Colorimetric and fluorescent probe for detection of nanomolar lysine in aqueous medium

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General information

Reagents and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD (300 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.16) or tetramethylsilane (TMS δ 0.00) was used as a reference. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), bs (broad

singlet). Coupling constants were reported in Hertz (Hz). High resolution mass spectra were obtained on a Micromass/Q-Toff. micro™ spectrometer. IR spectra were measured on Thermo Scientific Nicolet 380 instrument. For thin layer chromatography (TLC), Merck precoated TLC plates (Merck 60 F254) were used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine. Flash chromatography separations were performed on SRL 230-400 mesh silica gel.

Measurement of fluorescence quantum yield

For determination of the fluorescence quantum yields (Φ_{fl}), of BODIPY dye we used a Perkin-Elmer instrument, with fluorescein in 0.1 M NaOH as a fluorescence standard (fluorescence quantum yield of 0.85 in 0.1 N NaOH).

Fluorescence quantum yields (Φ_{fl}) were obtained by the following equation

$$\Phi_{fl}^{sample} = \Phi_{fl}^{standard} \, Abs^{standard} \, \Sigma[F^{sample}] / Abs^{sample} \, \Sigma[F^{standard}]$$

Where F denotes fluorescence intensity at each wavelength and Σ [*F*] was calculated by summation of fluorescence intensity.[1]

solvent	Absorbance maximum	Fluorescence maximum	Quantum yield (%)
CH_2Cl_2	504	517	80
Methanol	503	515	68

Equation used for calculating detection limit (DL)

DL = CL × CT; where CL = conc. of ligand; CT = conc. of Lys at which fluorescence enhanced.

Thus, DL = $1 \times 10^{-6} \times 0.001$ μM = 0.001×10^{-6} μM.

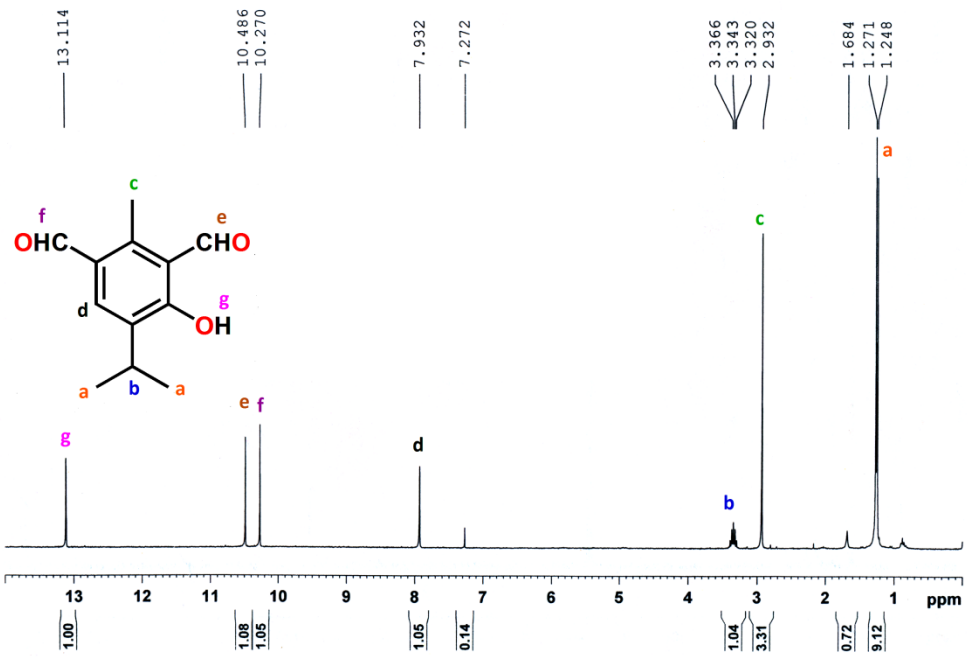


Figure S-1 ^{1}H NMR spectrum of **DFTH** in $CDCl_3$

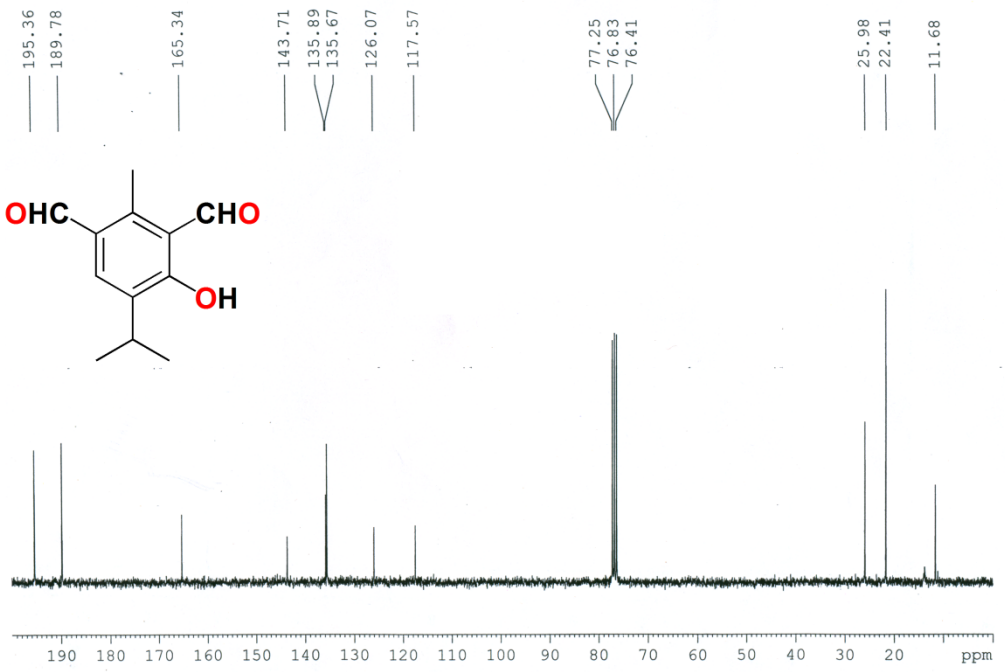


Figure S-2 ^{13}C spectrum of **DFTH** in $CDCl_3$

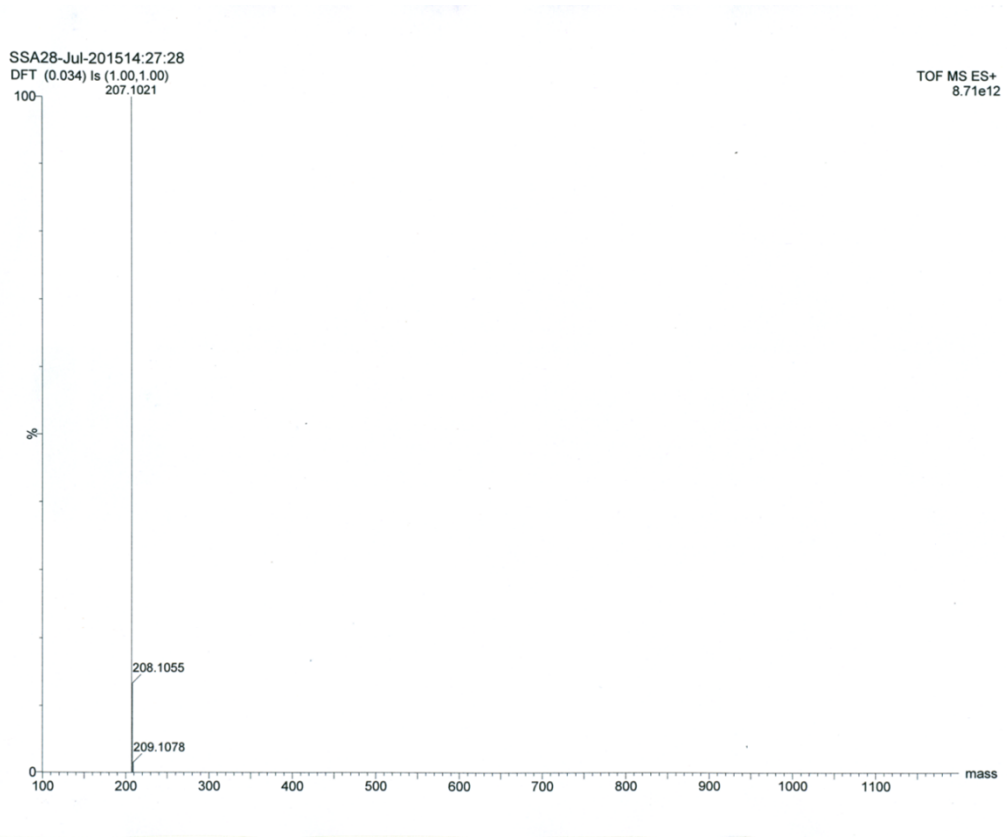


Figure S-3 HRMS spectrum of **DFTH** in methanol

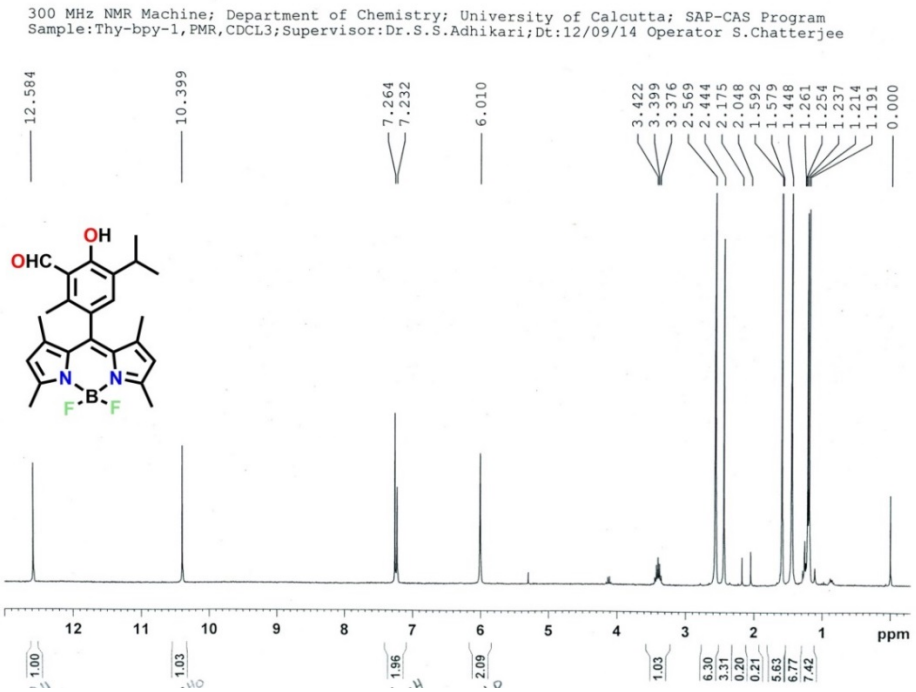


Figure S-4 ^{1}H NMR spectrum of **THBPY** in $CDCl_3$

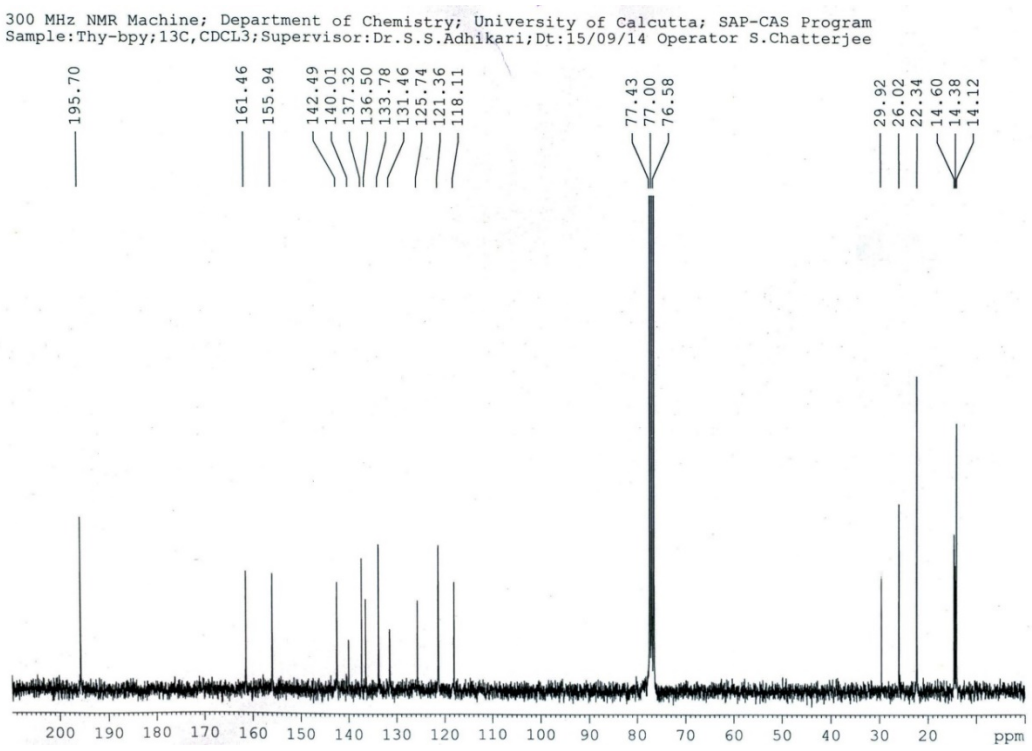


Figure S-5 ^{13}C NMR spectrum of **THBPY** in $CDCl_3$

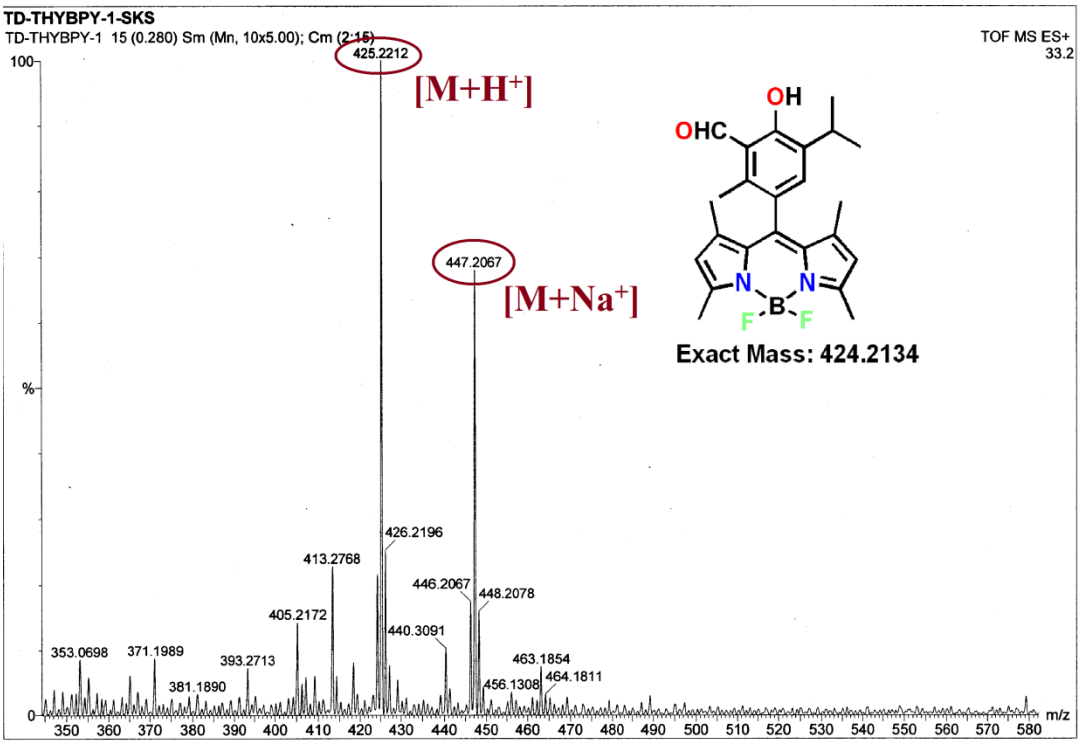


Figure S-6 HRMS spectrum of **THBPY** in methanol

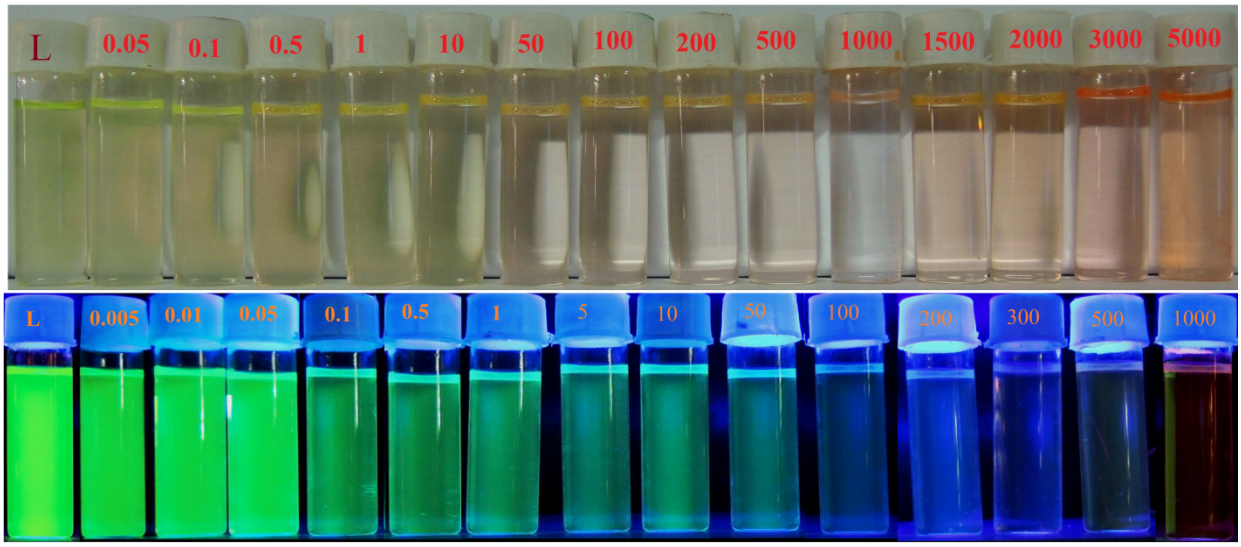


Figure S-7 Color changes of **THBPY** (10 μM) in DMSO–HEPES buffer (0.01 M, pH 7.4) (1: 9, v/v) media upon gradual addition of Lys from 0.05 to 5000.0 μM (bare eye, top) and under UV light for Lys 0.005 μM to 1000 μM (bottom).

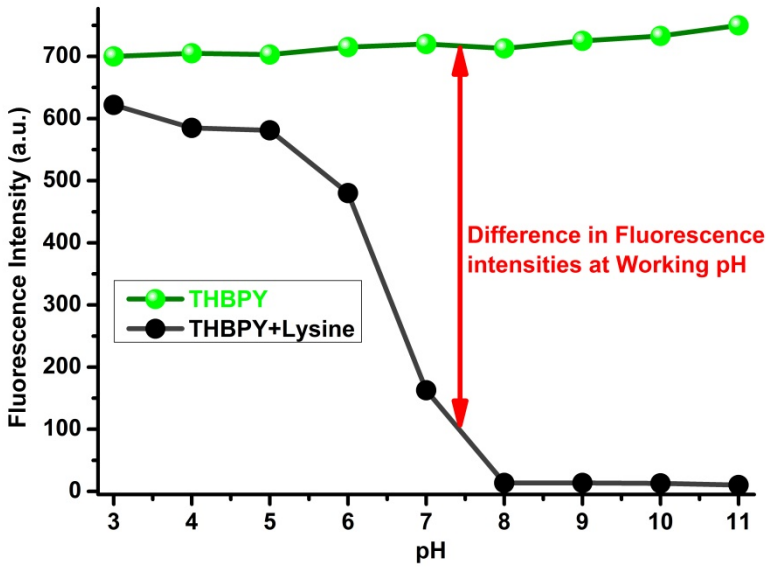


Figure S-8 Effect of pH on the emission intensity of **THBPY** and [**THBPY-**Lys] adduct

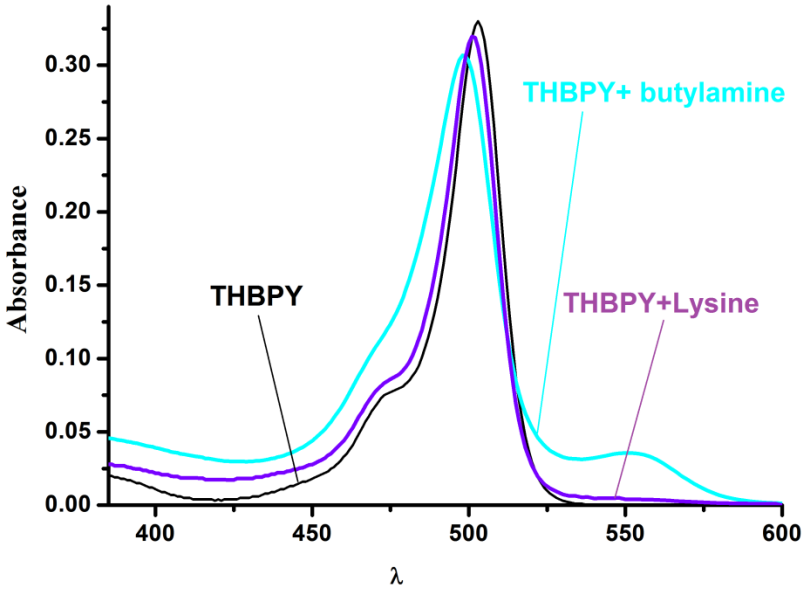


Figure S-9 Change in absorbance of **THBPY** upon addition of butyl amine in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4)

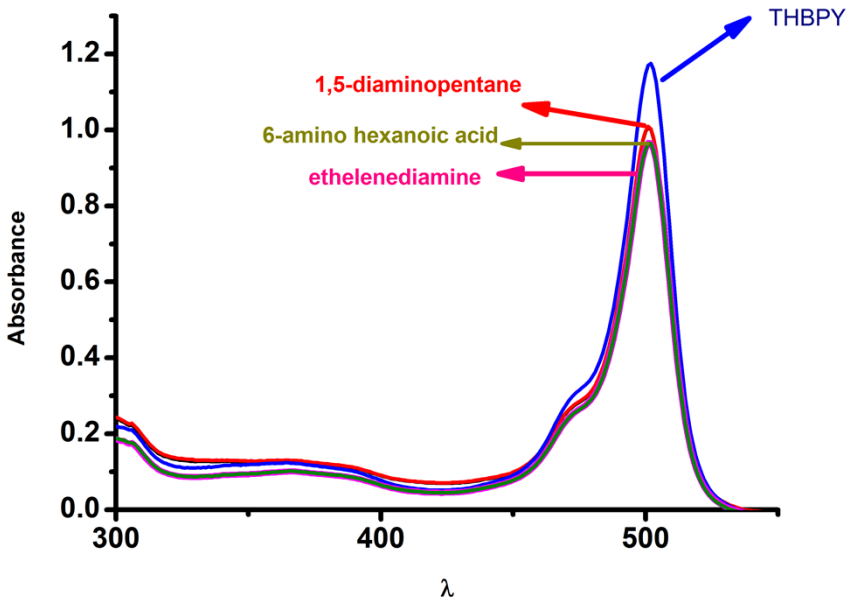


Figure S-10 Changes in absorbance of **THBPY** (10 μM) upon addition of 1,5-diaminopentane, 6-amino hexanoic acid and ethylenediamine (50 μM) in DMSO–HEPES buffer, 1: 9, v/v, 0.01 M, pH 7.4 .

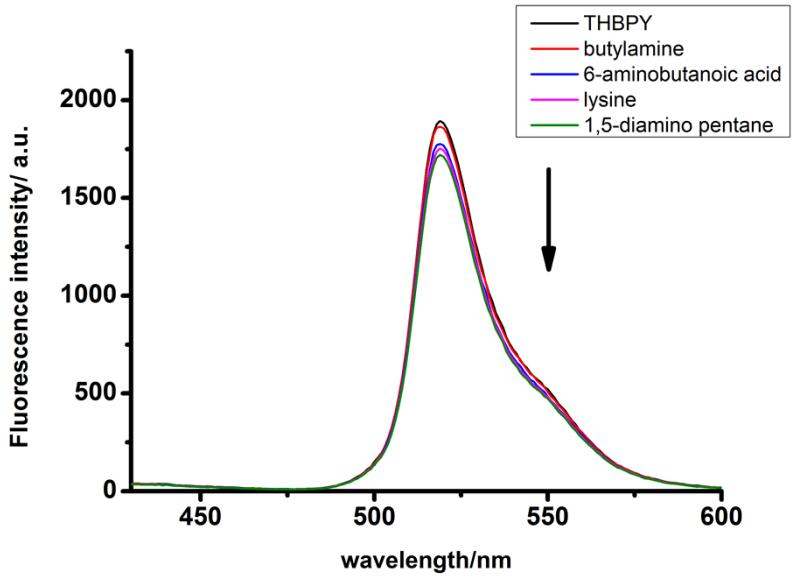


Figure S-11 Changes in fluorescence of **THBPY** (10 μM) upon addition of butyl amine, 1,5-diaminopentane, 6-amino hexanoic acid, ethelenediamine (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4) .

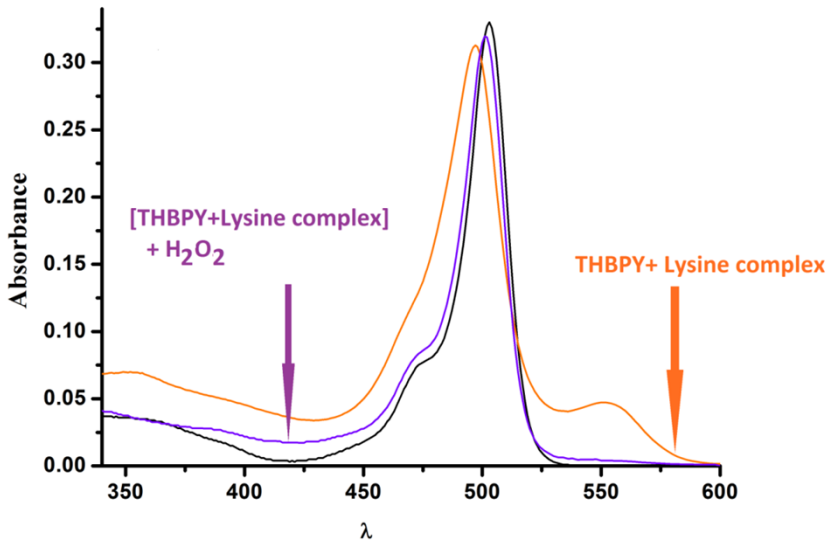


Figure S-12 Reversibility of absorbance of [**THBPY**+ Lys] adduct (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4) upon addition of 10,100 μM H_2O_2 solutions.

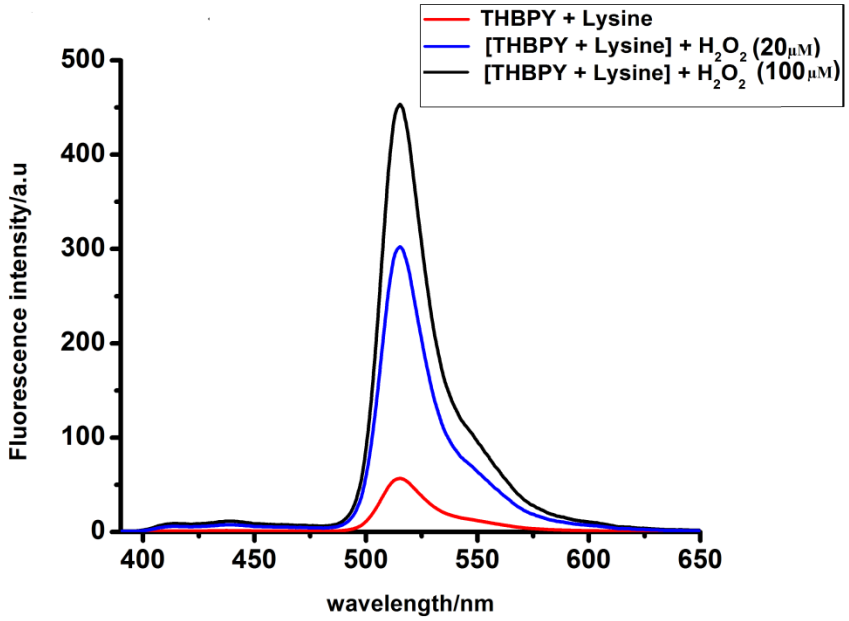


Figure S-13 Reversibility of fluorescence spectrum of the [**THBPY**+ Lys] adduct (10 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4) upon addition of 20,100 μM H_2O_2 solutions.

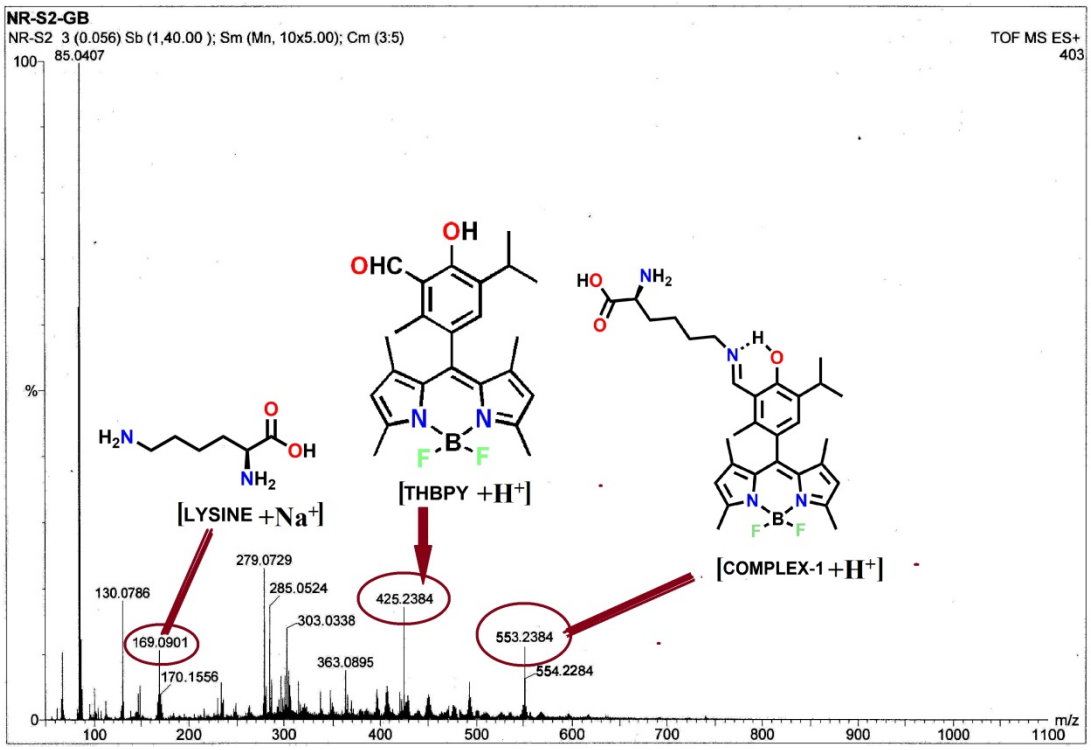


Figure S-14 HRMS spectrum of the solution of [**THBPY**+ Lys] adduct after treatment of H_2O_2.

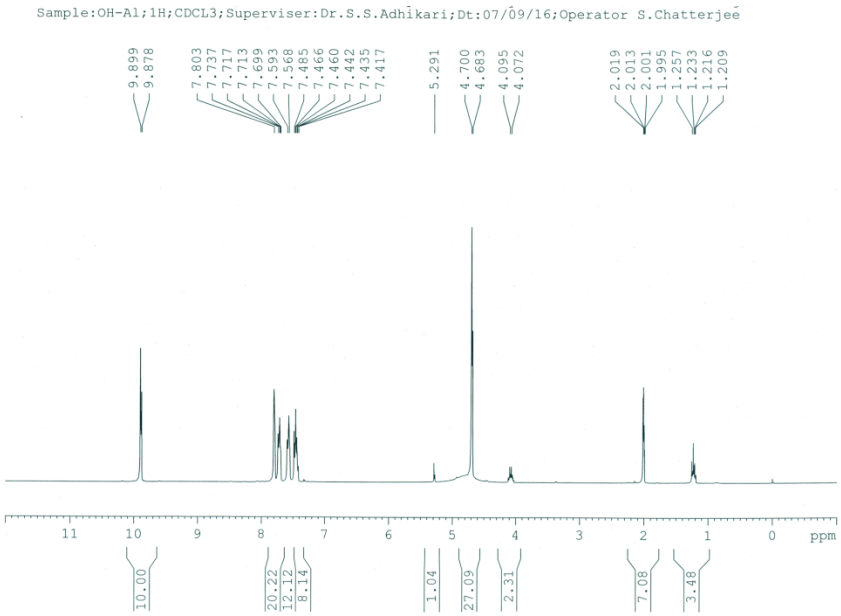


Figure S-15 ^{1}H NMR spectrum of **1** in $CDCl_3$

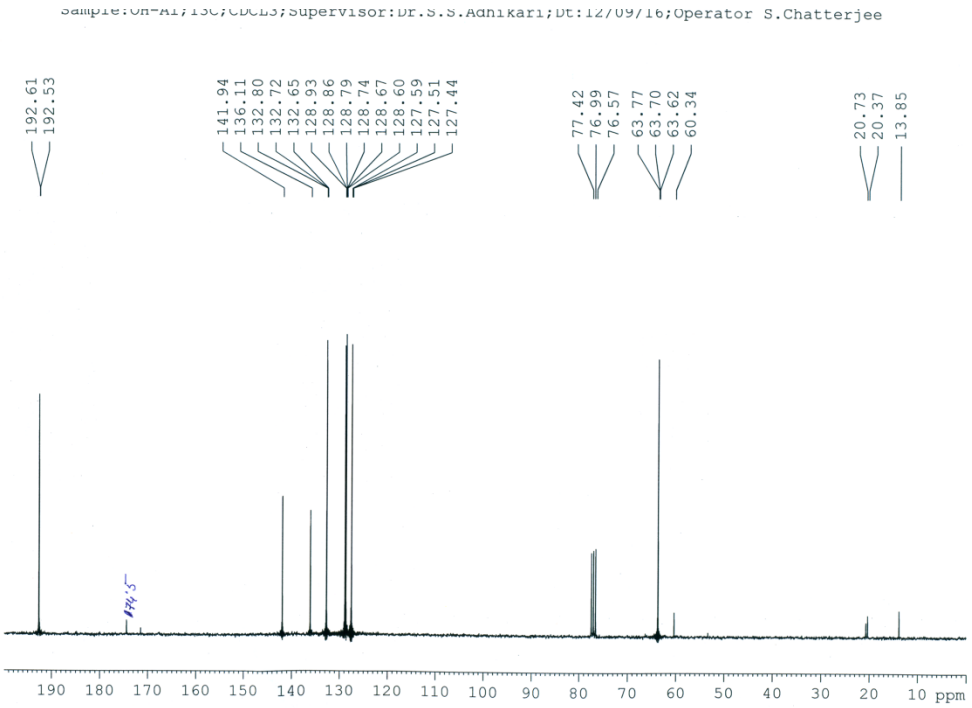


Figure S-16 ^{13}C NMR spectrum of **1** in $CDCl_3$

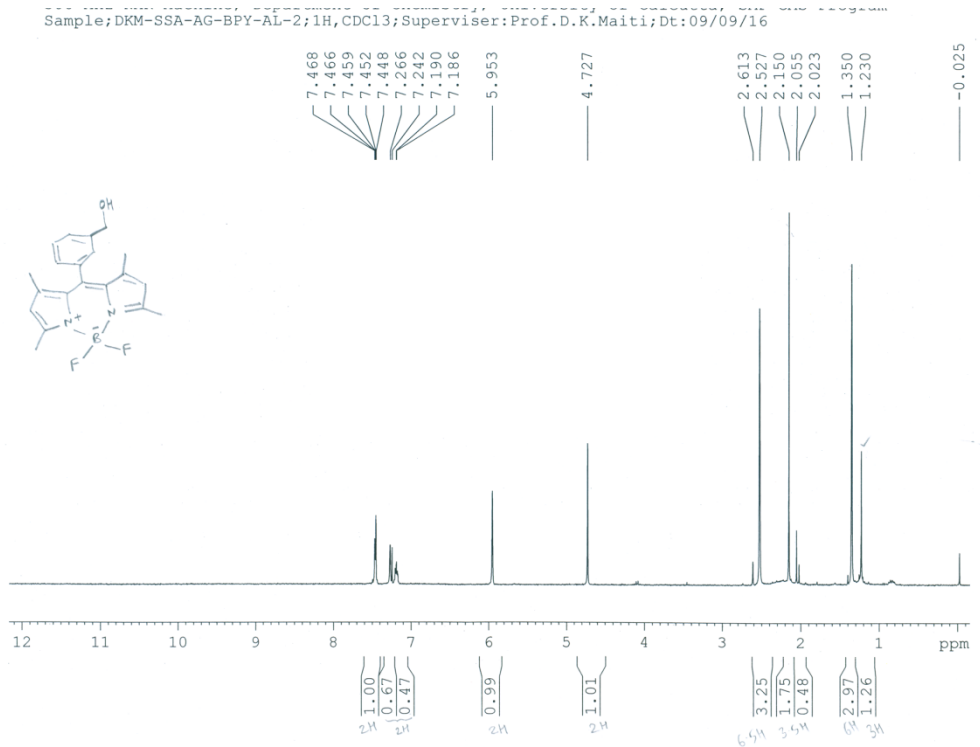


Figure S-17 ^{1}H NMR spectrum of **2** in $CDCl_3$

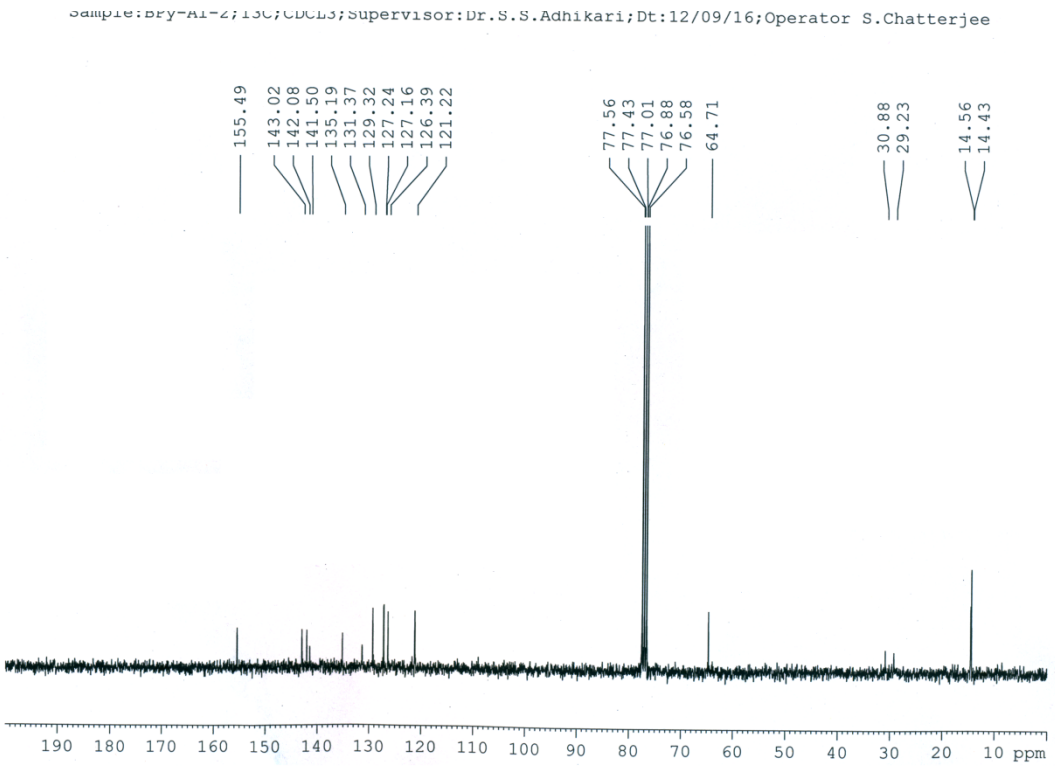


Figure S-18 ^{13}C NMR spectrum of **2** in $CDCl_3$

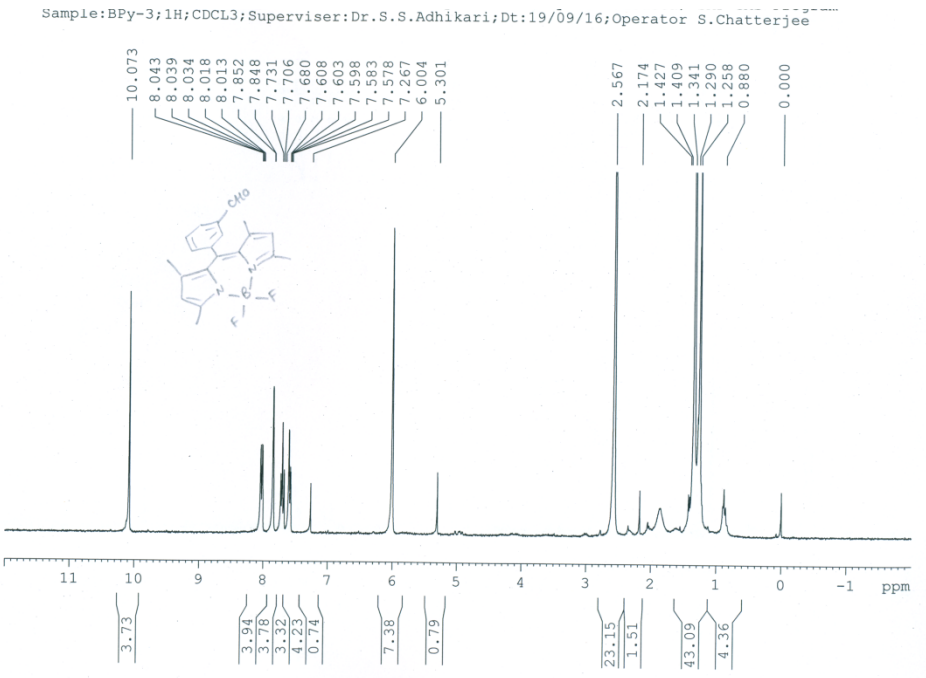


Figure S-19 ^{1}H NMR spectrum of **3** in $CDCl_3$

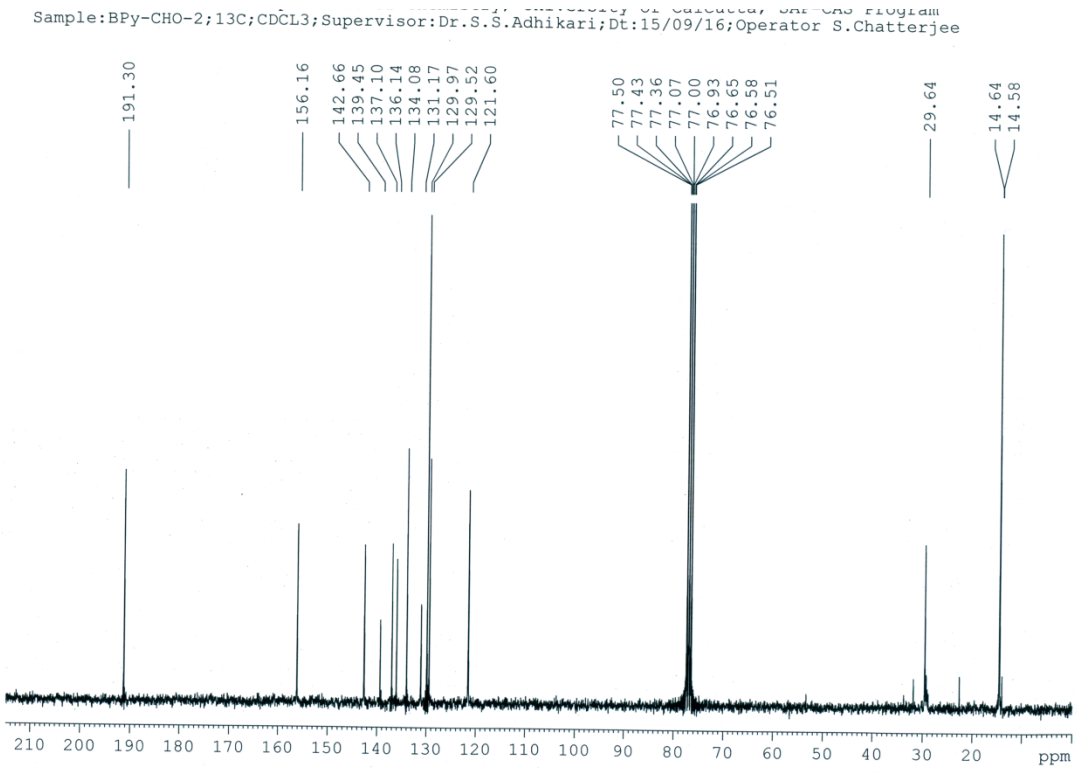


Figure S-20 ^{13}C NMR spectrum of **3** in $CDCl_3$

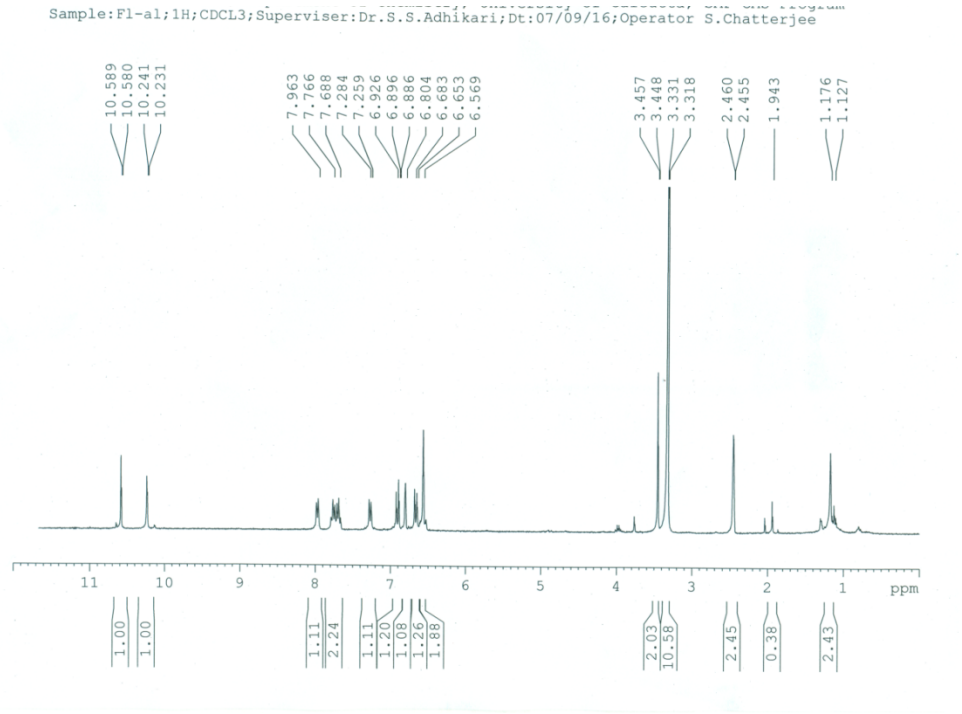


Figure S-21 ^{1}H NMR spectrum of **4** in DMSO-d_6

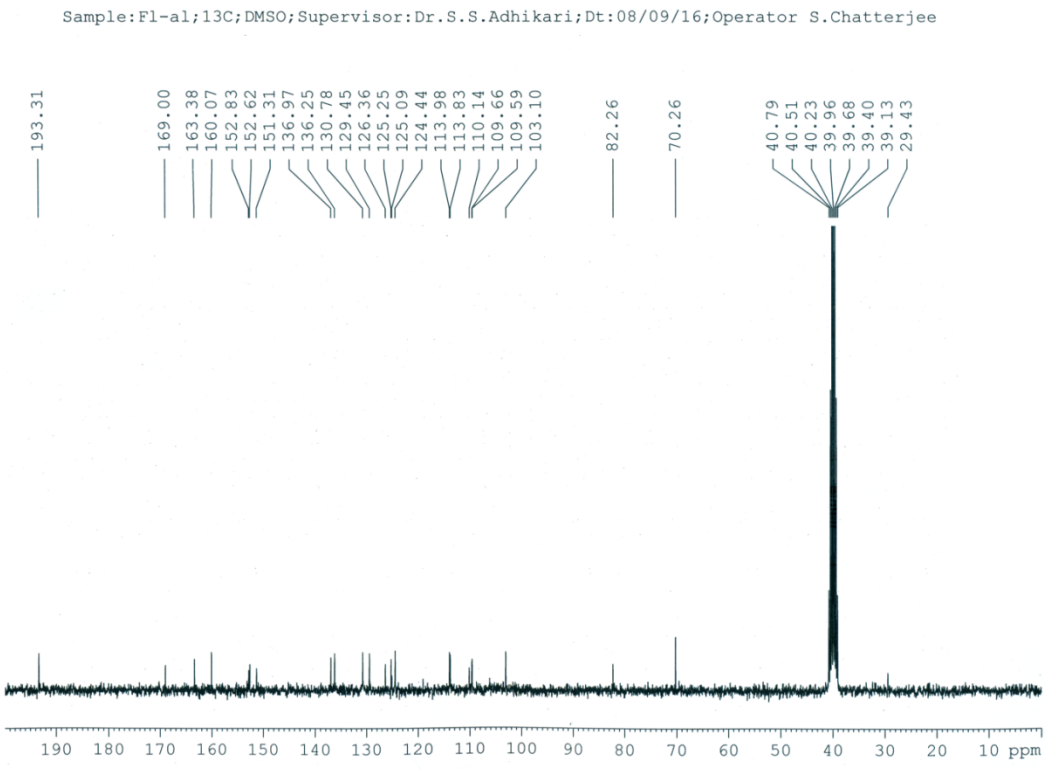


Figure S-22 ^{13}C NMR spectrum of **4** in DMSO-d_6

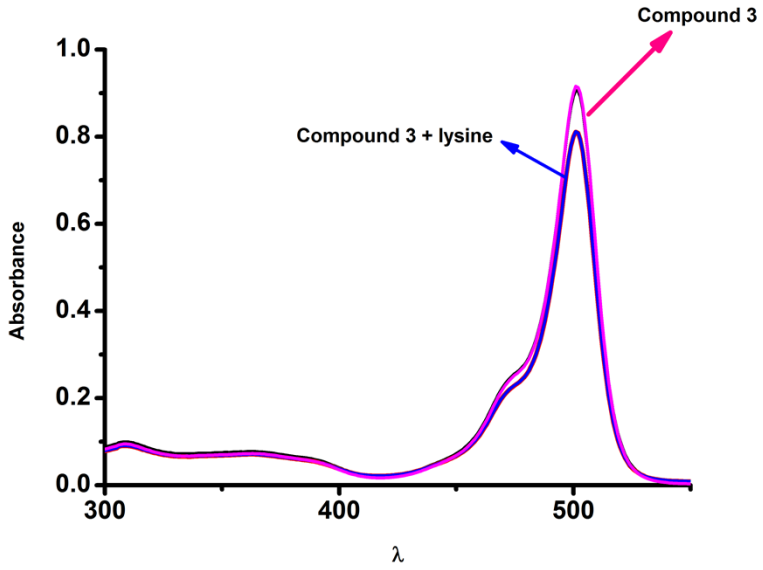


Figure S-23 Changes in the absorbance of **3** (10 μM) upon addition of Lys (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4).

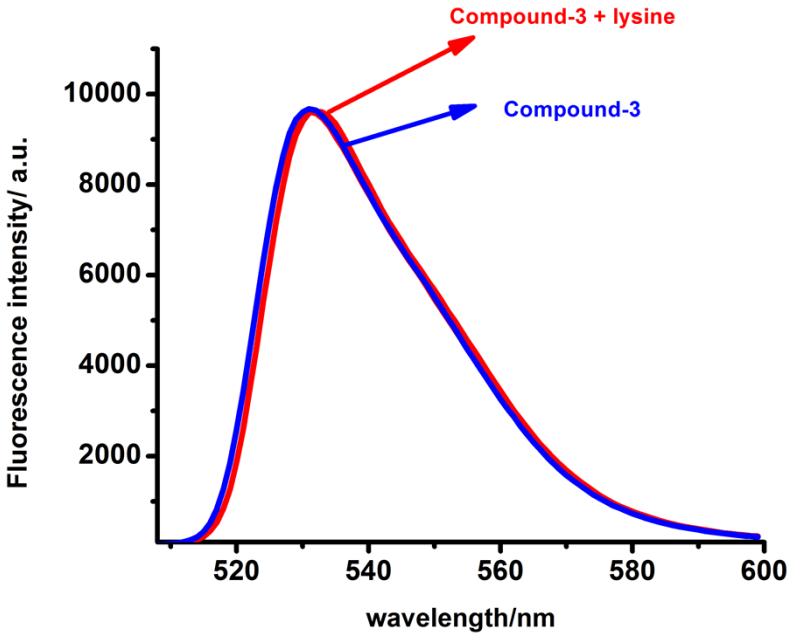


Figure S-24 Changes in the emission profile of **3** (10 μM) upon addition of Lys (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4).

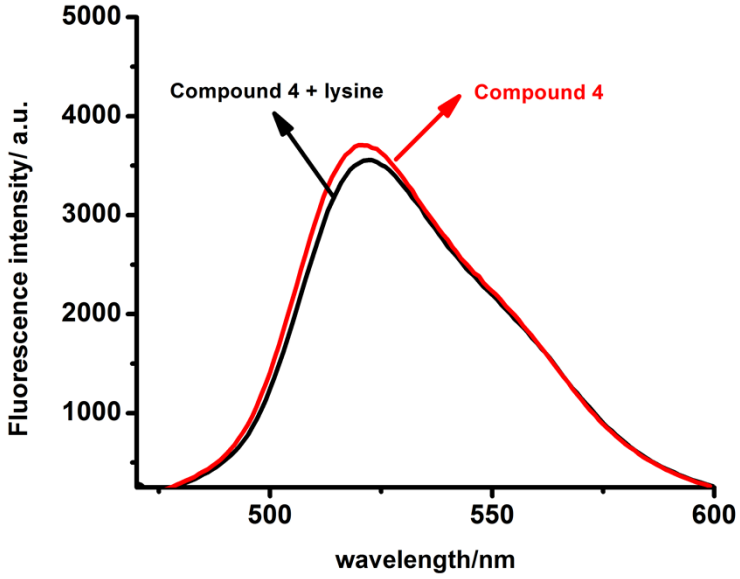


Figure S-25 Changes in emission profile of **4** (10 μM) upon addition of Lys (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4).

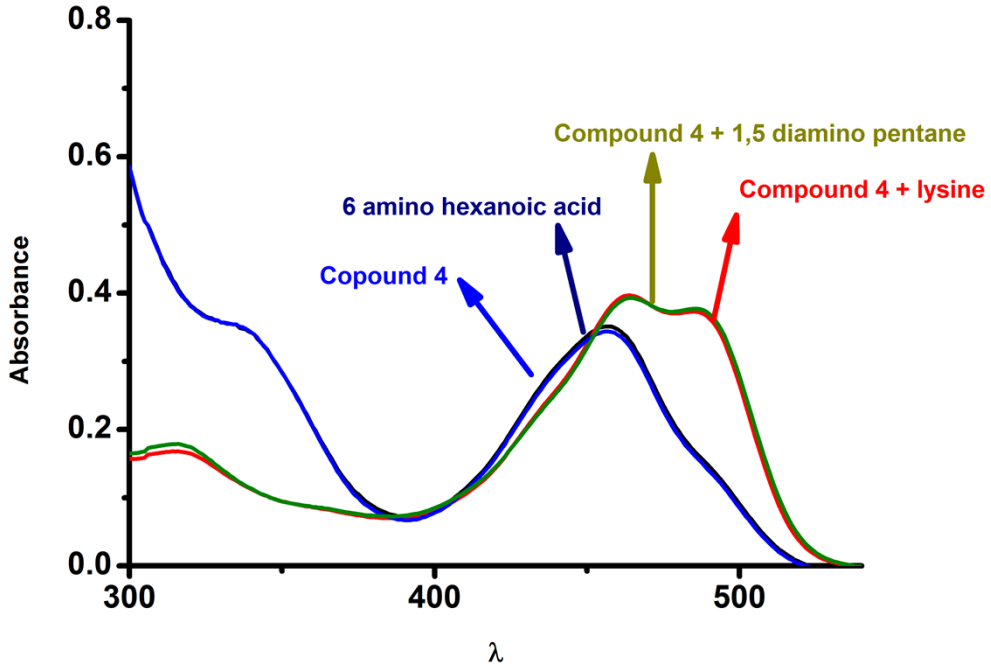


Figure S-26 Change in absorbance of 4 (10 μM) upon addition of butyl amine 1,5-diaminopentane, 6-amino hexanoic acid (50 μM in DMSO–HEPES buffer, 1: 9, v/v, 0.01 M, pH 7.4) .

Table S-1 Crystal data and structure refinement for THBPY (**CCDC No. 1409304**)

Bond lengths (Å)

(B1)-(F1)	1.380(3)	(C8)-(N2)	1.409(4)	(C7)-(C6)	1.406(3)
(F1)-(B1)	1.380(3)	(N2)-(C10)	1.344(3)	(C6)-(C7)	1.406(3)
(B1)-(F2)	1.402(4)	(C10)-(N2)	1.344(3)	(C7)-(C14)	1.494(4)
(F2)-(B1)	1.402(4)	(N1)-(C6)	1.409(4)	(C14)-(C7)	1.494(4)
(B1)-(N2)	1.545(4)	(C6)-(N1)	1.409(4)	(C8)-(C12)	1.437(3)
(N2)-(B1)	1.545(4)	(N1)-(C2)	1.340(3)	(C12)-(C8)	1.437(3)
(B1)-(N1)	1.552(4)	(C2)-(N1)	1.340(3)	(C6)-(C4)	1.416(3)
(N1)-(B1)	1.552(4)	(C7)-(C8)	1.389(3)	(C4)-(C6)	1.416(3)
(N2)-(C8)	1.409(4)	(C8)-(C7)	1.389(3)	(C12)-(C13)	1.499(4)

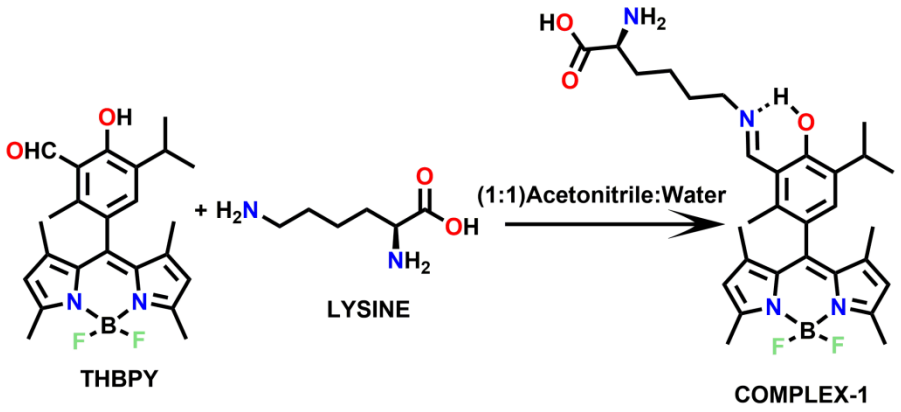
(C13)-(C12)	1.499(4)	(C13)-(H13C)	0.96	(C18)-(C19)	1.415(4)
(C12)-(C11)	1.373(4)	(H13C)-(C13)	0.96	(C19)-(C14)	1.390(4)
(C11)-(C12)	1.373(4)	(C3)-(H3)	0.93	(C14)-(C19)	1.390(4)
(C10)-(C9)	1.493(5)	(H3)-(C3)	0.93	(C19)-(C20)	1.507(4)
(C9)-(C10)	1.493(5)	(C5)-(H5A)	0.961	(C20)-(C19)	1.507(4)
(C10)-(C11)	1.396(4)	(H5A)-(C5)	0.961	(C18)-(C17)	1.413(4)
(C11)-(C10)	1.396(4)	(C5)-(H5B)	0.959	(C17)-(C18)	1.413(4)
(C4)-(C3)	1.381(4)	(H5B)-(C5)	0.959	(C18)-(C24)	1.454(5)
(C3)-(C4)	1.381(4)	(C5)-(H5C)	0.96	(C24)-(C18)	1.454(5)
(C4)-(C5)	1.505(4)	(H5C)-(C5)	0.96	(C15)-(H15)	0.929
(C5)-(C4)	1.505(4)	(C11)-(H11)	0.93	(H15)-(C15)	0.929
(C2)-(C3)	1.402(5)	(H11)-(C11)	0.93	(C15)-(C14)	1.409(4)
(C3)-(C2)	1.402(5)	(C1)-(H1A)	0.96	(C14)-(C15)	1.409(4)
(C2)-(C1)	1.500(5)	(H1A)-(C1)	0.96	(C17)-(O2)	1.353(4)
(C1)-(C2)	1.500(5)	(C1)-(H1B)	0.96	(O2)-(C17)	1.353(4)
(C9)-(H9A)	0.961	(H1B)-(C1)	0.96	(C24)-(H24)	0.93
(H9A)-(C9)	0.961	(C1)-(H1C)	0.961	(H24)-(C24)	0.93
(C9)-(H9B)	0.96	(H1C)-(C1)	0.961	(C24)-(O1)	1.233(4)
(H9B)-(C9)	0.96	(C16)-(C15)	1.375(4)	(O1)-(C24)	1.233(4)
(C9)-(H9C)	0.96	(C15)-(C16)	1.375(4)	(C20)-(H20A)	0.96
(H9C)-(C9)	0.96	(C16)-(C17)	1.404(5)	(H20A)-(C20)	0.96
(C13)-(H13A)	0.96	(C17)-(C16)	1.404(5)	(C20)-(H20B)	0.96
(H13A)-(C13)	0.96	(C16)-(C21)	1.518(4)	(H20B)-(C20)	0.96
(C13)-(H13B)	0.959	(C21)-(C16)	1.518(4)	(C20)-(H20C)	0.96
(H13B)-(C13)	0.959	(C19)-(C18)	1.415(4)	(H20C)-(C20)	0.96

(C21)-(H21)	0.981	(H2)-(O2)	0.82	(C22)-(H22A)	0.96
(H21)-(C21)	0.981	(C23)-(H23A)	0.96	(H22A)-(C22)	0.96
(C21)-(C23)	1.520(5)	(H23A)-(C23)	0.96	(C22)-(H22B)	0.959
(C23)-(C21)	1.520(5)	(C23)-(H23B)	0.96	(H22B)-(C22)	0.959
(C21)-(C22)	1.528(4)	(H23B)-(C23)	0.96	(C22)-(H22C)	0.96
(C22)-(C21)	1.528(4)	(C23)-(H23C)	0.96	(H22C)-(C22)	0.96
(O2)-(H2)	0.82	(H23C)-(C23)	0.96		

Bond angles (°)

(F1)-(B1)-(F2)	108.9(2)	(C7)-(C8)-(C12)	132.1(2)	(C10)-(C9)-(H9B)	109.5
(F1)-(B1)-(N2)	111.3(2)	(N1)-(C6)-(C7)	119.8(2)	(C10)-(C9)-(H9C)	109.5
(F1)-(B1)-(N1)	111.3(2)	(N1)-(C6)-(C4)	108.0(2)	(H9A)-(C9)-(H9B)	109.5
(F2)-(B1)-(N2)	109.2(2)	(C7)-(C6)-(C4)	132.1(3)	(H9A)-(C9)-(H9C)	109.5
(F2)-(B1)-(N1)	109.4(2)	(C8-(C12)-(C13)	129.7(2)	(H9B)-(C9)-(H9C)	109.5
(N2)-(B1)-(N1)	106.6(2)	(C8)-(C12)-(C11)	105.8(2)	(C12)-(C13)-(H13A)	109.4
(B1)-(N2)-(C8)	125.4(2)	(C13)-(C12)-(C11)	124.5(3)	(C12)-(C13)-(H13B)	109.5
(B1)-(N2)-(C10)	126.2(2)	(N2)-(C10)-(C9)	121.8(3)	(C12)-(C13)-(H13C)	109.4
(C8)-(N2)-(C10)	108.3(2)	(N2)-(C10)-(C11)	109.0(3)	(H13A)-(C13)-(H13B)	109.5
(B1)-(N1)-(C6)	125.5(2)	(C9)-(C10)-(C11)	129.2(3)	(H13A)-(C13)-(H13C)	109.5
(B1)-(N1)-(C2)	126.5(3)	(C6)-(C4)-(C3)	106.1(3)	(H13B)-(C13)-(H13C)	109.6
(C6)-(N1)-(C2)	108.0(2)	(C6)-(C4)-(C5)	128.7(3)	(C4)-(C3)-(C2)	108.8(3)
(C8)-(C7)-(C6)	121.5(2)	(C3)-(C4)-(C5)	125.3(3)	(C4)-(C3)-(H3)	125.6
(C8)-(C7)-(C14)	118.6(2)	(N1)-(C2)-(C3)	109.1(3)	(C2)-(C3)-(H3)	125.6
(C6-(C7)-(C14)	119.9(2)	(N1)-(C2)-(C1)	122.7(3)	(C4)-(C5)-(H5A)	109.5
(N2)-(C8)-(C7)	120.6(2)	(C3)-(C2)-(C1)	128.2(3)	(C4)-(C5)-(H5B)	109.5
(N2)-(C8)-(C12)	107.4(2)	(C10)-(C9)-(H9A)	109.4	(C4)-(C5)-(H5C)	109.5

(H5A)-(C5)-(H5B)	109.4	(C16)-(C17)-(18)	121.3(3)	(H23A)-(C23)-(H23B)	109.4
(H5A)-(C5)-(H5C)	109.4	(C16)-(C17)-(O2)	117.5(3)	(H23A)-(C23)-(H23C)	109.5
(H5B)-(C5)-(H5C)	109.5	(C18)-(C17)-(O2)	121.1(3)	(H23B)-(C23)-(H23C)	109.5
(C12)-(C11)-(C10)	109.5(3)	(C7)-(C14)-(C19)	121.0(2)	(C21)-(C22)-(H22A)	109.4
(C12)-(C11)-(H11)	125.2	(C7)-(C14)-(C15)	119.0(2)	(C21)-(C22)-(H22B)	109.5
(C10)-(C11)-(H11)	125.2	(C19)-(C14)-(C15)	119.9(2)	(C21)-(C22)-(H22C)	109.5
(C2)-(C1)-(H1A)	109.5	(C18)-(C24)-(H24)	117.7	(H22A)-(C22)-(H22B)	109.5
(C2)-(C1)-(H1B)	109.5	(C18)-(C24)-(O1)	124.6(3)	(H22A)-(C22)-(H22C)	109.4
(C2)-(C1)-(H1C)	109.5	(H24)-(C24)-(O1)	117.7	(H22B)-(C22)-(H22C)	109.5
(H1A)-(C1)-(H1B)	109.5	(C19)-(C20)-(H20A)	109.5		
(H1A)-(C1)-(H1C)	109.4	(C19)-(C20)-(H20B)	109.4		
(H1B)-(C1)-(H1C)	109.4	(C19)-(C20)-(H20C)	109.5		
(C15)-(C16)-(C17)	116.7(2)	(H20A)-(C20)-(H20B)	109.5		
(C15)-(C16)-(C21)	123.6(2)	(H20A)-(C20)-(H20C)	109.5		
(C17)-(C16)-(C21)	119.7(2)	(H20B)-(C20)-(H20C)	109.5		
(C18)-(C19)-(C14)	118.0(2)	(C16)-(C21)-(H21)	107.6		
(C18)-(C19)-(C20)	121.6(2)	(C16)-(C21)-(C23)	113.4(3)		
(C14)-(C19)-(C20)	120.4(2)	(C16)-(C21)-(C22)	110.0(3)		
(C19)-(C18)-(C17)	120.5(2)	(H21)-(C21)-(C23)	107.6		
(C19)-(C18)-(C24)	120.6(3)	(H21)-(C21)-(C22)	107.6		
(C17)-(C18)-(C24)	118.8(3)	(C23)-(C21)-(C22)	110.4(3)		
(C16)-(C15)-(H15)	118.3	(C17)-(O2)-(H2)	109.4		
		(C21)-(C23)-(H23A)	109.5		
(C16)-(C15)-(C14)	123.5(2)	(C21)-(C23)-(H23B)	109.5		
(H15)-(C15)-(C14)	118.2	(C21)-(C23)-(H23C)	109.5		



Scheme S-1 Synthesis of [**THBPY**- Lys] adduct (complex-1)

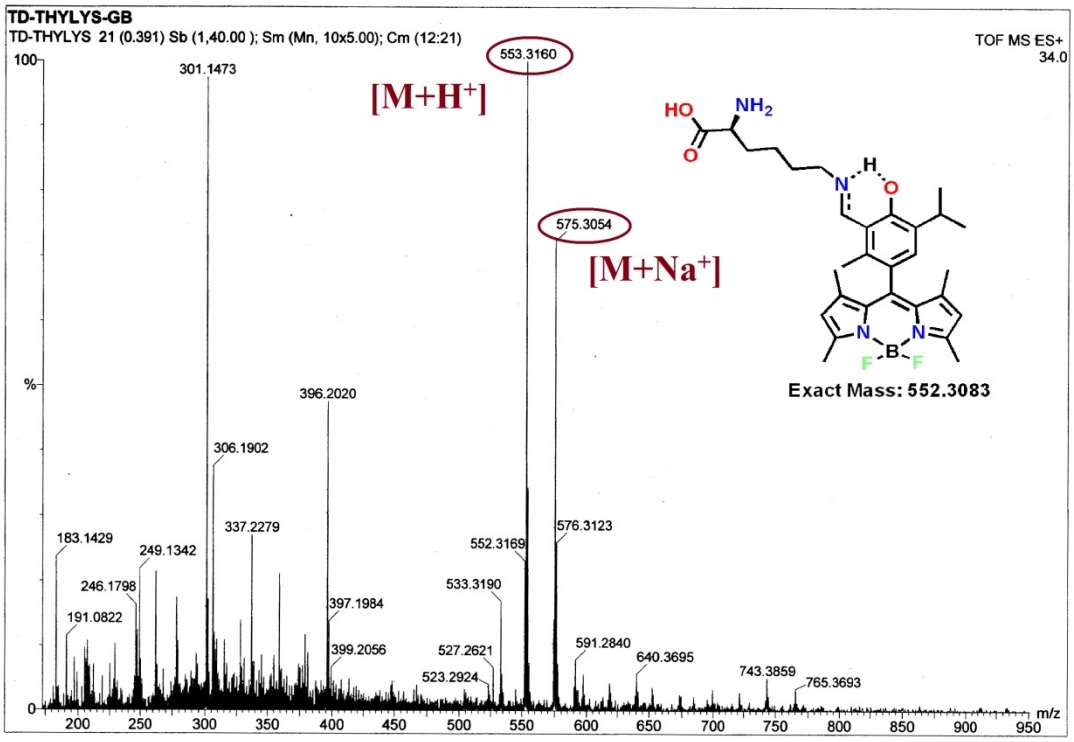
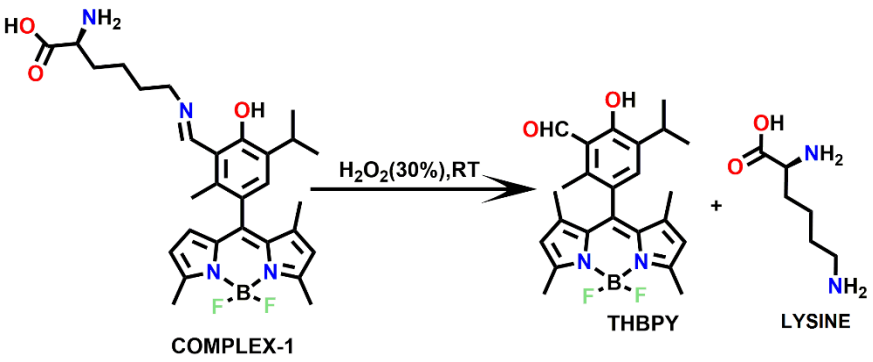
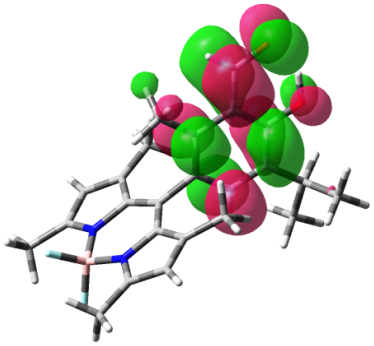


Figure S-27 HRMS spectrum of [**THBPY**- Lys] adduct in methanol.



Scheme S-2 Reversibility of [**THBPY**- Lys] adduct in presence of hydrogen peroxide

Table S-2 Frontier molecular orbitals (MOs) of **THBPY** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/6-31G/level using Gaussian 09

Frontier orbital	**Energy (a.u.)**	**Energy optimised geometry**
LUMO+1	-0.0498	

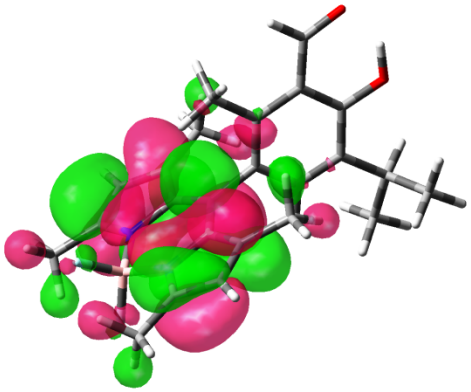
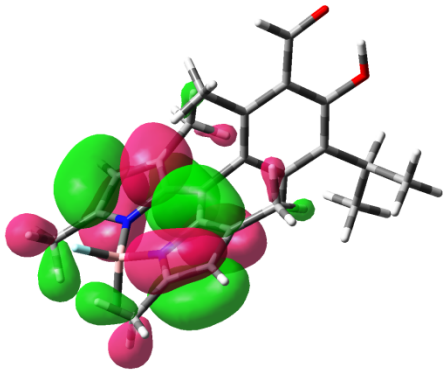
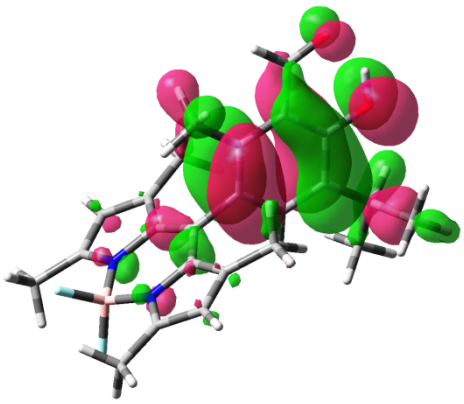
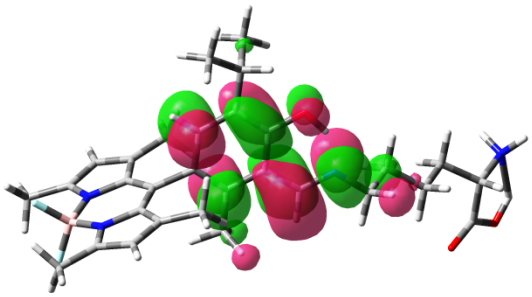
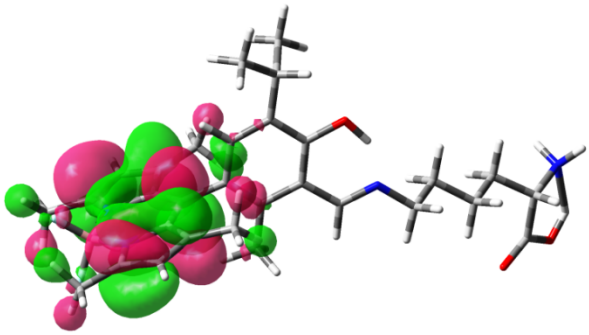
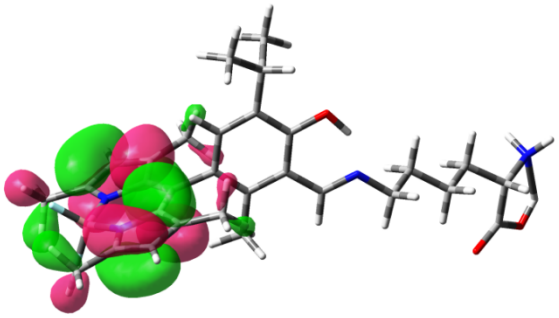
LUMO	-0.0985	
HOMO	-0.2084	
HOMO-1	-0.2410	

Table S-3 Frontier molecular orbitals (MOs) of **THBPY-lysine (neutral)** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/6-31G/level using Gaussian 09.

Frontier orbital	Energy (a.u.)	Energy optimised geometry
LUMO+1	0.0565	
LUMO	-0.0738	
HOMO	-0.1253	

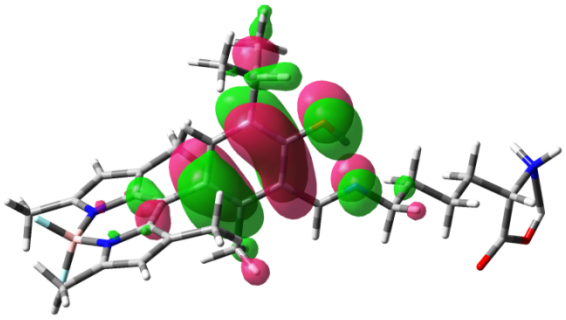
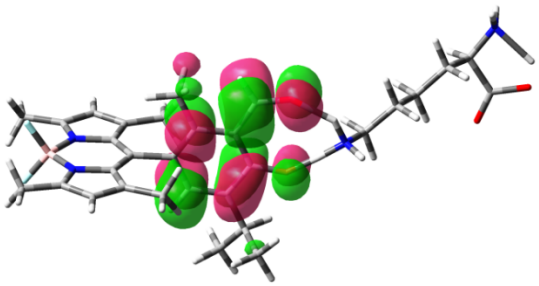
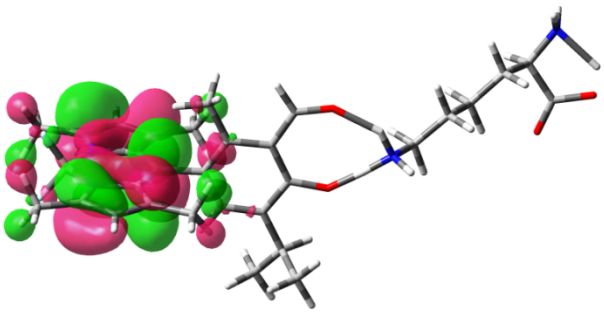
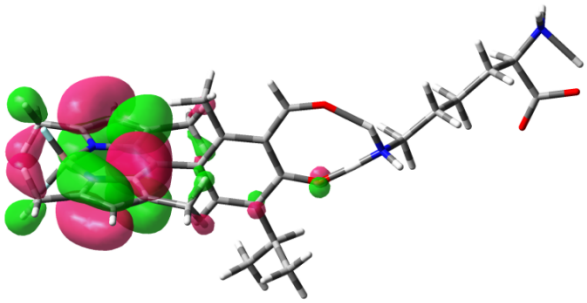
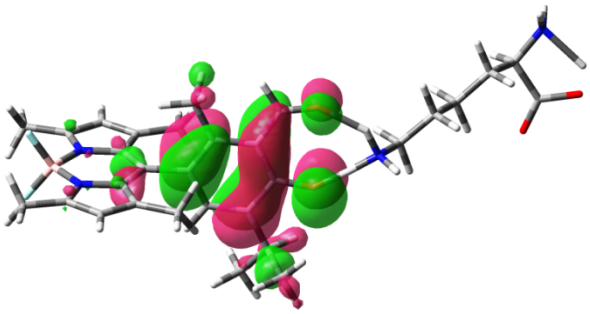
HOMO-1	-0.1433	

Table S-4 Frontier molecular orbitals (MOs) of **THBPY-lysine (cationic)** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/6-31G/level using Gaussian 09.

Frontier orbital	**Energy (a.u.)**	**Energy optimized geometry**
LUMO+1	0.0613	
LUMO	-0.08011	

HOMO	-0.1415	
HOMO-1	-0.1612	

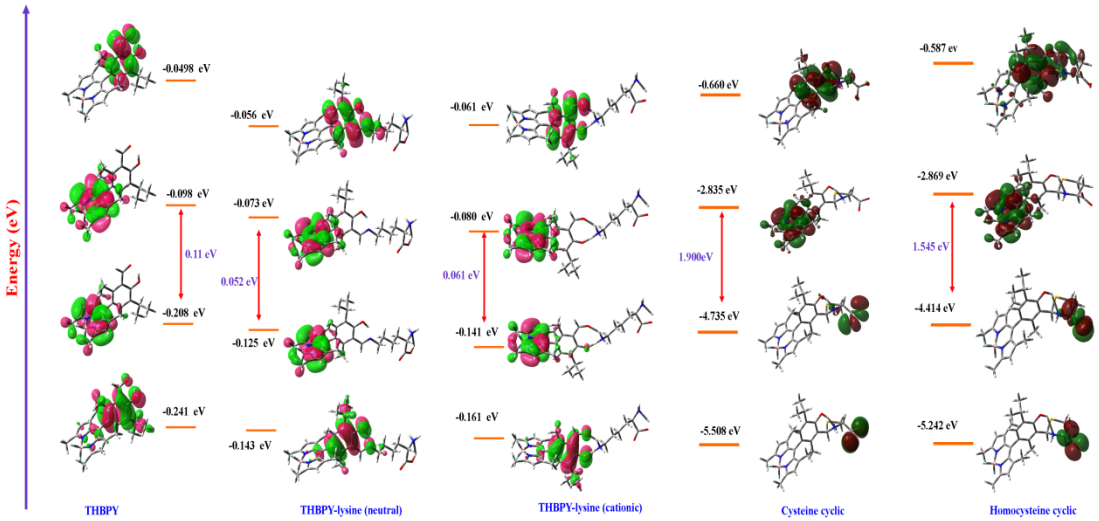


Figure S-28 Selected MOs of **THBPY**, [**THBPY** + cysteine], [**THBPY** + homo cysteine], (not to scale; isovalue = 0.02).

Cytotoxicity assay

In vitro cytotoxicity was measured by using the colorimetric methyl thiazolyltetrazolium (MTT) assay against MDA-MB 231 cells. Cells were seeded into 24-well tissue culture plate in presence of 500 µL Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ºC temperature and 5 % CO_2 atmosphere for overnight and then incubated for 12h in presence of **THBPY** at different concentrations (10-100 µM). Then cells were washed with PBS buffer and 500 µL supplemented DMEM medium was added. Subsequently, 50 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT (5 mg/mL) was added to each well and incubated for 4h. Next, violet formazan was dissolved in 500 µL of sodium dodecyl sulfate solution in water/DMF mixture. The absorbance of solution was measured at 570 nm using microplate reader. The cell viability was determined by assuming 100 % cell viability for cells without MDA-MB 231 cells.

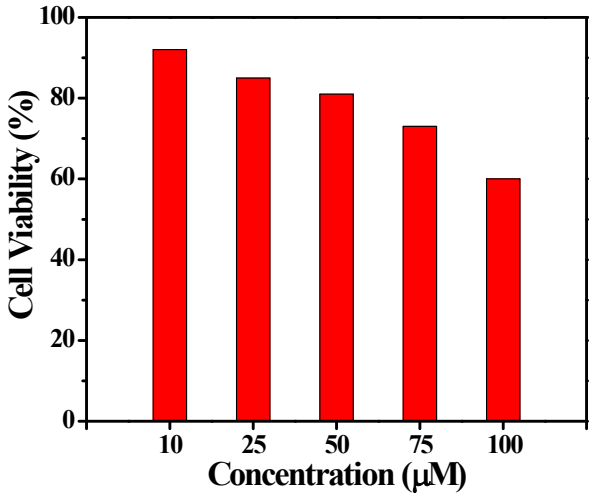


Figure S-29 Cell viability of **THBPY** at different concentration against MDA-MB 231 cells after 12h incubation.

Ref:

(1) Xu, J.; Li, Q.; Yue, Y.; Guo, Y.; Shao, S. *Biosens. Bioelectron.* **2014**, *56*, 58–63.