Supporting Information

Novel BODIPY-Based Fluorescence Turn-On Sensor for Fe^{3+} and its Bioimaging Application in Living Cells

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Experimental section

Reagents

All reagents and solvents were ordered from commercial suppliers and used without further purification. All metal cations were purchased as their chloride, sulfate or nitrate salts and used as received. Compound **2**, 2,2'-((2-(2-methoxyethoxy)phenyl)azanediyl)diethanol, was synthesized according to a known procedure.[1]

Instruments

^{1}H and ^{13}C NMR spectra were carried out in $CDCl_3$ solution on a Bruker AVANCE spectrometer (400 MHz). An Agilent 8453 spectrophotometer was used for UV-vis absorption spectra measurements. An FLS980 fluorescence spectrometer was used to record fluorescence emission spectra. Fluorescence microscopy images were recorded on an Olympus IX-81 DSU microscope equipped with a Hamamatsu EM-CCD C9100 digital camera using a FITC filter cube (Ex: 477/50; DM: 507; Em: 536/40) and a 20x (Olympus LUCplanFLN 20x, N.A. = 0.45) objective lens.

Synthesis

*Synthesis of compound **3***

To a solution of **2** (2.55 g, 10 mmol) in dry DMF (20 mL) was added $POCl_3$ (7.77 g, 50 mmol) dropwise under argon atmosphere at 0 °C. The resulting mixture was warmed to room temperature and then slowly heated to 60 °C. After stirring at 60 °C overnight, the reaction mixture was cooled to room temperature and then slowly poured into ice water. The excess $POCl_3$ was quenched by the addition of Na_2CO_3 powder. The mixture was extracted with CH_2Cl_2

(100 mL × 2) and the combined extracts were concentrated. The residue was then dissolved in ethyl acetate (100 mL) and the solution was washed with brine (100 mL × 2), dried over $MgSO_4$, and evaporated. The crude mixture was purified by flash column chromatography on silica gel using hexane/ethyl acetate (4/1), giving the desired compound **3** as a light yellow liquid (1.60 g, 50.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.44 (s, 3H), 3.64 – 3.73 (m, 8H), 3.76 – 3.80 (m, 2H), 4.16 – 4.22 (m, 2H), 6.94 (d, *J* = 8.2, 1H), 7.35 – 7.43 (m, 2H), 9.80 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 41.44, 55.04, 59.12, 67.96, 70.79, 111.36, 117.17, 126.69, 130.11, 144.00, 150.69, 190.67. HRMS (ESI, m/z): calcd for $C_{14}H_{19}Cl_2NO_3$ ($[M+H]^+$) 320.0815; found 320.0830.

*Synthesis of compound **4***

To a solution of 1,10-diaza-18-crown-6 (2.62 g, 10 mmol) in 5 mL triethylamine and 40 mL anhydrous CH_2Cl_2 was added *o*-acetylsalicyloyl chloride (4.97 g, 25 mmol) in one portion at room temperature. A white suspension formed rapidly. The reaction mixture was stirred for 2 h at room temperature and then was diluted with CH_2Cl_2 (60 mL). The solution was transferred to a separation funnel and washed with brine (100 mL × 2), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/methanol (50/1) as the eluent to give **4** as a yellow liquid (4.94 g, 84.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.25 (d, *J* = 8.5, 6H), 3.44 – 3.87 (m, 24H), 7.13 – 7.20 (m, 2H), 7.21 – 7.26 (m, 2H), 7.27 – 7.32 (m, 2H), 7.36 – 7.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 20.99, 45.91, 49.49, 49.57, 69.57, 70.20, 70.65, 70.72, 123.15, 126.06, 127.67, 130.04, 130.18, 146.92, 168.44, 169.00. HRMS (ESI, m/z): calcd for $C_{30}H_{38}N_2O_{10}$ ($[M+H]^+$) 587.2599; found 587.2605.

*Synthesis of compound **5***

80 ml aqueous methanol (1:1) was added to a mixture of compound **4** (4.11 g, 7 mmol), ammonium acetate (2.16 g, 28 mmol) and $NaHCO_3$ (2.35 g, 28 mmol). The resulting mixture was heated to reflux and stirred overnight. After cooling to room temperature, the reaction mixture was diluted with 40 mL water and then filtered. The white residue was washed with H_2O and dried under vacuum, providing the desired compound **5** as a white solid (3.24 g, 92.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.64 (s, 8H), 3.79 (t, J = 2.7, 16H), 6.81 – 6.86 (m, 2H), 6.97 (d, J = 8.0, 2H), 7.28 – 7.33 (m, 4H), 9.22 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 69.66, 70.74, 118.00, 118.59, 118.82, 127.86, 132.28, 157.90, 172.14. HRMS (ESI, m/z): calcd for $C_{26}H_{34}N_2O_8$ ($[M+H]^+$) 503.2388; found 503.2407.

*Synthesis of compound **6***

A solution of compound **3** (0.32 g, 1.0 mmol) and KI (0.37 g, 2.2 mmol) in 100 ml dry DMF was heated to 60 °C and stirred for 2 h at that temperature to make solution 1. A mixture of compound **5** (0.50 g, 1.0 mmol) and Cs_2CO_3 (0.98 g, 3.0 mmol) in 100 ml dry DMF was degased by argon for 30 min and then heated to 105 °C. After stirring for 1.5 h, the resulting mixture was added solution 1 dropwise over 4 h under argon atmosphere. The reaction mixture was stirred at 105 °C for 6 d. After cooling to room temperature, DMF was removed under reduced pressure. The residue was added 20 mL water and extracted with CH_2Cl_2 (20 mL × 2). The combined extracts were washed three times with water and then once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel using ethyl acetate/methanol (20/1) provided **6** as a yellow solid (0.15 g, 20.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.34 – 4.21 (m, 39H), 6.90 (d, J = 8.1,

2H), 6.95 – 7.03 (m, 3H), 7.16 – 7.21 (m, 2H), 7.27 – 7.32 (m, 2H), 7.35 – 7.41 (m, 2H), 9.77 (d, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 46.58, 49.22, 54.33, 59.02, 59.12, 67.68, 69.04, 69.59, 70.84, 70.91, 71.17, 71.32, 110.33, 112.60, 113.28, 115.56, 121.40, 121.85, 126.74, 127.29, 127.49, 127.77, 129.33, 130.47, 145.11, 150.19, 154.34, 154.57, 169.66, 169.86, 190.69. HRMS (ESI, m/z): calcd for $C_{40}H_{51}N_3O_{11}$ ($[M+H]^+$) 750.3596; found 750.3613.

*Synthesis of sensor **1***

To a solution of compound **6** (0.38 g, 0.5 mmol) and 2,4-dimethylpyrrole (0.11 g, 1.1 mmol) in degased CH_2Cl_2 (50 mL) was added 2 drops of trifluoroacetic acid under argon atmosphere. The mixture was stirred at ambient temperature overnight, then a mixture of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (0.14 g, 0.6 mmol) in CH_2Cl_2 (10 mL) was added. The reaction mixture was stirred continuously for another 4 h. After the addition of 3 mL triethylamine, 3 mL $BF_3{\cdot}OEt_2$ was added dropwise to the mixture. The mixture was kept stirring at ambient temperature overnight, then filtered through a celite pad. The residue was washed with 20 mL CH_2Cl_2 and the combined filtrate was rotary evaporated to dryness. The residue was dissolved in 30 mL CH_2Cl_2 and the solution was washed with 30 mL 5% aqueous $NaHCO_3$ solution followed with water (30 mL × 2). The organic portion was dried over anhydrous $MgSO_4$, filtered and then evaporated *in vacuo*. The crude product was purified by flash column chromatography on silica gel using CH_2Cl_2/methanol (25/1) as the eluent to give **1** as a red solid (0.063 g, 13.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.48 (s, 6H), 2.55 (s, 6H), 2.95 – 4.34 (m, 39H), 5.98 (s, 2H), 6.76 – 7.02 (m, 6H), 7.08 – 7.24 (m, 3H), 7.26 – 7.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 14.46, 14.57, 14.73, 46.64, 49.25, 49.64, 54.45, 59.12, 59.18, 67.79, 68.38, 68.98, 69.66, 69.95, 70.19, 70.82, 70.88, 70.99, 71.14, 71.24, 71.37, 112.40, 112.62, 112.97, 114.05, 119.75,

121.03, 121.18, 121.28, 121.51, 121.59, 126.83, 127.25, 127.62, 127.78, 128.63, 128.83, 130.37, 131.78, 138.75, 139.92, 141.66, 143.09, 152.45, 152.87, 154.49, 154.67, 155.54, 169.70, 169.83. HRMS (ESI, m/z): calcd for $C_{52}H_{64}N_5O_{10}BF_2$ ($[M+H]^+$) 968.4796; found 968.4832.

Cell culture for imaging

Epithelial colorectal carcinoma cells (HCT-116) were cultured in RPMI cell medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin at 37 °C and 5% CO_2. Then cells were seeded onto a 40 mm glass slide, which was later mounted onto a bioptics live cell imaging chamber after cells were incubated with a 20 μM solution of sensor **1** in MEM medium for 10 min. For the Fe^{3+} sensing experiment, a 30 μM Fe^{3+} solution in MEM medium was pumped into cell chambers. For the control experiment, only MEM medium was pumped into cell chambers. Cells were kept at 37 °C and imaged with Olympus IX-81 DSU microscope every 10 min.

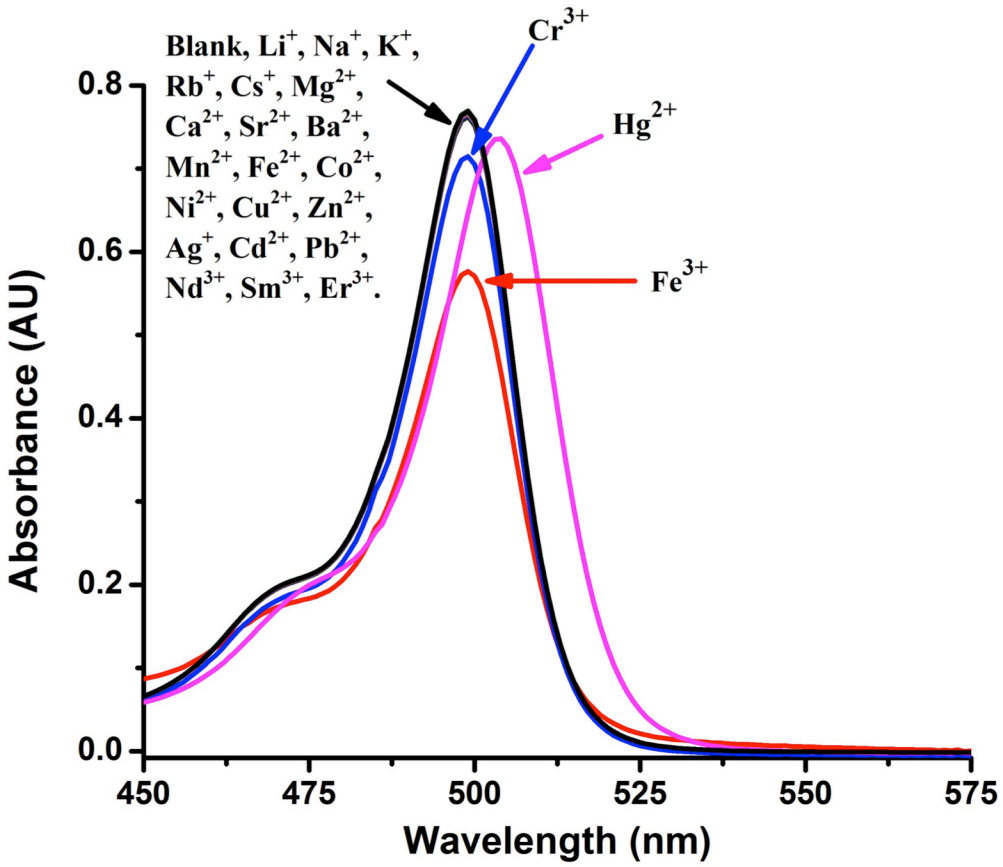

Figure S1. UV-vis absorption spectra of sensor **1** (7 μM) in H_2O-CH_3CN (9:1, v/v) upon addition of 100 equiv. of various metal cations.

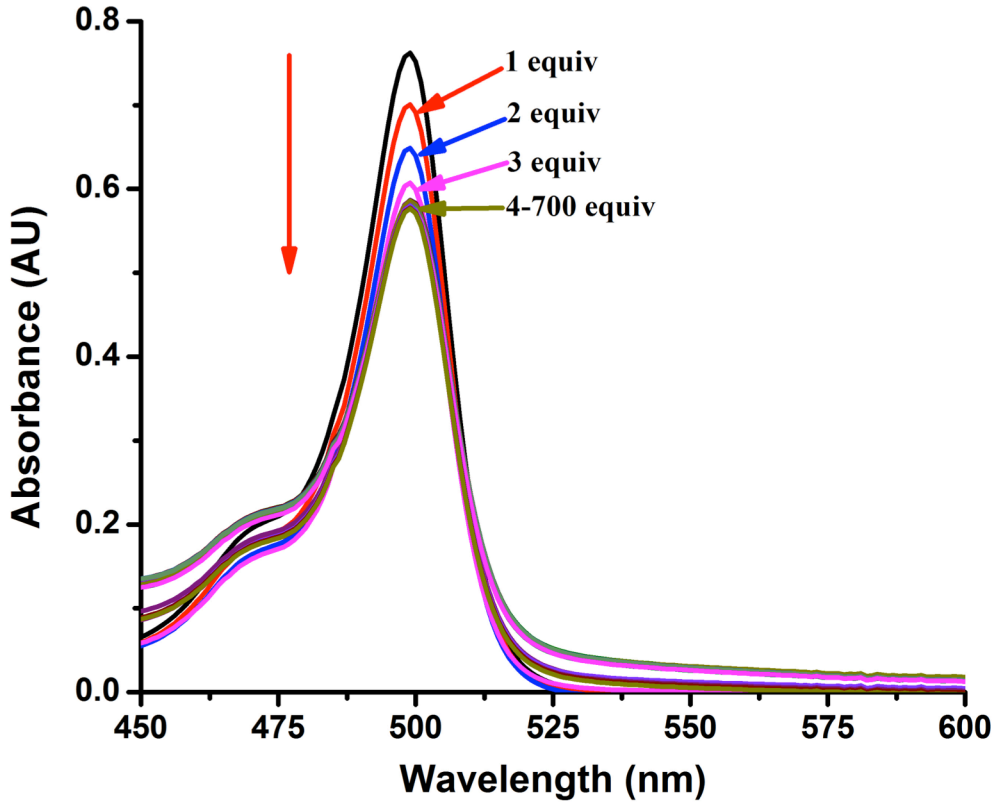

Figure S2. UV-vis spectral changes of sensor **1** (7 μM) in H_2O-CH_3CN (9:1, v/v) observed upon addition of 1-700 equiv. of Fe^{3+} ions.

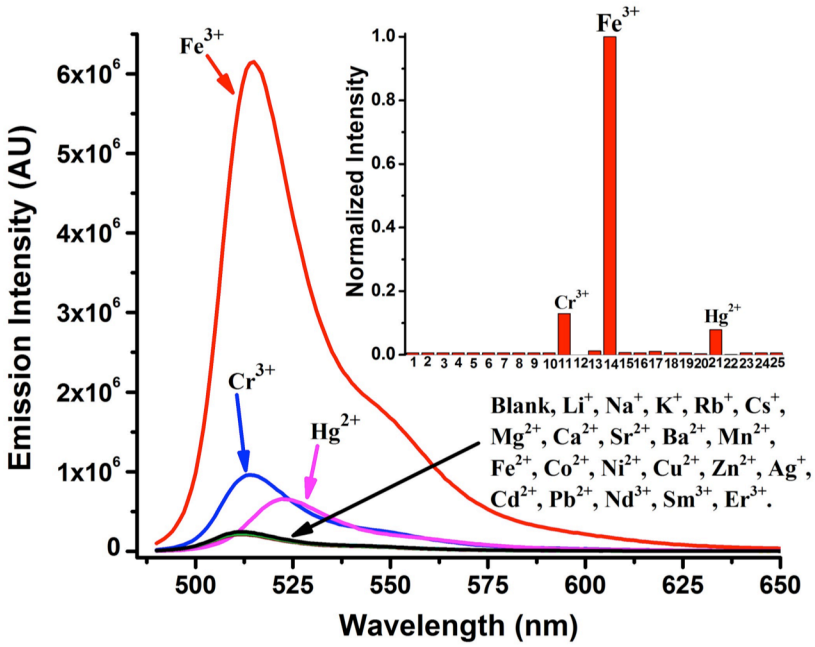

Figure S3. Fluorescence emission spectra of **1** (7 μM) in H_2O-CH_3CN (9:1, v/v) upon addition of 100 equiv. of various metal cations. Inset: normalized intensity of fluorescence emission triggered by different metal cations. 1, blank; 2, Li^+; 3, Na^+; 4, K^+; 5, Rb^+; 6, Cs^+; 7, Mg^{2+}; 8, Ca^{2+}; 9, Sr^{2+}; 10, Ba^{2+}; 11, Cr^{3+}; 12, Mn^{2+}; 13, Fe^{2+}; 14, Fe^{3+}; 15, Co^{2+}; 16, Ni^{2+}; 17, Cu^{2+}; 18, Zn^{2+}; 19, Ag^+; 20, Cd^{2+}; 21, Hg^{2+}; 22, Pb^{2+}; 23, Nd^{3+}; 24, Sm^{3+}; 25, Er^{3+}. λ_{ex} = 480 nm.

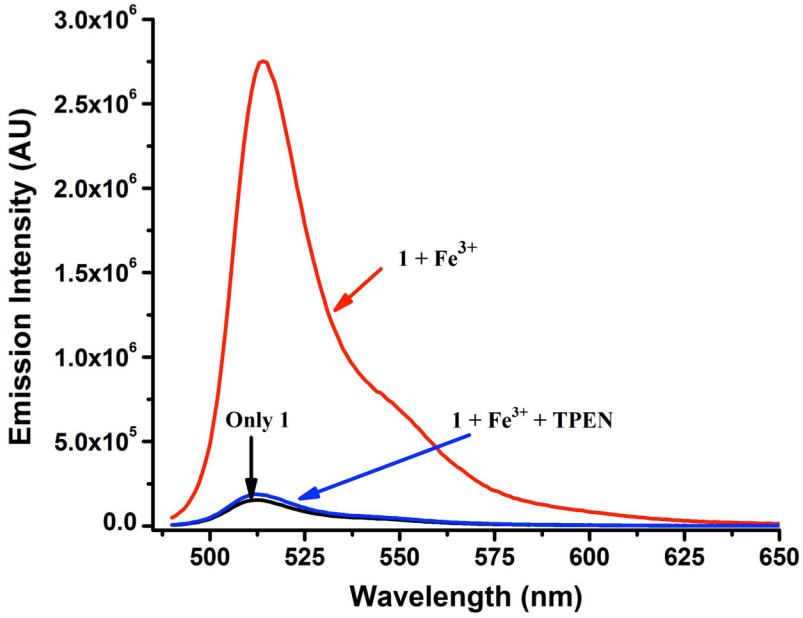

Figure S4. Fluorescence response reversibility of **1** (7 μM) in H_2O-CH_3CN (9:1, v/v). Fluorescence emission spectra of free sensor **1**, **1** with 20 equiv. of Fe^{3+}, and **1** with 20 equiv. of Fe^{3+} and 60 equiv. of TPEN. λ_{ex} = 480 nm.

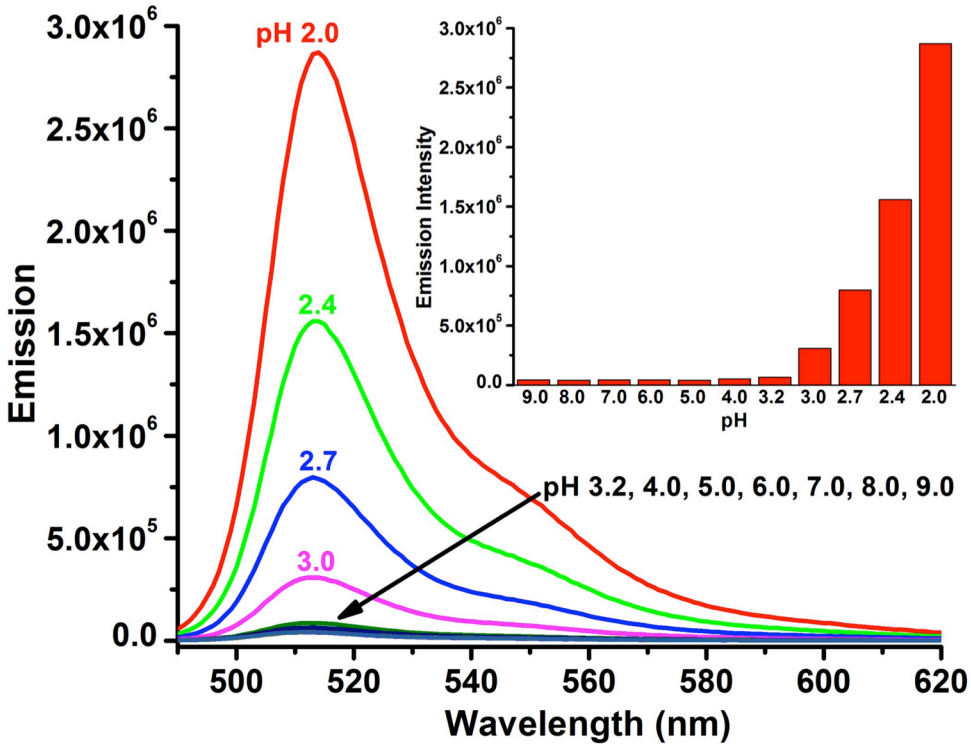

Figure S5. Fluorescence emission spectra of sensor **1** (7 μM) in H_2O-MeCN (9:1, v/v) at different pH, λ_{ex} = 480 nm.

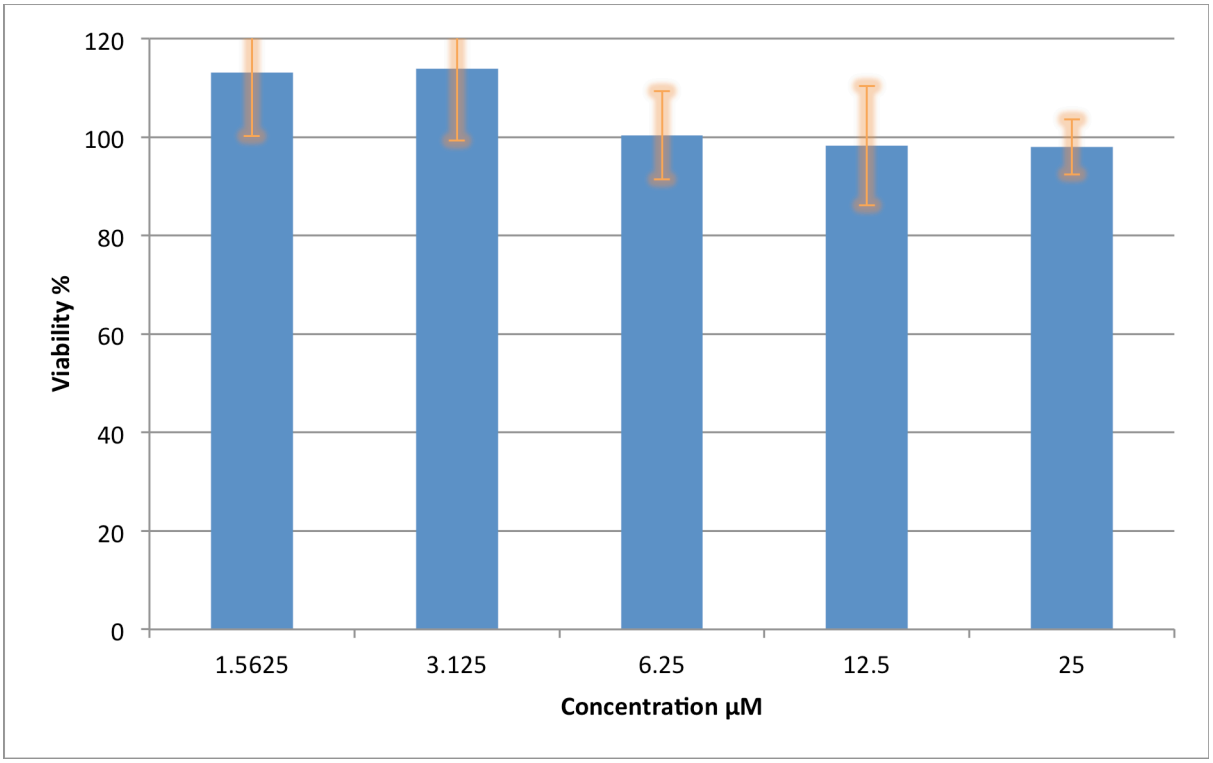

Figure S6. Viability of HCT-116 cells incubated with different concentrations of sensor **1**.

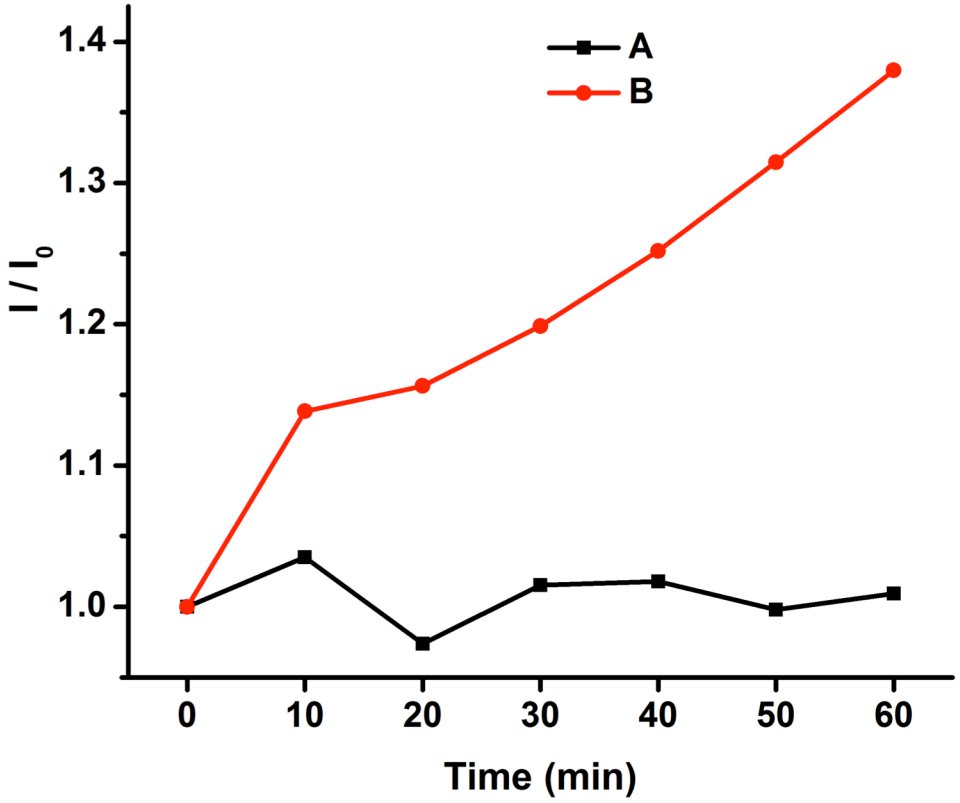

Figure S7. Average fluorescence intensity of cell images at different time. (A) Control experiment; (B) Fe^{3+} sensing experiment.

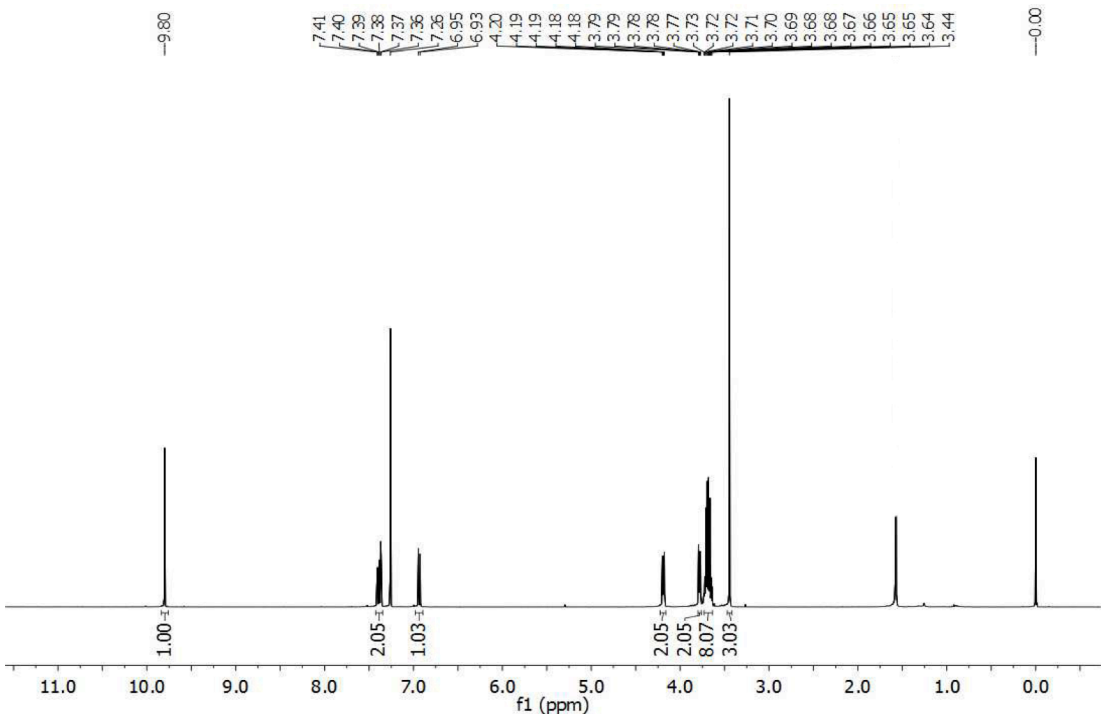

Figure S8. ^{1}H NMR spectrum (400 MHz) of **3** in $CDCl_3$.

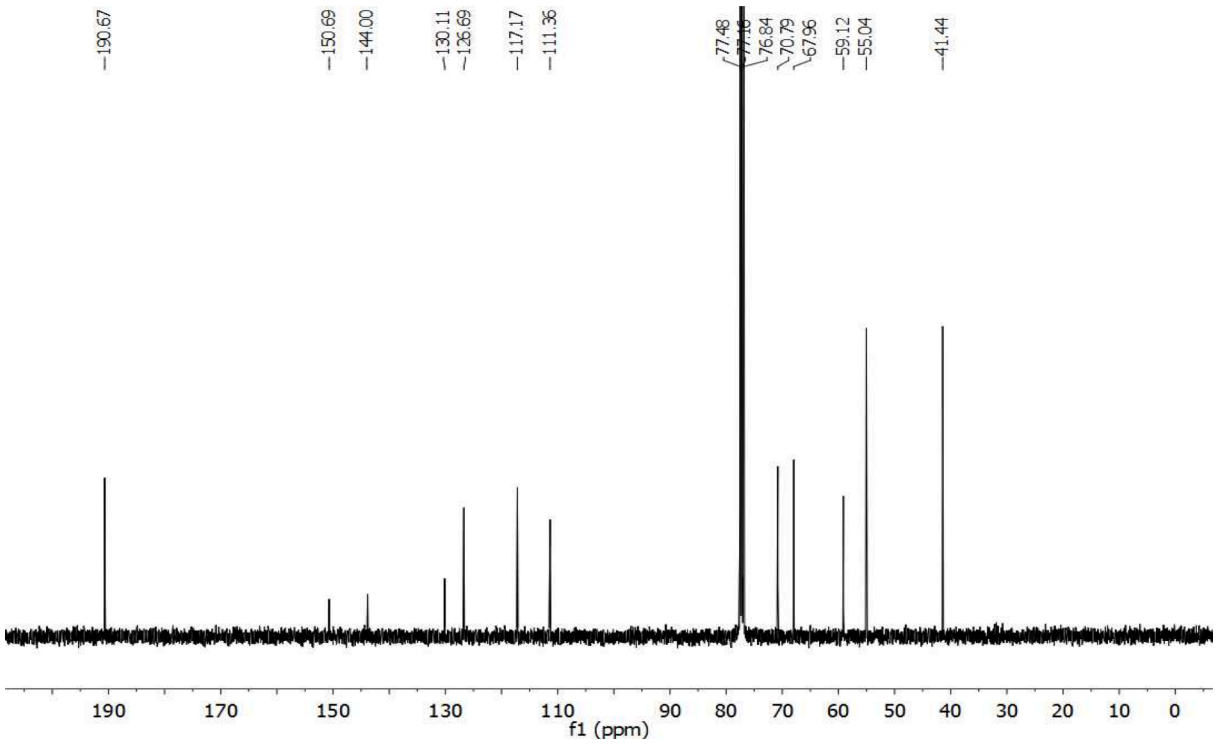

Figure S9. ^{13}C NMR spectrum (101 MHz) of **3** in $CDCl_3$.

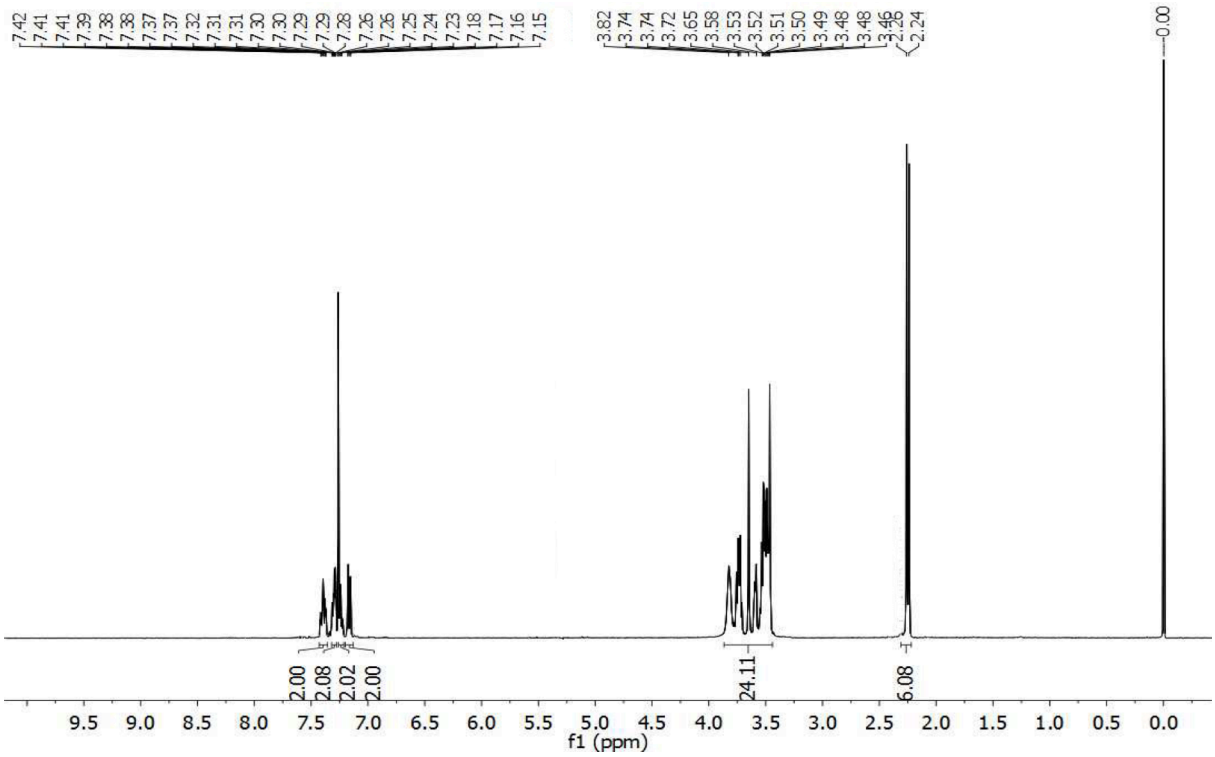

Figure S10. ^{1}H NMR spectrum (400 MHz) of **4** in $CDCl_3$.

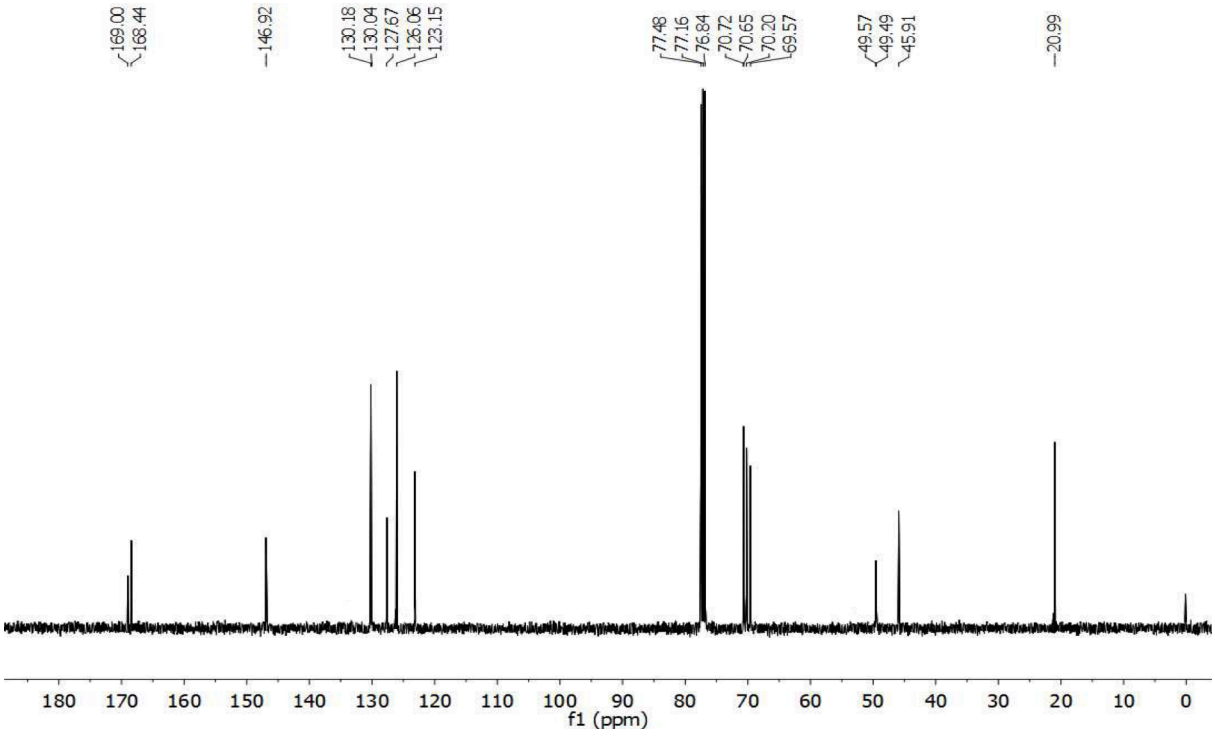

Figure S11. ^{13}C NMR spectrum (101 MHz) of **4** in $CDCl_3$.

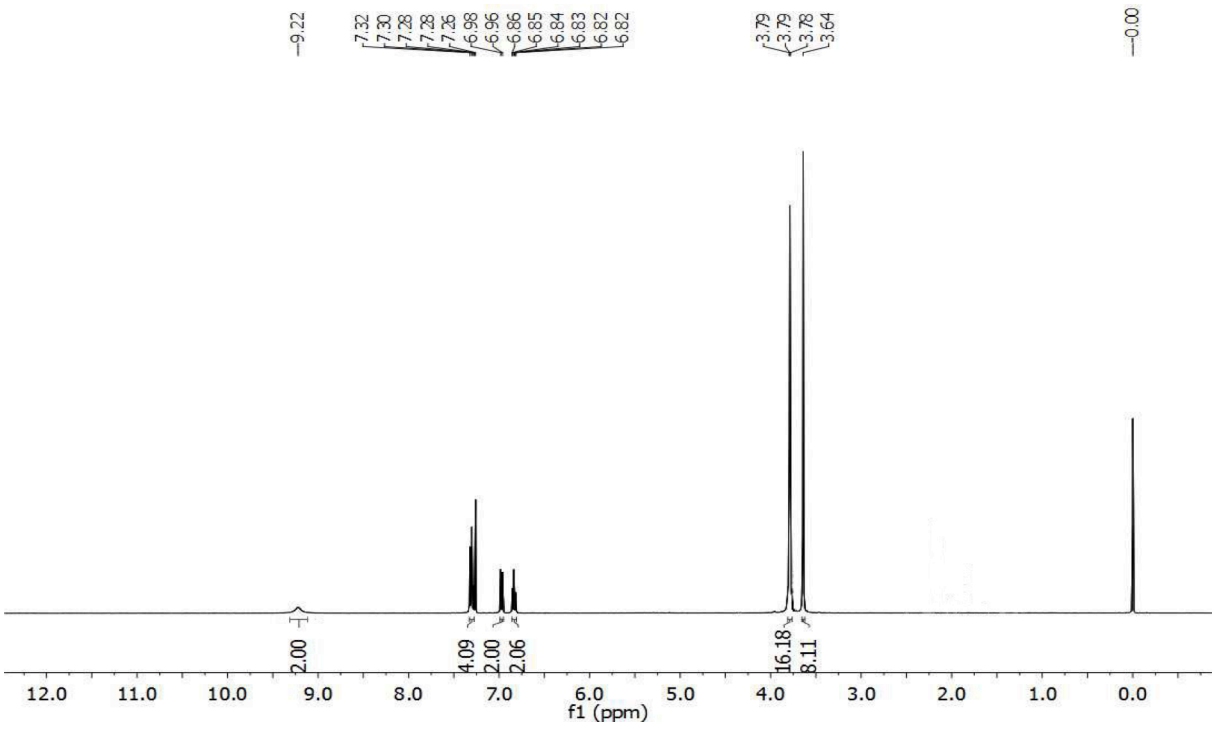

Figure S12. ^{1}H NMR spectrum (400 MHz) of **5** in $CDCl_3$.

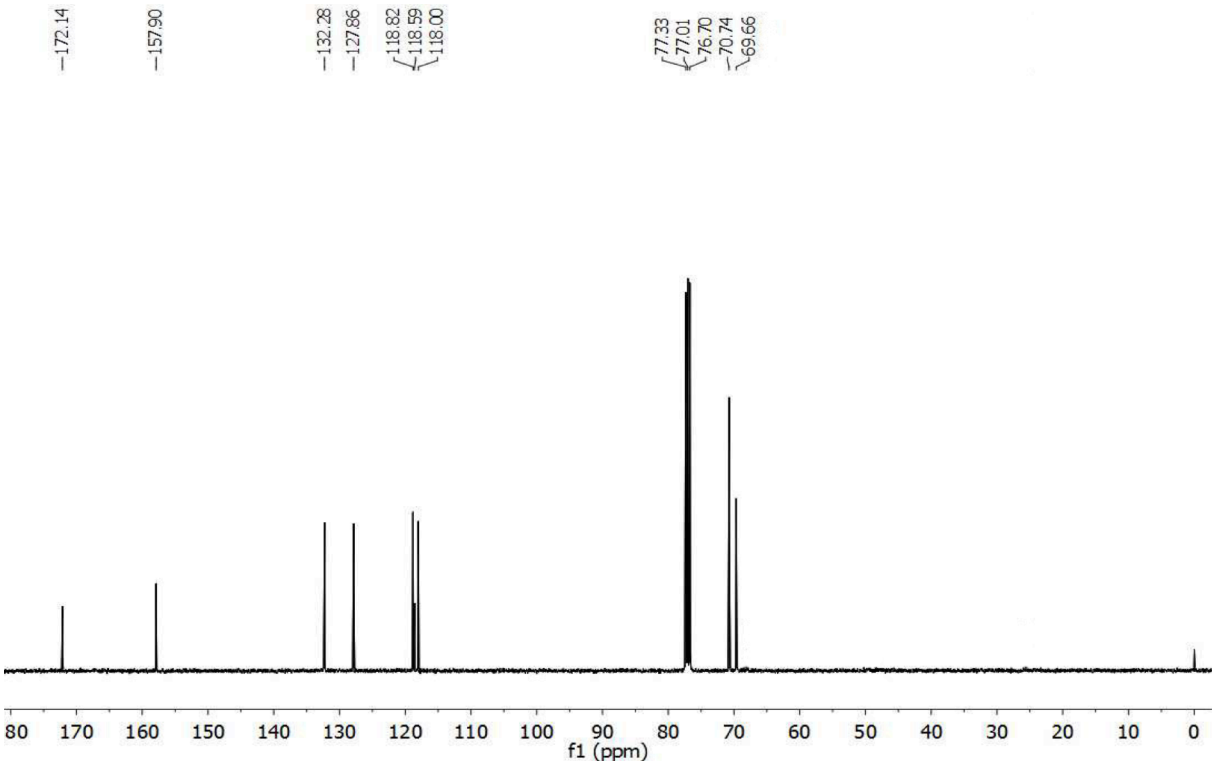

Figure S13. ^{13}C NMR spectrum (101 MHz) of **5** in $CDCl_3$.

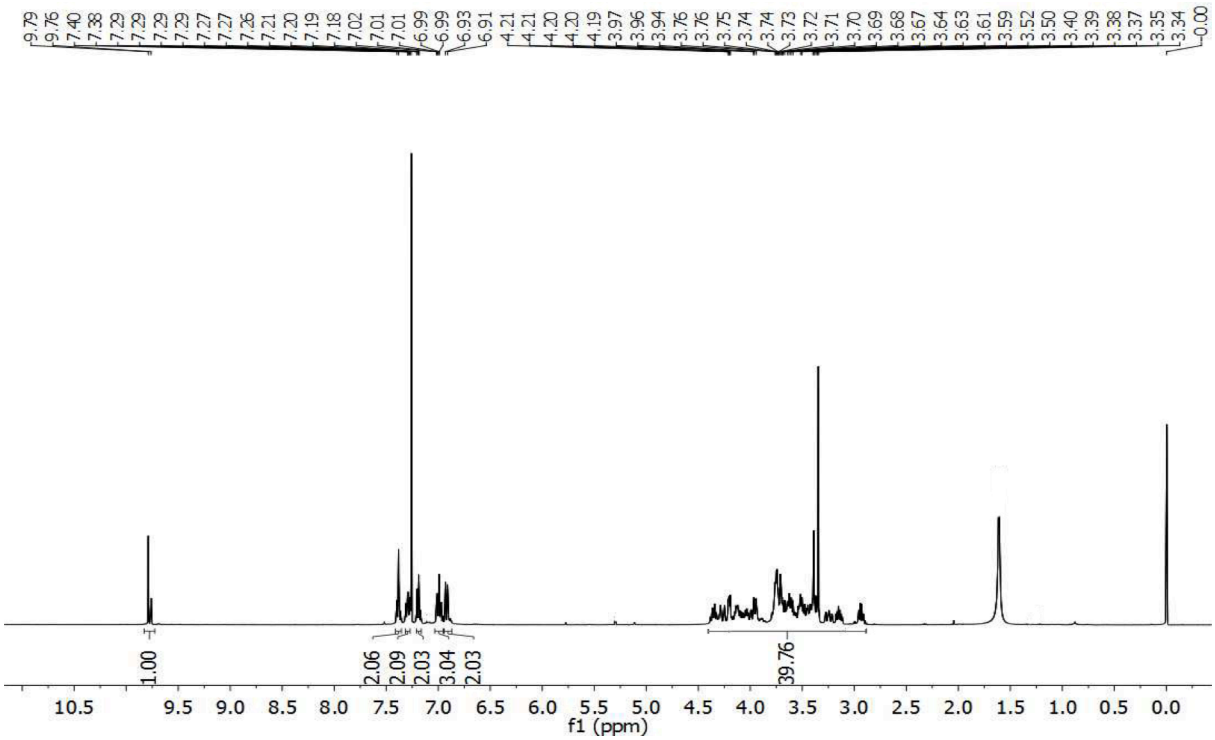

Figure S14. ^{1}H NMR spectrum (400 MHz) of **6** in $CDCl_3$.

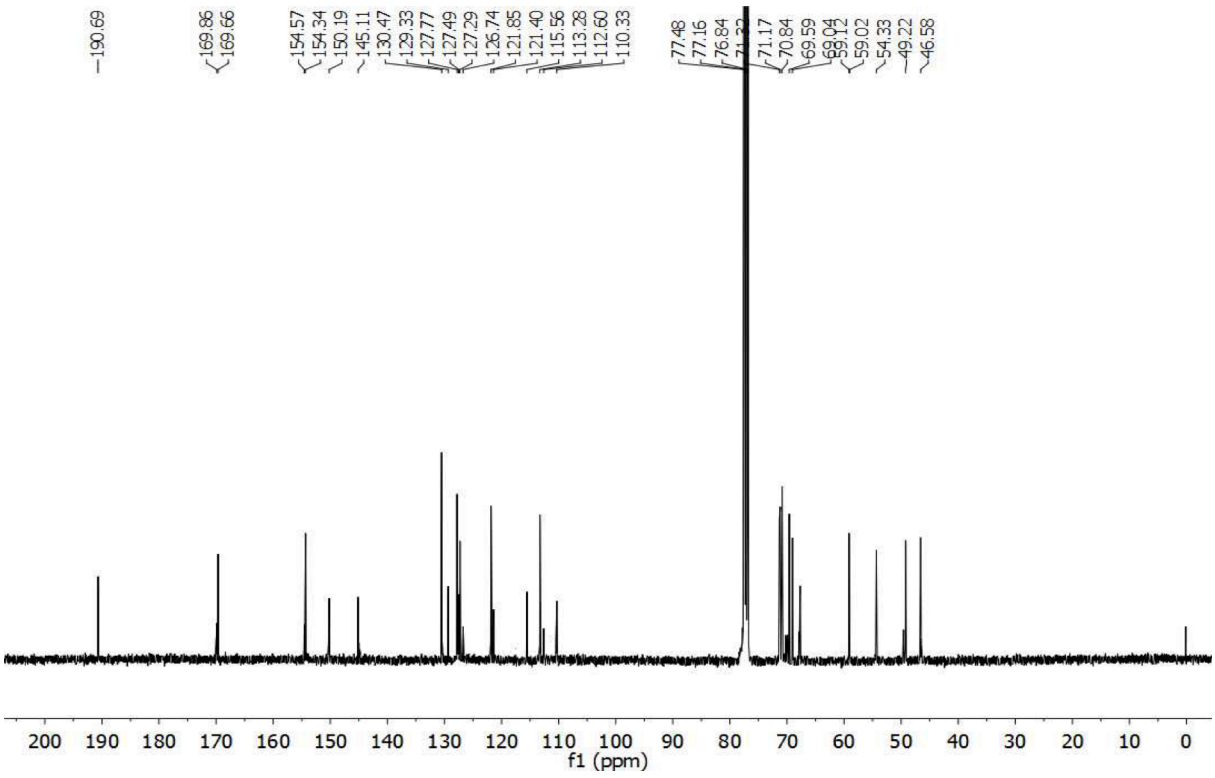

Figure S15. ^{13}C NMR spectrum (101 MHz) of **6** in $CDCl_3$.

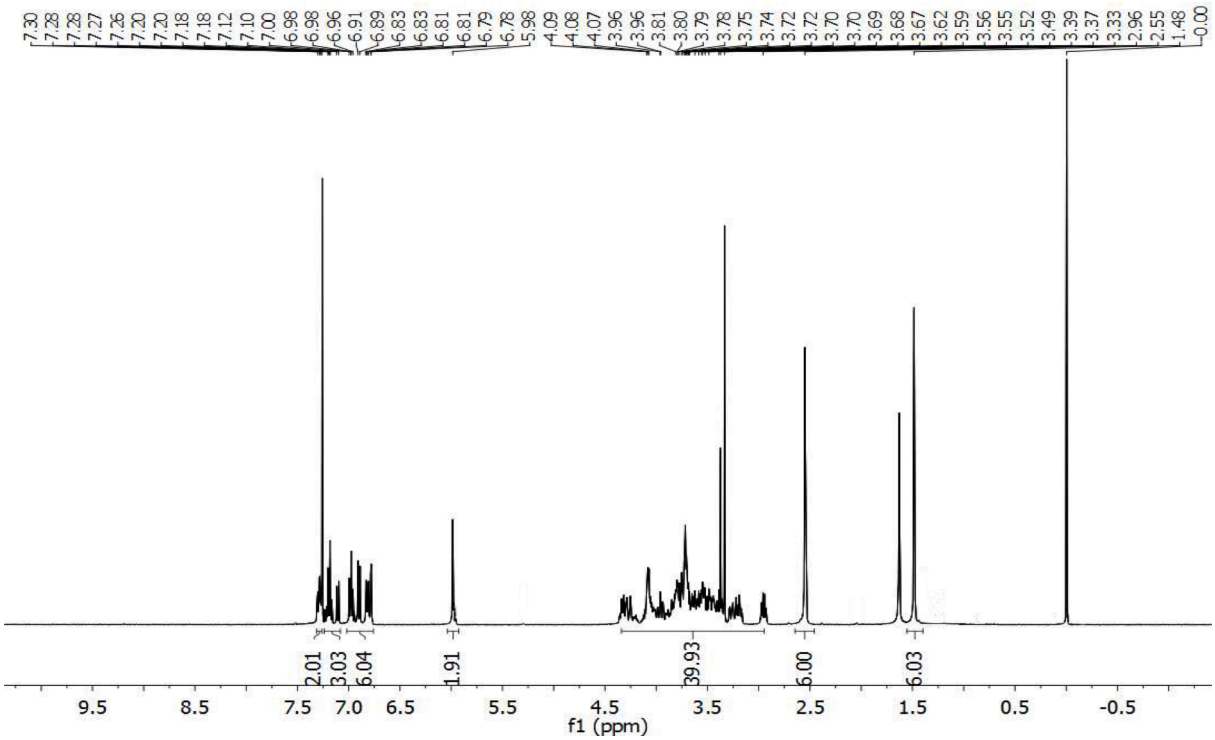

Figure S16. ^{1}H NMR spectrum (400 MHz) of **1** in $CDCl_3$.

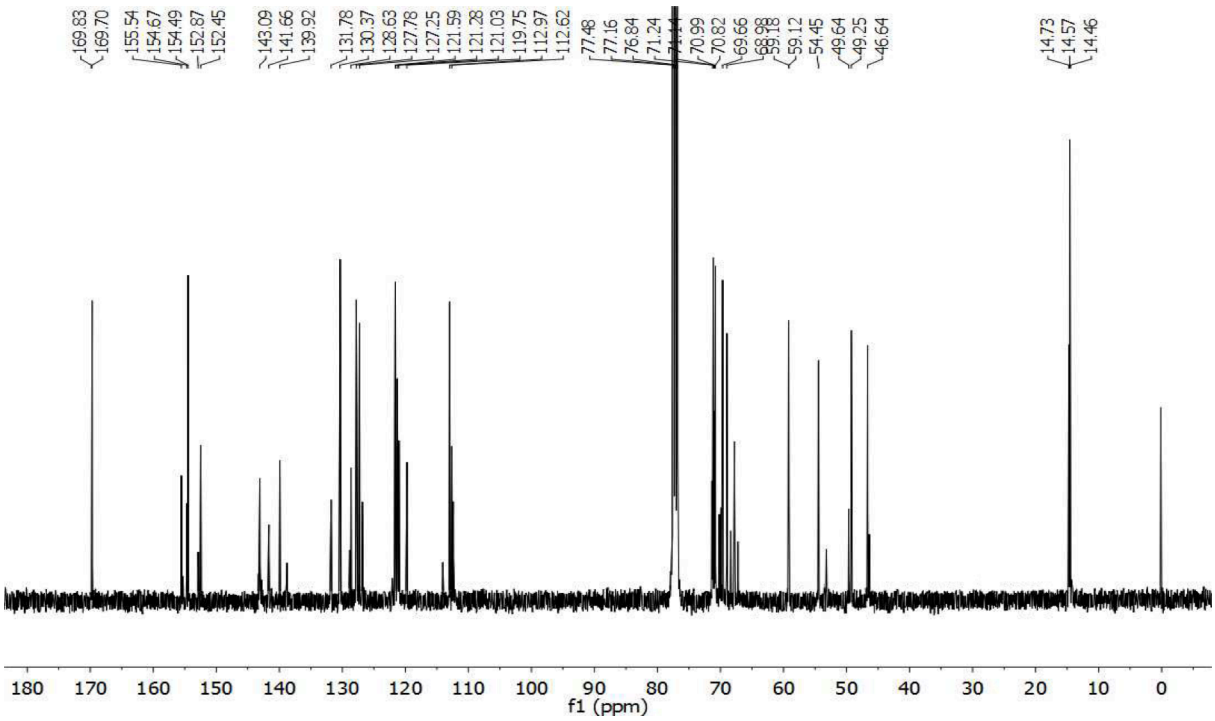

Figure S17. ^{13}C NMR spectrum (101 MHz) of **1** in $CDCl_3$.

References

(1) Ast, S.; Schwarze, T.; Müller, H.; Sukhanov, A.; Michaelis, S.; Wegener, J.; Wolfbeis, O. S.; Körzdörfer, T.; Dürkop, A.; Holdt, H.-J. A Highly K^+-Selective Phenylaza-[18]crown-6-Lariat-Ether-Based Fluoroionophore and Its Application in the Sensing of K^+ Ions with an Optical Sensor Film and in Cells. *Chem. Eur. J.* **2013**, *19*, 14911-14917.