A selective fluorescent turn-on NIR probe for cysteine†

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1 General

^{1}H NMR spectra were recorded on a VARIAN Mercury 400 MHz spectrometer. ^{1}H NMR chemical shifts (δ) are given in ppm downfield from Me_4Si, determined by chloroform ($\delta = 7.26$ ppm) and dimethyl sulfoxide ($\delta = 2.5$ ppm). ^{13}C NMR spectra were recorded on a VARIAN Mercury 100 MHz spectrometer. ^{13}C NMR chemical shifts (δ) are reported in ppm with the internal $CDCl_3$ and d_6-DMSO at δ 77.0 and 39.4 ppm as standard, respectively. Mass spectrometric measurements were performed by the mass spectrometry service of the ETHZ on a Bruker Reflex MALDI as matrix (20 kV). The refractive index of the medium was measured by 2 W Abbe's refractometer at 20 °C.

Tetrahydrofuran (THF) was freshly distilled from Na/benzophenone, *n*-hexane was distilled over Na, and other solvents were distilled over CaH_2. Merck silica gel 60 was used for the column chromatography.

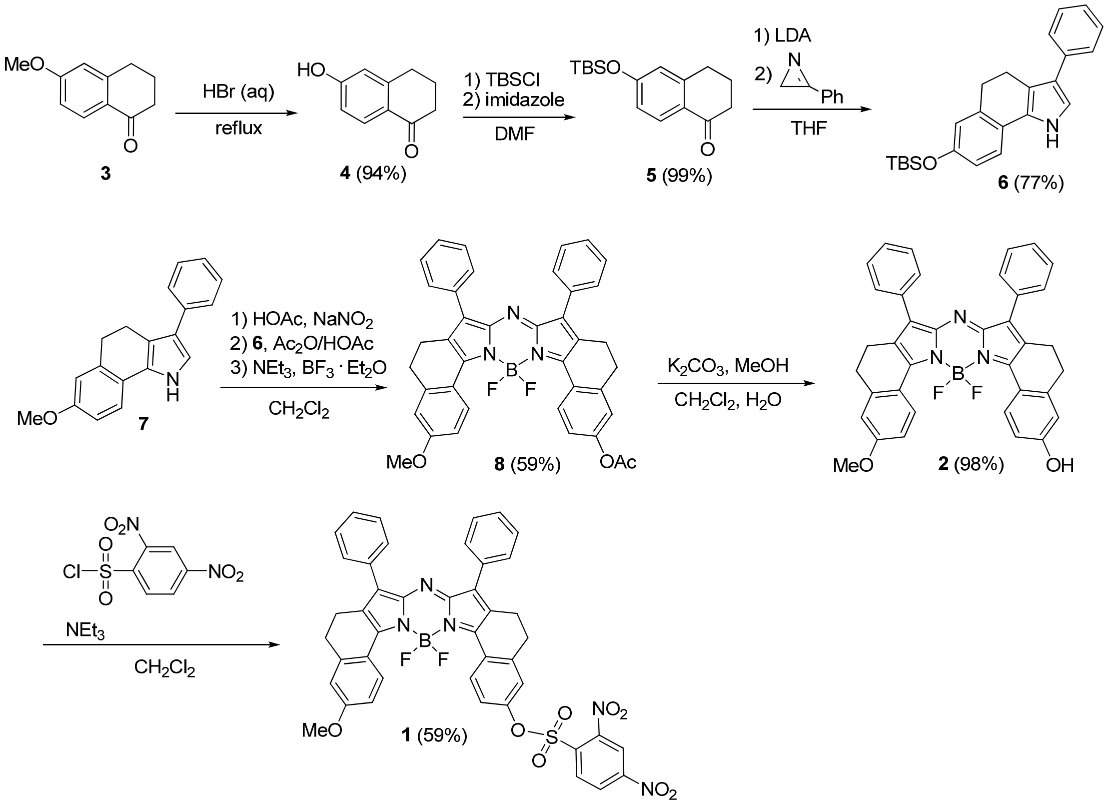
Fluorescence spectra were recorded on FluoroSENS spectrophotometer. UV/Vis spectra were recorded on Perkin-Elmer Lambda 35 UV/Vis spectrophotometer at room temperature.

The fluorescence quantum yields (Φ_f) of the BODIPY systems were calculated using the following relationship (equation 1):

$$\Phi_f = \Phi_{ref} F_{sampl} A_{ref} n^2_{sampl} / F_{ref} A_{sampl} n^2_{ref} \qquad (1)$$

Here, F denotes the integral of the corrected fluorescence spectrum, A is the absorbance at the excitation wavelength, and n is the refractive index of the medium (n = 1.4555 in $CHCl_3$; n = 1.3648 in $MeCN/H_2O/DMSO$ (v/v/v = 79/20/1)), ref and sampl denote parameters from the reference and unknown experimental samples, respectively. The reference systems used was boronazadipyrromethene compound aza-BODIPY ($\Phi_f = 0.36$ in chloroform).[1]

2 Synthesis



6-Hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (4). Compound **3** (5.0 g, 28.3 mmol) was added to 25 mL 47% hydrobromic acid solution and refluxed for 48 h. The reaction mixture was diluted with water (200 mL). A solid was precipitated, filtered, and washed with water until neutrality. After washing with *n*-hexane, the residue was purified by chromatography followed by recrystallization from CH_2Cl_2/*n*-hexane to afford **4** (4.32 g, 26.6 mmol, 94%).

6-(tert-Butyldimethylsilyloxy)-3,4-dihydronaphthalen-1(2*H*)-one (5). Compound **4** (4.0 g, 24.6 mmol) was added to imidazole (6.8 g, 100 mmol) and *tert*-butyl dimethyl chlorosilane (5.0 g, 33 mmol) in DMF (50 mL) and stirred overnight at room temperature. The mixture was extracted with MeCOOEt (2 × 60 mL), and the organic layer was washed with brine (2 × 50 mL) and dried over anhydrous $MgSO_4$. After removing the solvents by evaporation, the resulting crude mixture was separated by column chromatography (*n*-hexane : CH_2Cl_2 = 1 : 1) to afford **5** (6.72 g, 24.3 mmol, 99%) as yellow solid. **5**: ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.95 (d, *J* = 11.2 Hz, 1H), 6.74 (dd, *J* = 11.2, 3.2 Hz, 1H), 6.68–6.64 (m, 1H), 2.89 (t, *J* = 8.0 Hz, 2H), 2.60 (t, *J* = 8.0, Hz, 2H), 2.11 (quintet, *J* = 8.0 Hz, 2H), 0.99 (s, 9H), 0.24 (s, 6H).

7-(tert-Butyldimethylsilyloxy)-3-phenyl-4,5-dihydro-1H-benzo[g]indole (6). Under N_2, LDA (1.01 mL, 2.02 mmol) in THF (6 mL) was added to **5** (552.8 mg, 2.0 mmol) in THF (20 mL) at −78 °C. Then, 3-phenyl-2*H*-azirine (240.1 mg, 2.05 mmol) in THF (2 mL) was added and the resulting mixture was stirred for 2 h at the same temperature. The reaction was allowed to warm up to room temperature slowly. It was quenched with water, neutralized with dilute HCl to a pH about 7. The mixture was extracted with CH_2Cl_2 (2 × 60 mL), and the organic layer was washed with brine (2 × 50 mL) and dried over anhydrous $MgSO_4$. After removing the solvents by evaporation, the resulting crude mixture was separated by column chromatography (*n*-hexane : CH_2Cl_2 = 1 : 1) to afford **6** (578.3 mg, 1.54 mmol, 77%) as pale green solid. **6**: ^{1}H NMR (400 MHz , $CDCl_3$): δ (ppm) = 8.27 (br s, 1H), 7.47 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.26 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 2.4 Hz, 1H), 6.78 (s, 1H), 6.68 (dd, *J* = 8.0, 2.4 Hz, 1H), 2.93 (s, 4H), 1.03 (s, 9H), 0.24 (s, 6H).

Aza-BODIPY dye (8). Sodium nitrite (6.9 mg, 0.1 mmol) was added at 0 °C with stirring to a suspension of **7** (27.5 mg, 0.1 mmol) in acetic acid (1 mL), and was stirred for 10 min. The color changed from colorless to brown, then green, and finally brown was observed. The second pyrrole moiety **6** (37.5 mg, 0.1 mmol) was added, followed by addition of acetic anhydride (0.4 mL). The mixture turned blue immediately. After 0.5 h stirring, the mixture was heated at 80 °C for 0.5 h. Crushed ice was added to the cold reaction mixture, the resulted blue dye was filtered, washed with water. The blue dye was dissolved in CH_2Cl_2, filtered through a pad of alumina (activity III) and washed with CH_2Cl_2. Solvent was removed under reduced pressure, the residue was dissolved in dry 1,2-dichloroethane, triethylamine (0.24 mL) was added, followed by dropwise addition of $BF_3{\cdot}Et_2O$ (0.24 mL) with stirring at room temperature. The mixture was stirred for 0.5 h, then heated in 80 °C oil bath for 0.5 h, and was cooled down. The reaction was quenched with crushed ice, extracted with CH_2Cl_2, and was purified by chromatography on silica gel followed by recrystallization from CH_2Cl_2/*n*-hexane to afford **8** (37.9 mg, 0.0594 mmol, 59%) as coppery solid. **8**: ^1H NMR(400 MHz, $CDCl_3$): δ (ppm) = 8.82 (d, J = 8.8 Hz, 1H), 8.78 (d, J = 8.8 Hz, 1H), 7.70 (t, J = 6.8 Hz, 4H), 7.45–7.35 (m, 6H), 7.18 (dd, J = 8.8, 2.4 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 7.03 (dd, J = 8.8, 2.4 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 3.92 (s, 3H), 2.93 (s, 8H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 169.1, 162.5, 154.4, 151.8, 149.4, 146.8, 145.1, 144.8, 142.7, 138.0, 136.3, 132.4, 131.8, 130.3, 129.8, 128.4, 128.1, 128.0, 127.9, 125.7, 121.4, 120.6, 120.2, 114.6, 113.2, 55.5, 30.5, 30.4, 21.9, 21.5, 21.2. HRMS-MALDI (m/z): $[M + H]^+$ Calcd. For $C_{39}H_{31}BF_2N_3O_3$: 638.2426; found: 638.2421.

Aza-BODIPY dye (2). Compound **8** (24.7 mg, 0.0387 mmol) was added to K_2CO_3 (20 mg, 0.14 mmol) in a solution of CH_2Cl_2 (10 mL), MeOH (4 mL) and water (0.4 mL) at room temperature and stirred overnight. After removing the solvents by evaporation, the crude solid was dissolved in water (20 ml), and neutralized with dilute NaH_2PO_4 to a pH about 5. The solution was stirred until the solid was formed, and the precipitated solid was filtered and washed. The resulting crude mixture was purified by chromatography on silica gel followed by recrystallization from CH_2Cl_2/*n*-hexane to afford **2** (22.6 mg, 0.379 mmol, 98%) as coppery solid. **2**: m.p. > 250 °C. IR (KBr, cm^{-1}): 3448, 2924, 2862, 1603, 1514, 1450, 1394, 1281, 1236, 1186, 1141, 1053, 1028, 983, 774, 700, 624. ^1H NMR (400 MHz, d_6-DMSO): δ (ppm) = 10.6 (br s, 1H), 8.54 (d, J= 9.2 Hz, 1H), 8.51 (d, J= 9.2 Hz, 1H), 7.75–7.70 (m, 4H), 7.53–7.45 (m, 4H), 7.42–7.35 (m, 2H), 7.14 (dd, J = 9.2, 2.8 Hz, 1H), 7.03 (d, J = 2.8 Hz, 1H), 7.93 (dd, J = 9.2, 2.8 Hz, 1H), 6.84 (d, J = 2.8 Hz, 1H), 3.88 (s, 3H), 2.91–2.85 (m, 8H); ^{13}C NMR (100 MHz, d_6-DMSO): δ (ppm) = 161.4, 161.3, 152.1, 149.4, 145.1, 144.8, 144.0, 143.9, 136.2, 131.5, 131.2, 130.6, 130.2, 129.9, 129.8, 128.2, 128.1, 128.0, 119.9, 117.8, 115.8, 114.9, 114.3, 113.1, 55.4, 30.5, 29.5, 20.9, 20.8. HRMS-MALDI (m/z): $[M + H]^+$ Calcd. For $C_{37}H_{29}BF_2N_3O_2$: 596.2320; found:

596.2315.

Aza-BODIPY dye (1). Under N_2, triethylamine (0.1 mL) was added to **2** (59.5 mg, 0.1 mmol) in CH_2Cl_2 (10 mL) at room temperature, and the mixture was stirred for 5 min. A solution of 2,4-dinitrobenzenesulfonyl chloride (80.0 mg, 0.3 mmol) in CH_2Cl_2 (2 mL) was added dropwise at 0 °C. Then, the reaction mixture was stirred for 2 h at 50 °C. After removing the solvents by evaporation, the resulting crude mixture was purified by chromatography on silica gel followed by recrystallization from CH_2Cl_2/*n*-hexane to afford **1** (48.7 mg, 0.059 mmol, 59%) as coppery solid.

m.p. > 250 °C. **1**: IR (KBr, cm^{-1}): 2924, 2857, 1601, 1541, 1512, 1450, 1386, 1342, 1274, 1238, 1188, 1142, 1082, 1056, 1031, 918, 815, 699. ^1H NMR (400 MHz, d_6-DMSO): δ (ppm) = 9.15 (s, 1H), 8.64 (br s, 2H), 8.51 (d, J= 9.2 Hz, 1H), 8.38 (d, J= 9.2 Hz, 1H), 7.96 (s, 1H), 7.73–7.11 (m, 12H), 3.93 (s, 3H), 2.91-2.83 (m, 8H); ^{13}C NMR (100 MHz, d_6-DMSO): δ (ppm) = 163.4, 156.0, 151.4, 148.7, 148.0, 146.6, 145.5, 143.4, 138.3, 134.8, 133.7, 133.4, 131.5, 130.9, 130.6, 130.0, 128.8, 128.2, 128.1, 127.5, 127.2, 121.9, 121.1, 120.4, 118.5, 114.6, 113.9, 55.7, 45.5, 29.3, 29.1, 21.0, 20.3, 8.5, 7.0. HRMS-MALDI (m/z): $[M + Na]^+$ calcd for $C_{43}H_{30}BF_2N_5O_8SNa$: 848.1776; found: 848.1767.

3 Supplementary Figure S1

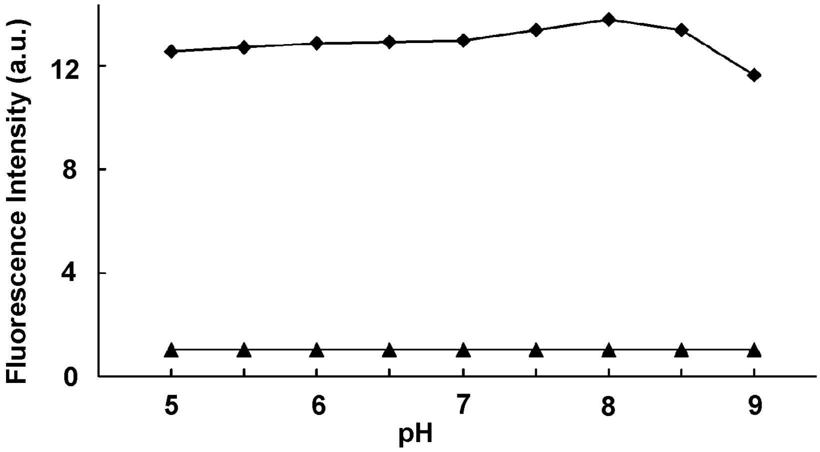
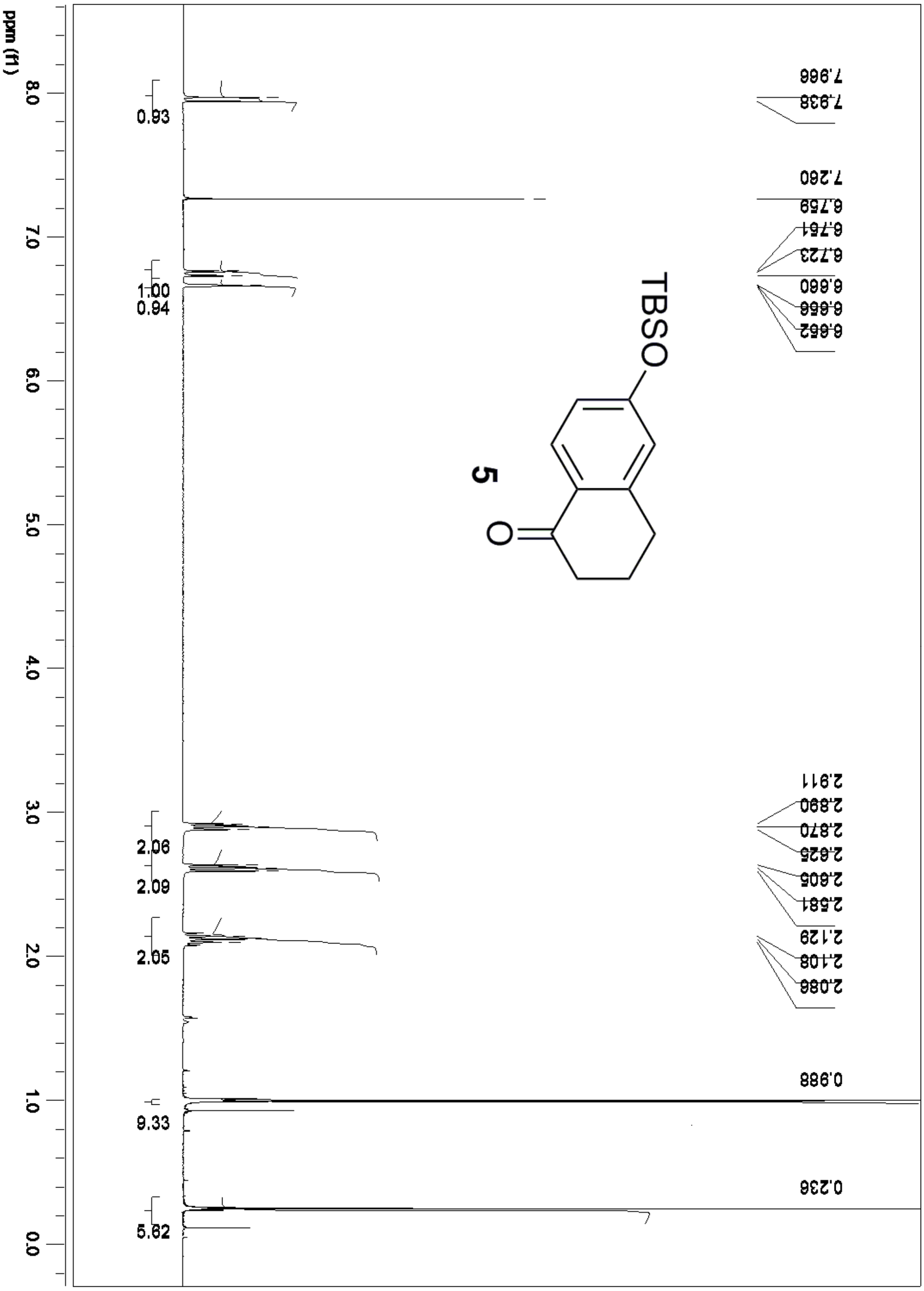
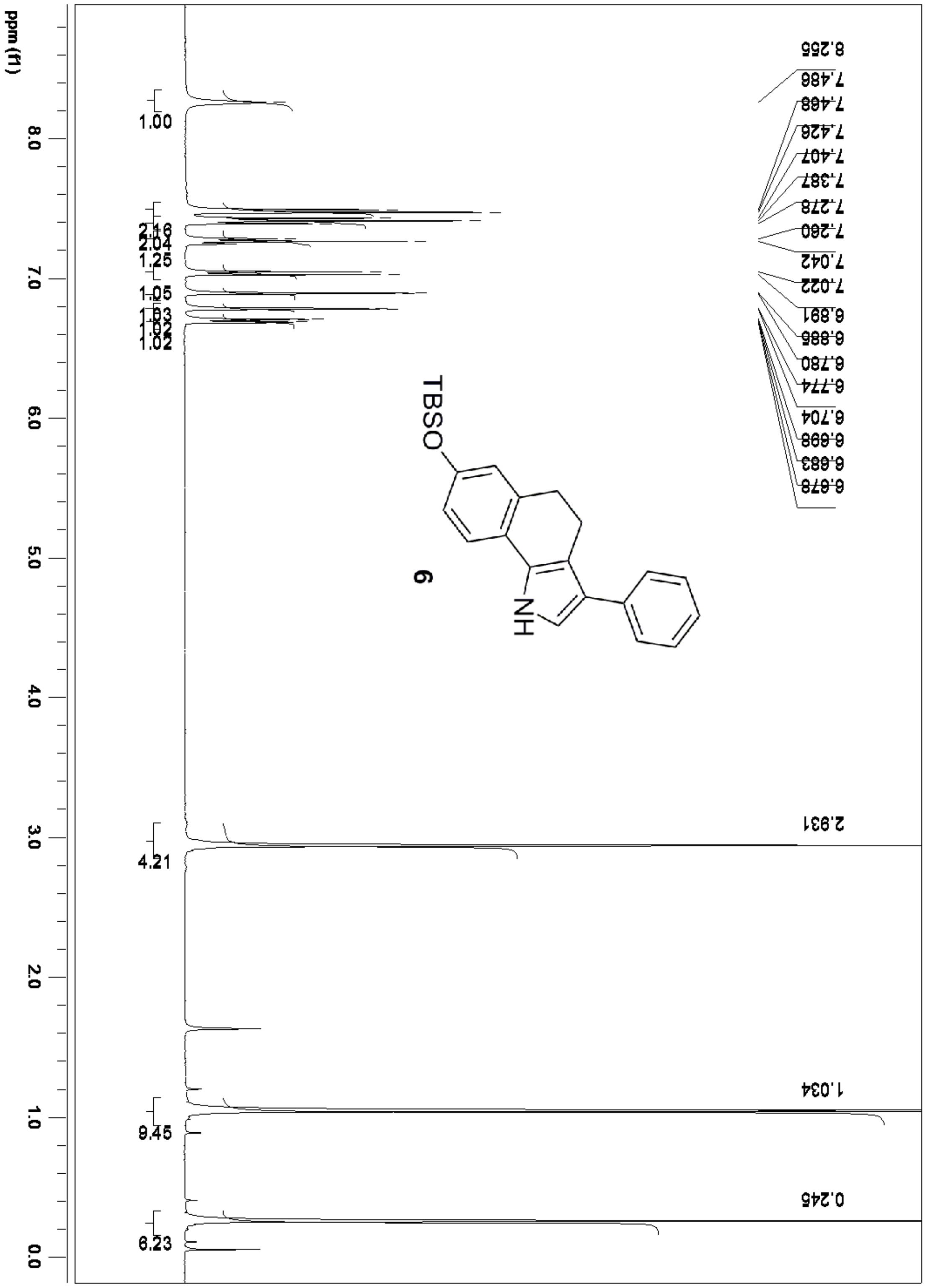


Fig. S1 The response of the emission of 20 μM probe **1** (MeCN/H_2O/DMSO = 79/20/1, v/v/v) before (▲) and after (◆) the addition of 20 mM cysteine in pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, respectively. For the study of its stability, the solutions of **1** at pH 5.0–9.0 were diluted with equal volume of MeCN/H_2O/DMSO (79/20/1, v/v/v; pH 5.0–9.0), respectively. For the examination of dependency of the present fluorometric assay on pH, the solutions of **1** (pH 5.0–9.0) were added equal volume of MeCN/H_2O/DMSO (79/20/1, v/v/v; pH 5.0–9.0) containing 20 mM of cysteine, respectively. The fluorescence intensity at λ_{ex} = 755 nm was plotted before or 1 h after the addition of cysteine at 20 °C. The excitation wavelength was 670 nm.

4 Spectroscopic data

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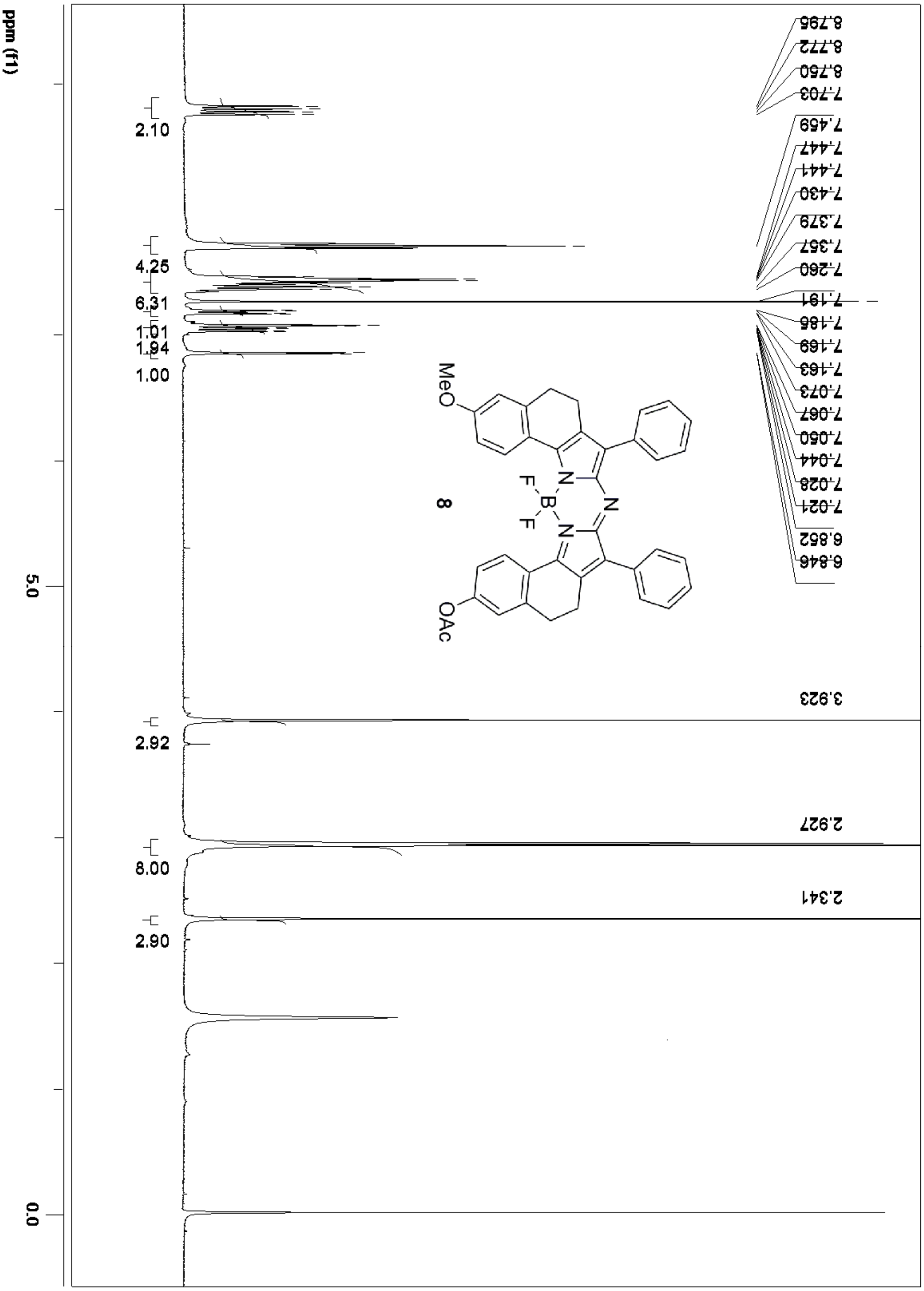
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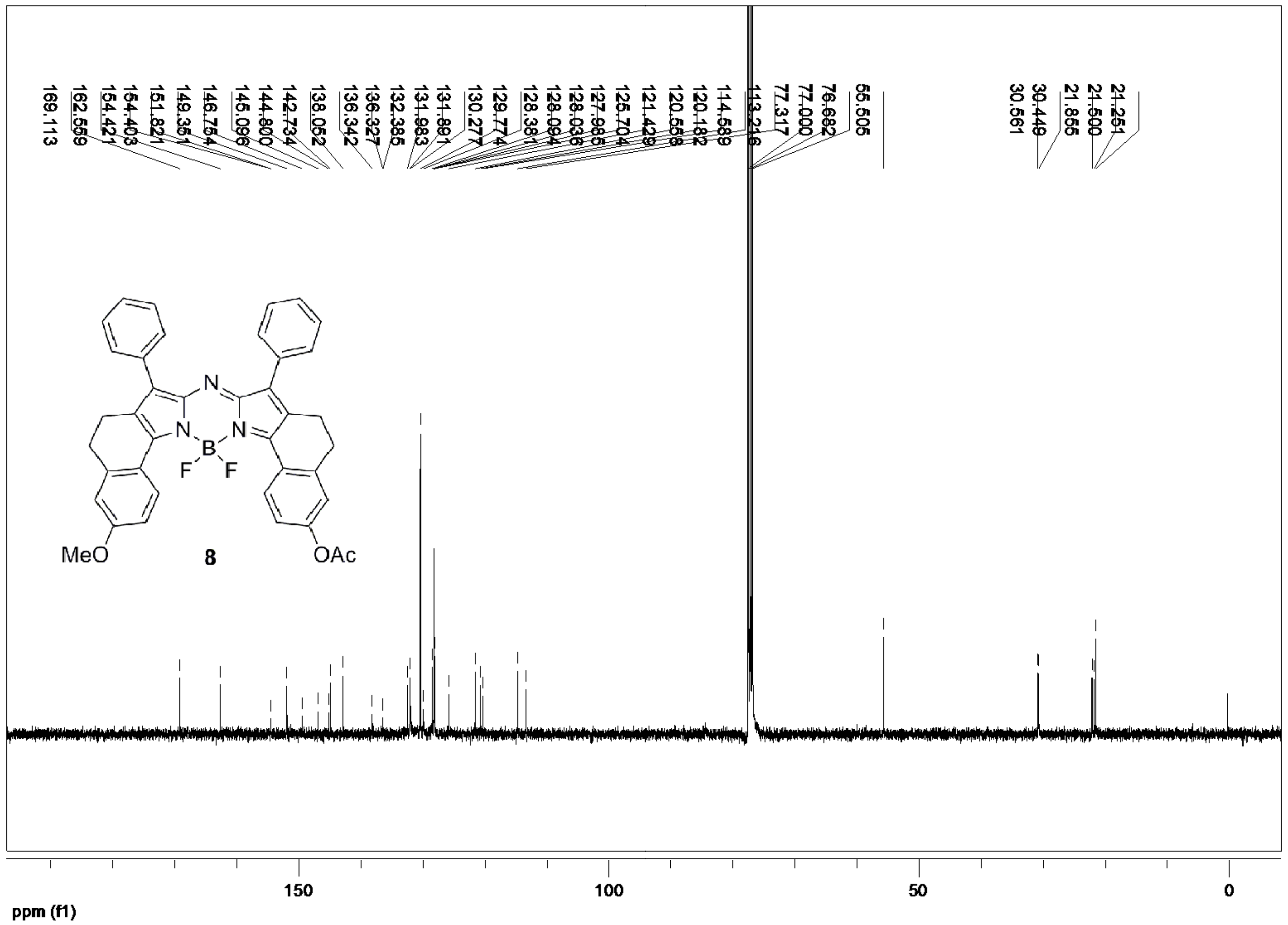
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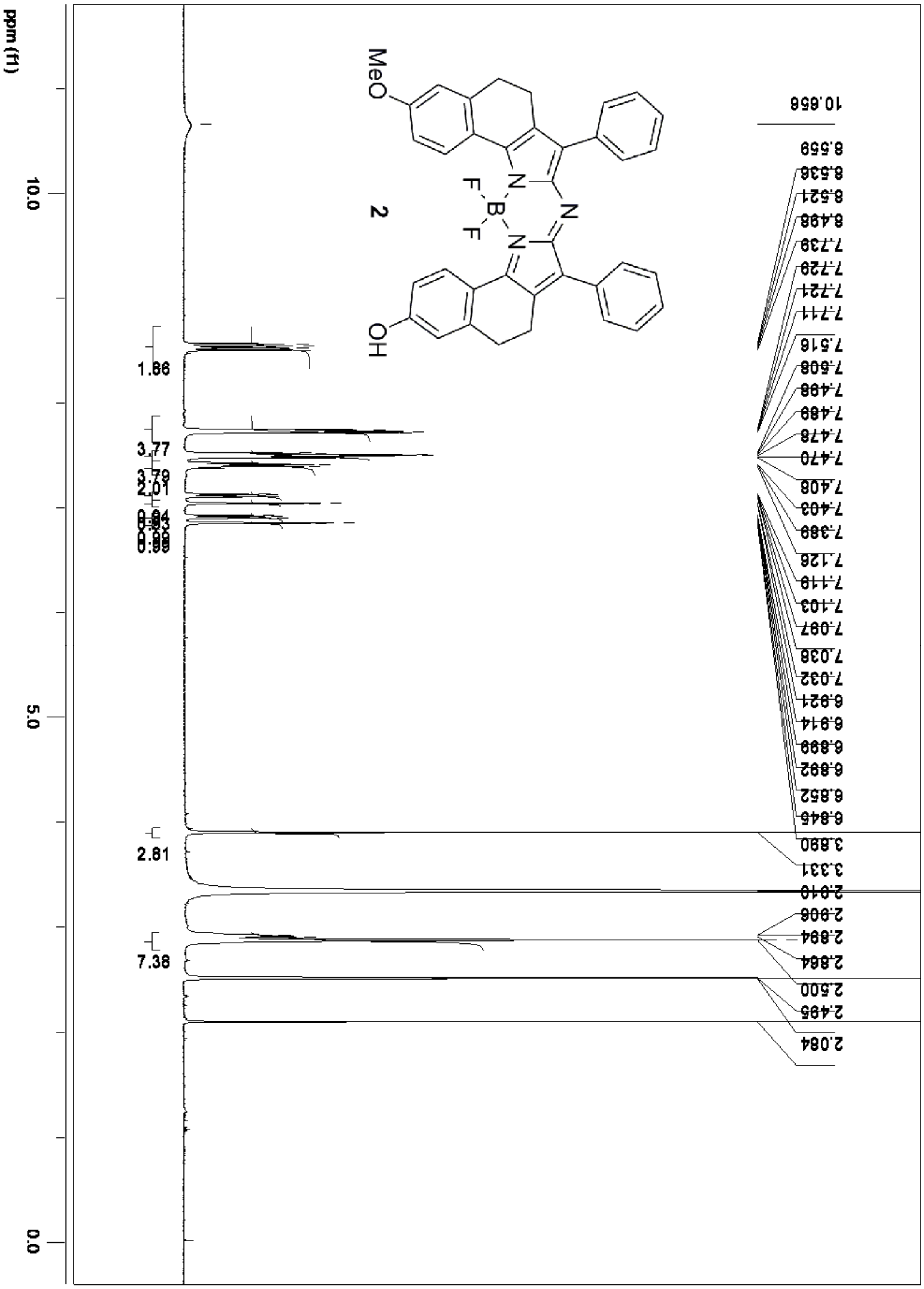
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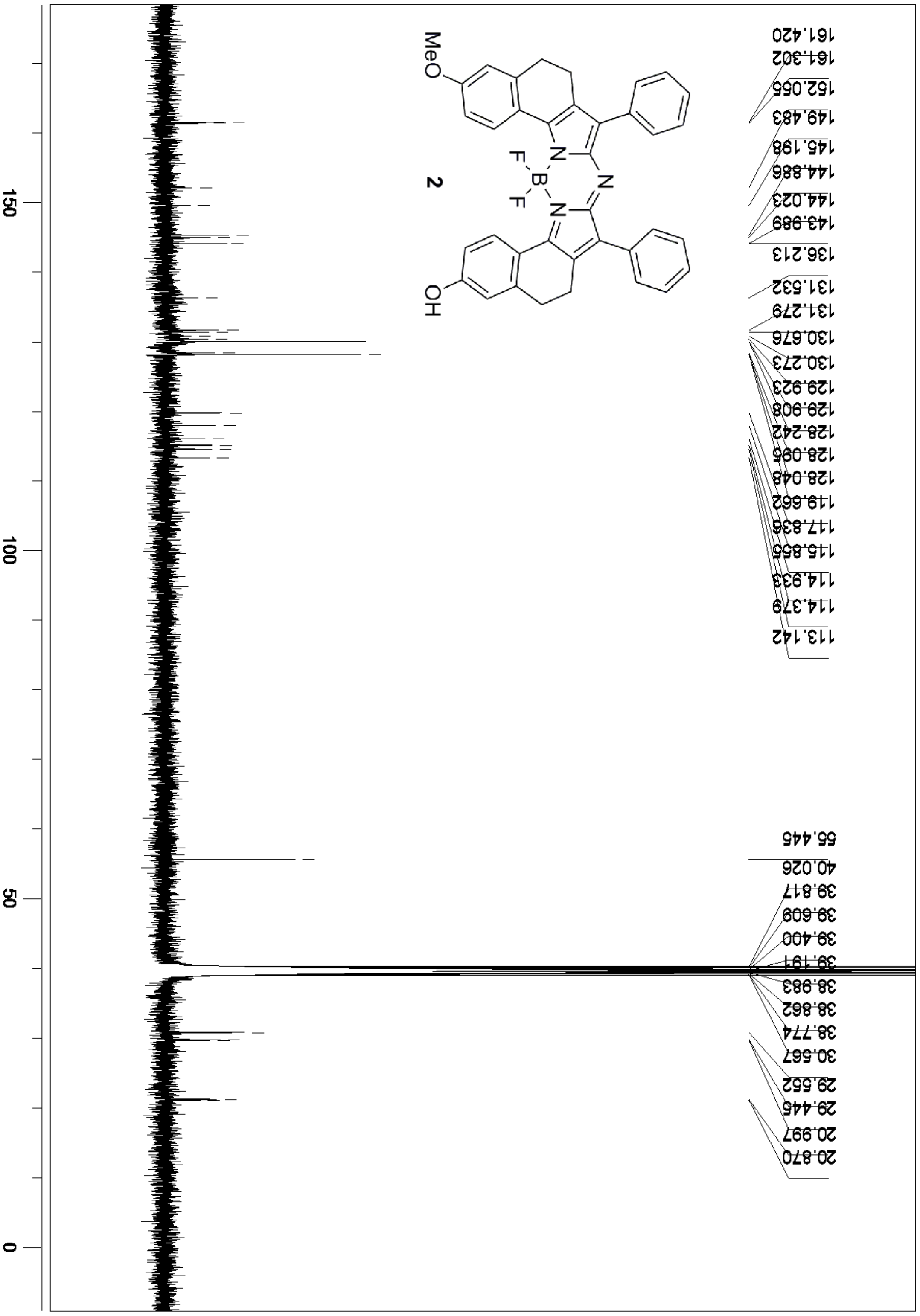
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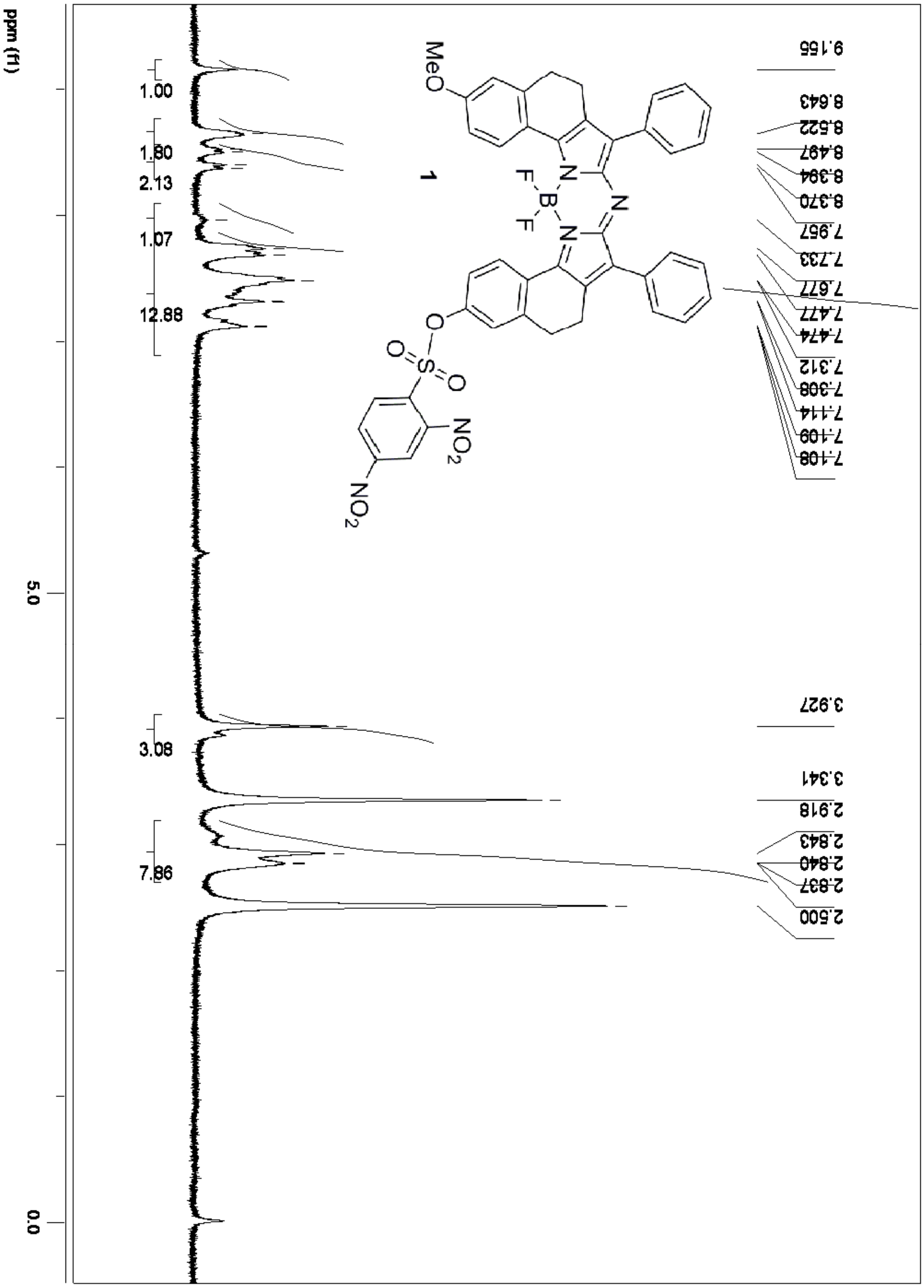
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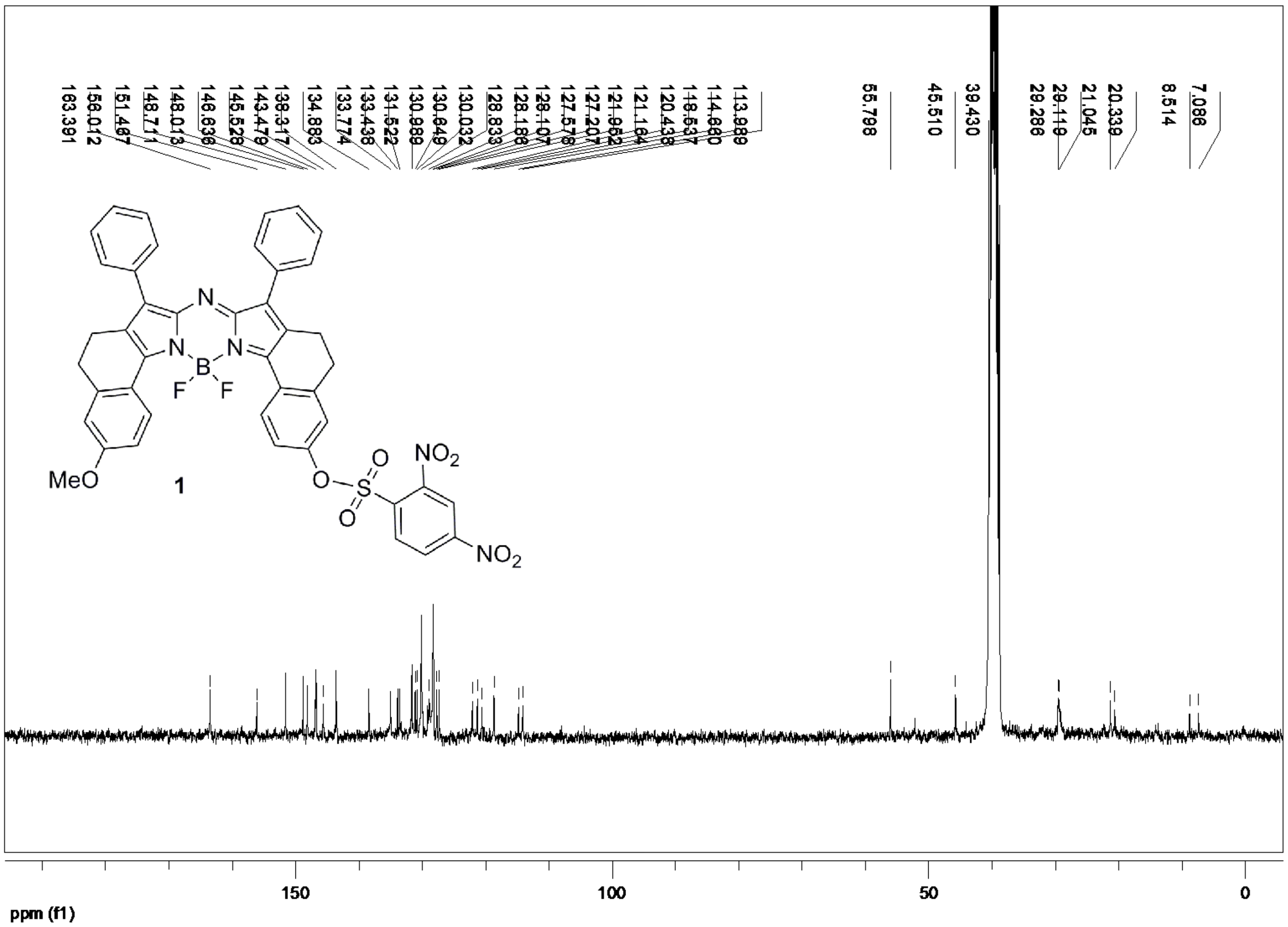
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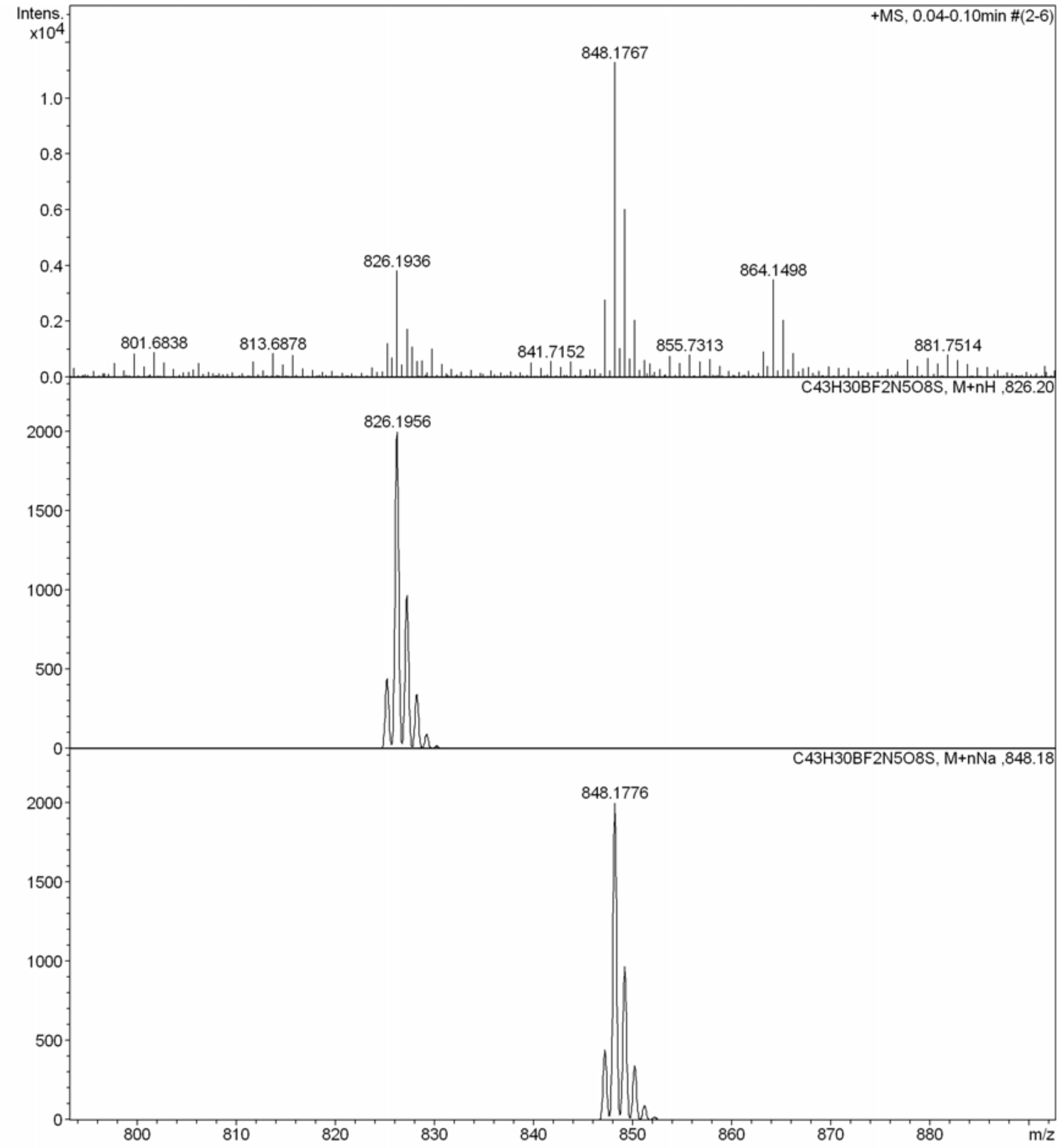
5 HRMS-MALDI of 1



HRMS-MALDI (m/z): $[M + H]^+$ calcd for $C_{43}H_{31}BF_2N_5O_8S$: 826.1956; found: 826.1936; $[M + Na]^+$ calcd for $C_{43}H_{30}BF_2N_5O_8SNa$: 848.1776; found: 848.1767.

6 References

1 A. Gorman, J. Killoran, C. O’Shea, T. Kenna, W. M. Gallagher and D. F. O’Shea, *J. Am. Chem. Soc.*, 2004, **126**, 10619.