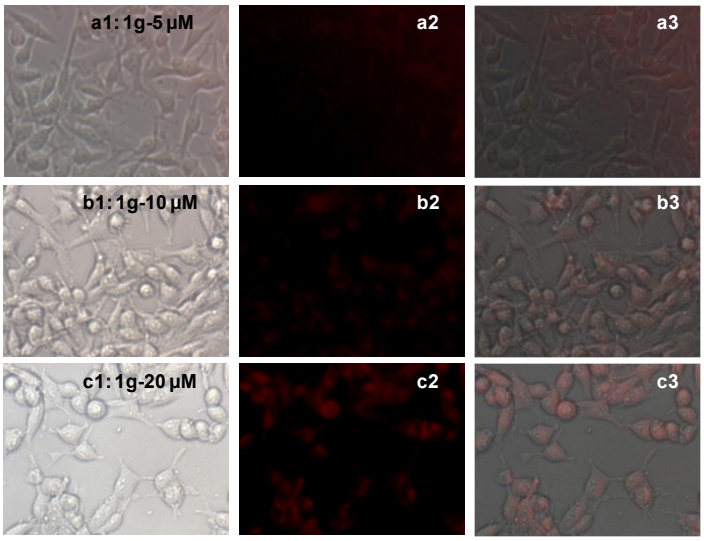



**SI-Fig.12:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): cells treated with 5 µM **1**; Row 2 (**b1-b3**): cells treated with 10 µM **1**; Row 3 (**c1-c3**): cells treated with 20 µM **1**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1** in B16 F10 cells is 6 h.

SI-Fig.13




SI-Fig.14




**SI-Fig.14:** Fluorescence microscopic images of B16 F10 cells. Row 1 (**a1-a3**): cells treated with 5 µM **1g**; Row 2 (**b1-b3**): cells treated with 10 µM **1g**; Row 3 (**c1-c3**): cells treated with 20 µM **1g**. Column 1: bright field images; Column 2: fluorescence images obtained using red filter; Column 3: merging of bright field and fluorescent images. The incubation time period of the compound **1g** in B16 F10 cells is 6 h.

# UV and Fluorescence Spectra

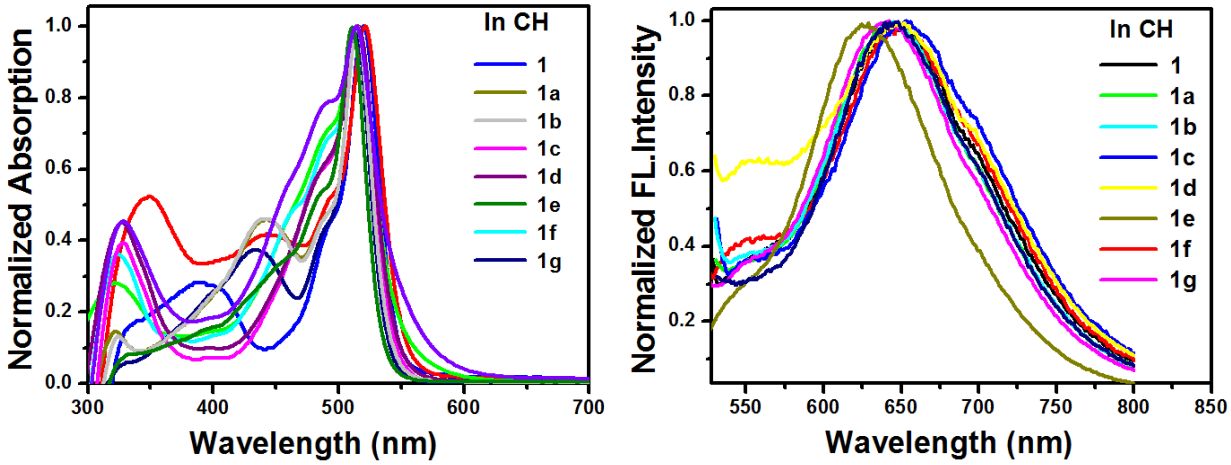



**Fig 15:** Normalized absorption and fluorescence (FL) emission spectra of BODIPY dyes **1**, **1a-1g** in cyclohexane (CH).

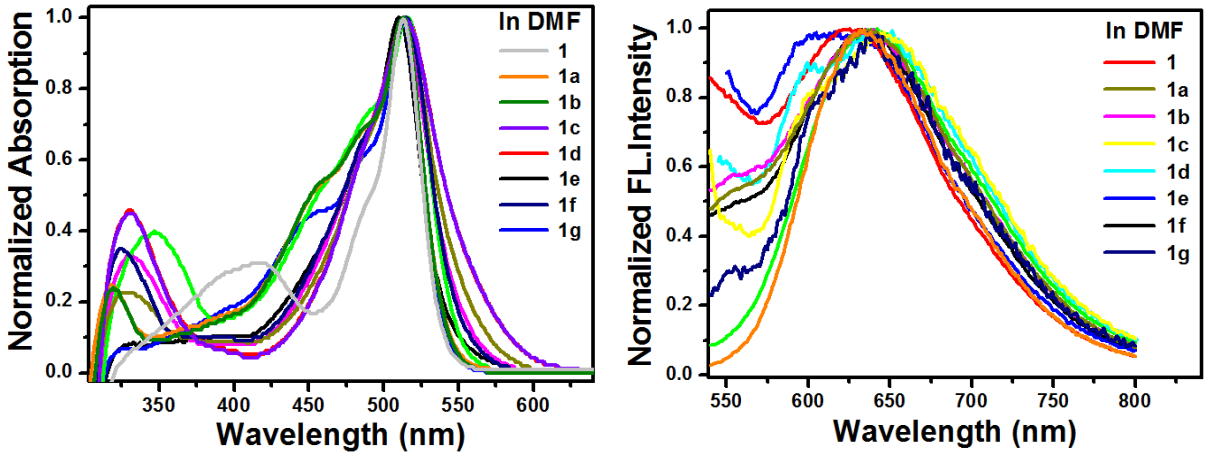



**Fig 16:** Normalized absorption and fluorescence (FL) emission spectra of BODIPY dyes **1**, **1a-1g** in dimethylformamide (DMF).

# $^{1}$H and $^{13}$C NMR Spectra

**Fig 17: $^{1}$H** NMR Spectrum of **1a**

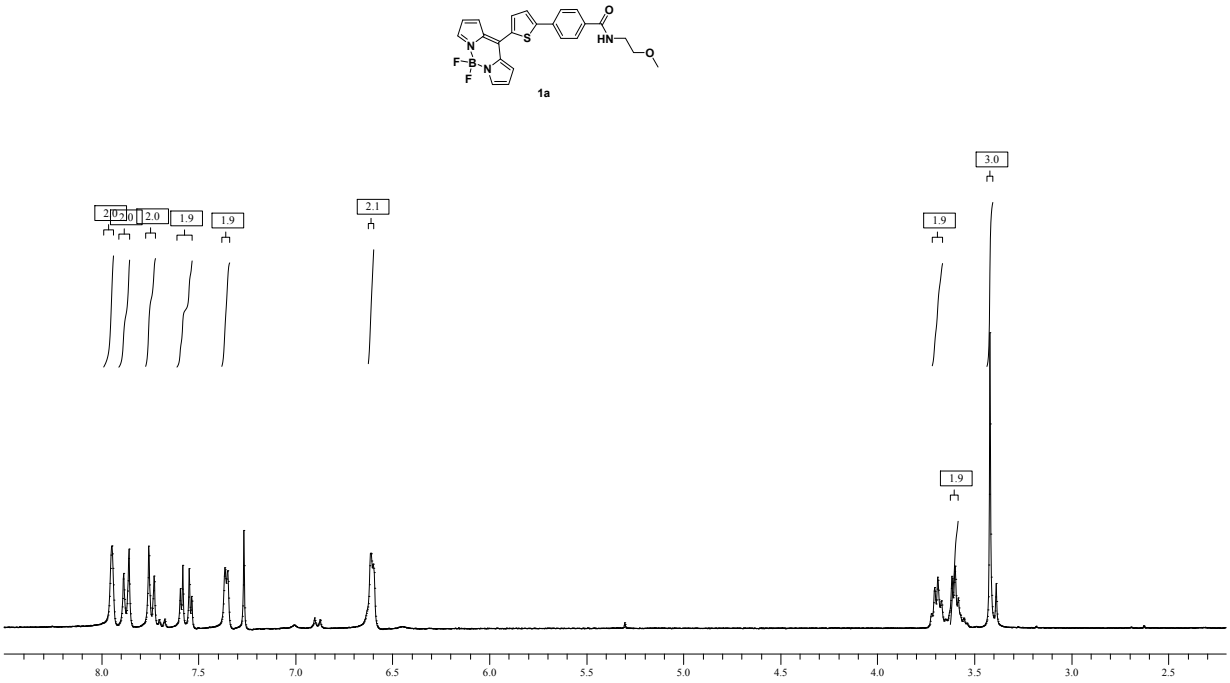



**Fig 18: $^{13}$C** NMR Spectrum of **1a**

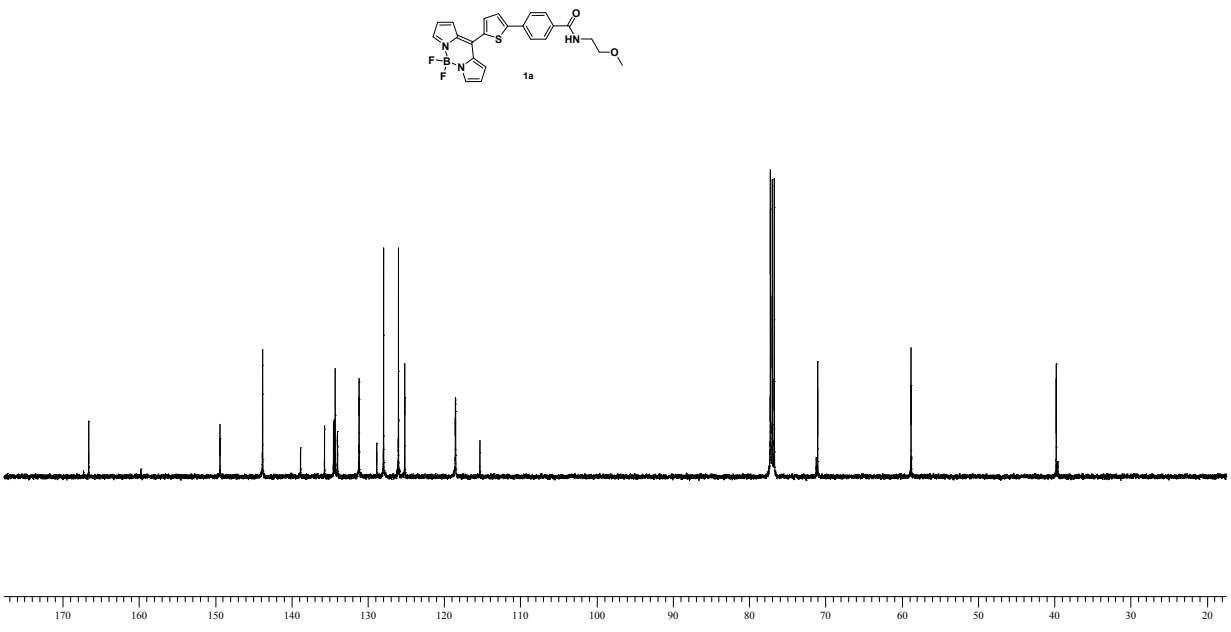


**Fig 19: $^{1}$H** NMR Spectrum of **1b**

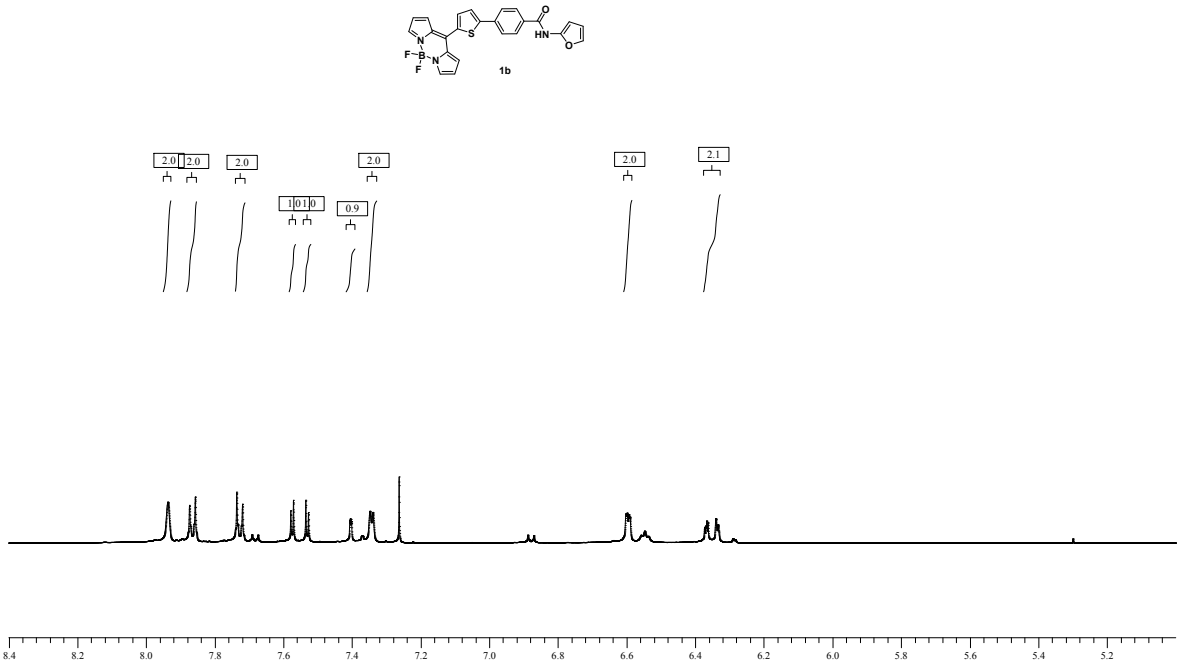



**Fig 20: $^{13}$C** NMR Spectrum of **1b**

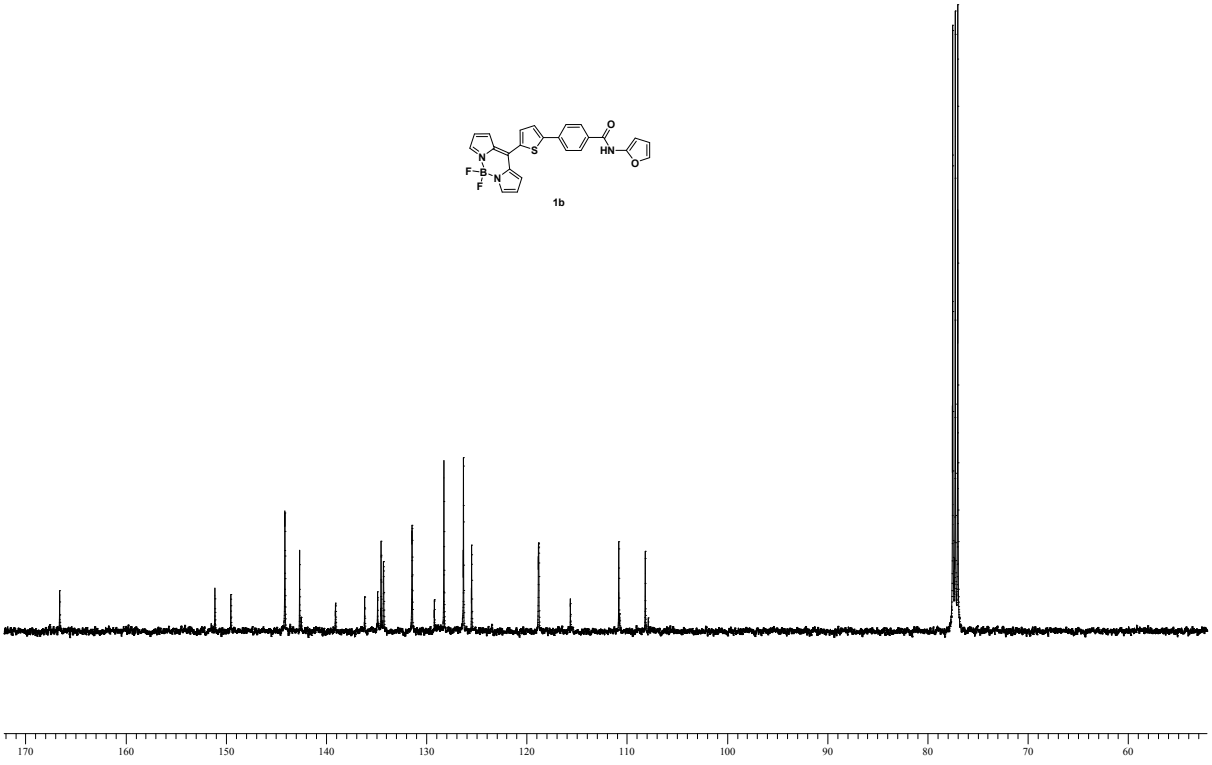


**Fig 21: $^{1}$H** NMR Spectrum of **1c**

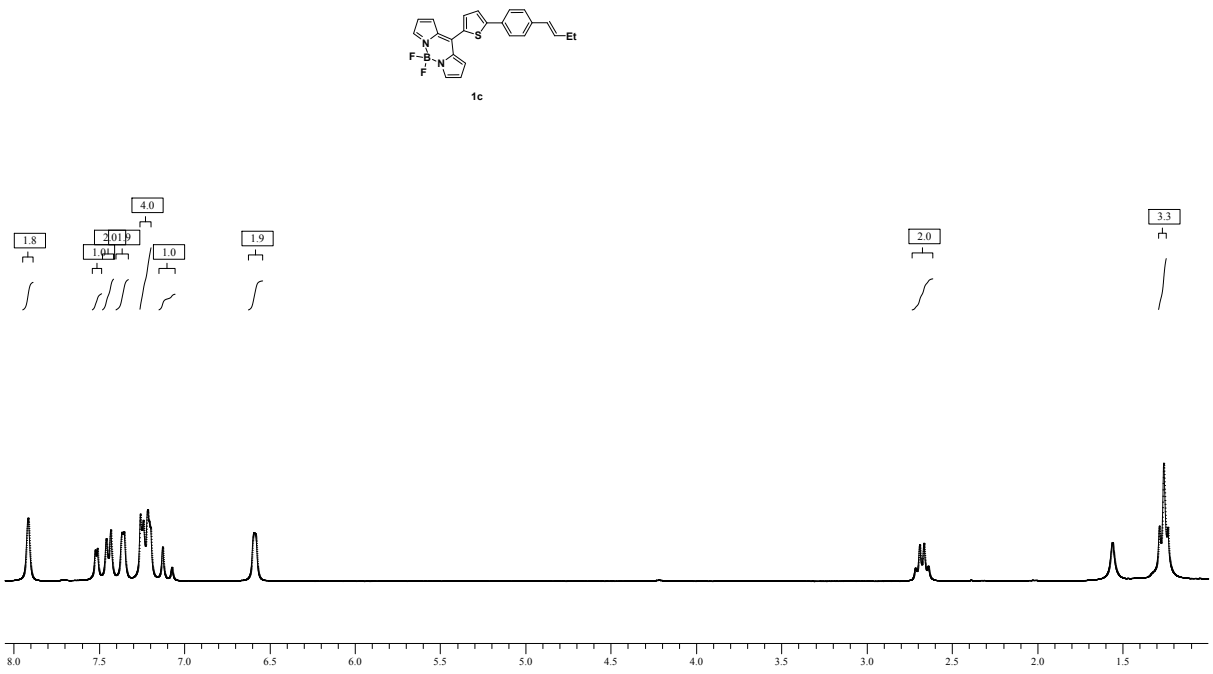



**Fig 22: $^{13}$C** NMR Spectrum of **1c**

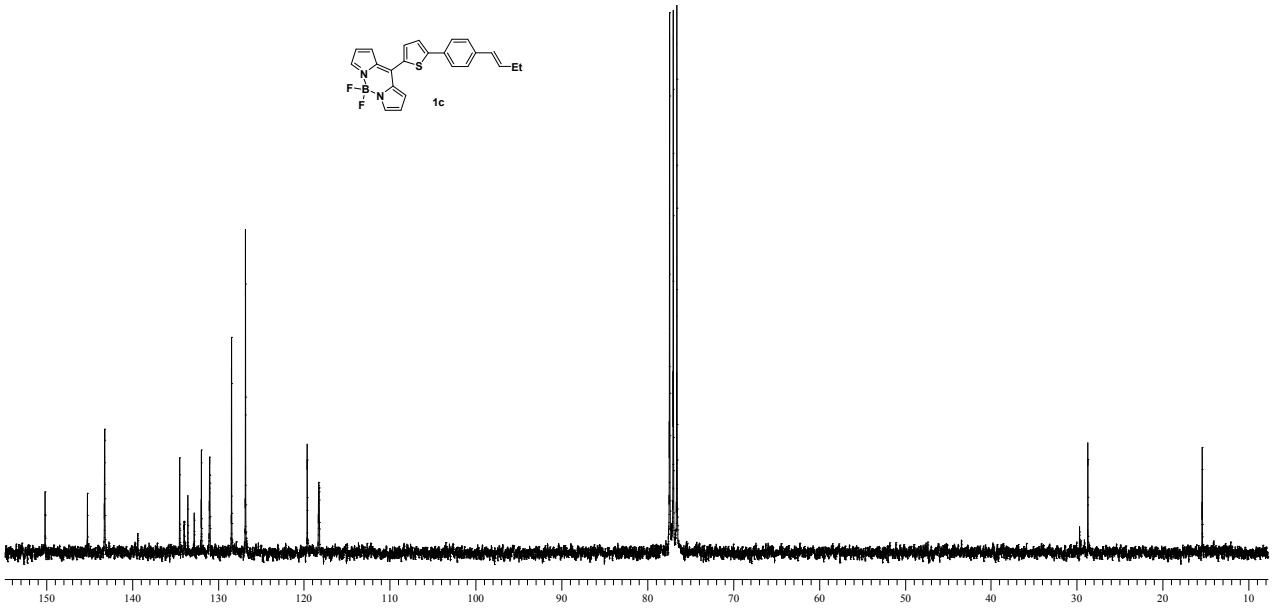


**Fig 23: $^{1}$H** NMR Spectrum of **1d**

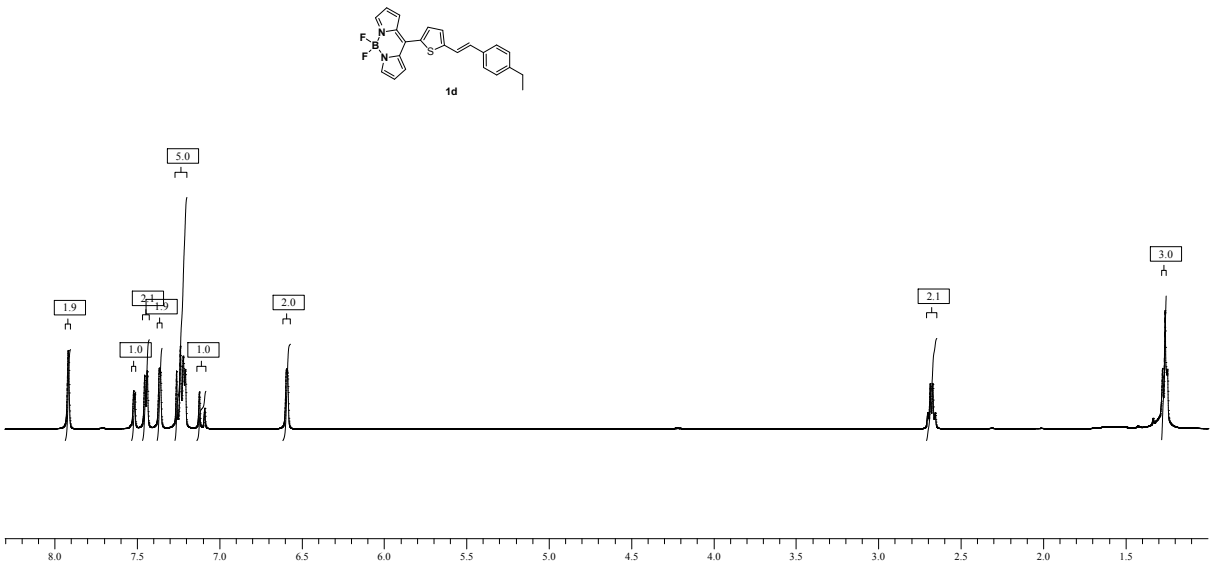



**Fig 24: $^{13}$C** NMR Spectrum of **1d**

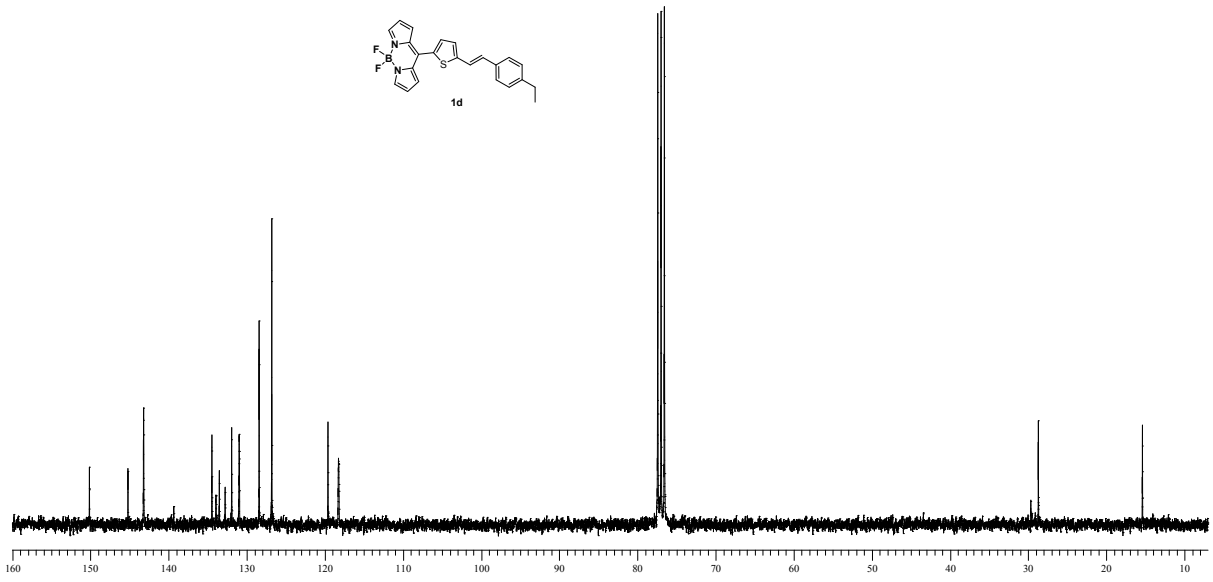


**Fig 25: $^{1}$H** NMR Spectrum of **1e**

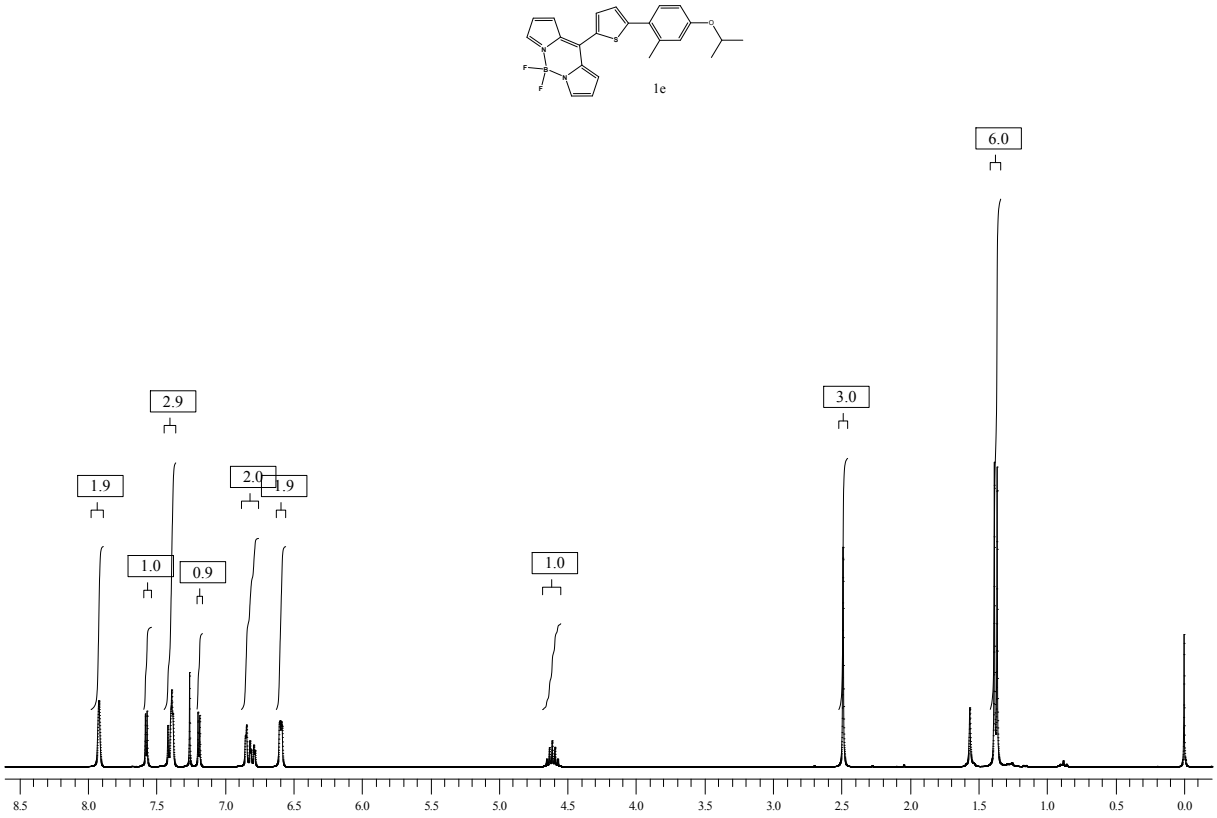



**Fig 26: $^{13}$C** NMR Spectrum of **1e**

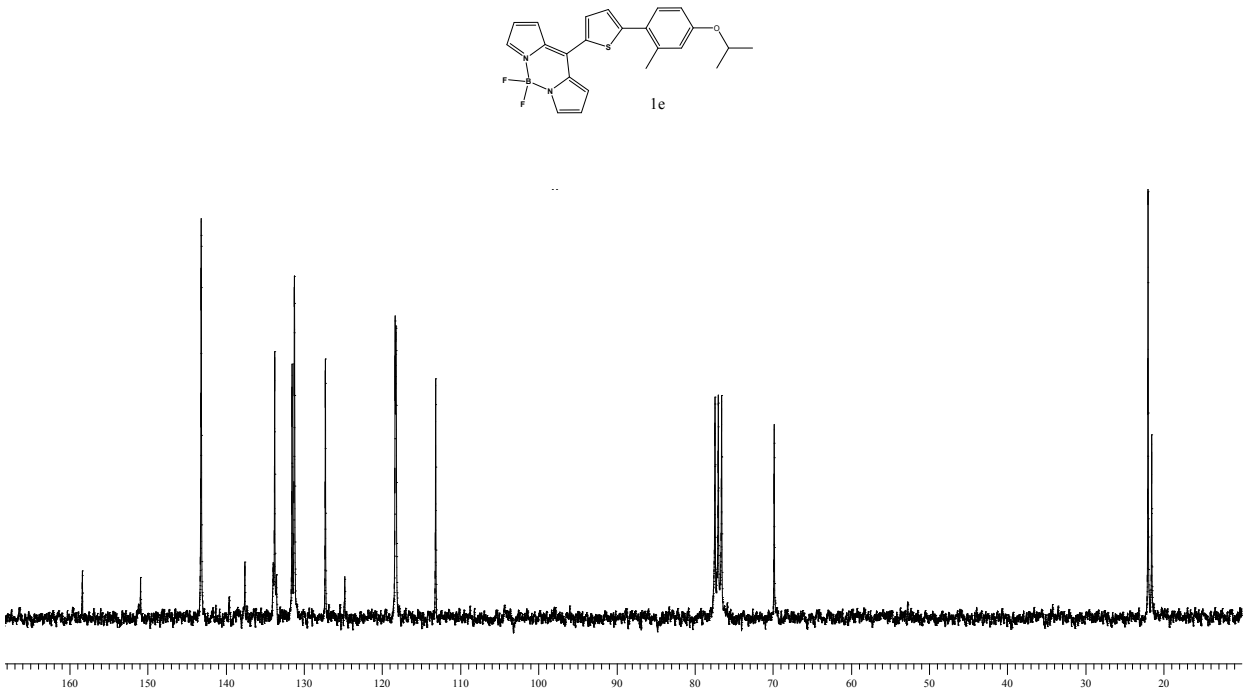


**Fig 27: $^{1}$H** NMR Spectrum of **1f**

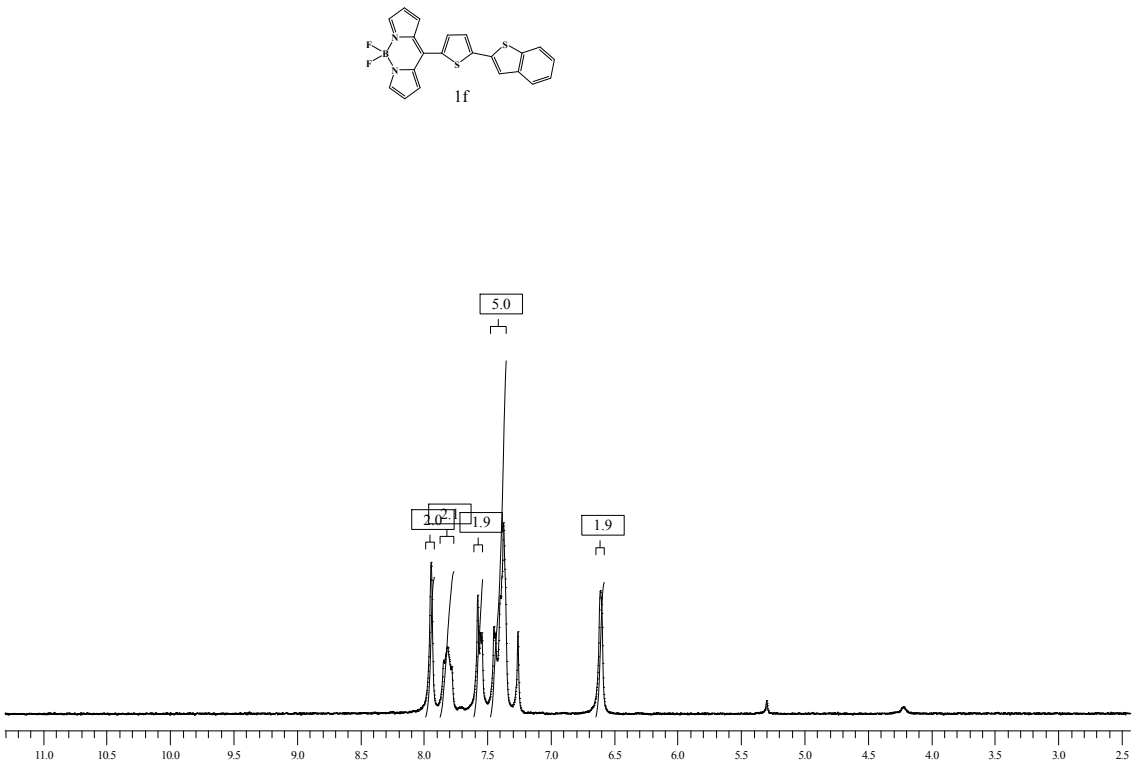



**Fig 28: $^{13}$C** NMR Spectrum of **1f**

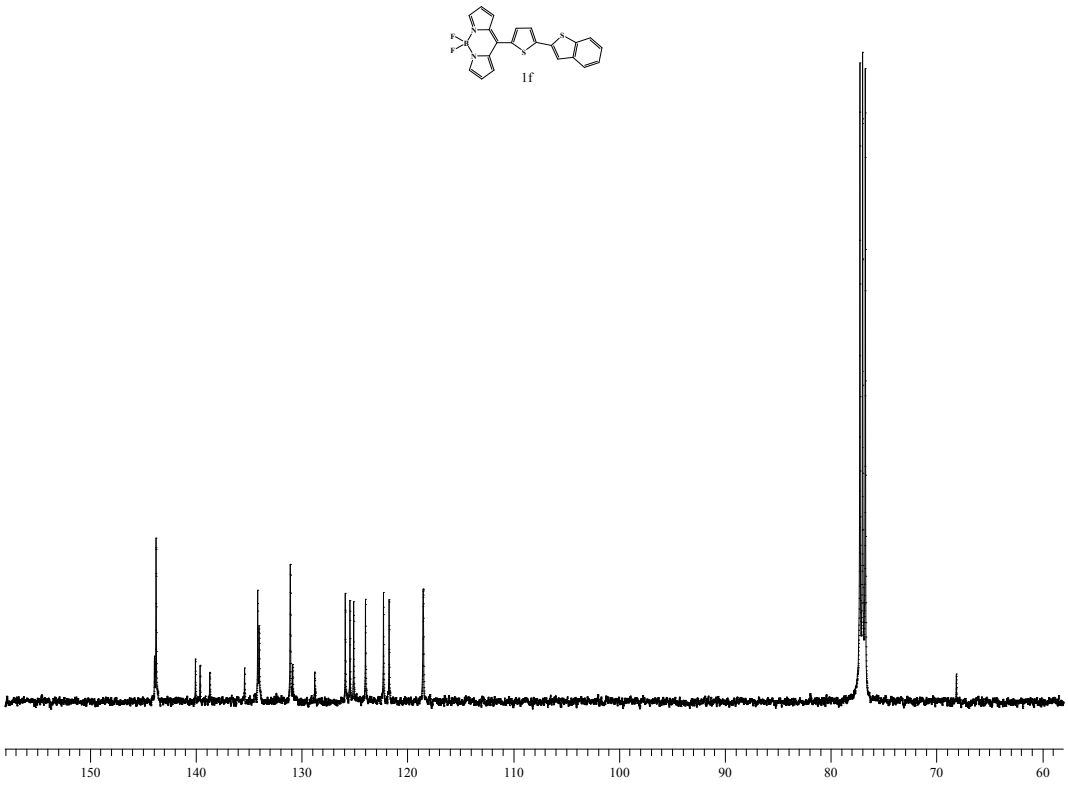


**Fig 29: $^{1}$H** NMR Spectrum of **1g**

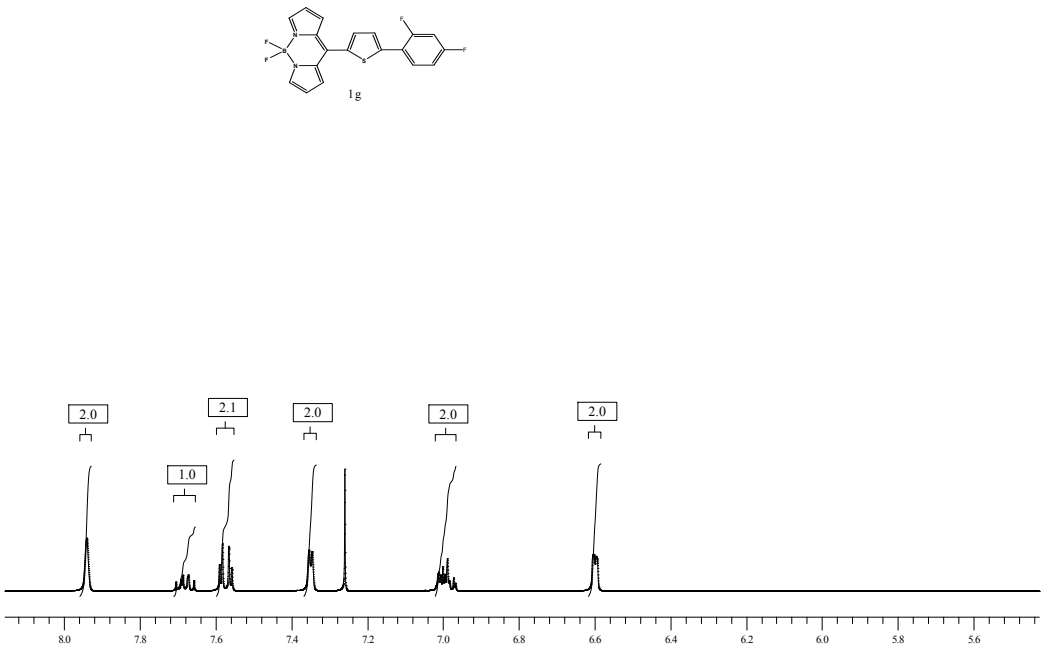



**Fig 30: $^{13}$C** NMR Spectrum of **1g**