Supporting Information

Extremely selective “turn−on” fluorescence detection for hypochlorite confirmed by neurological studies via esterase action in living cells

Atul P. Singh,[a] Olga G. Tsay,[a] Dhiraj P. Murale,[a] Taehong Jun,[a] Hyunjeong Liew, Yoo–Hun Suh,[b] and David G. Churchill[*,a]

a Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), 373–1 Guseong–dong, Yuseong–gu, Daejeon 305–701, Republic of Korea E–mail:dchurchill@kaist.ac.kr.

b Department of Pharmacology, College of Medicine, and Neuroscience Research Institute, MRC, Seoul National University, Seoul 110–799, Republic of Korea.

E–mail: dchurchill@kaist.ac.kr

Experimental Section

General Considerations. All chemicals used herein were used as received from commercial suppliers (Aldrich, Acros, and Junsei companies). The synthetic details for the preparation of the dipyrromethanes and for the BODIPY systems follow literature methods.[1] ^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance 400 MHz spectrometer. TMS was used as an internal standard. ^{1}H and ^{13}C NMR spectral signals were calibrated internally by the respective protio impurity or carbon resonance of the $CDCl_3$ (^{1}H: δ 7.24; ^{13}C: δ 77.0). A high resolution hybrid tandem LC–MS/MS spectrometer was used for mass data collection. Data was acquired by the research support staff at KAIST.

Absorption and Emission Spectroscopy. For cuvette determinations of photophysical properties, all compounds were dissolved in acetonitrile in preparing a BODIPY solution of concentration 1.0×10^{-6} M. UV–vis absorption and emission measurements were obtained using a CARY 300 Bio UV–vis spectrometer and a Shimatzu RF–5301 PC spectrophotometer, respectively. Emission spectra are obtained through the excitation at λ_{max} from the absorption spectrum of each compound. Fluorescein in 0.1 *N* NaOH (Φ_F = 0.92)[2] was used as a standard to calibrate quantum yield values. The compound has different solubilities, and measurements in water for ROS detection are described below.

Cell culture details. SH–SY5Y human neuroblastoma cells were maintained in a DMEM medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L–glutamine, and 10% (v/v) heat–inactivated fetal bovine serum at 37 °C and 5% (v/v) CO_2.

Every other day, cells were re–cultured with a fresh medium.

Protocol for Determinations of ROS in neuronal cultures. Briefly, SH–SY5Y cells were seeded onto coverslip in a six–well plate (~2×10^5 cells per well) 1 day before the experiment. Compound **4** (1.1×10^{-5} M) was pre–treated in culture media during 30 min and without washing, NaOCl (0.5×10^{-2} M) was added in culture media of live cells. All incubation was retained at 37° C in a CO_2 incubator. After this time, the cells were washed with Hank's Balanced Salt Solution (HBSS). Without mounting, fluorescence staining was promptly visualized by an Olympus fluorescence microscope (BX61; New Hyde Park, NY, USA).

Synthetic procedures.

For compounds 1 and 2. The synthesis of **1** was performed via a standard procedure.[3] Firstly, CuI (0.5 mol %) and benzotriazole (1.0 mol %) were mixed in 2–3 mL DMSO. Aryl halide (0.50 g, 1.9 mmol) was then added and stirred the for 10 min. Next, aryl–thiol (0.71 g, 4.75 mmol) and Cs_2CO_3 (0.85 g, 2.66 mmol) were added and the reaction mixture was stirred for 10–12 h at 90–95 °C. The reaction was then monitored by TLC. After completion of the reaction, 30 mL of ethylacetate was added and this layer was washed with water (3 times with 10 mL). Solution was dried on Na_2SO_4 and pumped off through use of a rotoevaporator. The viscous mass was then subjected to column chromatography (dichloromethane/methanol) to afford the desired pure compound **1 (**Yield = 0.350 g, 55.1%).

Synthesis of **2** was accomplished via an esterification reaction of compound **1**.[4] The reaction was carried out in a small round–bottom flask in DMF solvent (3–4 mL), by the

reaction of compound **1** (0.472 g, 1.4 mmol) with ethyl bromide (0.18 mL, 2.1 mmol) and potassium carbonate (0.23 g, 1.7 mmol) at room temperature with heating to 120 °C for 8–10 h. The reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the reaction mixture was cooled to room temperature; then, the compound was extracted with ethyl acetate / water. Ethyl acetate solution was kept on Na_2SO_4 for drying for 10–20 minutes, and was then filtered. The solvent was then pumped off through the use of a rotoevaporator to get crude compound **2**. The purification of compound **2** was completed by silica–gel column chromatography (hexane/dichloromethane = 1:1; Yield = 0.520 g, 100%.).

Compound 1 spectral data: Yield = 0.350 g, 55.1%. ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): δ 9.97 (s, 1H$_6$), 8.17 (dd, $^4J_{H–H}$ / $^3J_{H–H}$ = 1.5 / 7.9 Hz, 1H$_9$), 7.58 (s, 1H$_4$), 7.44 (ddd, $^4J_{H–H}$ / $^3J_{H–H}$ / $^3J_{H–H}$ = 1.6 / 8.1 / 8.1 Hz, 1H$_{11}$), 7.27 (ddd, $^4J_{H–H}$ / $^3J_{H–H}$ / $^3J_{H–H}$ = 1.1 / 7.5 / 7.5 Hz, 1H$_{10}$), 6.90 (dd, $^4J_{H–H}$ / $^3J_{H–H}$ = 1.1 / 8.5 Hz, 1H$_{12}$). ^{13}C NMR ($CDCl_3$, δ 77.0, 400 MHz) = 183.8, 170.4, 145.8, 143.5, 142.5, 134.2, 132.3, 129.6, 126.7, 125.8, 125.4, 118.2. ESI–MS (positive mode, $CHCl_3$ + CH_3OH) = = $[M + CH_3OH + Na]^+$ = 414.9285 (cal.), 414.9074 (exp.).

Compound 2 spectral data: Yield = 0.520 g, 100%. ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): δ 9.93 (s, 1H$_6$), 8.02 (ddd, $^5J_{H–H}$ / $^4J_{H–H}$ / $^3J_{H–H}$ = 0.4 / 1.6 / 7.8 Hz, 1H$_9$), 7.53 (s, 1H$_4$), 7.35 (ddd, $^4J_{H–H}$ / $^3J_{H–H}$ / $^3J_{H–H}$ = 1.6 / 7.4 / 8.2 Hz, 1H$_{11}$), 7.20 (ddd, $^4J_{H–H}$ / $^3J_{H–H}$ / $^3J_{H–H}$ = 1.1 / 7.4 / 8.0 Hz, 1H$_{10}$), 6.87 (ddd, $^5J_{H–H}$ / $^4J_{H–H}$ / $^3J_{H–H}$ = 0.4 / 1.4 / 8.1 Hz, 1H$_{12}$), 4.14 (q, 7.1 Hz, 2H$_{14}$), 1.40 (t, $^3J_{H–H}$ = 7.1 Hz, 3H$_{15}$). ^{13}C NMR ($CDCl_3$, δ 77.0, 400 MHz) = 183.8, 168.0, 145.5, 144.3, 141.2, 133.1, 131.2, 129.4, 127.0, 126.7, 125.7, 117.8, 61.6, 14.2. ESI–

MS (positive mode, $CHCl_3 + CH_3OH$) = $[M + CH_3OH + Na + 2H]^+$ = 426.9649 (cal.), 426.9454 (exp.).

General procedure for the one–pot synthesis of BODIPY. Aryl aldehyde and dimethylpyrrole were added to a two–neck flask in 20 mL of dichloromethane at ice–bath temperature; sparging with nitrogen for 10 min was then undertaken to remove atmospheric oxygen. Trifluoroacetic acid (TFA) was added dropwise and the materials were allowed to react under N_2 for ~1 h. Thin layer chromatography (TLC) assaying revealed the formation of a new spot at R_f = ~ 0.4 (for **3**, hexane/ethylacetate = 1:1), and 0.45 for **4** **(**hexane/dichloromethane = 1:1), thus indicating the consumption of the starting aldehyde and signifying the formation of dipyrromethane–based species. The resultant solution was neutralized with *N*,*N*–di–isopropylethylamine to maintain a pH of ~7; the solution volume was maintained to ~50 mL with dichloromethane. The solution temperature was retained at ice–cold temperature; *p*–chloranil (tetrachloro–*p*–benzoquinone) was added slowly and stirred for ~3 h to complete the oxidation reaction. The resultant solution was neutralized with *N*,*N*–di–isopropylethylamine, followed by 10 min of additional stirring. Boron trifluoride etherate was then added; the reaction was kept stirring for the next 2 h at room temperature. A thin layer chromatography (TLC) assay revealed the expected orange–red spot at R_f = ~ 0.2 for **3** (hexane/ethylacetate = 1:1) and R_f = 0.8 for **4** (in dichloromethane). The solvent of the reaction mixture was pumped off via rotoevaporation and the crude solid material was used for silica gel column chromatography (eluent: hexane/ethylacetate = 1:1 (for **3**), hexane/dichloromethane = 1:1 (for **4**)). Here, PTLC can sometimes be used as a last purification step to improve analytical purity if necessary, subsequent to column chromatography.

Compound 3 spectral data: Yield = 0.145 g, 16.2 %. ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): δ 7.94 (dd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ =1.6 / 7.8 Hz, $1H_{13}$), 7.48 (dd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ = 1.1 / 8.1 Hz, $1H_{16}$),7.33 (ddd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ / $^{3}J_{H–H}$ =1.6 / 7.3 / 8.0 Hz, $1H_{15}$), 7.22 (ddd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$/$^{3}J_{H–H}$ =1.2 / 7.6 / 7.6 Hz, $1H_{14}$), 6.95 (s, $1H_{10}$), 5.93 (s, $2H_2$), 2.3 (s, $6H_{18}$), 1.61 (s, $6H_{19}$). ^{13}C NMR ($CDCl_3$, δ 77.0, 400 MHz) = 169.2, 156.3, 142.3, 141.3, 138.5, 133.1, 132.7, 132.2, 131.9, 131.3, 131.1, 131.0, 128.6, 126.7, 121.6, 117.2,14.6, 13.0. ^{11}B–NMR ($CDCl_3$, BF_3·OEt_2, δ 0.00): 5.34 (t, 32.75 Hz). ESI–MS (positive mode, $CHCl_3$ + CH_3OH) = $[M + Na]^+$ = 585.0265 (cal.), 585.0071 (exp.); $[M + H–C_7H_6O_2S]^+$ = 408.0279 (cal.), 408.3075 (exp.).

Compound 4 spectral data: Yield = 0.220 g, 34.65 %. ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): δ 7.86 (dd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ = 1.6 / 7.8 Hz, $1H_{13}$), 7.43 (dd, 1.1 / 8.0, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ = $1H_{16}$), 7.29 (ddd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ / $^{3}J_{H–H}$ = 1.6 / 8.0 / 7.3 Hz, $1H_{15}$), 7.12 (ddd, $^{4}J_{H–H}$ / $^{3}J_{H–H}$ / $^{3}J_{H–H}$ = 1.2 / 7.7 / 7.7 Hz, $1H_{14}$), 6.94 (s, $1H_{10}$), 5.94 (s, $2H_2$), 4.31 (q, $^{3}J_{H–H}$ = 7.1, $2H_{18}$), 2.50 (s, $6H_{20}$), 1.65 (s, $6H_{21}$), 1.32 (q, $^{3}J_{H–H}$ = 7.1 Hz, $3H_{19}$). ^{13}C NMR ($CDCl_3$, δ 77.0, 400 MHz) = 166.0, 156.2, 142.2, 141.0, 138.1, 133.2, 132.5, 131.9, 131.3, 131.0, 131.0, 130.1, 129.6, 126.4, 121.5, 117.1, 61.0, 14.6, 14.1, 14.0. ^{11}B–NMR ($CDCl_3$, BF_3·OEt_2, δ 0.00): 5.34 (t, 32.8 Hz). ESI–MS (positive mode, $CHCl_3$ + CH_3OH) = $[M + Na + 2H]^+$ = 613.0578 (cal.), 613.0395 (exp.); $[M + 2H–F]^+$ = 571.0696 (cal.), 571.0517 (exp.).

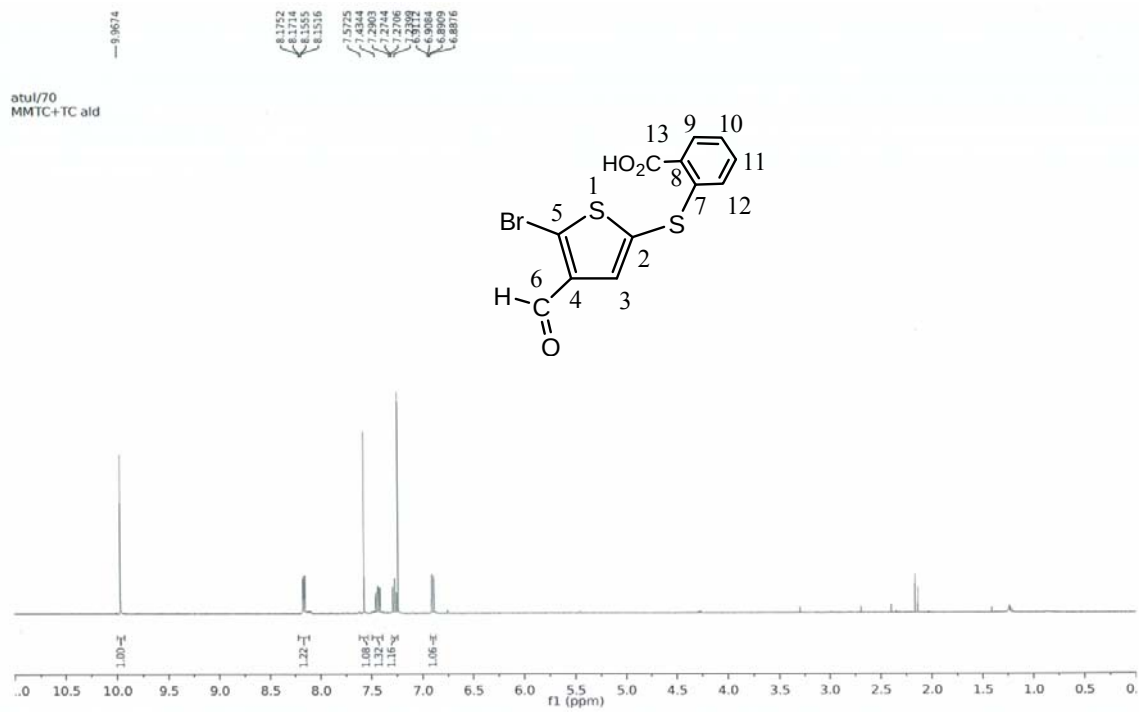

Figure S1.1: ^{1}H NMR spectrum of **1**.

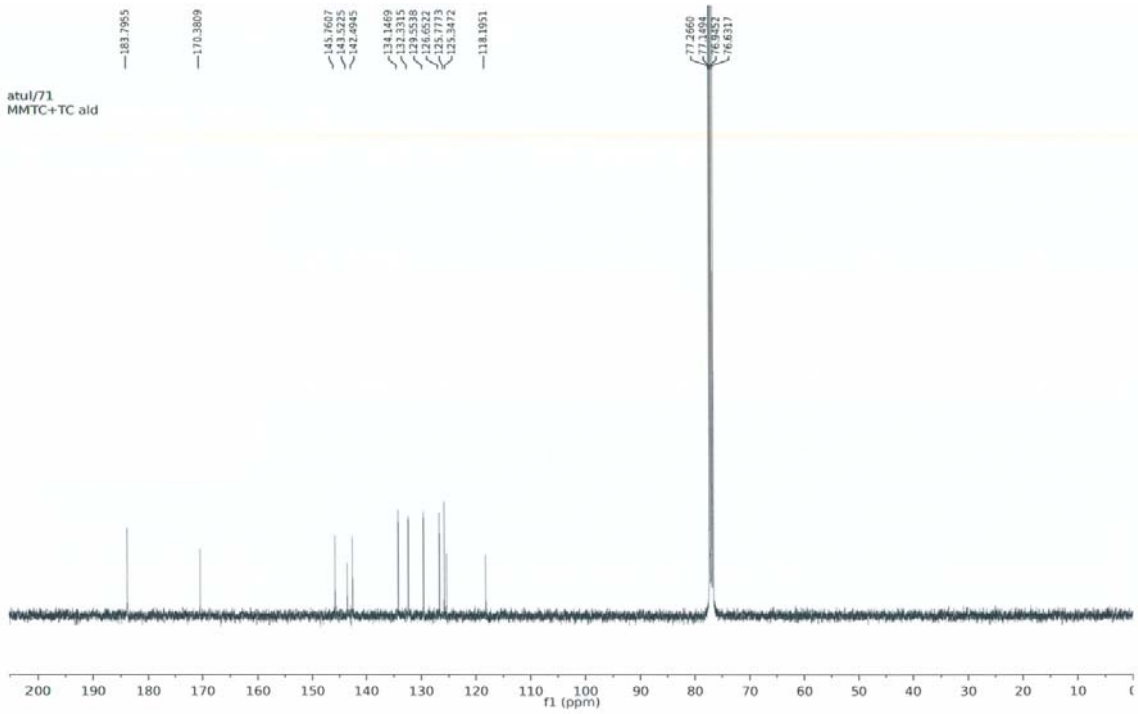

Figure S1.2: ^{13}C NMR spectrum of **1.**

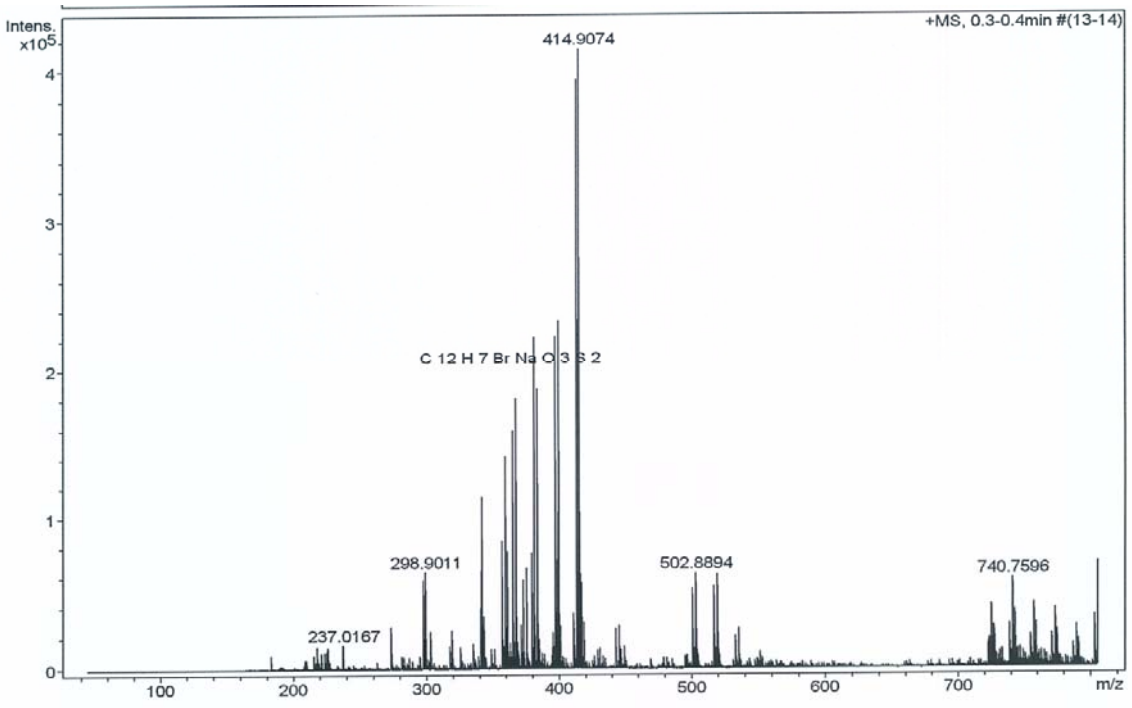

Figure S1.3: ESI Mass spectrum of **1**.

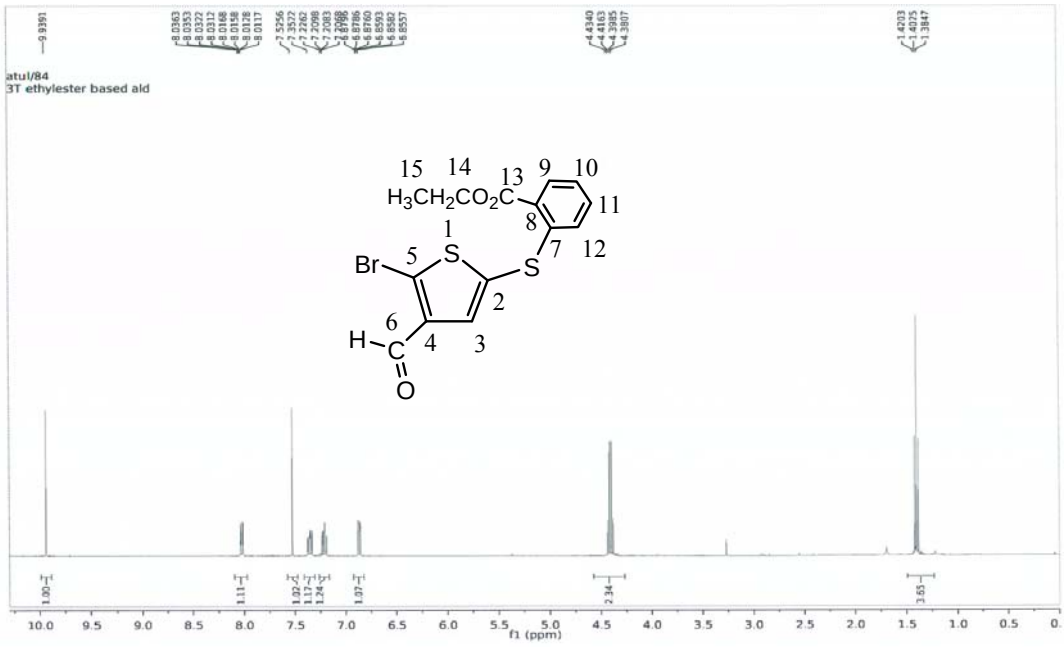

Figure S2.1: ^{1}H NMR spectrum of **2**.

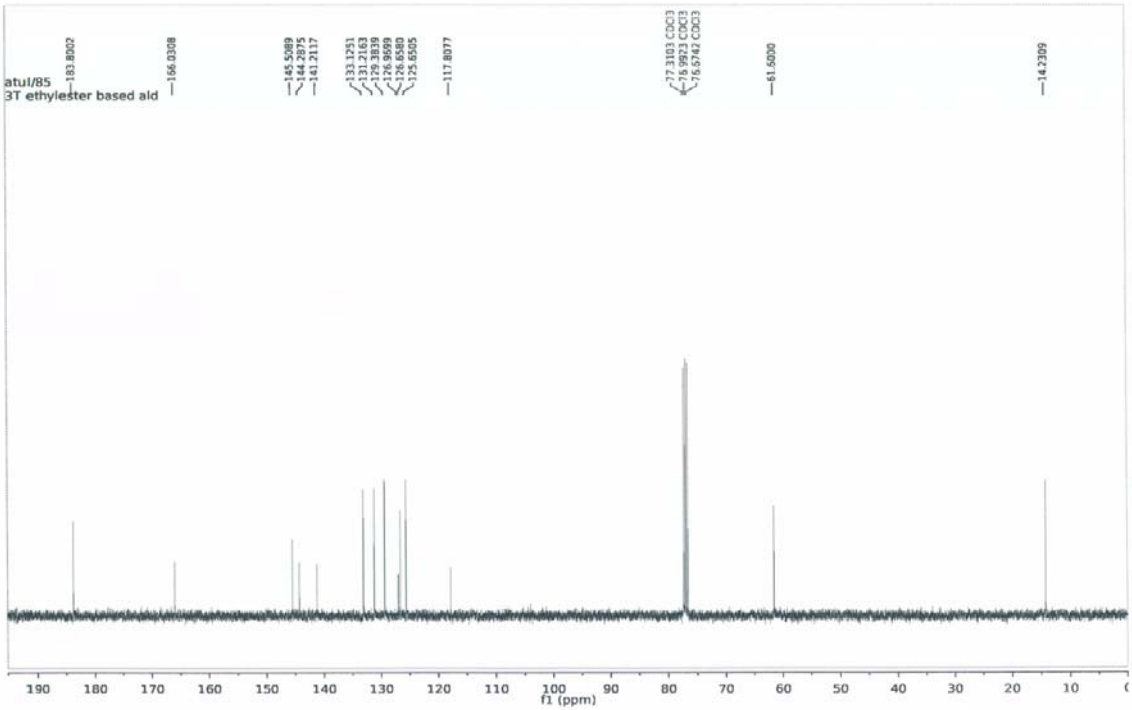

Figure S2.2: ^{13}C NMR spectrum of **2.**

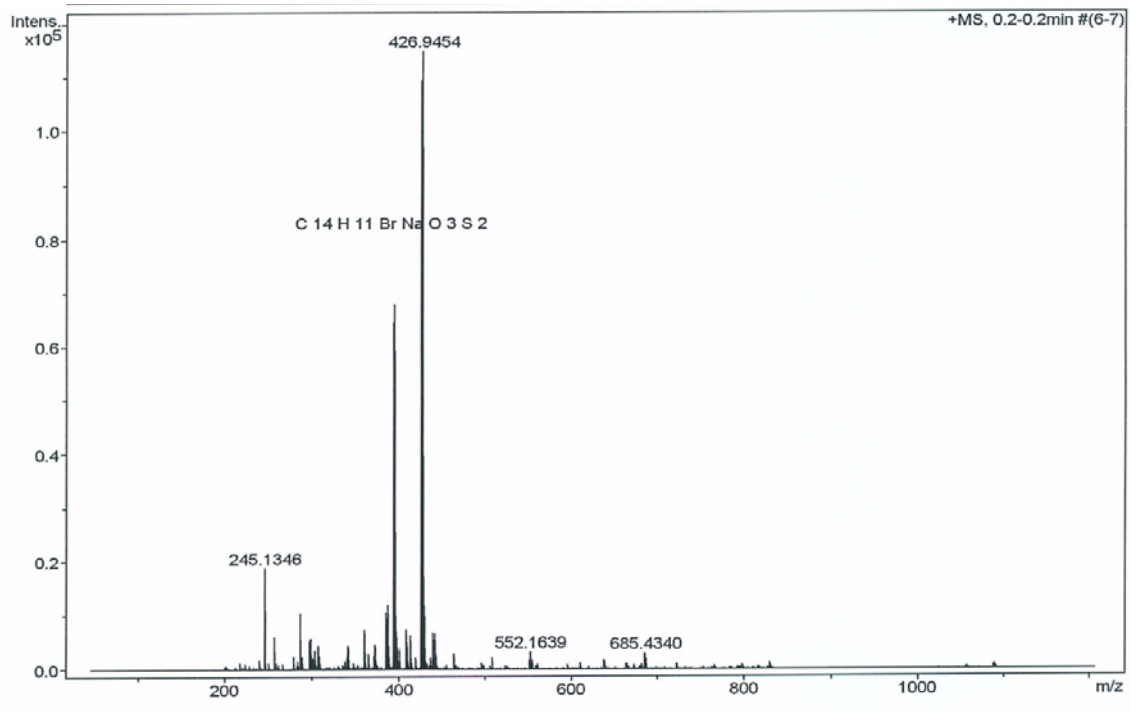

Figure S2.3: Mass spectrum of **2**.

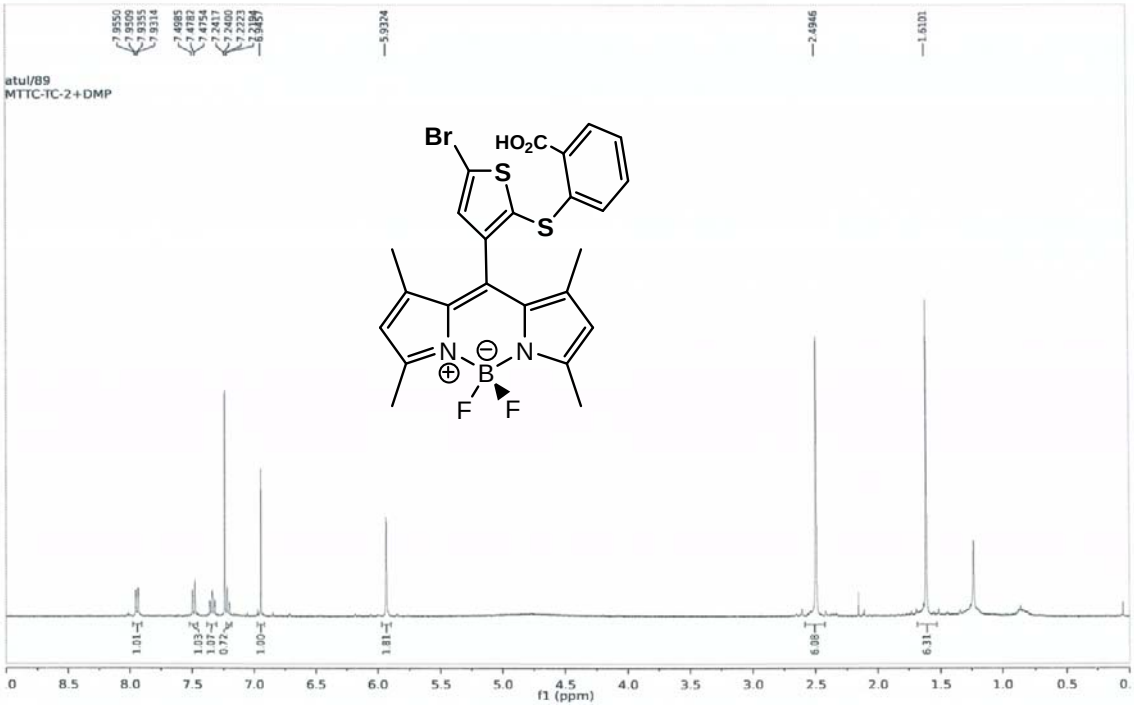

Figure S3.1: ^{1}H NMR spectrum of **3**.

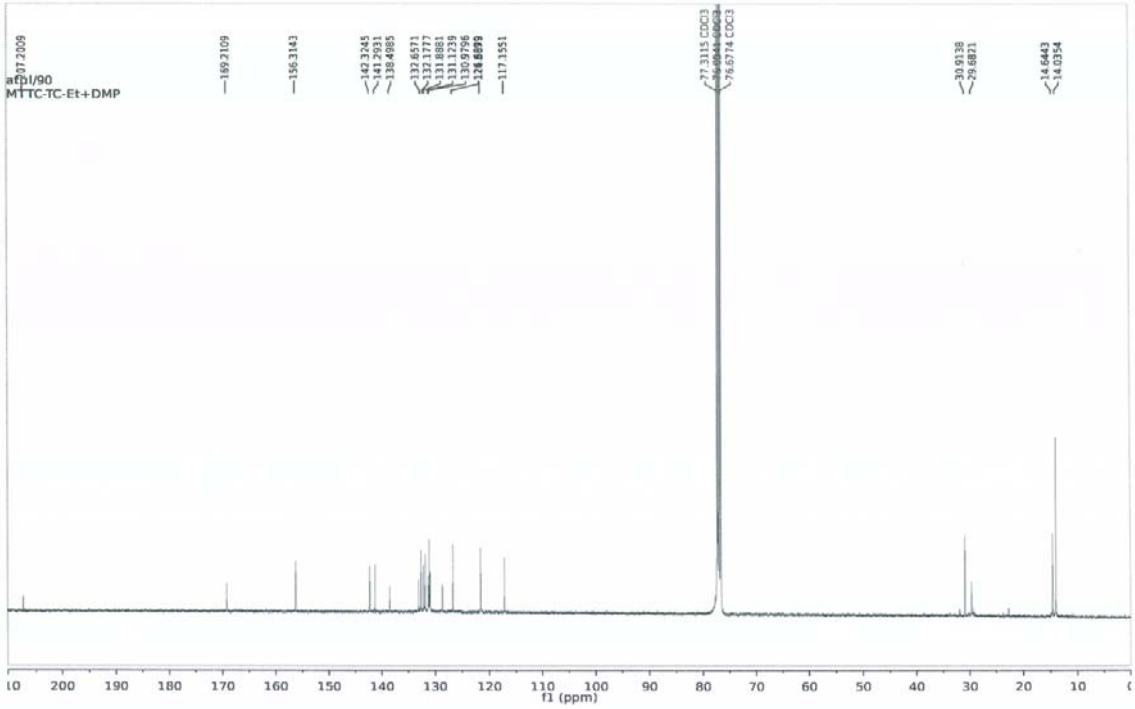

Figure S3.2: ^{13}C NMR spectrum of **3.**

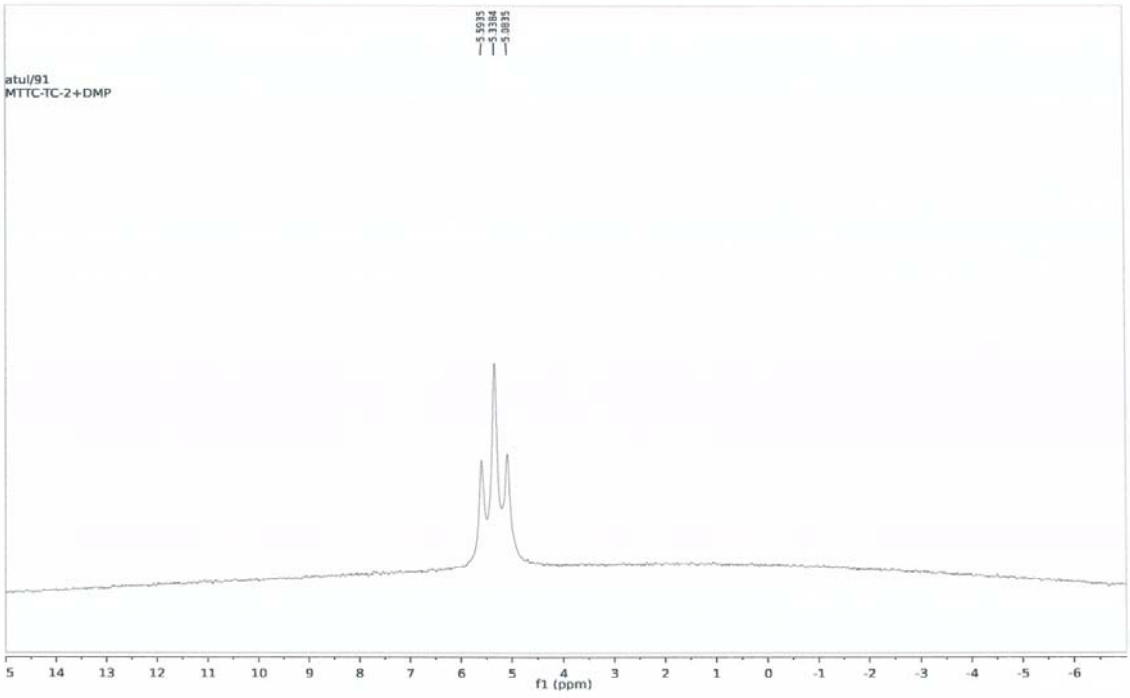

Figure S3.3: ^{11}B NMR spectrum of **3.**

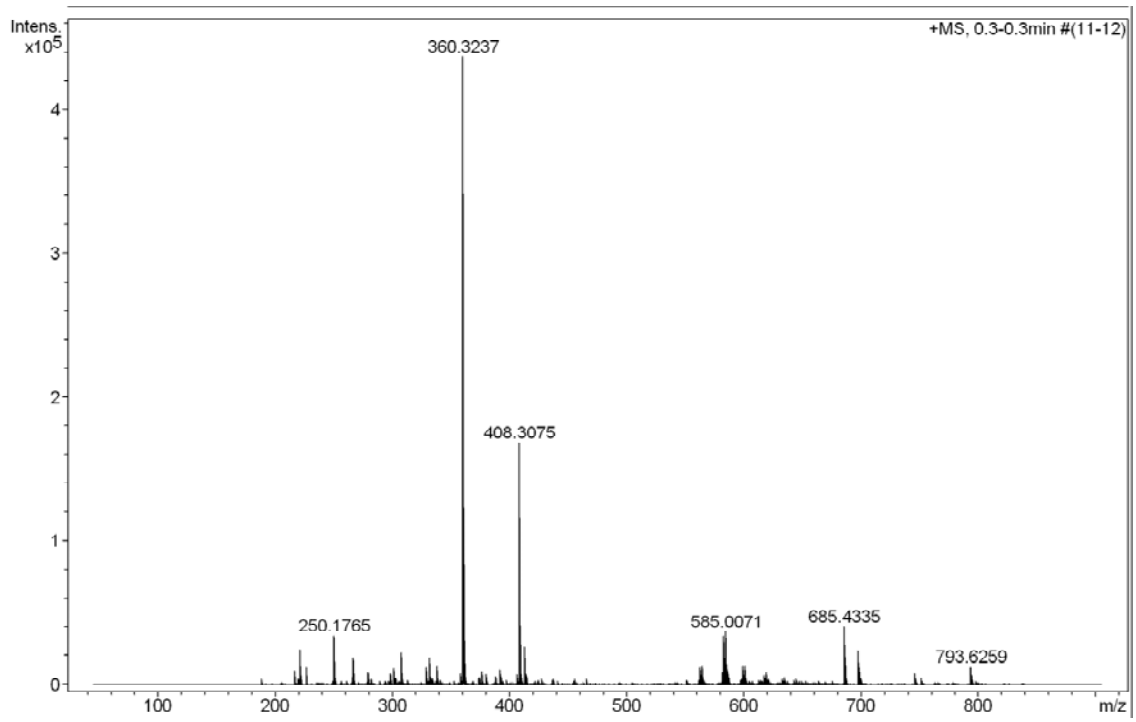

Figure S3.4: Mass spectrum of **3**.

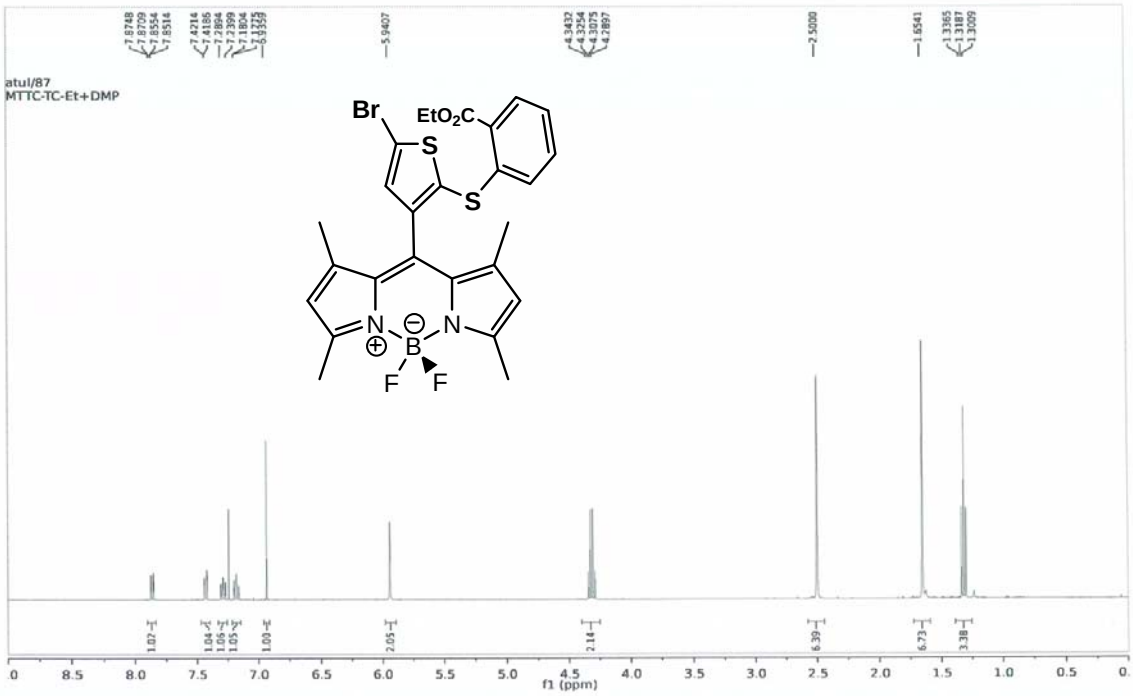

Figure S4.1: ^{1}H NMR spectrum of **4**.

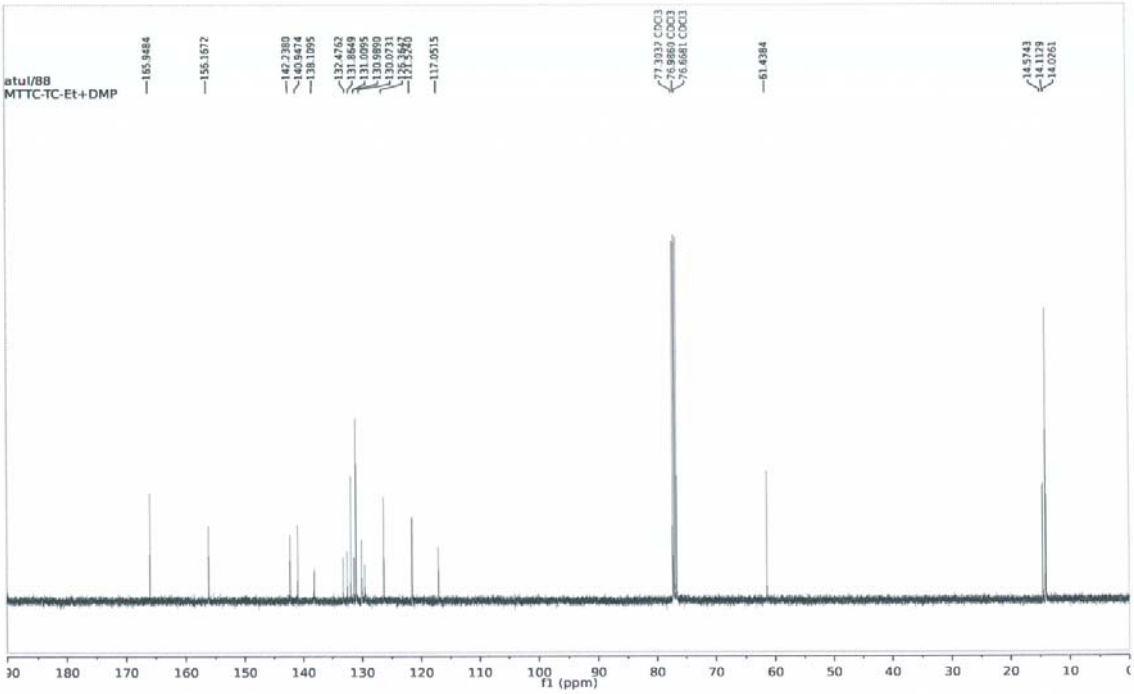

Figure S4.2: ^{13}C NMR spectrum of **4.**

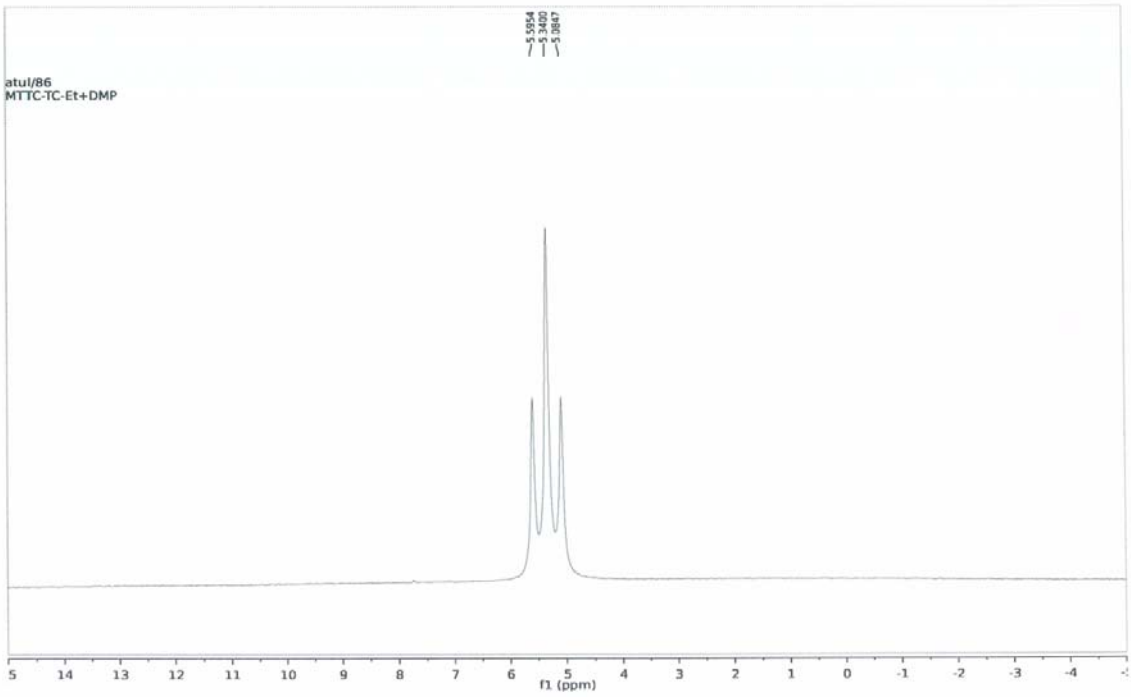

Figure S4.3: ^{11}B NMR spectrum of **4**

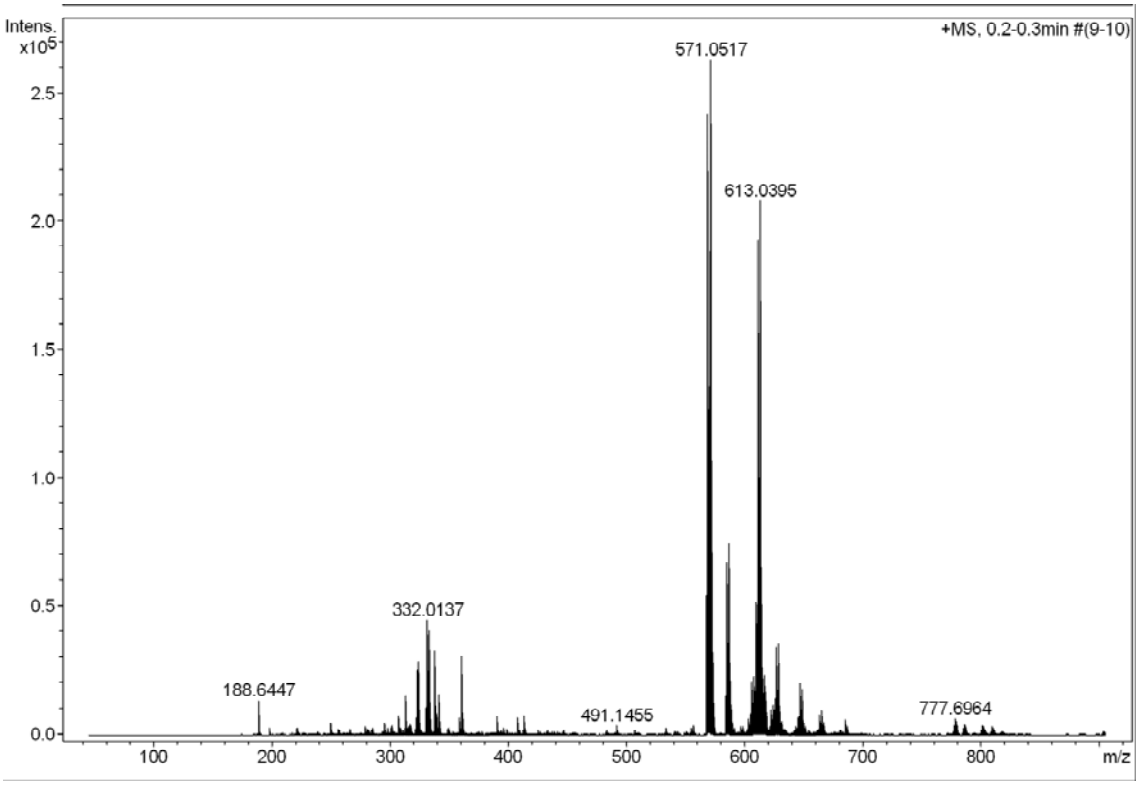

Figure S4.4: Mass spectrum of **4**.

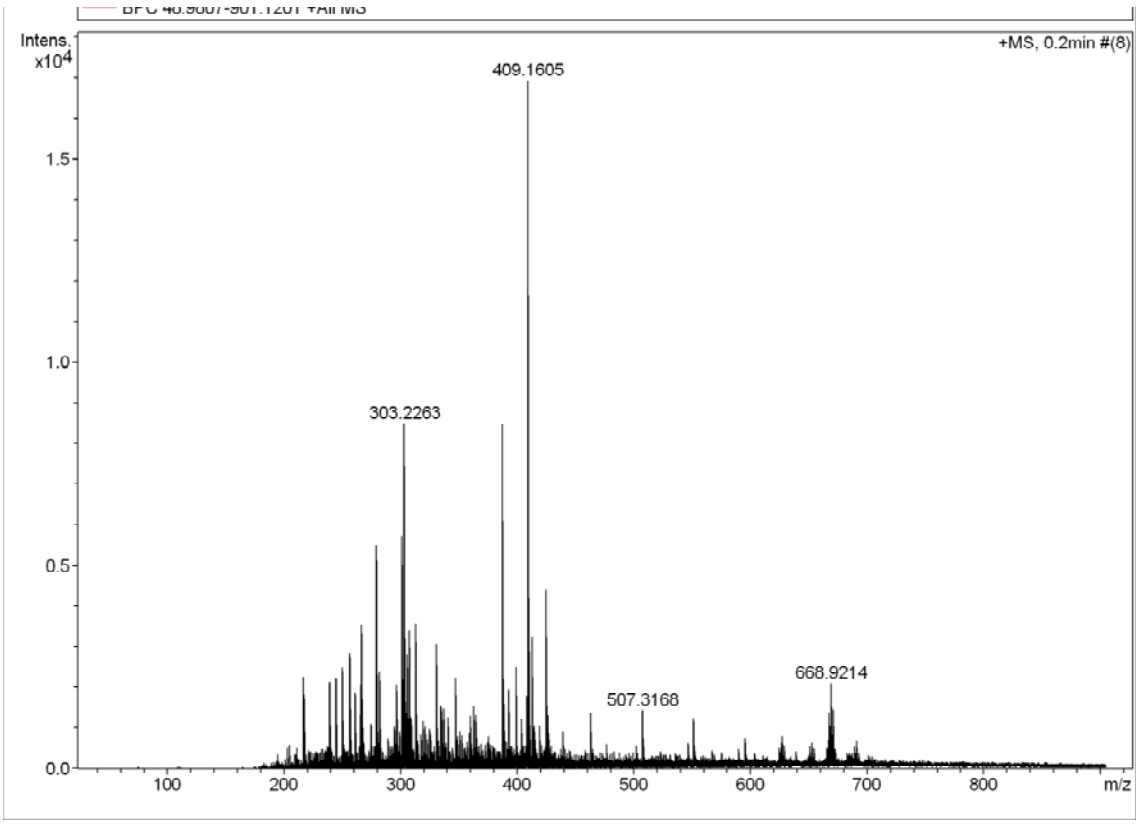

Figure S5: Mass spectrum of **3**+NaOCl (in H_2O and Acetone).

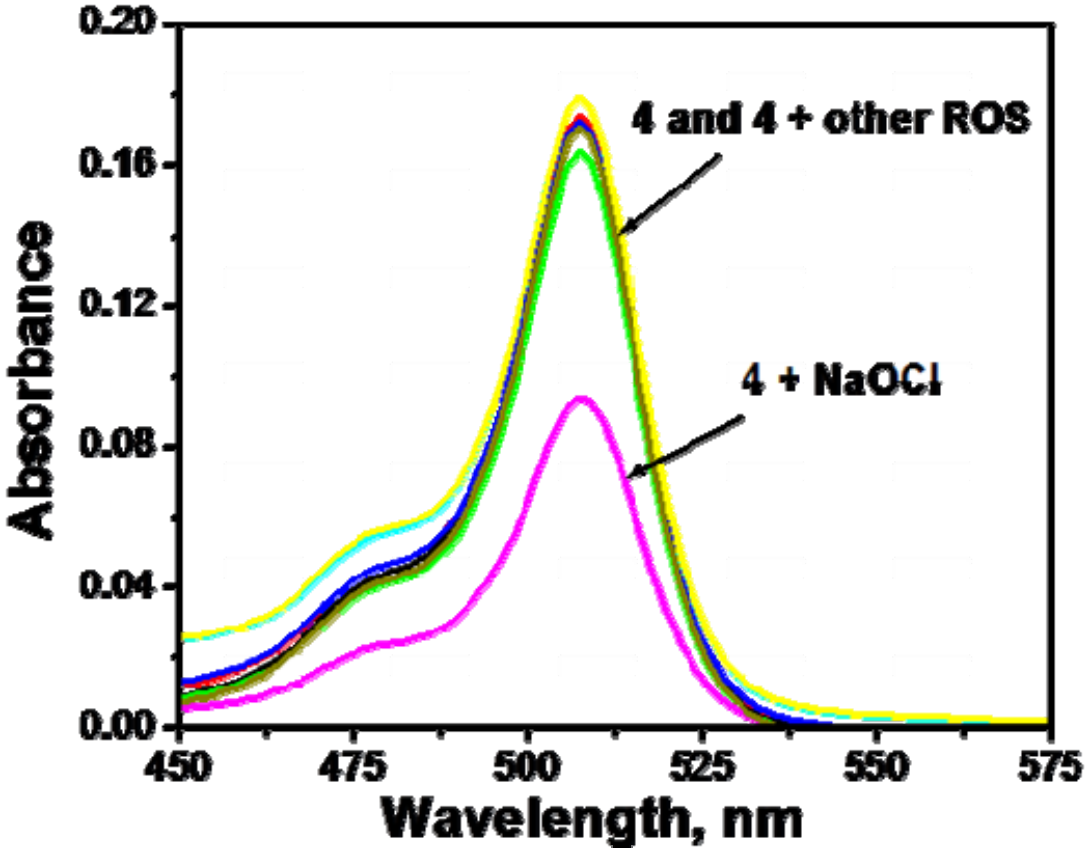

Figure S6. Absorption spectra of compound **4** (1×10^{-6} M) with hypochlorite ion (1×10^{-3} M, ~15 equiv.) in acetonitrile.

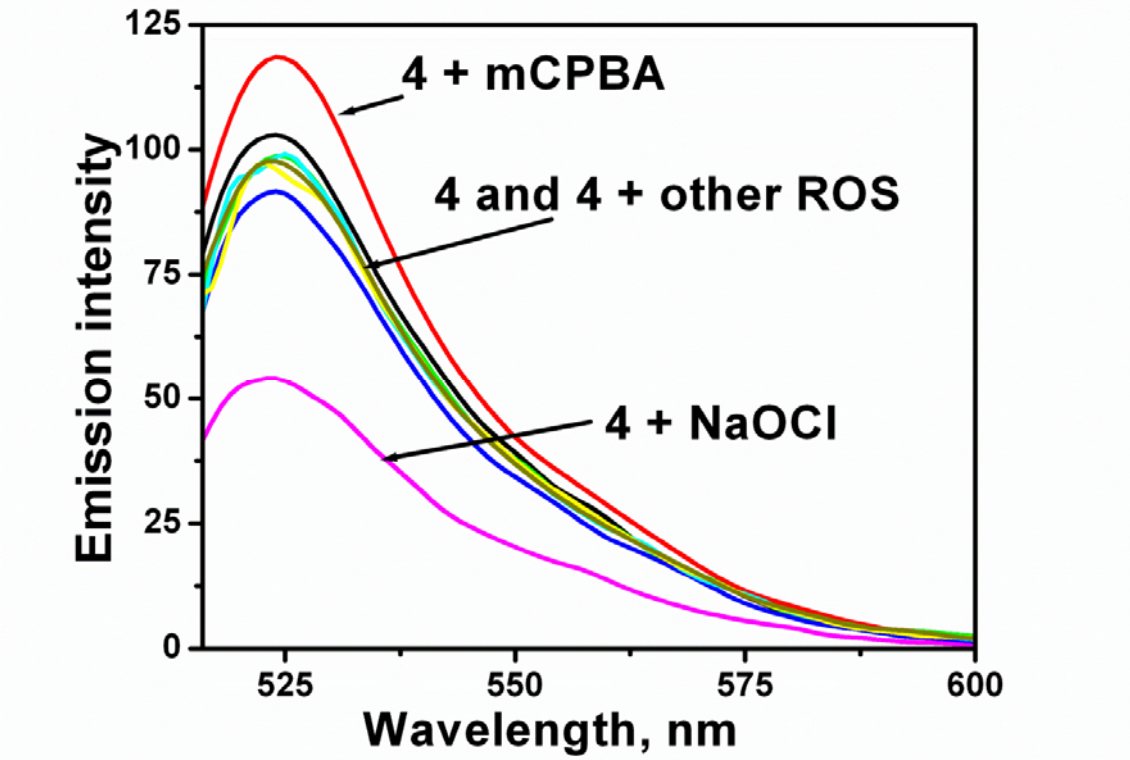

Figure S7. Emission spectra of compound **4** (1.0×10^{-6} M) with hypochlorite ion (1×10^{-3} M, ~15 equiv.) in acetonitrile (λ_{exc} = 506 nm).

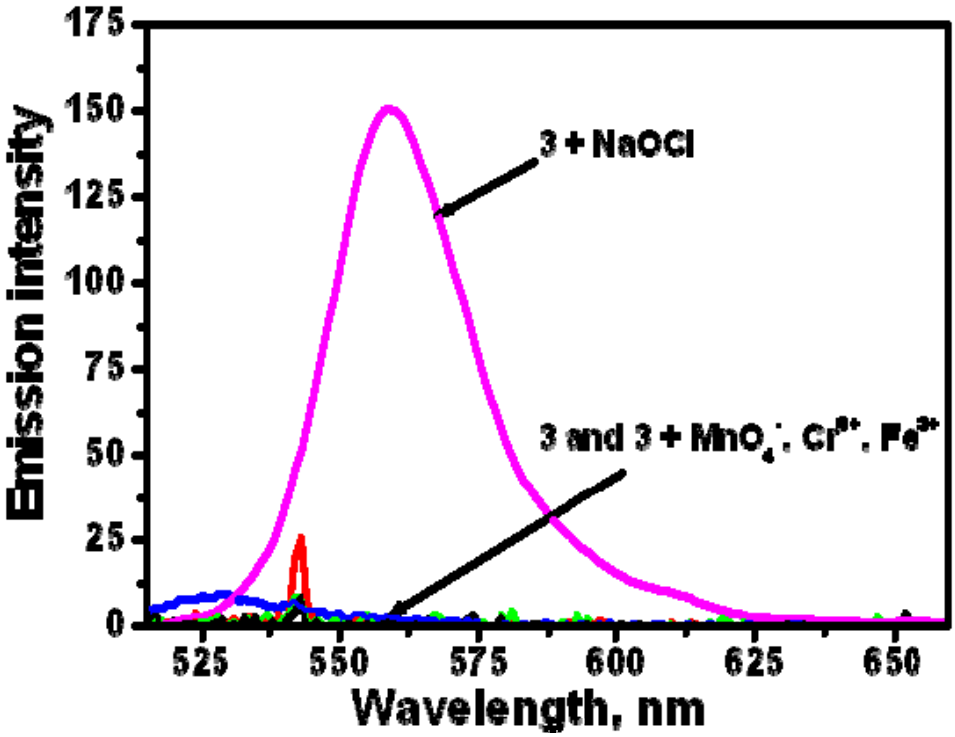

Figure S8. Emission spectra of compound **4** (1.0×10^{-6} M) with permanaganate, chromium VI, and iron III (1×10^{-3} M, ~15 equiv.) in acetonitrile (λ_{exc} = 506 nm).

Figure S9. Enlarged version of *Figure 4* from the text.

REFERENCES (Supporting Information)

1. (*a*) C. Brückner, V. Karunaratne, S. J. Rettig, D. Dolphin, *Can. J. Chem.* 1996, **74**, 2182. (*b*) R. W. Wagner, J. S. Lindsey, *Pure Appl. Chem.* 1996, **68**, 1373. (c) C. H. Lee, J. S. Lindsey, *Tetrahedron* 1994, **50**, 11427.

2. J. N. Demas, In Optical Radiation Measurements; K. D. Mielenz, Ed.; Academic Press: New York, **1982**; Vol. 3 (Measurment of Photoluminescence), 195–248.

3. A. K. Verma, J. Singh, R. Chaudhary, *Tetrahedron Letters* 2007, **48**, 7199.

4. I. –H. Kim, C. Morriseau, T. Watanabe, B. D. Hammock, *J. Med. Chem.* 2004, **47**, 2110.

— END —