Supplementary Information

For

Micelle-based fluorogenic sensing of trypsin: a sensitive method in pancreatic disease diagnosis

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Experimental

Materials

All chemicals used in the synthesis were of reagent grade and used without further purification. Normal human urine was purchased from Innovative Research Company. Zn (dust), Potassium carbonate, Sodium hydroxide, and sodium carbonate were purchased from Daejung Company. Titanium tetrachloride was purchased from Fujifilm. Tetrahydrofuran was purchased from Thermo Fisher Scientific. Trifluoroacetic acid was purchased from Acros Organics. Acetonitrile, dichloromethane, and methanol were bought from Samchun Company. Silica gel (Merck, 230-400 mesh) was used for chromatographic purification of all intermediate and target molecules. All other chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, or TCI and used as received.

Instrumentation and Methods

NMR spectra were recorded using a Bruker Ascend (AVANCE III 400), operating at 400 MHz for ^{1}H-NMR and at 100 MHz for ^{13}C-NMR. UV/Vis absorption spectra were recorded using a

Shimadzu UV-2600i UV spectrophotometer. Steady-state fluorescence spectra were obtained with a Shimadzu fluorometer RF-6000. Dynamic light scattering (DLS) experiments were performed with Zetasizer Nano S90 from Malvern Panalytical. Scanning Electron Microscopy (SEM) images were obtained with a FE-SEM S-4300 from Hitachi.

A 1 cm quartz cuvette was used for all spectral measurements. Stock solution (3.0 mM) of **1** was prepared in H_2O. The solution was kept at room temperature for one hour before use. Trypsin from bovine pancreas, alkaline phosphatase (ALP), bovine serum albumin (BSA), lysozyme, pepsin from porcine gastric mucosa, thrombin from bovine plasma, and glucose (stock solutions: [trypsin] = [BAS] = [lysozyme] = [pepsin] = [trhombin] = 1.0 mg mL^{-1} in H_2O; [ALP] = 3.0 U L^{-1} in H_2O; [glugose] = 3.0 mM in H_2O) were tested to evaluate the selectivity of **1**-protamine complexes. To obtain clear SEM patterns, **1**-protamine complex was filtered using a hydrophilic syringe filter (0.45 μm) to remove large particles. The films of the **1**-protamine complex were prepared by drop-casting onto the glass. Then, the films were sufficiently washed in a deionized water bath and dried at 40 °C for 24 hours to remove residual water.

Fluorescence quantum yield was determined relative to known standards (9,10-diphenylanthracene, Φ_{FL}=0.95 in cyclohexane).

Synthesis

4,4',4'',4'''-(Ethene-1,1,2,2-tetrayl)tetraphenol (3). 4,4-Dihydroxybenzo-phenone (**2**) (2.00 g, 9.34 mmol) was dissolved in 50 mL of THF. The resulting solution was deoxygenated with argon for 15 minutes. Then $TiCl_4$ (7.09 g, 37.36 mmol) and zinc powder (4.89 g, 74.72 mmol) were added to the stirred solution under the protection of argon. The reaction mixture was then heated up to 80 °C and stirred for 16 hours. The excess zinc residue was removed by flash chromatography using THF as a solvent and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using ethyl acetate:hexane (1:3) as the eluent Yield: 47%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.22 (S, 4H), 6.71 (d, 8H), 6.49 (d, 8H) ppm.; ^{13}C NMR (100 MHz, DMSO- d_6): δ 155.35, 137.68, 135.05, 131.95, 114.49 ppm.

Compound 4. To a solution of compound **3** (1.00 g, 2.52 mmol) in dried CH_3CN (30 mL), anhydrous K_2CO^3 (1.04 g, 7.56 mmol) was added. After stirring for 5 minutes, *tert*-butyl 2-bromoacetate (2.21 g, 11.34 mmol) was added to the reaction mixture. The resulting mixture was

vigorously stirred at 80 °C for 24 hours under argon gas. After the reaction mixture was allowed to r.t., the solvent was removed *in vacuo*. The reaction mixture was acidified with water and then extracted with ethyl acetate (200 mL). The organic layer was separated, washed with water (150 mL), and dried over anhydrous $MgSO_4$, and the solvent was evaporated to yield a yellow solid. The pure product was isolated by column chromatography on silica gel using ethyl acetate:hexane (1:4) as the eluent. Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.85 (d, 8H), 6.66 (d, 8H), 4.55 (s, 8H), 1.39 (s, 36H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.80, 155.94, 138.11, 136.64, 131.85, 113.79, 81.32, 64.94, 27.64 ppm; ESI(+)-MS (m/z): [M^+] calcd. for $C_{50}H_{60}O_{12}$, 852.4085; found, 852.4080.

Compound 5. Compound **4** (0.50 g, 0.59 mmol) was dissolved in 10 mL of dichloromethane and cooled in an ice/water bath. Then, trifluoroacetic acid (TFA, 0.18 mL) was added dropwise to the solution. Once the addition was completed, the reaction mixture was allowed to warm to room temperature and stirred for another 10 hours. The residual TFA and solvent were removed *in vacuo*, and the resulting solid was washed three times with dichloromethane. A white solid powder was collected by filtration. Yield: 72%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.95 (s, 4H), 6.85 (d, 8H), 6.68 (d, 8H), 4.58 (s, 8H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.14, 156.01, 138.09, 136.65, 131.93, 113.80, 64.41 ppm; ESI(+)-MS (m/z): [M^+] calcd. for $C_{34}H_{28}O_{12}$, 628.1581; found, 628.1578.

Compound 1. Compound **5** (0.30 g, 0.48 mmol) was treated with aqueous NaOH (0.02 g, 0.53 mmol) solution (2 mL) and stirred at room temperature for 1 hour. The solution was then poured into 50 mL of methanol, and the formed TPE precipitate was collected by filtration to obtain a white solid powder. Yield: 91%; ^{1}H NMR (400 MHz, methanol-d_4): δ 6.90 (d, 8H), 6.70 (d, 8H), 4.47 (S, 8H) ppm.; ^{13}C NMR (100 MHz, methanol-d_4): δ 177.11, 157.78, 139.84, 138.33, 135.45, 114.89, 68.04 ppm; ESI(+)-MS (m/z): [M^+] calcd. for $C_{34}H_{25}Na_4O_{12}$, 717.0937; found, 717.0935.

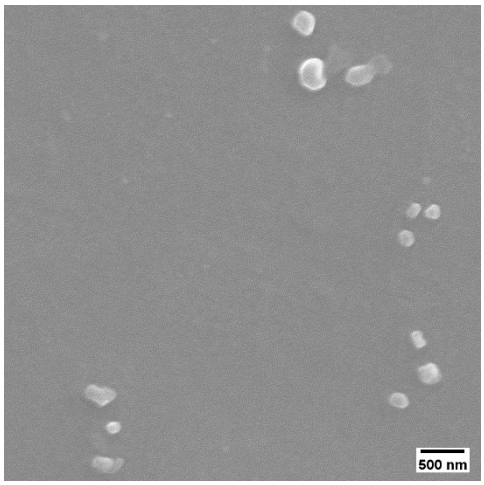


Figure S1. SEM image of **1** titrated with protamine on a glass (scale bars, 500 nm); average size is 198.2 nm. Conditions: [**1**] = 5.0×10^{-6} M and [protamine] = 5.0 μg mL^{-1}.

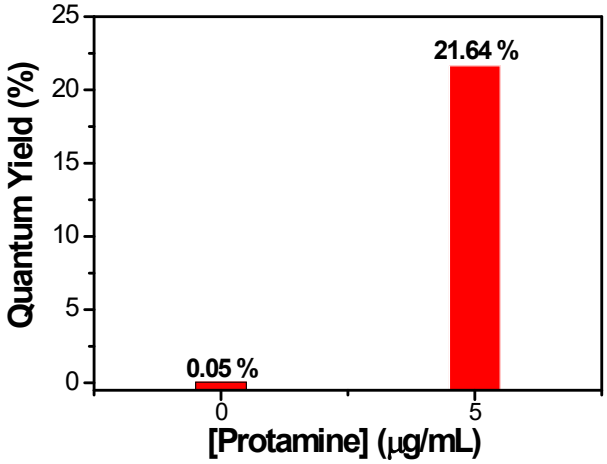


Figure S2. Fluorescence quantum yield of **1** (5.0×10^{-6} M) upon the addition of protamine (0 and 5 μg mL^{-1}) in Tris-HCl buffer (10 mM).

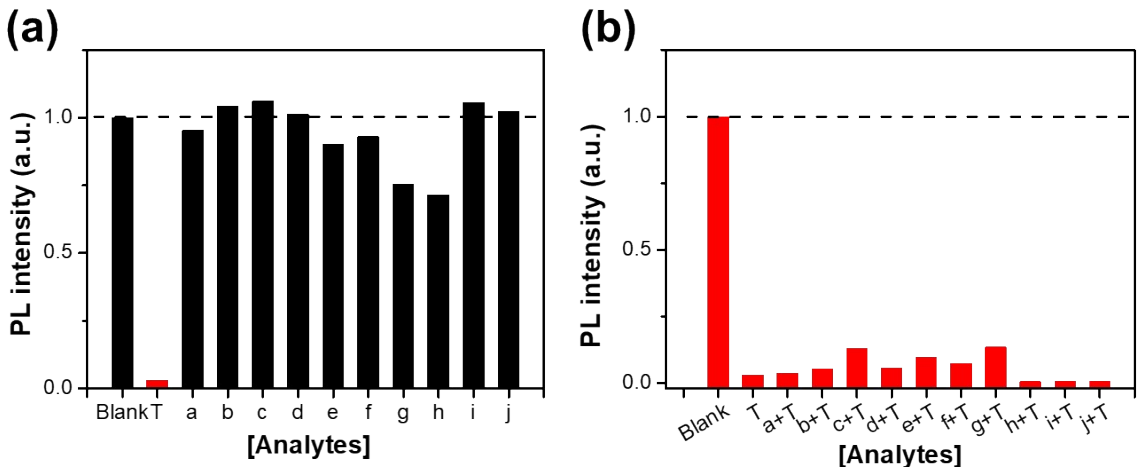


Figure S3. (a) Fluorescence intensity changes observed when the compound **1**-protamine complex was incubated with various competitive analytes; (b) fluorescence intensity changes of the complex to trypsin in the presence of competitive materials; T: trypsin, a: tryptophan, b: phenylalanine, c: cysteine, d: Na_2SO_4, e: NaCl, f: KCl, g: $MgCl_2$, h: $CaCl_2$, i: β–nicotinamide adenine dinucleotide, and j: urea. Conditions: Tris-HCl buffer (10 mM, pH 7.5); [**1**] = 5.0×10^{-6} M, [protamine] = 5.0 μg mL^{-1}, [trypsin] = 1.0 μg mL^{-1}, [a~c] = 1.0×10^{-4} M, [d~j] = 100.0 μg mL^{-1}; excitation wavelength was performed at 320 nm and emission was monitored at 479 nm.

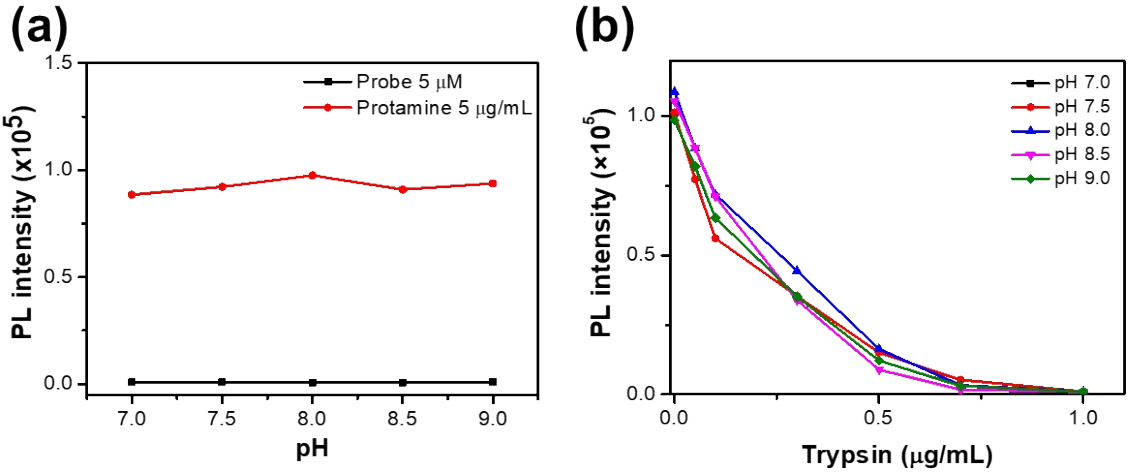
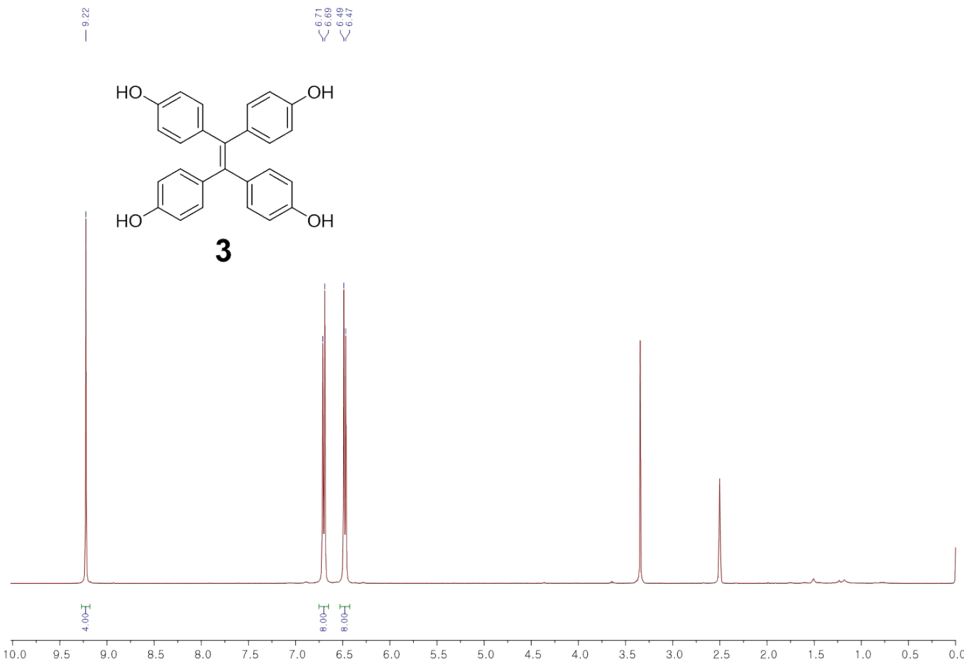
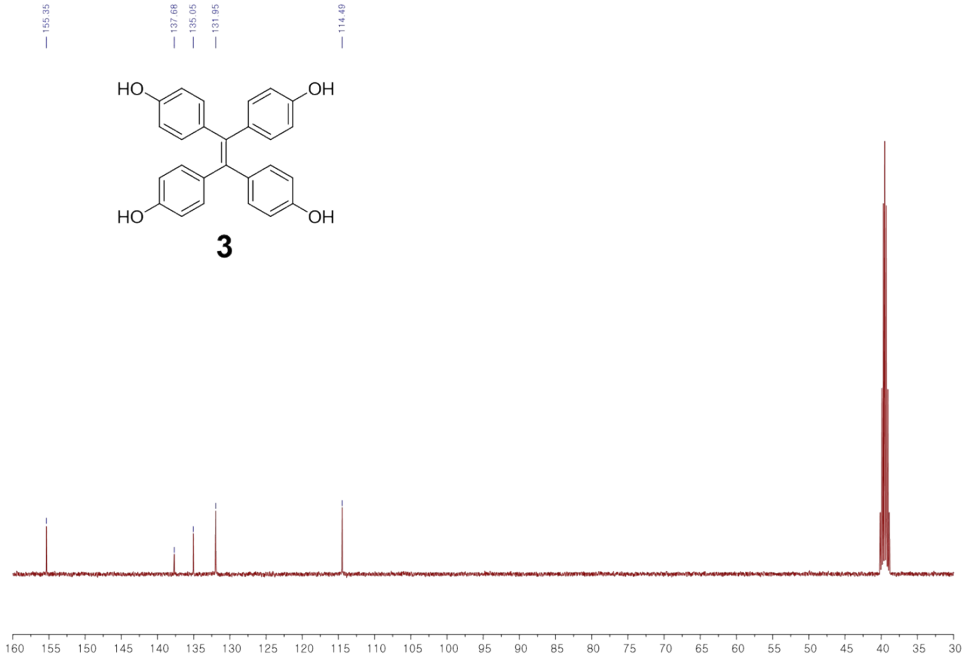


Figure S4. Fluorescence emission changes of **1** upon the addition of protamine at various pH (pH = 7.0, 7.5, 8.0, 8.5, and 9.0) in Tris-HCl buffer (10 mM); (b) Fluorescence intensity changes of the **1**-protamine complex when incubated with different concentrations of trypsin at various pHs (pH = 7.0, 7.5, 8.0, 8.5, and 9.0). Conditions: [**1**] = 5.0×10^{-6} M, [Protamine] = 5 μg mL^{-1}; excitation was performed at 320 nm and emission was monitored at 479 nm.

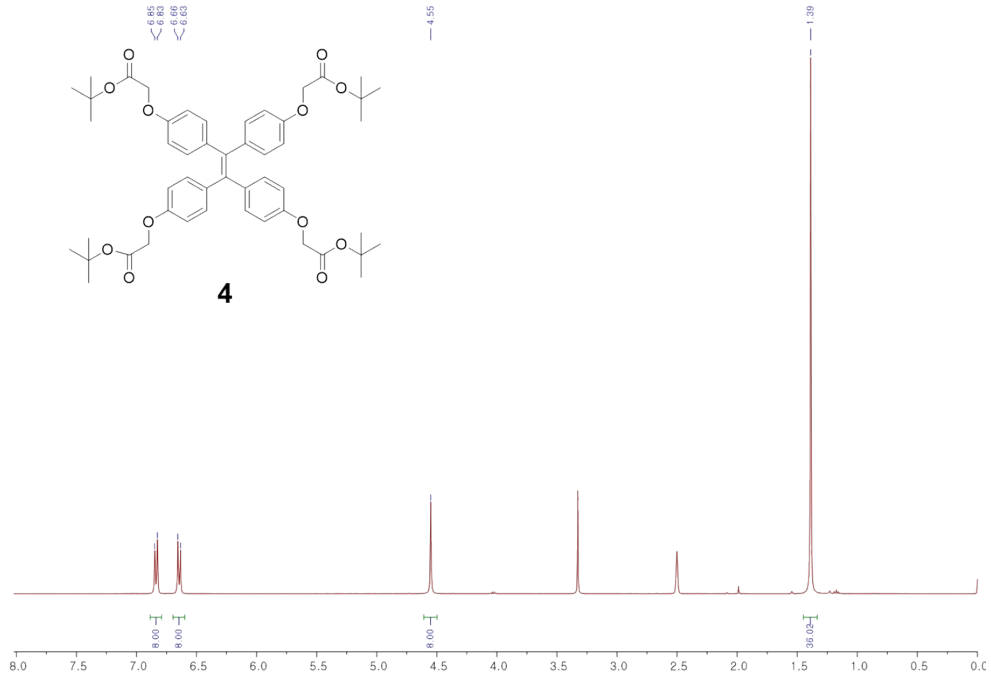
Appendix



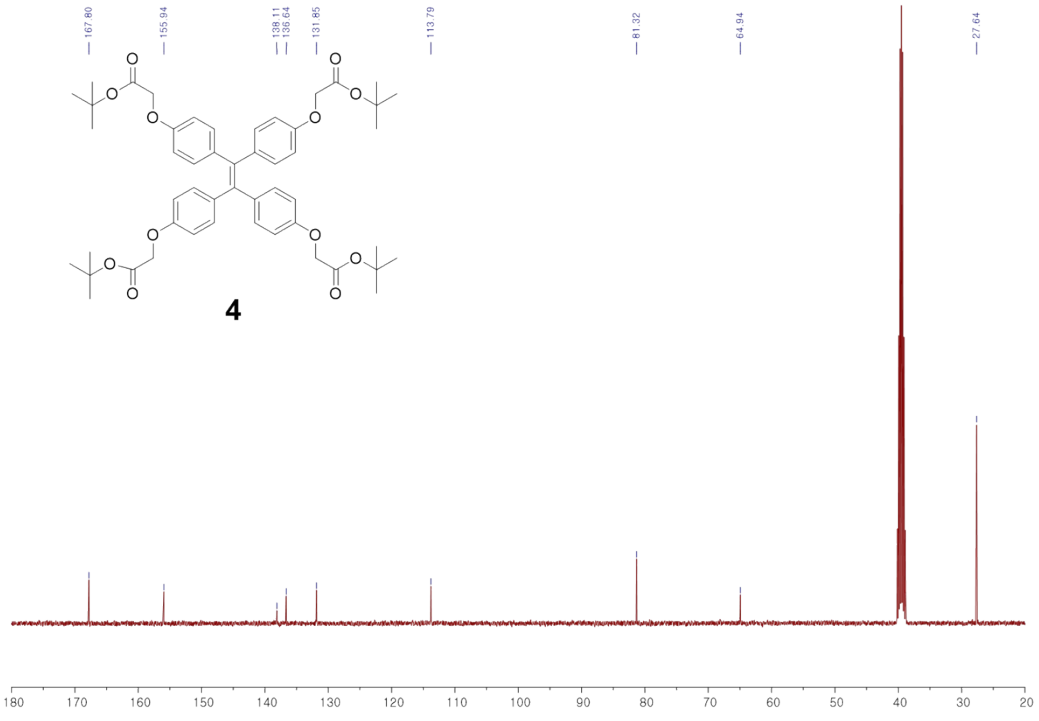
^{1}H NMR spectrum of **3** in DMSO-d_6



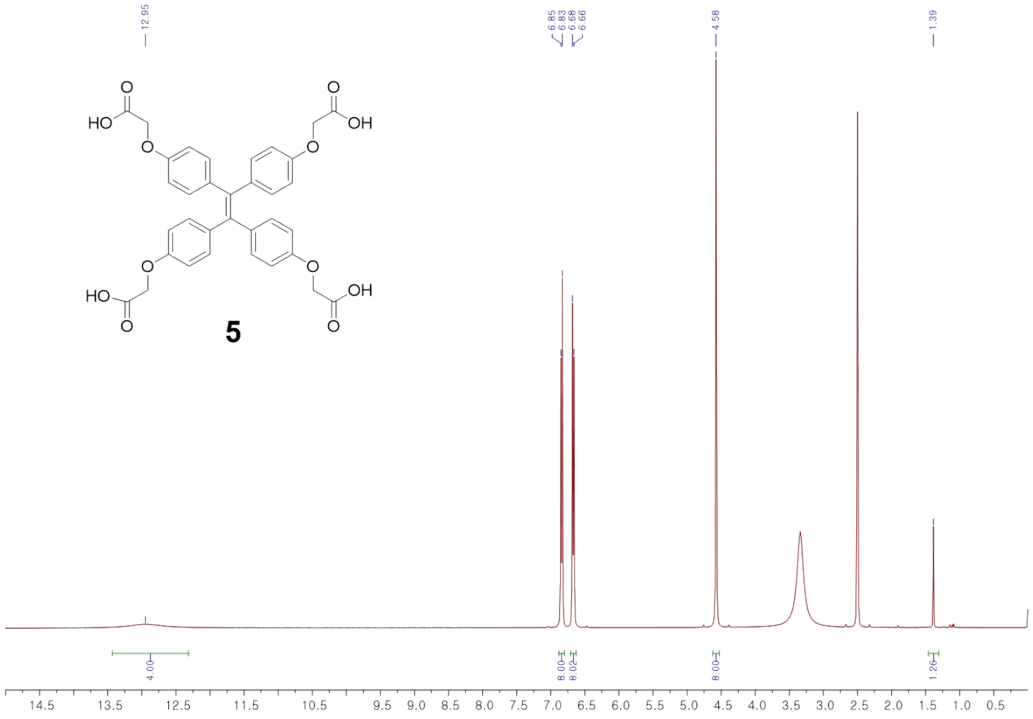
^{13}C NMR spectrum of **3** in DMSO-d_6



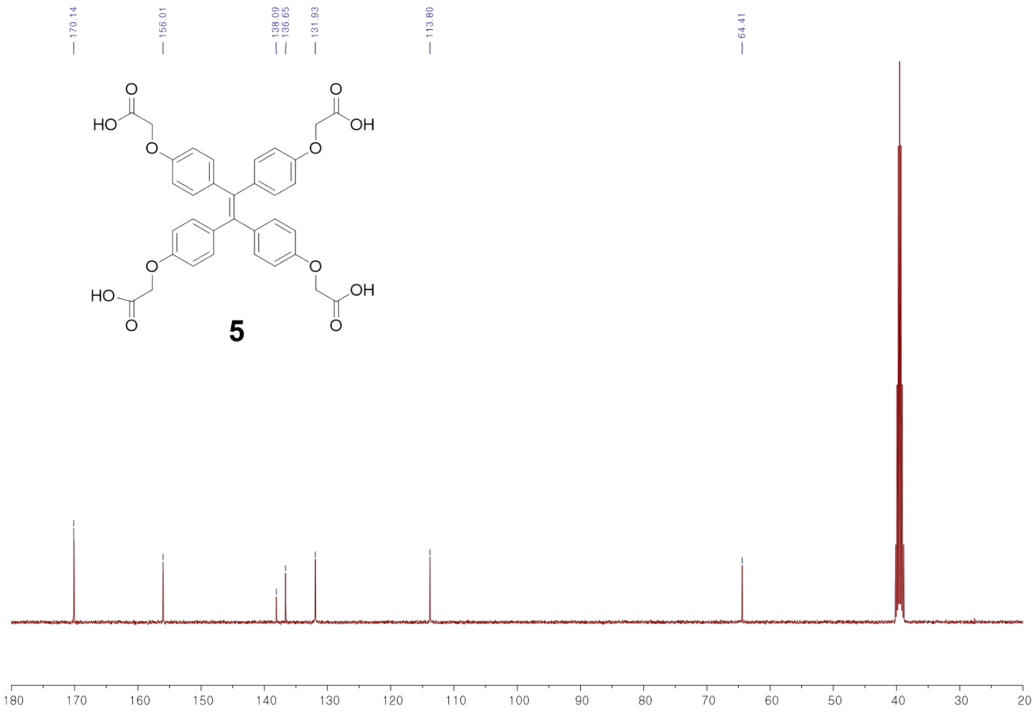
^{1}H NMR spectrum of **4** in DMSO-d_6



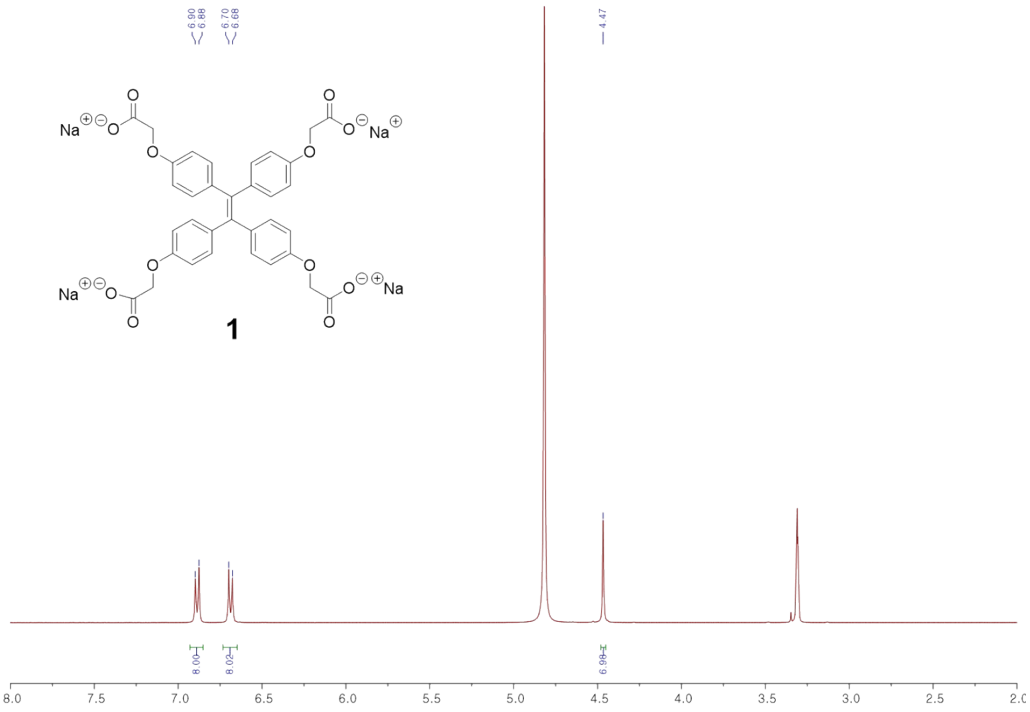
^{13}C NMR spectrum of **4** in DMSO-d_6



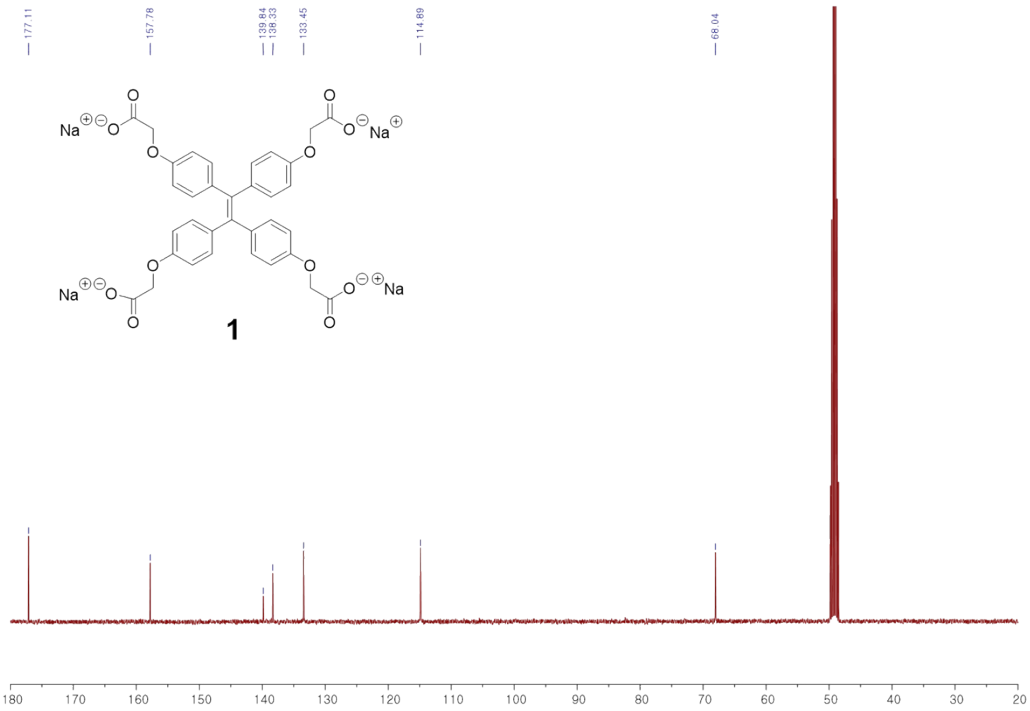
^{1}H NMR spectrum of **5** in DMSO-d_6



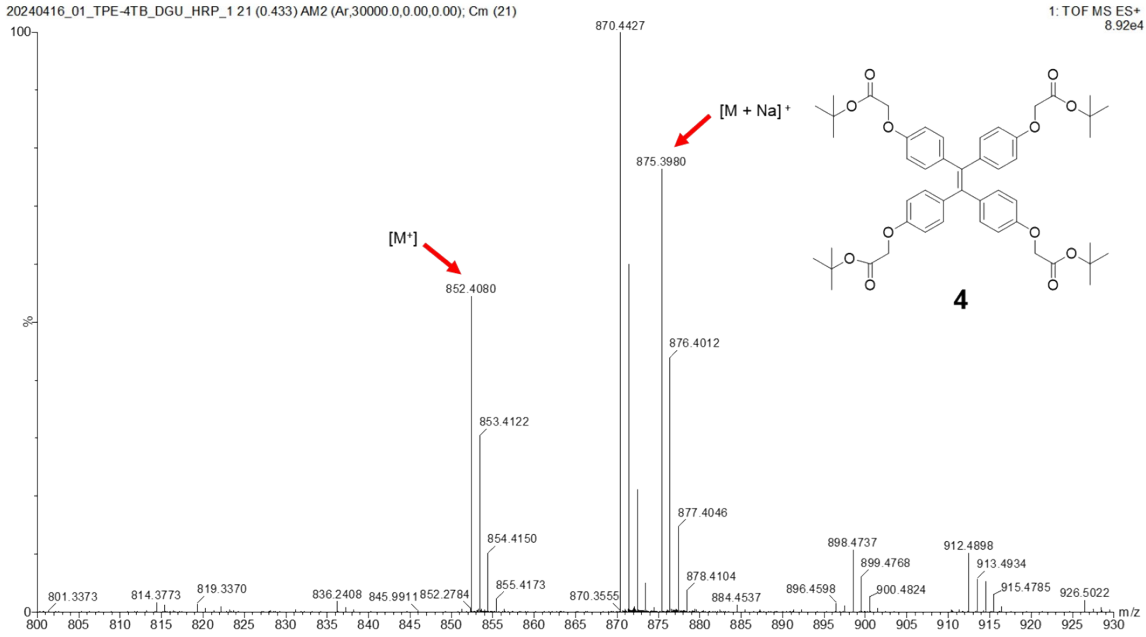
^{13}C NMR spectrum of **5** in DMSO-d_6



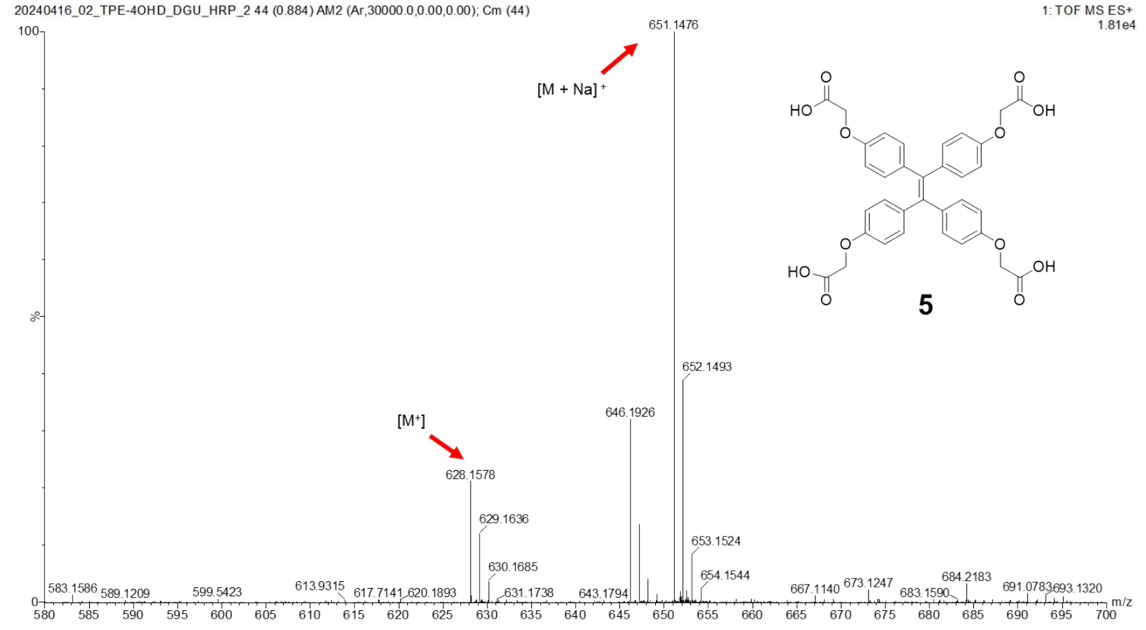
^{1}H NMR spectrum of **1** in Methanol-d_4



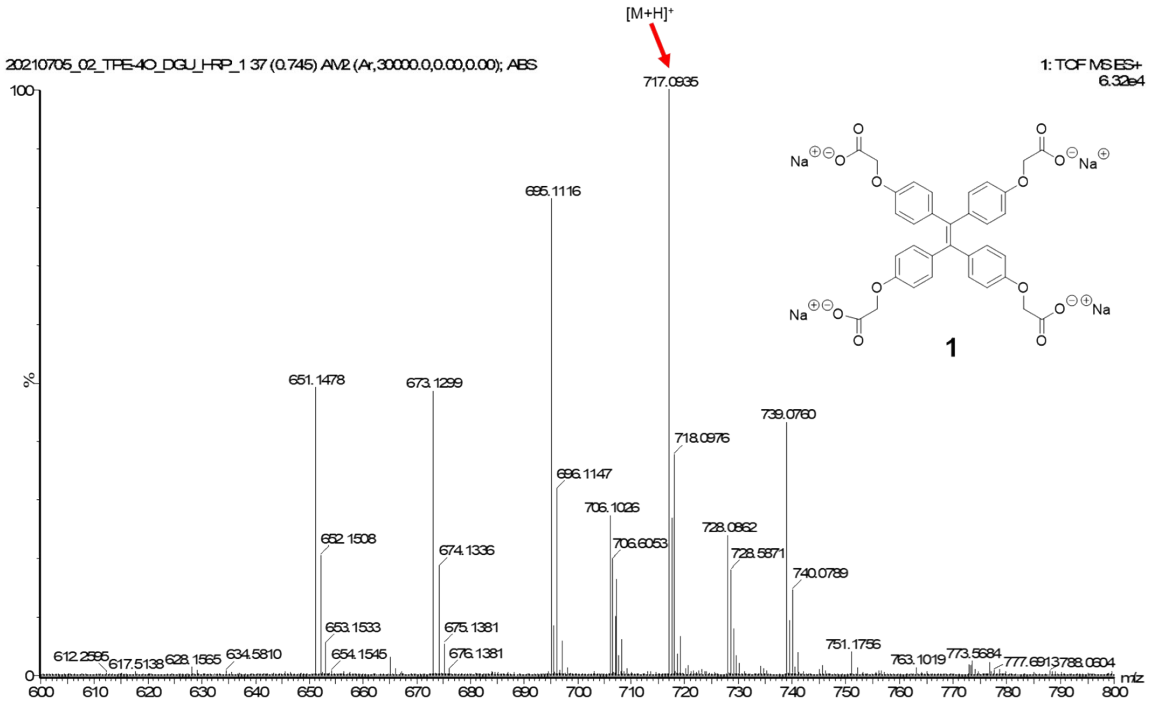
^{13}C NMR spectrum of **1** in Methanol-d_4



ESI Mass spectrum of **4**



ESI Mass spectrum of **5**



ESI Mass spectrum of **1**