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Supporting Information

A Fluorescent Indicator for Imaging Lysosomal Zinc(II) with Förster Resonance Energy Transfer (FRET)-Enhanced Photostability and a Narrow Band of Emission

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1. Materials and general methods. Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. All reactions were carried out in oven- or flame-dried glassware. Analytical thin-layer chromatography (TLC) was performed using pre-coated TLC plates with silica gel 60 F254 or with aluminum oxide 60 F254 neutral. Flash column chromatography was performed using 40-63 μm (230-400 mesh ASTM) silica gel or alumina (80-200 mesh, pH 9-10) as the stationary phases. Silica and alumina gel was flame-dried under vacuum to remove adsorbed moisture before use. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 125 MHz (on two different instruments), respectively. All chemical shifts were reported in δ units relative to tetramethylsilane. The preparations of compounds **1**[1] and **2**[2] were reported previously.

2. Syntheses and characterizations.

Compound 6. In a flame-dried round-bottom flask compounds **5** (100 mg, 0.27 mmol)[1] and **8** (82 mg, 0.32 mmol)[3] were dissolved in dry THF (20 mL) and cooled to -78 °C. Potassium hexamethyldisilazide (KHMDS) (640 μL, 0.5 M in toluene, 0.32 mmol) was added dropwise. Upon completing the addition, the stirring was continued for 3 h, while the temperature rose to rt. The reaction mixture was then diluted with ethyl acetate (50 mL) and filtered through a short pad of silica gel. The solvent was removed under reduced pressure. The crude product was dissolved in dichloromethane (2 mL) and added dropwise to methanol (20 mL). The precipitated product was filtered and dried under vacuum. The yield was 85 mg (67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.74 (d, *J* = 1.8 Hz, 1H), 8.65 (s, 1H), 8.39 (t, *J* = 7.2 Hz, 2H), 7.94 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.82 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 16.2 Hz, 1H), 6.99 (d, *J* = 16.2 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.69 (s, 2H), 4.24 (d, *J* = 2.4 Hz, 2H), 4.03 (t, *J* = 6.0 Hz, 2H), 3.51 (t, *J* = 6.0 Hz,

2H), 2.51 (t, *J* = 1.8 Hz, 1H), 2.04-2.12 (m, 2H), 1.94-2.01 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 159.2, 155.8, 154.4, 149.1, 148.1, 136.9, 133.6, 133.2, 132.8, 130.6, 129.7, 128.2, 122.6, 121.1, 120.9, 114.9, 79.4, 75.4, 69.0, 67.1, 57.6, 33.7, 29.6, 28.0; HRMS (ESI+) (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{26}BrN_2O_2$ 477.1177, found 477.1161.

Compound 7. Compound **6** (50 mg, 0.10 mmol) and azide **9** (37 mg, 0.10 mmol)[1] were dissolved in a dichloromethane/methanol (1:1) mixture (6.0 mL). Aqueous solutions of sodium ascorbate (0.5 M, 50 μL) and $Cu(OAc)_2{\cdot}H_2O$ (0.1 M, 50 μL) were mixed to produce an orange suspension containing the copper(I) catalytic species, which was subsequently added to the stirring solution. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, catalytic amount) was added, and the mixture was stirred for 12 h at rt. The reaction mixture was then partitioned between dichloromethane and a basic EDTA (0.1 M, pH = 10) solution. The organic fraction was washed with basic saturated brine (pH > 10) two more times before dried over anhydrous K_2CO_3. After filtration, the solvent was removed under reduced pressure, and the product was isolated by silica chromatography. Unreacted starting material was isolated using ethyl acetate in dichloromethane (0-50%). The subsequent elution with methanol in dichloromethane (gradient 0-2%) afforded the product in pure form. The yield was 68 mg (78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.72 (d, *J* = 1.8 Hz, 1H), 8.64 (s, 1H), 8.36 (t, *J* = 8.4 Hz, 2H), 7.92 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.82 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.54 (s, 1H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.18 (d, *J* = 16.8 Hz, 1H), 6.92 (d, *J* = 16.2 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.03 (s, 2H), 4.74 (s, 2H), 4.68 (s, 2H), 4.37 (t, *J* = 6.6 Hz, 2H), 4.03 (t, *J* = 6.0 Hz, 2H), 3.50 (t, *J* = 6.6 Hz, 2H), 2.91 (t, *J* = 8.4 Hz, 2H), 2.49 (s, 6 H), 2.36 (s, 6H), 1.92-2.12 (m, 6H), 1.60-1.72 (m, 2H), 1.40-1.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 159.3, 155.7, 154.3, 154.2, 148.9, 148.0, 145.8, 145.1, 140.4, 136.8, 133.7, 133.4, 133.3, 131.5,

130.7, 129.7, 128.2, 122.6, 121.9, 121.2, 120.9, 114.9, 70.1, 67.1, 64.2, 50.2, 33.6, 31.3, 30.3, 29.6, 28.2, 28.1, 27.1, 16.6, 14.6; HRMS (ESI+) (*m/z*): $[M+H]^+$ calcd for $C_{44}H_{50}BBrF_2N_7O_2$ 836.3270, found 836.3253.

Compound 3. Compound **7** (40 mg, 0.05mmol) and *n*-propylamine (3 mL, excess) were heated in acetonitrile (10 mL) at 50 °C. After 12 h, the reaction mixture was allowed to cool to rt and diluted with dichloromethane (20 mL). The organic layer was washed with water (50 mL × 2), separated, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. The crude product was then dissolved in dichloromethane (1.0 mL) and added dropwise to hexanes (15 mL). The precipitated orange powder was filtered and dried under vacuum. The yield was 23 mg (59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.72 (s, 1H), 8.64 (s, 1H), 8.36 (t, *J* = 8.4 Hz, 2H), 7.92 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.82 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.53 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 16.2 Hz, 1H), 6.97 (d, *J* = 16.2 Hz, 1H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.03 (s, 2H), 4.74 (s, 2H), 4.69 (s, 2H), 4.37 (t, *J* = 7.2 Hz, 2H), 4.00 (t, *J* = 6.6 Hz, 2H), 3.15 (br, s, 1H), 2.91 (t, *J* = 7.8 Hz, 2H), 2.66 (t, *J* = 7.2 Hz, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.49 (s, 6H), 2.34 (s, 6H), 1.96 (t, *J* = 7.2 Hz, 2H),1.43-1.87 (m, 10H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 159.4, 155.7, 154.3, 154.1, 148.9, 148.0, 145.7, 145.1, 140.3, 136.7, 133.6, 133.3, 133.2, 131.5, 130.7, 129.5, 128.2, 122.6, 122.4, 121.8, 121.1, 120.8, 114.9, 70.1, 67.1, 64.1, 51.9, 50.1, 49.7, 31.3, 30.2, 28.2, 27.2, 27.1, 26.7, 23.2, 16.5, 14.6, 11.9; HRMS (ESI+) (*m/z*): $[M+H]^+$ calcd for $C_{47}H_{58}BF_2N_8O_2$ 815.4743, found 815.4741.

Compound 4. Compound **7** (20 mg, 0.02 mmol) and morpholine (100 μL, excess) were dissolved in acetonitrile (10 mL), and the solution was heated at 60 °C. After 12 h, the reaction mixture was allowed to cool to rt and diluted with dichloromethane (20 mL). The organic layer was washed

with water (50 mL × 2), separated, and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure. The crude product was then dissolved in dichloromethane (1.0 mL) and added dropwise to hexanes (15 mL). The precipitated orange powder was filtered and dried under vacuum. The yield was 12 mg (62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.72 (s, 1H), 8.63 (s, 1H), 8.37 (t, *J* = 8.4 Hz, 2H), 7.93 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.80 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.54 (s, 1H), 7.46 (d, *J* = 9.0 Hz, 2H), 7.18 (d, *J* = 16.2 Hz, 1H), 6.97 (d, *J* = 16.2 Hz, 1H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.03 (s, 2H), 4.74 (s, 2H), 4.69 (s, 2H), 4.27 (t, *J* = 7.2 Hz, 2H), 4.02 (t, *J* = 6.0 Hz, 2H), 3.78 (s, br, 4H), 2.91 (t, *J* = 8.4 Hz, 2H), 2.49 (m, 12H), 2.36 (s, 6H), 1.94 (t, *J* = 7.2 Hz, 2H), 1.42-1.83 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 159.3, 155.8, 154.4, 154.2, 148.9, 148.1, 145.7, 145.1, 140.4, 136.7, 133.6, 133.4, 133.3, 131.5, 130.7, 129.6, 128.2, 122.6, 121.9, 121.1, 120.8, 114.9, 70.1, 67.7, 66.6, 64.1, 58.6, 53.6, 50.2, 31.3, 30.3, 28.2, 27.2, 27.1, 22.8, 16.6, 14.6; HRMS (ESI+) (*m/z*): $[M+H]^+$ calcd for $C_{48}H_{58}BF_2N_8O_3$ 843.4693, found 843.4678.

3. Absorption and Fluorescence Spectroscopies. Spectrophotometric and fluorometric titrations were conducted on a Varian Cary 100 Bio UV-Visible Spectrophotometer and a Varian Cary Eclipse Fluorescence Spectrophotometer, respectively, with a 1-cm semi-micro septum-capped quartz cuvette (Starna). For the zinc(II) titration experiment, samples A and B were first prepared. Both contained an equal amount of the indicator; but B solution had an excess amount of $ZnCl_2$. The spectra were acquired as the B sample was titrated into the A sample, so that the indicator concentration remained constant in the cuvette while the zinc(II) concentration increased. For the acquisition of the fluorescence spectra, the samples were excited at 405 nm, at which wavelength the absorbance value of the sample was kept under 0.1. The choice of the

excitation wavelength was based on the availability of the laser lines in the confocal fluorescence microscopy experiments.

For the fluorescence quantum yield (ϕ) measurements,[4] the excitation wavelength (λ_{ex}) was chosen at the maximum absorption wavelength of the FRET donor moiety in each compound or zinc(II) complex, where (1) the absorbance value at λ_{ex} was 0.1 or below, and (2) the complete emission spectrum could be collected. The absorbance value at λ_{ex} and integrated emission intensity of the substrate, as well as those of a standard sample (quinine bisulfate in 1 N sulfuric acid, ϕ = 0.546) were used in Equation S1 to calculate the ϕ value of the sample.

$$\phi = \frac{A_S \cdot F \cdot n^2}{A \cdot F_S \cdot {n_S}^2} \phi_S \qquad \text{Equation S1}$$

Where A_s and A are the absorbance values of the sample and reference solutions at their respective excitation wavelengths, F_s and F are the corresponding integrated fluorescence intensity, and n is the refractive index of the solvent of the sample (n) or of the standard (n_s).

4. Photostability measurements. The absorption spectra of compounds **2** and **4** (5 μM each in 1:1 water/CH_3CN mixture at pH 7.3, HEPES 25 mM, NaCl 25 mM) in the presence or absence of $ZnCl_2$ (20 molar equivalents) were first collected in an unlit room. The quartz cuvette containing the sample was then placed in front of a handheld UV lamp at a 15-cm distance and irradiated at 365 nm. The absorption spectra were acquired at 10-s intervals (Figure 5). The experiment was terminated at 1 min.

5. Confocal fluorescence microscopy. Confocal fluorescence microscopy was performed using an Olympus (Center Valley, PA, USA) FV1000 confocal laser point-scanning system equipped with Fluoview software and on an Olympus IX81 inverted stand. Imaging was performed using an Olympus 60x PlanApo NA = 1.4 oil immersion objective and 405-nm diode laser line. Green

fluorescence from the tested compounds was detected between 500-530 nm and red fluorescence from Fusion Red from 590-690 nm using a variable bandpass filter. Images were acquired and processed (using the Olympus Fluoview software) with identical parameter sets for them to be comparable.

6. Photobleaching experiment. Photobleaching comparisons of compounds **2**-**4** were conducted using a Nikon (Melville, NY, USA) widefield inverted TE-2000S microscope using an X-Cite eXacte mercury arc lamp (Lumen Dynamics, Mississauga, ON, Canada). A custom filter-set consisting of a 305-405 nm excitation filter, 410 nm long-pass dichroic mirror, and a 510-560 nm emission filter were used for all photobleaching experiments. Imaging was performed with a Nikon 40x Plan Apo NA = 0.95 dry objective and recorded with a QImaging (Surrey, BC, Canada) Retiga ExI charge-coupled device camera with a hardware gain = 4.222, readout speed of 20 MHz, exposure time of 150 ms, and without binning. Photobleaching was performed at three different lamp power outputs for each compound: 5% (400 µW), 10% (970 µW), and 25% (1910 µW). Lamp power was measured at the objective. Three acquisitions were acquired for each compound at each of the three specified lamp powers, and analyzed using the Nikon Elements software. Cells were prepared and incubated with the appropriate compound as described previously;[5] and kept at physiological temperature and appropriate gas phase using a Bioptechs (Butler, PA, USA) Delta-T culture dish controller.

7. Structured illumination microscopy. Structured illumination microscopy (SIM) was performed using a Zeiss (Thornwood, NY, USA) Elyra PS.1 superresolution system. Excitation was performed using a 488-nm argon-ion laser for green fluorescence from compound **4** and a 561-nm diode laser for red fluorescence from the fluorescent protein Fusion Red, and detected using

a green 495-550 nm and red 570-620 nm emission filter, respectively. Fluorescence was detected using a Zeiss 63x C-Apochromat NA = 1.2 water immersion objective and a pco.edge scientific CMOS camera (PCO AG, Kelheim, Germany). Cells were prepared with compound **4** as described and imaged live in culture medium under zinc(II)-deficient conditions. Three rotations of the SIM grid pattern was chosen to facilitate fast imaging of living cells. All SIM data were analyzed using the SIM module available in the Zeiss ZEN 2012 software. Lateral resolutions of 110-nm in the green channel and 130-nm in the red were determined using this software.

8. Additional figures.

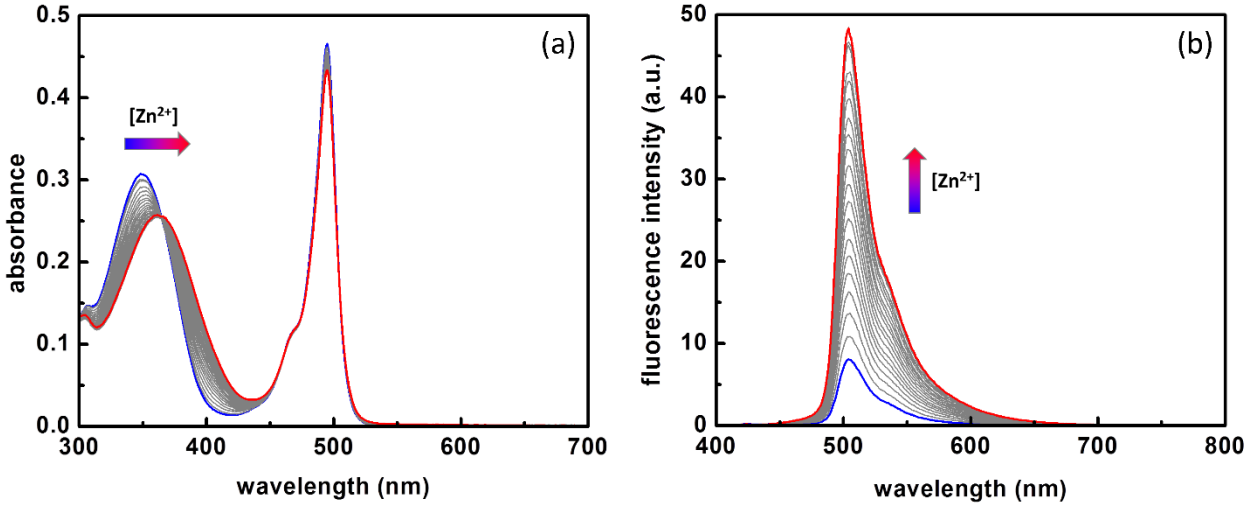


Figure S1. (a) Absorption spectral changes of **1** (10 μM) on addition of $ZnCl_2$ (0-10 molar equiv.) in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM) at pH 7.3. (b) Corresponding changes in the emission spectra. λ_{ex} = 405 nm.

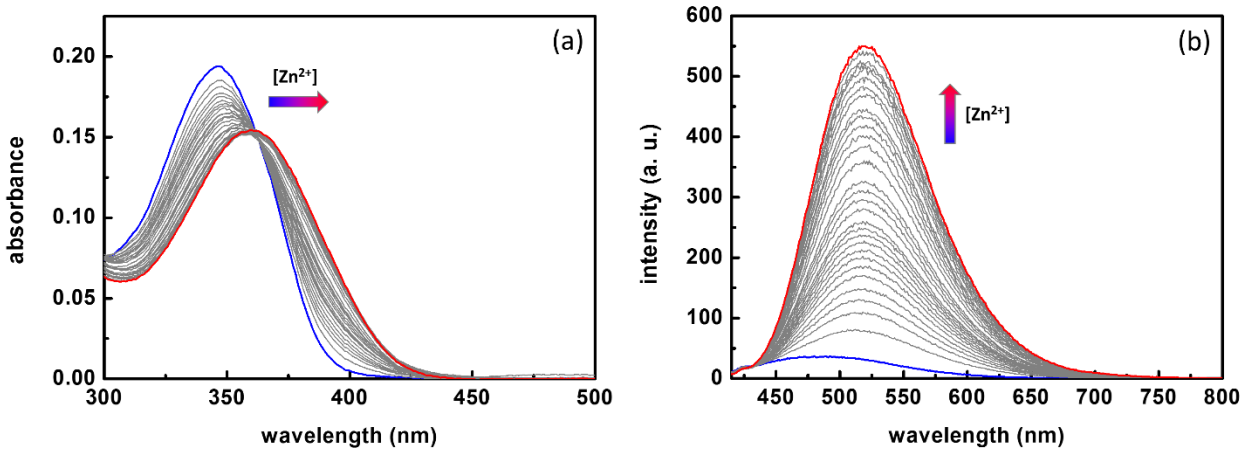


Figure S2. (a) Absorption spectral changes of **2** (5 μM) on addition of $ZnCl_2$ (0-20 molar equiv.) in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM) at pH 7.3. (b) Corresponding changes in the emission spectra. λ_{ex} = 405 nm.

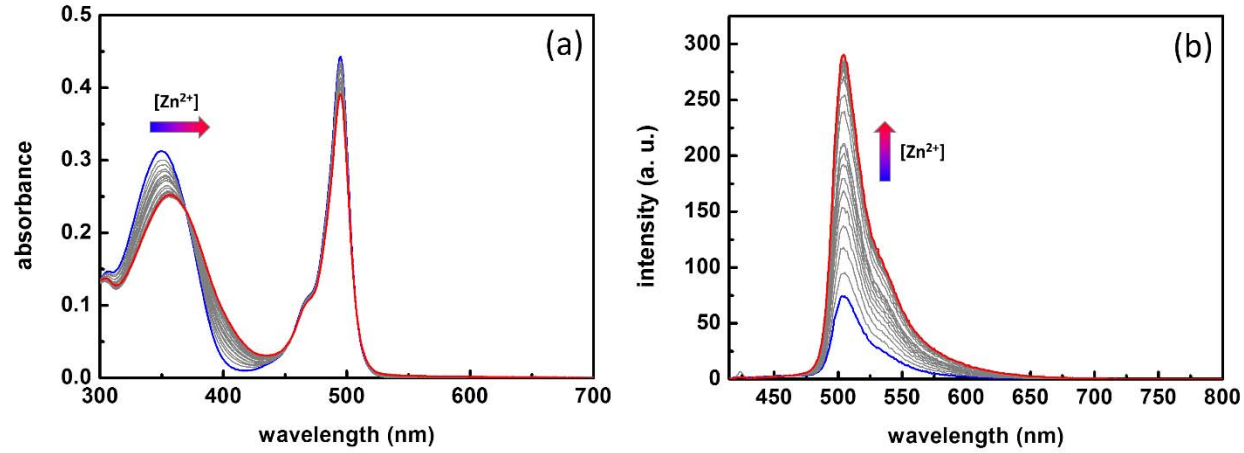


Figure S3. (a) Absorption spectral changes of **4** (10 μM) on addition of $ZnCl_2$ (0-20 molar equiv.) in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM) at pH 7.3. (b) Corresponding changes in the emission spectra. λ_{ex} = 405 nm.

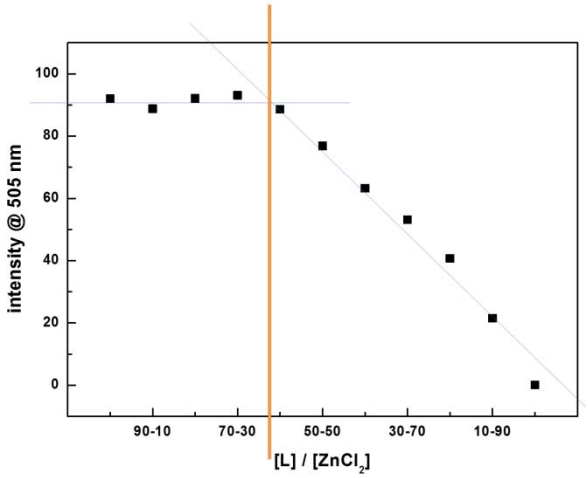


Figure S4. Job plot for compound **1** association with $ZnCl_2$ in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM) at pH 7.3. The total concentration of **1** and $ZnCl_2$ was maintained at 5 µM.

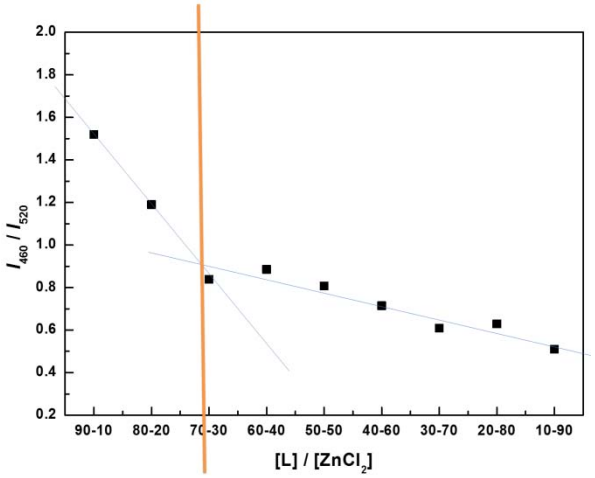


Figure S5. Job plot for compound **2** association with $ZnCl_2$ in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM) at pH 7.3. The total concentration of **2** and $ZnCl_2$ was maintained at 5 µM.

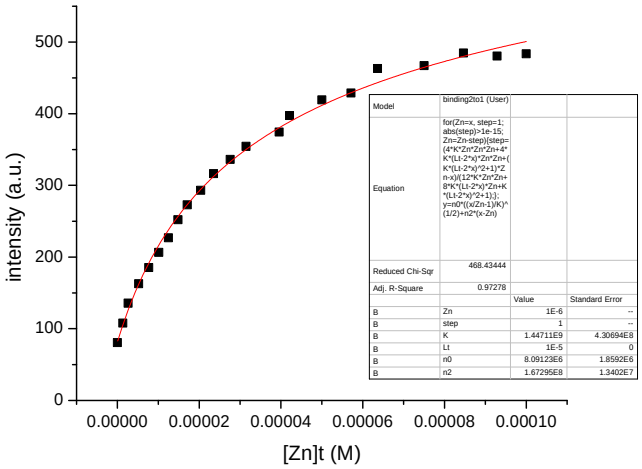


Figure S6. Solid squares: fluorescence intensity of compound **1** at 504 nm vs. total Zn(II) concentration ([Zn]t); red curve: the fitting curve based on a 2:1 (ligand/Zn(II)) binding model.

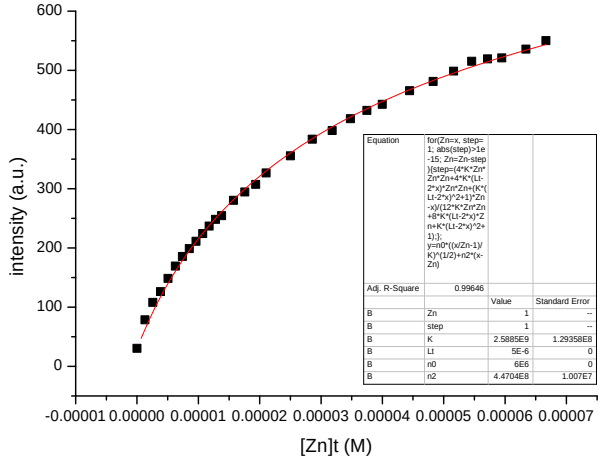


Figure S7. Solid squares: fluorescence intensity at 520 nm of compound **2** vs. total Zn(II) concentration ([Zn]t); red curve: the fitting curve based on a 2:1 (ligand/Zn(II)) binding model.

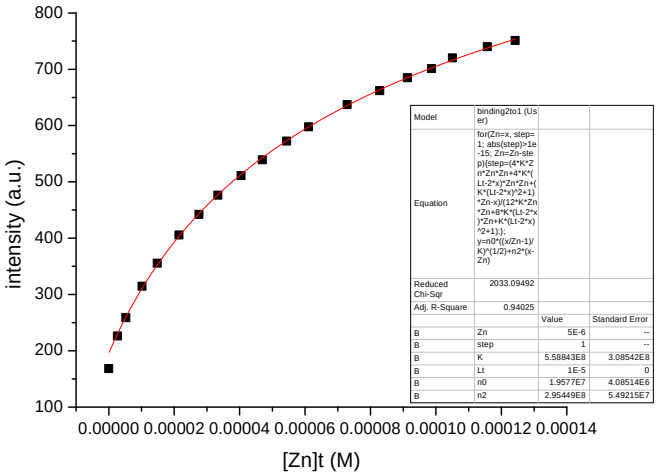


Figure S8. Solid squares: fluorescence intensity of compound **3** at 504 nm vs. total Zn(II) concentration ([Zn]t); red curve: the fitting curve based on a 2:1 (ligand/Zn(II)) binding model.

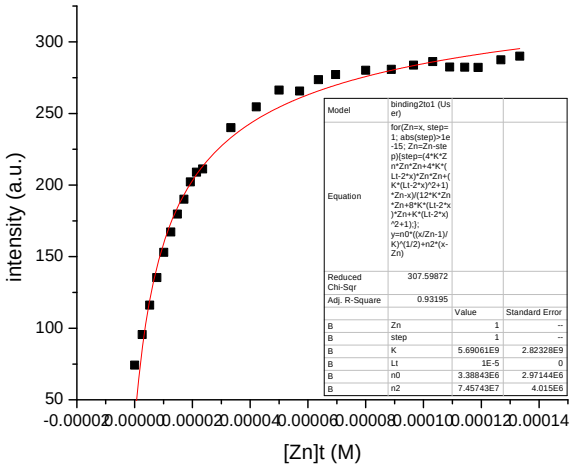


Figure S9. Solid squares: fluorescence intensity of compound **4** at 504 nm vs. total Zn(II) concentration ([Zn]t); red curve: the fitting curve based on a 2:1 (ligand/Zn(II)) binding model. The fluorescence intensity at 504 nm of compound **4** in the absence of Zn(II) under the conditions described in the caption of Figure S3 is 74.1 $\pm$ 3.47 (of 10 repeats). The limit-of-detection (lod) is considered as the Zn(II) concentration that leads to an intensity value of 74.1 + 3 $\times$ 3.47 = 84.5 on the calibration curve, which is 1.0 μM.

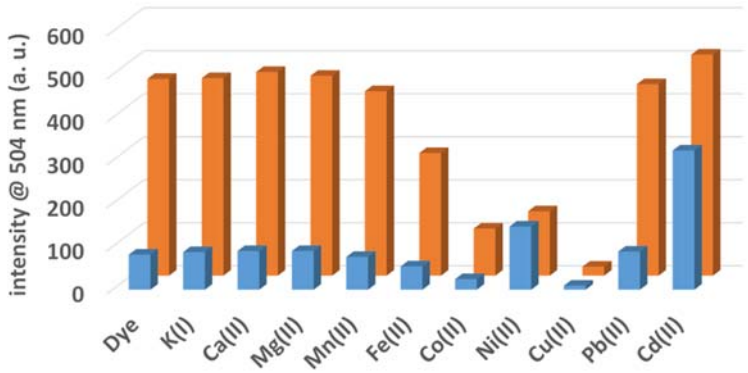


Figure S10. Fluorescence spectroscopic responses of compound **4** (10 μM, λ_{ex} = 405 nm) to various metal ions in 1:1 water/CH_3CN mixture at pH 7.3 (HEPES 25 mM, NaCl 25 mM). Blue bars represent the fluorescence intensity at 504 nm in the presence of various metal ions (chloride salts). Metal ion concentrations of K(I), Ca(II), Mg(II) were 1 mM, and for other ions 50 μM. Orange bars represent the intensity at 504 nm following the addition of $ZnCl_2$ (50 μM).

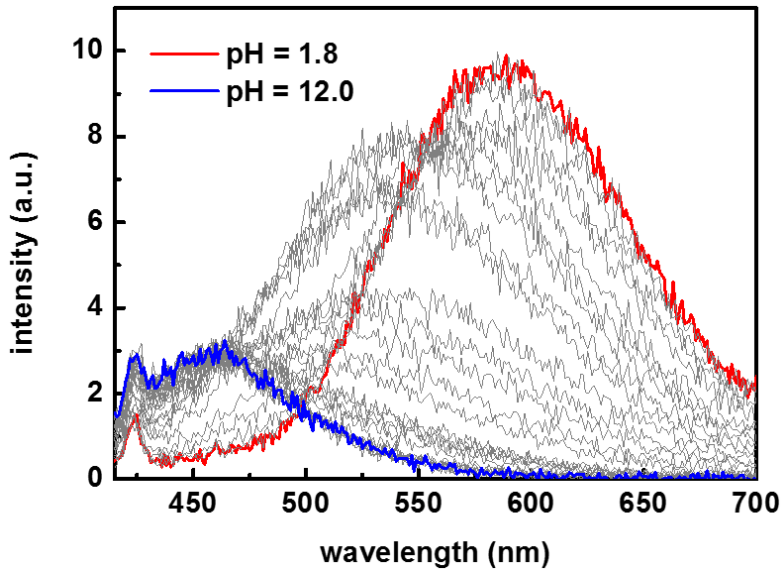


Figure S11. The emission spectra of **4** collected under 405-nm excitation using an otherwise identical parameter set to that of Figure 4b. The pH value is varied from basic (blue) to acidic (red). The overall intensity is very low.

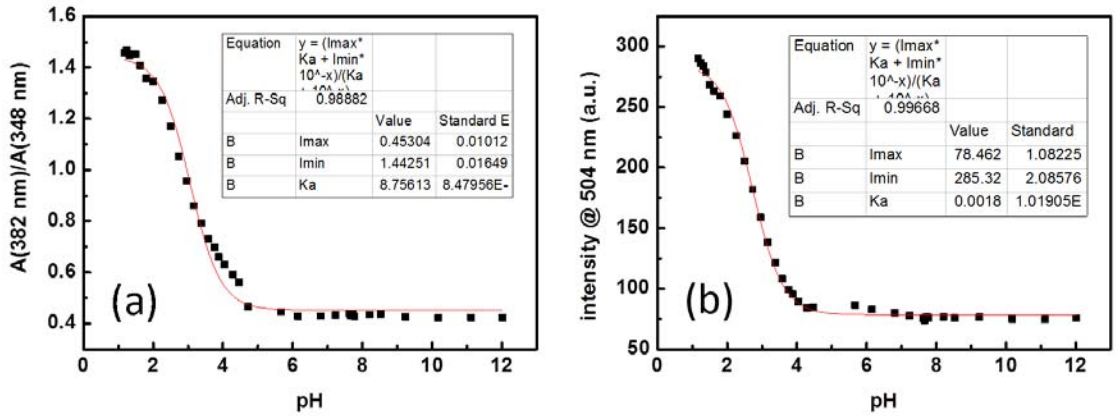


Figure S12. All spectra of compound **4** (2.0 μM, see Figure 4) are taken in water/CH_3CN mixture (1:1) containing HEPES (25 mM) and NaCl (25 mM). (a) The absorbance ratio at 382 nm and 348 nm vs. pH value. The fitted curve based on the Henderson–Hasselbalch equation is in red; (b) the emission intensity (λ_{ex} = 405 nm) at 504 nm vs. pH value. The fitted curve based on the Henderson–Hasselbalch equation is in red.

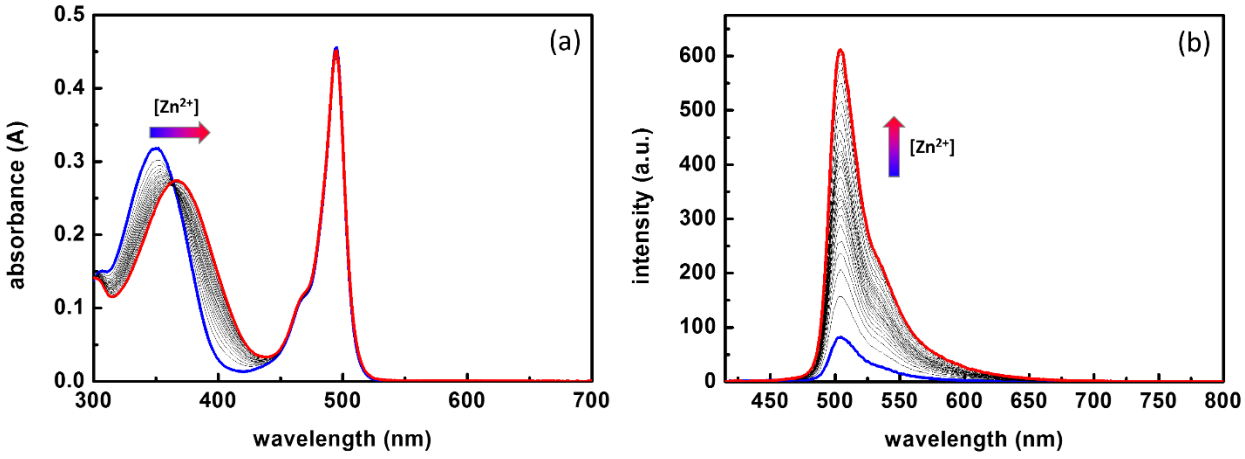


Figure S13. (a) Absorption spectral changes of **4** (10 μM) on addition of $ZnCl_2$ (0-20 molar equiv.) in water/CH_3CN mixture (1:1) containing HEPES (25mM) and NaCl (25 mM) at pH **4.8**. (b) Corresponding changes in the emission spectra. λ_{ex} = 405 nm.

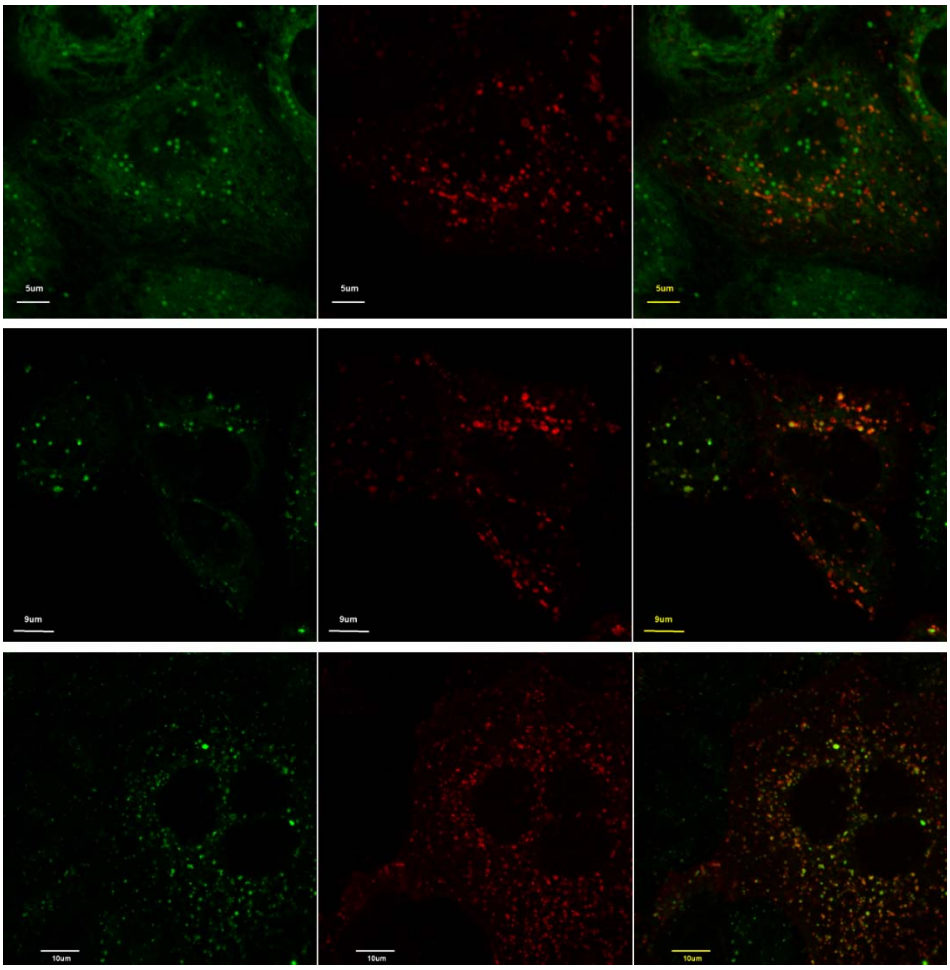


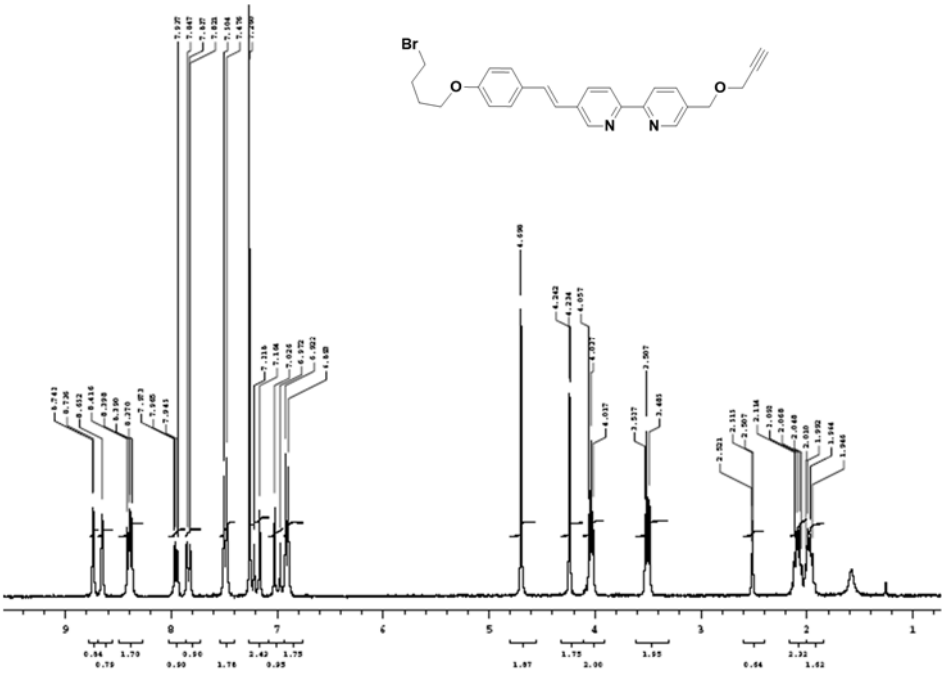
Figure S14. Green channel (left column): emission from compounds **1** (top row), **3** (middle row), and **4** (bottom row); Red channel (middle column): emission from FusionRed-LAMP; merged channels are on the right. The concentrations of the dyes at the incubation stage were 2 μM.

References:

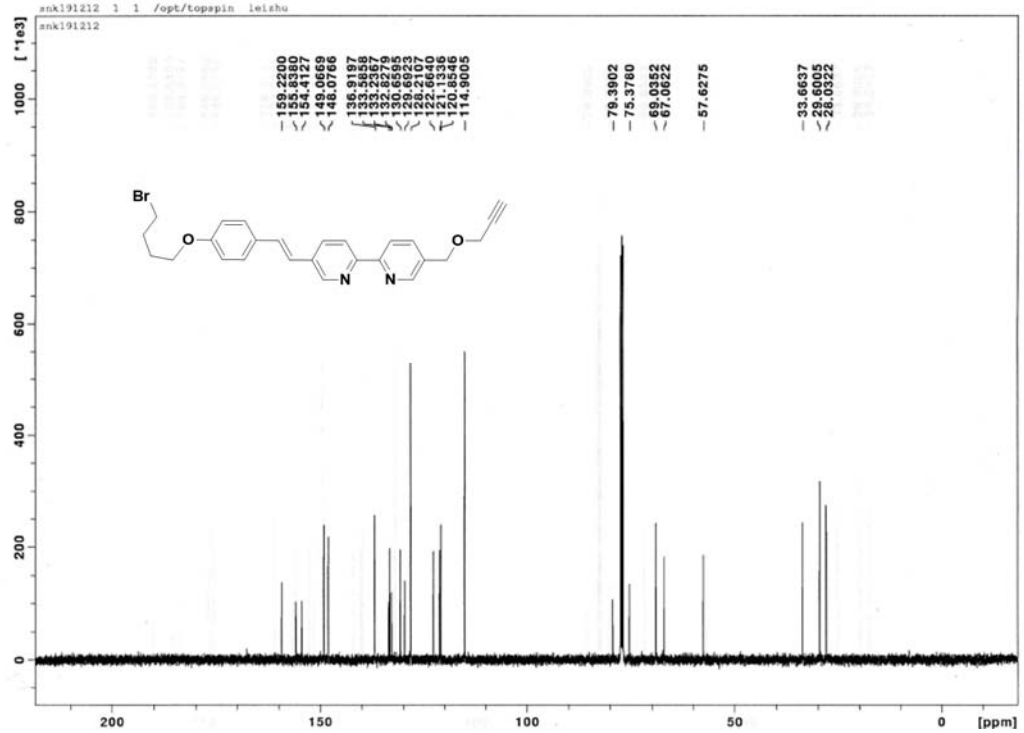
[1] K. Sreenath, C. Yi, J. K. L. Knappenberger, L. Zhu, *Phys. Chem. Chem. Phys.* **2014**, *16*, 5088-5092.
[2] A. H. Younes, L. Zhang, R. J. Clark, L. Zhu, *J. Org. Chem.* **2009**, *74*, 8761-8772.
[3] K. Sreenath, R. J. Clark, L. Zhu, *J. Org. Chem.* **2012**, *77*, 8268-8279.
[4] S. Fery-Forgues, D. Lavabre, *J. Chem. Ed.* **1999**, *76*, 1260-1264.
[5] a) K. Sreenath, J. R. Allen, M. W. Davidson, L. Zhu, *Chem. Commun.* **2011**, *47*, 11730-11732; b) J. T. Simmons, J. R. Allen, D. R. Morris, R. J. Clark, C. W. Levenson, M. W. Davidson, L. Zhu, *Inorg. Chem.* **2013**, *52*, 5838-5850.

Scanned copies of NMR spectra of new compounds.

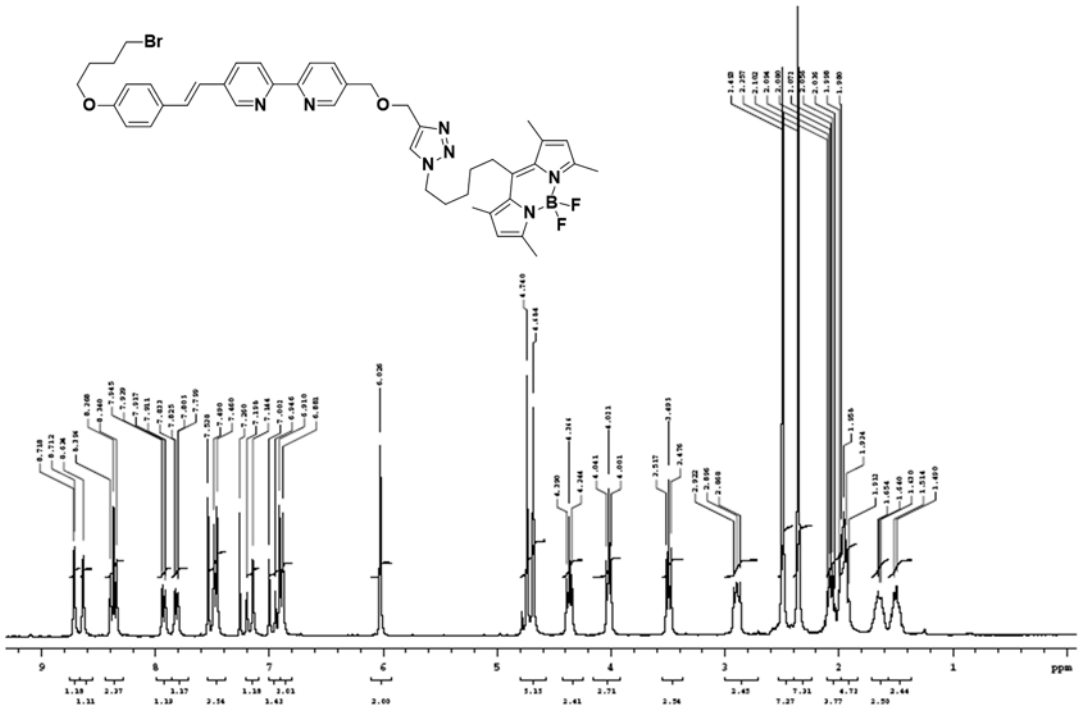
^{1}H NMR (300 MHz, $CDCl_3$) of compound **6**



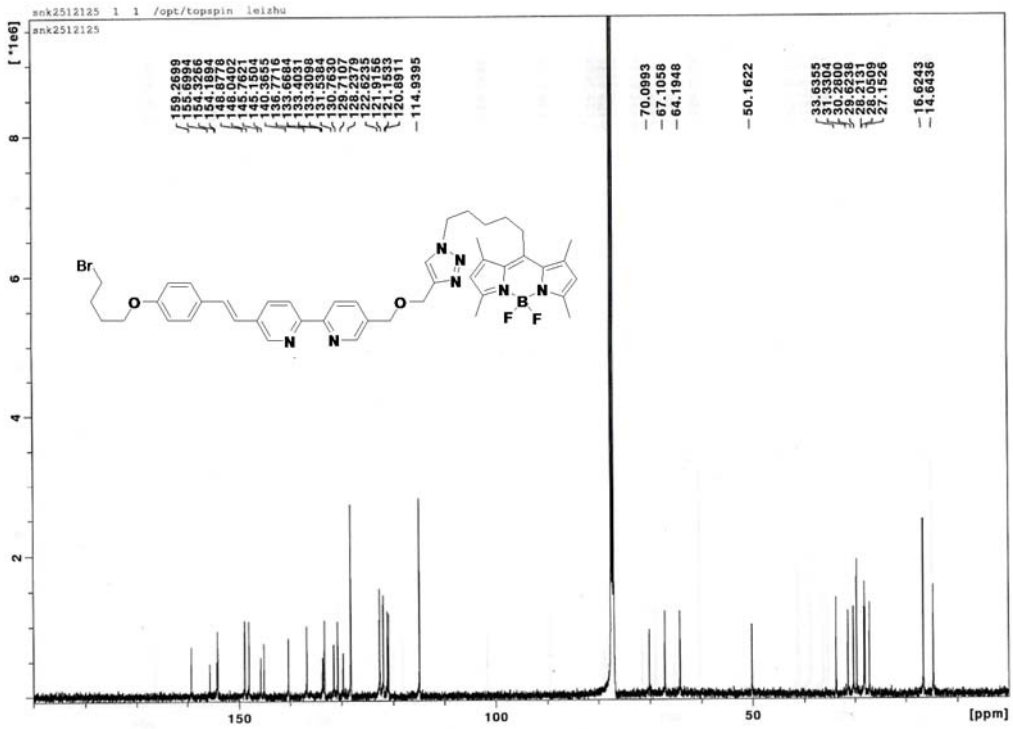
^{13}C NMR (125 MHz, $CDCl_3$) of compound **6**



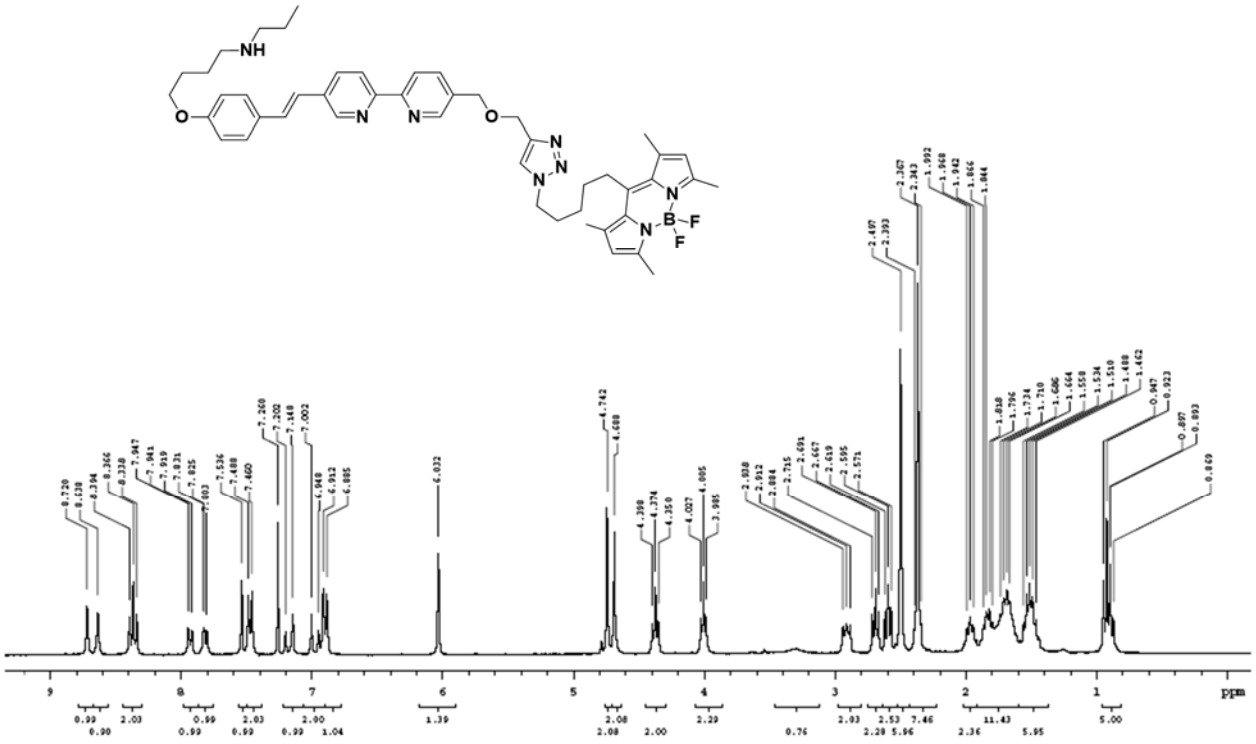
^{1}H NMR (300 MHz, $CDCl_3$) of compound **7**



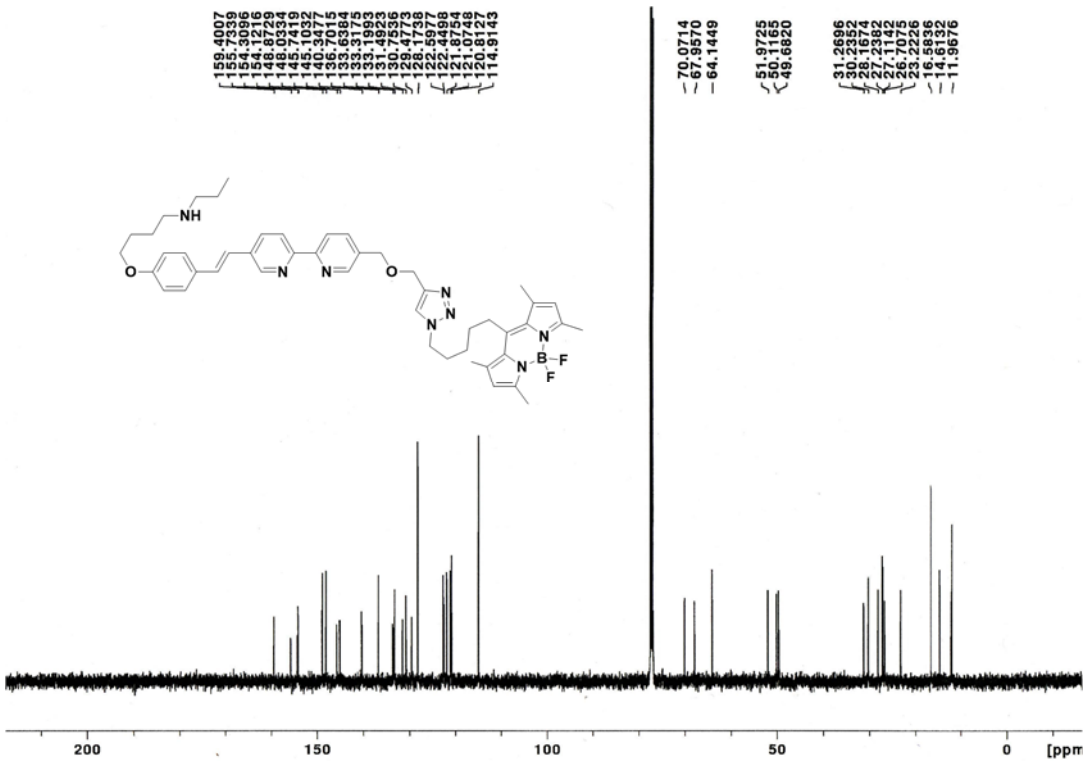
^{13}C NMR (125 MHz, $CDCl_3$) of compound **7**



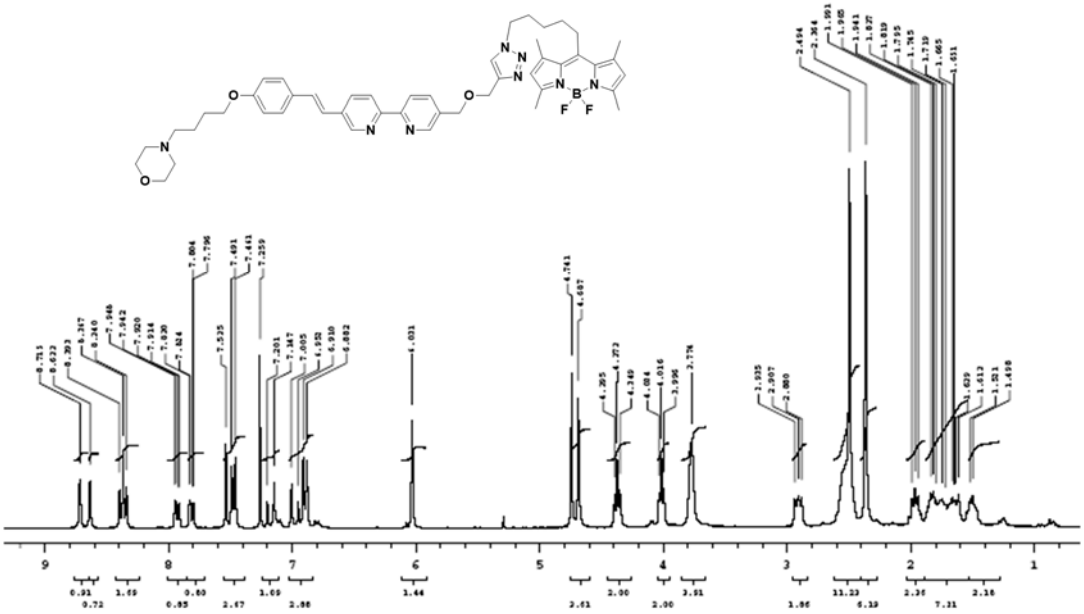
^{1}H NMR (300 MHz, $CDCl_3$) of compound **3**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **3**



^{1}H NMR (300 MHz, $CDCl_3$) of compound **4**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **4**