

## Supporting Information

# Colorimetric and Fluorometric BODIPY-Probe for Rapid, Selective Detection of H<sub>2</sub>S and Its Application in Live Cell Imaging

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### I. General Methods.

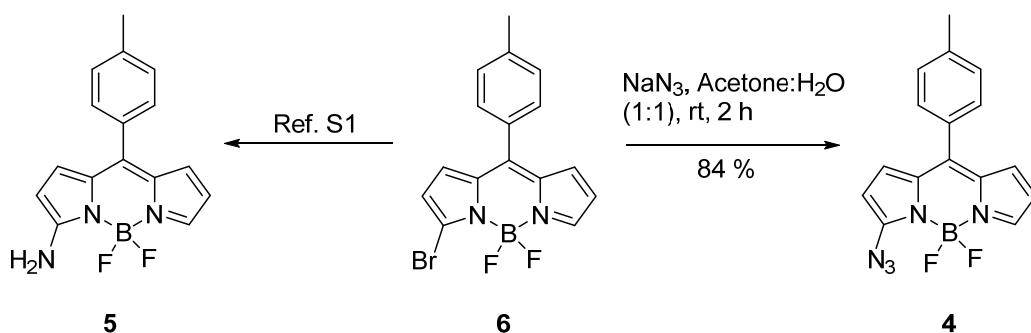
All reactions were conducted under the nitrogen atmosphere. All the chemicals were purchased from commercial sources and used as received unless stated otherwise. Solvents: petroleum ether and ethyl acetate (EtOAc) were distilled prior to thin layer and column chromatography. Dichloromethane (DCM) was pre-dried over calcium hydride and then distilled under vacuum. Column chromatography was performed on Merck silica gel (100–200 mesh). TLC was carried out with E. Merck silica gel 60-F<sub>254</sub> plates.

### II. Physical Measurements.

The <sup>1</sup>H and <sup>13</sup>C spectra were recorded on 400 MHz Jeol ECS-400 (or 100 MHz for <sup>13</sup>C) spectrometers using either residual solvent signals as an internal reference or from internal tetramethylsilane on the  $\delta$  scale (CDCl<sub>3</sub>  $\delta_{\text{H}}$ , 7.24 ppm,  $\delta_{\text{C}}$  77.0 ppm). The chemical shifts ( $\delta$ ) 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). 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<sup>-1</sup>. 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 structure and for processing cell image respectively.

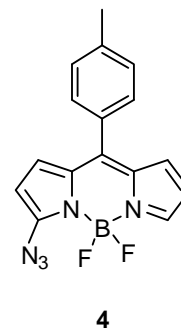
### III. Experimental Procedures.



**Scheme S1** Synthesis of probe 4 and amine 5.

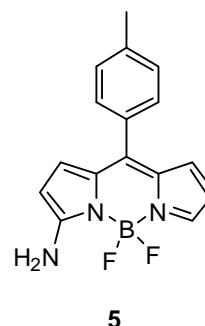
#### Synthesis of 7-azido-5,5-difluoro-10-(p-tolyl)-5H-dipyrrolo [1, 2-c:2',1'-f] [1, 3, 2] diazaborinin-4-ium-5-uide 4 ( $\text{C}_{16}\text{H}_{15}\text{BF}_2\text{N}_5$ ):

In a 50 mL round bottom flask monobromo BODIPY 6 (400 mg, 1.11 mmol) was dissolved in 20 mL Acetone/water (1:1) and  $\text{NaN}_3$  (80 mg, 1.22 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the reaction mixture was evaporated under reduced pressure to remove acetone and aqueous solution was extracted with Ethyl acetate (20 mL  $\times$  3). The combined organic layer was washed with water (10 mL  $\times$  3), brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . 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 (300 mg, 84%) as a red solid. **M.p.:** 190-191 °C (decomposed); **HPLC Purity:** 98.9%; **IR (KBr):**  $\nu/\text{cm}^{-1}$  3104, 2918, 2148, 1616, 1577, 1509, 1441, 1391, 1359, 1245, 1220, 1077, 1042.  **$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):**  $\delta$  7.80 (s, 1H), 7.43 (d,  $J = 8.0$  Hz, 2H), 7.33 (d,  $J = 8.0$  Hz, 2H), 6.99 (d,  $J = 4.6$  Hz, 1H), 6.83 (d,  $J = 4.0$  Hz, 1H), 6.49 (dd,  $J = 2.0$  and 4.0 Hz, 1H), 6.35 (d,  $J = 4.6$  Hz, 1H), 2.47 (s, 3H);  **$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):**  $\delta$  152.7, 144.4, 141.1, 134.1, 133.5, 133.0,



130.7, 130.6, 129.3, 129.0, 117.5, 110.1, 21.6; **HRMS (ESI):** Calc. for  $C_{16}H_{12}BF_2N_5Na^+$   $[M+Na]^+$ : 346.1052; Found: 346.1053.

**Synthesis of 7-amino-5,5-difluoro-10-(p-tolyl)-5H-dipyrrolo[1, 2-c:2',1'-f][1, 3, 2]diazaborinin-4-ium-5-uide 5 ( $C_{16}H_{14}BF_2N_3$ ):**<sup>S1</sup> In a 25 mL round bottomed flask were added monobromo BODIPY **6** (104 mg, 0.29 mmol), aqueous ammonia solution (2 mL) and dissolved in Acetonitrile (2 mL). The reaction mixture was stirred at room temperature for 2.5 h. After completion of the reaction, the reaction mixture was evaporated under reduced pressure to remove acetonitrile and aqueous solution was extracted with Ethyl acetate (10 mL × 3). The combined organic layer was washed with water (10 mL × 3), 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 **5** (82 mg, 96%) as a yellow solid. Obtained data was matched with the literature data.<sup>S1</sup>



#### IV. 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, pH = 7.4) with 1% DMSO (maximum). HEPES buffer was prepared by dissolving solid HEPES in deionized water followed by adjustment of pH by 0.5 (N) NaOH solution.

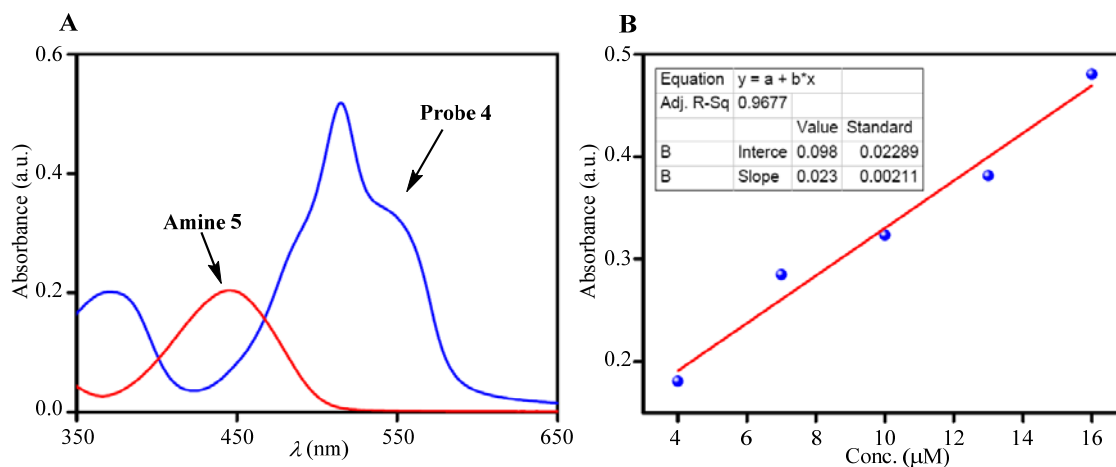
**Preparation of BSA solution:** BSA (5.0 g) was dissolved in 100 mL of HEPES buffer (10 mM, pH = 7.4) to afford 50 mg/mL concentration.

**Preparation of the solution of 4 and 5:** A stock solution of **4** (2000  $\mu$ M) and **5** (2000  $\mu$ M) was prepared in DMSO. Final concentration of each of **4** and **5** during each assay was 10  $\mu$ M with 1% DMSO (maximum).

**Preparation of the solution of analytes:** Stock solutions of NaCl, NaBr, NaI, NaF,  $Na_2SO_3$ ,  $Na_2SO_4$ ,  $Na_2S_2O_3$ , NaSCN, Cysteine (Cys), Glutathione (GSH), NaOH,  $NaNO_2$ ,  $NaNO_3$ ,  $H_2O_2$  were prepared in Deionized water (concentrations 20 mM). Calculated volumes of analytes were added from respective stock solutions to each fluorescence cuvette to provide 200  $\mu$ M. All spectral data were recorded at 10 min after the addition of analyte(s) by exciting at 444 nm. The excitation and emission slit width were 2 nm and 5 nm, respectively.

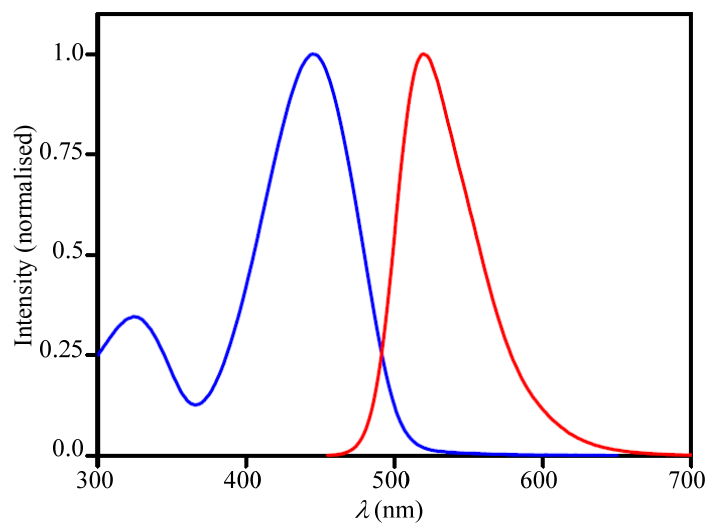
##### Absorption spectra, emission spectra and determination of molar extinction coefficient of probe 4 and amine 5:

Absorption spectra of probe **4** (10  $\mu$ M) and amine **5** (10  $\mu$ M) were recorded in HEPES buffer (10 mM, pH = 7.4, 1% DMSO) (Fig. S1). From absorption spectra molar extinction coefficient of probe **4** and amine **5** were determined using Beer-Lambert law.



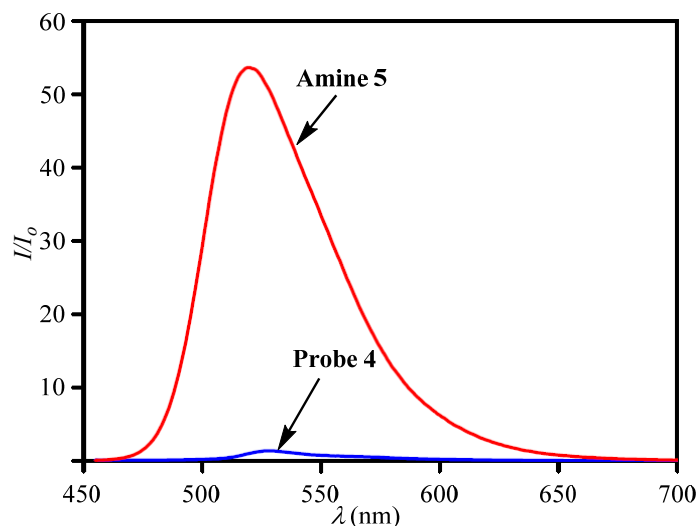
**Fig. S1** (A) UV-vis absorption spectra of probe **4** (15  $\mu$ M) and amine **5** (10  $\mu$ M) recorded in HEPES buffer (10 mM, pH = 7.4, 1% DMSO). (B) Absorbance *Vs* concentration plot for probe **4**.

Amine **5** was exhibited strong fluorescence when excited at 444 nm with emission maxima centered at 520 nm (Fig. S2).



**Fig. S2** Normalized UV-vis absorption and fluorescent emission spectra of amine **5** (10  $\mu$ M) recorded in HEPES buffer (10 mM, pH = 7.4, 1% DMSO).

When fluorescence intensities of probe **4** and amine **5** were compared at 520 nm, amine **5** was found stronger fluorescent than that of probe **4** (Fig. S3).

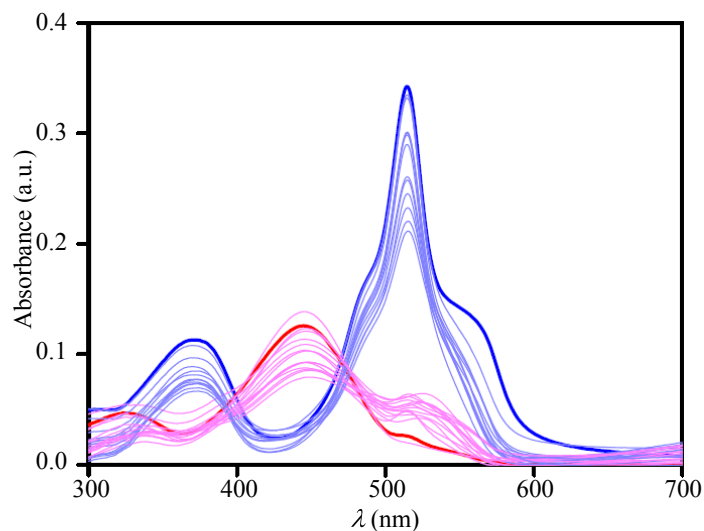


**Fig. S3** Comparison of fluorescence intensities of probe **4** (10  $\mu$ M) and amine **5** (10  $\mu$ M) recorded in HEPES buffer (10 mM, pH = 7.4, 1% DMSO).

## V. H<sub>2</sub>S Sensing

### Absorption spectra recorded for the selectivity studies of Probe 4:

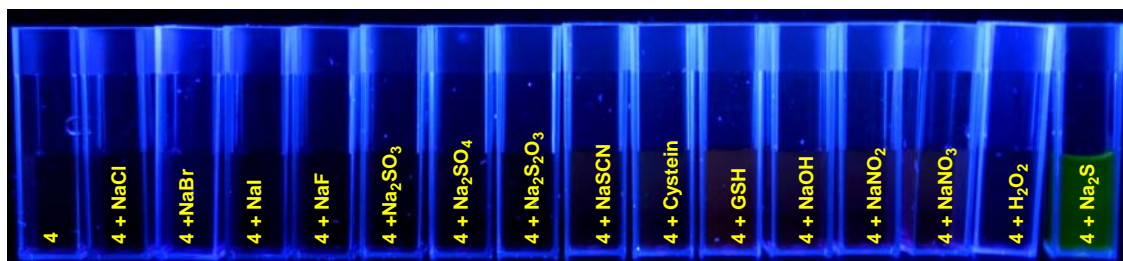
Selectivity and inertness of probe **4** towards other analytes was also confirmed by the UV-visible spectroscopy. Absorption spectra were recorded after 10 minutes stirring of probe **4** (10  $\mu$ M) with various analytes (200  $\mu$ M). The absorption profile was observed for each case was unchanged and identical with the absorption spectra of only probe **4** (Fig. S4). Addition of Na<sub>2</sub>S (200  $\mu$ M) in the same solutions resulted in the change in absorption profiles, blue shifted from the initial position and identical to the absorption spectra of amine **5**. This data also confirms the selectivity of probe **4** towards H<sub>2</sub>S in the presence of other analytes.



**Fig. S4** UV-vis absorption spectra of probe **4** (10  $\mu$ M) in presence of various analytes (200  $\mu$ M, blue colored) and in the same solution  $\text{Na}_2\text{S}$  (200  $\mu$ M, pink colored) in HEPES buffer.



**Fig. S5** Cuvette images of probe **4** in the presence of different analytes (Pink colored) and in  $\text{Na}_2\text{S}$  (Yellow colored) in HEPES buffer taken under ambient light.



**Fig. S6** Cuvette images of probe **4** in the presence of different analytes and in  $\text{Na}_2\text{S}$  (right hand side) in HEPES buffer taken under hand held UV lamp ( $\lambda = 365$  nm).

#### Determination of quantum yields:

The quantum yields of probe **4** and amine **5** were determined according to the following equation:

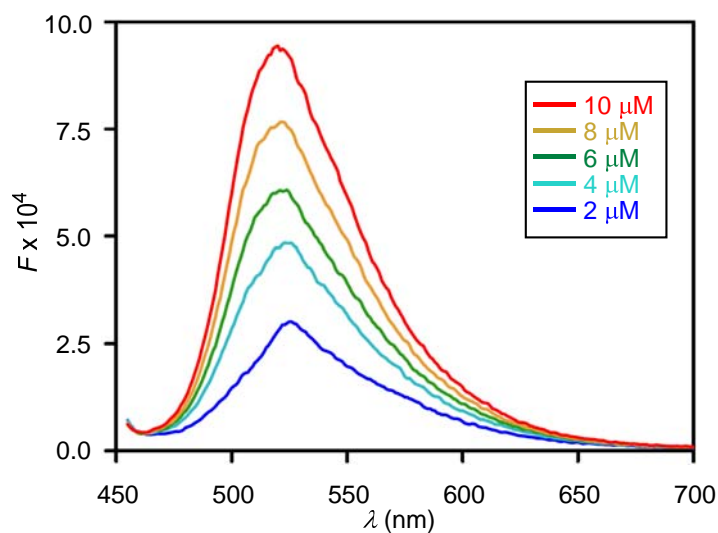
$$\Phi_1 = \Phi_B \times \frac{I_1 \times A_B \times \lambda_{exB} \times (\eta_1)^2}{I_B \times A_1 \times \lambda_{ex1} \times (\eta_B)^2} \quad (\text{Eq. 1})$$

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

#### Determination of detection limits of probe 4 towards H<sub>2</sub>S sensing:

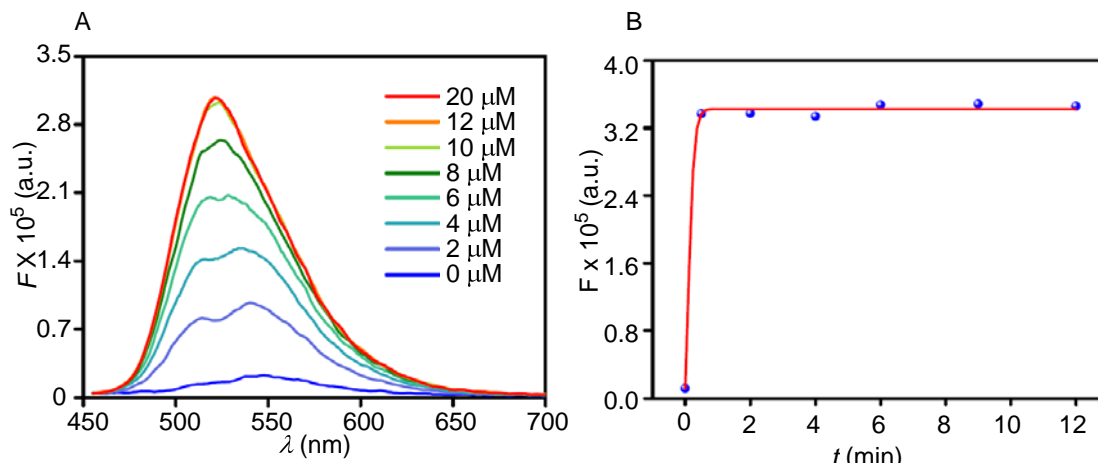
The detection limits were determined based on the fluorescence titrations. Probe 4 was employed at 10  $\mu\text{M}$ . To determine the  $S/N$  ratio, the emission intensity of probe 4 was measured without H<sub>2</sub>S by 7-times and the standard deviations of blank measurements were determined. Under these conditions, a good linear relationship between the fluorescence intensity and the H<sub>2</sub>S concentration (Fig. S7 and 7A) was obtained for probe 4 ( $R = 0.996$ ). The detection limit was then calculated with the equation: detection limit =  $3\sigma/m$ , where  $\sigma$  is the standard deviation of 7 blank measurements,  $m$  is the slope between intensity versus Na<sub>2</sub>S concentration. The detection limits of 4 towards H<sub>2</sub>S was calculated to be  $2.59 \times 10^{-7}$  M at  $S/N = 3$  (signal-to-noise ratio of 3:1).

Detection limit for probe 4 was also calculated in BSA solution using same protocol which was used in case of HEPES buffer (Fig. S8A, and 7B).



**Fig. S7** Fluorescence spectra of probe 4 (10  $\mu\text{M}$ ) with increasing concentrations (2, 4, 6, 8 and 10  $\mu\text{M}$ ) of Na<sub>2</sub>S in HEPES buffer.

### Detection of H<sub>2</sub>S in BSA solution:



**Fig. S8** Fluorescence spectra of probe **4** (10 μM) with increasing concentrations (2, 4, 6, 8 and 10 μM) of Na<sub>2</sub>S in BSA solution (A), time dependent fluorescence intensity of probe **4** at 520 nm in BSA solution after addition of 20 μM Na<sub>2</sub>S (B).



**Fig. S9** Cuvette images of probe **4** before and after addition of Na<sub>2</sub>S taken under ambient light (A), and under hand held UV- lamp (B) in BSA solution.

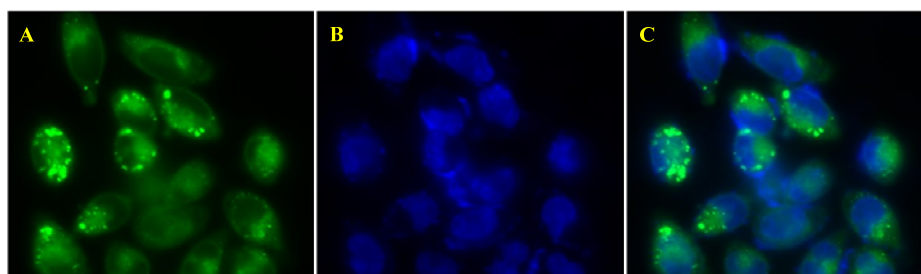
### VI. Cell Imaging:

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<sub>2</sub> at 37 °C. The cultured cells were subcultured twice in each week, seeding at a density of about 15 × 10<sup>3</sup> 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<sub>ex</sub> = 460-480 nm (by using GFP filter).



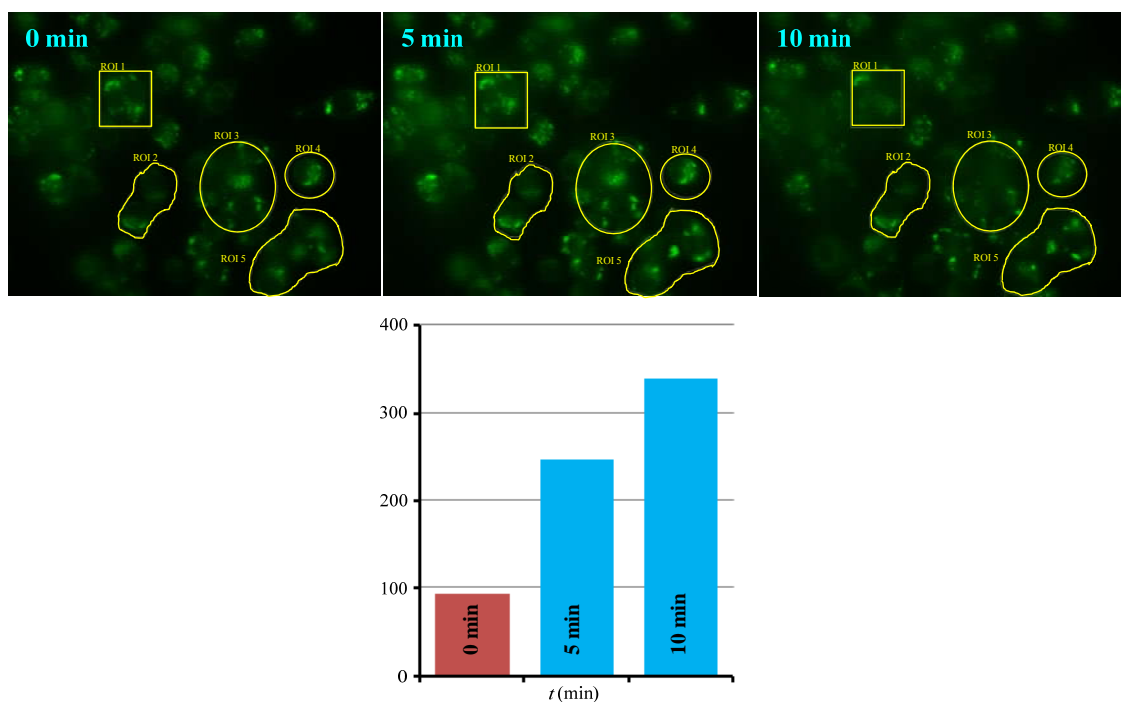
The HeLa cells were incubated with solution of the probe **4** (5  $\mu\text{M}$  in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37  $^{\circ}\text{C}$  for 30 min. After washing with PBS the fluorescence images were acquired. In this case less significant fluorescence was observed. Another set of HeLa cells were pre-incubated with probe **4** (5  $\mu\text{M}$  in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37  $^{\circ}\text{C}$  for 30 min followed by washing with PBS and incubation with  $\text{Na}_2\text{S}$  (100  $\mu\text{M}$  in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37  $^{\circ}\text{C}$  for 30 min. After washing with PBS the fluorescence images showed strong green fluorescence (Fig. 8).

To investigate the nuclear accumulation of probe **4** if any, the cells pre-incubated with probe **4** and  $\text{Na}_2\text{S}$  were again incubated with Hoechst nuclear staining dye (2  $\mu\text{M}$ ) at 37  $^{\circ}\text{C}$  for 10 min. Then washed with PBS thoroughly and fluorescence image were taken in both green (Fig. S10A) and blue channel (Fig. S10B). Probe **4** is permeable for cellular membrane but not for nuclear membrane, as no green fluorescence was observed inside the nucleus.



**Fig. S10** Cell image of HeLa cells: in green channel (A), blue channel (B), and overlay (C). Cells were pre incubated with probe **4** (5  $\mu\text{M}$ , at 37  $^{\circ}\text{C}$  for 30 min) and  $\text{Na}_2\text{S}$  (100  $\mu\text{M}$ , at 37  $^{\circ}\text{C}$  for 30 min) separately followed by incubation with Hoechst (2  $\mu\text{M}$ , at 37  $^{\circ}\text{C}$  for 10 min).

For measuring the intensity of cell fluorescence quantitatively, HeLa cells were first incubated with probe **4** (5  $\mu\text{M}$ , at 37  $^{\circ}\text{C}$  for 30 min) the plate was thoroughly washed with PBS and placed under the microscope fitted with an incubator. The images were taken in 5 min time intervals after addition of  $\text{Na}_2\text{S}$  (100  $\mu\text{M}$ ). Five different region of interest (ROI) were selected and the intensity was obtained by using *image j* software. The average intensity of ROIs for each image was plotted in bar diagram (Fig. 9E and Fig. S11). Fig. 9E and Fig. S11 corresponds to two independent set of experiments.



**Fig. S11** Fluorescence images of HeLa cells incubated with probe **4** (5  $\mu$ M) followed by incubation with  $\text{Na}_2\text{S}$  (100  $\mu$ M), acquired at different time intervals (0, 5, and 10 min). Bar diagram showing average intensity of ROI *V*s time.

## VII. NMR Spectra.

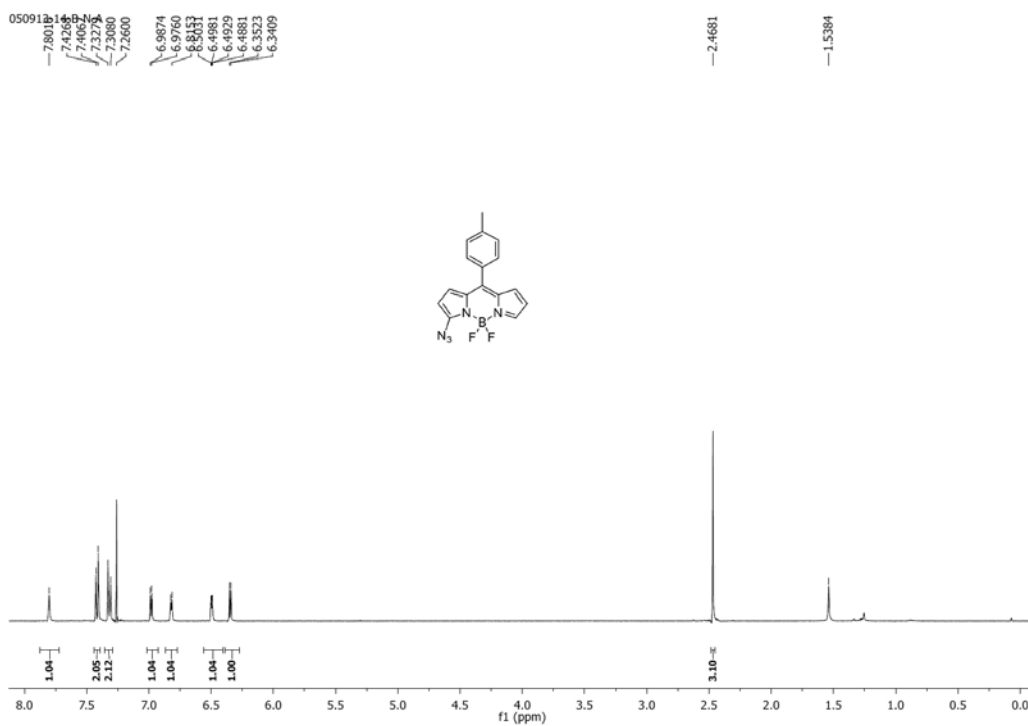


Figure S12. <sup>1</sup>H NMR spectra of **4** in CDCl<sub>3</sub>.

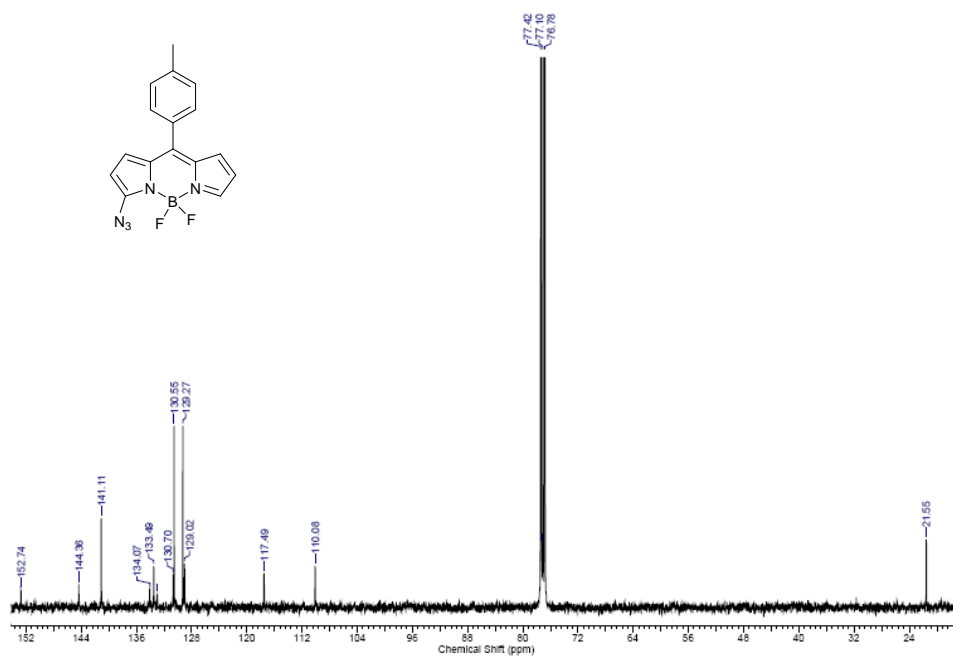
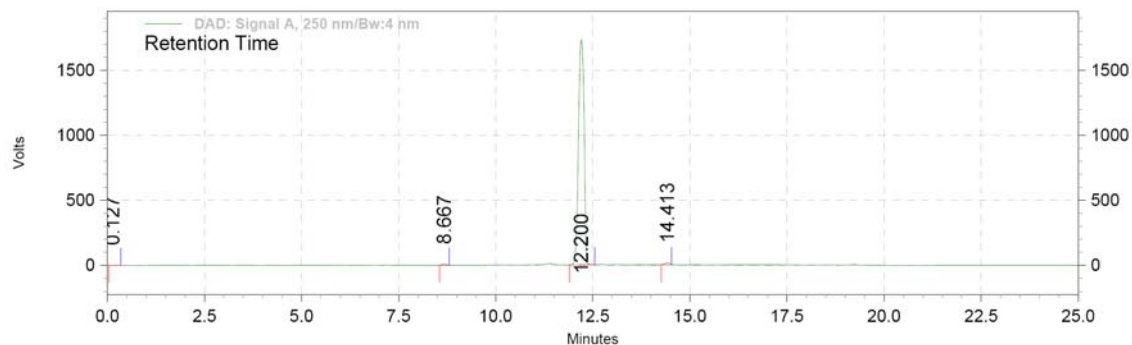


Figure S13. <sup>13</sup>C NMR spectra of **4** in CDCl<sub>3</sub>.

## VIII. HPLC Purity.

### Area % Report

Data File: C:\Users\HPLC agilent\Desktop\PT\HPLC Data\azide1.dat  
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\DK Azide.met  
Acquired: 10/16/2013 11:26:06 AM  
Printed: 10/16/2013 1:03:05 PM



### DAD: Signal A, 250 nm/Bw:4 nm Results

Retention Time	Area	Area %	Height	Height %
0.127	514	0.00	55	0.00
8.667	142066	0.39	17996	0.49
12.200	36291709	98.88	3629030	98.70
14.413	267829	0.73	29786	0.81
Totals	36702118	100.00	3676867	100.00

**Figure S14.** HPLC Data of Probe 4.

Column: Phenomenex (4.6 mm × 250 mm)

Flow: 1.0 mL/min

Method: Gradient

20 % Acetonitrile/water 0 min

100 % Acetonitrile 0 to 10 min

100 % Acetonitrile 10 to 15 min

20 % Acetonitrile/water 15 to 20 min

20 % Acetonitrile/water 20 to 25 min

Wavelength: 250 nm.

Retention time ( $t_R$ ) = 12.20 min.

## IX. References.

S1 D. Kand, P. K. Mishra, T. Saha, M. Lahiri and P. Talukdar, *Analyst*, 2012, **137**, 3921.