Supporting Information

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A fluorescent naphthalenediimide AND logic gate: A Pourbaix Sensor for two protons and one electron

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Experimental

Materials

4-(2-Aminoethyl)morpholine (98%, Tokyo Chemical Industry), 2,6-dibromonaphthalene -1,4,5,8-tetracarboxylic dianhydride (98%, Tokyo Chemical Industry), ferrocene carboxaldehyde (97%, Tokyo Chemical Industry), hydroxylamine (HPLC Grade, Sigma-Aldrich), lithium aluminium hydride (> 95%, Tokyo Chemical Industry), sodium persulfate (>= 96.0%, Fischer Scientific), methanesulfonic acid (> 99.0%, Tokyo Chemical Industry) were used as received. Acetic acid (glacial), dichloromethane (DCM), diethyl ether, *N*,*N*-dimethylformamide (DMF), ethanol, ethyl acetate, methanol and tetrahydrofuran were HPLC or Analysis grade from Carlo Erba. Chloroform, deuterated (99.8%, Carlo Erba), fluorescein (High Purity, BDH), magnesium sulfate, anhydrous (> 98%, Fluka), potassium bromide (Spectroscopy Grade, Fischer), potassium hydroxide (Analysis Grade, Carlo Erba), sodium chloride (Extra Pure, Scharlau), sodium hydroxide (Laboratory Reagent Grade, Biochem Chemopharma) and triethylamine (TEA, HPLC Grade, Fischer) were used as received. Molecular sieves 3 Å (1.5 mm, Biochem Chemopharma) were activated in an oven at 200 °C for at least 48 hours before use. Silica gel 60 Å (60-200 μm, Acros Organics) and silica on aluminium for TLC (silica gel matrix with fluorescent indicator at 254 nm, Sigma-Aldrich) were used for column and thin-layer chromatography, respectively.

Instrumentation

Reactions were carried out in 50-, 100- or 150-mL round-bottom flasks heated in a mineral oil bath with an IKA C-MAG HS 7 hotplate and fitted with a reflux condenser. The temperature was regulated with an IKA ETS-D5 digital temperature probe. A Stuart RE400/MS rotary evaporator was used to remove solvents. TLC plates were observed using an Analytik Jena UVGL-58 handheld UV lamp emitting at 254 nm or 365 nm. The pH titrations were carried out using a Hanna instrument pH 210 microprocessor pH meter calibrated with pH 4.0 and pH 7.0 buffers.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance III HD spectrometer operating at 500.13 MHz and 125.76 MHz, respectively. The spectrometer is equipped with an Ascend 500 MHz superconducting magnet. Spectral data were collected using a 5 mm PABBO probe with TopSpin 3.2 software and processed with TopSpin 4.4.0. Chemical shifts of ^{1}H

NMR were referenced against tetramethylsilane (TMS) at δ = 0.00 ppm and for ^{13}C NMR spectra were referenced against the $CDCl_3$ signal at δ = 77.00 ppm.

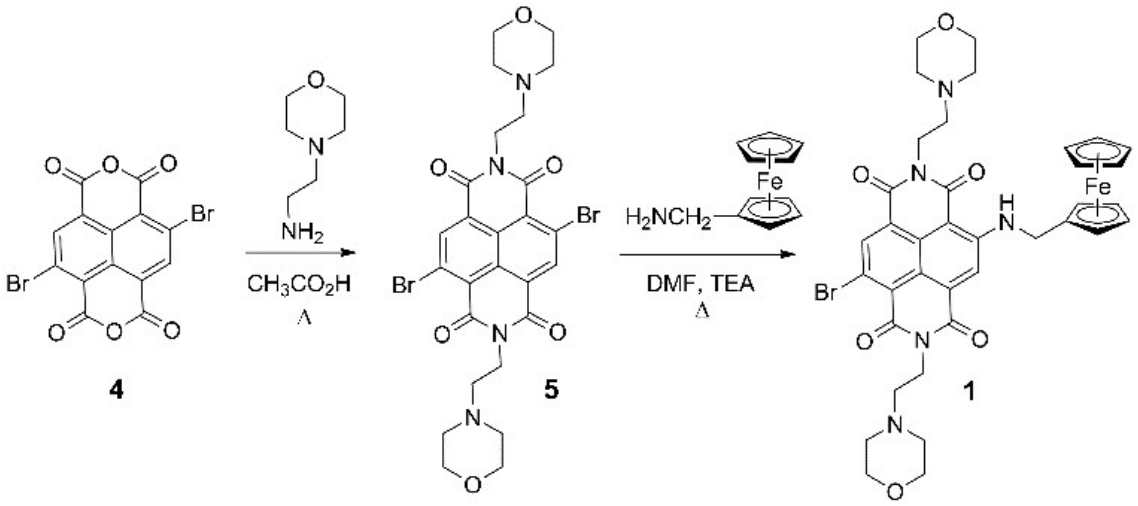
IR spectra were recorded using a Shimadzu IR-Affinity-I spectrometer over the 4000-400 cm^{-1} region. Spectral data was acquired and processed with SpectraGraph version 1.2. The spectrometer was calibrated against the polystyrene 1601 cm^{-1} absorption band. The IR spectra were taken as pressed KBr discs.

UV-visible absorption spectra were measured on a Jasco V-650 spectrophotometer using a 10 mm path length Suprasil quartz cuvette. The measurement was carried out at medium sensitivity with a bandwidth of 2.0 nm and a scan speed of 200 nm min^{-1}. Spectra were recorded from 250 to 750 nm at a scan interval of 0.5 nm. Baseline correction was applied by scanning a blank of the same solvent prior to sample analysis.

Fluorescence experiments were performed on a Jasco FP-8300 spectrofluorometer in fluorescence Suprasil quartz cuvettes with 10 mm path length. The slit widths for the excitation and emission slits were 2.5 nm and 5.0 nm, respectively. The parameters were set at the medium response setting with a scanning speed at 200 nm min^{-1} and data interval of 0.5 nm.

Melting points were determined using a Stuart SMP40 automated melting point apparatus. Calibration was done against vanillin and phenacetin.

Syntheses



4 **5** **1**

Synthesis of **5**, 2,6-dibromo-N,N'-bis(2-morpholinoethyl)naphthalenediimide

The synthesis of **5** was adapted from a published procedure.[S1] 2,6-Dibromonaphthalene-1,4,5,8-tetracarboxylic dianhydride **4** (0.500 g, 1.17 mmol) was mixed with 15 mL of glacial acetic acid and heated at 100 °C for 2 hours, and then 4-(2-aminoethyl)morpholine (0.40 mL,

2.9 mmol) was added. The reaction mixture was stirred with a magnetic stir bar and refluxed at 100 °C for 16 hours. The mixture was filtered, and then the solution pH was raised to pH 8.7 with dilute aqueous KOH solution. The vessel was cooled and a suspension formed, which was collected in a sintered funnel and washed with dilute aqueous KOH solution, then with diethyl ether. The precipitate was dissolved in a 1:1 (*v/v*) ethanol/ethyl acetate solution to remove residual water, which was removed by the rotary evaporator. A yellow-orange powder was obtained in 79.1% yield.

Compound **5**: R_f = 0.44 (98:2 (*v/v*) DCM/MeOH); m.p. = 290 °C (dec.); ^{1}H NMR ($CDCl_3$, ppm): δ_H 9.00 (s, 1H, Ar-H), 4.37 (t, *J* = 6.6 Hz, 2H, N-CH_2-CH_2-Mor), 3.65 (m, 4H, Mor-H), 2.71 (t, *J* = 6.7 Hz, 2H, N-CH_2-CH_2-Mor), 2.58 (m, 4H, Mor-H); ^{13}C NMR ($CDCl_3$, ppm): δ_C 160.82 (-), 160.78 (-), 139.07(↑), 128.39 (-), 127.83 (-), 125.38 (-), 124.19 (-), 67.00 (↓), 55.90 (↓), 53.81 (↓), 38.16 (↓); FTIR (KBr, cm^{-1}): 3059 (=C-H), 2970, 2968, 2813, 2810, 1709 (C=O), 1666 (C=O), 1558, 1439, 1366, 1310, 1213, 1113, 866, 785.

Synthesis of **1**, 2-bromo-6-ferrocenylmethyl-*N*,*N*'-bis(2-morpholinoethyl)naphthalenediimide

Intermediate **5** (0.100 g, 0.154 mmol) and ferrocenemethylamine (0.082 g, 0.36 mmol) were dissolved in 3 mL of DMF. Ferrocenemethylamine was synthesised using a published procedure.[S2] Three drops of triethylamine (TEA) were added, and the reaction mixture was stirred and refluxed at 80 °C for 22 hours. The mixture was diluted with 20 mL of DCM and washed with brine (5×20 mL). The organic layer was then collected, dried over anhydrous $MgSO_4$, vacuum filtered using a Hirsch funnel and the solvent removed by a rotary evaporator. The product was separated by column chromatography with silica gel and eluted with 2:98 (*v/v*) DCM/MeOH. The third band was eluted, and the solvent was removed by rotary evaporator to obtain a purple-pink powder in 18.0 % yield.

Compound **1**: R_f = 0.65 (98:2 (*v/v*) DCM/MeOH); m.p. > 400 °C; ^{1}H NMR ($CDCl_3$, ppm): δ_H 10.32 (t, *J* = 5.4 Hz, 1H, Fc-CH_2-NH-Ar), 8.87 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 4.47 (d, *J* = 5.2 Hz, 2H, Fc-CH_2-NH), 4.37 (m, 4H, N-CH_2-CH_2-Mor), 4.32 (t, *J* = 1.8 Hz, 2H, Fc-H), 4.29 (s, 5H, Fc-H), 4.25 (t, *J* = 1.9 Hz, 2H, Fc-H), 3.67 (m, 8H, Mor-H), 2.71 (t, *J* = 6.8 Hz, 4H, N-CH_2-CH_2-Mor), 2.59 (m, 8H, Mor-H); ^{13}C NMR ($CDCl_3$, ppm): δ_C 165.84 (-), 162.06 (-), 161.92 (-), 161.47 (-), 151.13 (-), 138.38 (↑), 128.81 (-), 127.48 (-), 123.62 (-), 123.46 (-), 121.66 (-), 120.68 (↑), 120.52 (-), 100.14 (-), 83.68 (-), 68.97 (↑), 68.91 (↑), 68.48 (↑), 67.56

(↑), 67.05 (↓), 67.04 (↓), 55.96 (↓), 53.86 (↓), 53.83 (↓), 42.86 (↓), 38.04 (↓), 37.26 (↓) ; FTIR (KBr, cm^{-1}): 3686 (N-H), 2924, 2808, 1684 (C=O), 1636 (C=O), 1578, 1508, 1443, 1308, 1261, 1080, 957; HRMS (ESI): $C_{37}H_{39}BrFeN_5O_6^+$ $[M+H]^+$ calculated 784.1428, observed 784.1445.

Spectroscopic Measurements

A solution of **1** was prepared in 1:1 (*v*/*v*) water/methanol and methanol with an absorbance < 0.1. NaOH and CH_3SO_3H aqueous solutions were used to adjust the solution pH. $Na_2S_2O_8$ was used as the oxidant for the redox titrations. The isosbestic point at 461 nm observed during the pH titration without oxidant was used as the excitation wavelength The pH was measured in 1:1 (*v*/*v*) water/methanol using a pH meter calibrated with buffers at pH 4.0 and pH 7.0. The solution was constantly stirred using a magnetic stirrer during pH measurements.

For the fluorescence quantum yield measurements, a solution of **1** was prepared in 1:1 (*v*/*v*) water/methanol and methanol using 5-10 mg of solid to give an absorbance < 0.2. Fluorescein in 1 M NaOH was used as the fluorescence standard for measurements at 461 nm ($\Phi_F = 0.95$). Measurements were taken with no input, at pH 3.6 with no oxidant, at pH 10 with 100 μM oxidant, and at pH 3.6 with 100 μM oxidant.

The acid dissociation constant (pK_a) of **1** was determined using the Henderson-Hasselbalch equation for UV-vis absorbance (eq. 1) or fluorescence emission (eq. 2) measurements. The log ratios were plotted on the y-axis against the pH on the x-axis. The y-intercept gives the pK_a. Ideally the slope is unity. To correct for the slope of the trendline, the negative of the y-intercept is divided by the slope to obtain the observed pK_a value.

$$pK_a = pH - \log\left(\frac{A_{F_{max}} - A}{A - A_{F_{min}}}\right) \quad (1)$$

$$pK_a = pH - \log\left(\frac{I_{F_{max}} - I}{I - I_{F_{min}}}\right) \quad (2)$$

The molar absorption coefficients (ε) were calculated using the Beer-Lambert equation (eq. 3) using the absorbance at a specific wavelength (A), the cell pathlength of 1.0 cm (b) and the solution concentration (c).

$$A = \varepsilon bc \quad (3)$$

The fluorescence quantum yields (Φ_f) were calculated relative to fluorescein as the standard using the area under the fluorescence spectra (I), the absorbance of the excited wavelength (A) and the solvent refractive index ($\eta = 1.33$). The values denoted with 'ref' refer to the reference. Fluorescein in aqueous 1 M NaOH ($\Phi_{ref} = 0.95$) was used as the reference.

$$\phi_f = \phi_{ref}\left(\frac{I}{I_{ref}}\right)\left(\frac{A_{ref}}{A}\right)\left(\frac{\eta^2}{\eta^2{}_{ref}}\right) \quad (4)$$

References

S1 S. Mpima, S. A. Ohnmacht, M. Barletta, J. Husby, L. C. Pett, M. Gunaratnam, S. T. Hilton and S. Neidle, *Bioorg. Med. Chem.*, 2013, **21**, 6162–6170.

S2 P. D. Beer and D. K. Smith, *J. Chem. Soc., Dalton Trans.*, 1998, 417–424.

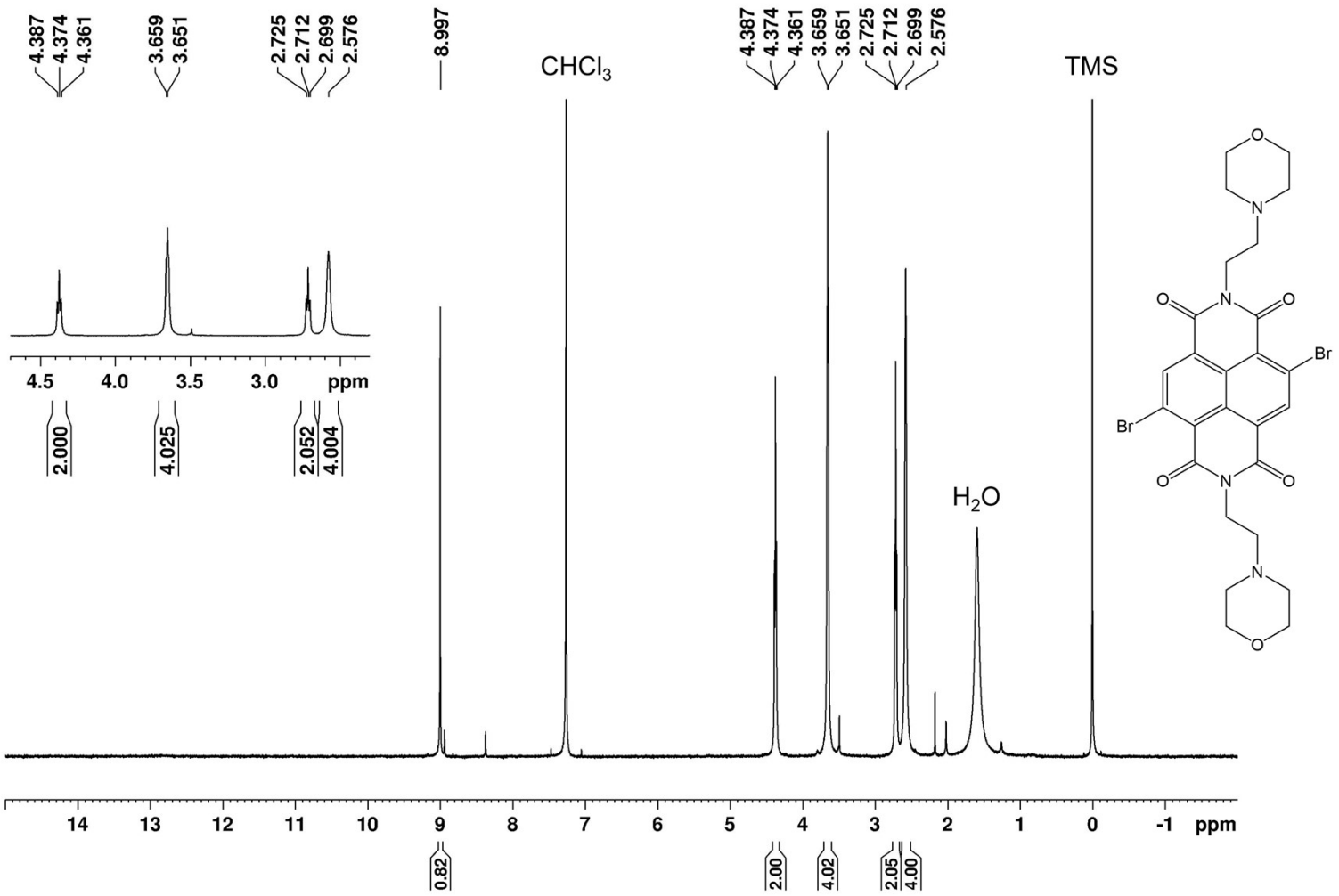


Fig. S1. ^{1}H NMR spectrum of **5** in $CDCl_3$ at 500 MHz.

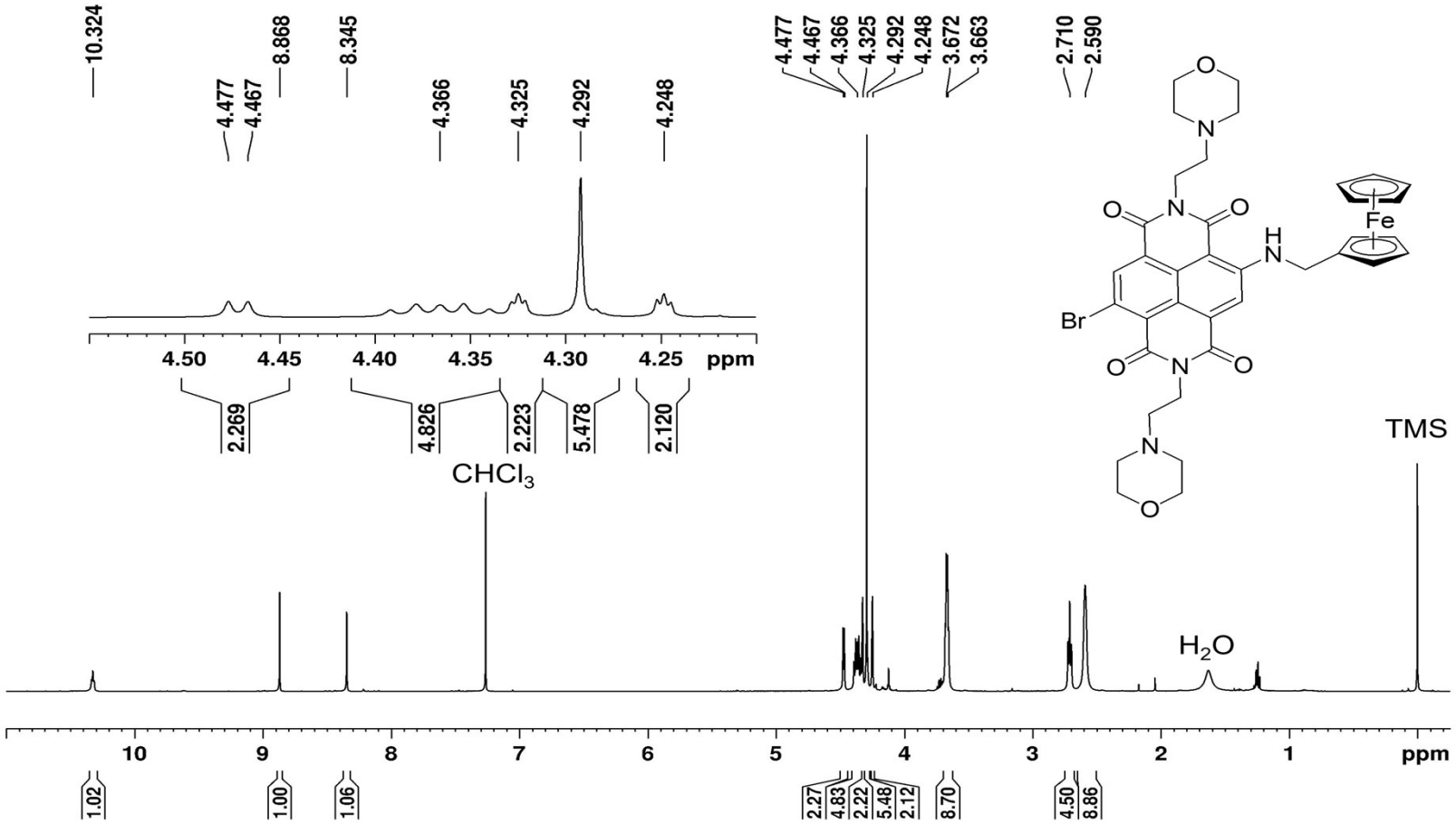


Fig. S2. ^{1}H NMR spectrum of **1** in $CDCl_3$ at 500 MHz.

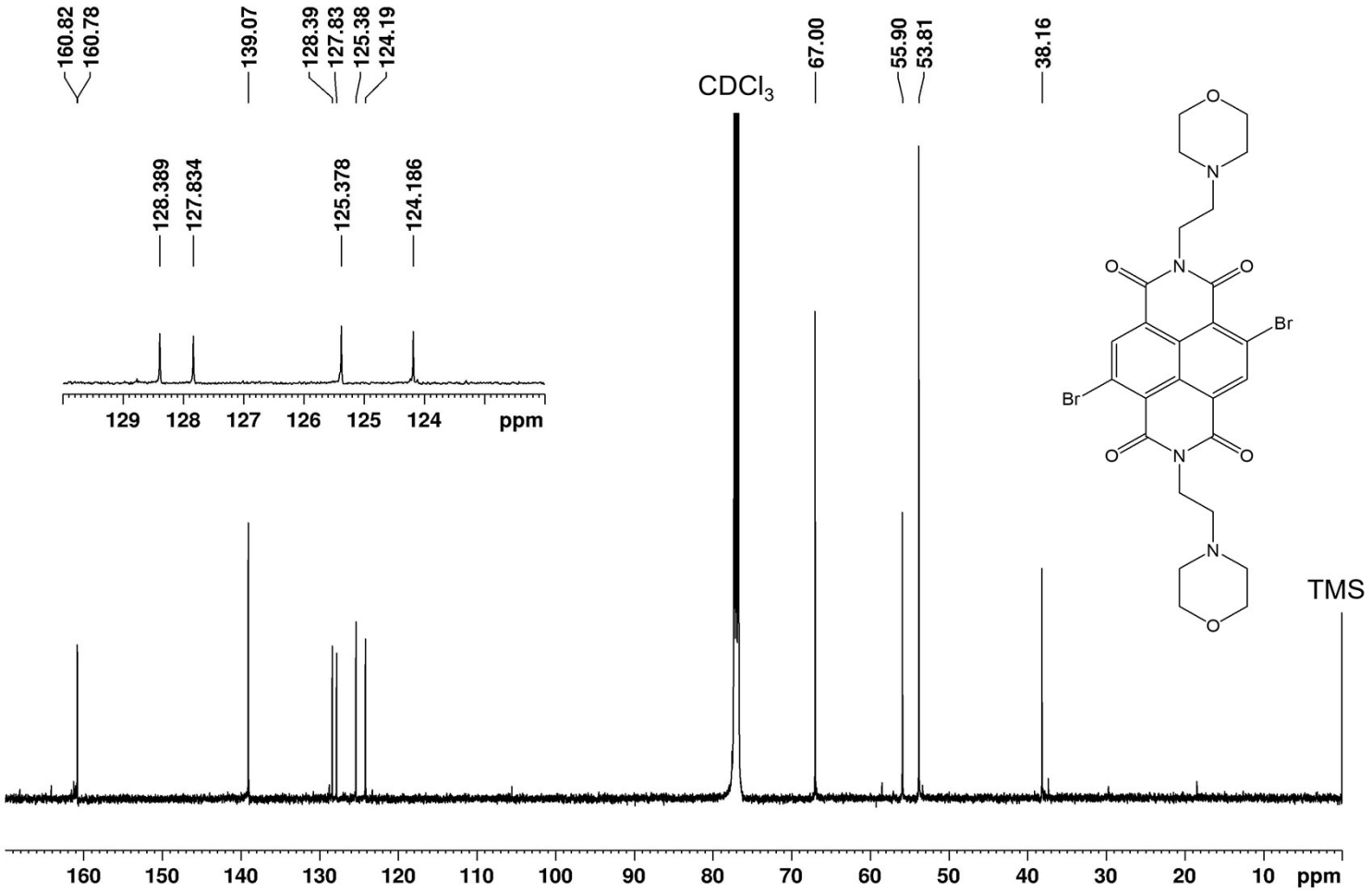


Fig. S3. ^{13}C NMR spectrum of **5** in $CDCl_3$ at 126 MHz.

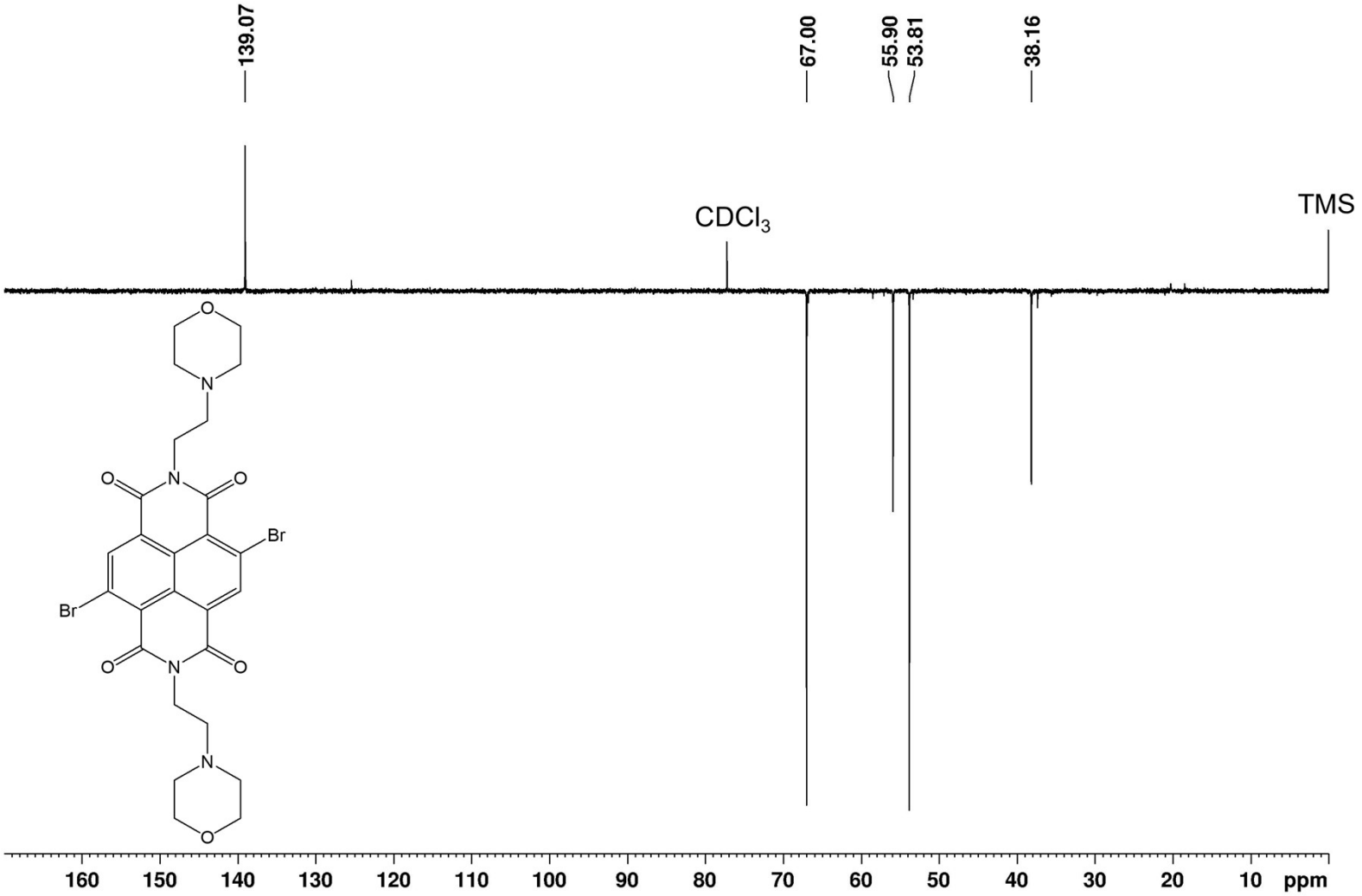


Fig. S4. ^{13}C DEPT NMR spectrum of **5** in $CDCl_3$ at 126 MHz.

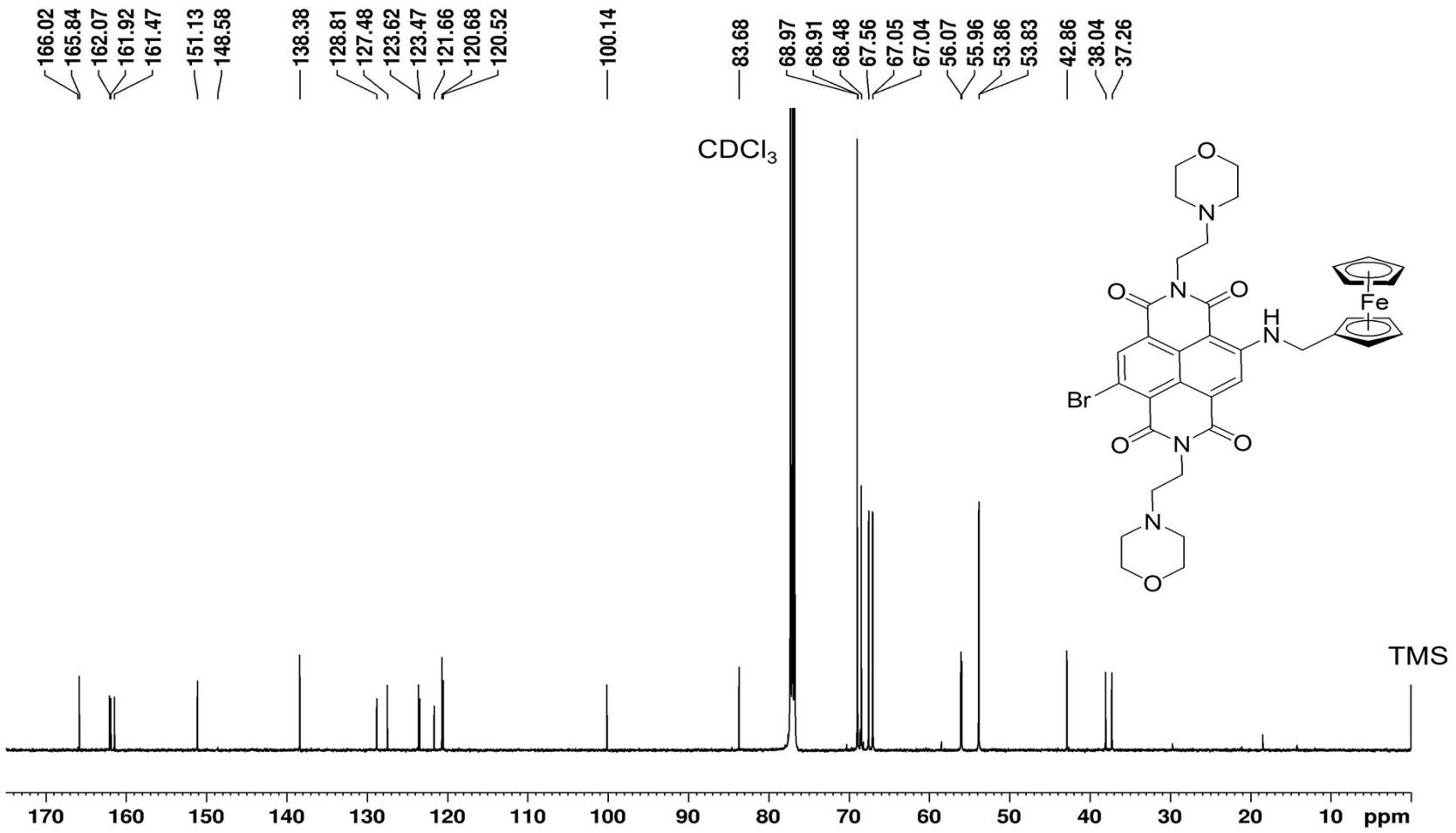


Fig. S5. ^{13}C NMR spectrum of **1** in $CDCl_3$ at 126 MHz.

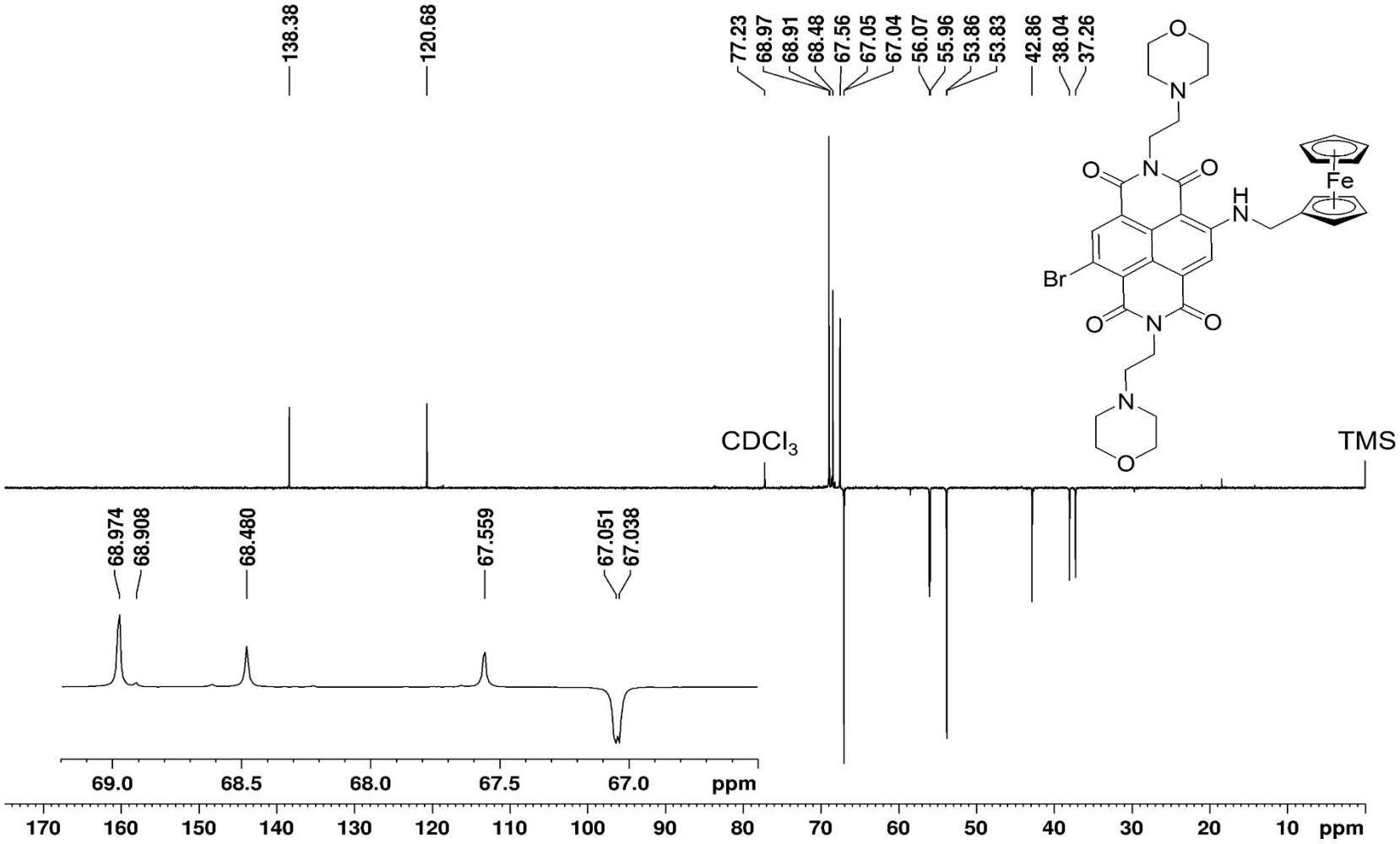


Fig. S6. ^{13}C DEPT NMR spectrum of **1** in $CDCl_3$ at 126 MHz.

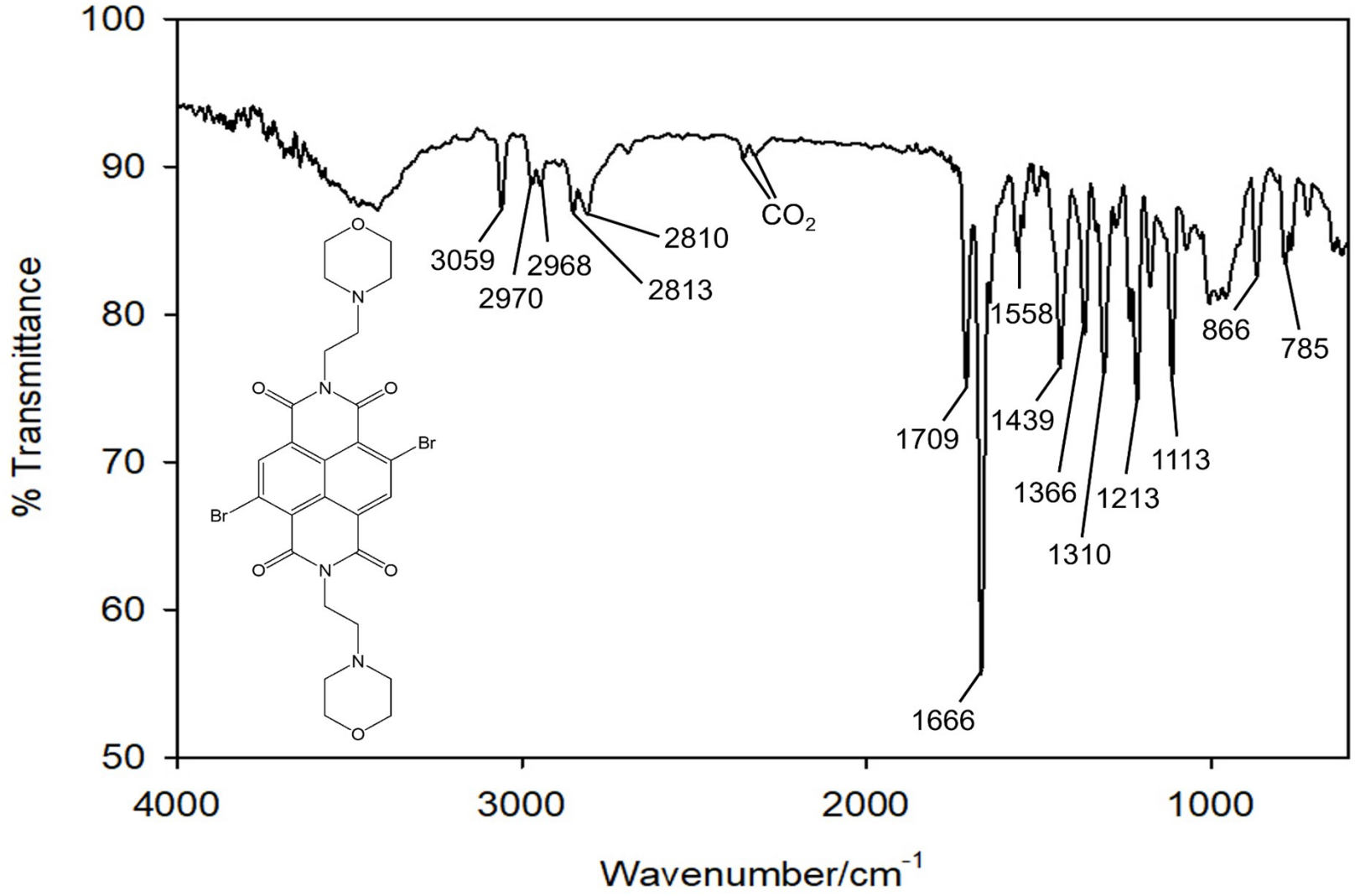


Fig. S7. IR spectrum of **5** (KBr disc).

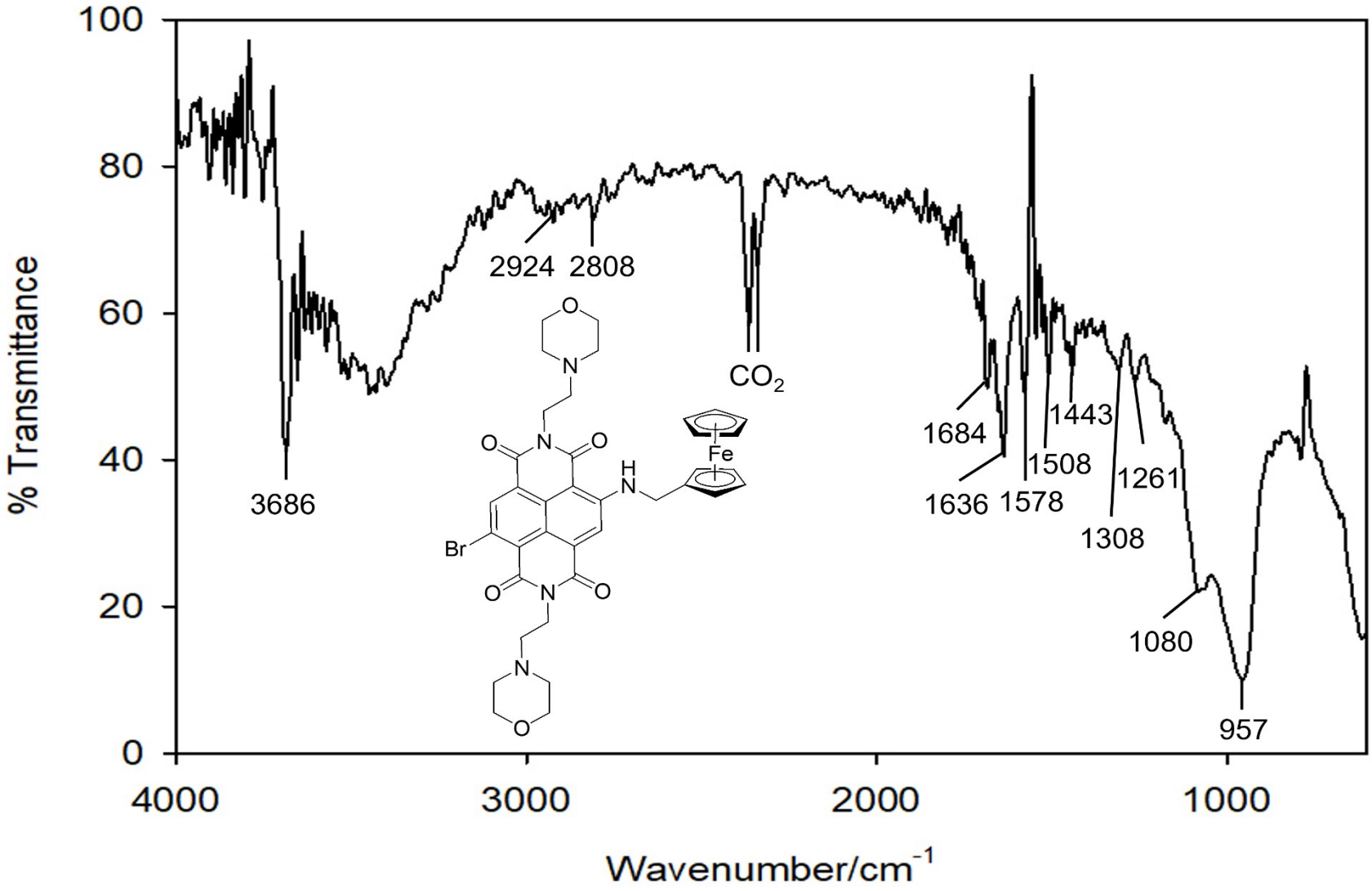


Fig. S8. IR spectrum of **1** (KBr disc).

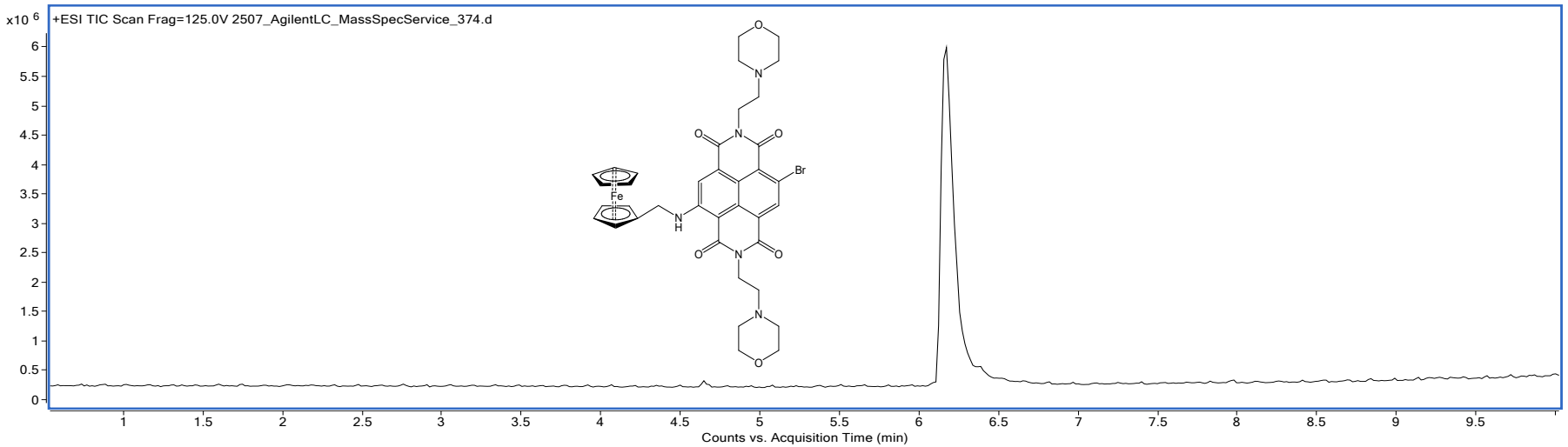


Figure S9. Total ion chromatogram of **1**.

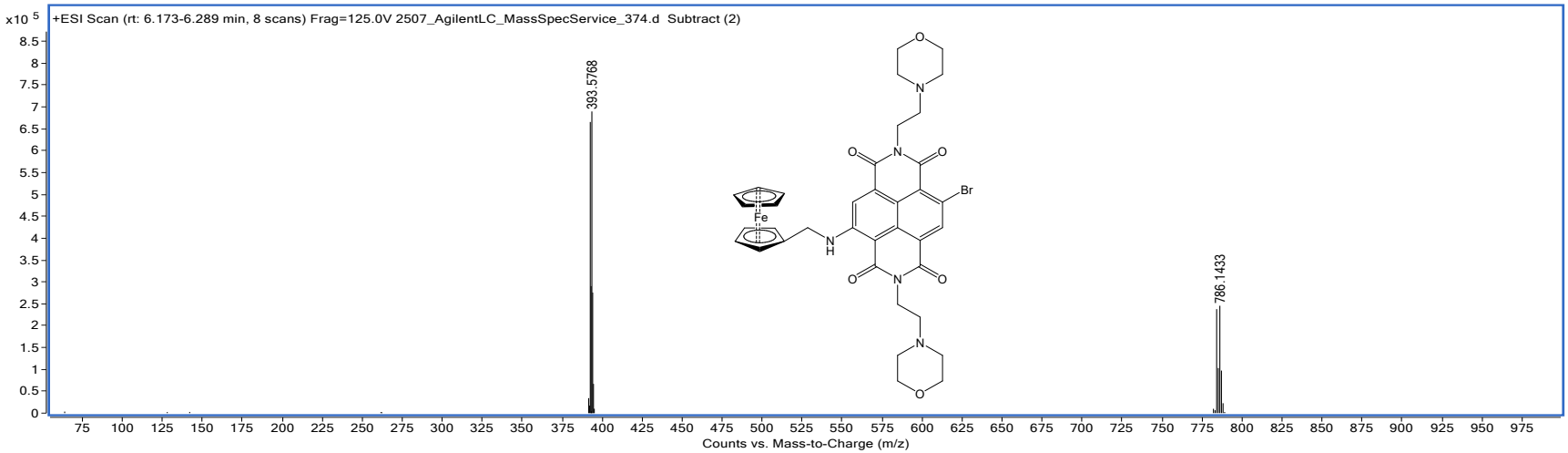


Fig S10. Mass spectrum of peak of **1** at RT 6.2 mins.

Accurate Mass Report1

Formula	C37H39N5O6Br[56Fe]+
Calculated Mass, MH+	784.1428
Observed Mass, MH+	784.1445
Mass Difference (ppm)	2.17

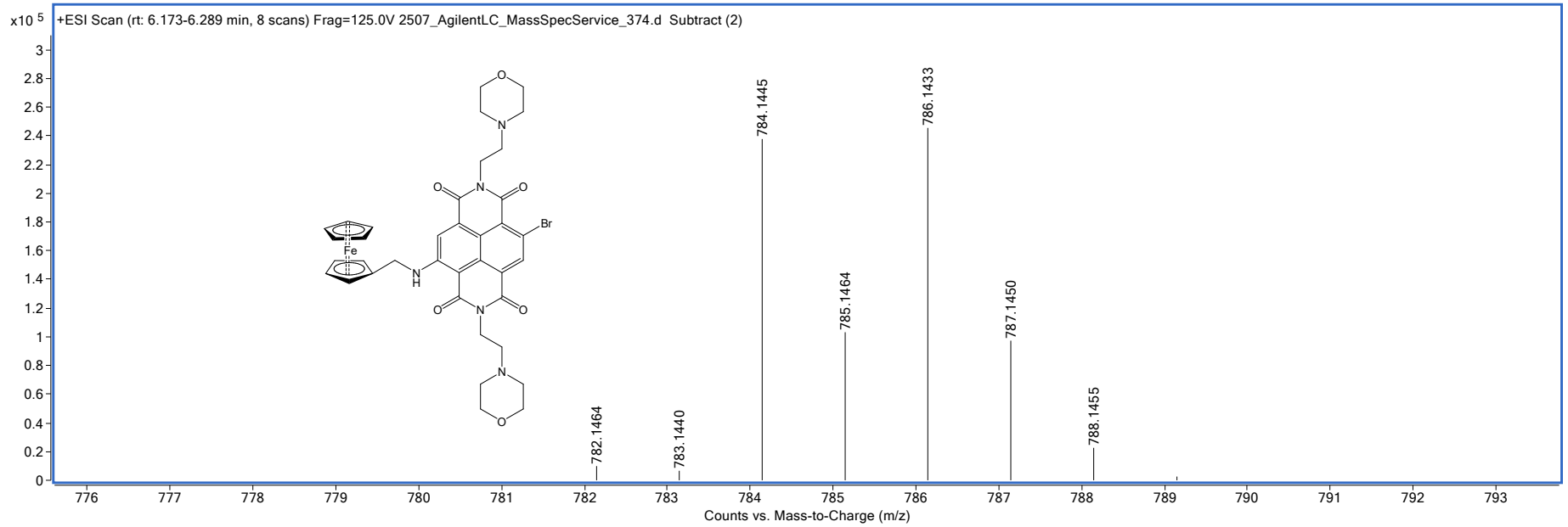


Fig S11. Accurate mass determination of **1**.

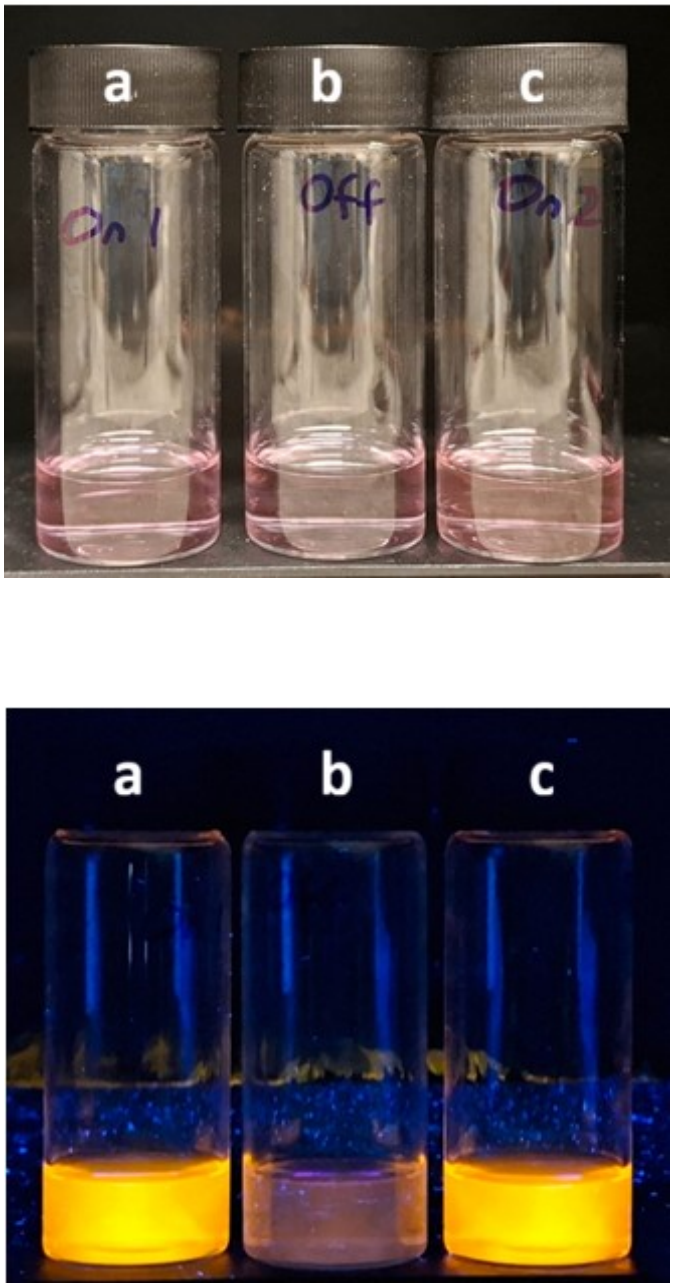


Fig S12. Solutions of **1** in methanol in the presence of: (a) acid and persulfate, (b) subsequent addition of thiosulfate and base, and (c) subsequent addition of persulfate and acid under room lighting (top) and irradiated with a 365 nm UV lamp (bottom).

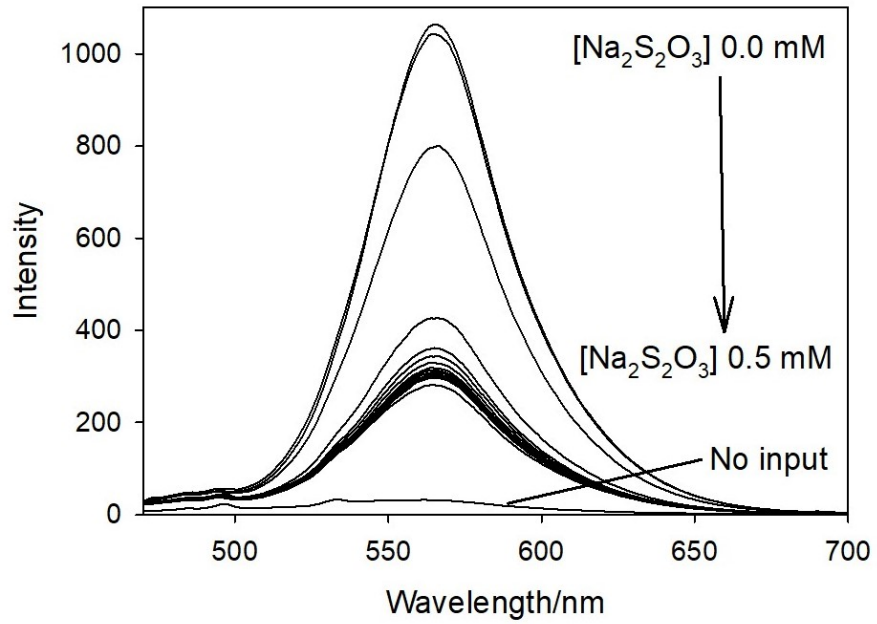


Fig. S13. Thiosulfate titration (0.5 mM) of **1** in presence of 0.2 mM acid and 0.1 mM sodium persulfate in MeOH (λ_{ex} = 461 nm).

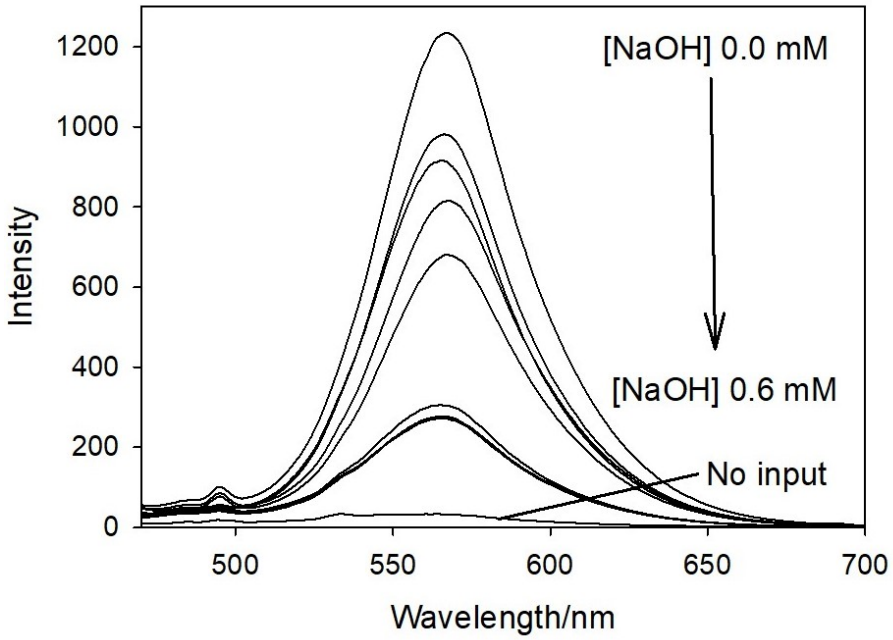


Fig. S14. Base titration (0.6 mM) of **1** in presence of 0.2 mM acid and 0.1 mM sodium persulfate in MeOH (λ_{ex} = 461 nm).

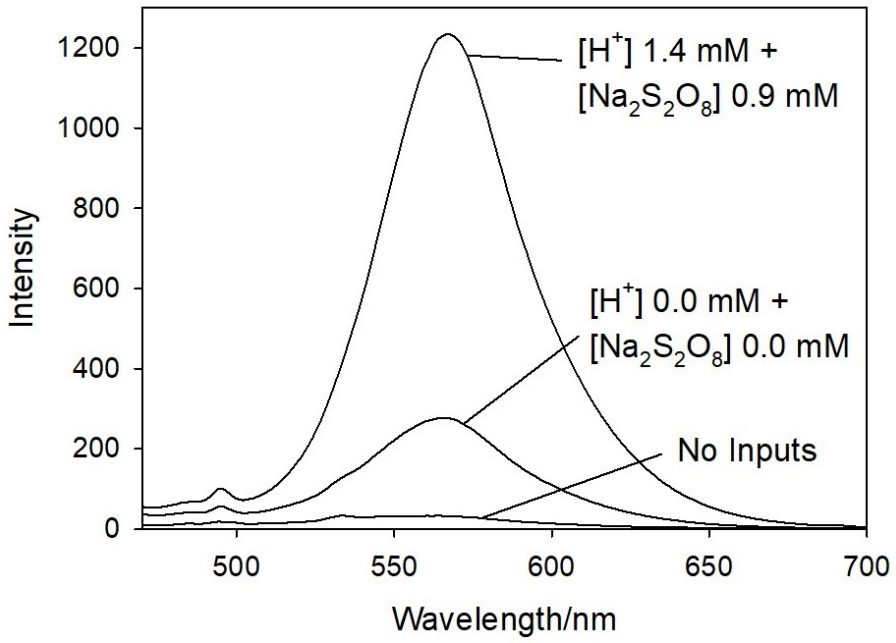


Fig. S15. Turning 'on' of **1** with 1.4 mM acid and 0.9 mM sodium persulfate in MeOH (λ_{ex} = 461 nm) after reduction with thiosulfate and deprotonation with base.