Host–guest interaction enhanced aggregation-induced emission and its application in cell imaging

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Electronic Supplementary Information (12 pages)

1. Materials and methods

All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature. Compounds **1**[S1] and **2**[S2] were synthesized according to published literature procedures. NMR spectra were recorded with a Bruker Avance DMX 400 spectrophotometer, a Bruker Avance DMX 500 spectrophotometer, or a Bruker Avance DMX 600 spectrophotometer with the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Low-resolution electrospray ionization mass spectra (LRESI-MS) were obtained on a Bruker Esquire 3000 Plus spectrometer (Bruker-Franzen Analytik GmbH Bremen, Germany) equipped with an ESI interface and an ion trap analyzer. High-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Bruker 7-Tesla FT-ICR mass spectrometer equipped with an electrospray source (Billerica, MA, USA). The melting points were collected on a SHPSIC WRS-2 automatic melting point apparatus. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan).

Fabrications of NPs. The NPs were obtained through reprecipitation method. Briefly, **H** (8.16 mg) and 1 equiv. of **G** (5.18 mg) were dissolved in acetone (10 mL). A 50 μL acetone solution of the host−guest system was injected into a certain amount of water with controlled stirring for 12 h to afford the corresponding NPs.

Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) Studies. The nanostructures of NPs were revealed using TEM. TEM samples were prepared by drop-coating the solution onto a carbon-coated copper grid. TEM experiments were performed on an HT-7700 instrument. The corresponding solution was left to stand overnight and the insoluble precipitate was eliminated by using a microporous membrane before DLS tests. Dynamic light scattering (DLS) measurements were carried out using a 200 mW polarized laser source Nd:YAG (λ = 532 nm). The polarized scattered light was collected at 90° in a self-beating mode with a Hamamatsu R942/02 photomultiplier. The signals were sent to a Malvern 4700 submicrometer particle analyzer system.

Cell Culture. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells grew as a monolayer and were detached upon confluence using trypsin (0.5% *w*/*v* in PBS). The cells were harvested from cell culture medium by incubating in trypsin solution for 5 min. The cells were centrifuged, and the supernatant was discarded. A 3 mL portion of serum-supplemented DMEM was added to neutralize any residual trypsin. The cells were resuspended in serum-supplemented DMEM at a concentration of

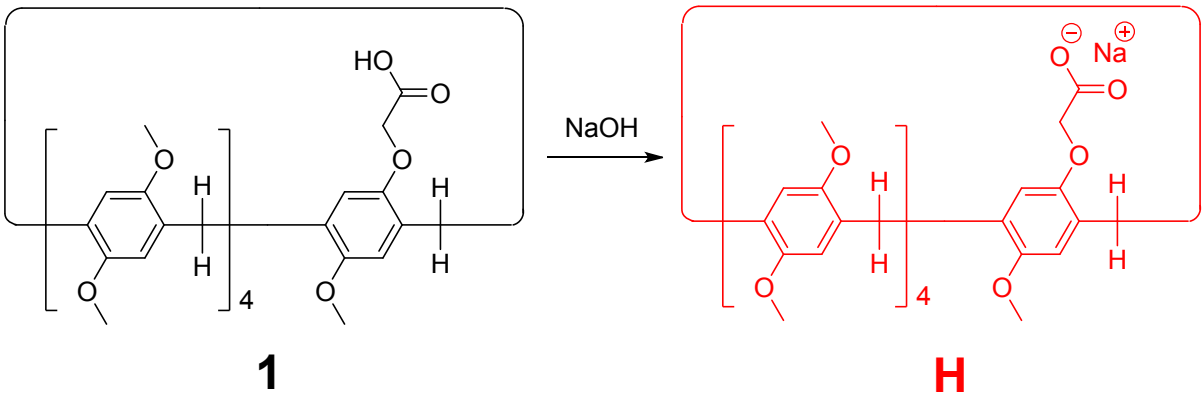
1×10^4 cells/mL. Cells were cultured at 37 °C and 5% CO_2.

Evaluation of Cytotoxicity. The cytotoxicity of the NPs against HeLa cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays in a 96-well cell culture plate. All solutions were sterilized by filtration with a 0.22 μm filter before tests. HeLa cells were seeded at a density of 1×10^4 cells/well in a 96-well plate, and incubated for 24 h for attachment. Cells were then incubated with NP at various concentrations for 4 h and 24 h. After washing the cells with PBS buffer, 20 μL of a MTT solution (5 mg/mL) was added to each well. After 4 h of incubation at 37 °C, the MTT solution was removed, and the insoluble formazan crystals that formed were dissolved in 100 μL of dimethylsulfoxide (DMSO). The absorbance of the formazan product was measured at 570 nm using a spectrophotometer (Bio-Rad Model 680). Untreated cells in media were used as a control. All experiments were carried out with five replicates.

In Vitro **Cell Accumulation of the NPs Determined by Confocal Laser Scanning Microscopy (CLSM).** HeLa cells were treated with the NPs (the concentration of the NPs was kept at 5 μM) in the culture medium at 37 °C for 4 h. The cells were washed three times with PBS and fixed with fresh 4.0% formaldehyde at room temperature for 15 min. After washing with PBS, the cells were stained with DAPI (1 μg/mL) for 15 min. The images were taken using a LSM-510 confocal laser scanning microscope (CLSM, ZEISS LSM780).

*2. Syntheses of compounds **H**, **G** and **G'***

*2.1. Synthesis of compound **H***



1 **H**

Synthesis of **H**: **1** (300 mg, 0.380 mmol) and NaOH (152 mg, 3.80 mmol) were added to H_2O (25 mL) and stirred at room temperature for 12 h. Water was removed under reduced pressure to give **H** as a white solid (310 mg, 100%). Mp 165.2−166.5 °C. The proton NMR spectrum of **H** is shown in Fig. S1. ^{1}H NMR (600 MHz, acetone-d_6/D_2O = 5:3, 293 K) δ (ppm): 7.17 (s, 1H), 6.87–6.85 (m, 9H), 4.25 (s, 2H), 3.87 (s, 2H), 3.76–3.71 (m, 35H). The ^{13}C NMR spectrum of **H** is shown in Fig. S2. ^{13}C NMR (100 MHz, chloroform-*d*, 293 K) δ (ppm): 152.16, 151.08, 150.90, 150.75, 150.47, 148.68, 128.66, 128.38, 128.18, 127.67, 114.59, 114.04, 113.78, 55.84, 55.25, 52.99, 29.63, 29.37. LRESIMS is shown in Fig. S3: *m*/*z* 793.3 [M − Na]$^-$. HRESIMS: *m*/*z* calcd for [M − Na]$^-$ $C_{46}H_{49}O_{12}^-$, 793.3224, found 793.3236, error −2 ppm.

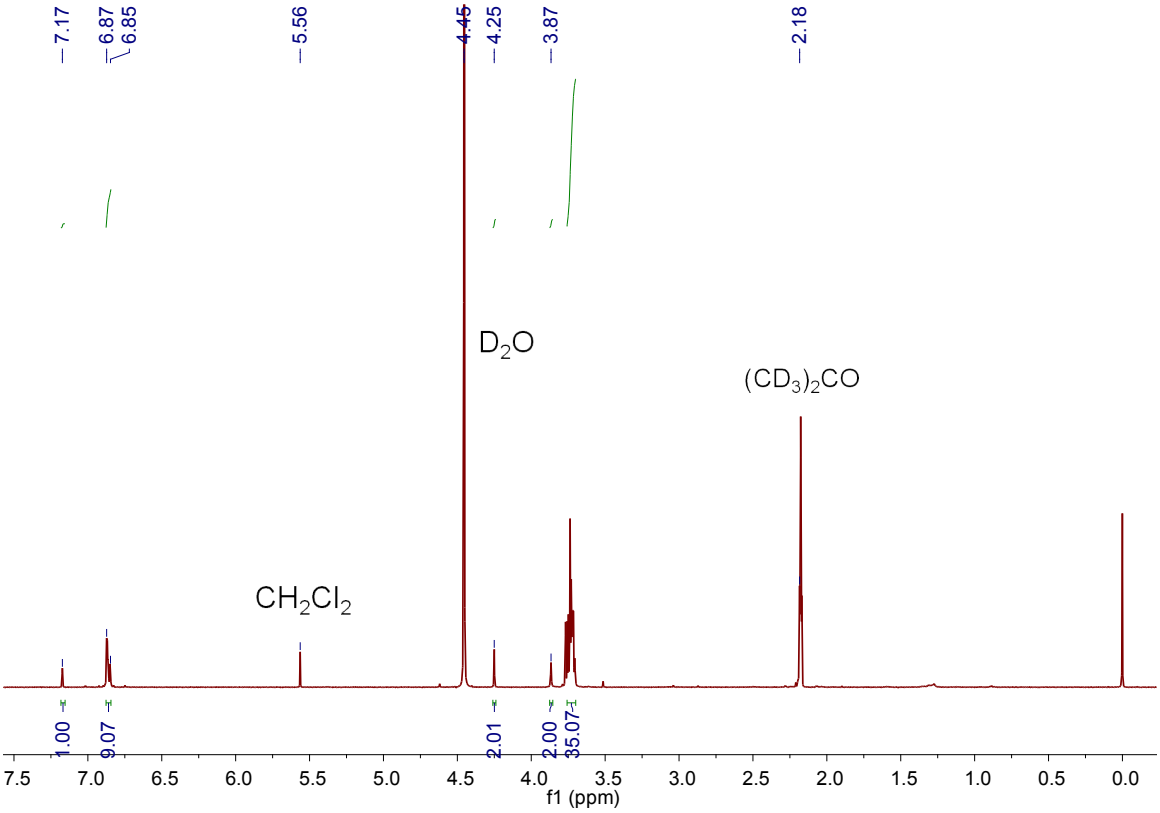


Fig. S1 ^{1}H NMR spectrum (600 MHz, acetone-d_6/D_2O = 5:3, 293K) of **H**.

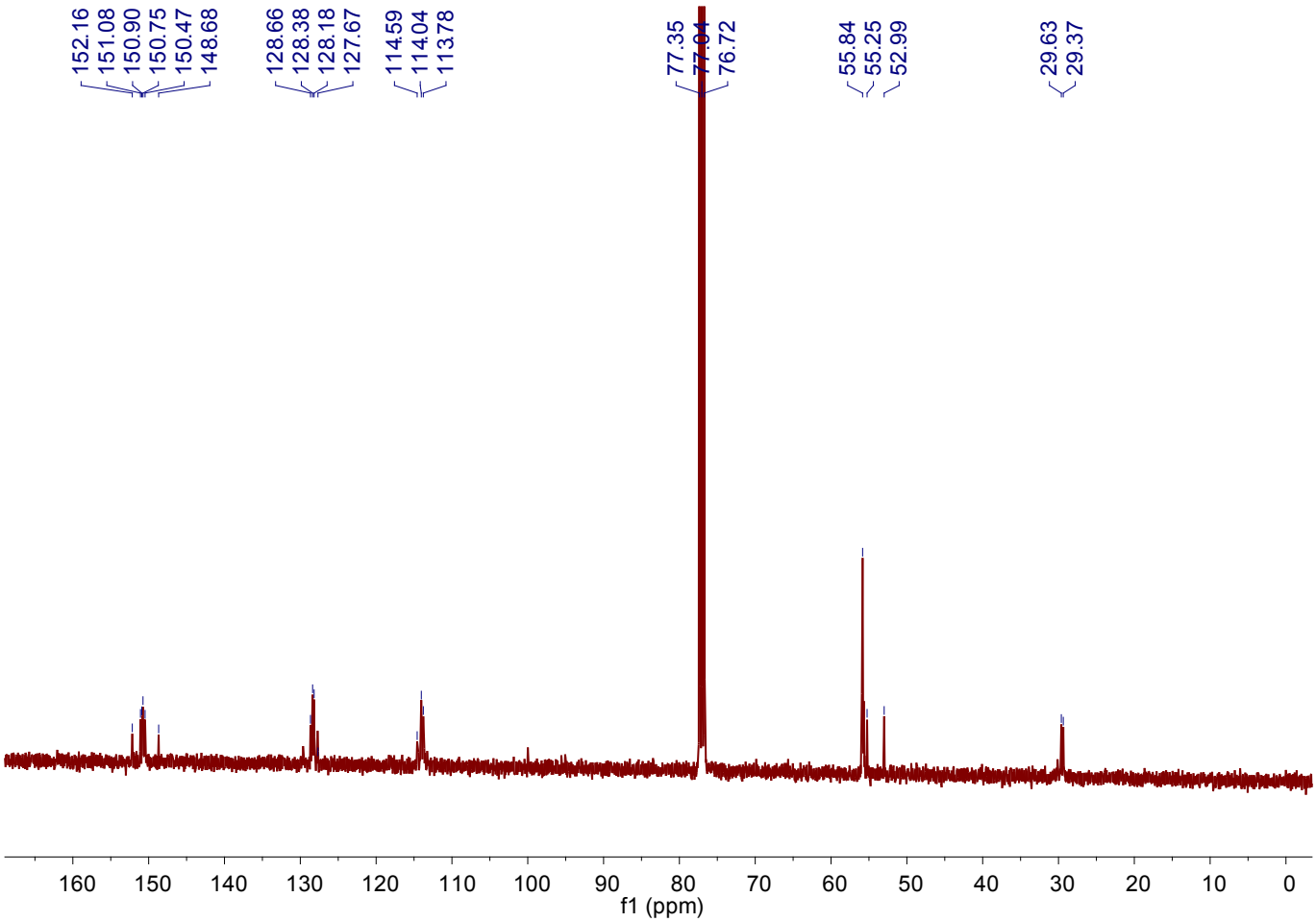


Fig. S2 ^{13}C NMR spectrum (100 MHz, chloroform-*d*, 293K) of **H**.

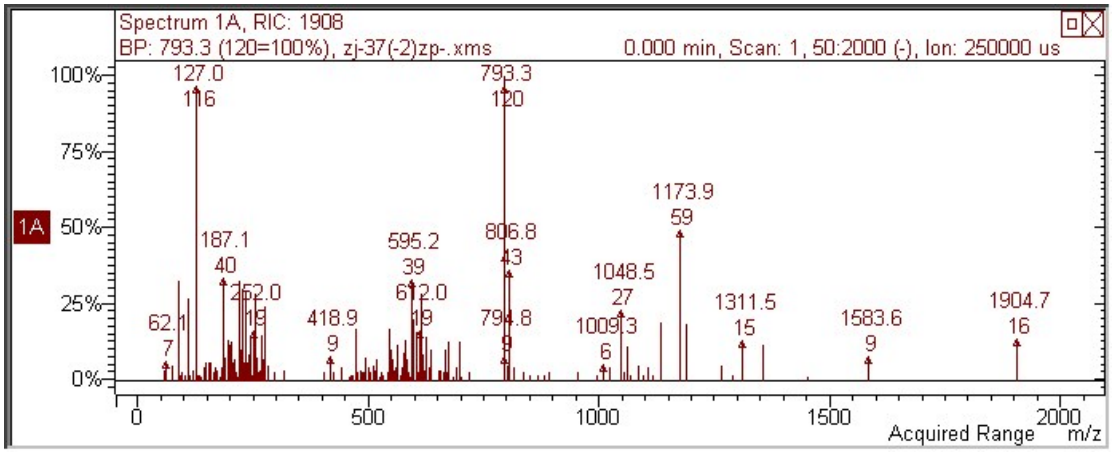
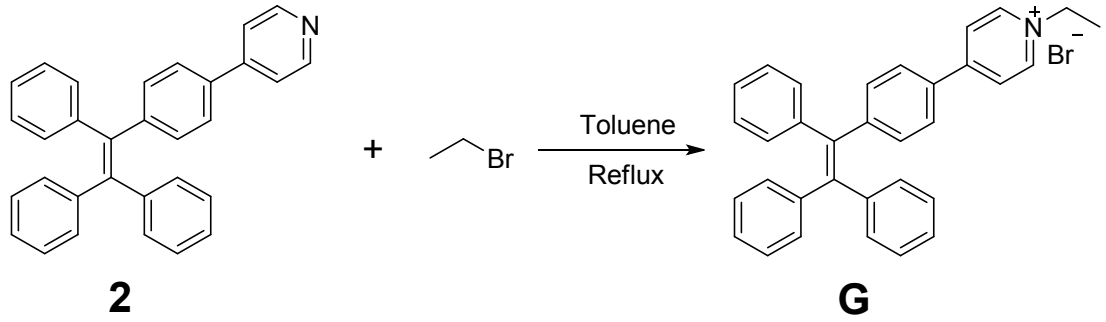


Fig. S3 Electrospray ionization mass spectrum of **H**. Assignment of the main peak: *m*/*z* 793.3 $[M - Na]^-$.

2.2. Synthesis of compound ***G***



Synthesis of **G**: Bromoethane (5.24 g, 49.0 mmol) was added to a solution of **2** (0.200 g, 0.490 mmol) in toluene (30 mL). The mixture was heated under nitrogen at reflux for 24 h. The cooled reaction mixture was evaporated under vacuum to yield **G** as a yellow solid (0.250 g, 100%). Mp 181.7−183.7 °C. The proton NMR spectrum of **G** is shown in Fig. S4. ^{1}H NMR (400 MHz, chloroform-*d*, 293K) δ (ppm): 9.33 (d, J = 8 Hz, 2H), 8.12 (d, J = 8 Hz, 2H), 7.53 (d, J = 8 Hz, 2H), 7.23–7.03 (m, 17H), 5.01–4.95 (m, 2H), 1.73 (t, J = 8 Hz, 3H). The ^{13}C NMR spectrum of **G** is shown in Fig. S5. ^{13}C NMR (100 MHz, chloroform-*d*, 293K) δ (ppm): 155.64, 148.81, 142.86, 139.24, 132.87, 131.03, 127.76, 127.18, 126.97, 124.44, 56.30, 17.16. LRESIMS is shown in Fig. S6: *m*/*z* 438.3 [M − Br]$^{+}$. HRESIMS: *m*/*z* calcd for [M − Br]$^{+}$ $C_{33}H_{28}N^{+}$, 438.2222, found 438.2207, error 3 ppm.

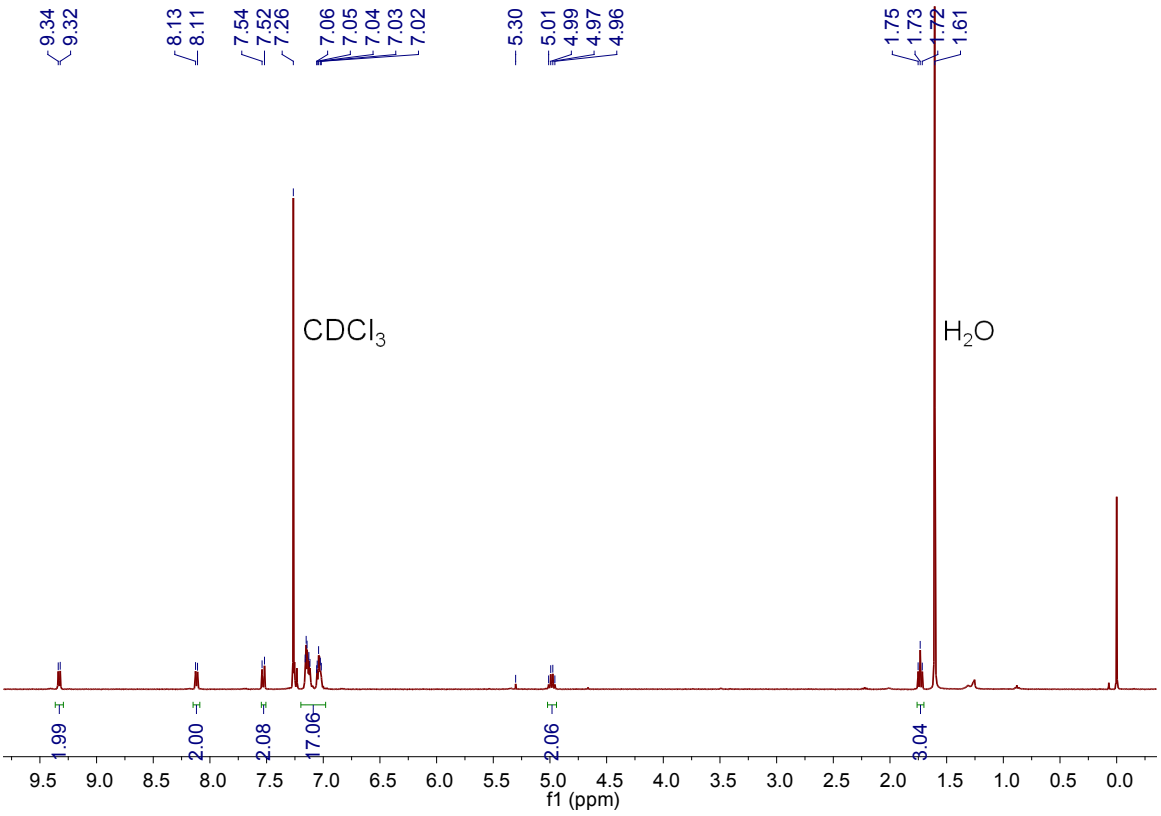


Fig. S4 ^{1}H NMR spectrum (400 MHz, chloroform-*d*, 293K) of **G**.

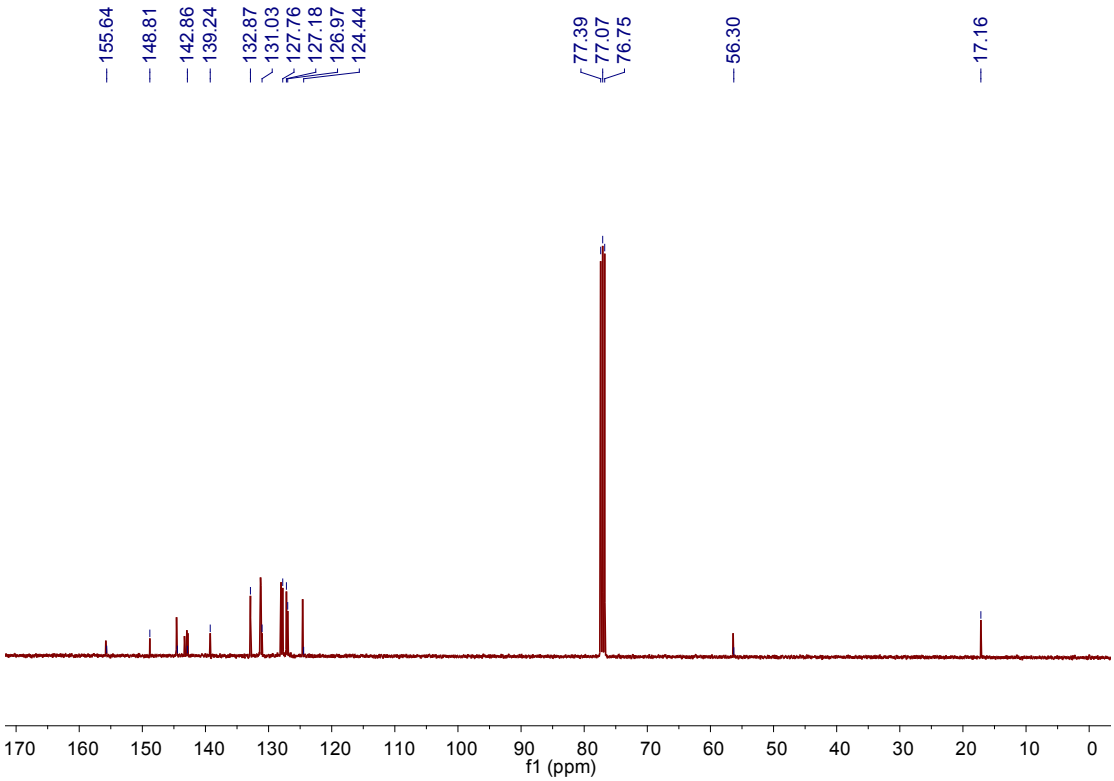


Fig. S5 ^{13}C NMR spectrum (100 MHz, chloroform-*d*, 293K) of **G**.

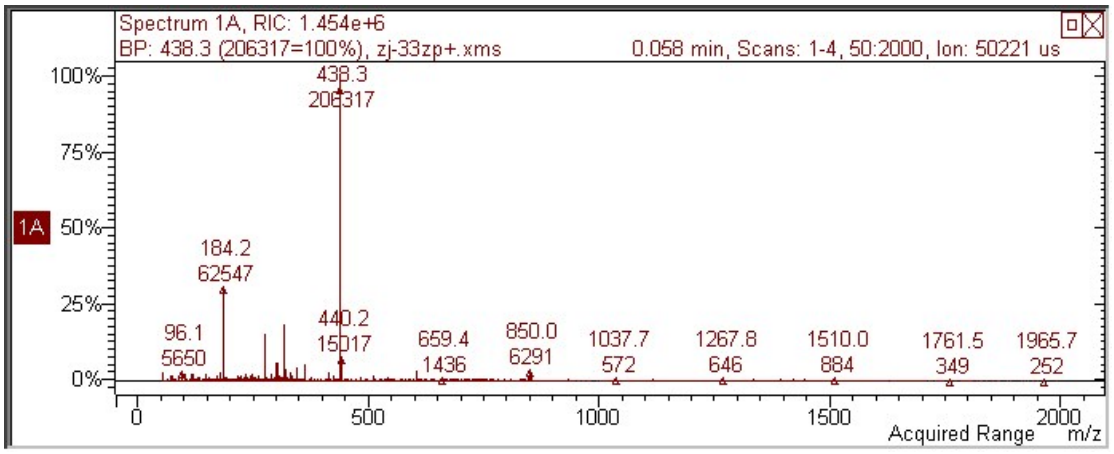
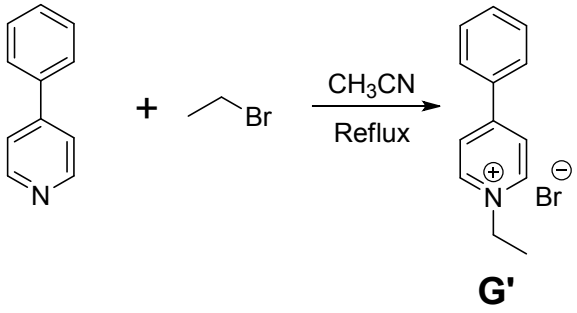


Fig. S6 Electrospray ionization mass spectrum of **G**. Assignment of the main peak: *m*/*z* 438.3 $[M - Br]^+$.

2.3. Synthesis of model compound ***G′***



G'

Synthesis of **G′**: Bromoethane (4.28 g, 40.0 mmol) was added to a solution of 4-phenylpyridine (0.310 g, 2.00 mmol) in acetonitrile (30 mL). The mixture was heated under nitrogen at reflux for 24 h. The cooled reaction mixture was evaporated under vacuum to yield **G′** as a liquid. The proton NMR spectrum of **G′** is shown in Fig. S7. ^{1}H NMR (400 MHz, D_2O, 293 K) δ (ppm): 8.73 (d, J = 8 Hz, 2H), 8.09 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 7.62 (t, J = 8 Hz, 1H), 7.55 (t, J = 8 Hz, 2H), 4.64–4.58 (m, 2H), 1.69 (t, J = 8 Hz, 3H). The ^{13}C NMR spectrum of **G′** is shown in Fig. S8. ^{13}C NMR (100 MHz, D_2O, 293 K) δ (ppm): 155.34, 143.49, 133.09, 132.10, 129.58, 127.70, 124.47, 56.29, 15.64. LRESIMS is shown in Fig. S9: *m*/*z* 184.1 $[M - Br]^+$. HRESIMS: *m*/*z* calcd for $[M - Br]^+$ $C_{33}H_{28}N^+$, 184.1126, found 184.1122, error 2 ppm.

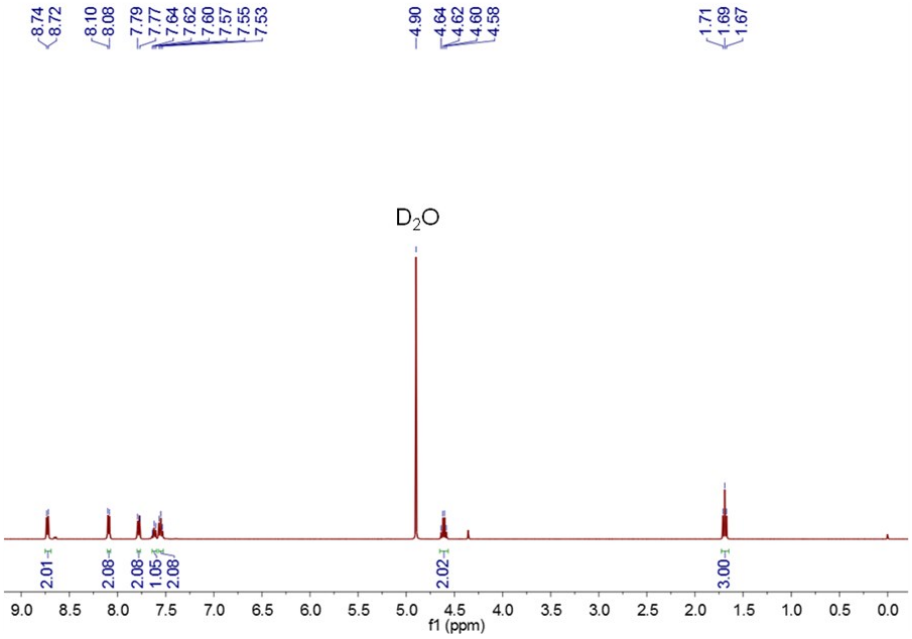


Fig. S7 ^{1}H NMR spectrum (400 MHz, D_2O, 293K) of **G′**.

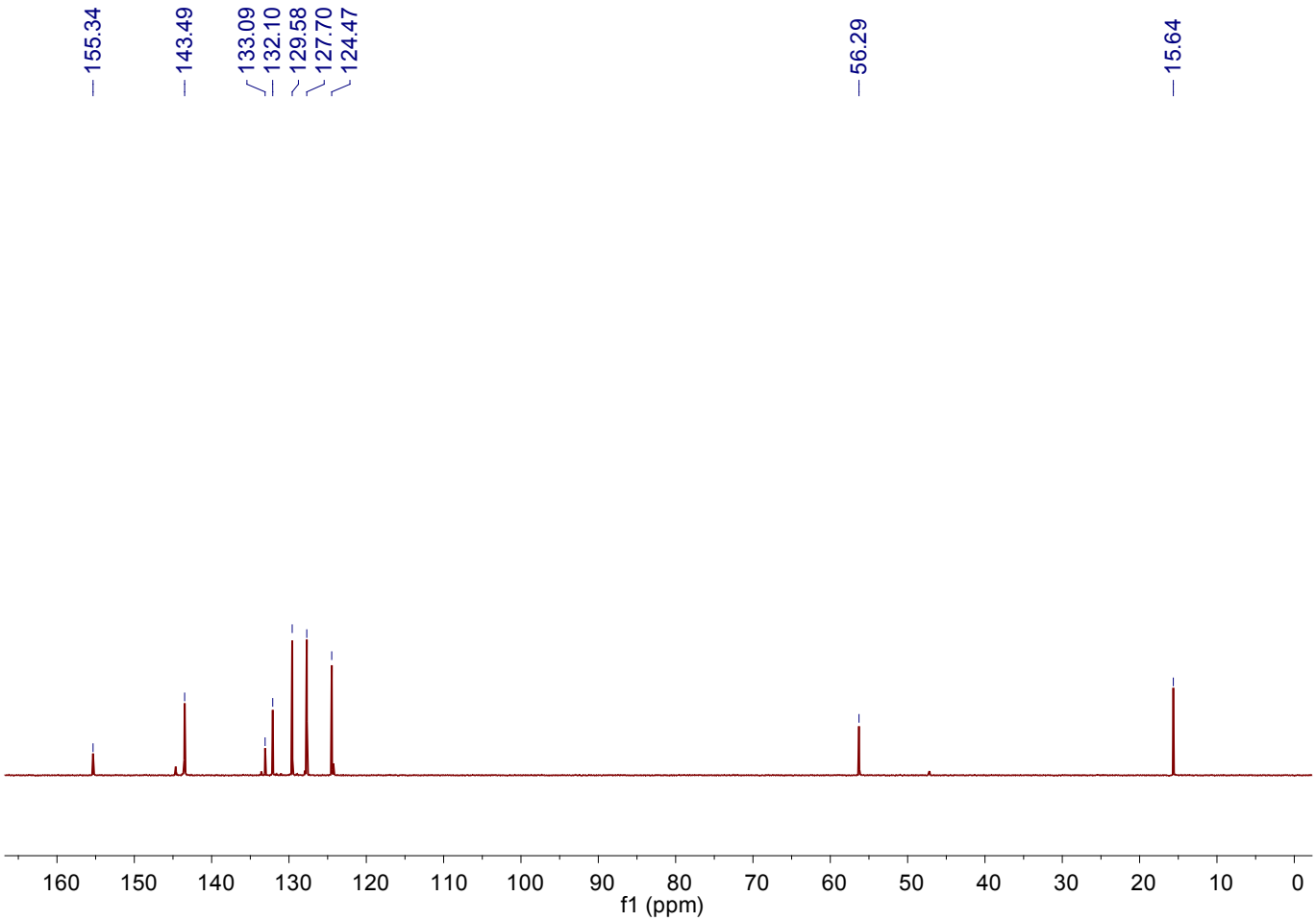


Fig. S8 ^{13}C NMR spectrum (400 MHz, D_2O, 293K) of **G′**.

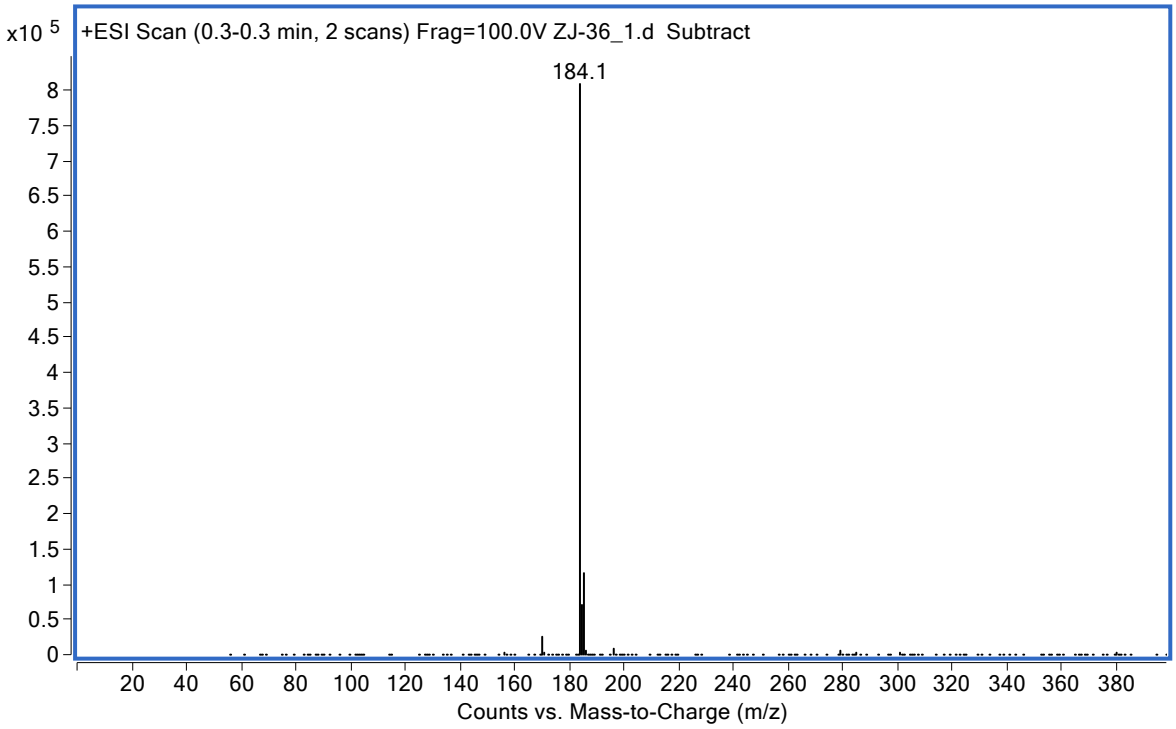
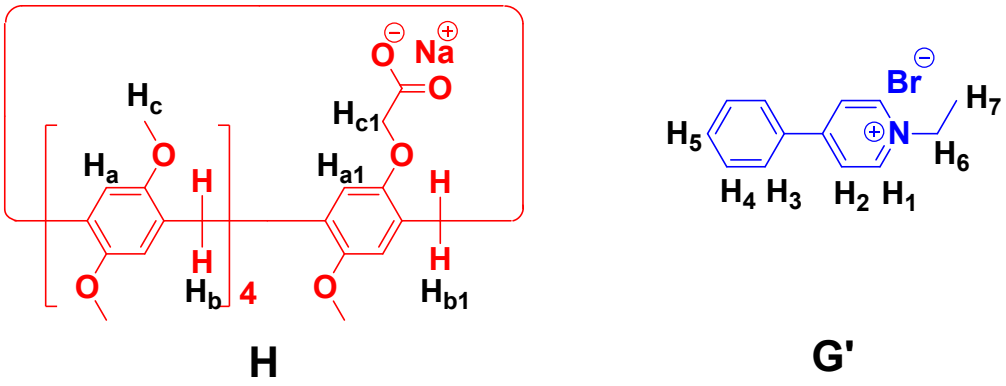


Fig. S9 Electrospray ionization mass spectrum of **G′**. Assignment of the main peak: *m*/*z* 184.1 $[M - Br]^+$.

*3. Investigation of the interactions between model compound **G'** and **H***



H **G'**

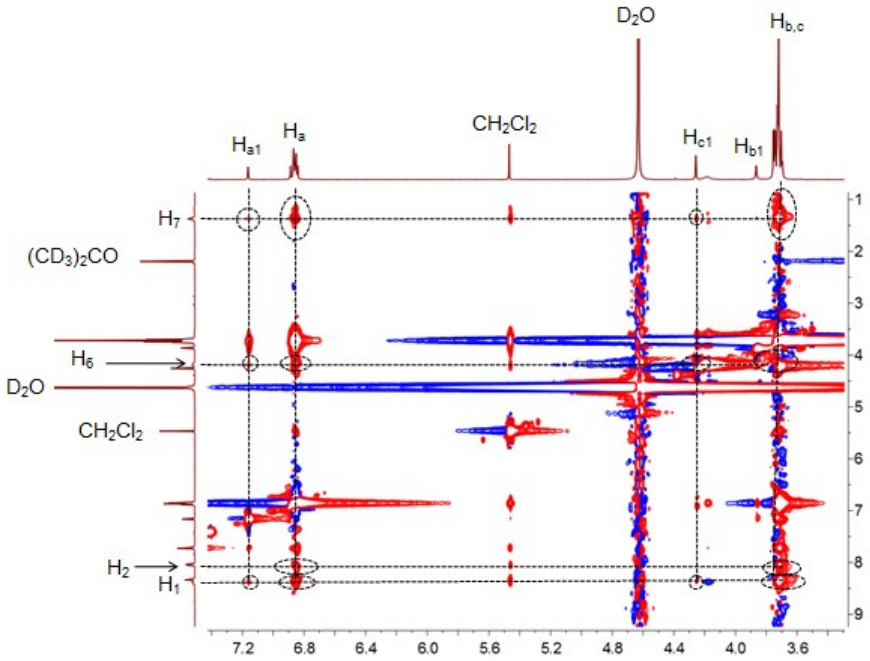


Fig. S10 NOESY NMR spectrum (500 MHz, acetone-d_6/D_2O = 5:3, 293 K) of **H** (10.0 mM) and **G′** (10.0 mM).

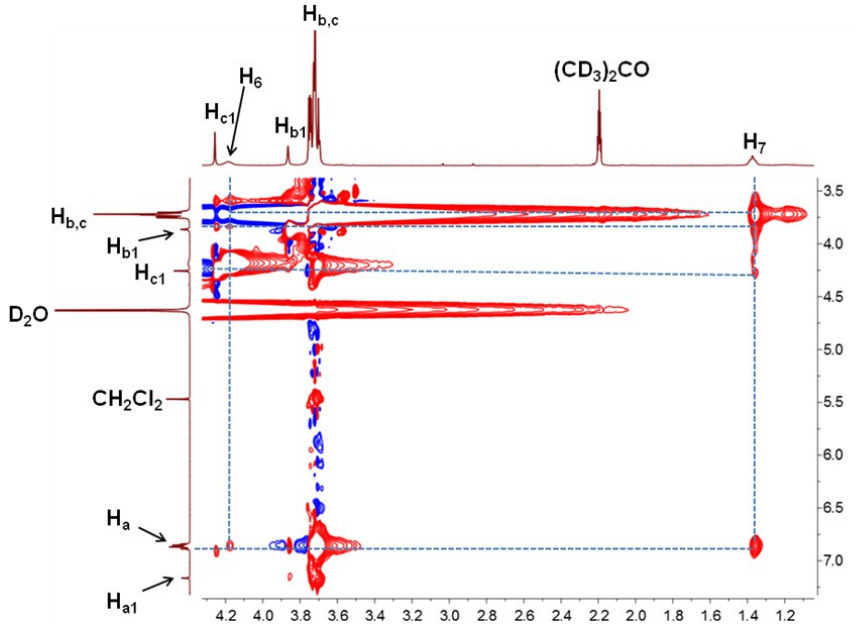


Fig. S11 Partial NOESY NMR spectrum (500 MHz, acetone-d_6/D_2O = 5:3, 293 K) of **H** (10.0 mM) and **G′** (10.0 mM).

*4. Fluorescence spectra of **G** in THF/water mixtures with different water fractions*

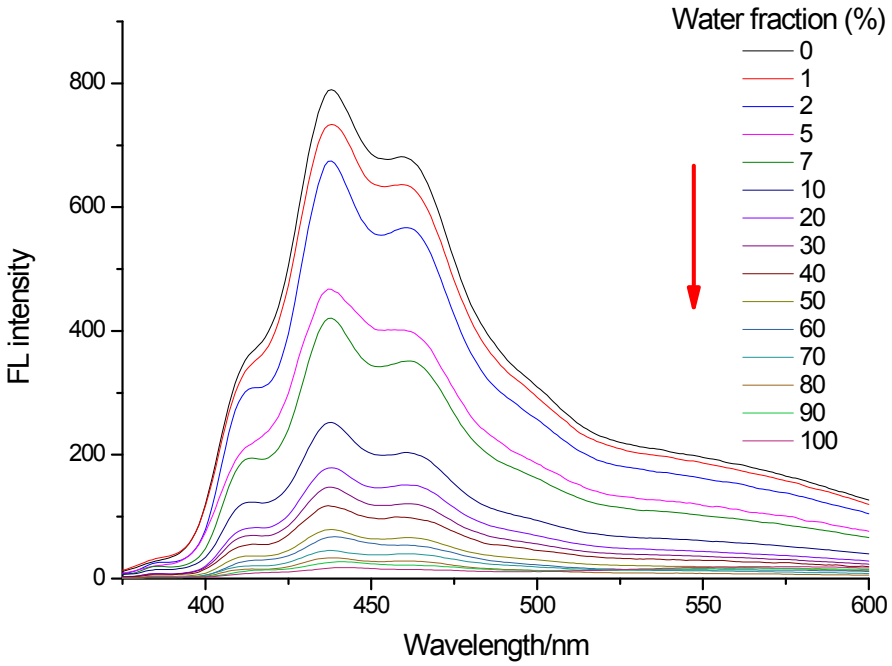


Fig. S12 Fluorescence spectra of **G** in THF and THF/water mixtures with different water fractions. The concentration of **G** was 1.00×10^{-4} M; excitation wavelength = 350 nm.

5. Tyndall effect of a solution containing ***H⊃G***

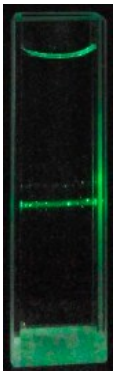


Fig. S13 Tyndall effect of a solution containing **H⊃G**

References:

S1. B. Xia and M. Xue, *Chem. Commun.*, 2014, **50**, 1021–1023.

S2. (a) M. Banerjee, S. J. Emond, S. V. Lindeman and R. Rathore, *J. Org. Chem*., 2007, **72**, 8054–8061; (b) M. Wang, Y.-R. Zheng, K. Ghosh and P. J. Stang, *J. Am. Chem. Soc*., 2010, **132**, 6282–6283.