Aggregation Induced Emission of Cyanostilbene Amphiphile as a Novel Platform for FRET-Based Ratiometric Sensing of Mercury Ion in Water

*Xiao Wang, Zongchun Gao, Junlong Zhu, Zhao Gao, and Feng Wang**

CAS Key Laboratory of Soft Matter Chemistry, Chinese Academy of Sciences,
Department of Polymer Science and Engineering,
University of Science and Technology of China,
Hefei, Anhui 230026 (P. R. China)
Fax: (+86) 551 3606 095; E-mail: drfwang@ustc.edu.cn.

Supporting Information

1. Materials and methods

All reagents and solvents were reagent grade from Adamas Reagent and used as received. Perchlorate salts (Li^{+}, Na^{+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Al^{3+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+} and Mg^{2+}) were used for the detection selectivity experiments.

^{1}H NMR spectra were collected on a Varian Unity INOVA-300 spectrometer with TMS as the internal standard. Transmission electron microscopy (TEM) images were recorded on Tecnai G2 Spirit BioTWIN (120 kV, FEI Company, USA). Electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Esquire 3000 plus mass spectrometer (Bruker-Franzen Analytik GmbH Breman, Germany) equipped with an ESI interface and ion trap analyzer. UV/Vis spectra were recorded on a UV-1800 Shimadzu spectrometer. Solution excitation and steady-state fluorescence emission spectra were recorded on a FluoroMax-4 spectrofluorometer (Horiba Scientific) and analyzed with an Origin (v8.1) integrated software FluoroEssence (v2.2).

2. ^1H NMR measurements of ***1***

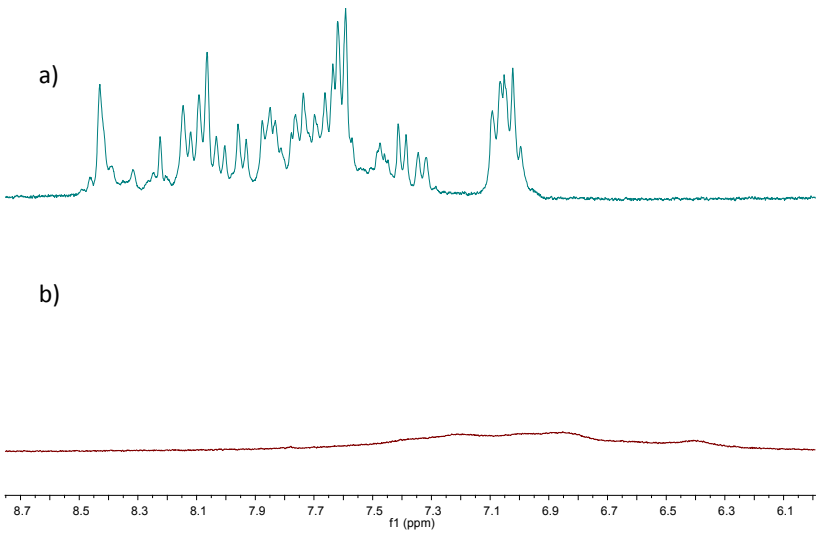


Figure S1. ^{1}H NMR spectra of **1** (2.00 mM, 293 K) in a) d_6-DMSO and b) D_2O. In d_6-DMSO solution, the aromatic resonances show sharp and discernable peaks, which are typical for the dominance of molecularly dissolved state. On the contrary, significantly upfield chemical shift appear for the aromatic resonances in D_2O solution, together with severe line broadening phenomenon, denoting the tendency for **1** to aggregate.

3. *Concentration-dependent fluorescent measurements of **1***

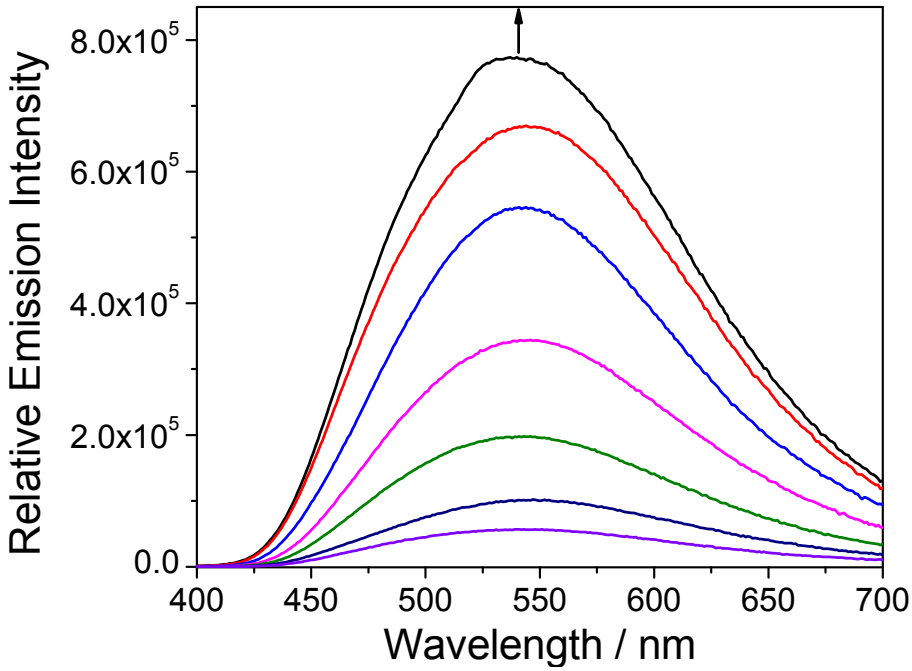


Figure S2. Concentration-dependent fluorescent spectra of **1**. Upon increasing the monomer concentration from 2×10^{-6} mol/L to 2×10^{-3} mol/L in water, the emission intensity exhibits a gradual enhancement. Hence, **1** shows strong tendency to form supramolecular assemblies in aqueous solution with prominent AIE behaviors.

*4. FRET measurement between **1** and Nile Red acceptor.*

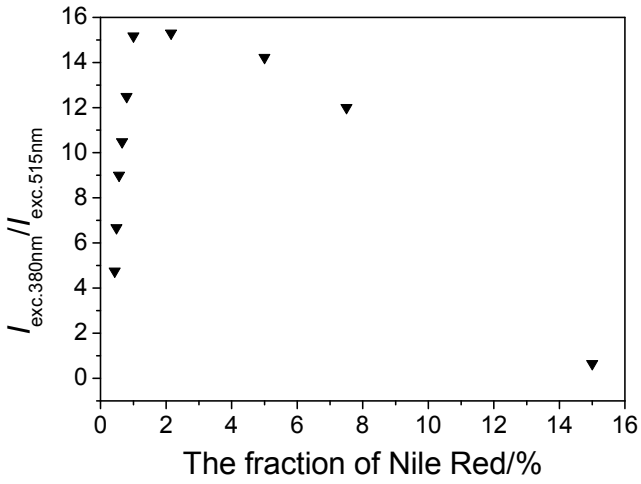


Figure S3. Plot of the Nile Red (acceptor unit) fraction against the emission intensity at 628 nm with two different excitation wavelengths ($I_{exc.380nm}$/ $I_{exc.515nm}$). Exciting at 380 nm represents the indirect excitation (dominance of the absorption for **1**), whilst exciting at 515 nm denotes the direct excitation (dominance of the absorption for Nile Red). The dramatic amplification of fluorescence by indirect excitation *versus* direct excitation demonstrates that, due to the entrapping of hydrophobic Nile Red dye into the self-assembled micelles of **1**, FRET takes place from excess amount of donor **1** to the encapsulated acceptor. Herein it is apparent that 2% fraction of the Nile red is capable of triggering efficient FRET between donor/acceptor pairs.

*5. ^{1}H NMR measurement for **2** upon addition of Hg^{2+} ion*

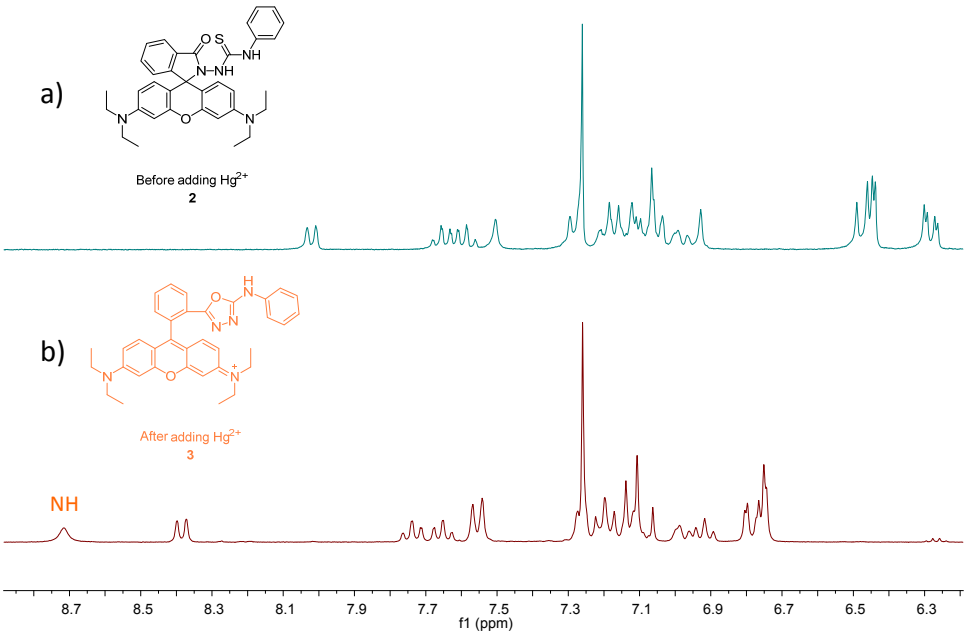


Figure S4. ^{1}H NMR spectra of **2** (a) before and (b) after addition of Hg^{2+} ion. The

complete conversion of **2** to the ring-opening product **3** can be achieved within 1 min.

*6. Control FRET experiment by titrating Hg^{2+} into **1***

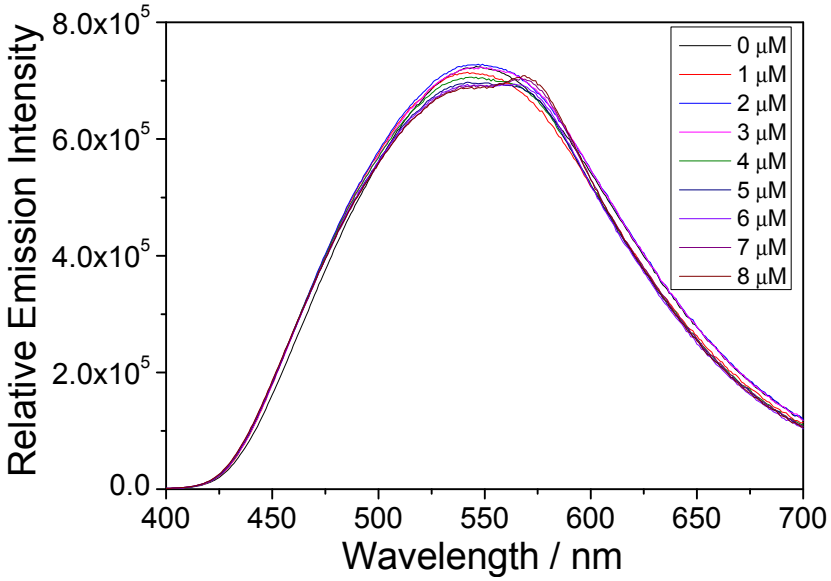


Figure S5. Fluorescent spectra (λ_{ex} =380 nm) of **1** (50 μM) in water upon gradual titrating of Hg^{2+}. No emission band shifting occurs, which is different from the experiment of titrating Hg^{2+} into **2⊂1** in water (see Figure 3b in the main text). It is highly plausible since the green emission (λ_{max} = 545 nm) originates from the self-assembled state of **1**. Upon addition of Hg^{2+}, it induces the ring-opening of **2**, accompanying with the successive FRET from donor **1** to the Rhodamine B acceptor **3**. Hence, the self-assembled AIE micelles possess dual functionalities (nano-carriers and fluorescent probes), both of which are necessary to induce FRET signals.

7. Determination of Hg^{2+} ion detection limit

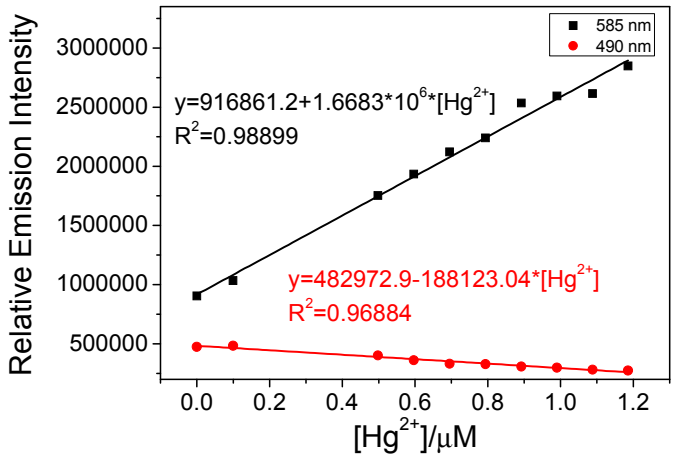


Figure S6. Changes of fluorescent intensities at 585 nm (black) and 490 nm (red) of **2⊂1** as a function of [Hg^{2+}] (λ_{ex} = 380 nm).

Method for determining the detection limit (DL):

The calibration curve was first obtained from the plot of $I_{585\ nm}/I_{490\ nm}$, as a function of Hg^{2+} ion concentration. The regression curve equation was then obtained.

The detection limit = 3 × S.D./k;

where k is the slope of the curve equation, and S.D. represents the standard deviation for intensity ratio of the sensor dispersion in the absence of mercury ion.

$I_{585\ nm}/I_{490\ nm}$ = 1.36825 + 7.29412 × [Hg^{2+}] (R^2=0.98238)

DL = 3 × 0.270/7.29412 μM= 0.11 μM (22 ppb)

8. Competition experiment towards Hg^{2+} ion

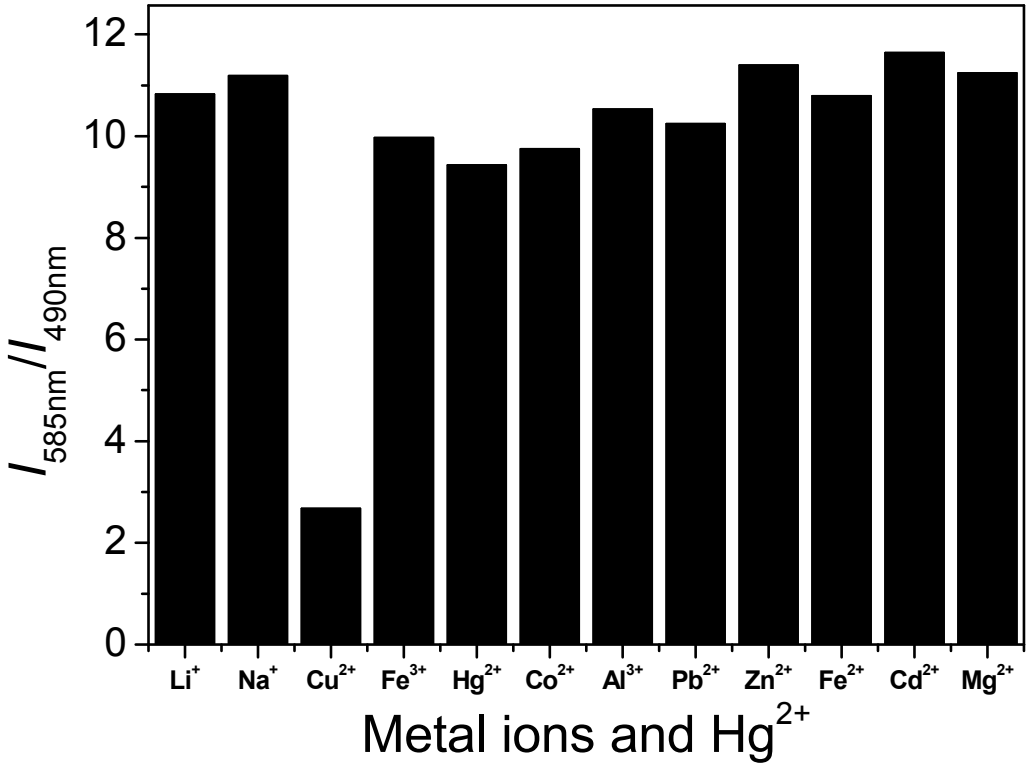
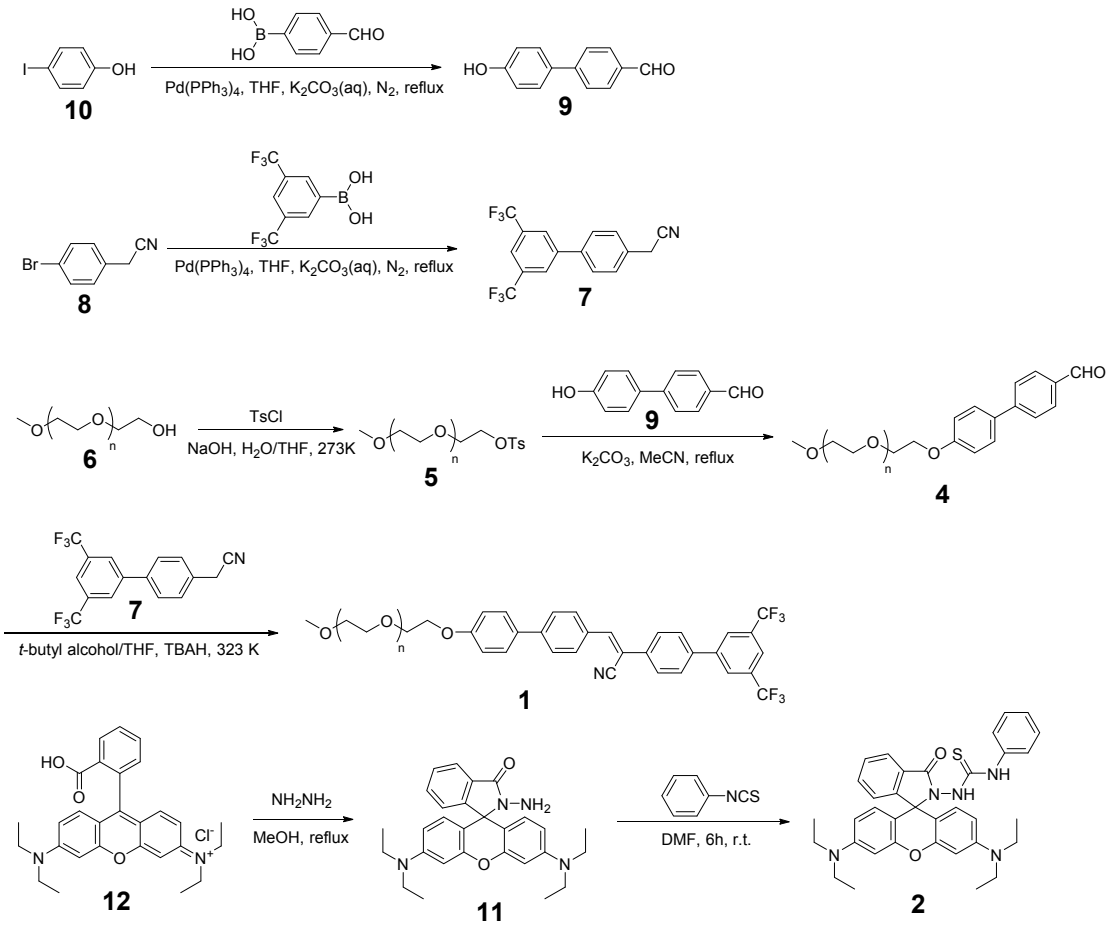


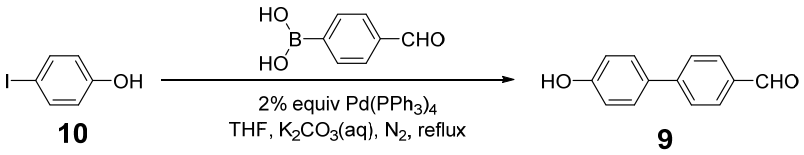
Figure S7. Fluorescence intensity ratio ($I_{585\ nm}/I_{490\ nm}$) of **2⊂1** in water in the presence of 5 equiv. of Li^+, Na^+, Cu^{2+}, Fe^{3+}, Hg^{2+}, Co^{2+}, Al^{3+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+} and Mg^{2+} ions in the presence of Hg^{2+}. As can be seen, the values of $I_{585\ nm}/I_{490\ nm}$ maintain high levels when mixing Hg^{2+} and various metal ions. The only exception is Cu^{2+}, which shows the decrease for $I_{585\ nm}/I_{490\ nm}$ value. Similar phenomenon is also encountered in the previous example (*Langmuir* **2010**, *26*, 724).

*9. Synthetic routes to compounds **1–2***



Scheme S1. Synthetic routes to the targeted compounds **1**–**2**.

*9.1. Synthesis of compound **9***



Compound **10** (6.00 g, 27.3 mmol), (4-formylphenyl) boronic acid (3.70 g, 24.8 mmol), and tetrakis-(triphenylphosphine) palladium (0.20 g, 0.20 mmol) were dissolved in THF (100 mL). The reaction mixture was mixed with degassed aqueous K_2CO_3 (50 mL, 2 M), and then refluxed overnight at 90 °C with vigorous stirring under nitrogen. After cooling to room temperature, the phase-separated aqueous layer was extracted three times with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, and the filtrate was dried by vacuum

evaporation. The crude product was purified by column chromatography on silica gel (petroleum ether/dichloromethane = 8/1, *v*/*v* as the eluent) to afford **9** as a colorless solid.[1] Yield 3.20 g (85%). ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 10.0 (s, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 5.02 (s, 1H).

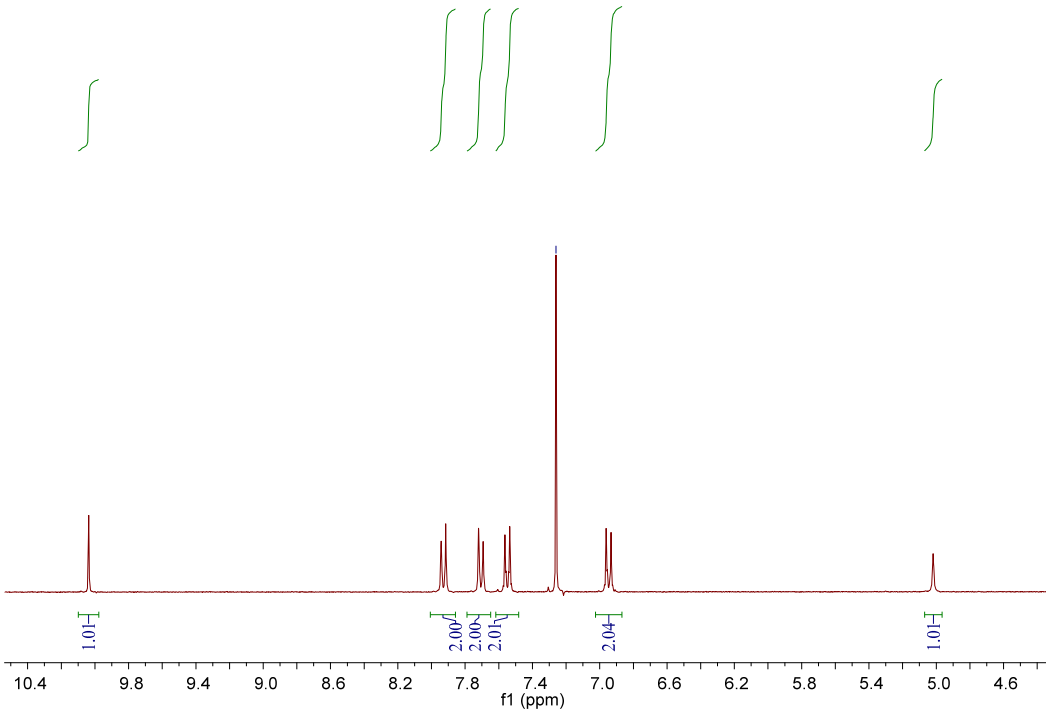
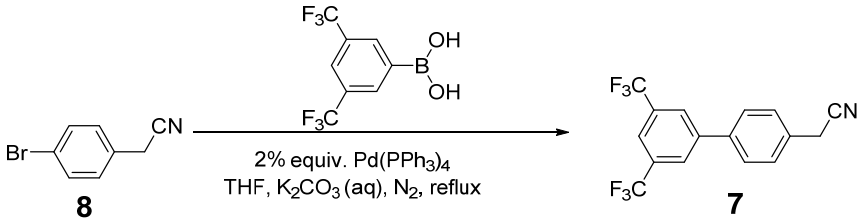


Figure S8. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of compound **9**.

9.2. Synthesis of compound 7



Compound **8** (5.00 g, 25.5 mmol), 3,5-bis(trifluoromethyl)phenyl boronic acid (7.20 g, 28.1 mmol), and tetrakis-(triphenylphosphine) palladium (0.50 g, 0.50 mmol) were dissolved in THF (150 mL). The reaction mixture was mixed with degassed aqueous K_2CO_3 (50 mL, 2 M) and then refluxed overnight at 90 °C with vigorous stirring under nitrogen. After cooling to room temperature, the phase-separated

aqueous layer was extracted three times with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, and the filtrate was dried by vacuum evaporation. The crude product was purified by column chromatography on silica gel (petroleum ether/dichloromethane = 10/1, *v*/*v* as the eluent) to afford **7** as a peak green solid.[2] Yield 5.80 g (88%). ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 8.00 (s, 2H), 7.88 (s, 1H), 7.64 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 3.84 (s, 2H).

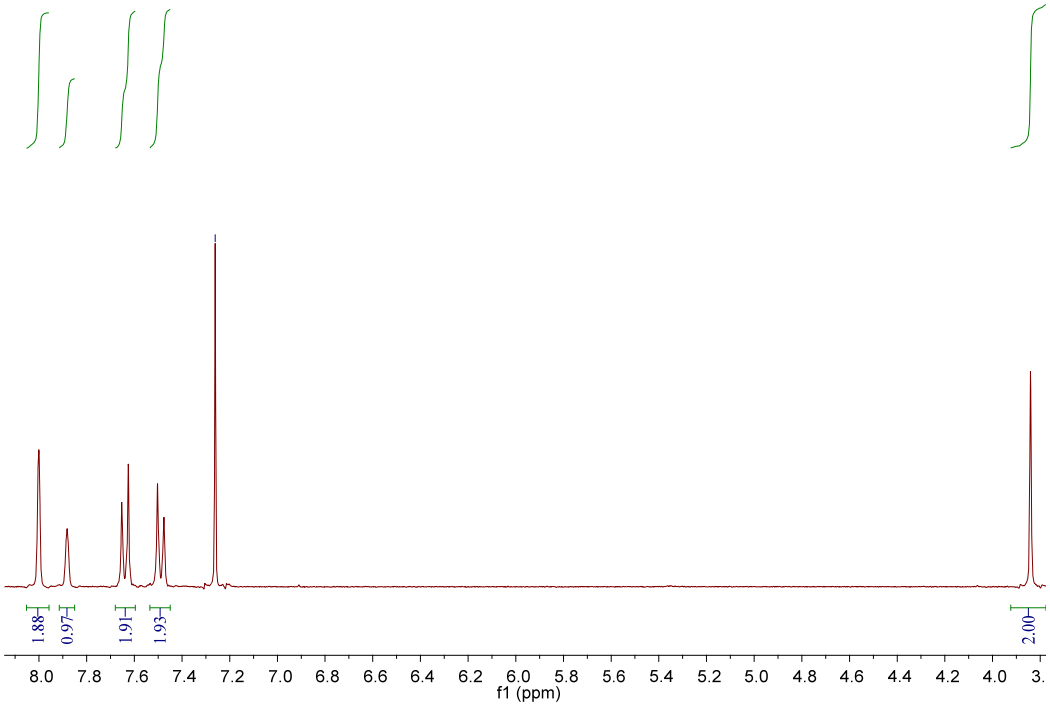
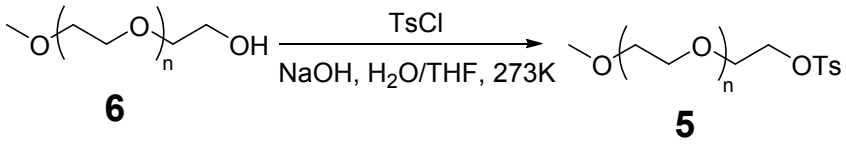


Figure S9. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of compound **7**.

9.3. Synthesis of compound ***5***



6 **5**

Compound **6** (5.00 g, 9.10 mmol) and NaOH (1.10 g, 27.5 mmol) were dissolved in the mixture solvents of water (7 mL) and THF (10 mL) under ice-cold conditions. The resulting solution was stirred for 30 min. Subsequently, *p*-tosyl chloride (2.00 g, 10.5 mmol) in 5 mL of THF was added drop-wise to this solution. The mixture was then stirred at room temperature overnight. The organic solvent was evaporated and

water (20 mL) was then added. The aqueous layer was extracted three times with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, and the filtrate was dried by vacuum evaporation. The crude product was purified by column chromatography on silica gel (ethyl acetate/dichloromethane = 1 : 1, *v*/*v* as the eluent) to afford **5** as a colorless oil (5.10 g, 79%).[2] ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 7.78 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 4.19–4.10 (m, 2H), 3.78–3.44 (m, 54H), 3.36 (s, 3H), 2.43 (s, 3H).

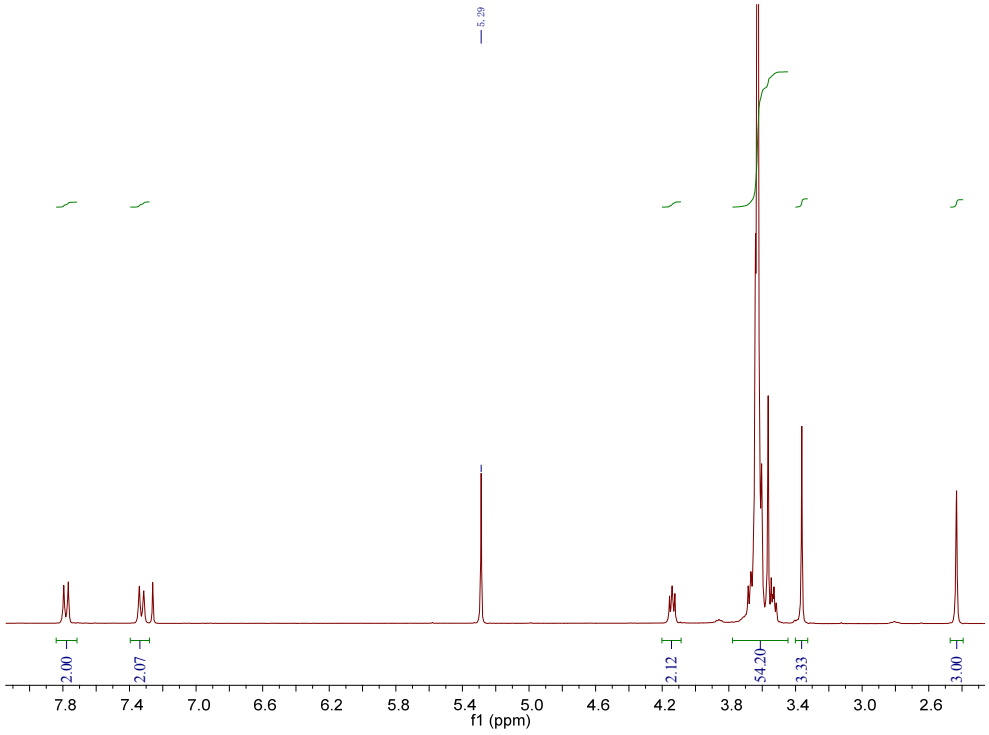
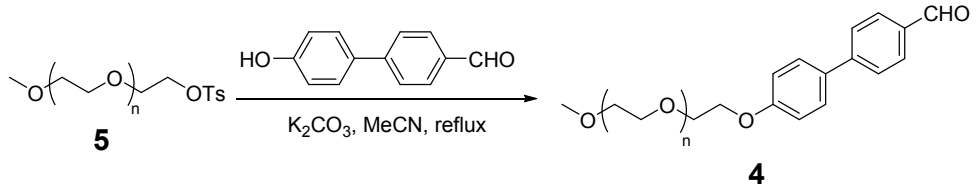


Figure S10. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of compound **5**.

*9.4. Synthesis of compound **4***



Compound **5** (2.40 g, 3.40 mmol), **9** (0.70 g, 3.80 mmol) and K_2CO_3 (1.90 g, 13.6 mmol) were added into a 100 mL round-bottom flask. CH_3CN (30 mL) was added and the mixture was refluxed at 90°C overnight. The organic solvents were removed with a rotary evaporator. The residue was then extracted with water/DCM for three times. The organic extracts were dried over anhydrous Na_2SO_4 and the

solvent was removed with a rotary evaporator. The residue was purified by flash column chromatograph (acetone/ethyl acetate = 1/2, *v*/*v* as the eluent) to afford compound **4** as colorless oil (2.20 g, 87%).[2] ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 10.0 (s, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 4.24–4.16 (m, 2H), 3.95–3.84 (m, 2H), 3.80–3.46 (m, 48H), 3.37 (s, 3H).

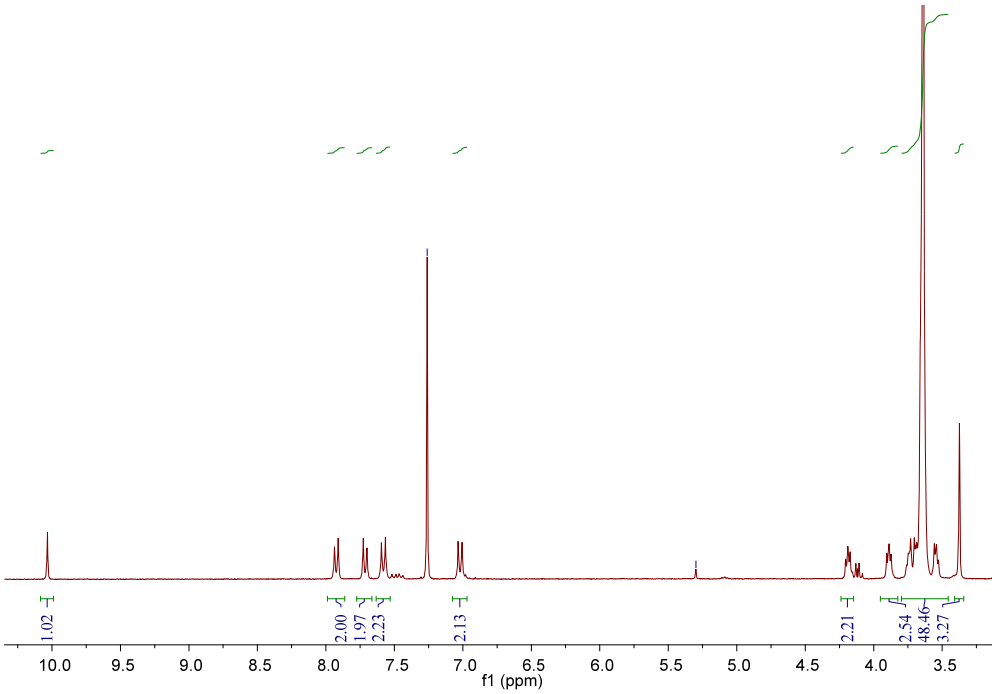
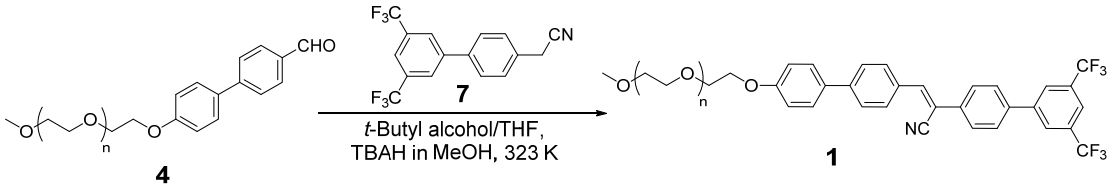


Figure S11. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of **4**.

9.5. Synthesis of compound 1



A mixture of compound **4** (0.70 g, 1.00 mmol) and **7** (0.50 g, 1.50 mmol) in *t*-butyl alcohol (5 mL) and THF (1 mL) was stirred at 50 °C for 1 h. Tetrabutylammonium hydroxide (TBAH, 1 M solution in methanol, 0.1 mL) was then added dropwise into the mixture. After stirring for 1 h, a yellow precipitate was collected by filtration and further purified by flash column chromatograph (acetone/ethyl acetate = 1/3, *v*/*v* as the eluent) to afford compound **1** as a pale green

oil (0.60 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 8.10–7.94 (m, 2H), 7.94–7.80 (m, 2H), 7.80–7.42 (m, 8H), 7.37 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 7.9 Hz, 2H), 4.25–4.11 (m, 2H), 3.88 (s, 3H), 3.82–3.49 (m, 50H), 3.37 (s, 3H). ESI–MS *m*/*z*: $[M + NH_4]^+$, 1069.4906.

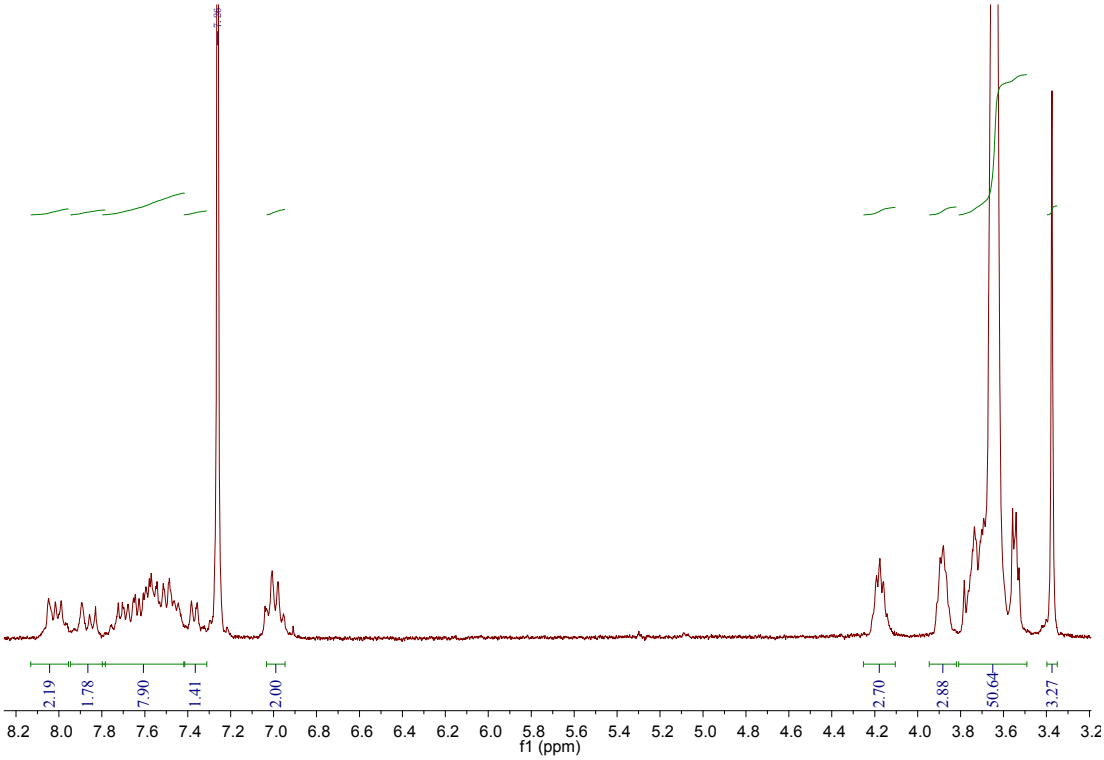


Figure S12. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of compound **1**.

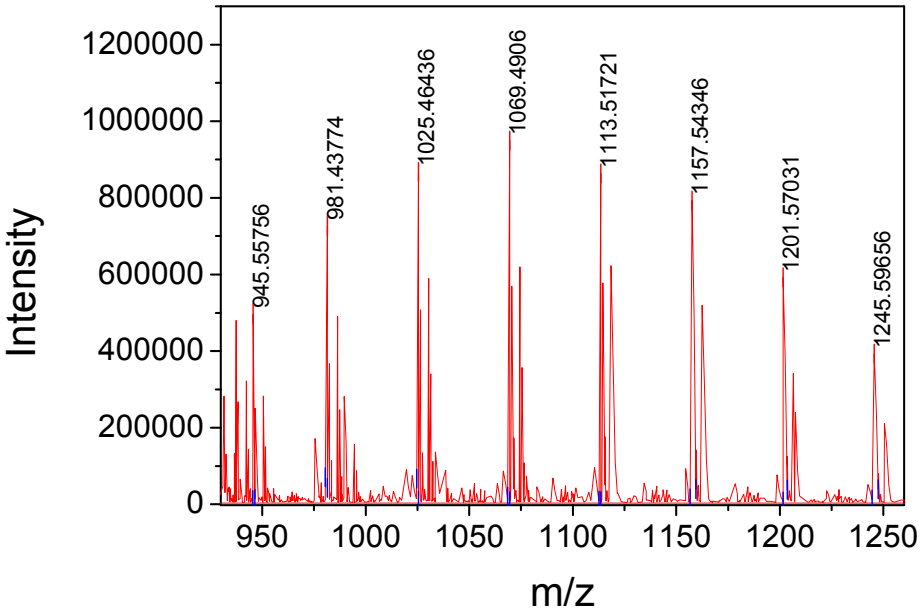
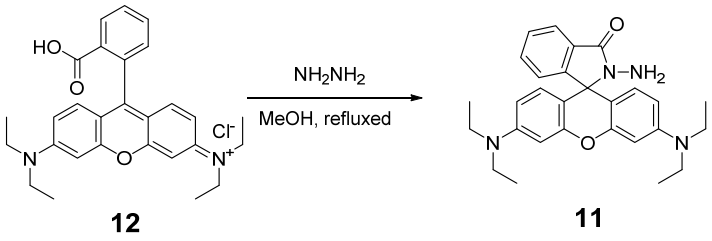


Figure S13. ESI-MS spectrum of compound **1**.

*9.6. Synthesis of compound **11***



12 **11**

To a Rhodamine B derivative **12** (1.00 g, 2.09 mmol) in MeOH (10 mL) was added hydrazine monohydrate (0.3 mL, 6.30 mmol). The reaction solution was refluxed for 6 hours and diluted with ethyl acetate (30 mL). The solution was washed with H_2O (10 mL) and 1 N NaOH (10 mL). The organic phase was dried over Na_2SO_4, concentrated and column chromatographed on silica gel (ethyl acetate/DCM = 1/5, *v*/*v* as the eluent) to afford compound **11** (0.80 g, 90%).[3] ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 7.94 (dd, J = 5.6, 2.7 Hz, 1H), 7.45 (dd, J = 5.2, 3.3 Hz, 2H), 7.14–7.07 (m, 1H), 6.50–6.39 (m, 4H), 6.29 (dd, J = 8.9, 2.5 Hz, 2H), 3.61 (s, 2H), 3.34 (q, J = 7.0 Hz, 8H), 1.16 (t, J = 7.0 Hz, 12H).

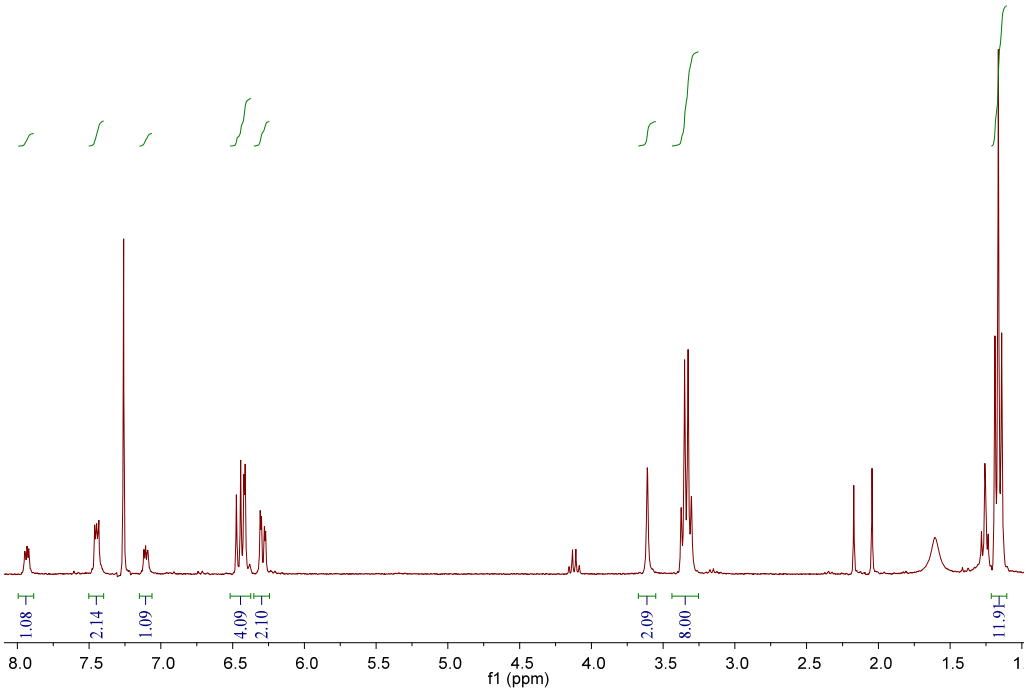
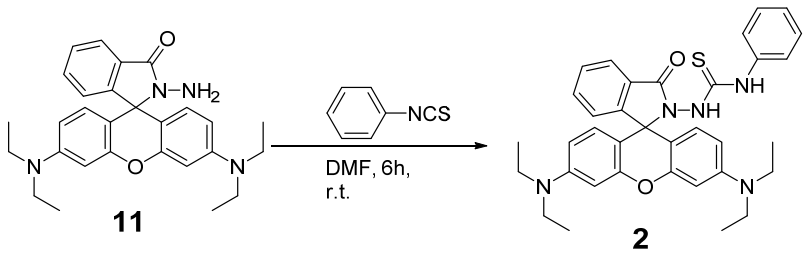


Figure S14. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of compound **11**.

9.7. Synthesis of compound ***2***



Compound **11** (200 mg, 0.44 mmol) in DMF (2 mL) was added to a solution of phenyl isothiocyanate (0.1 mL, 0.65 mmol) in DMF (1 mL). The reaction mixture was stirred for 6 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica gel (petroleum ether/dichloromethane = 5/1, *v*/*v* as the eluent) to give 234 mg (90%) of **2**.[3] ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 8.02 (d, J = 7.2 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.4 Hz, 1H), 7.50 (s, 1H), 7.28 (d, J = 6.6 Hz, 1H), 7.22 – 7.14 (m, 2H), 7.12 (t, J = 4.1 Hz, 1H), 7.05 (d, J = 9.0 Hz, 2H), 6.93 (s, 1H), 6.49 (s, 1H), 6.46 (s, 1H), 6.44 (d, J = 2.5 Hz, 2H), 6.30 (d, J = 2.6 Hz, 1H), 6.27 (d, J = 2.6 Hz, 1H), 3.34 (q, J = 7.1 Hz, 8H), 1.16 (t, J = 7.1 Hz, 12H).

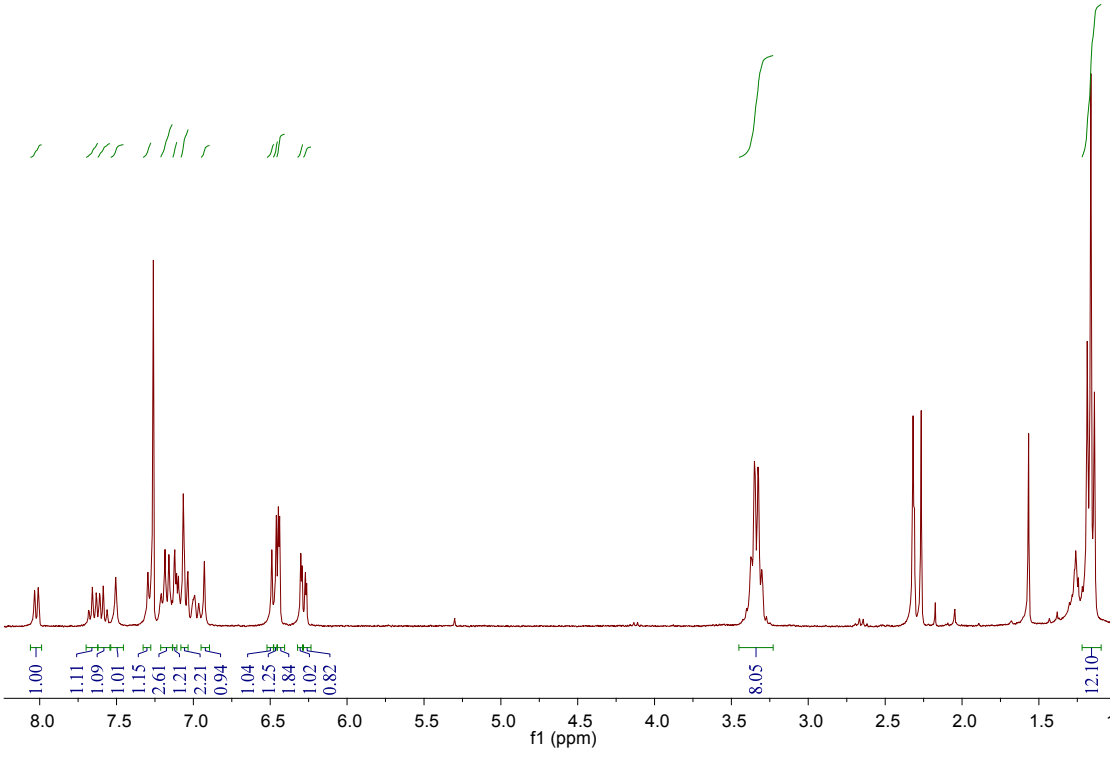


Figure S15. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of compound **2**.

References:

1. J. D. Revell, A. Ganesan, *Org. Lett.*, 2002, **4**, 3071
2. S. Shin, S. H. Gihm, C. R. Park, S. Kim, Y. S. Park, *Chem. Mater.*, 2013, **25**, 3288
3. L. E. Page, X. Zhang, A. M. Jawaid, P. T. Snee, *Chem. Commun.*, 2011, **47**, 7773