Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information for

Phenanthroline Bridged Bis(*β*-cyclodextrin)s/Adamantane-carboxylic Acid Supramolecular Complex as an Efficient Fluorescence Sensor to Zn^{2+}

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Experimental Section

Materials. All chemicals were reagent grade unless noted. *β*-Cyclodextrin was recrystallized twice from water and dried in vacuo at 90 °C for 24 h before use. Crude *N*,*N*-dimethylformamide (DMF) was stirred with CaH_2 for 3 days and then distilled under reduced pressure prior to use. 2,9-Bis(hydroxymethyl)-1,10-phenanthroline[1], and mono-(6-deoxyl-6-azido)- *β*-cyclodextrin[2] were prepared according to the reported methods. Column chromatography was performed on 200-300 mesh silica gel.

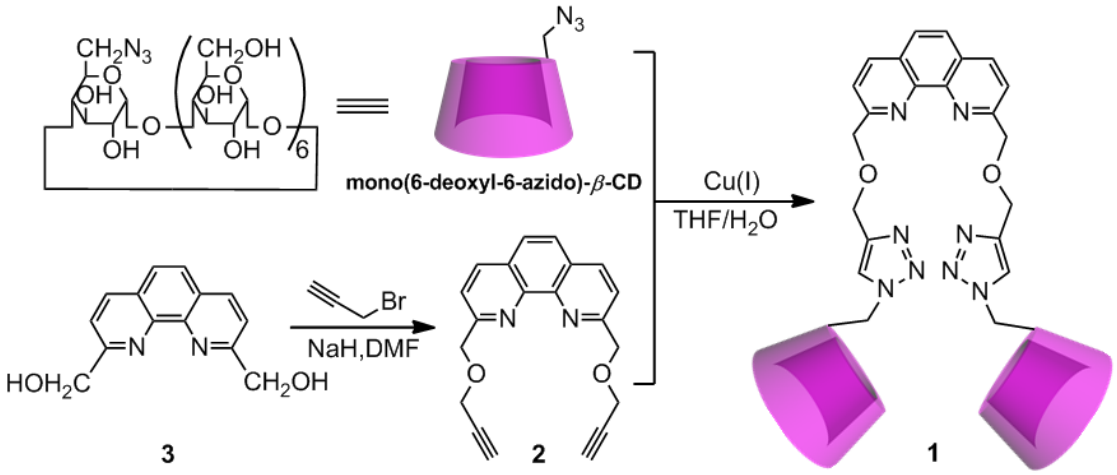
Instruments. Elemental analysis was performed on a Perkin-Elmer-2400C instrument. NMR spectra were recorded on Bruker AV400 instruments. The fluorescent spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) on a Varin Cary Eclipse equipped with a Varian Cary single-cell peltier accessory to control temperature at 25 °C. Circular dichroism spectra were collected in a conventional quartz cell (10 × 10 × 45 mm) on a MOS-500 spectropolarimeter (Bio-Logic) at 25 °C. Fluorescence stopped-flow kinetics was measured using a Bio-Logic SFM-3000 (Bio-Logic) device equipped with the MOS-500 spectrometer and with a 150 W xenon-mercury lamp as excitation source at 25 °C. Three shots were performed successively for each mixing scenario and an average dynamic curve was obtained. Dynamic data were fitted using the Biokine software (Bio-Logic). The excited wavelength and slits were set as 272 nm and 8 nm, respectively. FC-08 flowing cell was used, and the typical dead time of the stopped flow is approximately 1.0 ms. The confocal fluorescent images were captured with a fluorescence-inverted microscope (Olympus

FV1000S-I × 81).

Synthesis of 2,9-dipropargyl-1,10-phenanthroline (2). To 20 mL dry DMF was added 2,9-bis(hydroxymethyl)-1,10-phenanthroline (481 mg, 2 mmol), and the solution was cooled to 0 °C, then NaH (4 mmol, 100 mg) was added into the solution. The mixture was stirred at 0 °C for 0.5 h, and then propargyl bromide (80% w/w solution in toluene, 500 μL, 4 mmol) was added. The reaction mixture was stirred for 3 h in an ice bath. Then 20 mg NaH was added to complete the reaction, and the mixture was further stirred for another 12 h at room temperature. The reaction mixture was dried under reduced pressure to remove the solvent. The residue was dissolved in chloroform (100 mL) and washed with water (3 × 50 mL), then the organic phase was dried over $MgSO_4$. The solvent was removed under reduced pressure and compound **2** was obtained by column chromatography (silica gel) using dichloromethane/ethyl acetate (5:3 v/v) as the eluent to give pale yellow solid (198.5 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 2.51 (s, 2H, CH≡C-), 4.38 (d, J = 4 Hz, 4H, -CH_2-), 5.16 (s, 4H, -CH_2-), 7.79 (s, 2H, H of phenanthroline), 7.89 (d, J = 12 Hz, 2H, H of phenanthroline), 8.29 (d, J = 8 Hz, 2H, H of phenanthroline); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 58.5, 73.6, 75.0, 79.5, 121.0, 126.2, 128.1, 136.9, 145.1, 159.0 ppm; HR-MS (ESI), $C_{20}H_{16}N_2O_2$: $[M + Na]^+$ m/z: calcd 339.1109, found: 339.1108.

Cell culture and confocal fluorescent imaging. Human cervical carcinoma (HeLa) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and gentamicin (80 μg mL^{-1}) in 6-well plates (2 × 10^4 cells mL^{-1}, 1 mL per well) for 24h. The cells were incubated with 0.1 mM

$Zn(ClO_4)_2$ for 0.5 h, and then washed with PBS buffer for three times and further incubated with 50 μM **1** or **1**/AdCA for 3 h at 37 °C, respectively. The cells were washed twice with PBS buffer and then performed confocal fluorescent imaging.



Scheme S1. Synthetic route of **1**.

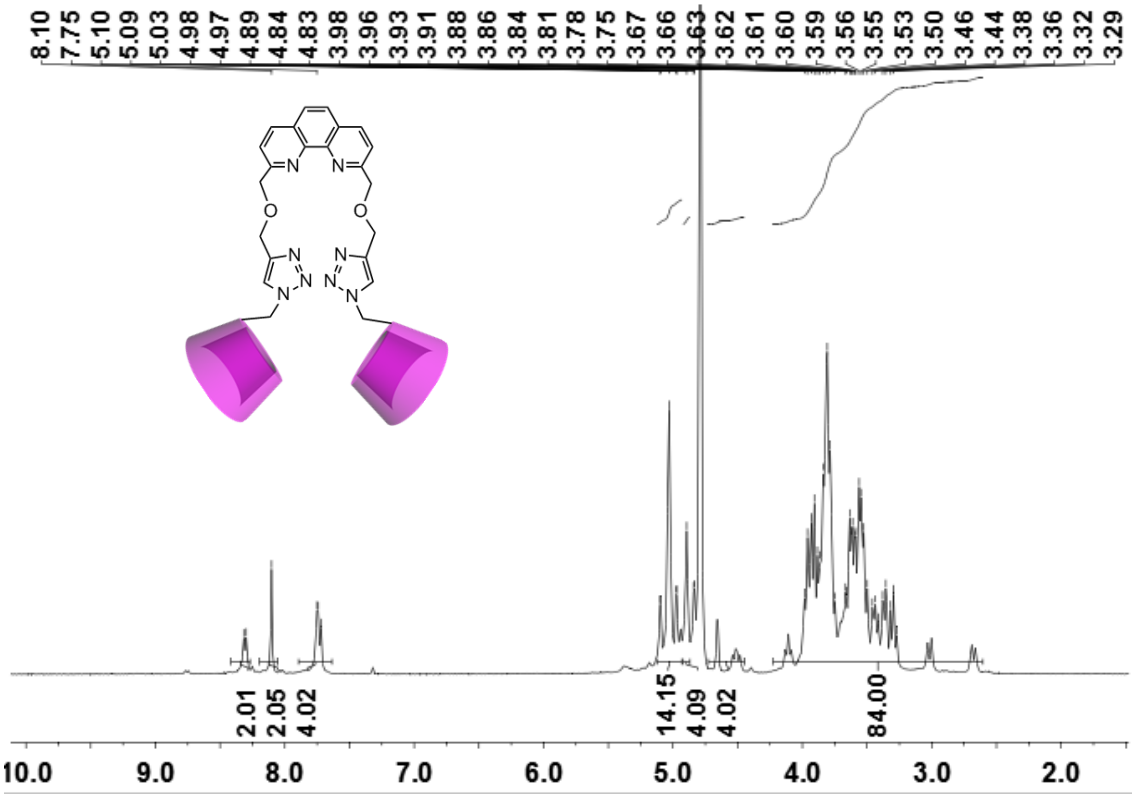


Figure S1. ^{1}H NMR (400 MHz) spectrum of **1** in D_2O at 25 °C.

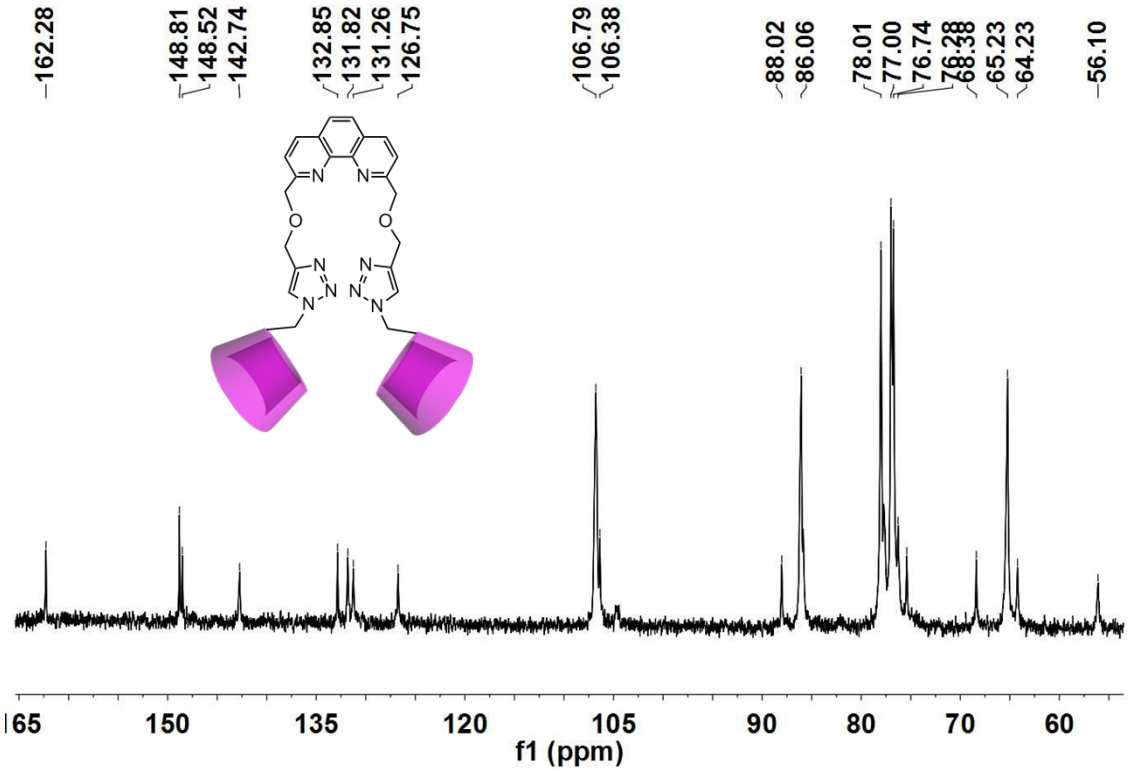


Figure S2. ^{13}C NMR (100 MHz) spectrum of **1** in D_2O at 25 °C.

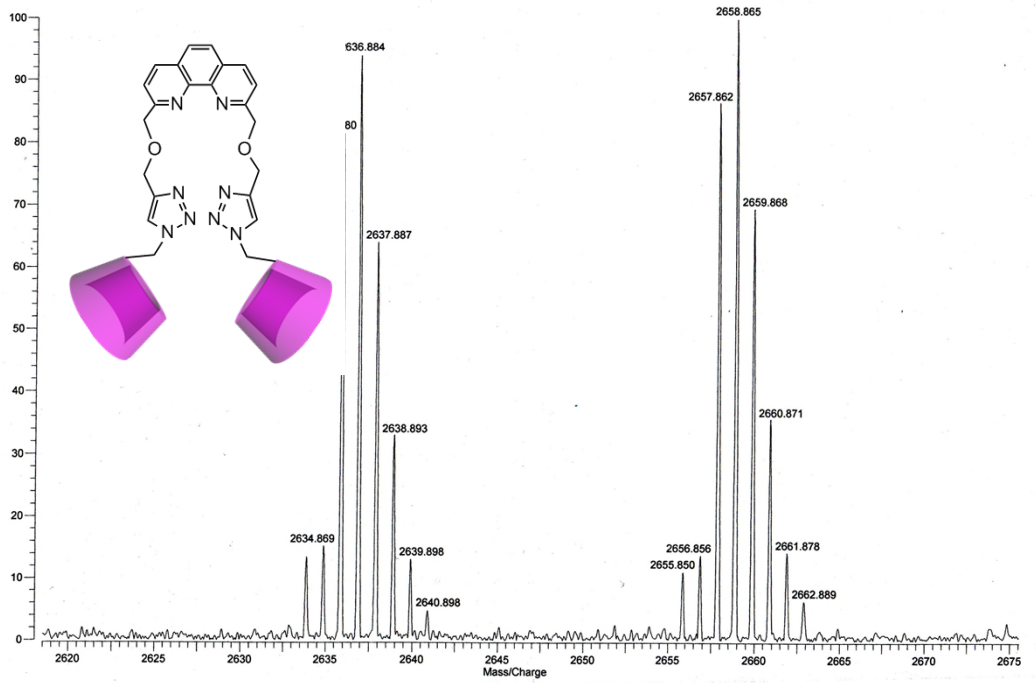


Figure S3. MALDI-TOF mass spectrum of **1**.

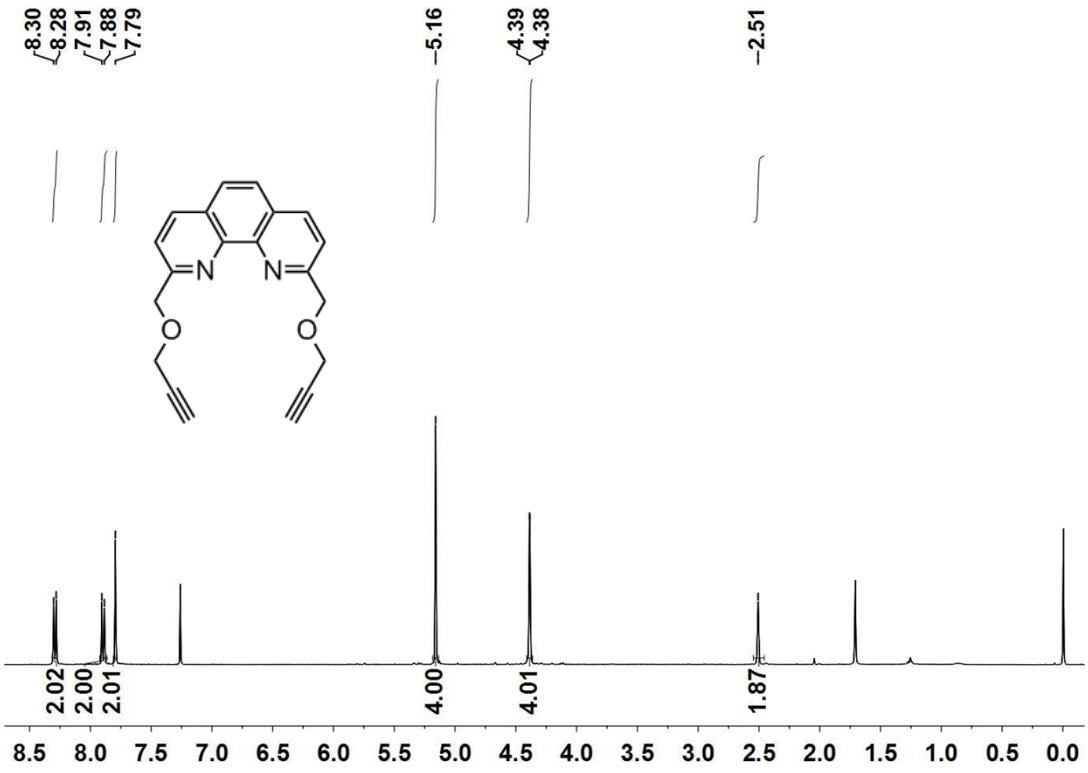


Figure S4. ^{1}H NMR (400 MHz) spectrum of **2** in $CDCl_3$ at 25 °C.

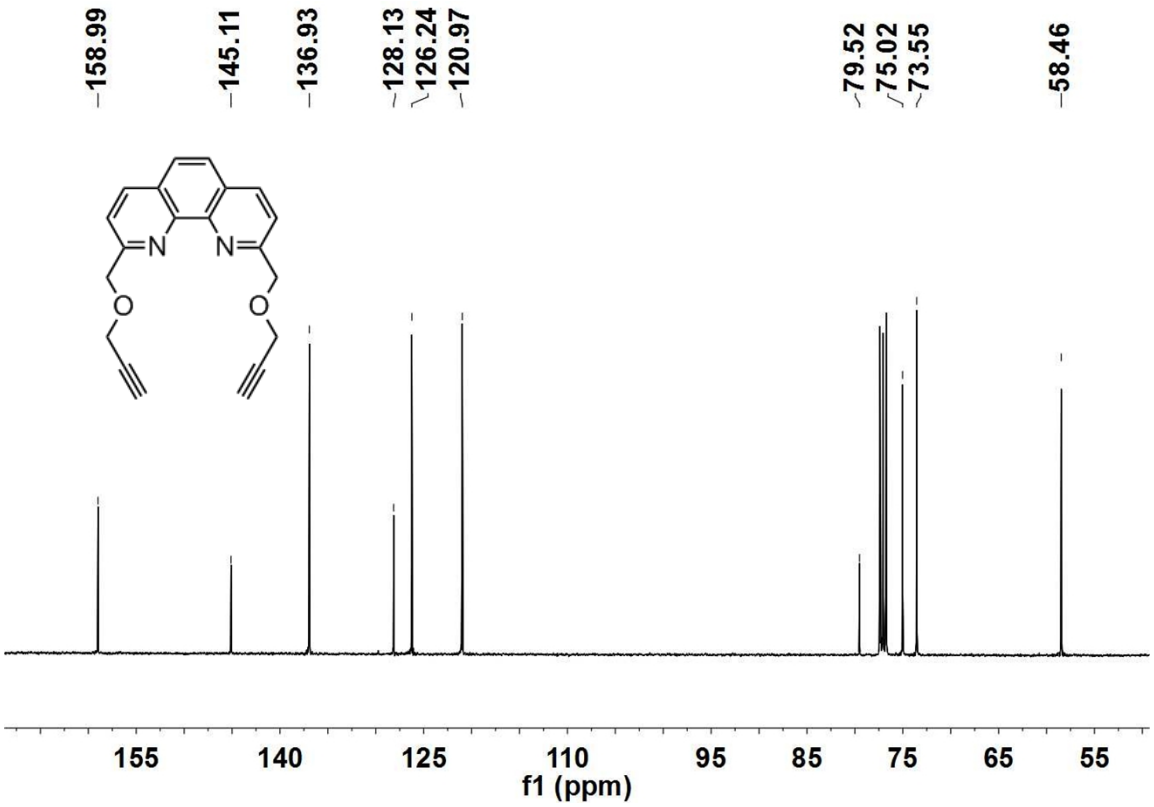


Figure S5. ^{13}C NMR (100 MHz) spectrum of **2** in $CDCl_3$ at 25 °C.

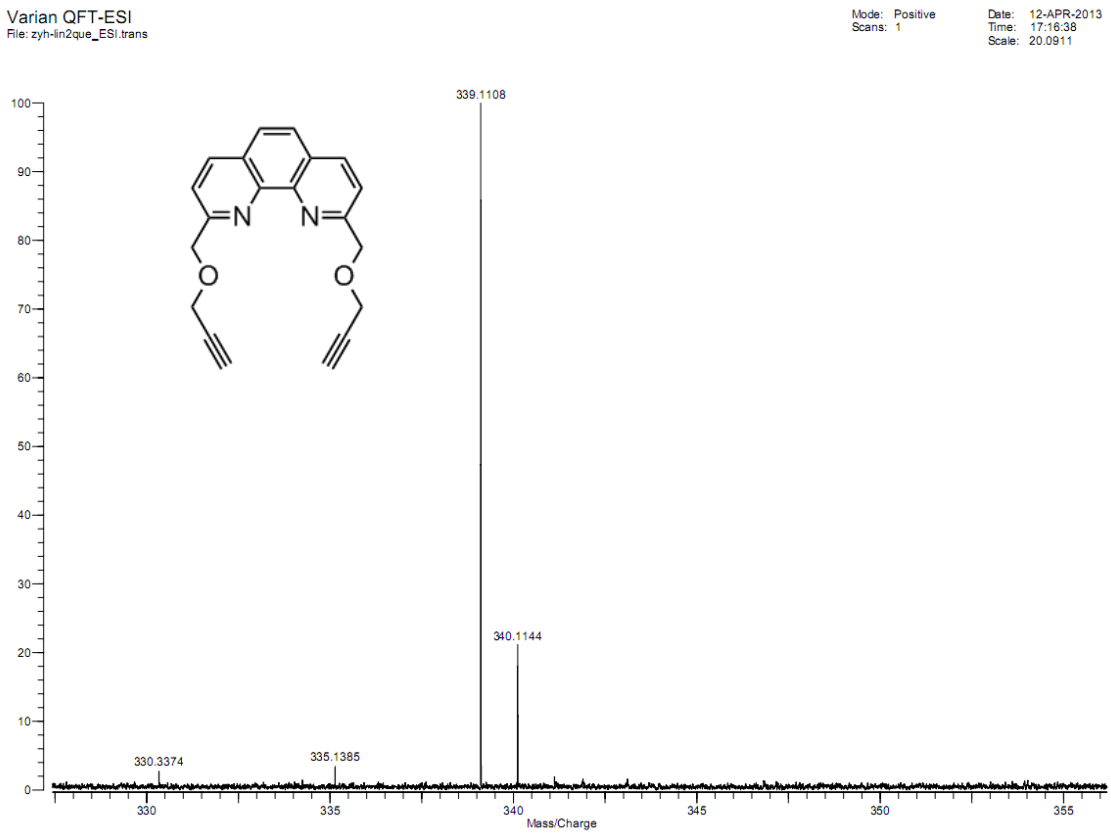


Figure S6. High-resolution mass spectrum of **2**.

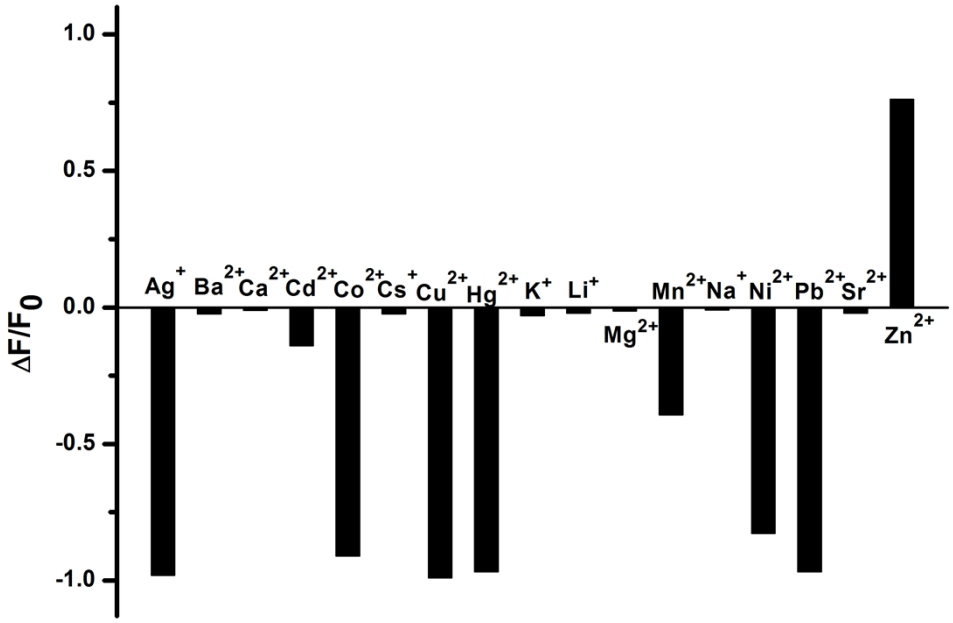


Figure S7. Relative fluorescence change ($\Delta F/F_0$) of **1** at 377 nm in the presence of different metal cations in HEPES buffer (10 mM, pH = 7.2, [**1**] = 1.5×10^{-5} M, [AdCA] = 1.0×10^{-3} M, [M^{n+}] = 3.0×10^{-5} M, λ_{ex} = 272 nm).

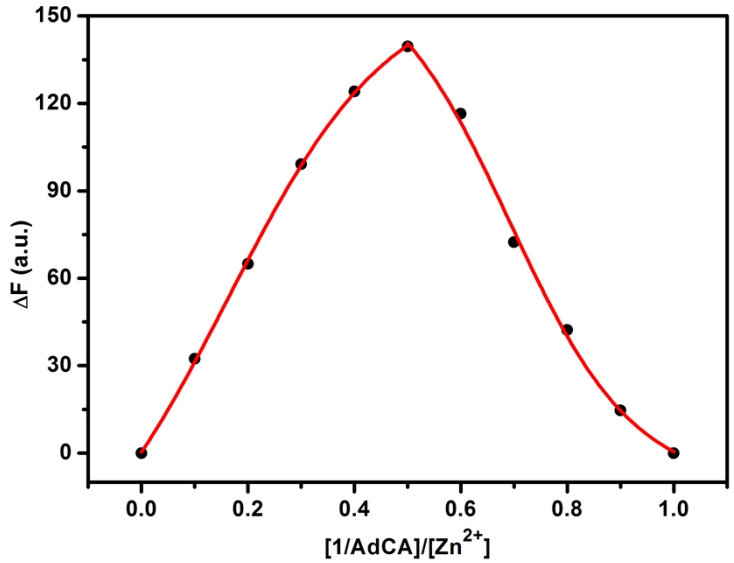


Figure S8. Job's plot of **1**/AdCA/Zn^{2+} system in HEPES buffer solution (10 mM, pH = 7.2) at 25 °C ([**1**] + [Zn^{2+}] = 2.0×10^{-5} M).

Determination of complex stoichiometry and binding constant of 1/AdCA system:[3]

In our case, the fluorescence intensity F and other binding parameters obey Hill plot:

$\log((F - F_{min})/(F_{max} - F)) = n\log[M] + B$ ($B = \log\beta$)

where F is the fluorescence intensity of **1** in the presence of a certain concentration of AdCA; F_{max} is the fluorescence intensity of **1** when the titration reaches equilibrium; F_{min} is the fluorescence intensity of **1** without addition of AdCA; and n is the binding stoichiometry of **1** with AdCA; and β is the binding constant of **1** with AdCA.

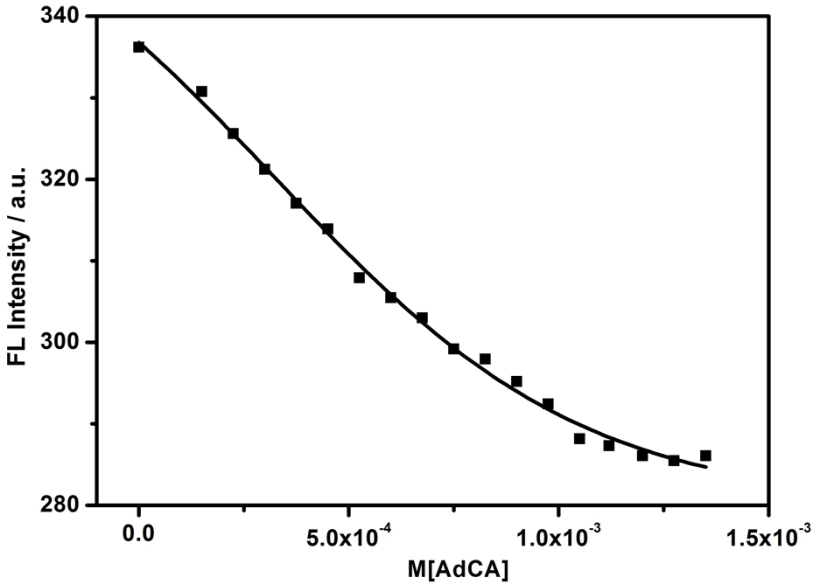


Figure S9. Fluorescence intensity changes of **1** upon addition of AdCA in HEPES buffer solution (10 mM, pH = 7.2) at 25 ºC ([**1**] = 1.5 × 10^{-5} M, λ_{ex} = 272 nm, and λ_{em} = 368 nm).

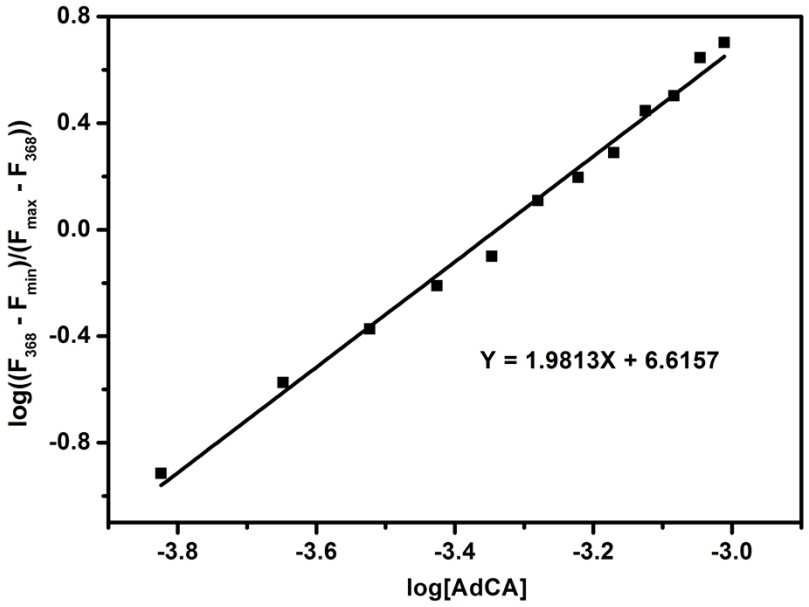


Figure S10. Linear fitting of $\log((F_{368} - F_{min})/(F_{max} - F_{368}))$ versus log[AdCA] ([**1**] = 1.5×10^{-5} M, $F_{max} = 286$, and $F_{min} = 336$). From the slope ($n = 1.98$) and intercept ($\log\beta = 6.62$), it can be seen that the binding stoichiometry and $\log K_S$ value between **1** and AdCA are 2 and 4.2×10^6 M^{-2}, respectively.

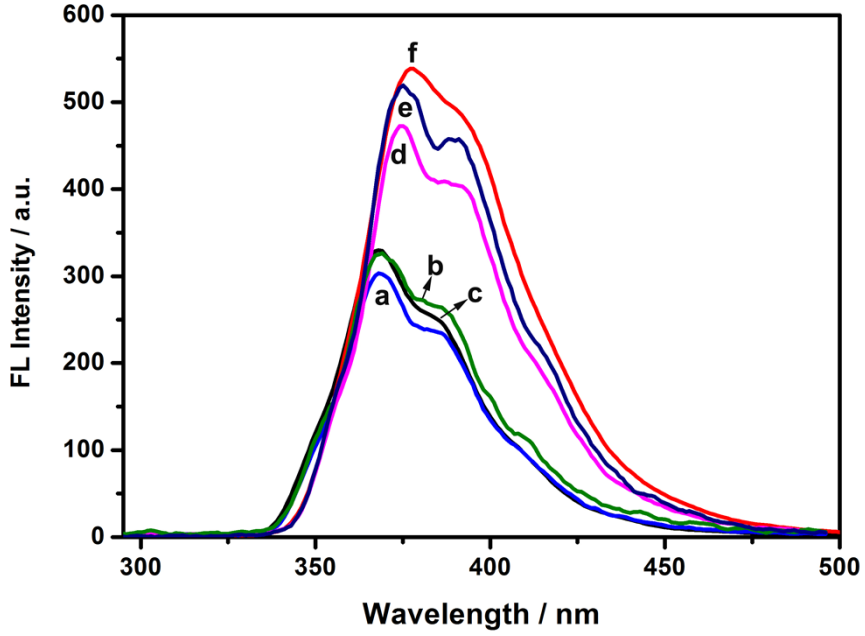


Figure S11. Fluorescence emission spectra of (a) **1**/AdCA complex with 90%

encapsulation ratio ([AdCA] = 1 × 10^{-3} M), (b) **1**/AdCA complex with 50% encapsulation ratio ([AdCA] = 4 × 10^{-5} M), (c) free **1**, (d) **1**/AdCA complex in (a) with Zn^{2+}, (e) **1**/AdCA complex in (b) with Zn^{2+} , and (f) **1**/Zn^{2+} complex in HEPES buffer solution at 25 °C ([**1**] = 1.5 × 10^{-5} M, [Zn^{2+}] = 3 × 10^{-5} M, λ_{ex} = 272 nm, and λ_{em} = 377 nm).

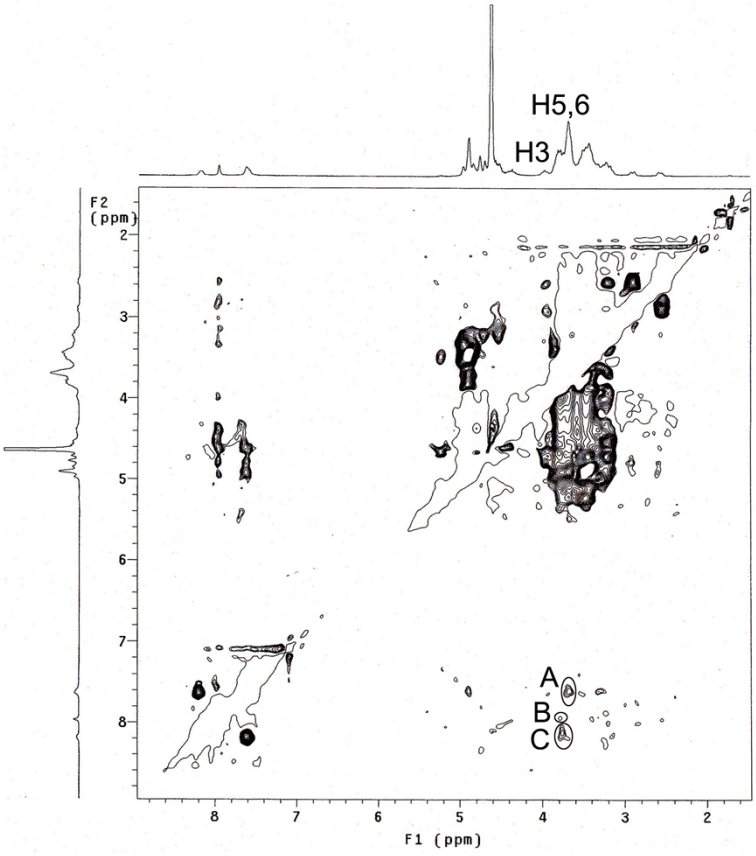


Figure S12. ROESY spectrum of **1** in D_2O at 25 °C. ([**1**] = 5 × 10^{-3} M).

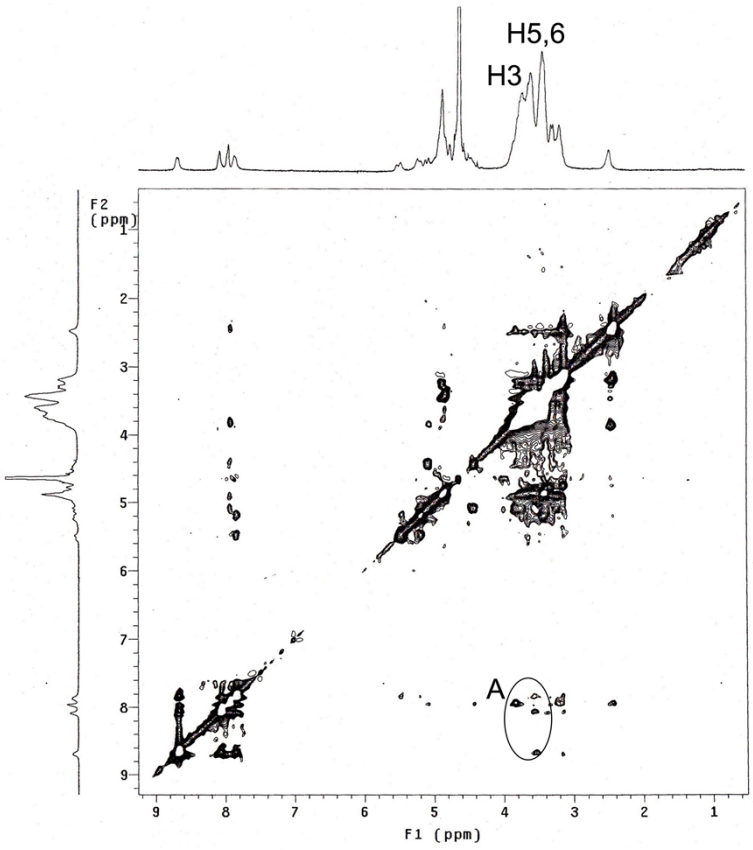


Figure S13. NOESY spectrum of **1**/Zn^{2+} system in D_2O at 25 °C. [**1**] = 2.5×10^{-3} M, [Zn^{2+}] = 5.0×10^{-3} M).

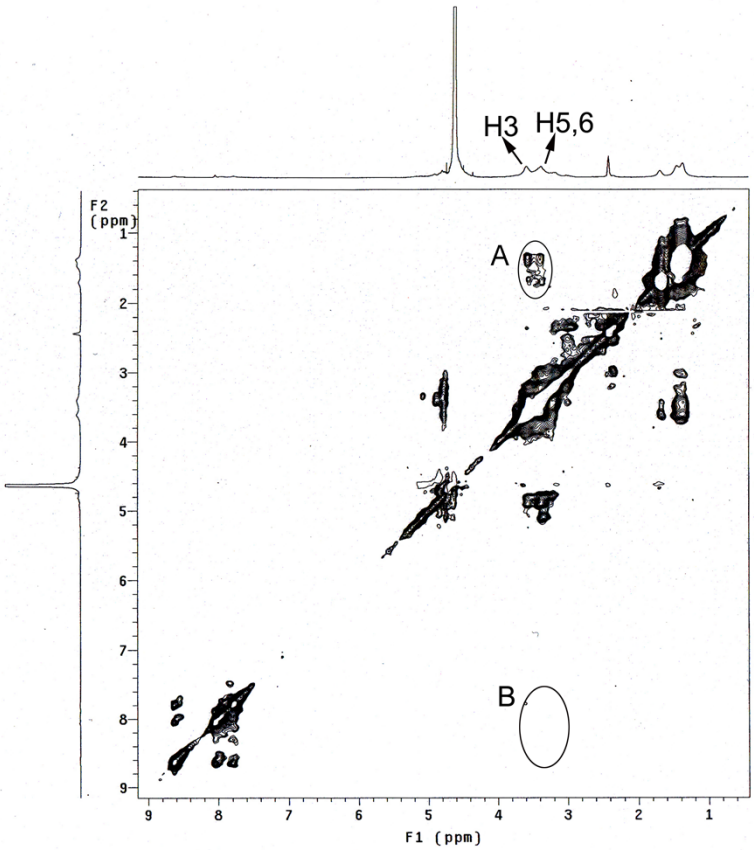


Figure S14. NOESY spectrum of **1**/AdCA/Zn^{2+} system in D_2O at 25 °C. ([**1**] = 2.5 × 10^{-3} M, [AdCA] = 7.4 × 10^{-3} M, [Zn^{2+}] = 5.0 × 10^{-3} M. Under this concentration, more than 99% of **1** and AdCA were converted to **1**/AdCA complex through a calculation based on the binding constant between CD and AdCA).

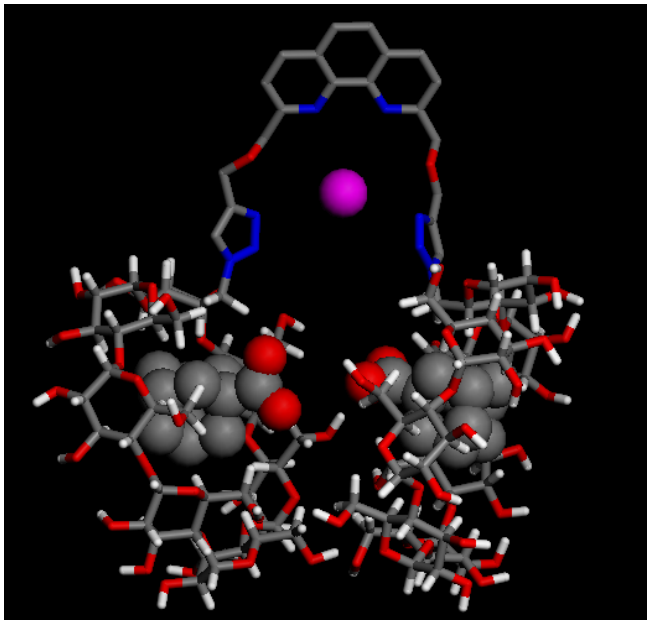


Figure S15. Energy minimization structure of **1**/AdCA/Zn^{2+} system obtained by molecular modeling study. The geometry of **1**/AdCA/Zn^{2+} complex was optimized by the molecular mechanics method with dreiding forcefield.

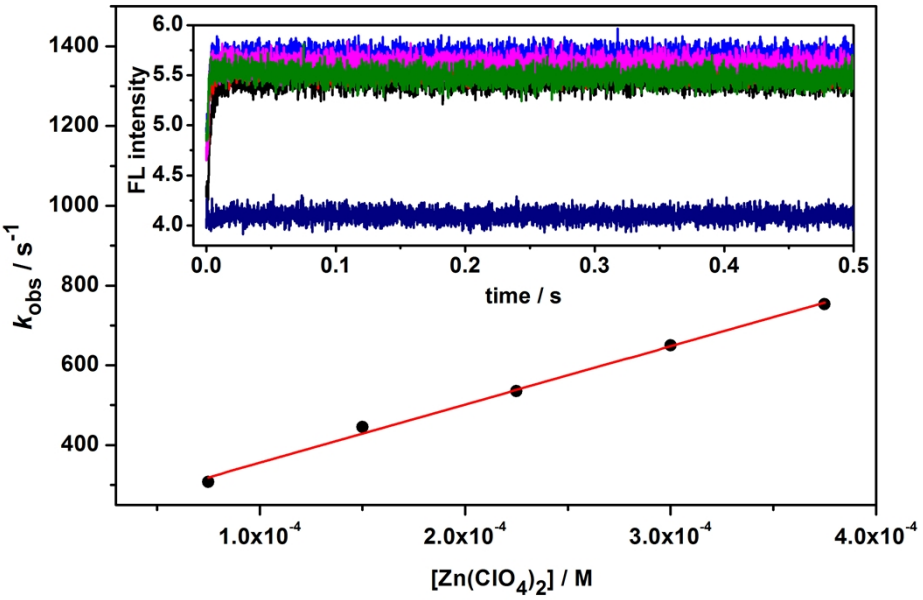


Figure S16. Dependence of observed rate constant k_{obs} of **1**/AdCA ([**1**] = 1.5×10^{-5} M, [AdCA] = 2×10^{-3} M) with different concentrations of Zn^{2+} in HEPES buffer solution

(10 mM, pH = 7.2). Inset: Dynamic experiments of the rapid mixing of **1**/AdCA with different concentrations of $Zn(ClO_4)_2$ (0, 0.75, 1.5, 2.25, 3.0, and 3.75×10^{-4} M). All concentrations mentioned above are the final ones after mixing.

References

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2. C. Hocquelet, J. Blu, C. K. Jankowski, S. Arseneau, D. Buisson and L. Mauclaire, *Tetrahedron* 2006, **62**, 11963-11971.

3. X.-L. Tang, X.-H. Peng, W. Dou, J. Mao, J.-R. Zheng, W.-W. Qin, W.-S. Liu, J. Chang and X.-J. Yao, *Org. Lett*., 2008, **10**, 3653-3656.