Supporting Information

Lysosome targeting fluorescence probe for imaging intracellular thiols

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I. General Methods.

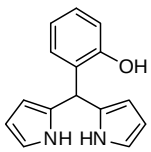
All reactions were conducted under the nitrogen atmosphere. All the chemicals were purchased from commercial sources and used as received unless stated otherwise. Solvents: petroleum ether and ethyl acetate (EtOAc) were distilled prior to thin layer and column chromatography. Tetrahydrofuran (THF) was pre-dried over Na wire and then refluxed over Na (1% w/v) and benzophenone (0.2% w/v) under an inert atmosphere until the blue color of the benzophenone ketyl radical anion persists. Dichloromethane (CH_2Cl_2) was pre-dried over calcium hydride and then distilled. Column chromatography was performed on Merck silica gel (100–200 mesh). TLC was carried out with E. Merck silica gel 60-F_{254} plates.

II. Physical Measurements.

The ^{1}H and ^{13}C spectra were recorded on 400 MHz Jeol ECS-400 (or 100 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal reference or from internal tetramethylsilane on the δ scale (CD_3CN δ_H 1.93 ppm, $CDCl_3$ δ_H 7.24 ppm, δ_C 77.0 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: m (multiplet), s (singlet), br s (broad singlet), d (doublet), t (triplet) dd (doublet of doublet). Low-resolution mass spectra were recorded on an Applied Biosystems 4800 Plus MALDI TOF/TOF analyzer. High-resolution mass spectra were obtained from MicroMass ESI-TOF MS spectrometer. (FT-IR) spectra were obtained using NICOLET 6700 FT-IR spectrophotometer as KBr disc and reported in cm^{-1}. Melting points were measured using a VEEGO Melting point apparatus. All melting points were measured in open glass capillary and values are uncorrected. Absorption spectra were recorded on a PerkinElmer, Lambda 45 UV-Vis spectrophotometer. Steady State fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on a Fluoromax 4 instrument (Horiba Jobin Yvon). Cell images were taken in 35 mm (diameter) dishes. The media (DMEM) and PBS buffer were purchased from commercial sources. Fluorescence images were taken using Olympus Inverted IX81 equipped with Hamamatsu Orca R2 microscope. ChemBio Draw Ultra and Image *J* software were used for drawing structures and for processing cell images respectively.

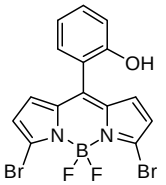
III. Experimental Procedures.

Synthesis of 2-(di(1*H*-pyrrol-2-yl)methyl)phenol ($C_{15}H_{14}N_2O$) 3: In a 1000 mL round bottom flask salicylaldehyde **2** (2.0 g, 16.4 mmol) and pyrrole (5.7 mL, 82 mmol) were dissolved in 500 mL CH_2Cl_2. To this solution, catalytic (2-3 drops) TFA was added and the reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, solvent was removed under reduced pressure to obtain a residue which was purified by column chromatography over silica gel (Eluent: 25 % EtOAc in petroleum ether) to furnish the pure **3** (2.5 g, 64 %) as gray solid. **M.p.:** 117 – 118 ^{o}C; **IR (KBr):** ν/cm^{-1} 3427, 3393, 1635, 1559, 1501, 1454, 1384, 1280, 1257, 1200, 1189, 1171, 1118, 1085; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.17 (s, 2H), 7.18 (td, *J* = 7.8, 1.7 Hz, 1H), 7.07 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.90 (td, *J* = 7.4, 0.9 Hz, 1H), 6.88 – 6.84 (m, 1H), 6.71 (dd, *J* =

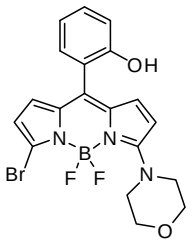


4.1, 2.5 Hz, 2H), 6.15 (dd, *J* = 5.8, 2.9 Hz, 2H), 6.05 – 5.95 (m, 2H), 5.54 (s, 1H), 5.18 (s, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 153.7, 131.1, 130.2, 128.8, 128.5, 121.6, 118.1, 117.6, 108.6, 107.1, 40.3; **HRMS (ESI):** Calc. for **$C_{15}H_{15}N_2O^+$** $[M+H]^+$: 239.1184; Found: 239.1173.

Synthesis of 3,7-dibromo-5,5-difluoro-10-(2-hydroxyphenyl)-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide ($C_{15}H_9BBr_2F_2N_2O$) 4: In a 100 mL round bottom flask dipyrromethane **3** (500 mg, 2.1 mmol) was dissolved in 40 mL dry THF and cooled to -78 ^oC. To this solution *N*-bromosuccinimide (781 mg, 4.2 mmol) was added and the reaction mixture was stirred at -78 ^oC for 2 h. To this reaction mixture 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) was added at room temperature and stirred at room temperature for 2 h. THF was evaporated under reduced pressure and residue obtained was dissolved in CH_2Cl_2 and filtered through silica column using CH_2Cl_2 Eluent was evaporated to reduce the volume to 100 mL. To this Et_3N (6.13 mL, 44.0 mmol) and $BF_3{\cdot}Et_2O$ (5.5 mL, 44.0 mmol) were added at room temperature and stirred for 3 h. After completion of the reaction, the reaction mixture was diluted with H_2O and CH_2Cl_2 layer was separated. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was washed with water (20 mL × 1), brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain a brown residue which was purified by column chromatography over silica gel (Eluent: 20 % EtOAc in petroleum ether) to furnish the pure **4** (555 mg, 60 %) as brown solid. **M.p.:** 190 – 192 ^oC; **IR (KBr):** ν/cm^{-1} 3491, 1610, 1553, 1449, 1409, 1384, 1308, 1255, 1183, 1093; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.47 – 7.35 (m, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.04 – 6.98 (m, 2H), 6.76 (d, *J* = 4.2 Hz, 2H), 6.49 (d, *J* = 4.2 Hz, 2H), 5.25 (s, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 153.5, 138.6, 135.9, 133.3, 132.2, 131.7, 131.6, 123.1, 120.6, 118.8, 117.0; **HRMS (ESI):** Calc. for $C_{15}H_9BBr_2FN_2O^+$ $[M-F]^+$: 422.9138; Found: 422.9139.

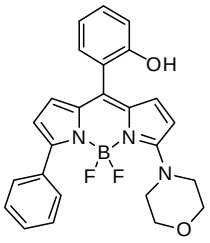


Synthesis of 7-bromo-5,5-difluoro-10-(2-hydroxyphenyl)-3-morpholino-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide ($C_{19}H_{17}BBr_2F_2N_3O_2$) 5: In a 25 mL round bottomed flask were added compound **4** (100 mg, 0.23 mmol), Et_3N (80 µL, 0.58 mmol) and morpholine (20 µL, 0.23 mmol) dissolved in CH_3CN (8 mL). The reaction mixture was stirred at room temperature for 6 h. After completion of the reaction, the reaction mixture was evaporated under reduced pressure to remove CH_3CN to obtain residue which was purified by column chromatography over silica gel (Eluent: 1 % methanol in chloroform) to



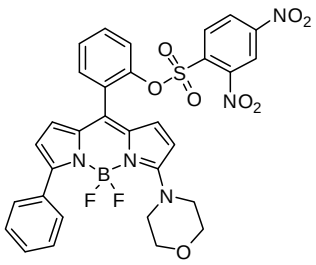
furnish the pure **5** (80 mg, 80%) as brown solid. **M.p.:** 185 – 186 ^{o}C (decomposed); **IR (KBr):** ν/cm^{-1} 3436, 1654, 1561, 1504, 1449, 1384, 1289, 1103, 1061; **^{1}H NMR (400 MHz, CD_3OD):** δ 7.87 (s, 1H), 7.27 (td, *J* = 7.9, 1.7 Hz, 1H), 7.14 (dd, *J* = 7.8, 1.6 Hz, 1H), 6.88 (d, *J* = 7.8 Hz, 2H), 6.79 (d, *J* = 5.3 Hz, 1H), 6.54 (d, *J* = 5.2 Hz, 1H), 6.18 (d, *J* = 3.7 Hz, 1H), 6.04 (d, *J* = 3.7 Hz, 1H), 4.07 – 3.93 (m, 4H), 3.90 – 3.70 (m, 4H); **^{13}C NMR (100 MHz, DMSO-d_6):** δ 162.4, 155.9, 136.1, 136.0, 133.1, 132.2, 130.7, 125.7, 120.9, 119.2, 117.3, 117.0, 116.4, 116.4, 111.8, 66.7, 51.1; **HRMS (ESI):** Calc. for $C_{19}H_{18}BBrF_2N_3O_2^+$ $[M+H]^+$: 447.0565; Found: 447.0592.

Synthesis of 5,5-difluoro-10-(2-hydroxyphenyl)-3-morpholino-7-phenyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide ($C_{25}H_{22}BN_3O_2$) 6: In a 100 mL round bottomed flask compound **5** (350 mg, 0.78 mmol) was dissolved in 1:1 mixture of 1M Na_2CO_3/ Toluene (40 mL) and degassed with N_2 gas. To this solution phenylboronic acid (95 mg, 0.78 mmol) and tetrakis (triphenylphosphine) palladium (91 mg, 0.08 mmol) were added at room temperature. The reaction mixture was refluxed for 12 h. After completion of the reaction, the reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with water (20 mL × 1), brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain a brown residue which was purified by column chromatography over silica gel (Eluent: 25 % EtOAc in petroleum ether) to furnish the pure **6** (275 mg, 79%) as dark brown solid. **M.p.:** 204 – 206 ^{o}C; **IR (KBr):** ν/cm^{-1} 3452, 2926, 2851, 1577, 1561, 1451, 1384, 1299, 1267, 1167, 1121, 1049; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.83 – 7.67 (m, 2H), 7.45 – 7.29 (m, 4H), 7.26 – 7.22 (m, 1H), 7.00 (td, *J* = 8.4, 4.2 Hz, 2H), 6.82 (d, *J* = 5.1 Hz, 1H), 6.41 (dd, *J* = 12.3, 3.9 Hz, 2H), 6.23 (d, *J* = 5.2 Hz, 1H), 5.27 (s, 1H), 3.94 – 3.85 (m, 4H), 3.84 – 3.70 (m, 4H); **^{13}C NMR (100 MHz, DMSO-d_6):** δ 162.0, 155.9, 145.6, 135.4, 135.2, 133.9, 132.2, 130.6, 129.1, 128.4, 127.7, 127.7, 121.7, 119.1, 118.6, 116.4, 115.9, 115.8, 66.7, 50.9; **HRMS (ESI):** Calc. for $C_{25}H_{23}BF_2N_3O_2^+$ $[M+H]^+$: 446.1851; Found: 446.1843.



Synthesis of 10-(2-(((2,4-dinitrophenyl)sulfonyl)oxy)phenyl)-5,5-difluoro-3-morpholino-7-phenyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide ($C_{31}H_{24}BF_2N_5O_8S$) 1: In a 25 mL round bottomed flask compound **6** (100 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to -78 ^{o}C. To this solution Et_3N (35 µL, 0.25 mmol) and 2,4-

dinitrobenzenesulfonyl chloride (67 mg, 0.25 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the reaction mixture was diluted with H_2O and CH_2Cl_2 layer was separated. The aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with water (15 mL × 1), brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain a brown residue which was purified by column chromatography over silica gel (Eluent: 20 % EtOAc in petroleum ether) to furnish the pure **1** (138 mg, 91 %) as brown solid. **M.p.:** 248 – 249 ^oC; **HPLC Purity:** 97.9%; **IR (KBr):** ν/cm^{-1} 3433, 3096, 2920, 1545, 1508, 1451, 1383, 1353, 1302, 1198, 1125; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.48 (s, 1H), 8.07 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.60 (dd, *J* = 4.6, 1.9 Hz, 2H), 7.41 – 7.33 (m, 1H), 7.42 – 7.30 (m, 4H), 6.69 (d, *J* = 5.2 Hz, 1H), 6.25 (d, *J* = 5.2 Hz, 1H), 6.23 (d, *J* = 3.8 Hz, 1H), 6.04 (d, *J* = 3.8 Hz, 1H), 3.89 – 3.79 (m, 8H); **^{13}C NMR (100 MHz, DMSO-D_6):** δ 162.1, 150.8, 147.5, 146.9, 145.8, 135.6, 135.1, 134.6, 133.7, 132.9, 132.6, 132.4, 131.7, 128.9, 128.8, 128.3, 127.9, 127.8, 125.3, 122.0, 121.4, 118.4, 117.6, 115.8, 66.6, 51.2; **HRMS (ESI):** Calc. for $C_{31}H_{25}BF_2N_5O_8S^+$ $[M+H]^+$: 676.1485; Found: 676.1483.



IV. Crystal Structure Parameters.

Crystal structure of 5 (CCDC 980654): Compound **5** was crystallized from methanol at room temperature. A red cube shaped crystal with approximate dimensions 0.300 × 0.250 × 0.130 mm gave an orthorhombic with space group *P b c n*; *a* = 14.8291(4) *b* = 12.6719(3) *c* = 21.3493(6) Å, α = 90° β = 90° γ = 90°; *V* = 4011.81(18) Å^3; *T* = 100 K; *Z* = 8; ρ_{calc}= 1.537 Mgm^{-3}; $2\theta_{max}$= 72.325; $CuK_\alpha\lambda$ = 1.54178 Å. Fine-focus sealed tube source with graphite monochromator. *R* = 0.0784 (for 3551 reflection $I>2\sigma(I)$), *wR*= 0.2836 which was refined $|F2|$ and S = 2.411 for 263 parameters and 3963 unique reflections. The structure was obtained by direct methods using SHELXS-97.[S4] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded μ = 3.188^{-1}.

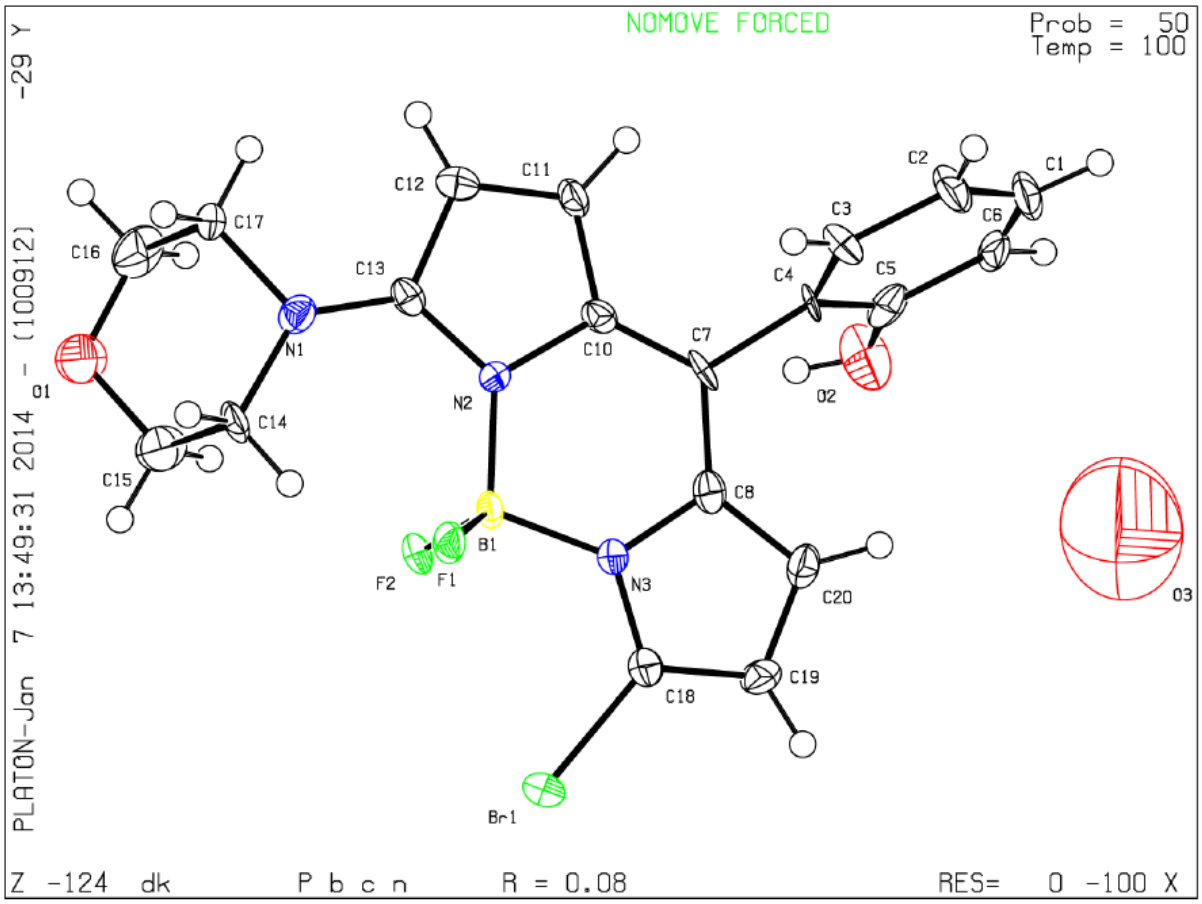


Fig. S1 ORTEP diagram of compound **5**.

Crystal structure of 1 (CCDC 980654): Compound **1** was crystallized from chloroform at room temperature. A brown cube shaped crystal with approximate dimensions 0.195 × 0.170 × 0.090 mm gave an Monoclinic with space group *P2(1)/n*; *a* = 17.4761(5) *b* = 8.0976(2) *c* = 22.1564(7) Å, α = 90° β = 111.715(1)° γ = 90°; *V* = 2912.95(14)Å^3; *T* = 296 (2) K; *Z* = 4; ρ_{calc}= 1.540 Mgm^{-3}; $2\theta_{max}$= 64.980; $CuK_{\alpha}\lambda$ = 1.54178 Å. Fine-focus sealed tube source with graphite monochromator. *R* = 0.0434 (for 4530 reflection $I>2\sigma(I)$), *wR*= 0.1156 which was refined |*F2*| and S = 1.045for 433 parameters and 4680 unique reflections. The structure was obtained by direct methods using SHELXS-97.[S4] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded μ = 1.663 mm^{-1}.

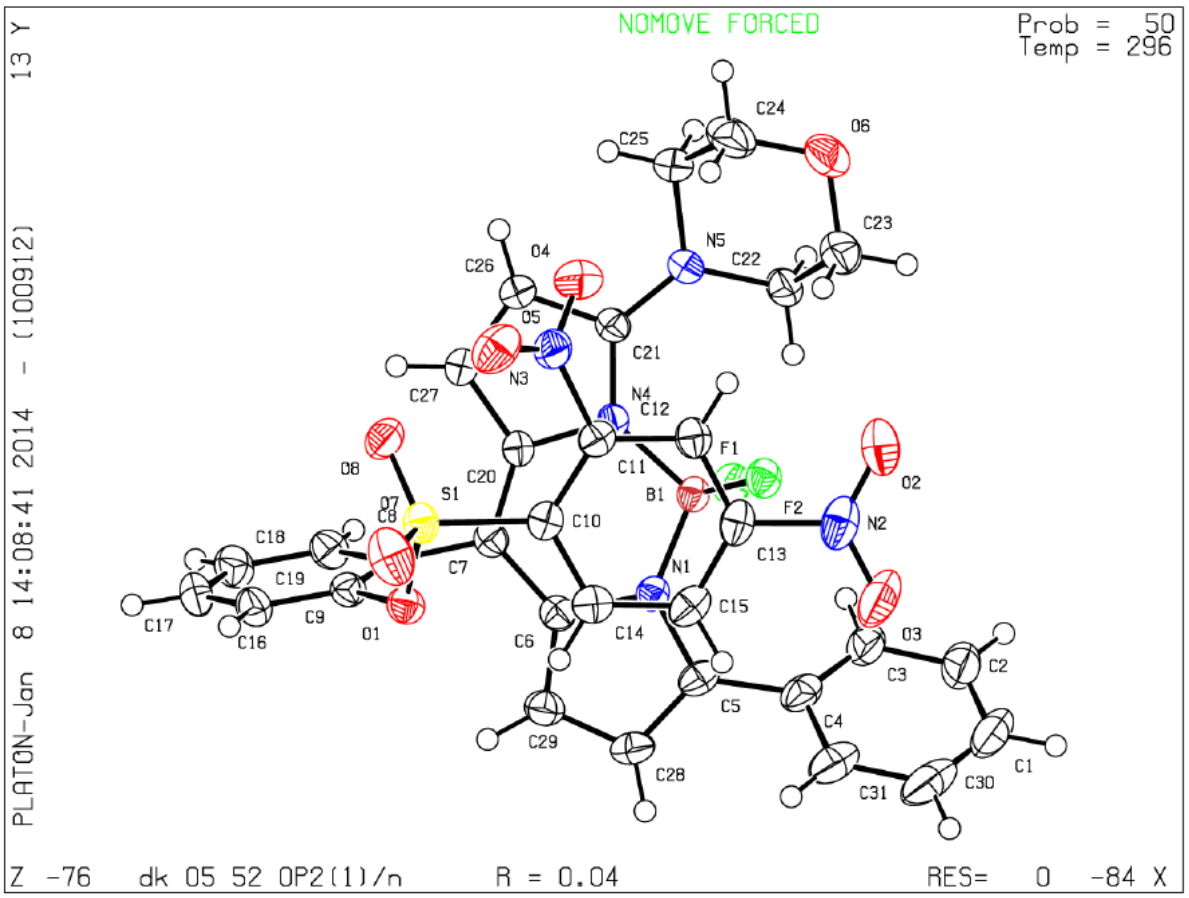


Fig. S2 ORTEP diagram of compound **1**.

V. Photophysical Studies.

Procedures:

Preparation of the medium: Deionized water was used throughout all experiments. All experiments were carried out in a HEPES buffer (10 mM, 1 mM CTAB, pH = 7.4) with 1% DMSO (maximum). HEPES buffer was prepared by dissolving solid HEPES in deionized water followed by adjustment of pH with 0.5 (N) NaOH.

Preparation of the solution of 1: A stock solution of **1** (2000 μM) was prepared in DMSO. Final concentration of each of **1** during each assay was 10 μM with 1% DMSO (maximum).

Preparation of the solution of analytes: Stock solutions of Ala, Arg, His, NaCl, Na_2SO_4, NaSCN, $NaNO_3$, Ser, GSH, Cys and Hcy were prepared in deionized water (concentrations 20 mM). Stock solutions of *n*-$BuNH_2$ was prepared in DMSO (concentration 40

mM).Calculated volumes of analytes were added from respective stock solutions to each fluorescence cuvette to provide 100 μM. All spectral data were recorded at 5 min after the addition of analyte(s) by exciting at 510 nm. The excitation and emission slit width were 2 nm and 3 nm, respectively.

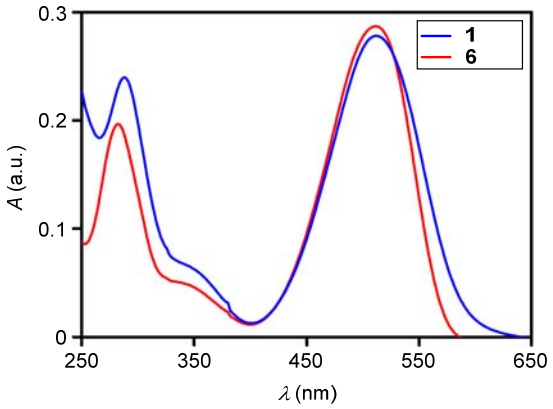


Fig. S3 Absorbance spectra of probe **1** (10 μM) and compound **6** (10 μM) in HEPES buffer (10 mM, 1 mM CTAB, pH 7.4).

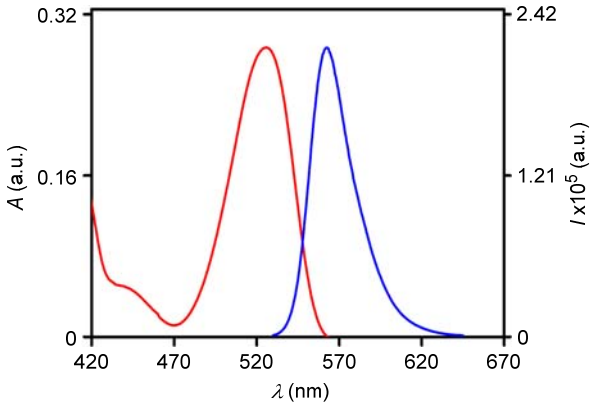


Fig. S4 Normalized Absorbance and Fluorescence spectra of probe **1** (10 μM) in HEPES buffer (10 mM, 1 mM CTAB, pH 7.4).

VI. Thiol Sensing.

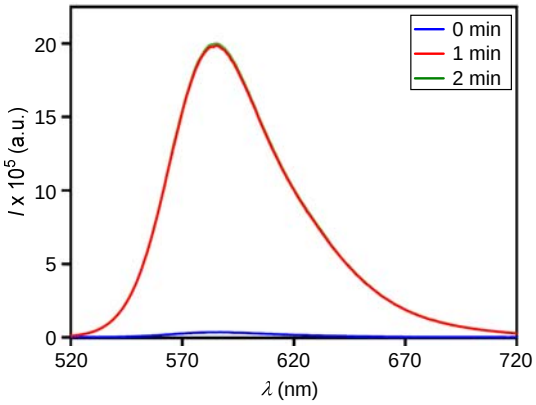


Fig. S5 Fluorescence spectra of probe **1** (10 μM) in the presence of Cys (100 μM) with time in HEPES buffer (10 mM, 1 mM CTAB, pH 7.4).

Determination of quantum yields:

The quantum yields of probe **1** and compound **6** were determined according to the Eq. 1:

$$\Phi_1 = \Phi_B \times \frac{I_1 \times A_B \times \lambda_{exB} \times (\eta_1)^2}{I_B \times A_1 \times \lambda_{ex1} \times (\eta_B)^2} \qquad \textbf{Eq. 1}$$

where, Φ is quantum yield; I is integrated area under the corrected emission spectra; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts 1 and B refer to the unknown and the standard, respectively.

Determination of reaction rate (k) and half-life ($t_{1/2}$) for probe 1:

Reaction rate (k), half-life ($t_{1/2}$) and response time for probe **1** was determined by fluorescence kinetics experiments. Experiments were carried out at pH 7.4 and 5.

Kinetics at pH 7.4: In each fluorescence kinetics experiment, a solution of **1** (10 μM) in HEPES buffer (10 mM, 1mM CTAB, pH = 7.4) was placed in a fluorescence cuvette and fluorescence intensity at λ = 585 nm (upon λ_{ex} = 510 nm) was monitored. At t = 30 s an analyte (100 μM of either of Cys or Hcy, GSH, Cys and nBu-NH_2) was added. A control

trace was also generated without adding any analyte. Rate constant, k for each reaction was calculated according to the following Eq. 2:

$$Y = a \times [1 - e^{(-kt)}] \quad \textbf{Eq. 2}$$

where, Y = fractional fluorescence intensity, a = arbitrary constant, k = pseudo first order rate constant, t = time.

Half-life of the reaction ($t_{1/2}$) was calculated using Equation 3:

$$t_{1/2} = 0.693/k \quad \textbf{Eq. 3}$$

where k = pseudo first order rate constant.

Kinetics at pH 5: In each fluorescence kinetics experiment, a solution of **1** (10 μM) in phosphate buffer (10 mM, 1 mM CTAB, pH = 5.0) was placed in a fluorescence cuvette and fluorescence intensity at λ = 585 nm (upon λ_{ex} = 510 nm) was monitored. At t = 30 s Cys (100 μM) was added. A control trace was also generated without adding any analyte. Based on this study, k and $t_{1/2}$ values were calculated according to Eq. 2 and Eq. 3.

Determination of detection limits of probe 4 towards Cysteine sensing:

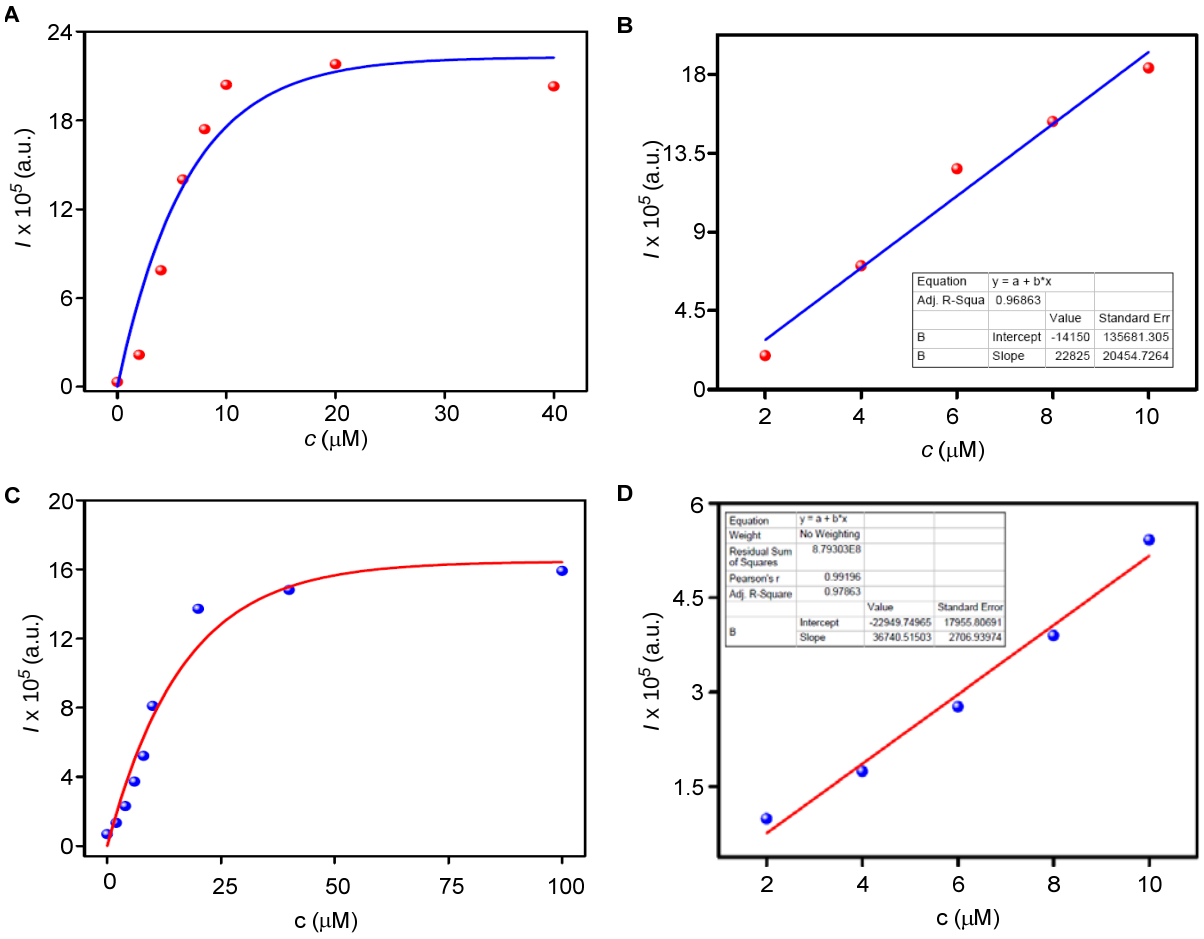


Fig. S6 The concentration *vs* intensity plot probe **1** (10 μM) with increasing concentration of a Cys recorded in HEPES buffer (10 mM, 1 mM CTAB, pH = 7.4) (**A**), linier fitting plot of the same up to 1 equivalent of Cys addition (**B**), concentration *versus* intensity plot probe **1** (10 μM) with increasing concentration of a Cys recorded in Phosphate buffer (10 mM, 1 mM CTAB, pH = 5.0) (**C**)and linier fitting plot of the same up to 1 equivalent of Cys addition (**D**).

The detection limits were determined based on the fluorescence titrations. Experiments were carried out at pH 7.4 and 5.

Detection limit at pH 7.4: Probe **1** was employed at 10 μM. To determine the *S/N* ratio, the emission intensity of probe **1** was measured without Cys by 7-times and the standard deviations of blank measurements were determined. Under these conditions, a good linear relationship between the fluorescence intensity and the Cys concentration (Fig. S6A) was obtained for probe **1** (R^2 = 0.9686). The detection limit was then calculated with the Eq. 4:

$$\text{Detection limit} = 3\sigma/m \qquad \textbf{Eq. 4}$$

where σ is the standard deviation of 7 blank measurements, m is the slope between intensity versus Cys concentration.

The detection limit of **1** towards Cys at pH 7.4 was calculated to be 8.2×10^{-9} M with $S/N = 3$ (signal-to-noise ratio of 3:1).

Detection limit at pH 5: Probe **1** was employed at 10 μM. To determine the *S/N* ratio, the emission intensity of probe **1** was measured without Cys by 7-times and the standard deviations of blank measurements were determined. Under these conditions, a good linear relationship between the fluorescence intensity and the Cys concentration (Fig. S6B) was obtained for probe **1** (R^2 = 0. 0.9787). The detection limit was then calculated with the Eq. 4:

The detection limit of **1** towards Cys at pH 7.4 was calculated to be 95.7×10^{-9} M with $S/N = 3$ (signal-to-noise ratio of 3:1).

VII. Cell Imaging and MTT Assay.

HeLa cells were purchased from National Centre for Cell Science, Pune (India). HeLa cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. The cultured cells were subcultured twice in each week, seeding at a density of about 15×10^3 cells/mL. Typan blue dye exclusion method was used to determine Cell viability. The fluorescence images were taken using Olympus Inverted IX81 equipped with Hamamatsu Orca R2 microscope by exciting at λ_{ex} = 460 – 480 nm and emission at λ_{em} = 495 – 540 nm. (by using GFP filter) and at λ_{ex} = 535 – 555 nm and emission at λ_{em} = 570 – 625 nm (by using RFP filter).

Live-cell imaging:

The HeLa cells were incubated with solution of the probe **1** (5 μM in 1:100 DMSO–DMEM v/v, pH = 7.4) at 37 ^{o}C for 10 min. After washing with PBS the fluorescence images were acquired. In this case significant red fluorescence was observed (Fig. S7A-C). In another set of experiment, HeLa cells were incubated with Lysosensor Green (1.0 μM in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37 ^{o}C for 10 min followed by washing with PBS

After washing with PBS the fluorescence images showed strong green fluorescence (Fig. S7D). These cells were then incubated with Probe **1** (5 μM in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37 ^{o}C for 10 min followed by washing with PBS After washing with PBS the fluorescence images showed strong red fluorescence (Fig. S7E). Excellent co localization of probe **1** and Lysosensor Green was observed (Fig. S7F). Cell Images shown in Fig. S7D-S7F were acquired at 60X.

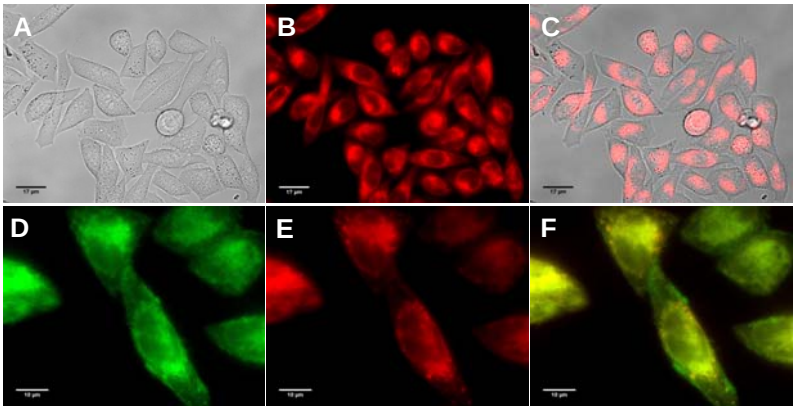


Fig. S7 Brightfield (**A**), fluorescence (**B**), and overlay (**C**) images of HeLa cells incubated with probe **1** (5.0 μM) for 10 min. Fluorescence images of cells taken in GFP filter (**D**) and RFP filter (**E**) after incubating with probe **1** (5.0 μM) for 10 min followed by incubation with LysoSensor Green (1.0 μM) for 10 min. Overlay image of images **D** and **E** (**F**).

MTT cell viability assay:

Cells were dispersed in a 96-well microtiter plate at density of 10^4 cells per 100 μL an incubated at 37 ^{o}C in a 5% CO_2 for 16 hours. Probe was added to each well in different concentration by maintaining maximum amount of DMSO at 2 μL and incubated for another 30 min. DMEM solution of probe in each well was replaced by 110 μL of MTT-DMEM mixture (0.5 mg MTT / mL of DMEM) and incubated for 4 h in identical condition. After 4 h remaining MTT solution was removed and 100 μL of DMSO was added in each well to dissolve the formazan crystals. The absorbance was recorded in a microplate reader (Varioskan Flash) at the wavelength of 570 nm. All experiments were performed in quadruplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells (Fig. S8).

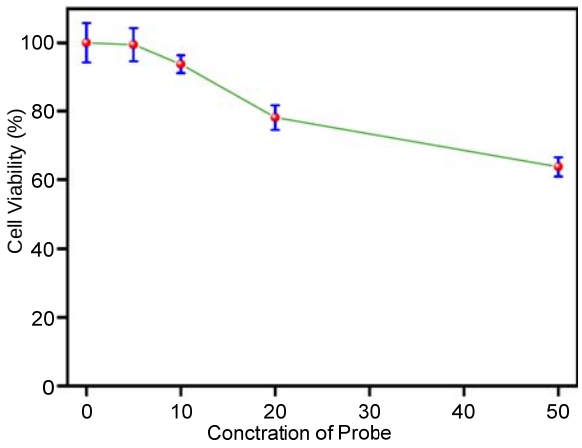


Fig. S8 Cell viability detected by MTT assay. HeLa cells were treated with probe **1** (0, 5, 10, 20 and 50 μM) before adding MTT. Results are expressed as % of the control (100%). Values are the average of three separate experiments in quadruplicate and are expressed as mean ± *slandered error*.

VIII. Mass Analysis of Cys Titration with Probe 1:

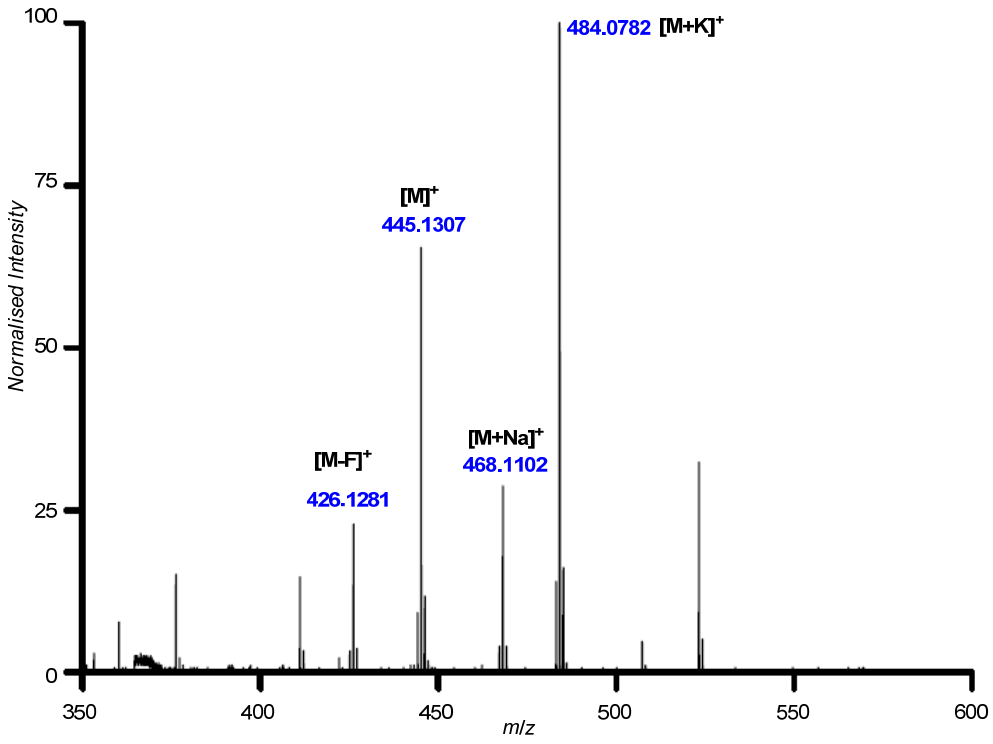


Fig. S9 MALDI-TOF spectrum probe **1** (10 μM) in the presence of a Cys (100 μM) recorded in HEPES buffer (10 mM, 1 mM CTAB, pH = 7.4).

IX. NMR Spectra.

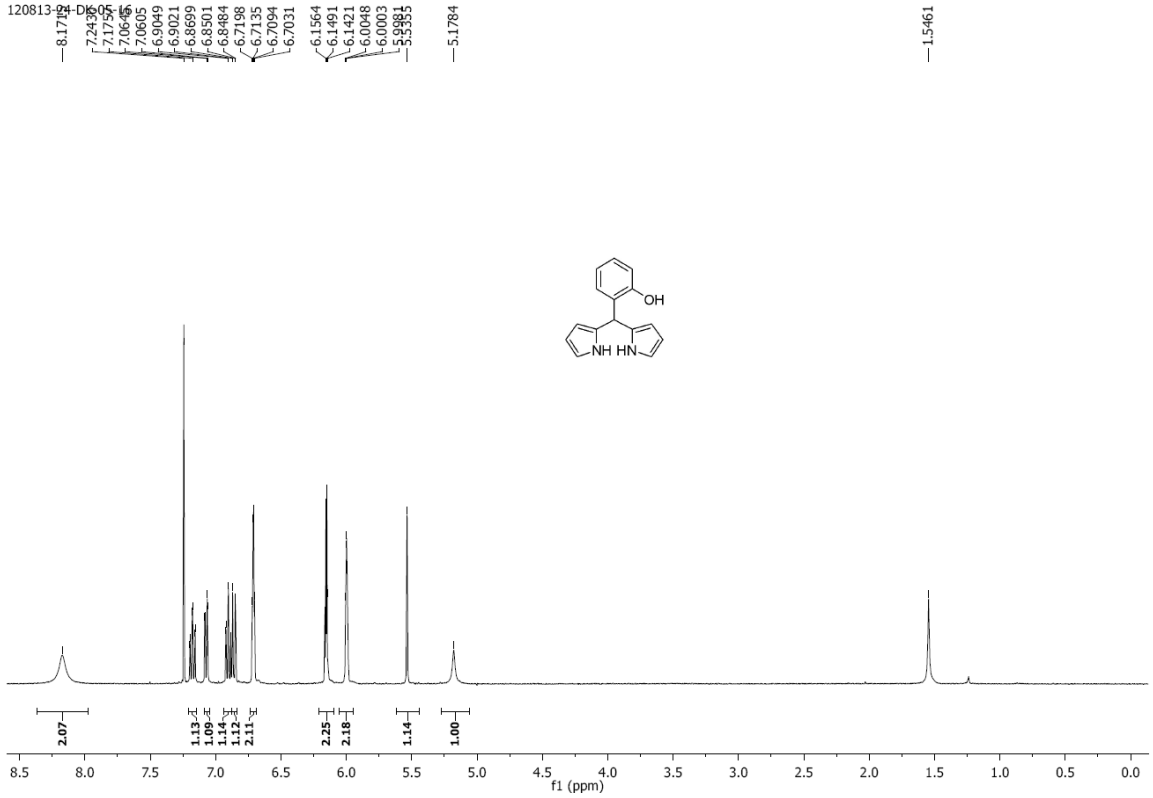


Fig. S10 ^{1}H NMR (400 MHz) spectrum of **3** in $CDCl_3$.

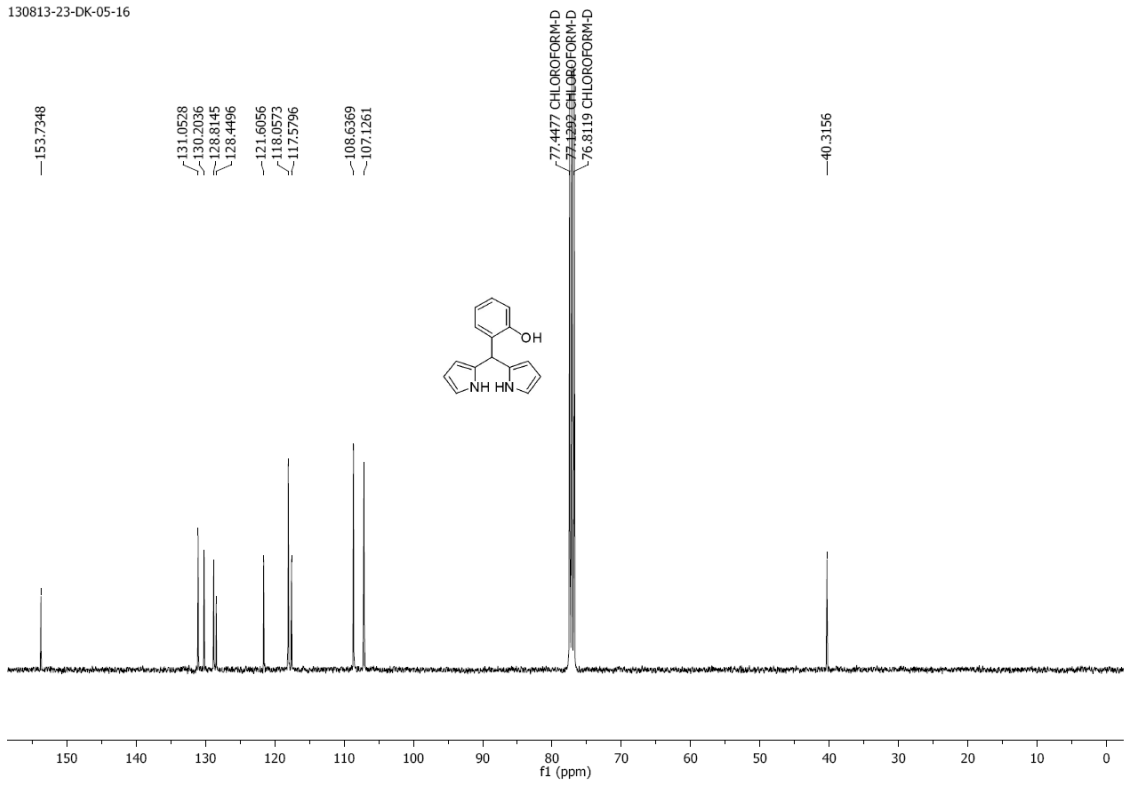


Fig. S11 ^{13}C NMR (100 MHz) spectrum of **3** in $CDCl_3$.

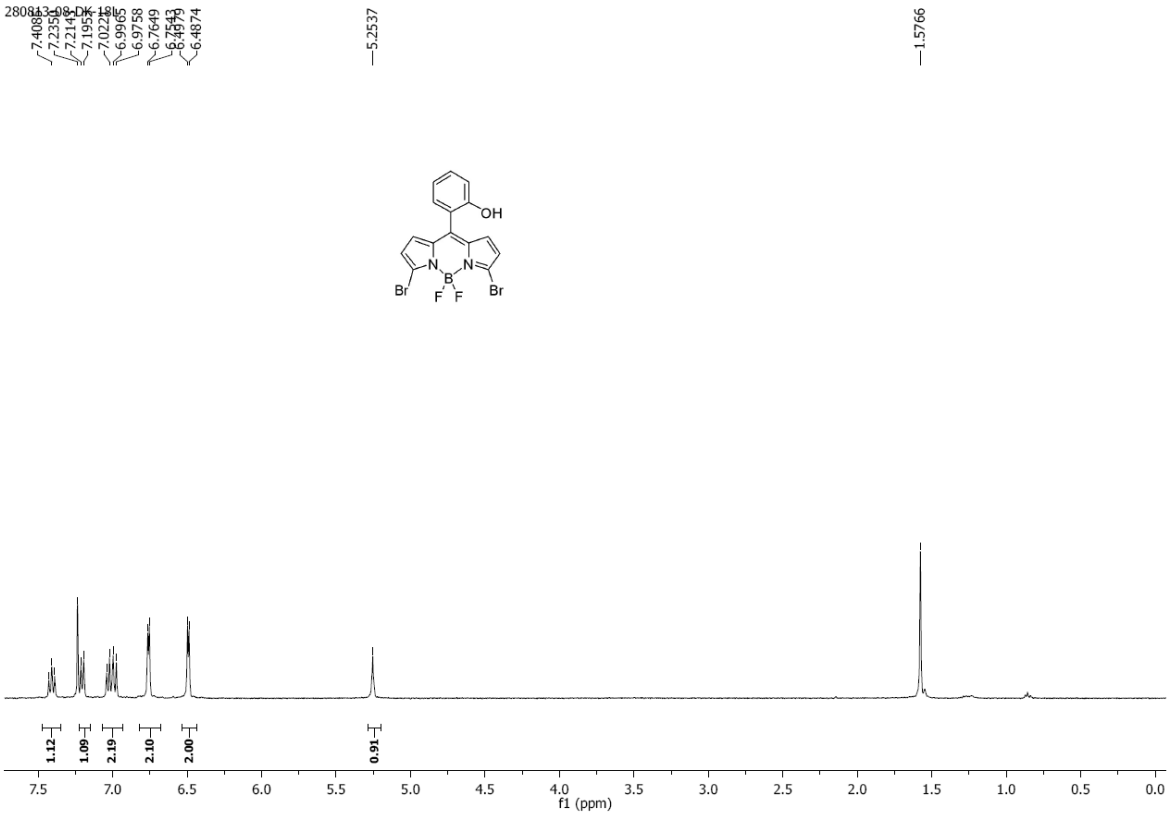


Fig. S12 ^{1}H NMR (400 MHz) spectrum of **4** in $CDCl_3$.

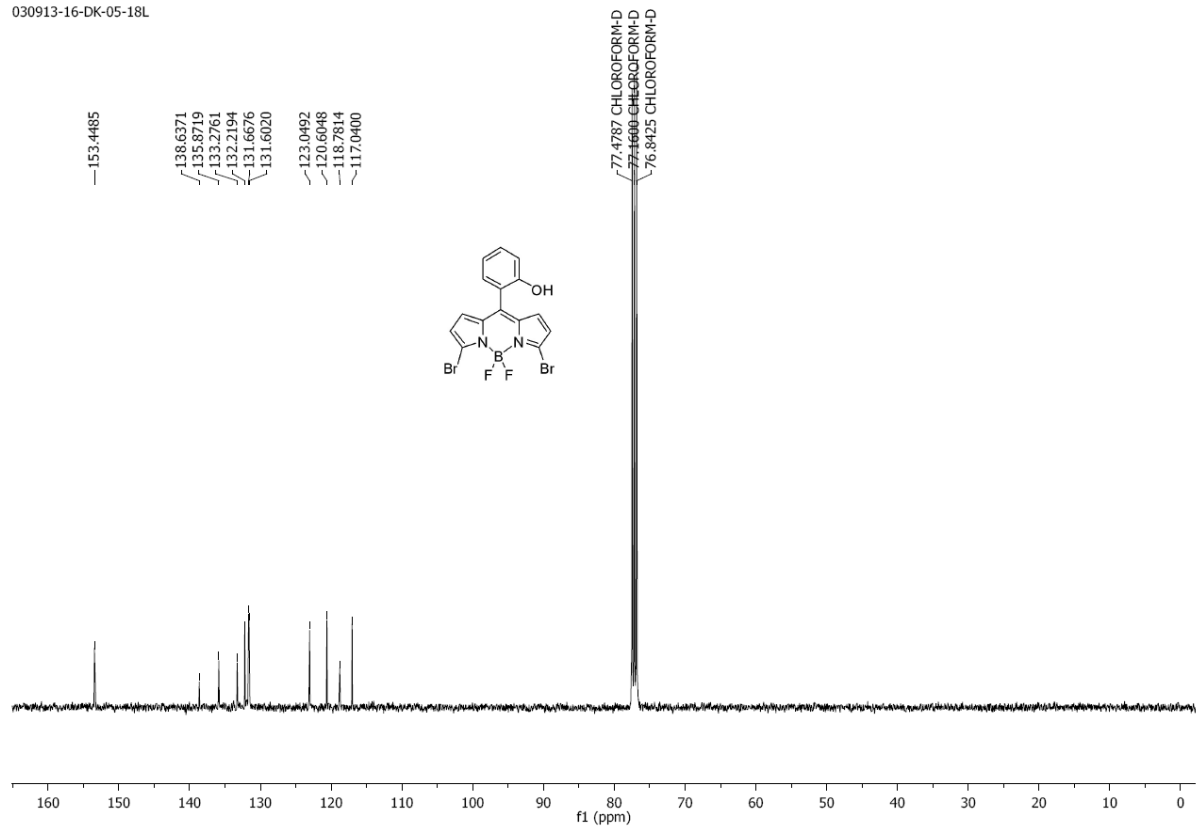


Fig. S13 ^{13}C NMR (100 MHz) spectrum of **4** in $CDCl_3$.

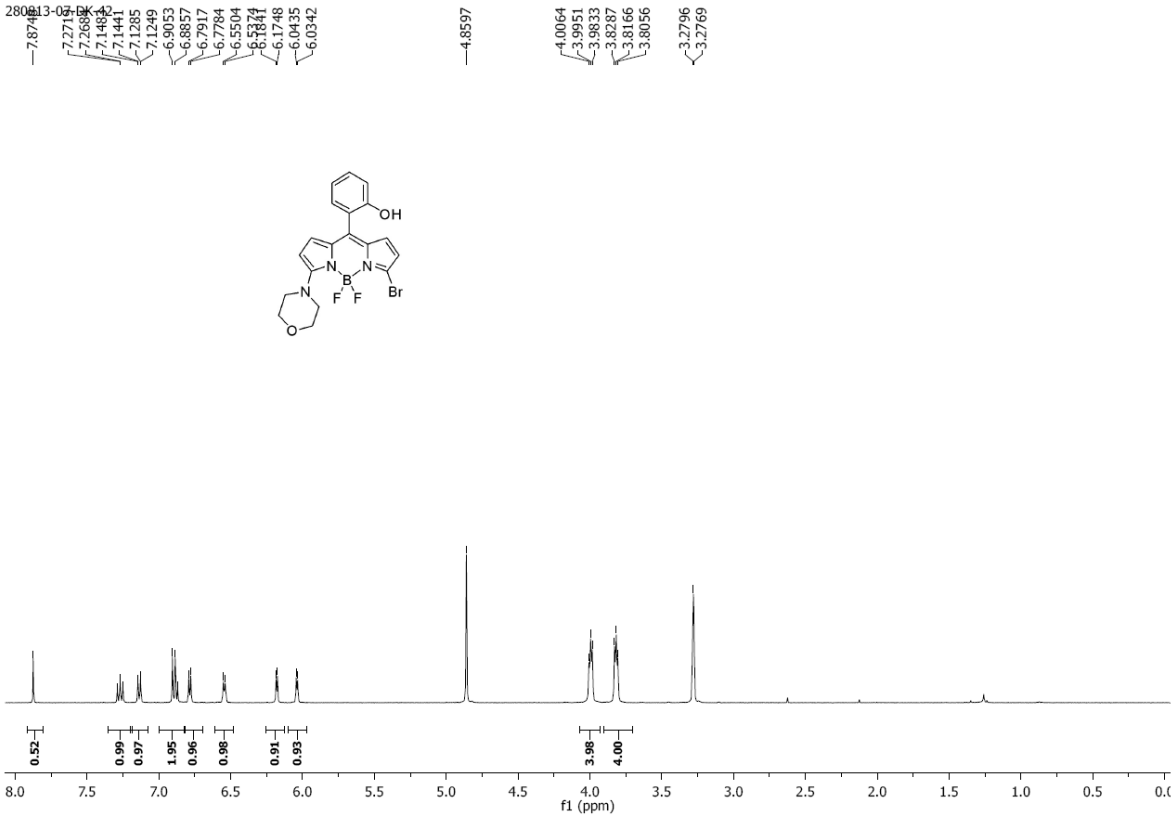


Fig. S14 ^{1}H NMR (400 MHz) spectrum of **5** in CD_3OD.

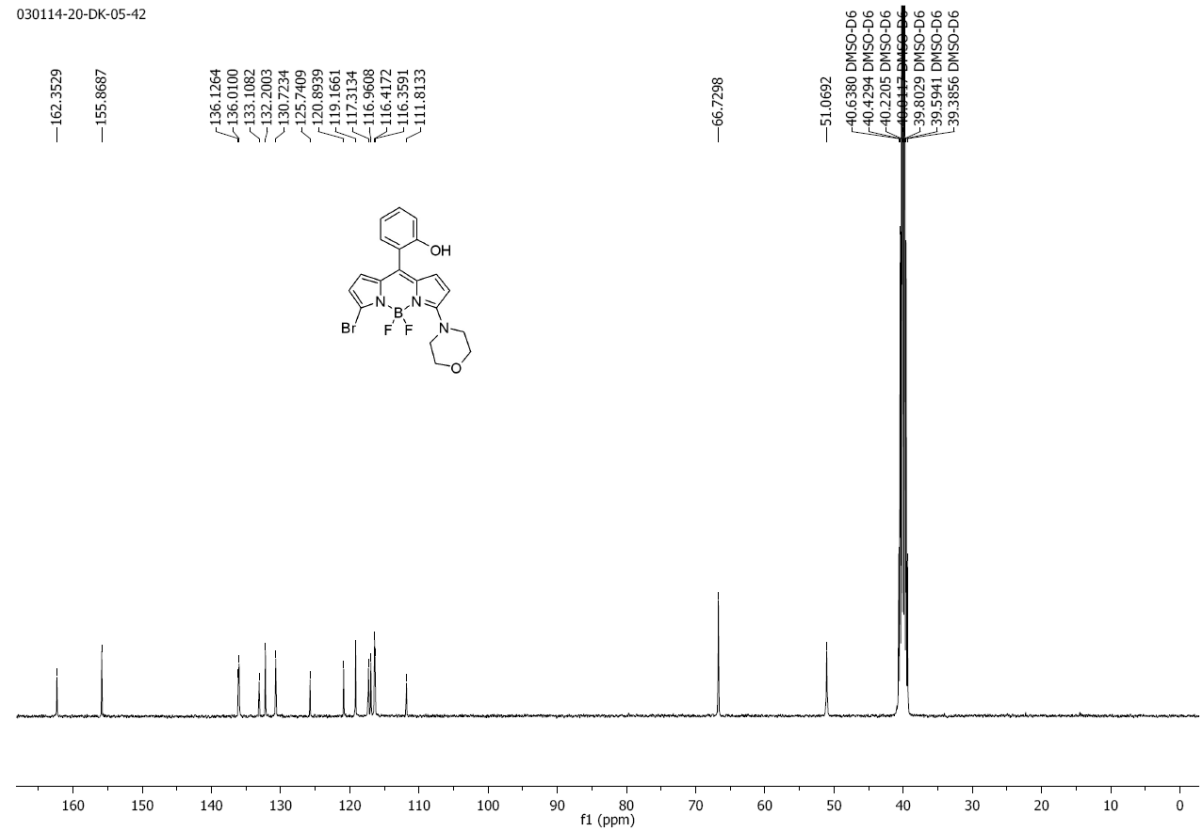


Fig. S15 ^{13}C NMR (100 MHz) spectrum of **5** in DMSO-d_6.

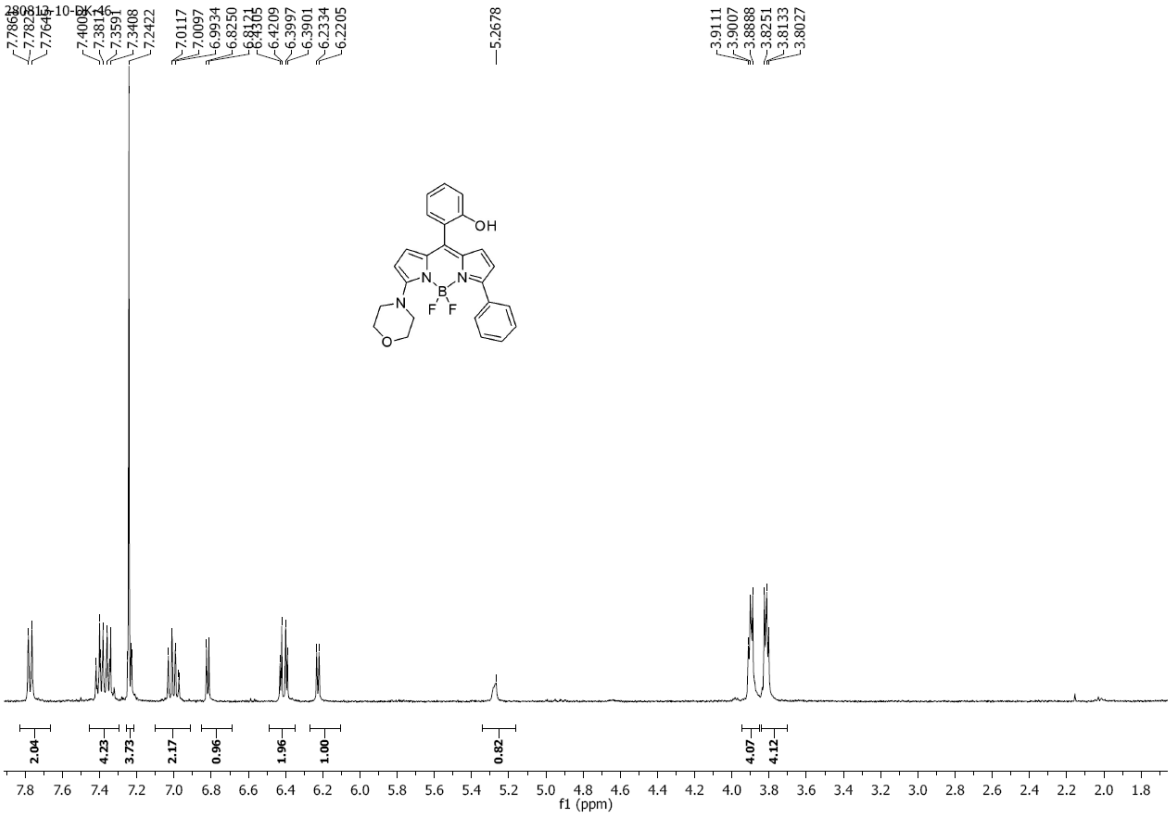


Fig. S16 ^{1}H NMR (400 MHz) spectrum of **6** in $CDCl_3$.

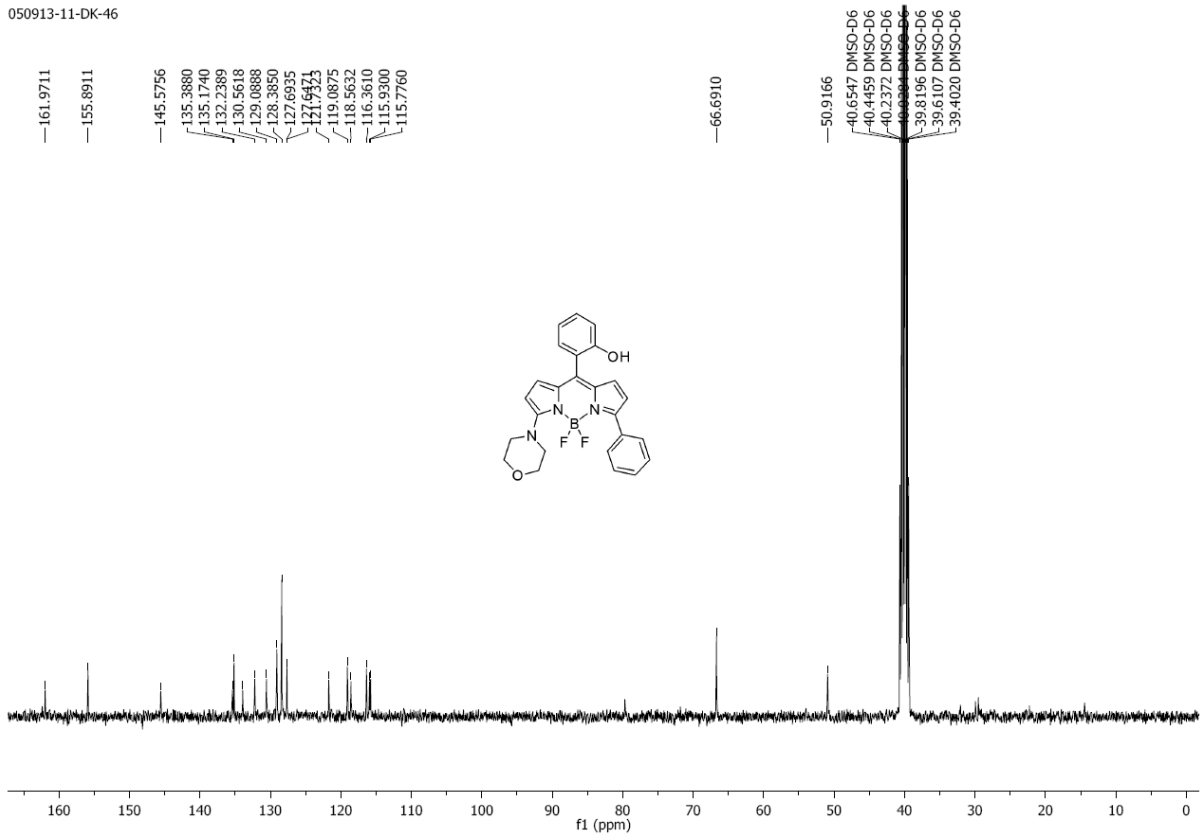


Fig. S17 ^{13}C NMR (100 MHz) spectrum of **6** in DMSO-d_6.

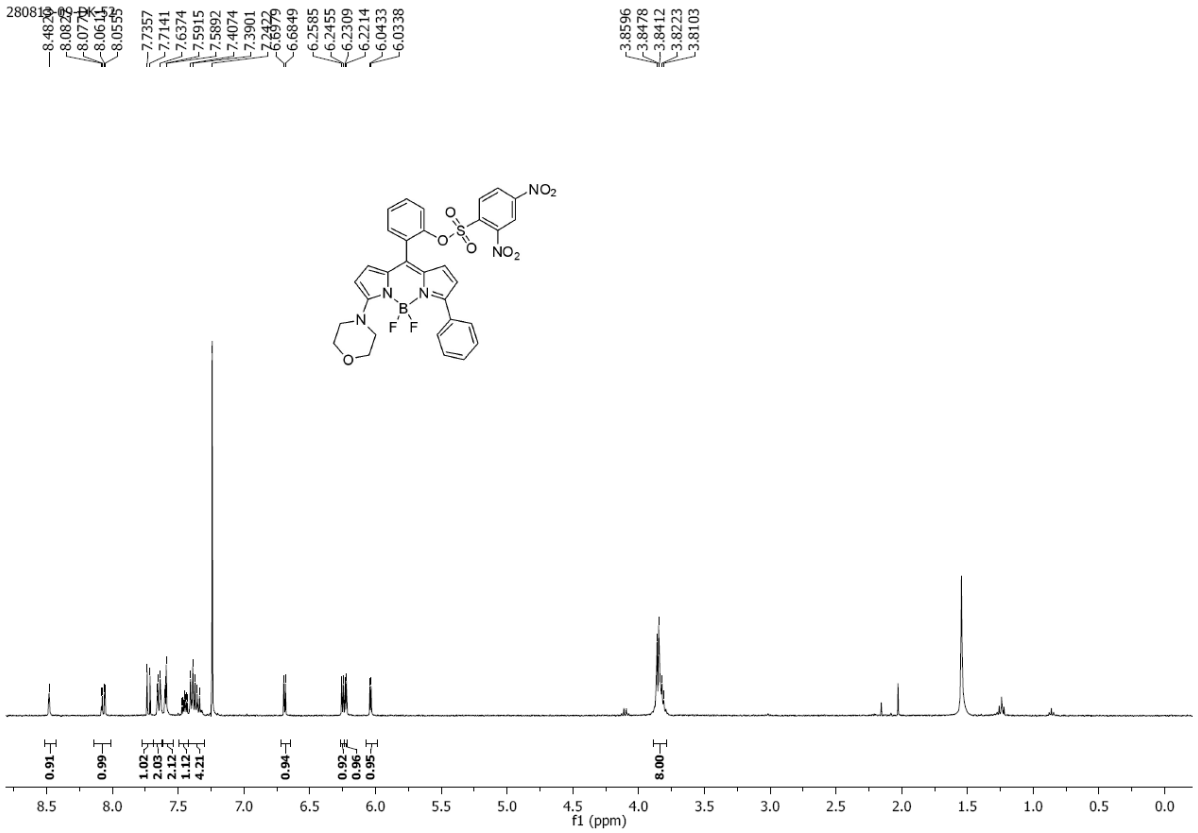


Fig. S18 ^{1}H NMR (400 MHz) spectrum of **1** in $CDCl_3$.

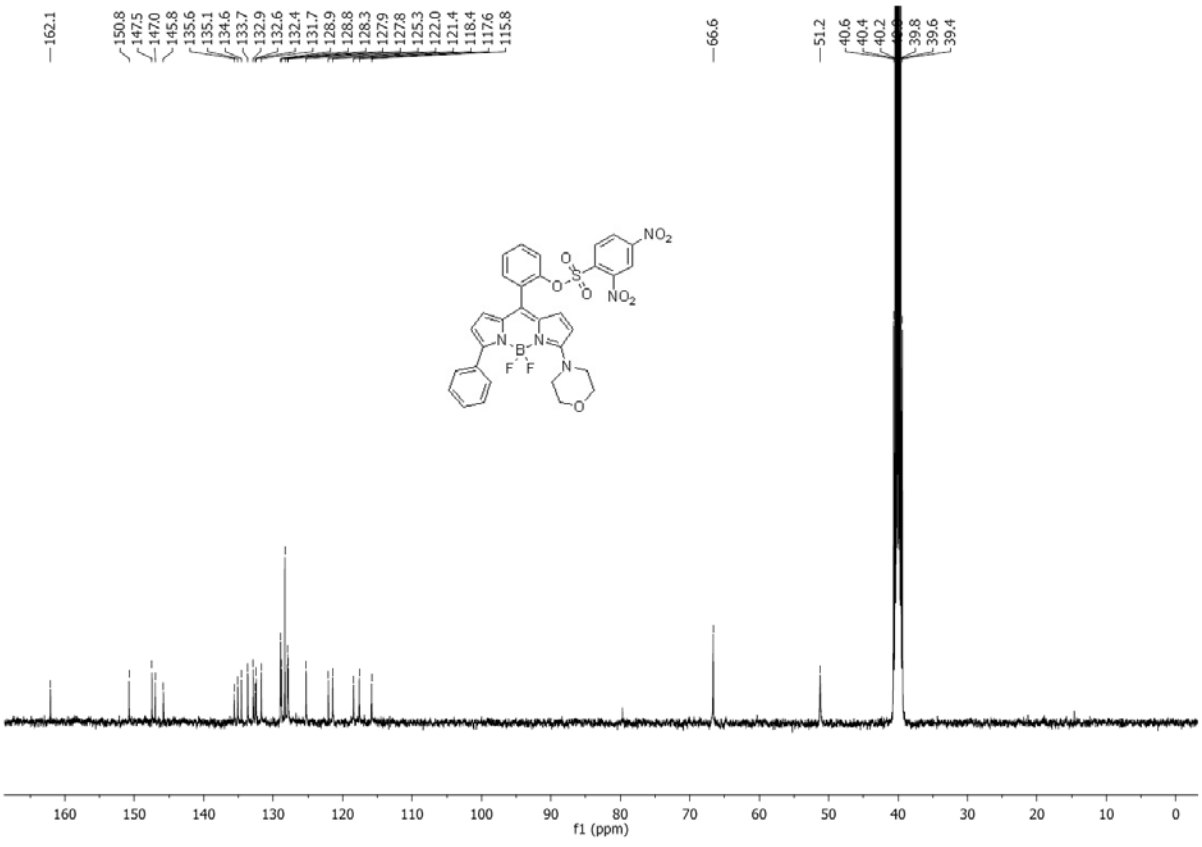


Fig. S19 ^{13}C NMR (100 MHz) spectrum of **1** in DMSO-d_6.

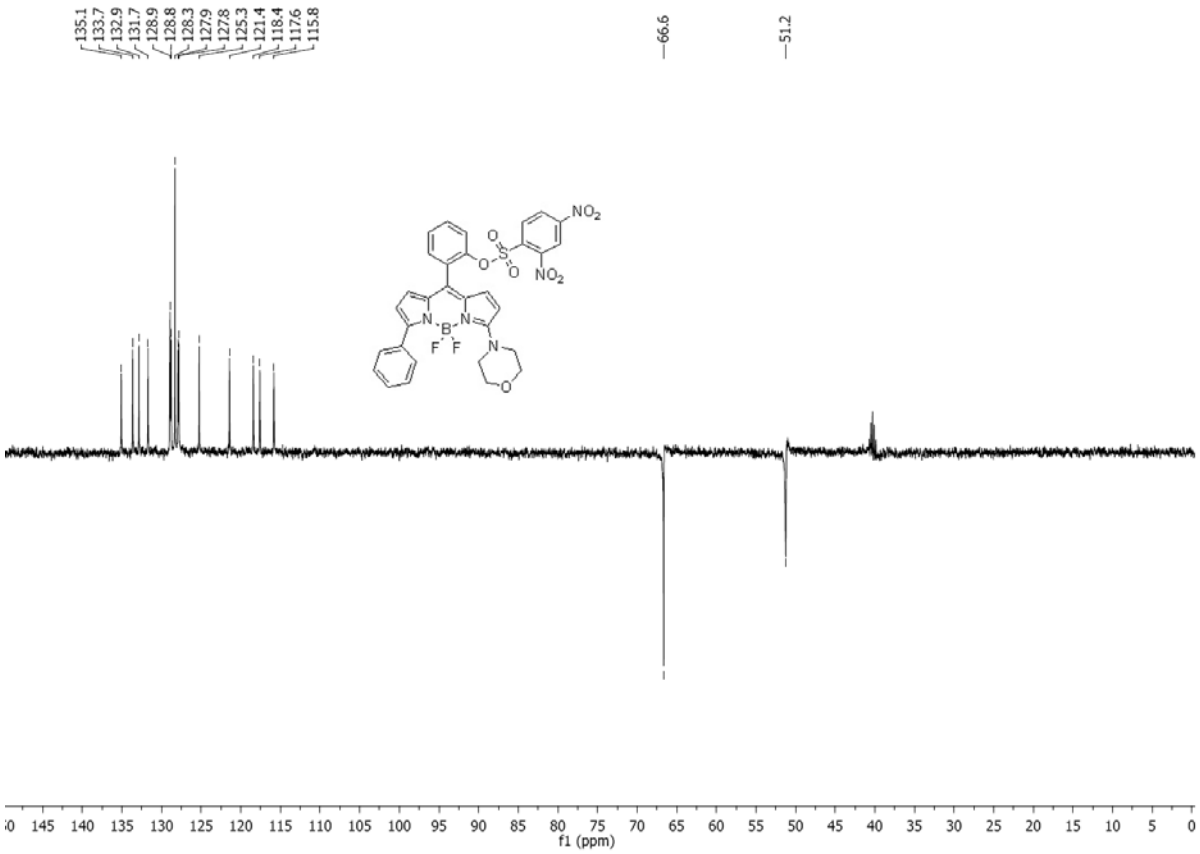


Fig. S20 DEPT-135 NMR (100 MHz) spectrum of **1** in DMSO-d_6.

X. HPLC Analysis.

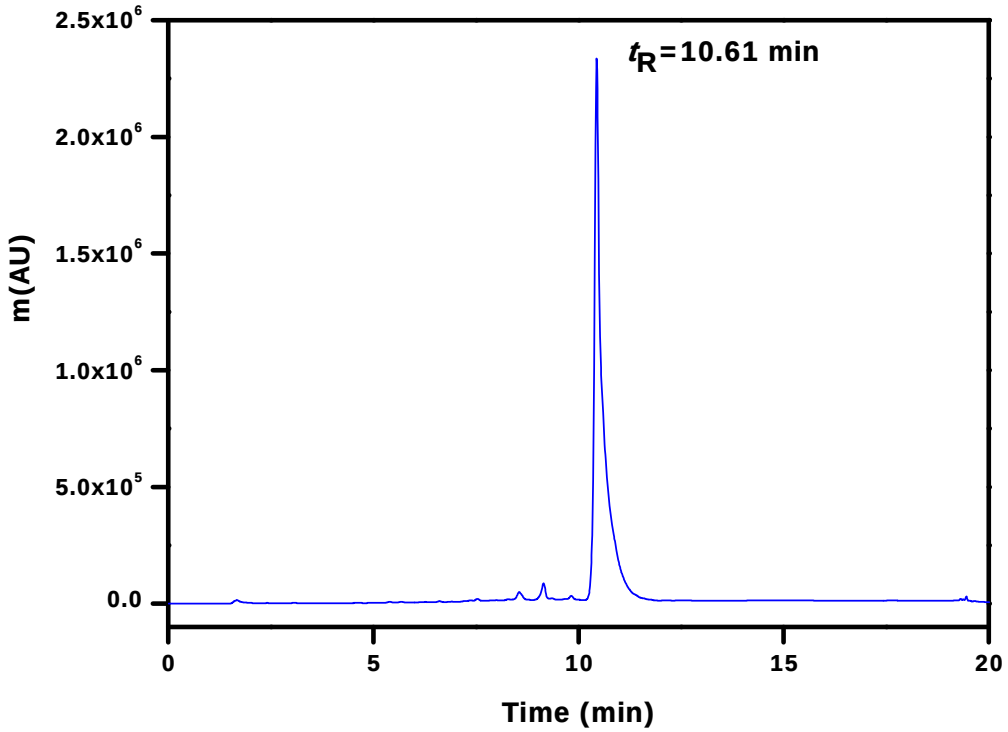


Fig. S21 HPLC data of probe **1**.

Column: Phenomenex (4.6 mm × 250 mm)

Flow: 1.0 mL/min

Method: Gradient

50 % Acetonitrile/water	0 min
100 % Acetonitrile	0 to10 min
100 % Acetonitrile	10 to 15 min
50 % Acetonitrile/water	15 to 20 min
50 % Acetonitrile/water	20 to 25 min

Wavelength: 254 nm.

Retention time (t_R) = 10.61 min.

HPLC conditions for assay:

Reverse Phase HPLC analysis was performed to confirm that the reaction of probe **1** with Cys results in the Cys mediated cleavage of DNs to form compound **6**. HPLC

chromatographs were obtained by treating probe **1** (10 μM) with increasing equivalents of Cys (0.5 eqiv and 1.0 eqiv) in mixture of acetonitrile/HEPES (10 mM, containing 1mM CTAB, pH = 7.4). HPLC gradient used for the analysis was as follows:

Column: Phenomenex (4.6 mm × 250 mm)
Flow: 1.0 mL/min
Method: Gradient

50 % Acetonitrile/water	0 min
50 % Acetonitrile	0 to15 min
100 % Acetonitrile	15 to 18 min
50 % Acetonitrile/water	18 to 25 min
50 % Acetonitrile/water	25 to 30 min

Wavelength: 254 nm.