Supporting Information

Highly Water-soluble BODIPY-based Fluorescent Probes for Sensitive Fluorescent Sensing of Zinc (II)

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Optical measurements and buffer conditions:

All buffer solutions were prepared by using deionized distilled water. Three different buffers at concentration of 10 mM were used to evaluate effect of pH on the fluorescent properties of fluorescent probes **A**, **B** and **C**. These buffers are citric acid buffer (acidic range), HEPES buffer (neutral range), tris buffer (basic range). No meaurable changes of optical property of fluorescent probe **A**, **B** or **C** were found when differnent buffers (citric, HEPES or tris) were used at same pH values.

Rhodamine 6G ($\Phi_f = 0.95$ in ethanol)[1, 2] was used as a reference standard to determine the fluorescence quantum yields of BODIPY dye **3**, **5** and probe **A**, **B**, **C**. The corresponding fluorescence spectra of fluorescent probes **A**, **B** and **C** were collected at the excitation wavelength of 470 nm. Both the samples and references were prepared fresh under identical conditions. The quantum yields were calculated using the following equation:

$$\Phi_X = \Phi_{st}(\mathrm{Grad}_X/\mathrm{Grad}_{st})(\eta_X^2/\eta_{st}^2)$$

Where the subscripts ‘st’ and ‘x’ stand for standard and test, respectively, Φ is the fluorescence quantum yield, Grad represents the gradient from the plot of integrated fluorescence intensity versus absorbance and η is the refractive index of the solvent

Synthetic Approaches to BODIPY dyes:

Materials. Unless otherwise indicated, all reagents and solvents were obtained from commercial suppliers (Aldrich, Sigma, Fluka, Acros Organics, Fisher Scientific, Lancaster) and used without further purification. Air- and moisture-sensitive reactions were conducted in oven-dried glassware using a standard Schlenk line or drybox techniques under an inert atmosphere of dry nitrogen. Aldehyde derivative (**1**) and highly water-soluble BODIPY dye (**2**) were prepared according to our reported procedure.[3]

4,4-Difluoro-8-[3,4-bis(3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}-2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxymethyl}propoxy)phenyl]-1,3,5,7-tetramethyl-2-formyl-4-bora-3a,4a-diaza-s-indacene (3).

A mixture of DMF (2.0 mL) and $POCl_3$ (1.0 mL) was stirred on ice for five minutes under argon atmosphere. The mixture was warmed to room temperature and was stirred further for 30 minutes. After adding compound **2** (300 mg, 0.27 mmol) in dichloroethane (50 mL) to the reaction mixture, the resulting mixture was stirred at 50 ^oC for two hours. The reaction mixture was cooled down to room temperature and then was slowly poured into saturated $NaHCO_3$ aqueous solution at 0 ^oC on ice. The mixture was warmed to room temperatureand was further stirred for 30 minutes and then washed with water. The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by using a mixed mobile phase (hexane: CH_2Cl_2: EtOAc: MeOH= 5:3:1:0.5, V/V) to attain BODIPY **3** (275 mg, 89 %) as red oil. ^1H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 6.09 (s, 1H), 4.03 (d, *J* = 5.6 Hz, 2H), 3.94 (d, *J* = 5.2 Hz, 2H), 3.61-3.15 (m, 68H), 2.84 (s, 3H), 2.56 (s, 3H), 2.43-2.37 (m, 1H), 2.23-2.17 (m, 1H), 1.75 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (100 For MHz, $CDCl_3$): δ 186.1, 162.0, 161.0, 156.6, 156.0, 147.2, 142.3, 141.6, 135.0, 130.5, 129.6, 126.1, 123.6, 115.5, 106.6, 100.6, 72.1, 70.8, 70.8, 70.8, 70.7, 70.7, 70.6, 70.5, 69.5, 69.2, 69.1, 66.7, 66.4, 59.2, 40.2, 39.7, 15.2, 14.8, 13.1, 11.3. HRMS (FAB) calcd for $C_{56}H_{92}BF_2N_2O_{19}$ $[M+H]^+$, 1145.6355; found, 1145.6335.

4,4-Difluoro-8-[3,4-bis(3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}-2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxymethyl}propoxy)phenyl]-1,3,5,7-tetramethyl-2,6-diformyl-4-bora-3a,4a-diaza-s-indacene (5).

BODIPY **5** was prepared using BODIPY dye **3** by a procedure similar to that used for preparing BODIPY dye **3** but the mixture was stirred at a higher temperature of 60 ^oC instead of 50 ^oC for five hours (82% of reaction yield). ^1H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 2H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.64 (dd, J = 8.8, 2.0 Hz, 1H), 6.59 (d, *J* = 2.0 Hz, 1H), 4.07 (d, *J* = 6.0 Hz, 2H), 3.97 (d, *J* = 5.2 Hz, 2H), 3.63-3.15 (m, 68H), 2.84 (s, 6H), 2.44-2.40 (m, 1H), 2.23-2.20 (m, 1H), 1.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.0, 162.5, 160.2, 156.5, 148.1, 145.6, 132.7, 129.3, 127.9, 114.8, 107.0, 100.9, 72.1, 70.9, 70.8, 70.8, 70.7, 70.5, 69.5, 69.1, 66.8, 66.5, 59.3, 59.2, 40.2, 39.6, 13.9, 11.8. HRMS (FAB) calcd for $C_{57}H_{92}BF_2N_2O_{20}$ $[M+H]^+$, 1173.6305; found, 1173.6310.

4,4-Difluoro-8-[3,4-bis(3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}-2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxymethyl}propoxy)phenyl]-1,3,5,7-tetramethyl-2-bis(2-pyridylmethyl)methanamino-4-bora-3a,4a-diaza-s-indacene (A).

After 10 mL of dry $ClCH_2CH_2Cl$ (10 mL) was added to a 50-mL flask containing BODIPY dye **3** (110 mg, 0.1 mmol) and 2-pyridinyl-N-(2-pyridinylmethyl)methanamine **4** (47 mg, 0.2 mmol), the mixture was stirred at 40 ^oC for four hours. When $NaBH(OAc)_3$ (40 mg, 0.18 mmol) and acetic acid (1 drop) were added to the flask at room temperature, the mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 and washed with water and brine, respectively. The organic layer was collected, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by TLC plate (hexane/EtOAc/CH_2Cl_2/MeOH, 5/1/3/0.5, v/v) to obtain BODIPY dye **A** (97 mg, 74%) as red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, *J* = 4.8 Hz, 2H), 7.59 (td, *J* = 7.6, 1.6 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 2H), 7.10-7.07 (m, 2H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.54 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.49 (d, *J* = 2.0 Hz, 1H), 5.87 (s, 1H), 4.01 (d, *J* = 5.6 Hz, 2H), 3.94-3.86 (m, 2H), 3.64-3.08 (m, 70H), 2.46 (s, 3H), 2.45 (s, 3H), 2.43-2.37 (m, 1H), 2.17-2.11 (m, 1H), 1.41 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.5, 159.9, 156.7, 155.7, 154.2, 149.0, 142.4, 141.4, 139.2, 136.5, 132.1, 131.7, 129.9, 127.1, 123.3, 122.2, 120.6, 116.7, 106.3, 100.5, 72.1, 70.8, 70.7, 70.7, 70.5, 70.5, 69.6, 69.2, 69.1, 66.6, 66.4, 60.7, 59.2, 48.4, 40.2, 39.6, 14.7, 14.2, 13.0, 11.9. HRMS (FAB) calcd for $C_{68}H_{105}BF_2N_5O_{18}$ $[M+H]^+$, 1328.7516; found, 1328.7502.

4,4-Difluoro-8-[3,4-bis(3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}-2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxymethyl}propoxy)phenyl]-1,3,5,7-tetramethyl-2,6-bis[bis(2-pyridylmethyl)-methanamino]-4-bora-3a,4a-diaza-s-indacene (B).

When 15 mL of dry $ClCH_2CH_2Cl$ (15 mL) was added to a 50-mL flask containing BODIPY dye **5** (100 mg, 0.085 mmol) and 2-pyridinyl-N-(2-pyridinylmethyl)methanamine **4** (90 mg, 0.45 mmol), The mixture was stirred at 40 ^{o}C for four hours. After NaBH(OAc)$_3$ (80 mg, 0.36 mmol) and acetic acid (2 drop) were added to the flask at room temperature, the mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 and washed with water and brine, respectively. The organic layer was collected, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by TLC plate using a mixed mobile phase (hexane/acetone/CH_2Cl_2/MeOH, 5/1/3/0.6, v/v) to obtain amino BODIPY dye **B** (68 mg, 52%) as red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (d, *J* = 4.8 Hz, 4H), 7.55 (t, *J* = 7.6 Hz, 4H), 7.32 (d, *J* = 7.6 Hz, 4H), 7.06-7.03 (m, 4H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.52 (d, *J* = 8.4 Hz, 1H), 6.46 (s, 1H), 4.00 (d, *J* = 5.2 Hz, 2H), 3.82 (d, *J* = 5.6 Hz, 2H), 3.61-3.01 (m, 72H), 2.43-2.35 (m, 7H), 2.06-2.03 (m, 1H), 1.35 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.5, 159.9, 156.7, 155.1, 149.0, 141.1, 138.9, 136.5, 131.6, 129.9, 126.9, 123.3, 122.2, 116.9, 106.4, 100.5, 72.1, 72.0, 70.8, 70.8, 70.7, 70.6, 70.6, 70.4, 70.4, 69.6, 69.1, 66.6, 66.5, 60.5, 59.2, 59.1, 48.3, 40.2, 39.6, 12.9, 11.8. HRMS (FAB) calcd for $C_{81}H_{118}BF_2N_8O_{18}$ $[M+H]^+$, 1539.8625; found, 1539.8622.

4,4-Difluoro-8-(4-methoxyphenyl)-3,5-dimethyl-2,6-bis[bis(2-pyridylmethyl)methanamino]-1,7-bis[(1E)-3,4-bis(3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}-2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxymethyl}-propoxy)phenyl]-4-bora-3a,4a-diaza-s-indacene (C).

A mixed solution of **8** (60 mg, 0.028 mmol) and 2-pyridinyl-N-(2-pyridinylmethyl)methanamine **4** (33 mg, 0.168 mmol) in 10 mL of dry $ClCH_2CH_2Cl$ was stirred at 50 ^{o}C for four hours. When NaBH(OAc)$_3$ (30 mg, 0.112 mmol) and acetic acid (1 drop) were added to the flask at room temperature, the mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 and washed with water and brine, respectively. The organic layer was collected, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by TLC plate (hexane/acetone/CH_2Cl_2/MeOH, 5/1/3/1, v/v) to obtain amino BODIPY dye **C** (46 mg, 65%) as dark purple oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, *J* = 5.2 Hz, 2H), 7.52 (t-like, *J* = 8.0Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.8 Hz ,2H), 7.05 (t-like, *J* = 6.0 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 16.0 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.50 (s, 2H), 6.44 (d, *J* = 8.4 Hz, 2H), 5.67 (d, *J* = 16.4 Hz, 2H), 3.98 (d, *J* = 5.6 Hz, 4H), 3.90 (d, *J* = 5.6 Hz, 4H), 3.73 (s, 3H), 3.62-3.44 (m, 108H), 3.34-3.31 (m, 28H), 2.51 (s, 6H), 2.43-2.36 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 164.6, 161.5, 153.6, 153.4, 146.5, 144.2, 140.1, 139.9, 137.9, 135.2, 134.7, 134.0, 128.4, 126.1, 125.7, 124.6, 122.4, 121.8, 116.9, 115.2, 114.0, 71.2, 71.1, 69.8, 69.7, 69.6, 69.6, 68.4, 58.6, 57.4, 57.3, 37.2, 37.1. HRMS (MAIDL) calcd for $C_{132}H_{200}BF_2N_8O_{37}$ $[M+H]^+$, 2538.4076; found, 2538.7638.

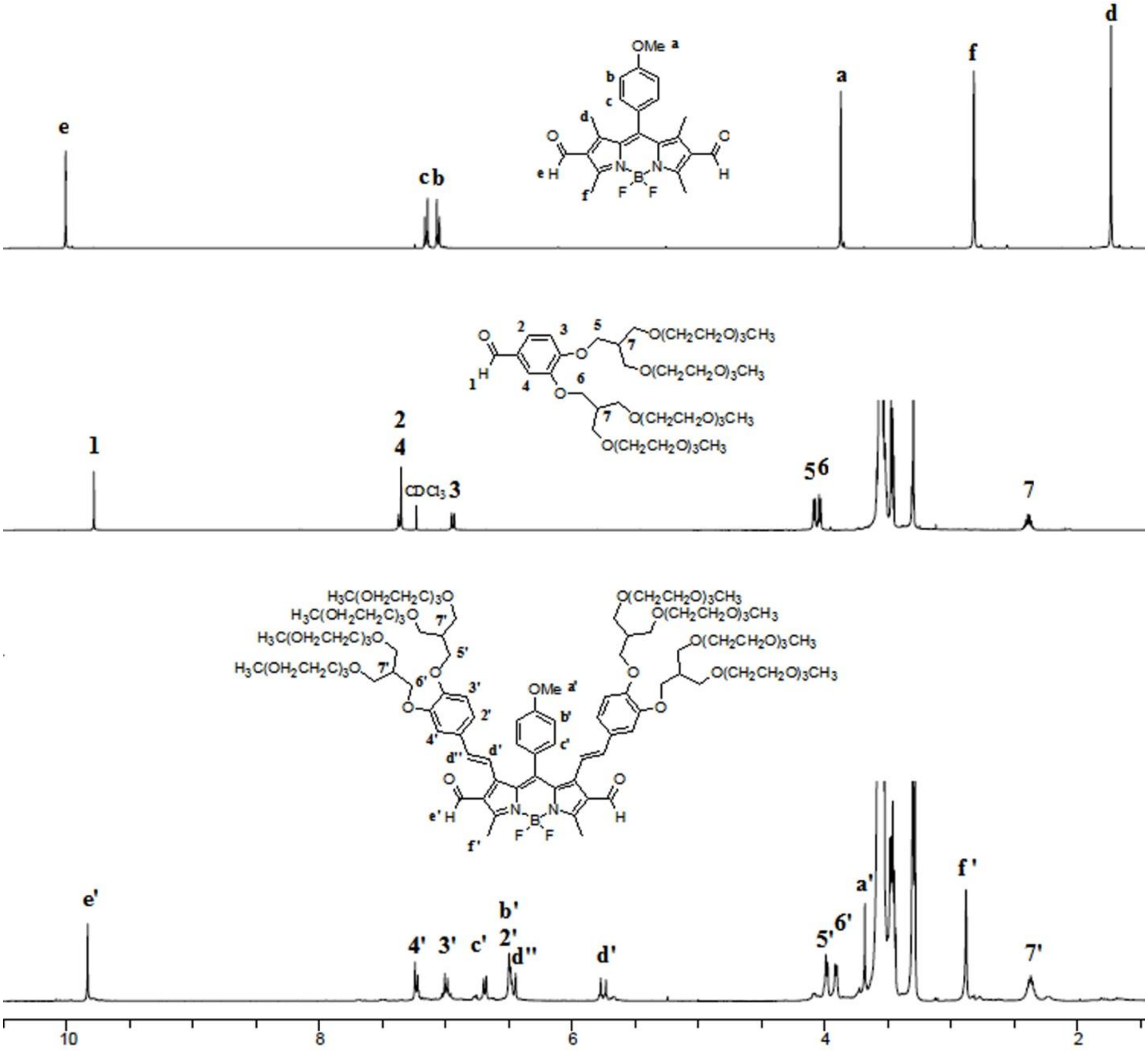

Figure S1. Comparing of ^{1}H spectra of BODIPY **6**, and **8** and compound **7**

^{1}H NMR spectra of BODIPY dye **6** and **8** were published in *RSC Advances*, 2011, 2, 404-407 (reproduction, copyright by Royal Chemical Society),[4] and ^{1}H NMR spectrum of compound **7** was published in *Org. Lett.*, 2011, 13, 438-441 (reproduction, copyright by the American Chemistry Society)[3]

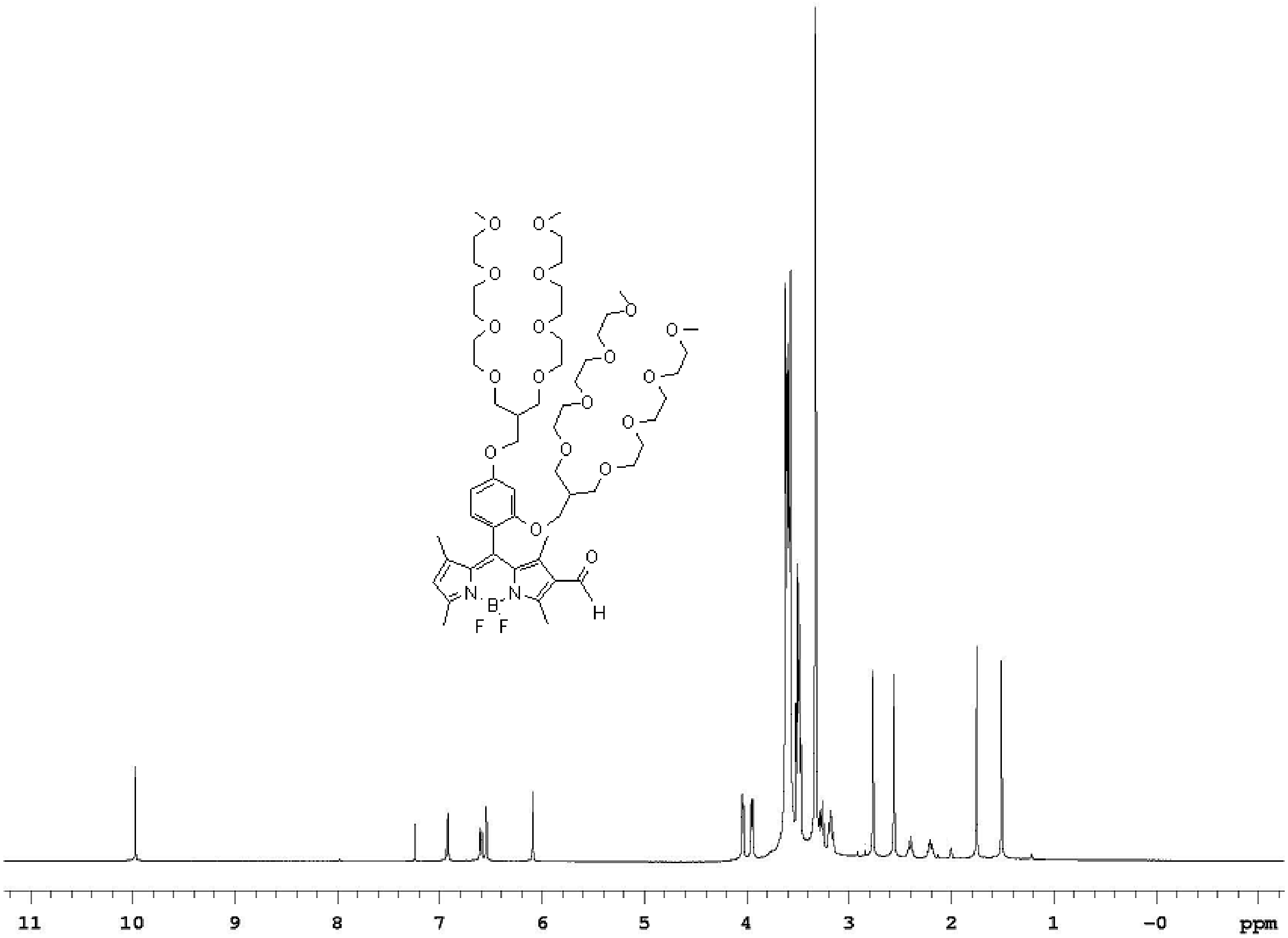

Figure S2. ^{1}H NMR spectrum of BODIPY dye **3** in $CDCl_3$ solution.

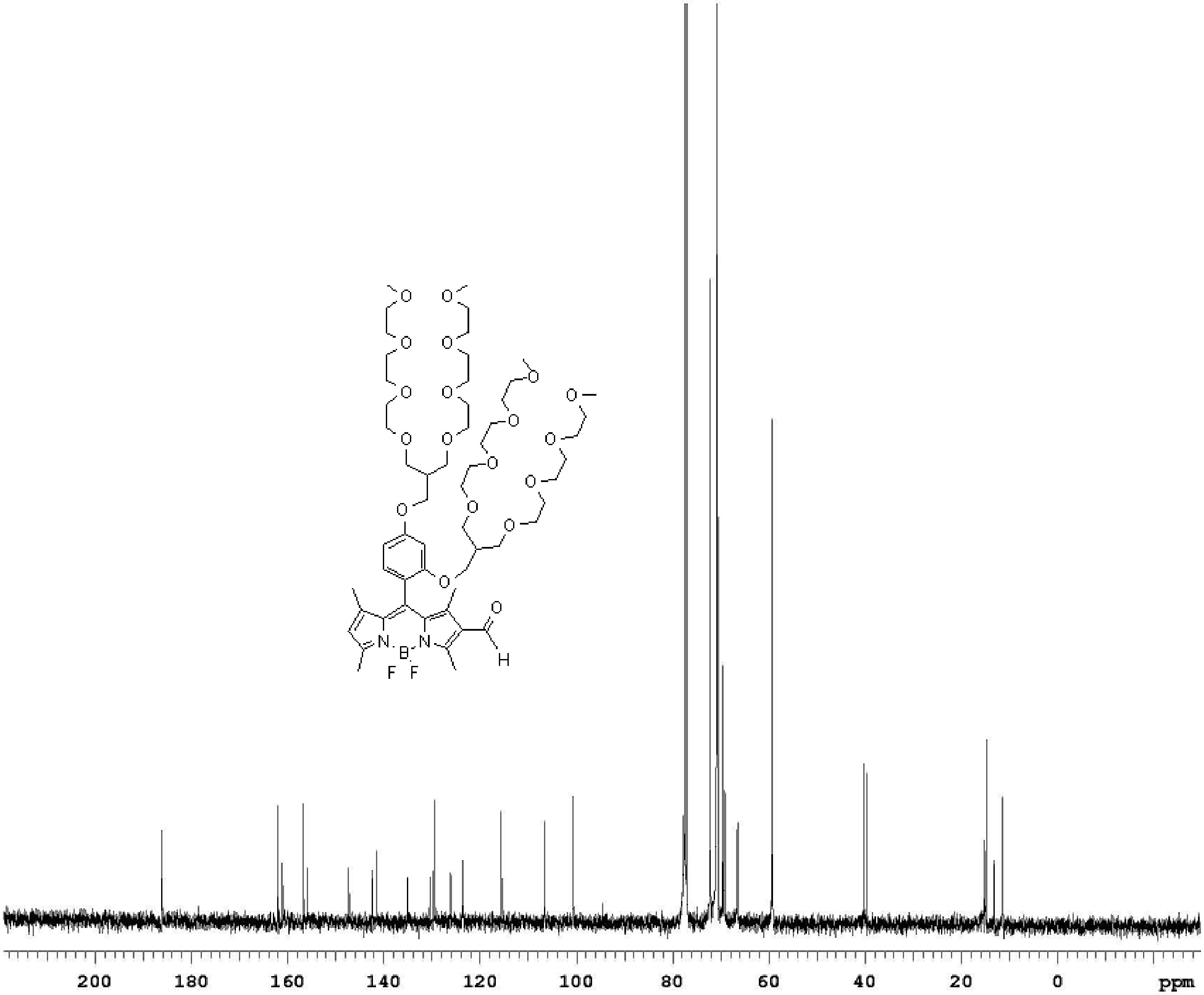

Figure S3. ^{13}C NMR spectrum of BODIPY dye **3** in $CDCl_3$ solution.

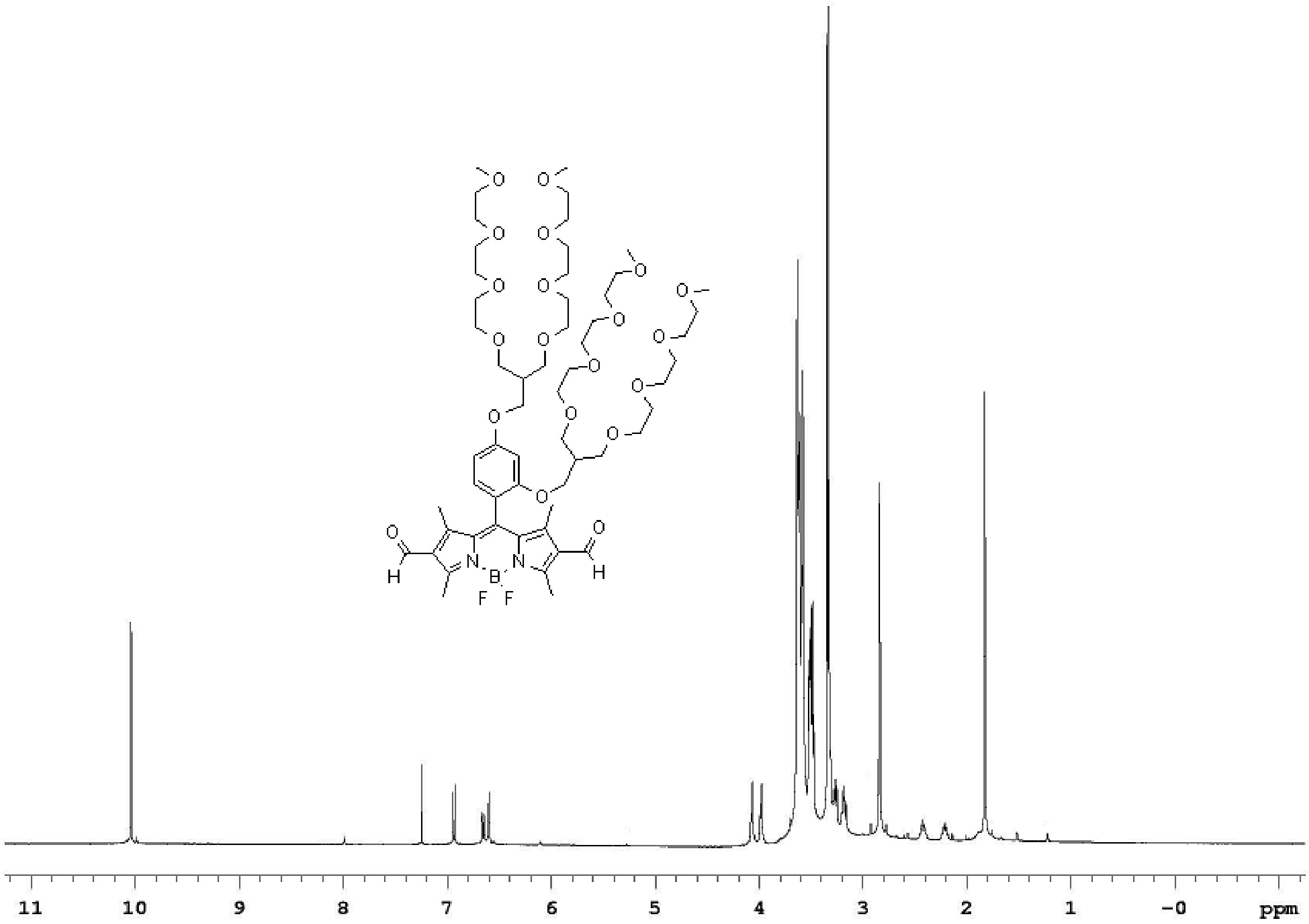

Figure S4. ^{1}H NMR spectrum of BODIPY dye **5** in $CDCl_3$ solution.

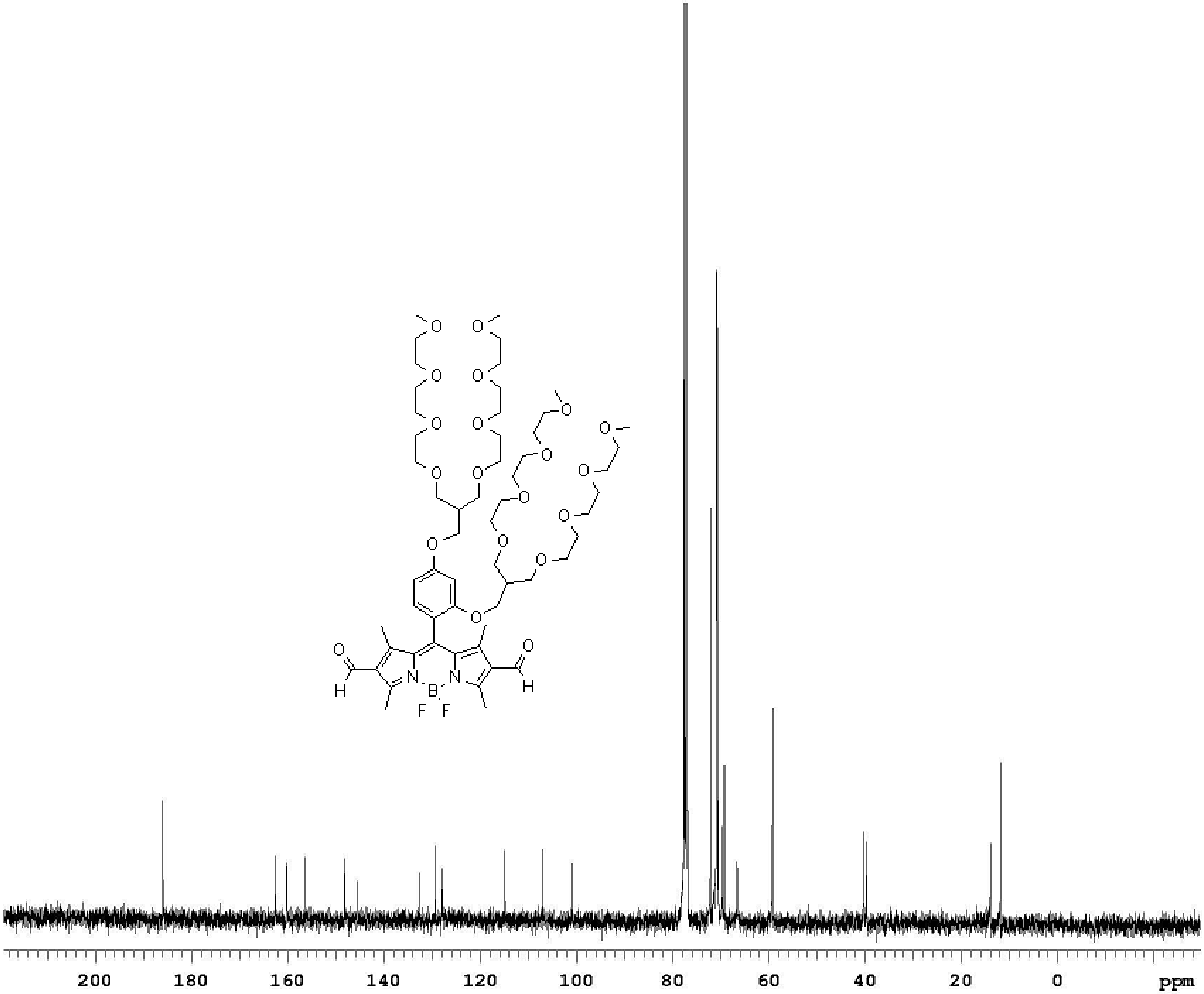

Figure S5. ^{13}C NMR spectrum of BODIPY dye **5** in $CDCl_3$ solution.

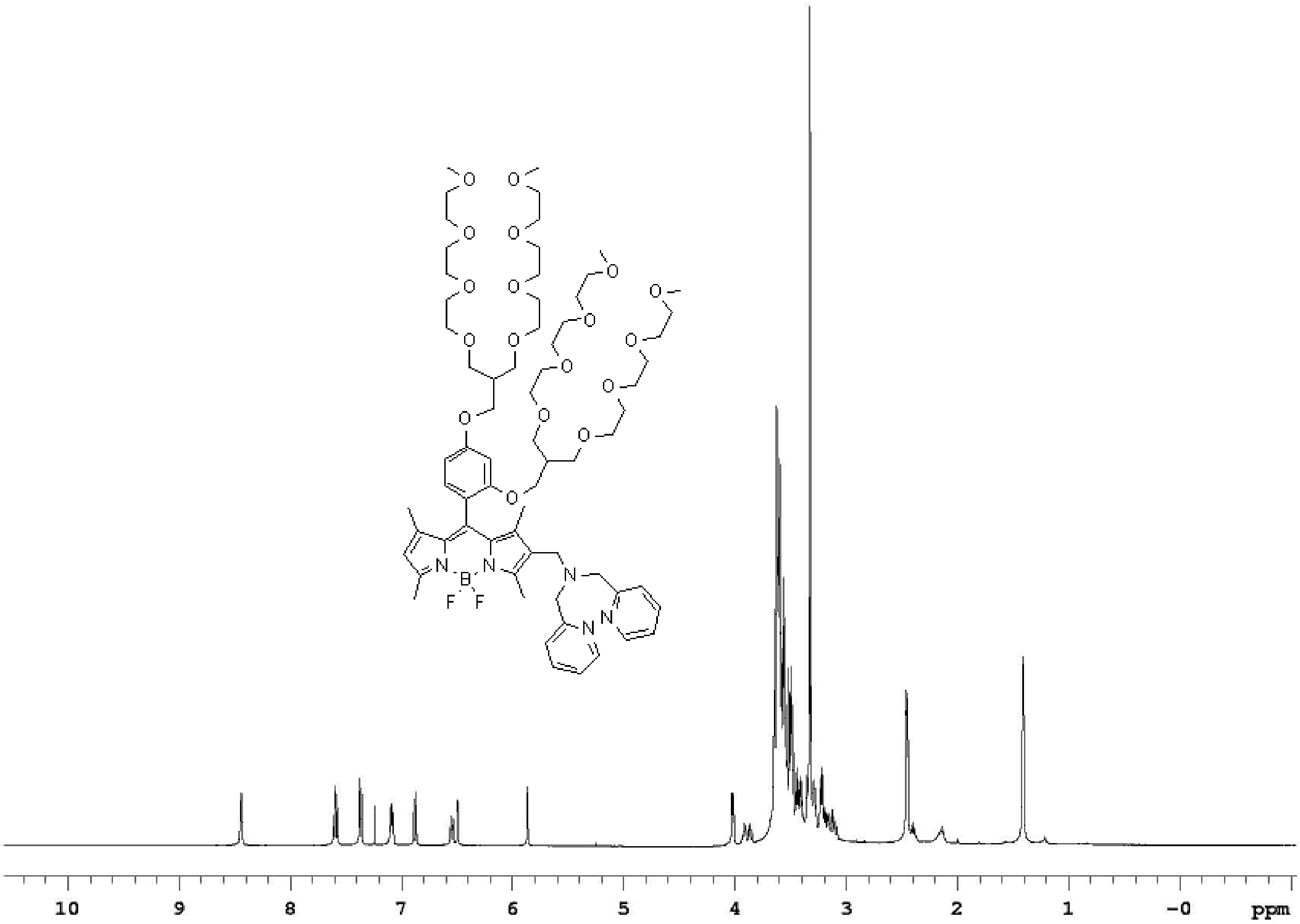

Figure S6. ^{1}H NMR spectrum of fluorescent probe **A** in $CDCl_3$ solution.

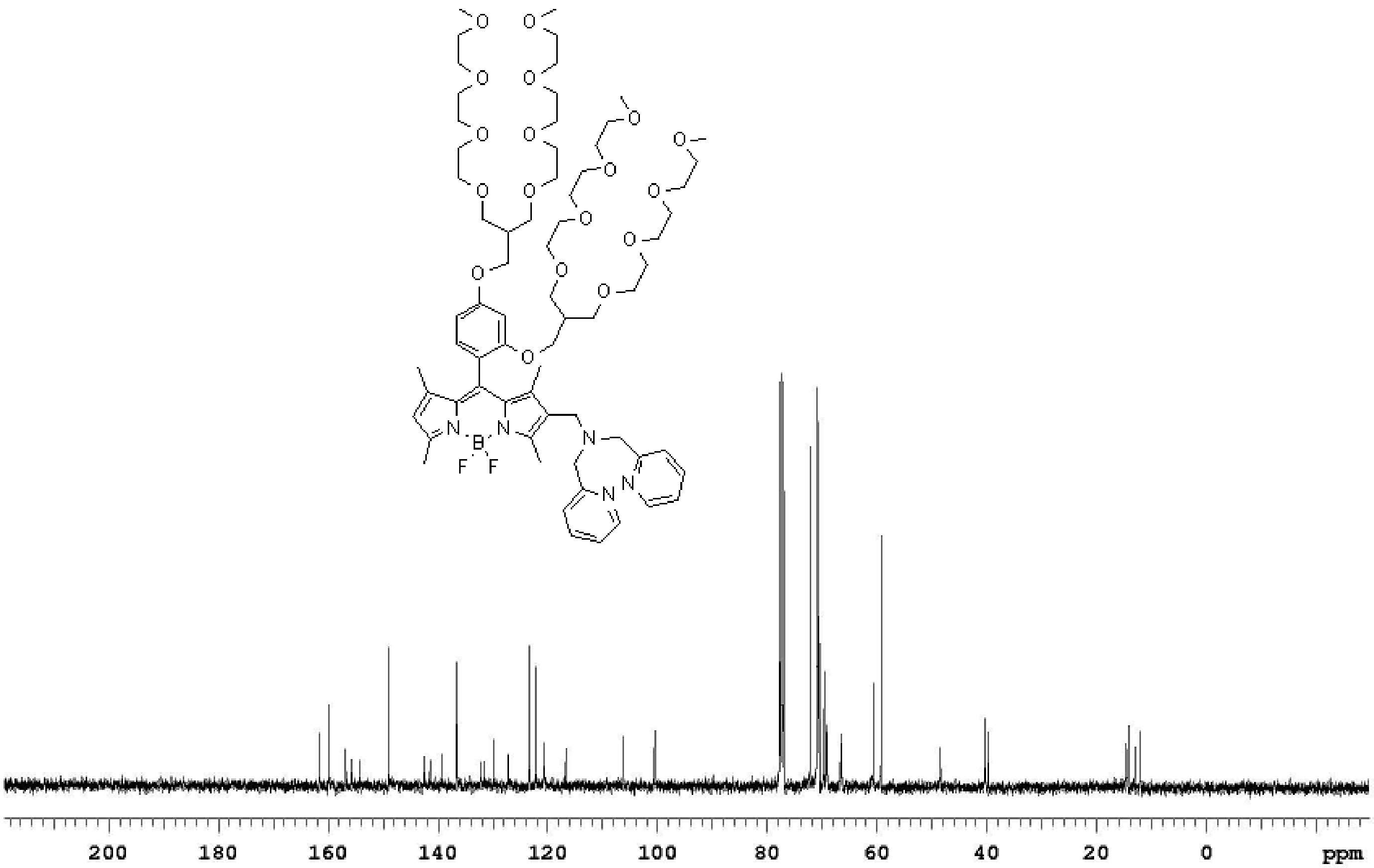

Figure S7. ^{13}C NMR spectrum of fluorescent probe **A** in $CDCl_3$ solution.

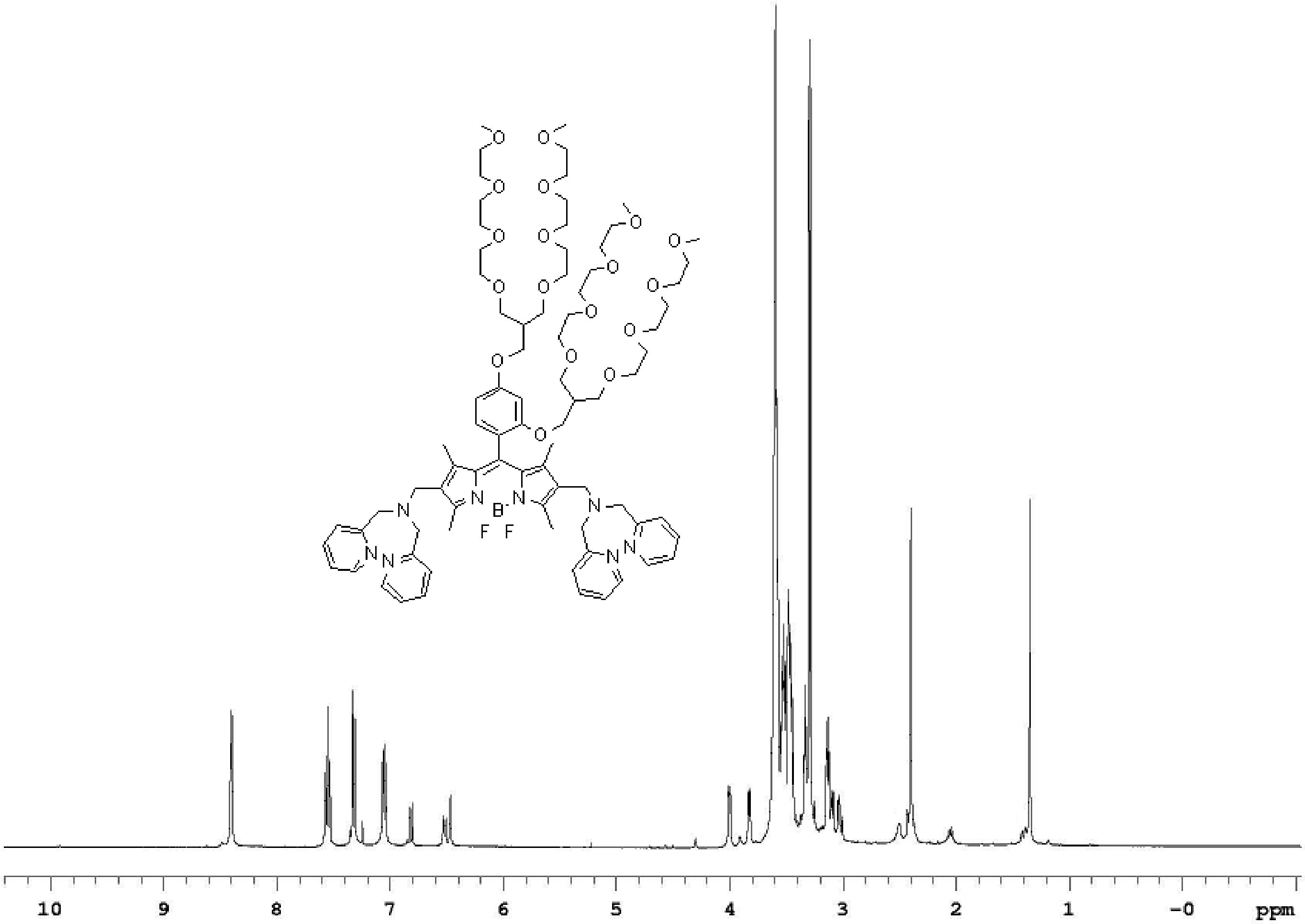

Figure S8. ^{1}H NMR spectrum of fluorescent probe **B** in $CDCl_3$ solution.

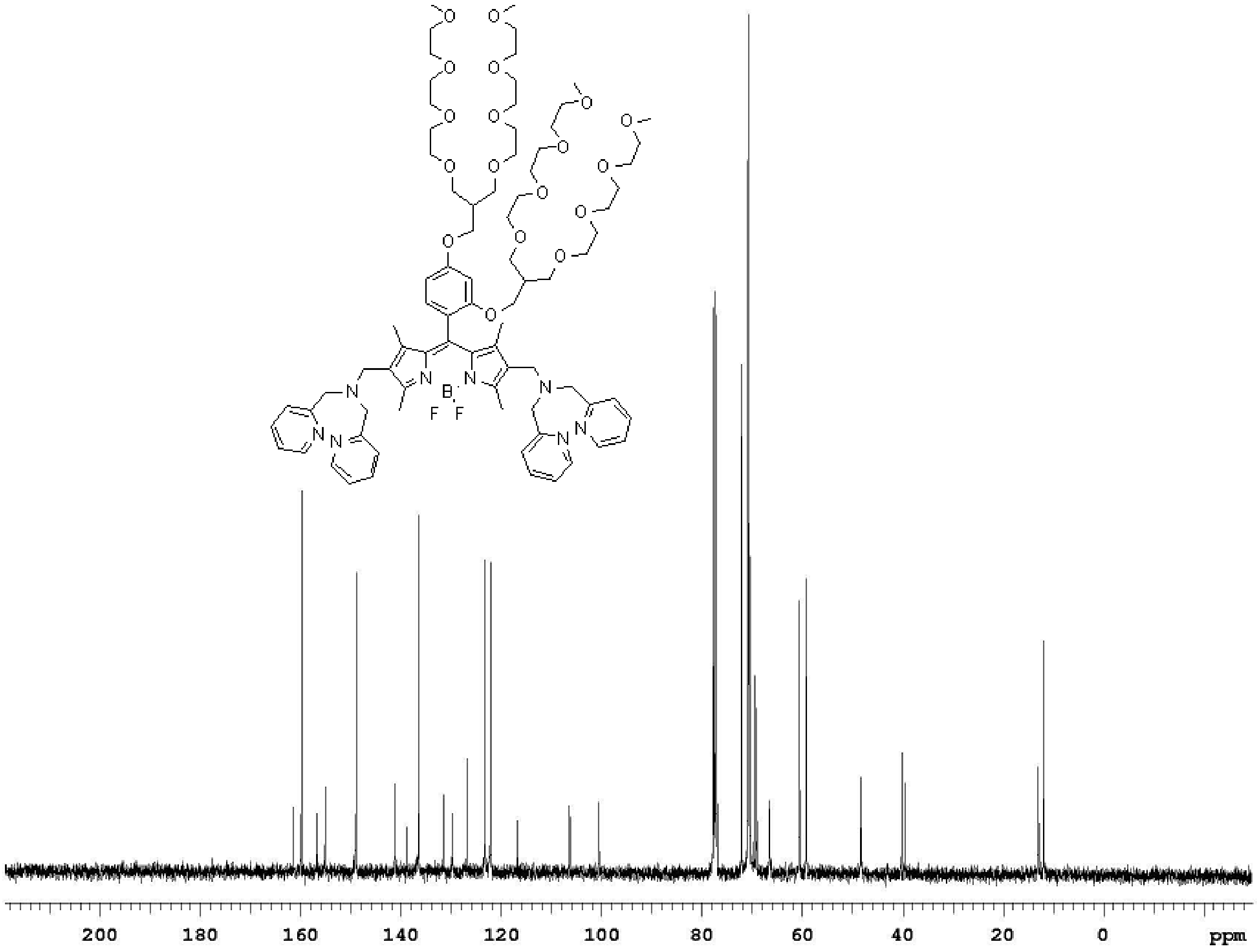

Figure S9. ^{13}H NMR spectrum of fluorescent probe **B** in $CDCl_3$ solution.

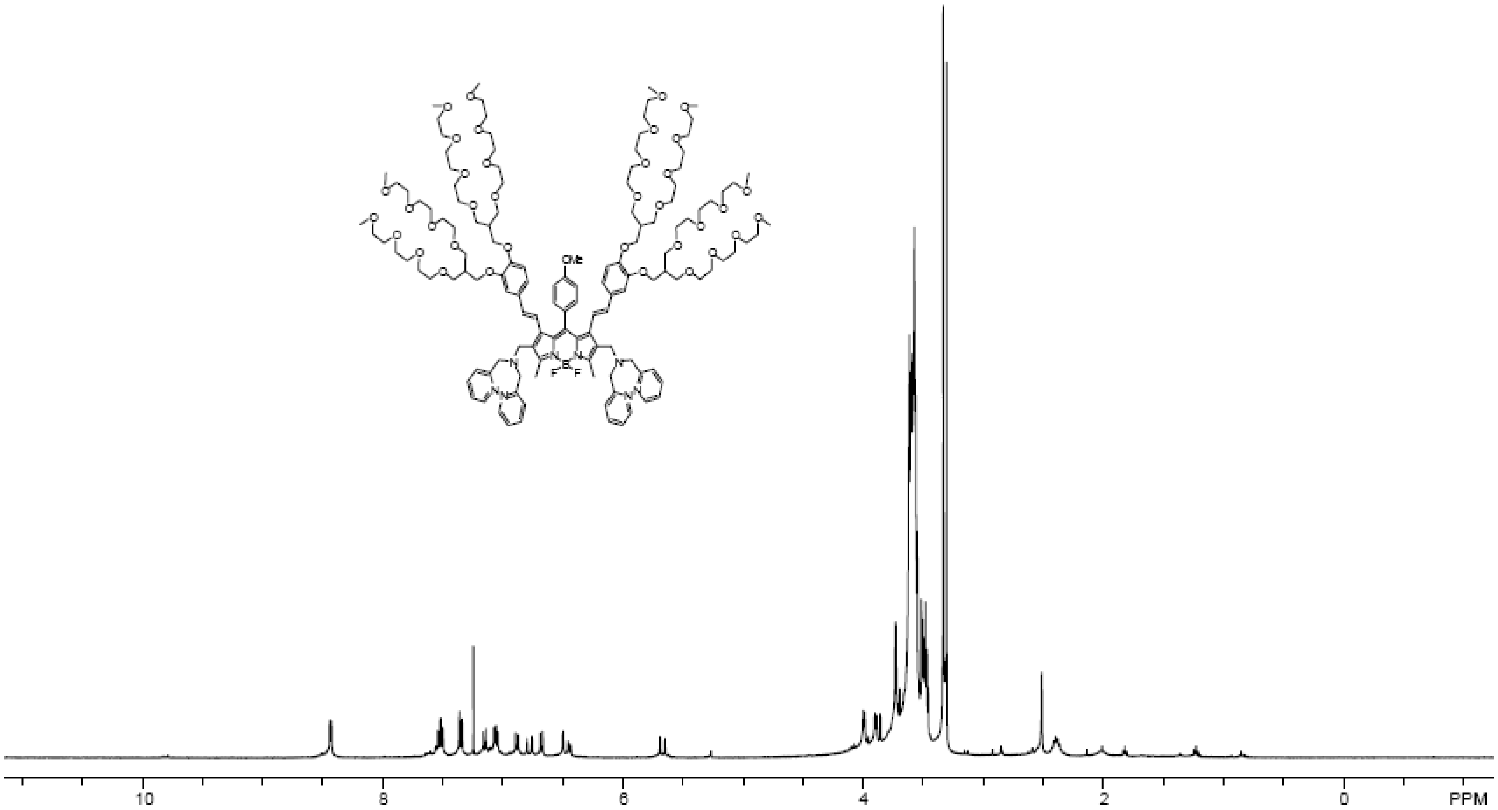

Figure S10. ^{1}H NMR spectrum of fluorescent probe **C** in $CDCl_3$ solution.

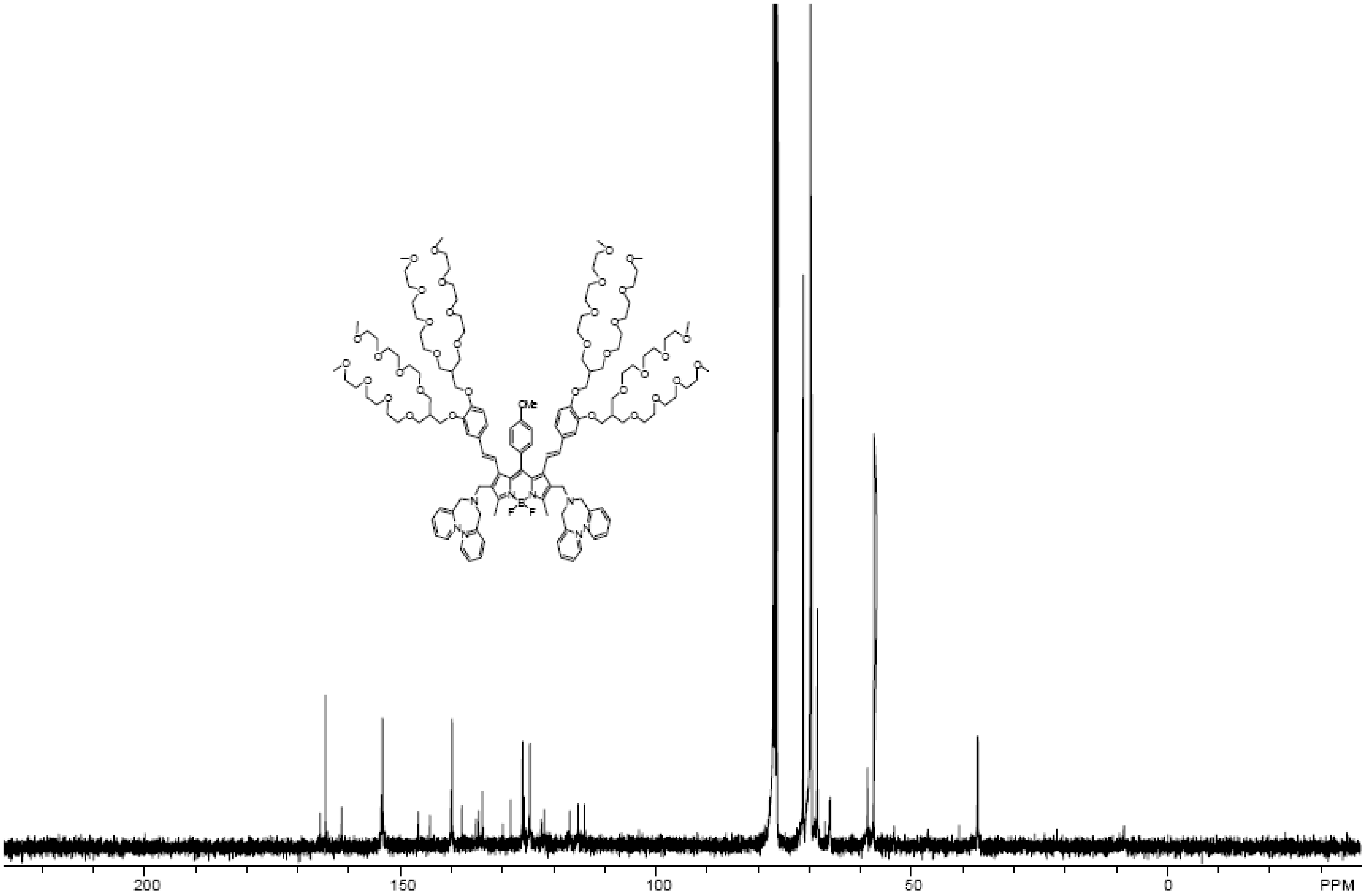

Figure S11. ^{13}C NMR spectrum of fluorescent probe **C** in $CDCl_3$ solution.

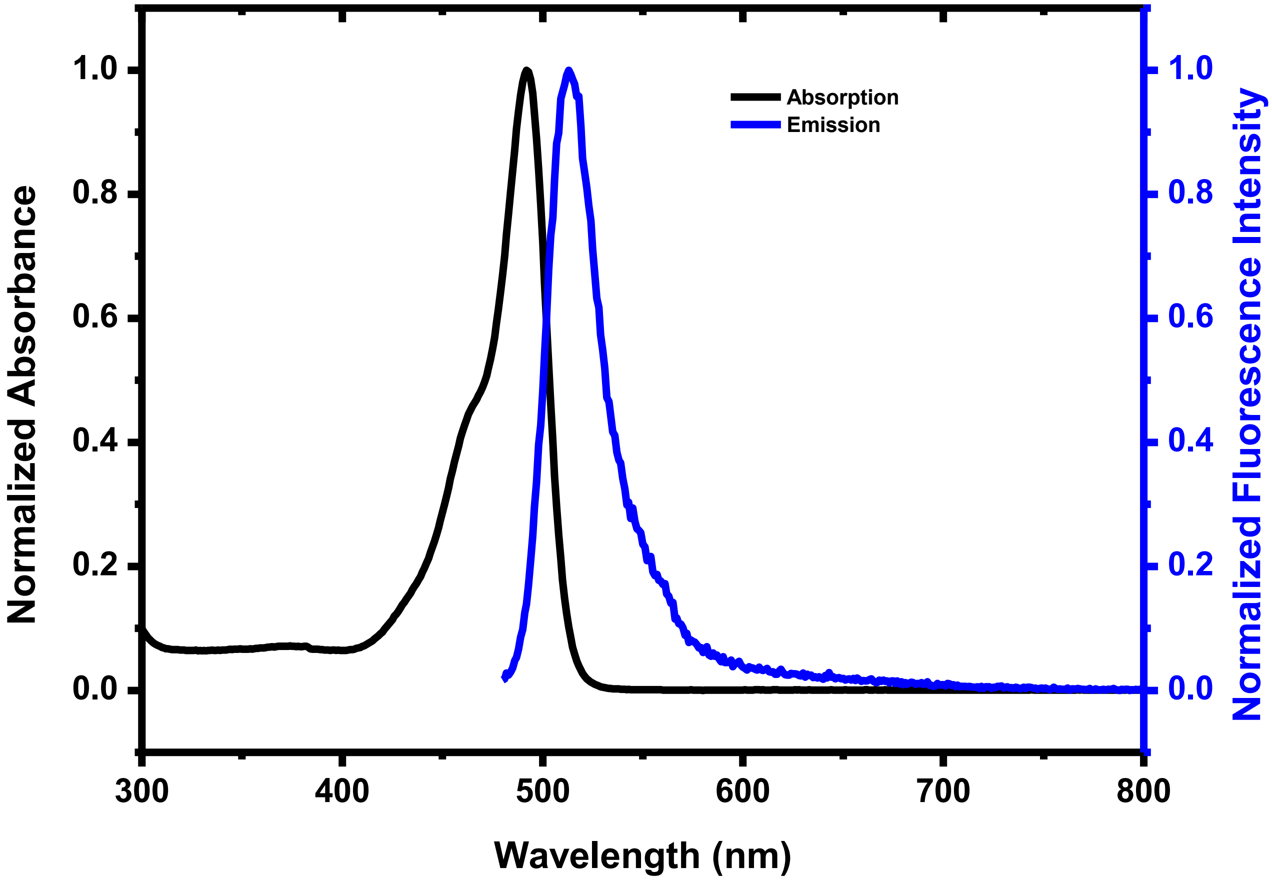

Figure S12. Normalized absorption and emission spectra of fluorescent probe **3** in 10 mM HEPES buffer solution pH 7.0.

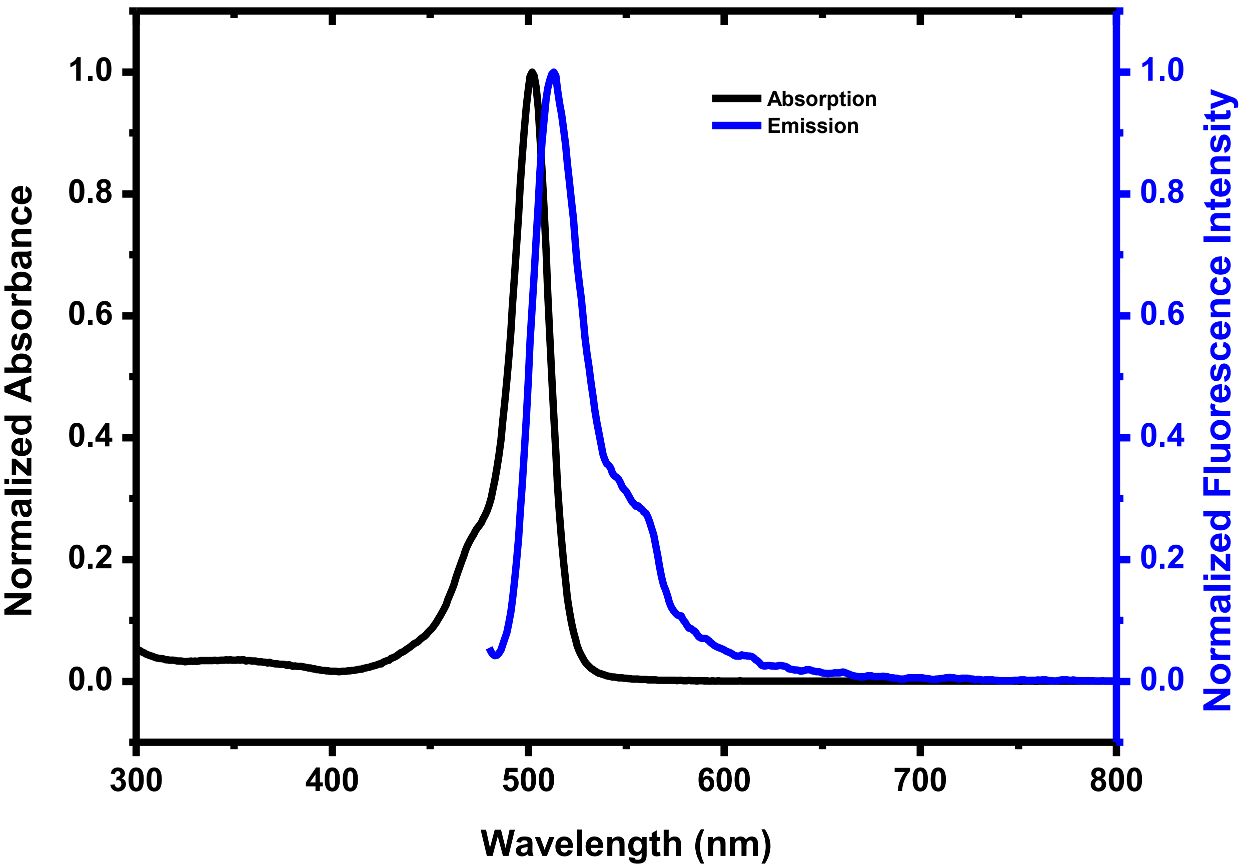

Figure S13. Normalized absorption and emission spectra of fluorescent probe **5** in 10 mM HEPES buffer solution pH 7.0.

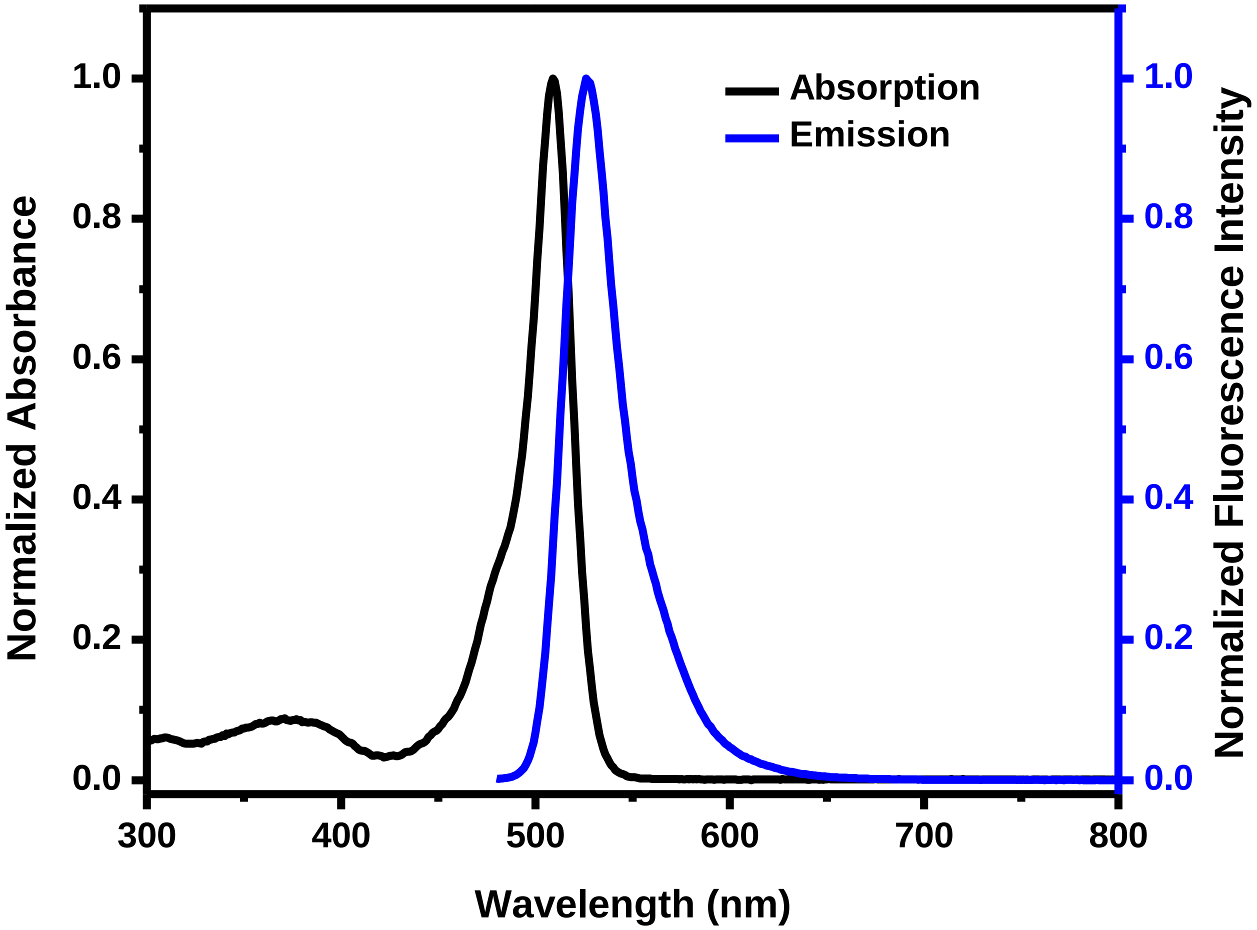

Figure S14. Normalized absorption and emission spectra of fluorescent probe **A** in 10 mM HEPES buffer solution pH 7.0.

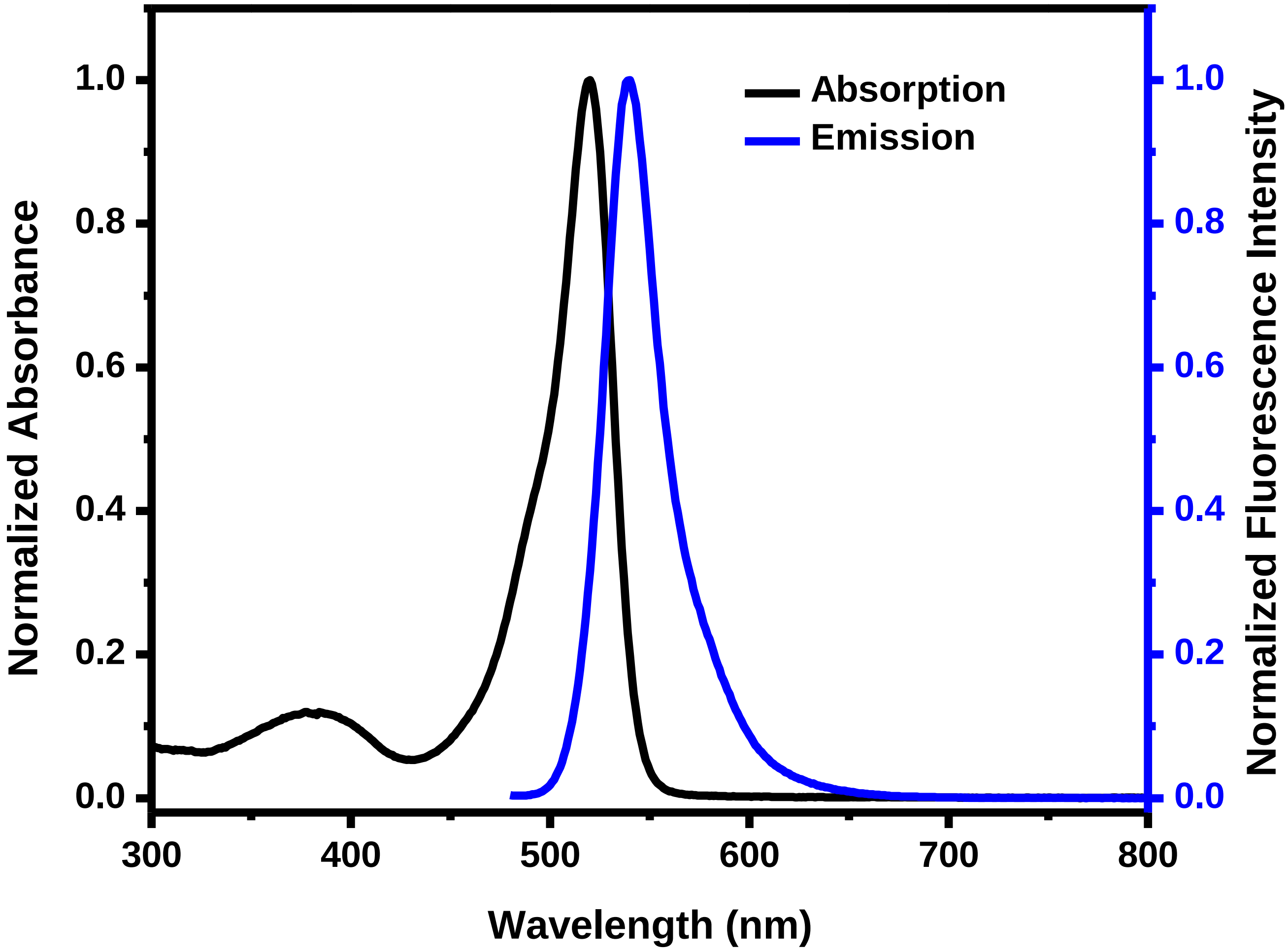

Figure S15. Normalized absorption and emission spectra of fluorescent probe **B** in 10 mM HEPES buffer solution pH 7.0.

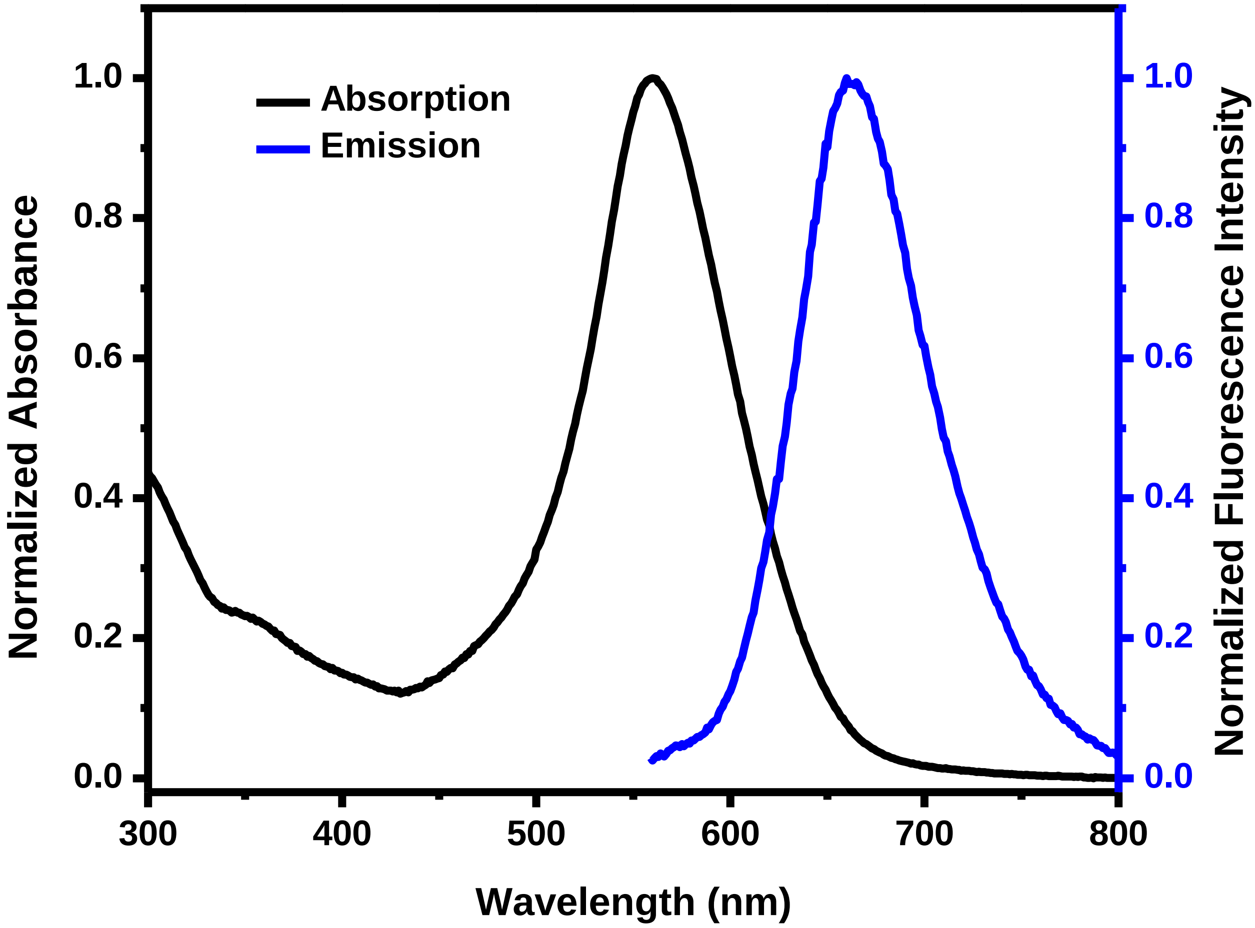

Figure S16. Normalized absorption and emission spectra of fluorescent probe **C** in 10 mM HEPES buffer solution pH 7.0.

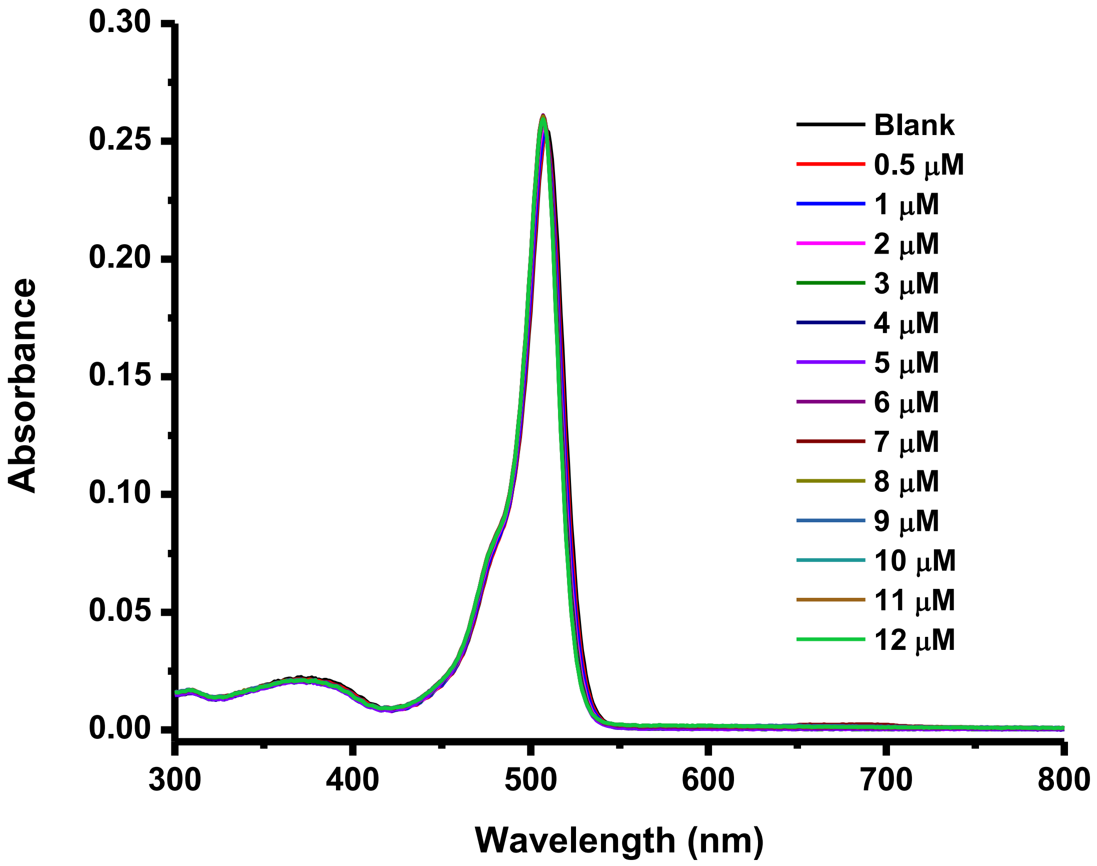

Figure S17. Absorption spectra of fluorescent probe **A** upon addition of different amount of $ZnCl_2$ in 10 mM HEPES buffer pH 7.0.

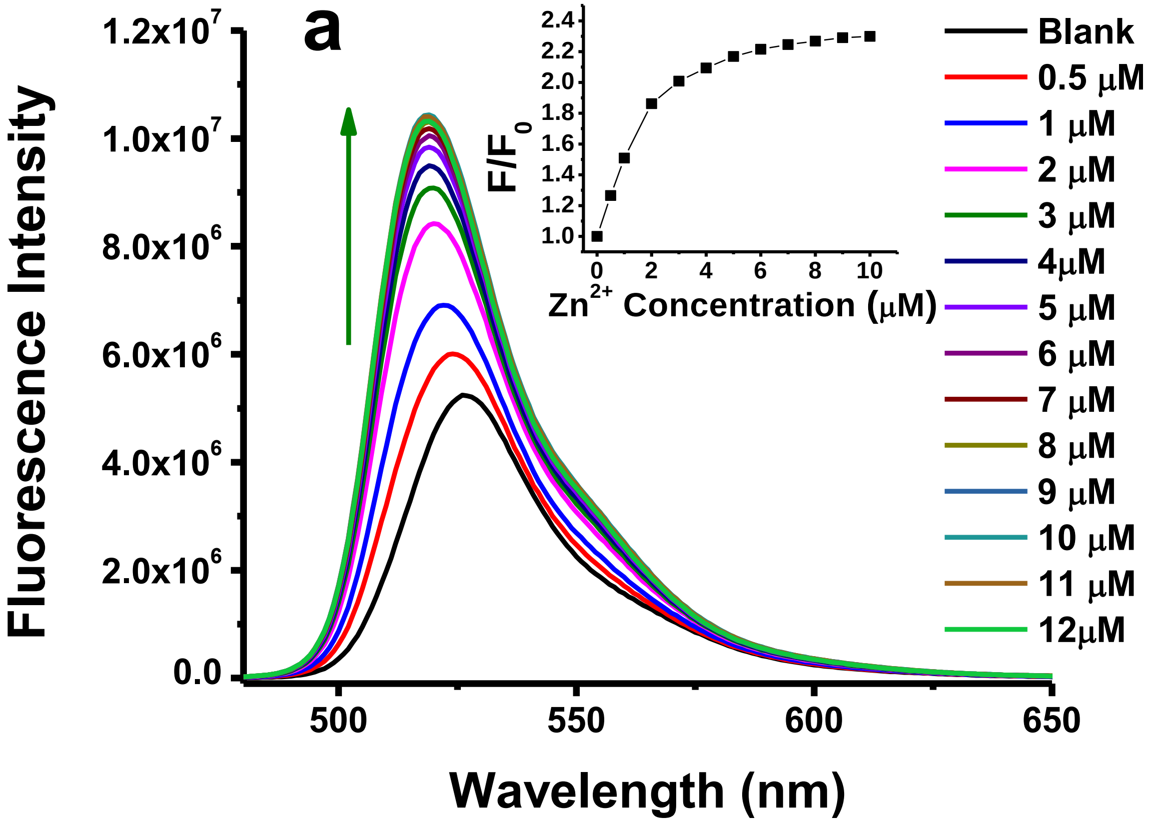

Figure S18. Fluorescence spectra of 5 μM fluorescent probe **A** in 10 mM HEPES buffer (pH 7.0) in the absence and presence of different concentrations of $ZnCl_2$. Inset: fluorescence response curves at 520 nm.

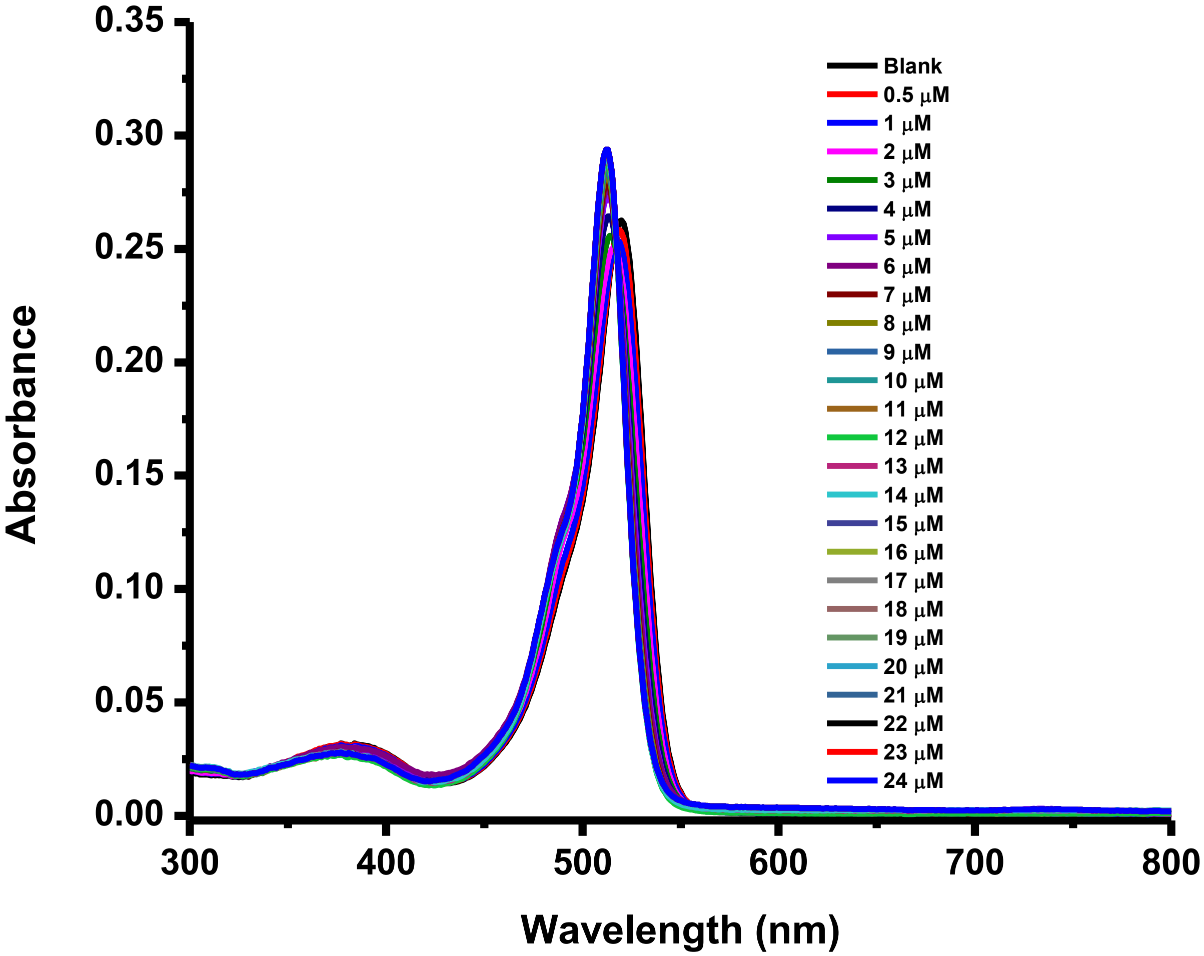

Figure S19. Absorption spectra of fluorescent probe **B** upon addition of different amount of $ZnCl_2$ in 10 mM HEPES buffer pH 7.0.

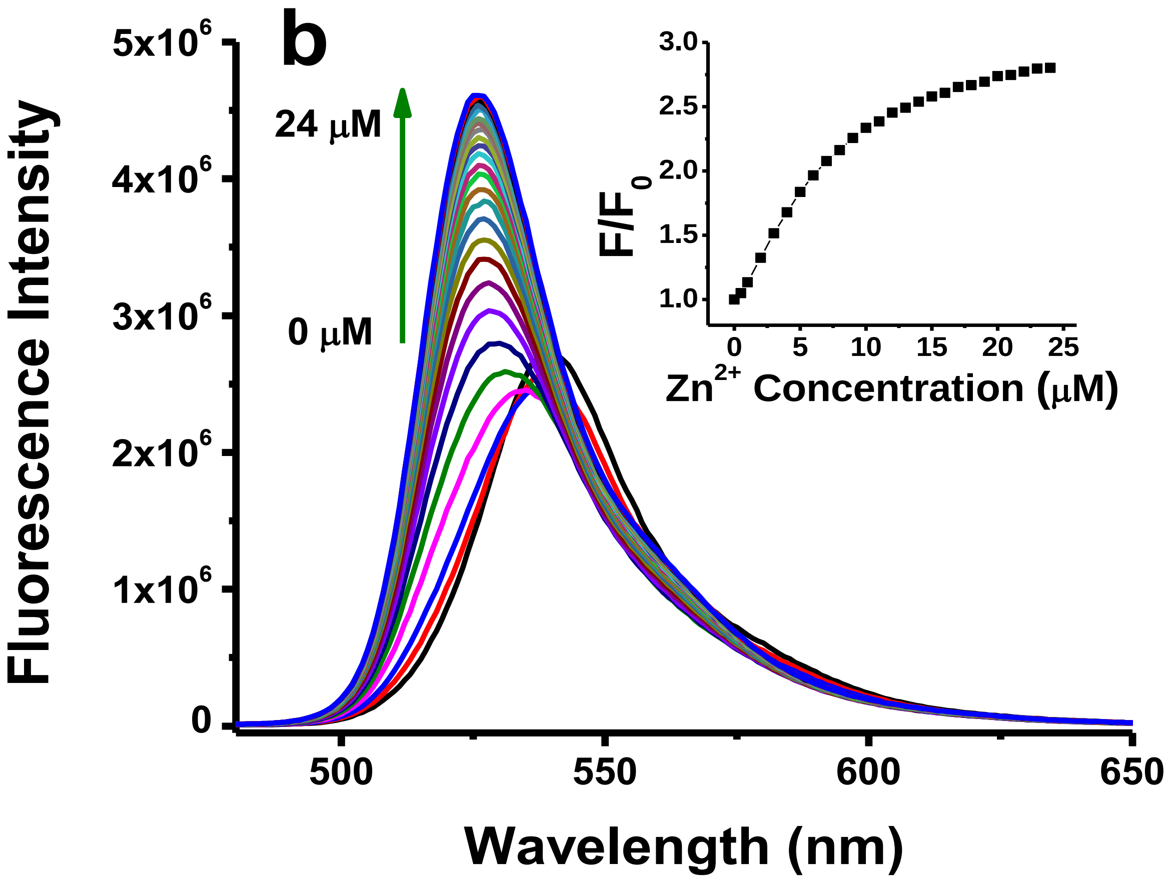

Figure S20. Fluorescence spectra of 5 μM fluorescent probe **B** in 10 mM HEPES buffer (pH 7.0) in the absence and presence of different concentrations of $ZnCl_2$. Inset: fluorescence response curves at 527 nm.

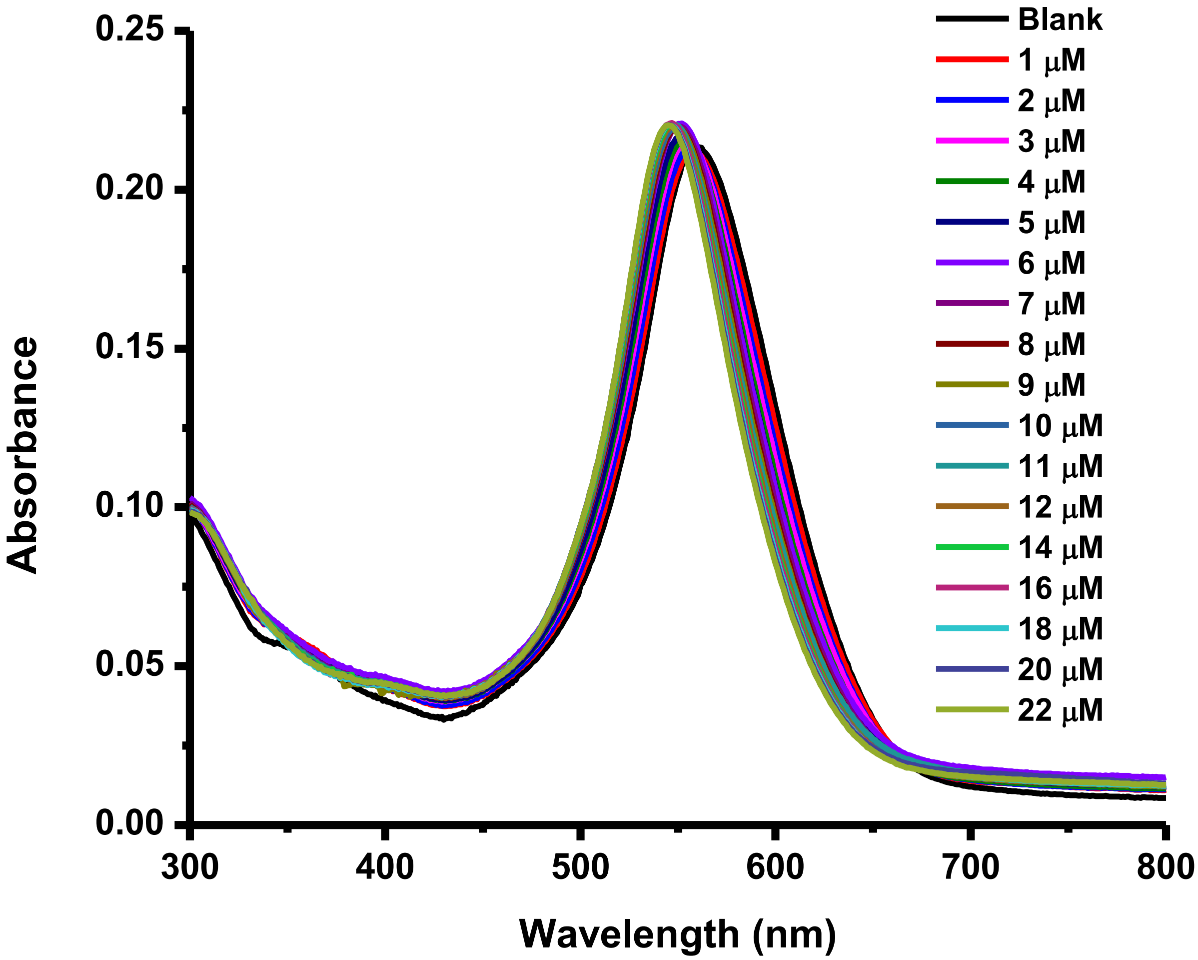

Figure S21. Absorption spectra of fluorescent probe **C** upon addition of different amount of $ZnCl_2$ in 10 mM HEPES buffer pH 7.0.

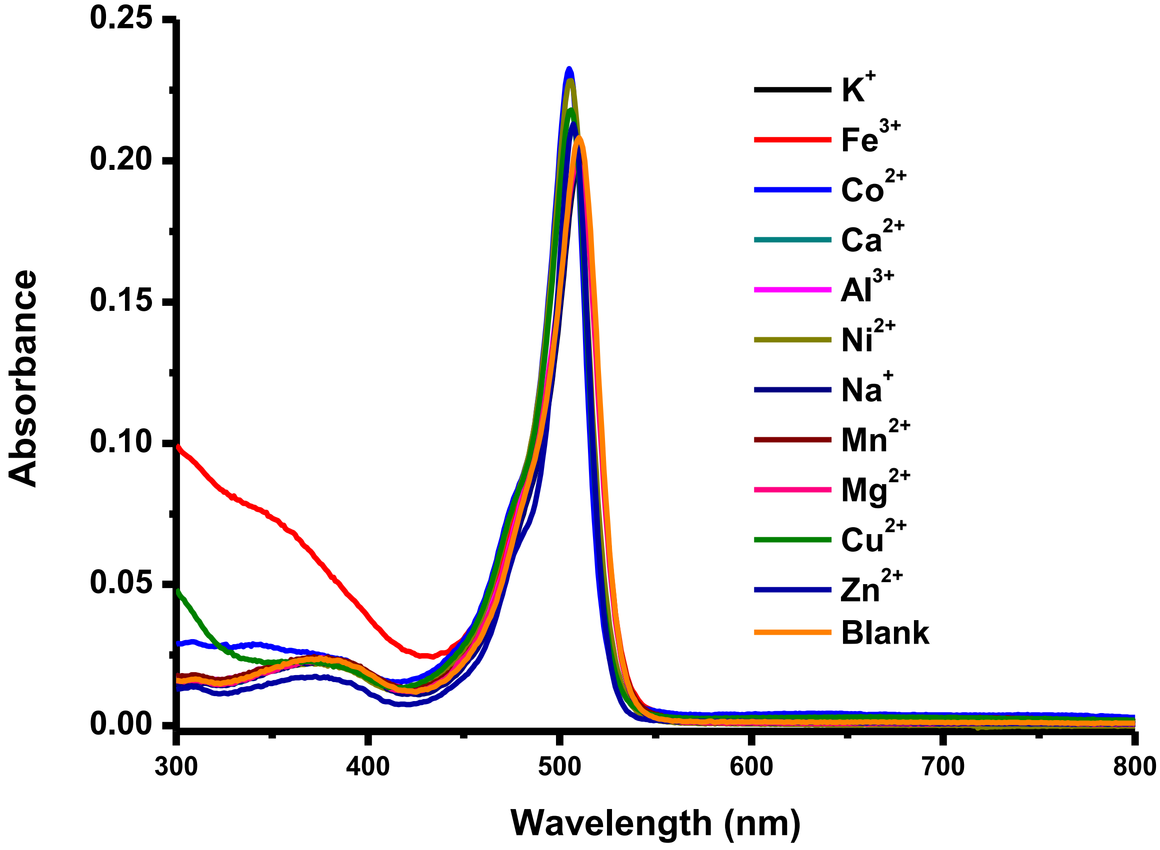

Figure S22. Absorption spectra of fluorescent probe **A** upon addition of different cations (20 μM Zn^{2+}, other ions at 40 μM) in 10 mM HEPES buffer pH 7.0.

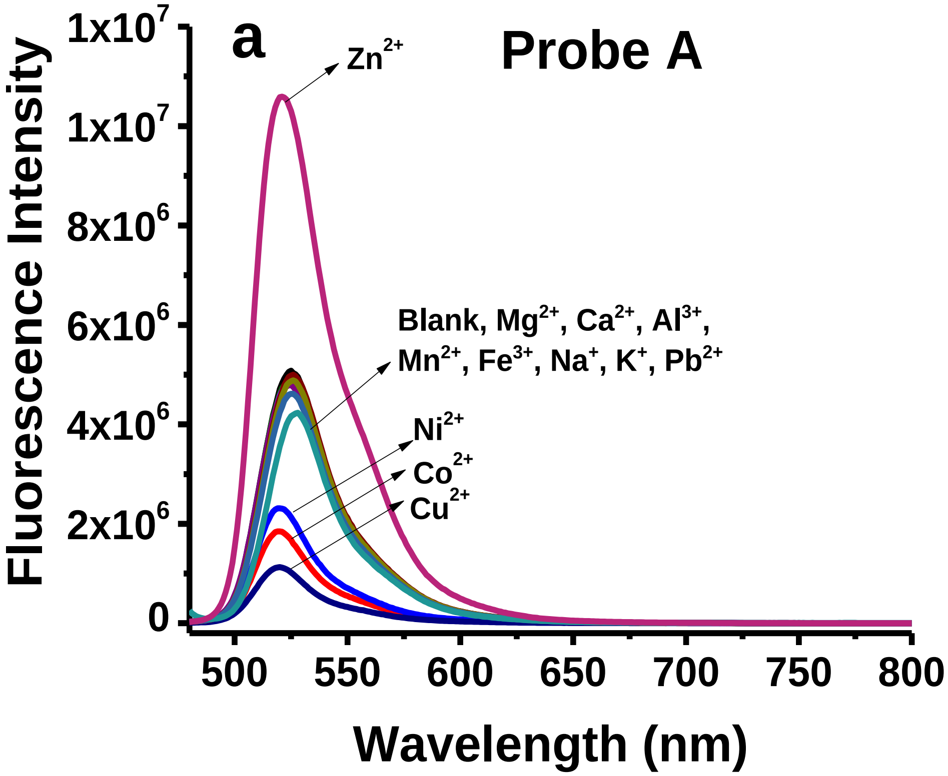

Figure S23. Fluorescent responses of 5 μM fluorescent probe **A** to different metal ions (20 μM Zn^{2+}, all other ions at 40 μM) in 10 mM HEPES buffer (pH 7.0).

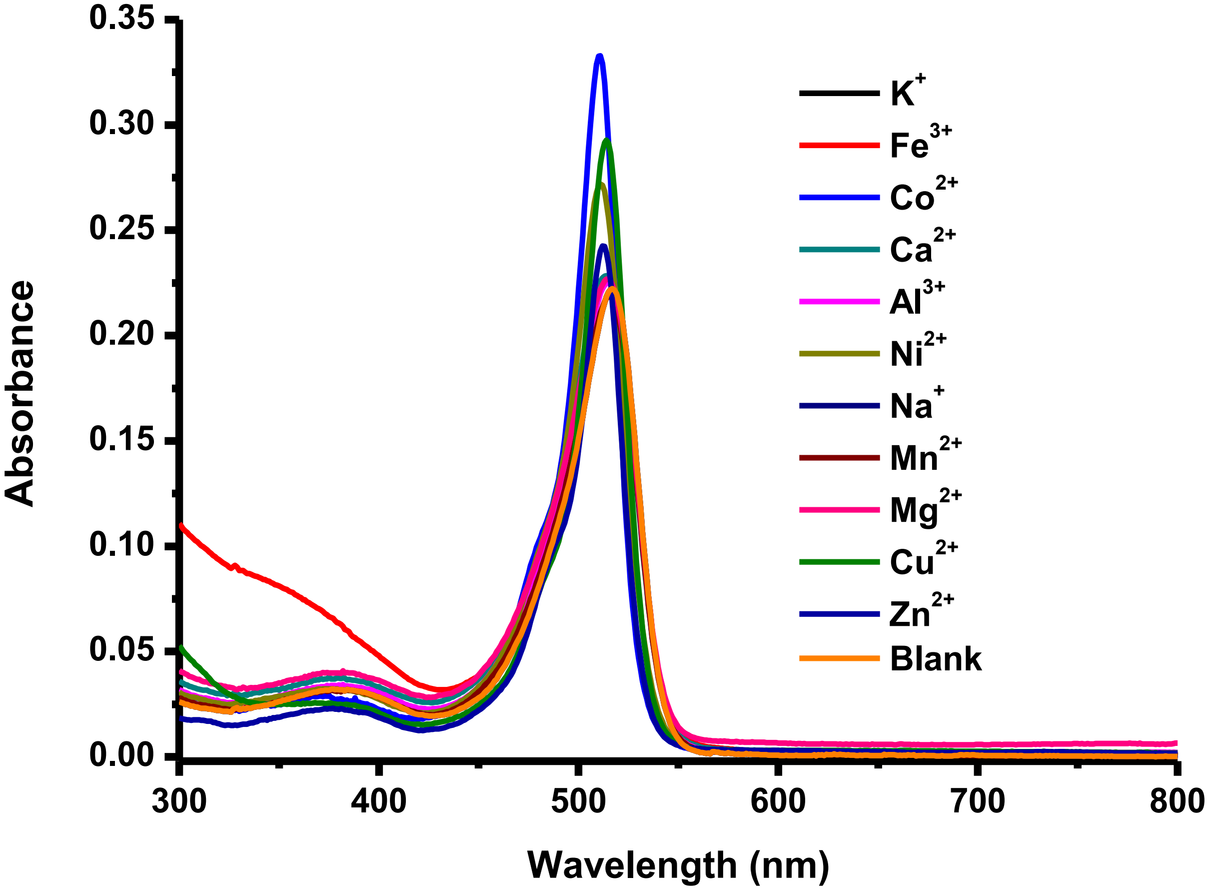

Figure S24. Absorption spectra of fluorescent probe **B** upon addition of different cations (20 μM Zn^{2+}, other ions at 40 μM) in 10 mM HEPES buffer pH 7.0.

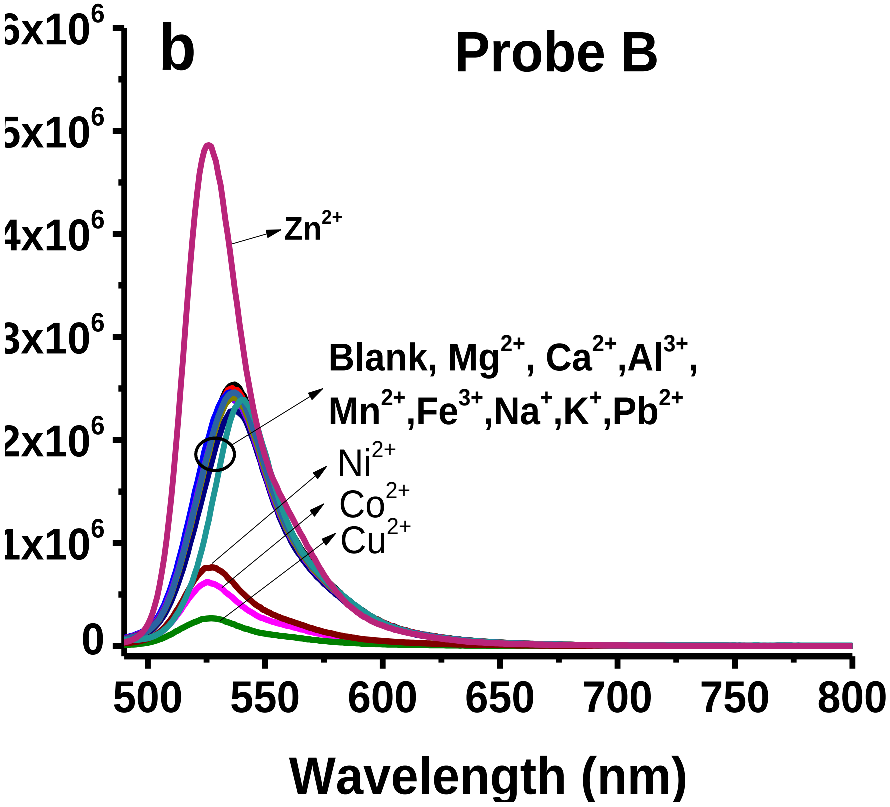

Figure S25. Fluorescent responses of 5 μM fluorescent probe **B** to different metal ions (20 μM Zn^{2+}, all other ions at 40 μM) in 10 mM HEPES buffer (pH 7.0).

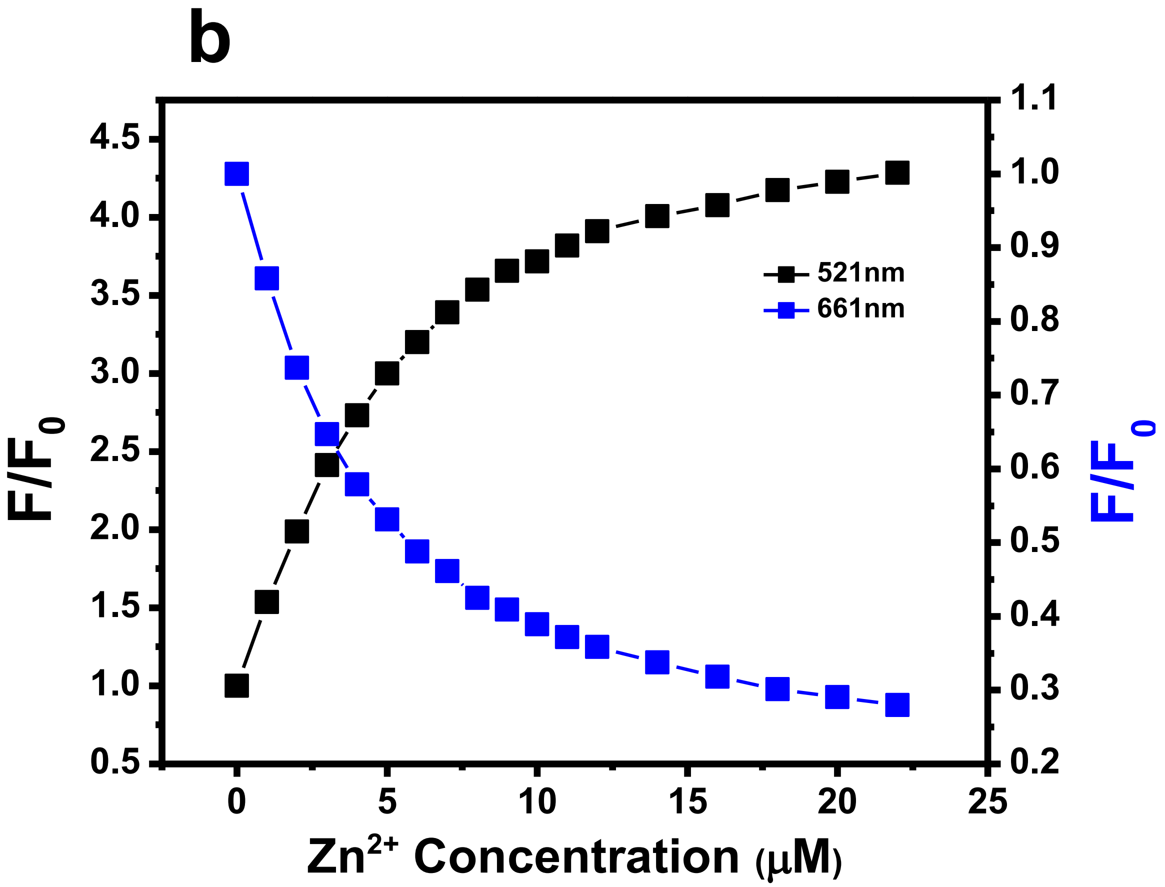

Figure S26. Fluorescence response curve (b) of 5 μM fluorescent probe **C** in 10 mM HEPES buffer (pH 7.0) in the absence and presence of different concentrations of $ZnCl_2$.

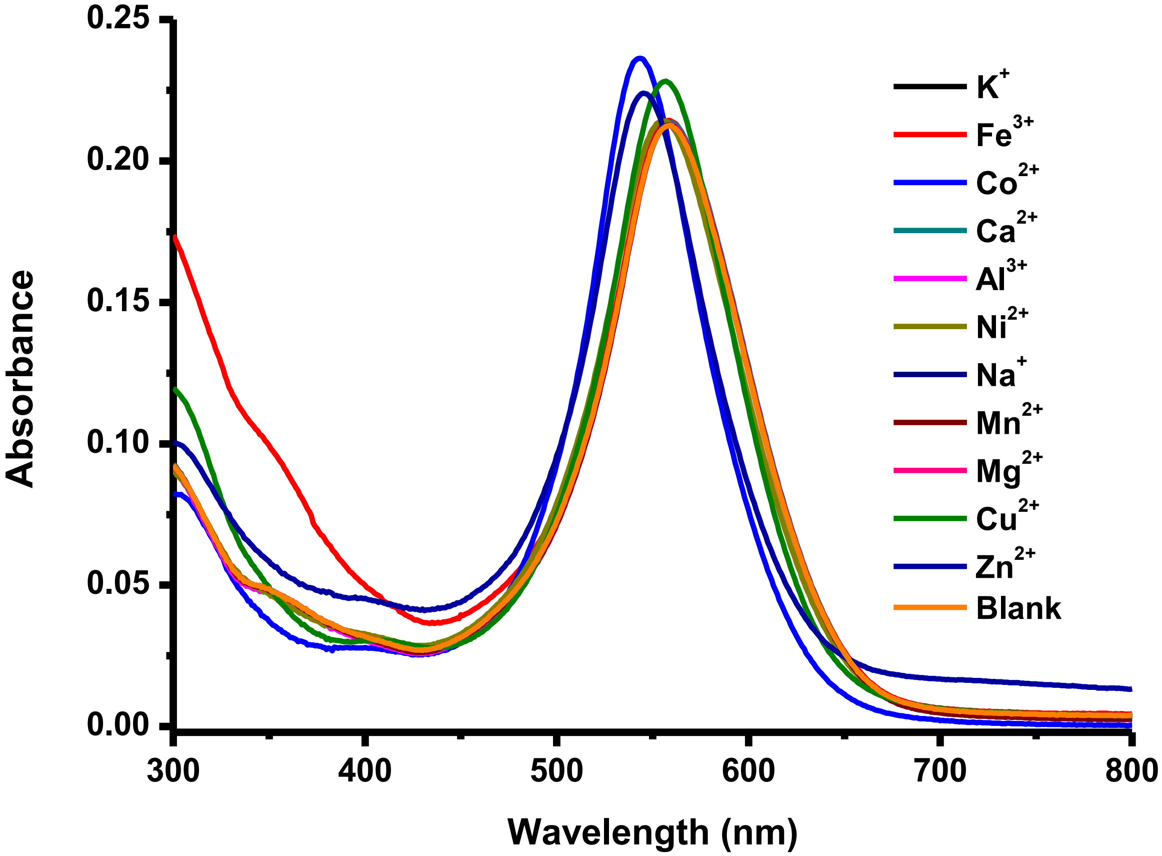

Figure S27. Absorption spectra of fluorescent probe **C** upon addition of different cations (20 μM Zn^{2+}, other ions at 40 μM) in 10 mM HEPES buffer pH 7.0.

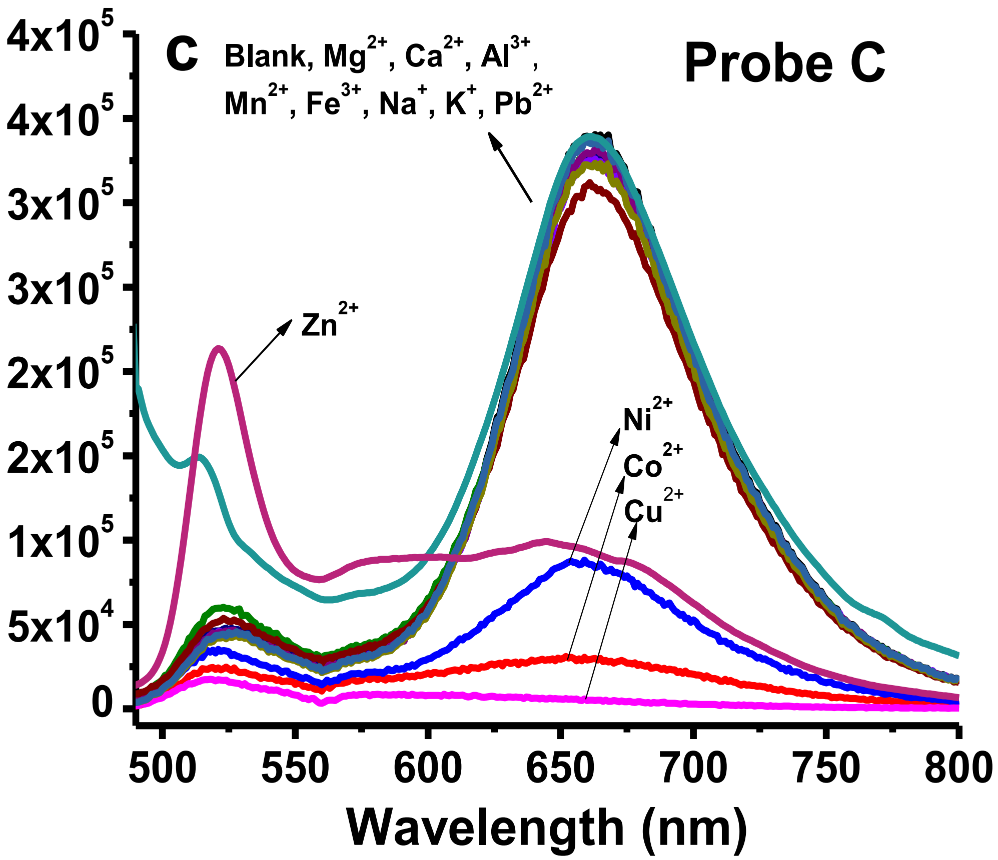

Figure S28. Fluorescent responses of 5 μM fluorescent probe **C** to different metal ions (20 μM Zn^{2+}, all other ions at 40 μM) in 10 mM HEPES buffer (pH 7.0).

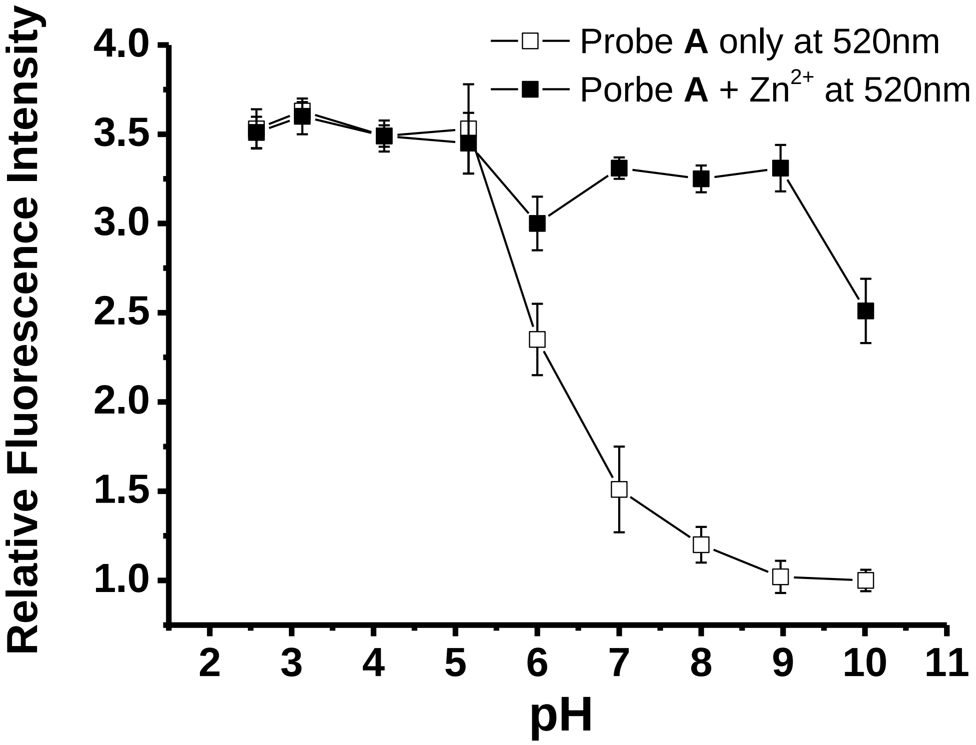

Figure S29. Fluorescence responses of 5 μM fluorescent probe **A** at different pHs in absence and presence of 6 μM Zn^{2+}.

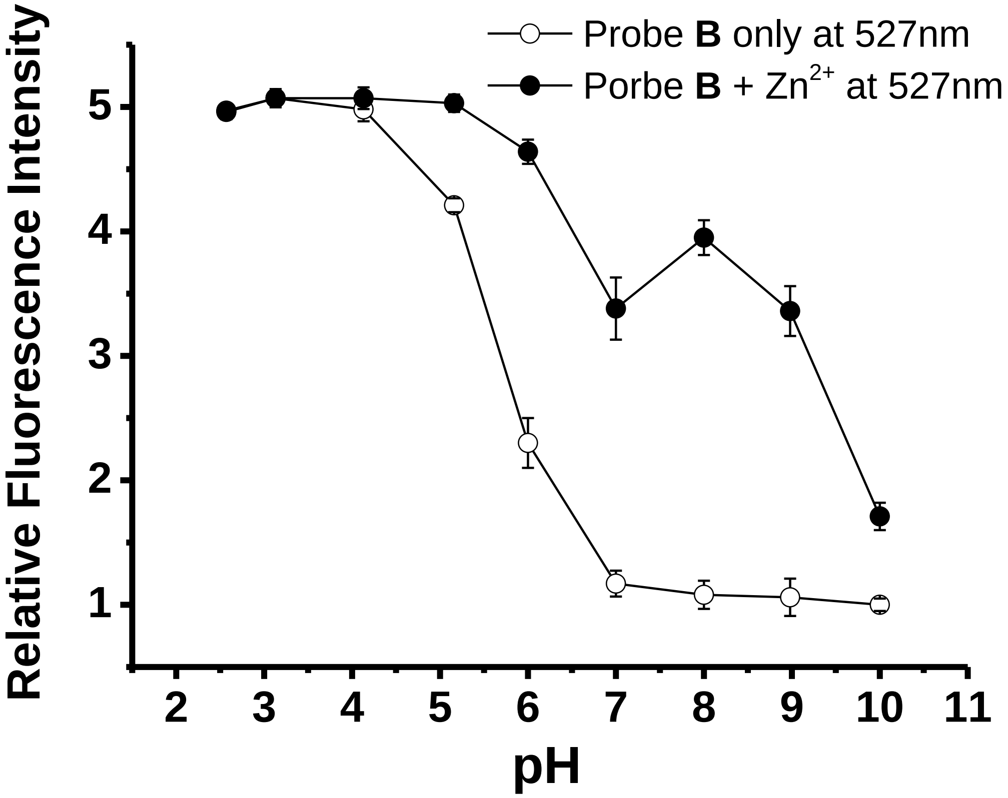

Figure S30. Fluorescence responses of 5 μM fluorescent probe **B** at different pHs in absence and presence of 6 μM Zn^{2+}.

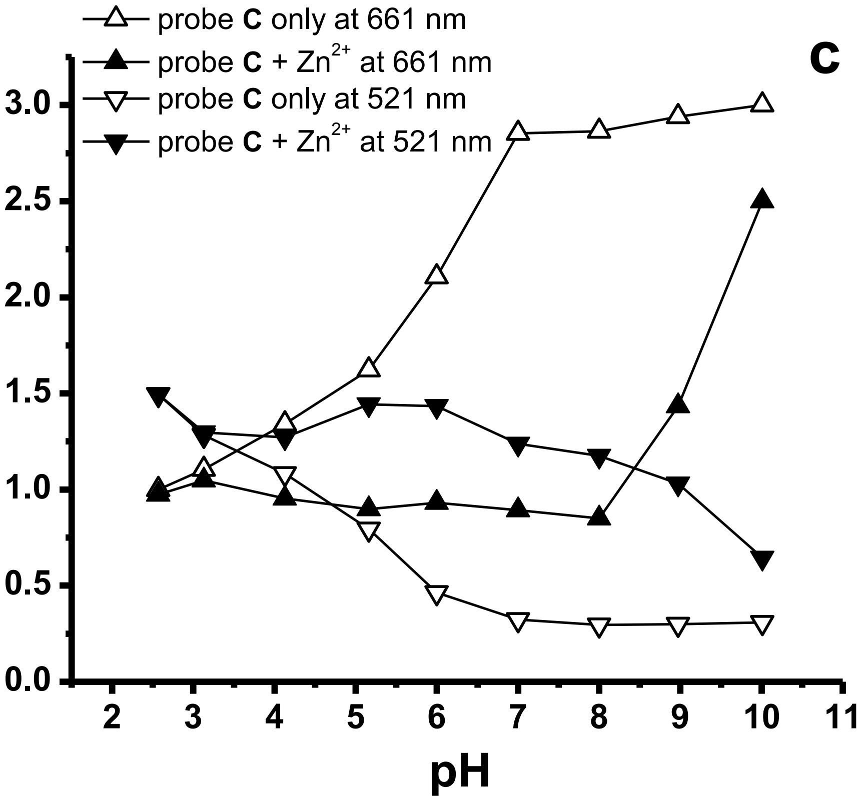

Figure S31. Fluorescence responses of 5 μM fluorescent probe **C** at different pHs in absence and presence of 22 μM Zn^{2+}.

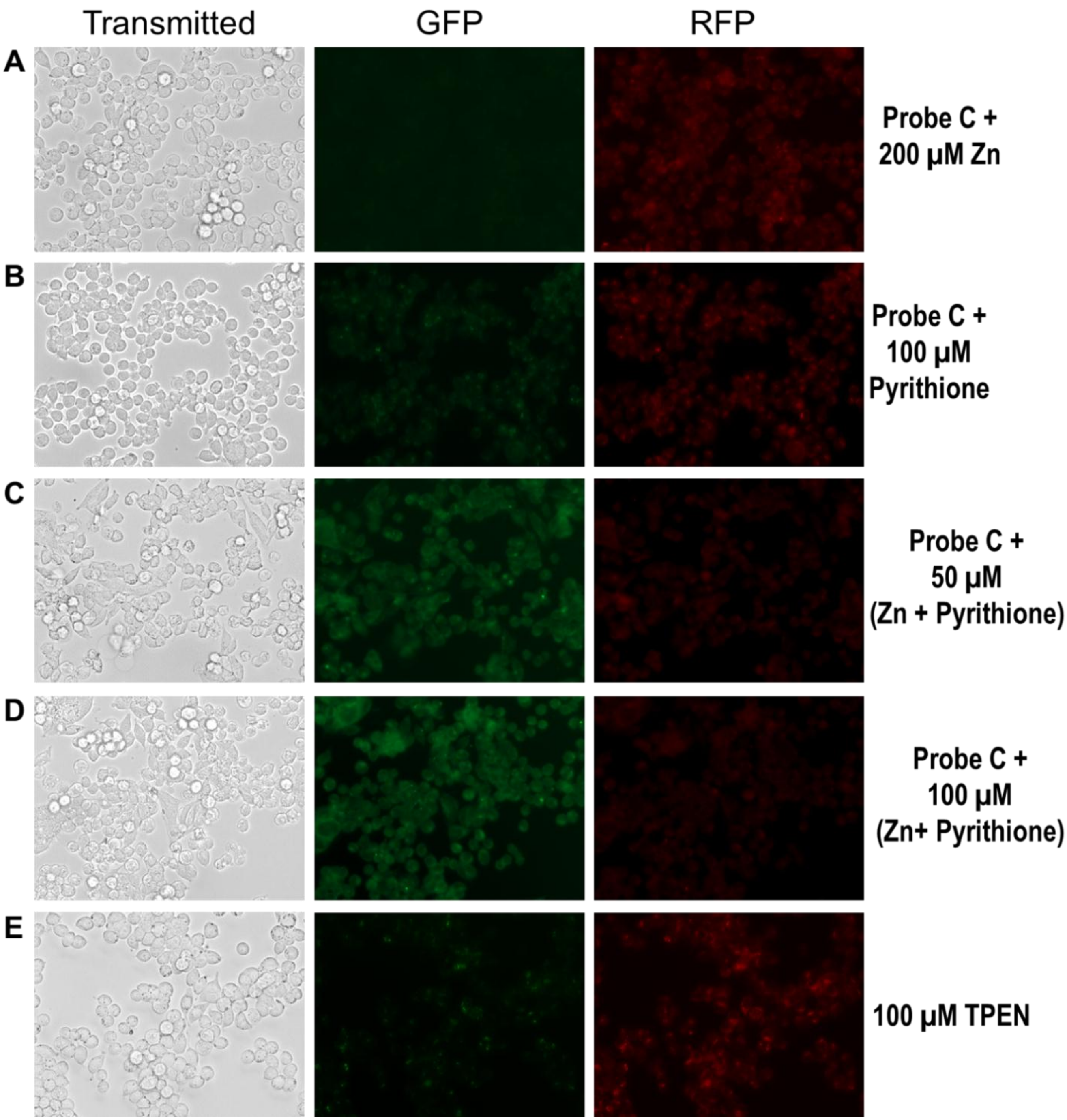

Figure S33. Effect of zinc and pyrithione concentrations on fluorescence probe C in SW620 cells. The SW620 cells incubated with probe C and indicated concentrations of zinc and pyrithione before imaging (**A**-**D**). The cells imaged in (**D**) were further incubated with 100 µM of TPEN a zinc chelator for 10 min before re-imaging (**E**). The bright field images were acquired at 40X magnification with transmitted light and the fluorescence images were acquired using green (GFP) and red (RFP) light cubes.

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