Supplementary Material (ESI) for New Journal of Chemistry

Metal-Coordination-Mediated Sequential Chelation-Enhanced Fluorescence (CHEF) and Fluorescence Resonance Energy Transfer (FRET) in a Heteroditopic Ligand System

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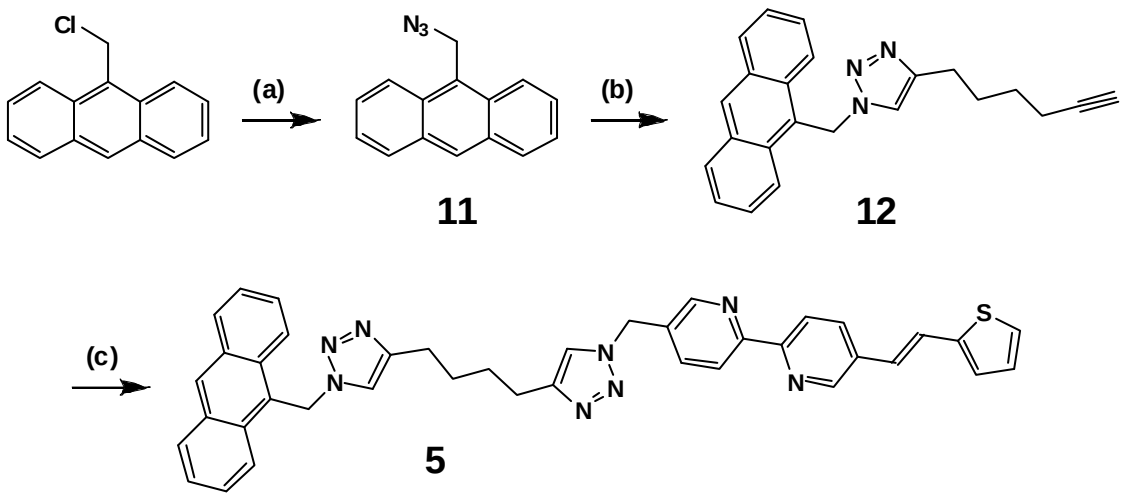
Supporting Information

Materials and General Methods.

Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. CH_3CN (OmniSolv, EMD) were directly used in titration experiments without purification. All reactions were carried out in oven- or flame-dried glassware in an inert atmosphere of argon. Analytical thin-layer chromatography (TLC) was performed using pre-coated TLC plates with silica gel 60 F254 (EMD) or with aluminum oxide 60 F254 neutral (EMD). Flash column chromatography was performed using 40-63 μm (230-400 mesh ASTM) silica gel (EMD) or alumina (80-200 mesh, pH 9-10, EMD) as the stationary phases. Silica and alumina gel was flame-dried under vacuum to remove absorbed moisture before use. THF was dried by distilling from sodium-benzophenone in a continuous still under argon protection. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, on a Varian Mercury spectrometer. All chemical shifts were reported in δ units relative to tetramethylsilane. $CDCl_3$ was treated with alumina gel prior to use. High resolution mass spectra were obtained at the Mass Spectrometry Laboratory at FSU: ESI spectra were obtained on a JEOL AccuTOF spectrometer. Spectrophotometric and fluorometric tritrations were conducted on a Varian Cary 100 Bio UV-Visible Spectrophotometer and a Varian Cary Eclipse Fluorescence Spectrophotometer, respectively.

Synthesis.



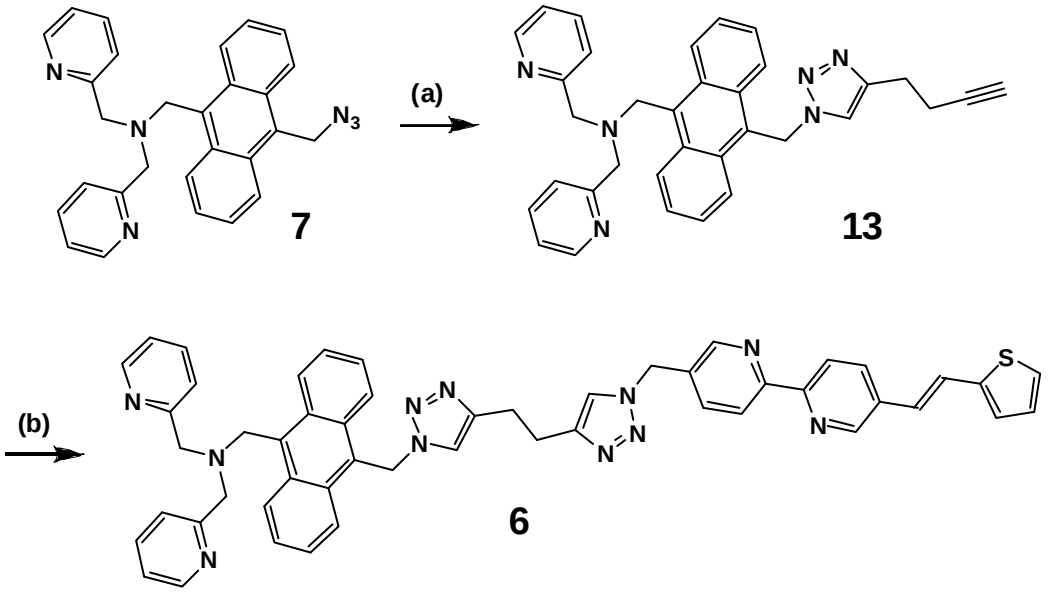
Scheme S1. (a) NaN_3, 18-crown-6, tetrabutylammonium iodide, CH_3CN, rt, 16 h, > 95%; (b) 1,7-octadiyne, $Cu(OAc)_2$, sodium ascorbate, CH_3OH, rt, 16 h, 42%; (c) **10**, $Cu(OAc)_2$, sodium ascorbate, CH_3OH, rt, 16 h, 35%.

Compound 11. 9-(Chloromethyl)anthracene (453 mg, 2 mmol) was dissolved in THF (10 mL) followed by the sequential addition of NaN_3 (260 mg, 4 mmol), 18-crown-6 (catalytic amount), and tetrabutylammonium iodide (catalytic amount). The reaction mixture was stirred at rt for overnight. The residue was diluted with CH_2Cl_2 and washed three times with water. The organic fraction was then dried over K_2CO_3 before the solvent was removed under vacuum. The yield was 95-100%. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.51 (s, 1H), 8.30 (d, J = 9.0 Hz, 2H), 8.06 (d, J = 9.0 Hz, 2H), 7.54 (m, 4H), 5.33 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 131.4, 130.7, 129.3, 129.0, 126.9, 125.8, 125.2, 123.5, 46.3.

Compound 12. Compound **11** (230 mg, 1.02 mmol) and 1,7-octadiyne (500 μL, 3.76 mmol) were dissolved in CH_3OH (40 mL). Aqueous solutions of sodium ascorbate (0.5 M, 1 mL) and $Cu(OAc)_2$ (0.1 M, 1 mL) were mixed to produce an orange suspension containing the Cu(I) catalytic species, which was subsequently added to the stirring methanolic solution. The mixture was stirred for 2 days before CH_3OH was removed under vacuum. The residue was partitioned between ethyl acetate and basic brine (pH = 12). The organic fraction was washed with the basic brine solution two more times before dried over K_2CO_3. The solvent was removed, and

compound **12** was isolated by alumina chromatography eluted by 10-40% ethyl acetate in CH_2Cl_2. The yield was 42%. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.58 (s, 1H), 8.32 (d, J = 9.0 Hz, 2H), 8.08 (d, J = 8.1 Hz, 2H), 7.58 (m, 4H), 6.85 (s, 1H), 6.50 (s, 2H), 2.55 (t, J = 7.4 Hz, 2H), 2.11 (td, J = 6.9, 2.6 Hz, 2H), 1.82 (t, J = 2.6 Hz, 1H), 1.62 (m, 2H), 1.47 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 148.1, 131.5, 130.9, 129.8, 129.5, 127.7, 125.5, 124.2, 123.2, 120.2, 84.4, 68.5, 46.4, 28.4, 28.0, 25.3, 18.2. HRMS (ESI+): calcd. ($M+H^+$) 340.1814, found 340.1806.

Compound 5. Compound **12** (72 mg, 0.21 mmol) and compound **10** (68 mg, 0.21 mmol) were dissolved in CH_3OH (12 mL). Aqueous solutions of sodium ascorbate (0.5 M, 1 mL) and $Cu(OAc)_2$ (0.1 M, 1 mL) were mixed to produce an orange suspension containing the Cu(I) catalytic species. The orange Cu(I) suspension (1.38 mL) was added to the stirring mixture. The mixture was stirred for overnight followed by the addition of an EDTA solution (0.1 M, 1.38 mL) while the stirring was continued for 1 h. After CH_3OH was removed under vacuum, the residue was diluted with ethyl acetate, washed 3 times with basic brine, and dried over K_2CO_3. The solvent was removed and compound **5** was isolated by alumina chromatography eluted by 60-80% ethyl acetate in CH_2Cl_2. Compound **5** was further purified by washing with diethyl ether. The yield was 35%. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.72 (d, J = 2.0 Hz, 1H), 8.59 (d, J = 2.0 Hz, 1H), 8.57 (s, 1H), 8.38 (t, J = 8.4 Hz, 2H), 8.31 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 8.0 Hz, 2H), 7.92 (dd, J = 8.3, 2.2 Hz, 1H), 7.66 (dd, J = 8.2, 2.3 Hz, 1H), 7.68-7.49 (m, 4H), 7.36 (d, J = 16.1 Hz, 1H), 7.27 (m, 1H), 7.18 (s, 1H), 7.14 (d, J = 3.5 Hz, 1H), 7.04 (dd, J = 3.6, 1.4 Hz, 1H), 6.93 (d, J = 16.1 Hz, 1H), 6.84 (s, 1H), 6.49 (s, 2H), 5.53 (s, 2H), 2.63 (t, J = 7.0 Hz, 2H), 2.54 (t, J = 7.2 Hz, 2H), 1.58 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 148.8, 148.5, 148.0, 147.8, 142.2, 136.6, 133.2, 133.1, 131.5, 130.8, 129.4, 127.8, 127.6, 127.2, 125.4, 124.3, 124.1, 124.0, 123.1, 121.2, 121.1, 120.5, 120.2, 114.3, 51.3, 46.3, 41.0, 29.7, 28.8, 25.3. HRMS (ESI+): calcd. ($M+Na^+$) 681.2525, found 681.2544.



Scheme S2. (a) 1,5-hexadiyne, $Cu(OAc)_2$, sodium ascorbate, CH_3OH, rt, 16 h, 44%; (b) **10**, $Cu(OAc)_2$, sodium ascorbate, CH_3OH, rt, 16 h, 29%.

Compound 13. Compound **7** (483 mg, 1.09 mmol) and 1,5-hexadiyne (537 μL, 5.5 mmol) were dissolved in CH_3OH (60 mL). Aqueous solutions of sodium ascorbate (0.5 M, 1 mL) and $Cu(OAc)_2$ (0.1 M, 1 mL) were mixed to produce an orange suspension containing the Cu(I) catalytic species, which was subsequently added to the stirring methanolic solution. The mixture was stirred for 2 days followed by the addition of an aqueous solution of EDTA (0.1 M, 7 mL) while the stirring was continued for 1 h. After CH_3OH was removed under vacuum, the residue was partitioned between ethyl acetate and basic brine (pH = 12). The organic fraction was washed with basic brine two more times before dried over K_2CO_3. The solvent was removed, and compound **13** was isolated by alumina chromatography eluted by 20-50% ethyl acetate in CH_2Cl_2. The yield was 44%. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.52 (d, J = 8.5 Hz, 2H), 8.49 (d, J = 4.8 Hz, 2H), 8.27 (d, J = 8.2 Hz, 2H), 7.61-7.47 (m, 6H), 7.31 (d, J = 7.8 Hz, 2H), 7.12 (m, 2H), 6.94 (s, 1H), 6.45 (s, 2H), 4.73 (s, 2H), 3.90 (s, 4H), 2.74 (t, J = 7.4 Hz, 2H), 2.39 (td, J = 7.3, 2.5 Hz, 2H), 1.73 (t, J = 2.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 159.5, 149.0, 146.3, 136.4, 133.5, 131.5, 130.6, 127.1, 126.3, 125.6, 124.7, 123.6, 122.2, 120.9, 83.4, 69.0, 60.7, 51.2, 46.7, 25.1, 18.7. HRMS (ESI+): calcd. ($M+H^+$) 523.2610, found 523.2586.

Compound 6. Compound **13** (90 mg, 0.17 mmol) and compound **10** (55 mg, 0.17 mmol) were dissolved in t-butanol (2 mL). Aqueous solutions of sodium ascorbate (0.5 M, 1 mL) and $Cu(OAc)_2$ (0.1 M, 1 mL) were mixed to produce an orange suspension containing the Cu(I) catalytic species. The orange Cu(I) suspension (1 mL) was added to the stirring mixture. The mixture was stirred for overnight followed by the addition of an EDTA solution (0.1 M, 1 mL) while the stirring was continued for 1 h. The residue was diluted with ethyl acetate, washed three times with basic brine (pH = 12), then dried over K_2CO_3. The solvent was removed and compound **6** was isolated by alumina chromatography using 100% ethyl acetate followed by 1-4% CH_3OH in CH_2Cl_2. The product was further purified by washing with diethyl ether. The yield was 29%. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 8.70 (s, 1H), 8.54 (s, 1H), 8.50 (m, 4H), 8.36 (dd, J = 8.2, 3.6 Hz, 2H), 8.28 (d, J = 8.4 Hz, 2H), 7.90 (dd, J = 10.5, 2.0 Hz, 1H), 7.61-7.48 (m, 8H), 7.32 (m, 5H), 7.14 (m, 2H), 7.07 (s, 1H), 7.03 (dd, J = 3.7, 1.4 Hz, 1H), 6.92 (d, J = 13.5 Hz, 1H), 6.83 (s, 1H), 6.44 (s, 2H), 5.35 (s, 2H), 4.73 (s, 2H), 3.90 (s, 4H), 2.95 (d, J = 6.2 Hz, 2H), 2.90 (d, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 159.4, 156.3, 154.0, 148.9, 148.4, 147.9, 147.6, 146.8, 142.2, 136.4, 136.2, 133.4, 133.2, 133.1, 130.5, 130.4, 127.8, 127.1, 127.0, 126.3, 125.5, 125.3, 124.7, 124.6, 124.2, 123.5, 123.4, 122.0, 121.2, 121.0, 120.7, 60.6, 51.1, 46.5, 31.6, 25.3, 25.2. HRMS (ESI+): calcd. ($M+H^+$) 842.3502, found 842.3471.

Additional Spectra.

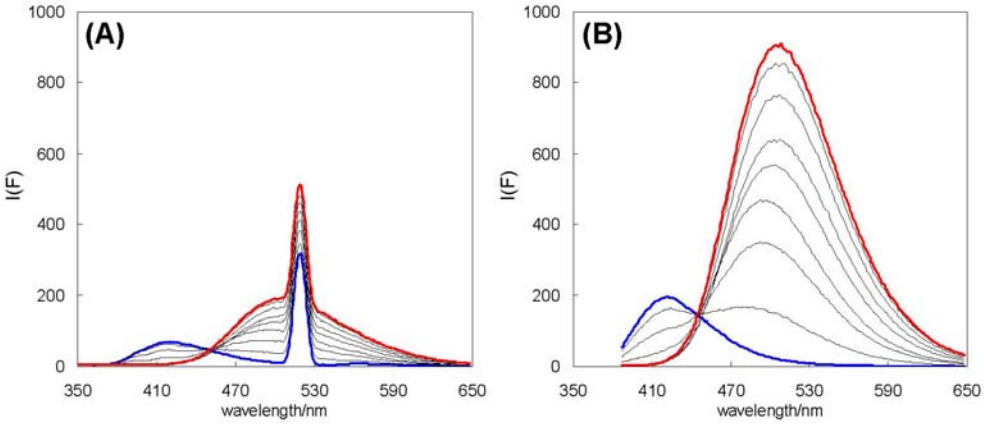


Fig. S1 Fluorescence spectra of **3** (5 μM) in the presence of $Zn(ClO_4)_2$ from 0 (blue) to 17 μM (red) in CH_3CN. (A) λ_{ex} = 260 nm (emission at 520 nm is due to the second-order scattering); (B) λ_{ex} = 375 nm.

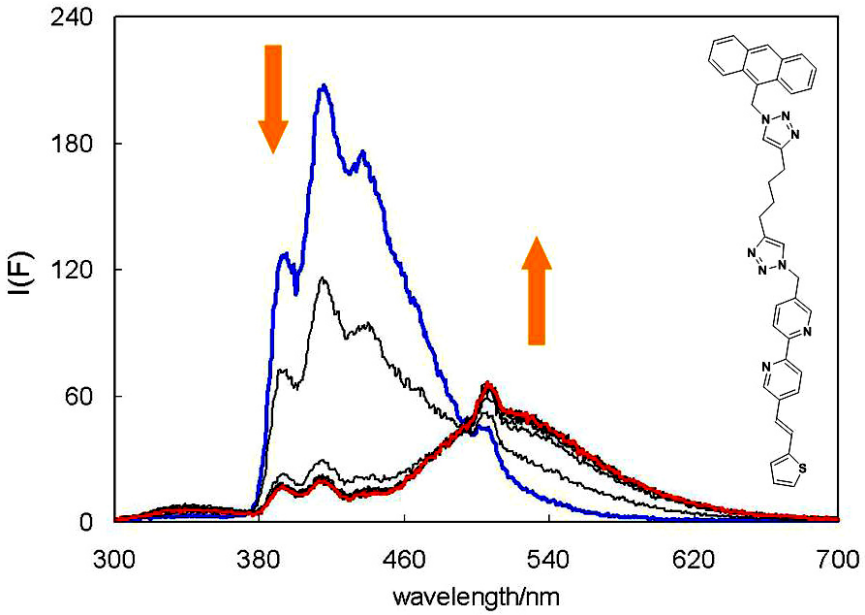


Fig. S2 Fluorescence spectra of **5** (12 μM, λ_{ex} = 260 nm) in the presence of $Zn(ClO_4)_2$ from 0 (blue) to 199 μM (red) in CH_3CN.

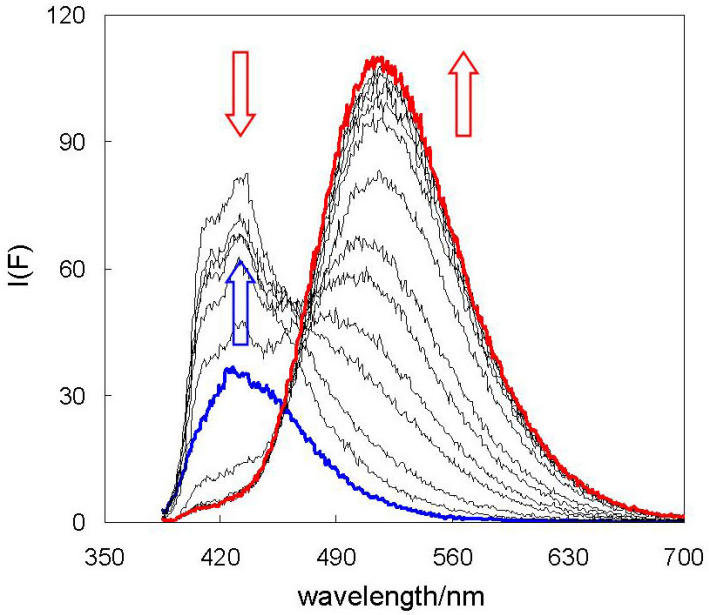


Fig. S3 Fluorescence spectra of **4** (5.1 μM, λ_{ex} = 375 nm) in the presence of $Zn(ClO_4)_2$ from 0 (blue) to 38 μM (red) in CH_3CN.

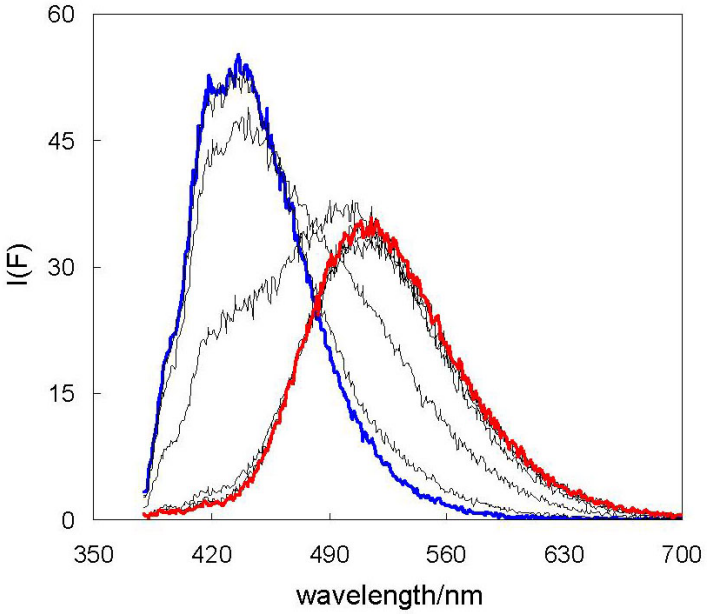


Fig. S4 Fluorescence spectra of **5** (12 μM, λ_{ex} = 375 nm) in the presence of $Zn(ClO_4)_2$ from 0 (blue) to 19 μM (red) in CH_3CN.

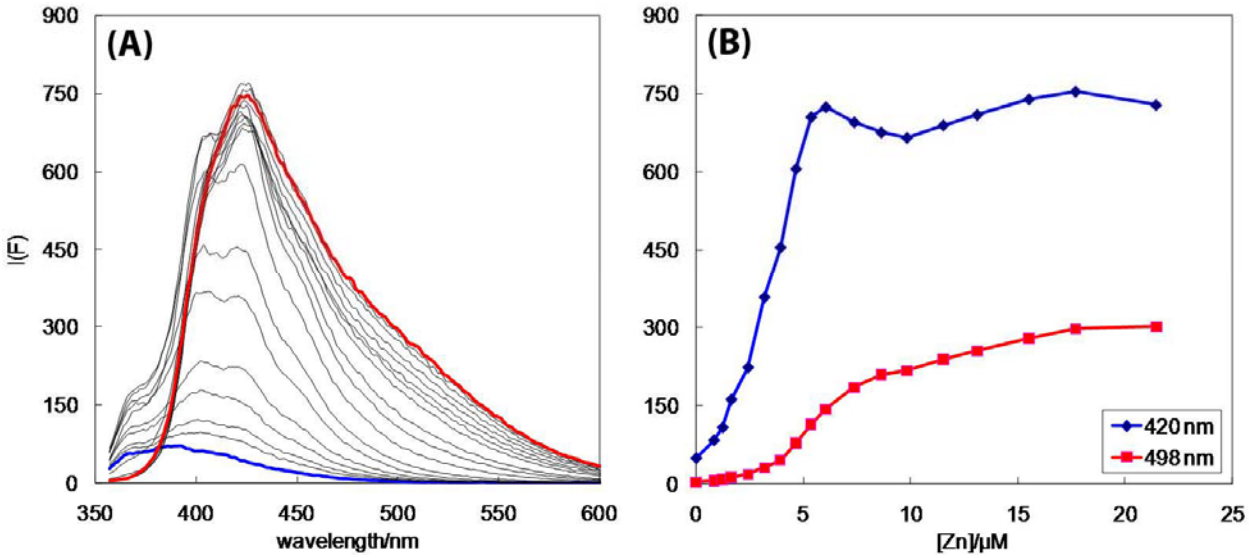


Fig. S5 (A) Fluorescence spectra (λ_{ex} = 345 nm) of the mixture of **2** (3 μM) and **3** (3 μM) in the presence of $Zn(ClO_4)_2$ from 0 (blue) to 21 μM (red) in CH_3CN. (B) The fluorescence intensity of the mixed sample at 420 nm (blue) and 498 nm (red) at various $[Zn^{2+}]$.

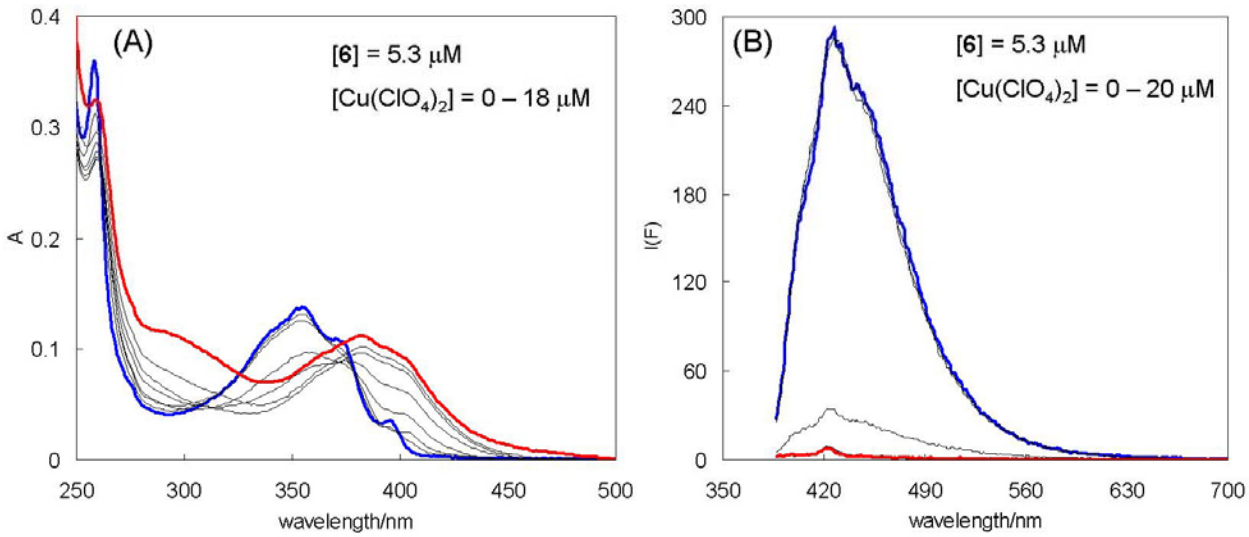


Fig. S6 Absorption (A) and fluorescence (B) spectra of **6** (λ_{ex} = 375 nm) in the presence of $Cu(ClO_4)_2$ in CH_3CN. The spectra taken in the absence of Cu^{2+} are coded blue; the spectra in the presence of highest concentrations of Cu^{2+} are coded red.

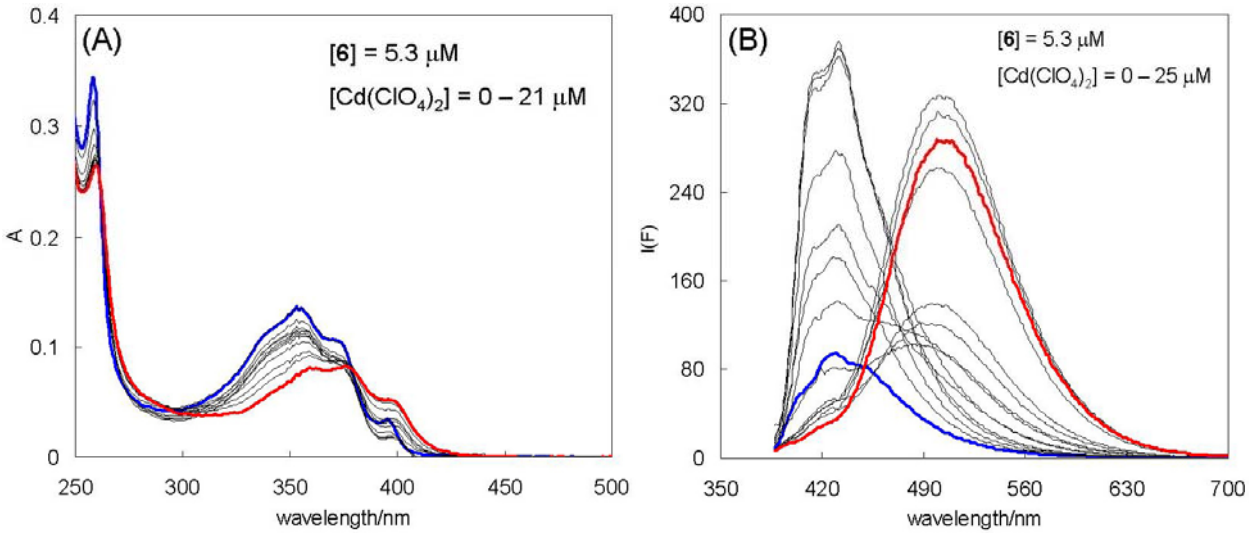


Fig. S7. Absorption (A) and fluorescence (B) spectra of **6** (λ_{ex} = 375 nm) in the presence of $Cd(ClO_4)_2$ in CH_3CN. The spectra taken in the absence of Cd^{2+} are coded blue; the spectra in the presence of highest concentrations of Cd^{2+} are coded red.

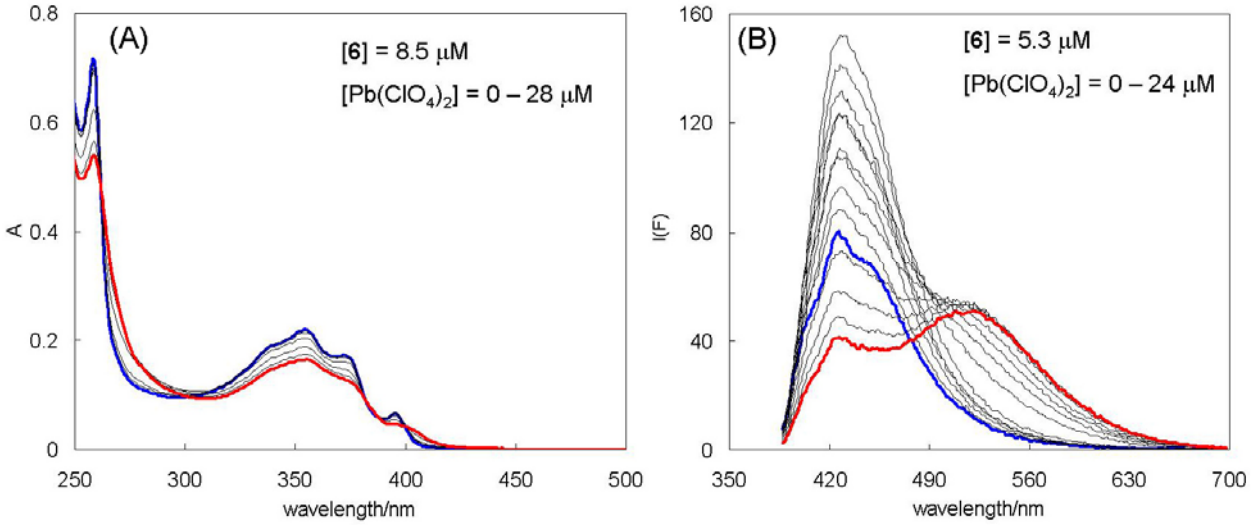


Fig. S8. Absorption (A) and fluorescence (B) spectra of **6** (λ_{ex} = 375 nm) in the presence of $Pb(ClO_4)_2$ in CH_3CN. The spectra taken in the absence of Pb^{2+} are coded blue; the spectra in the presence of highest concentrations of Pb^{2+} are coded red.

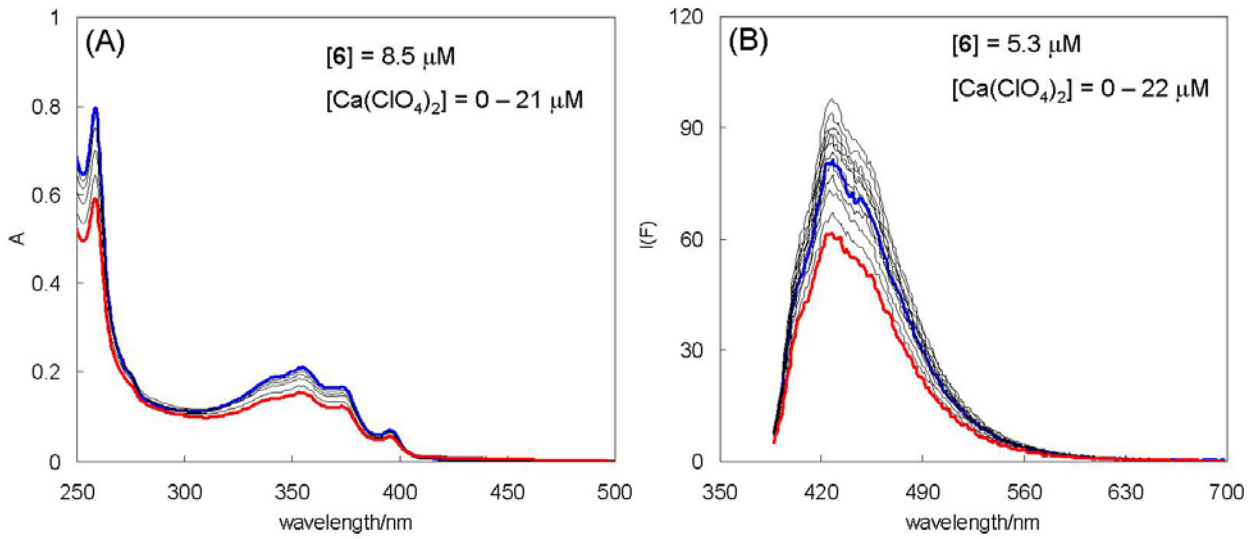


Fig. S9. Absorption (A) and fluorescence (B) spectra of **6** (λ_{ex} = 375 nm) in the presence of $Ca(ClO_4)_2$ in CH_3CN. The spectra taken in the absence of Ca^{2+} are coded blue; the spectra in the presence of highest concentrations of Ca^{2+} are coded red.

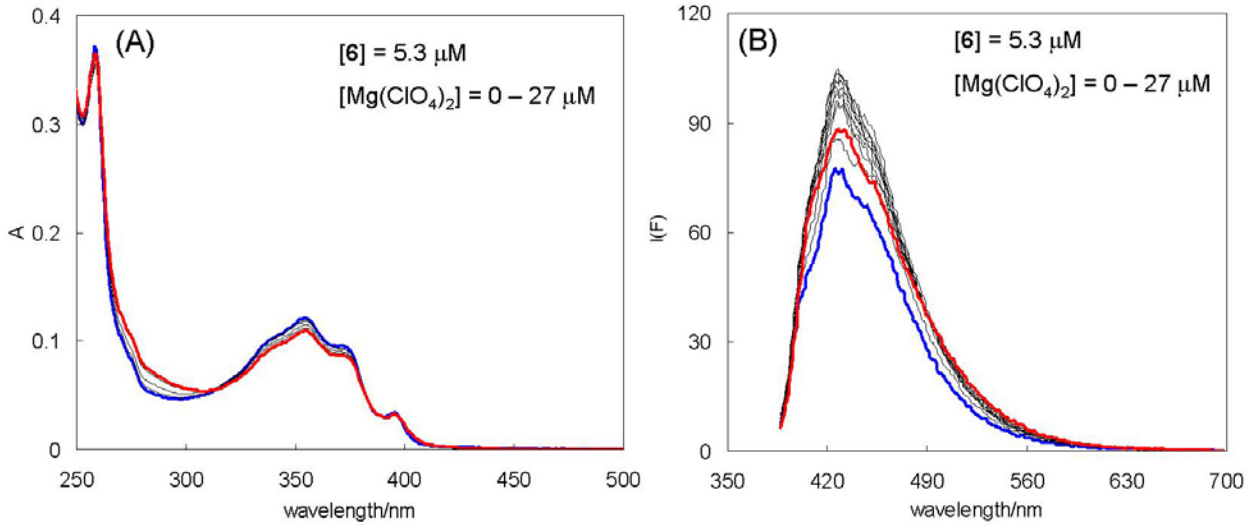


Fig. S10. Absorption (A) and fluorescence (B) spectra of **6** (λ_{ex} = 375 nm) in the presence of $Mg(ClO_4)_2$ in CH_3CN. The spectra taken in the absence of Mg^{2+} are coded blue; the spectra in the presence of highest concentrations of Mg^{2+} are coded red.

Fluorescence Quantum Yield Measurements.

All samples were bubbled with argon to avoid the quenching effect of molecular oxygen. The fluorescence quantum yield (ϕ) of each sample was recorded by adjusting the absorbance at the excitation wavelength (the maximum absorbance wavelength of the sample) below 0.1 and measuring the integrated emission intensity. The ϕ_u of the sample was determined in reference to quinine sulfate in 0.05 M H_2SO_4 (ϕ_s = 0.55). Obtained data was substituted in equation S1 to provide the fluorescence quantum yield for each sample,

$$\phi_u = [(A_s F_u n^2)/(A_u F_s n_0^2)]\phi_s \qquad \textbf{Equation S1}$$

where A_s and A_u are the absorbance of the sample and reference solutions at their respective excitation wavelengths, F_s and F_u are the corresponding integrated fluorescence intensity, and n is the refractive index of the solvent of the sample (n) or of the standard (n_0).

The ϕ values of monozinc complexes of **4** and **6** were determined using a previously reported method (*J. Org. Chem.* **2008**, *73*, 8321-8330).

Fluorescence Lifetime Measurements.

Time correlated single photon counting (TCSPC) technique was used to determine the lifetimes of the reported compounds. The samples were excited using an 370 nm light emitting diode (LED) operating at a repetition rate of 1 MHz. Lifetimes were recorded at the emission maxima of each sample with a band width of 2 nm and 10,000 counts in the peak channel. The timescale of the experiment was adjusted to 100 ns (115.3 ps/channel). The recorded data were analyzed using a DAS6 software and the τ values were obtained with a $\chi^2 < 1.3$.

Table S1 Individual components of lifetimes (τ)[a] and χ^2 of **4-6** and their Zn^{2+} complexes in CH_3CN.

	τ_1/ns (RA%)[b]	τ_2/ns (RA%)[b]	τ_3/ns (RA%)[b]	τ/ns (avg)	χ^2
4	0.14 (87.6)	1.25 (9.54)	6.26 (2.85)	3.05	0.73
$[Zn_2(\mathbf{4})]^{4+}$	0.67 (19.2)	2.36 (47.3)	7.80 (33.6)	5.99	0.99
5	0.17 (63.3)	0.63 (31.2)	3.59 (5.5)	1.70	0.63
$[Zn(\mathbf{5})]^{2+}$	0.23 (32.3)	1.61 (52.7)	3.97 (15.0)	2.47	0.96
6	0.13 (72.2)	1.83 (12.5)	6.64 (15.3)	5.36	1.05
$[Zn_2(\mathbf{6})]^{4+}$	0.77 (25.9)	2.18 (66.4)	12.1 (7.7)	5.68	0.95

[a] Fluorescence decay traces of free ligands and Zn^{2+} complexes were observed at 431 nm and 517 nm, respectively. A 370 nm LED was used as the excitation source. [b] RA%: relative abundances of individual exponentials.

Representative Fluorescence Lifetime Traces.

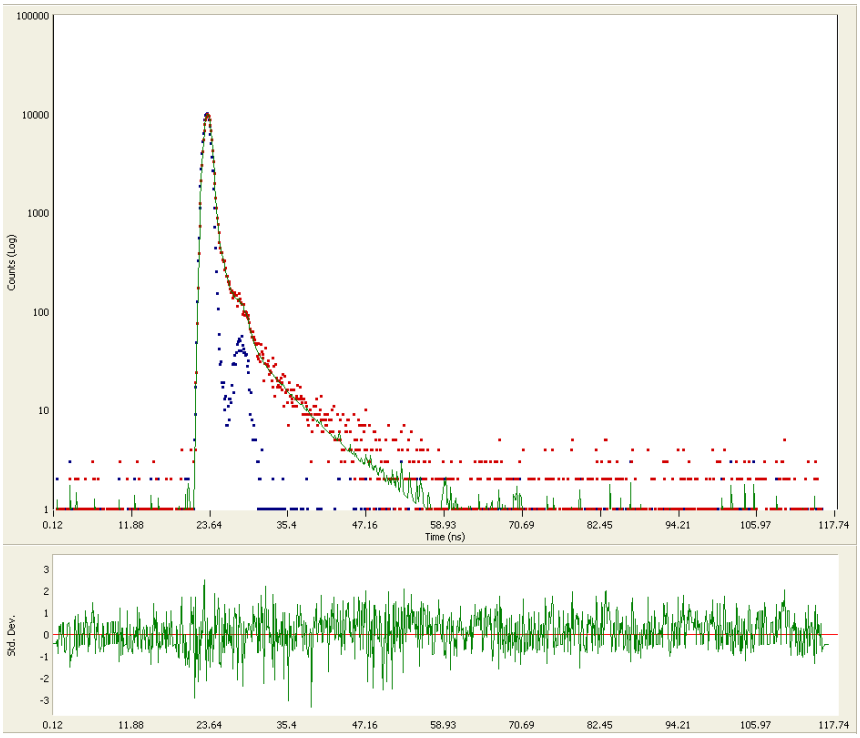


Fig. S11 The fluorescence decay trace of compound **4** in MeCN ($\chi^2 = 0.73$).

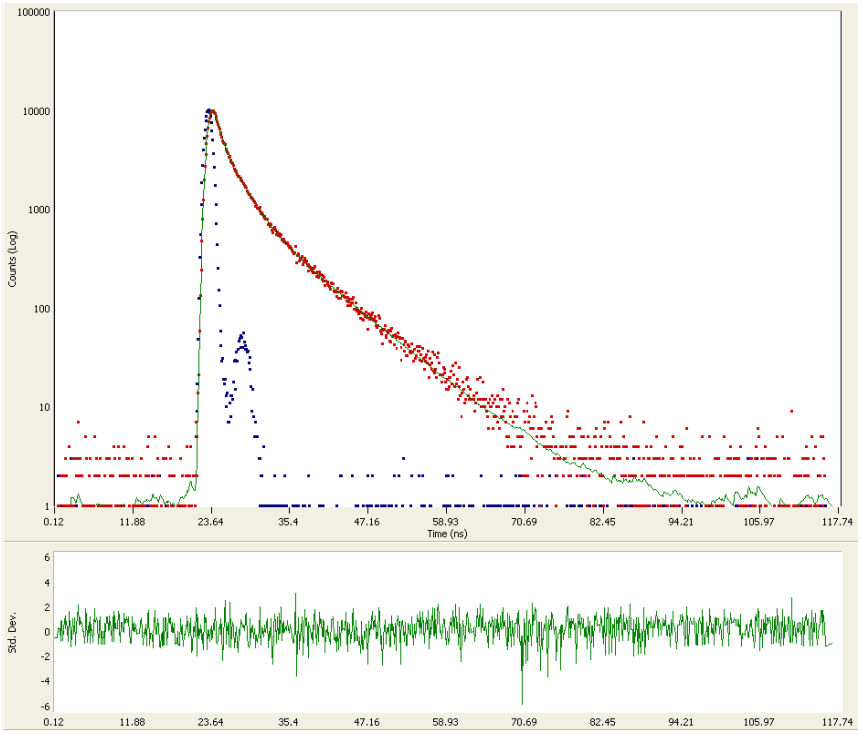


Fig. S12 The fluorescence decay trace of the zinc complex of compound **4** in MeCN ($\chi^2 = 0.99$).

Calculation of Average Fluorescence Lifetimes.

The average lifetime τ_{av} was calculated using equation S2. (See Principles of Fluorescence Spectroscopy, Third Edition, Chapter 4),

$\tau_{av} = f_1\tau_1 + f_2\tau_2$ **Equation S2**

where f_i represents the fractional contribution of each decay time to the steady state intensity and can be determined using equation S3, with $\alpha_i\tau_i$ being proportional to the area under the decay curve under each decay time.

$f_i = \alpha_i\tau_i/(\Sigma_j\alpha_j\tau_j)$ **Equation S3**