Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Selective fluorescent probe for cysteine and its imaging in live cells

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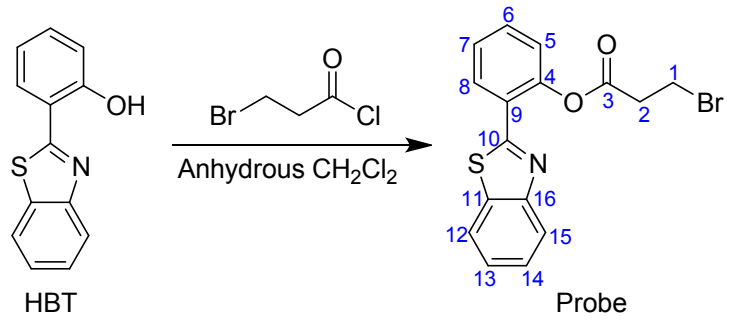
EXPERIMENTAL SECTION

General considerations. All chemicals used herein were used as received from commercial suppliers (Aldrich, Tokyo Chemical Industry). ^{1}H, ^{13}C NMR spectra were acquired using a Bruker Avance 600 MHz spectrometer. TMS was used as external standard. ESI-mass spectrometry was performed on a BRUKER micro-TOF-QⅡ by the research support staff at KAIST. Time-of-Flight mass spectrometer was operated at a resolution of 20,000. Absorption spectra were measured using a JASCO V–530 UV/Vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer.



Synthetic procedure of the probe. 2-(2-hydroxyphenyl)benzothiazole (300 mg, 1.32 mmol) and 3-Bromopropionyl Chloride (453 mg, 2.64 mmol) were added in anhydrous methylene chloride (10 mL) under nitrogen gas. The mixture were stirred for 60 h. The reaction was monitored by TLC. After completion of the reaction, the solution was washed with water (10 mL) three times. The organic layer was dried over anhydrous sodium sulphate and evaporated. The crude was purified by silica gel column chromatography (ethylacetate/hexane eluant) to afford the desired product. Yield = 241 mg, 50.4 %. ^{1}H NMR ($CDCl_3$, δ 7.24, 600 MHz): 8.23 (dd, $^{3}J_{H\text{-}H}$ = 7.9 Hz, $^{4}J_{H\text{-}H}$ = 1.6 Hz, 1 H_5), 8.07 (dd, $^{3}J_{H\text{-}H}$ = 7.9 Hz, $^{4}J_{H\text{-}H}$ = 1.1 Hz, 1 H_{15}), 7.92 (td, $^{3}J_{H\text{-}H}$ = 8.2 Hz, $^{4}J_{H\text{-}H}$ = 0.9 Hz, 1 H_{12}), 7.51 (m, 2 $H_{6,\ 13}$), 7.41 (m, 2 $H_{8,\ 14}$), 7.27 (dd, $^{3}J_{H\text{-}H}$ = 8.2 Hz, $^{4}J_{H\text{-}H}$ = 1.3 Hz, 1 H_7), 3.73 (t, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 2 H_1), 3.37 (t, $^{3}J_{H\text{-}H}$ = 7.0 Hz, 2 H_2). ^{13}C NMR ($CDCl_3$, δ 77.0, 150 MHz) = 168.9 (C_3), 162.6 (C_{10}), 153.1 (C_{11}), 147.9 (C_4), 135.1 (C_{16}), 131.5 (C_6), 130.5 (C_5), 126.7 (C_8), 126.4 (C_{13}), 126.0 (C_9), 125.5 (C_{14}), 123.7 (C_7), 123.3 (C_{15}), 121.3 (C_{12}), 38.3 (C_2), 25.2 (C_1). ESI-MS (positive mode, CH_3OH - calculated 360.9772 obtain 385.9653 [C 16, H 12, Br, N, Na, O 2, S] *Probe* + *Na.*

Cell culture and probe treatment. Hep3B cells from the Korean Cell Line Bank were maintained as a monolayer in a humidified incubator (5% CO_2) at 37℃ in Dulbecco's Modified Eagle's Medium (DMEM, WELGENE) supplemented with 10% (v/v) fetal bovine serum (FBS, WELGENE), 100 IU/mL penicillin-streptomycin solution (WELGENE). Before treatment of the probe, the cells were incubated with media containing 0.5 mM and 1 mM of N-Ethylmaleimide (NEM) for 1 h. Finally, probe (20 μM) in PBS was treated and after 10 min incubation, confocal fluorescence images were recorded using a 405 nm Argon laser and a 410-585 nm band pass emission filter.

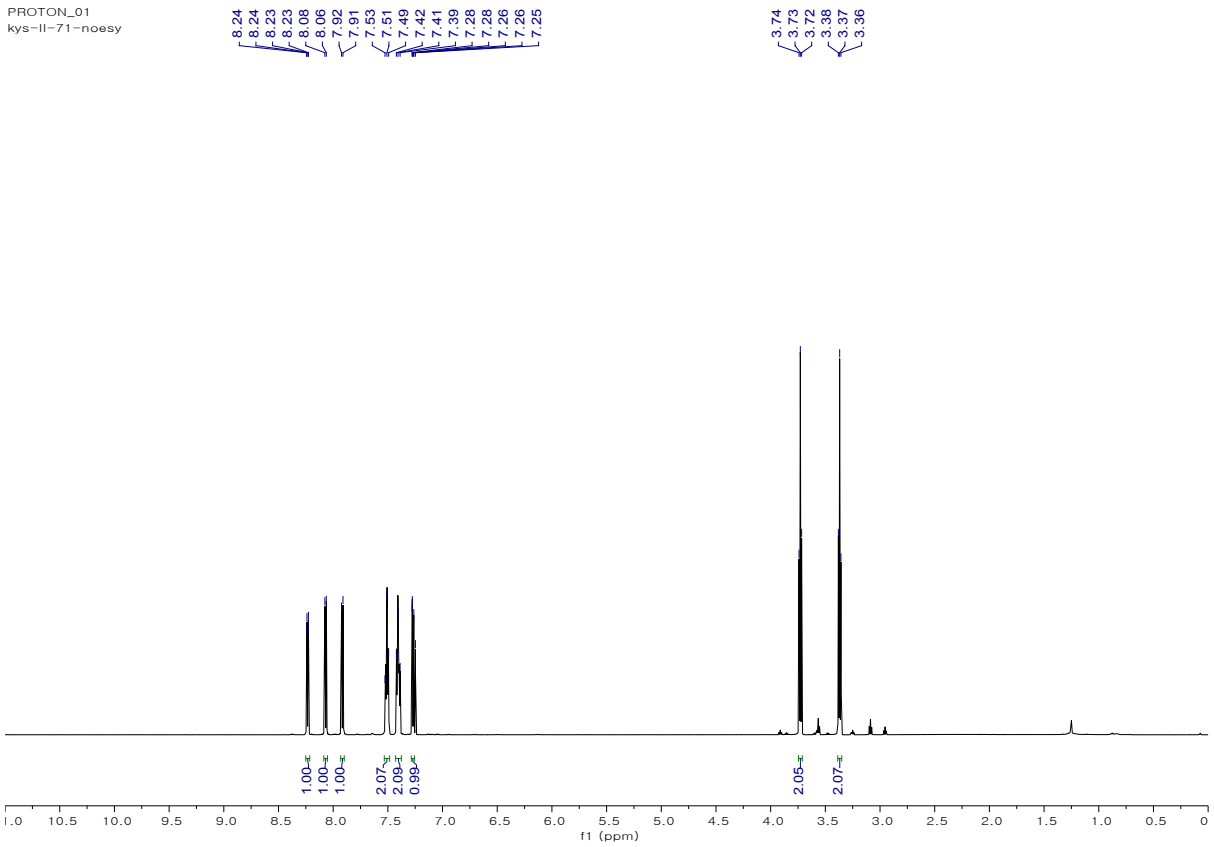


Fig. S1. ^{1}H NMR spectrum of the probe.

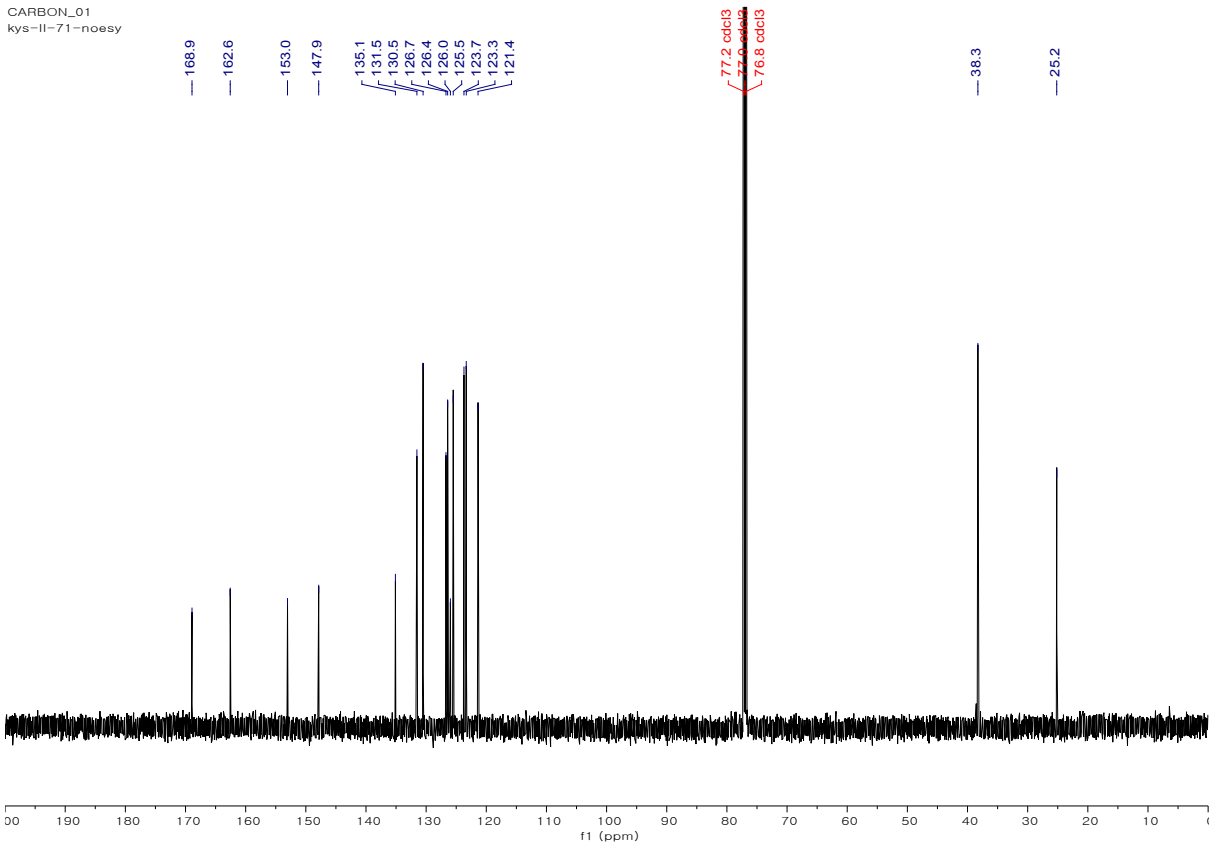


Fig. S2. ^{13}C NMR spectrum of the probe.

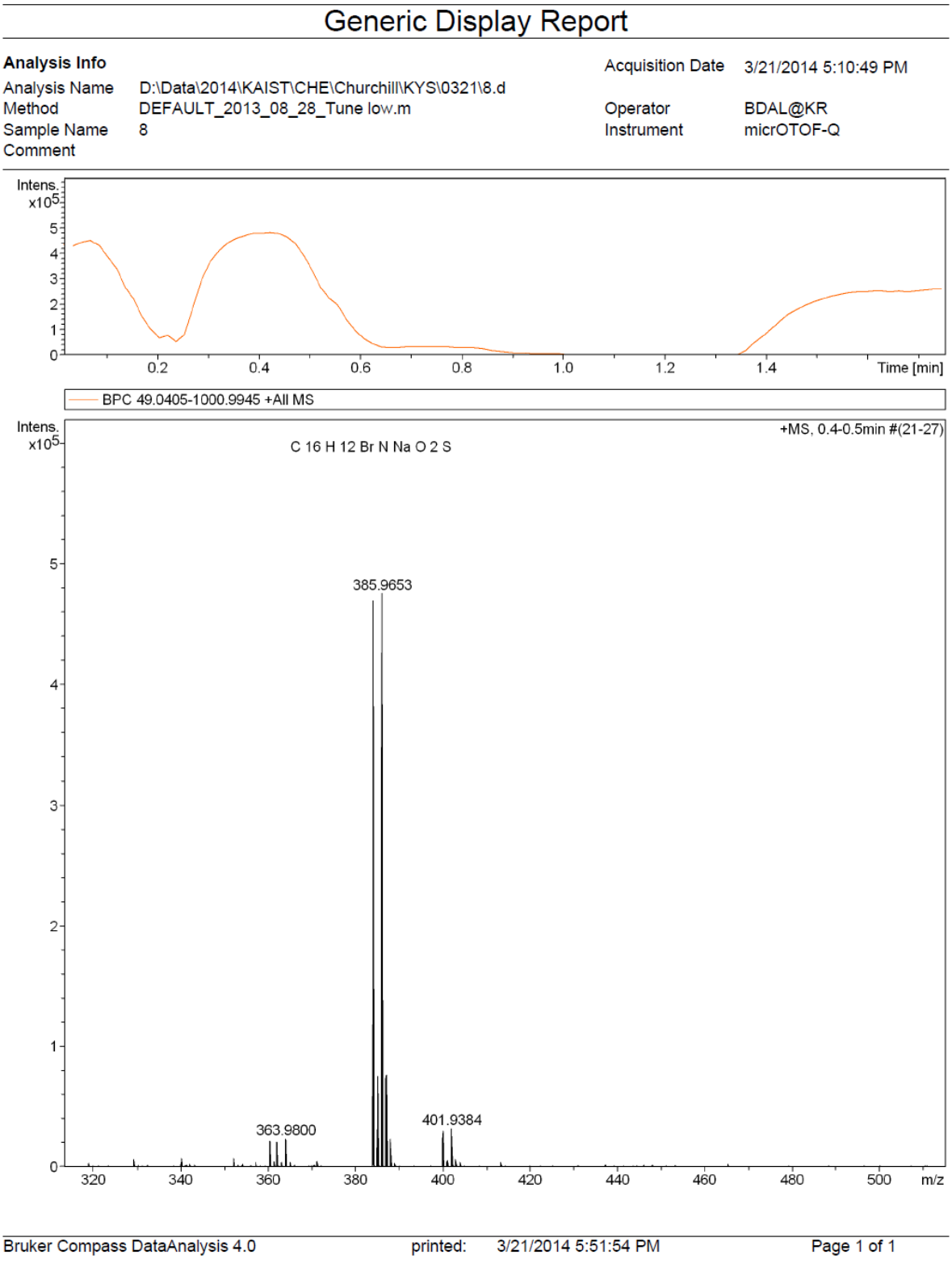


Fig. S3. ESI – mass spectrum of the probe.

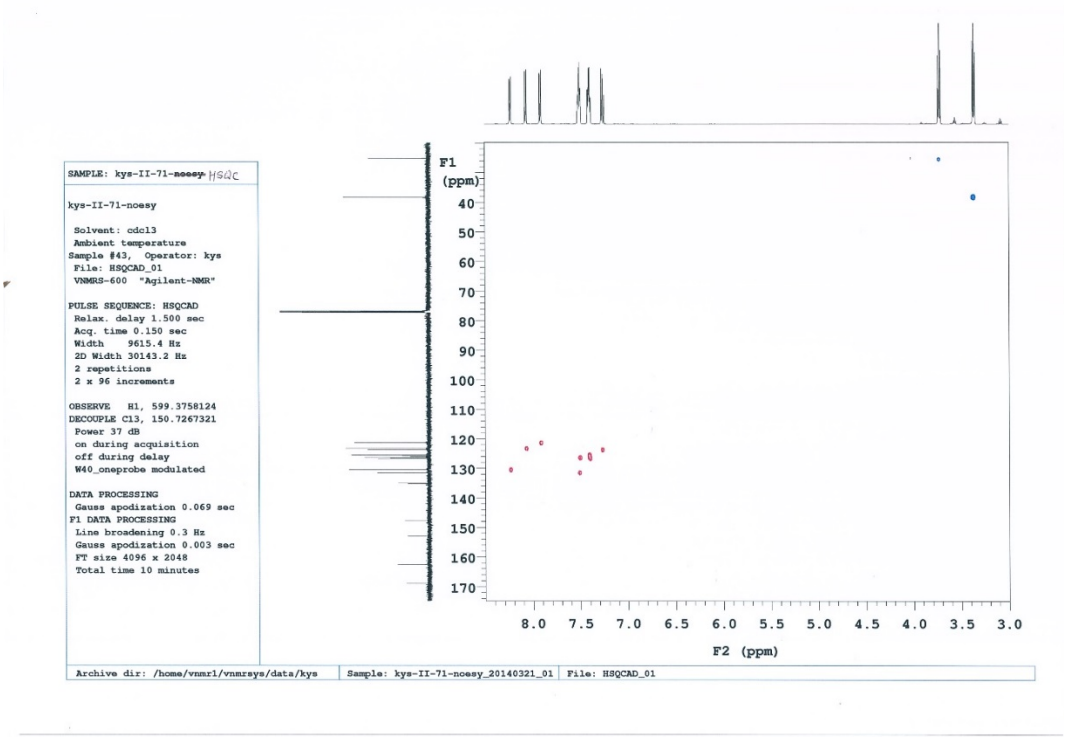


Fig. S4. ^{1}H – ^{13}C HSQC NMR spectrum of the probe.

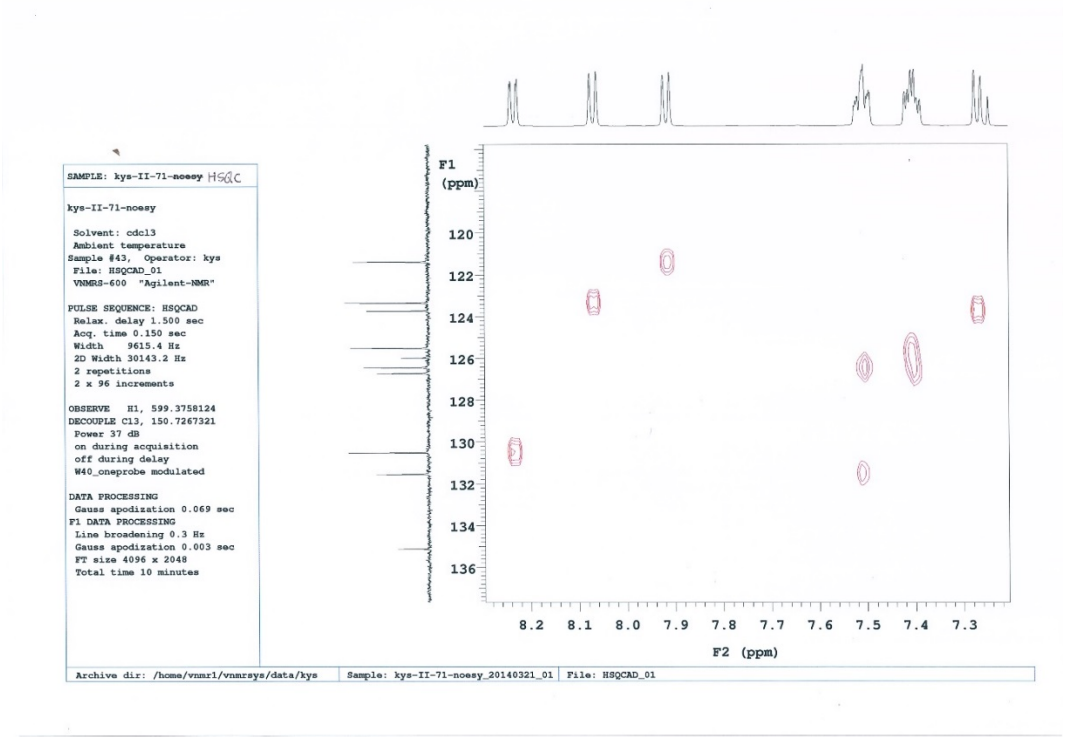


Fig. S5. ^{1}H – ^{13}C HSQC NMR spectrum of the probe (Expanded aromatic region).

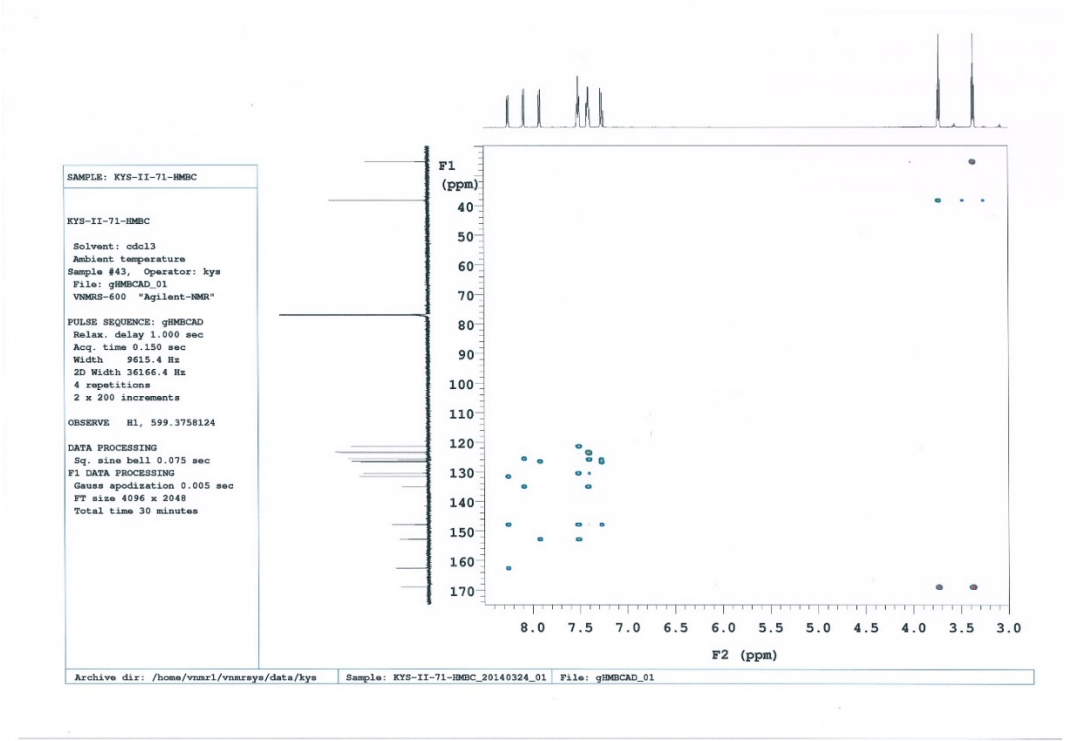


Fig. S6. ^{1}H – ^{13}C HMBC NMR spectrum of the probe.

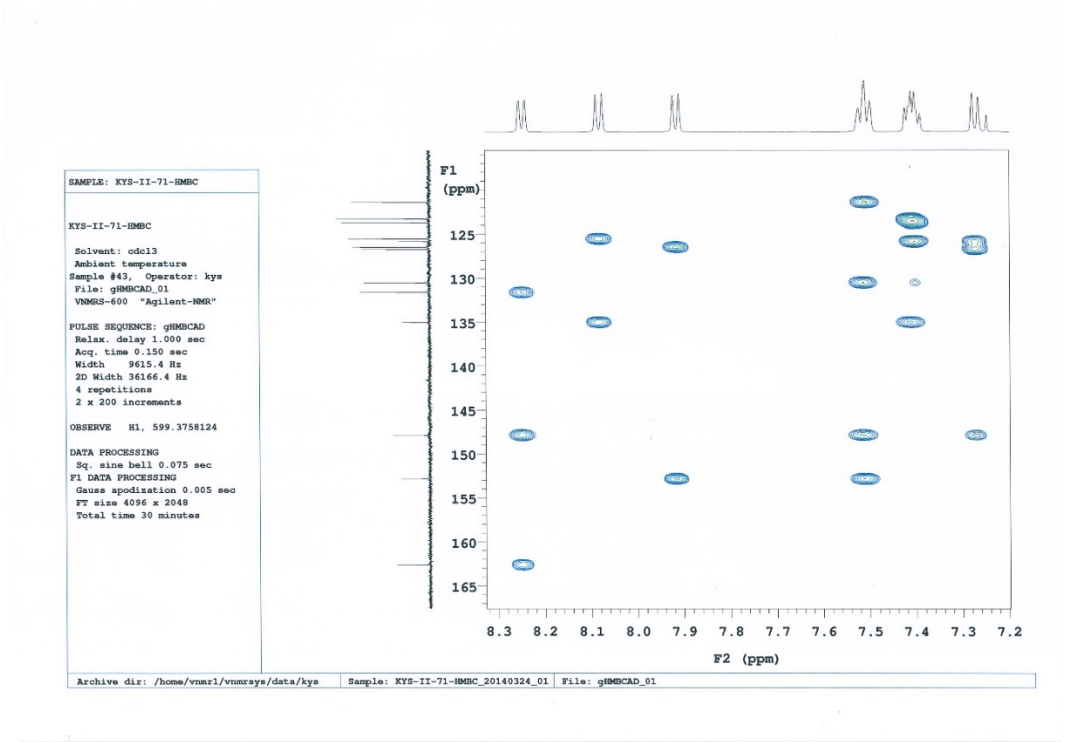


Fig. S7. ^{1}H – ^{13}C HMBC NMR spectrum of the probe (Expanded aromatic region).

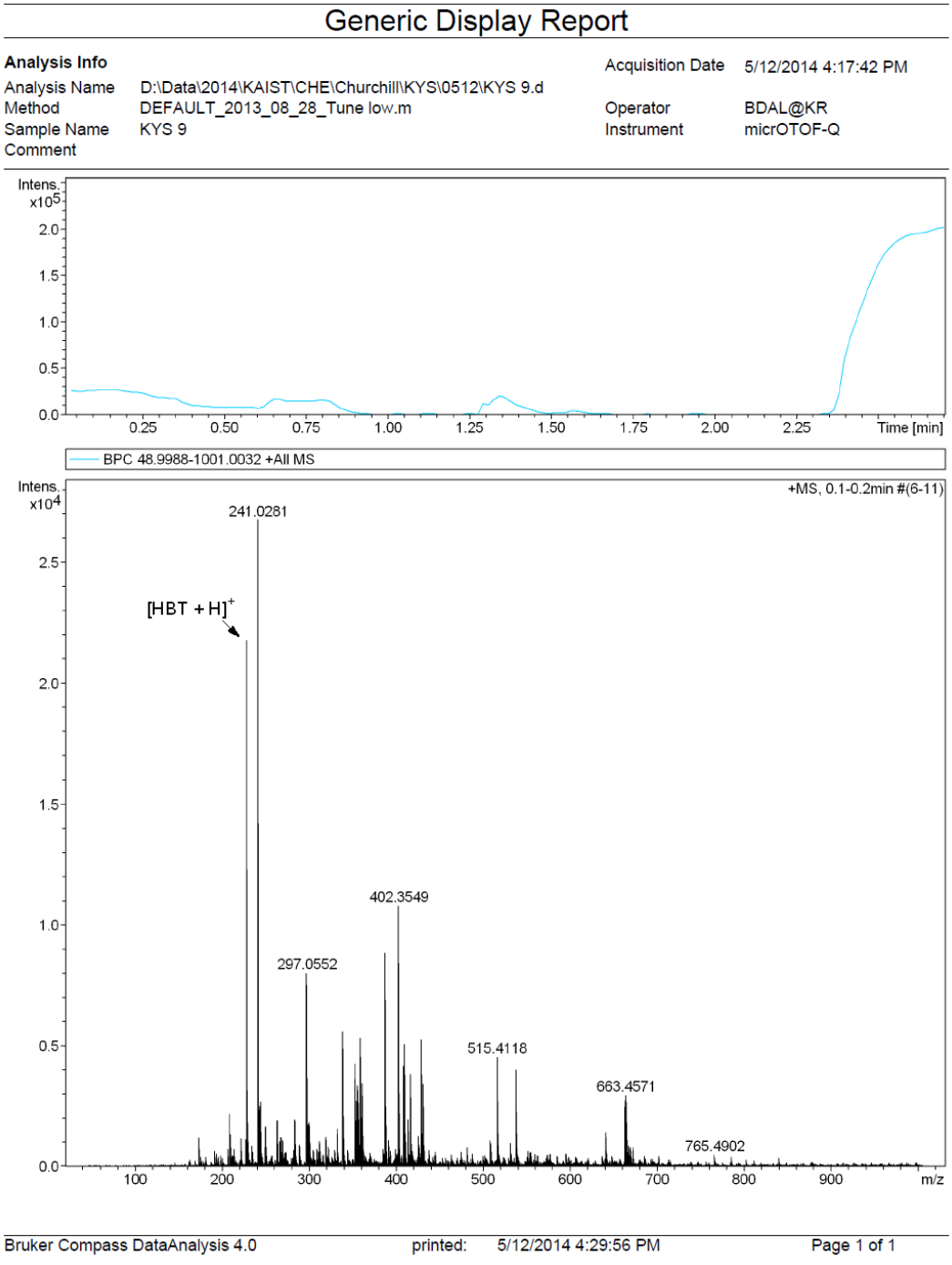


Fig. S8. ESI – mass spectrum of the probe + Cys (1 eq).

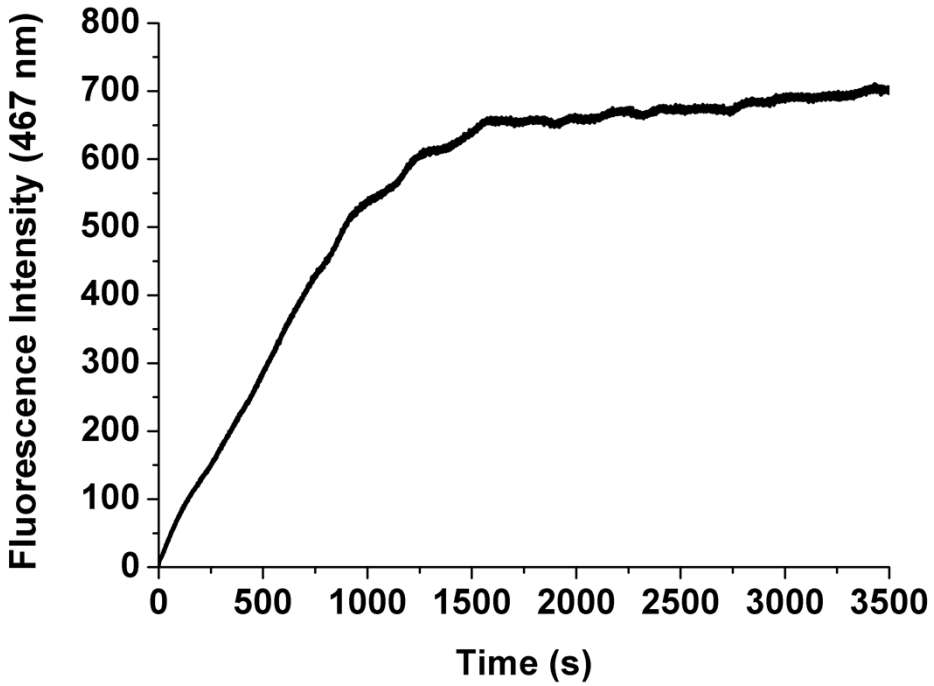


Fig. S9. Time-dependant intensity of the probe + Cys (1 equiv) (excitation wavelength : 330 nm / emission wavelength : 467 nm).

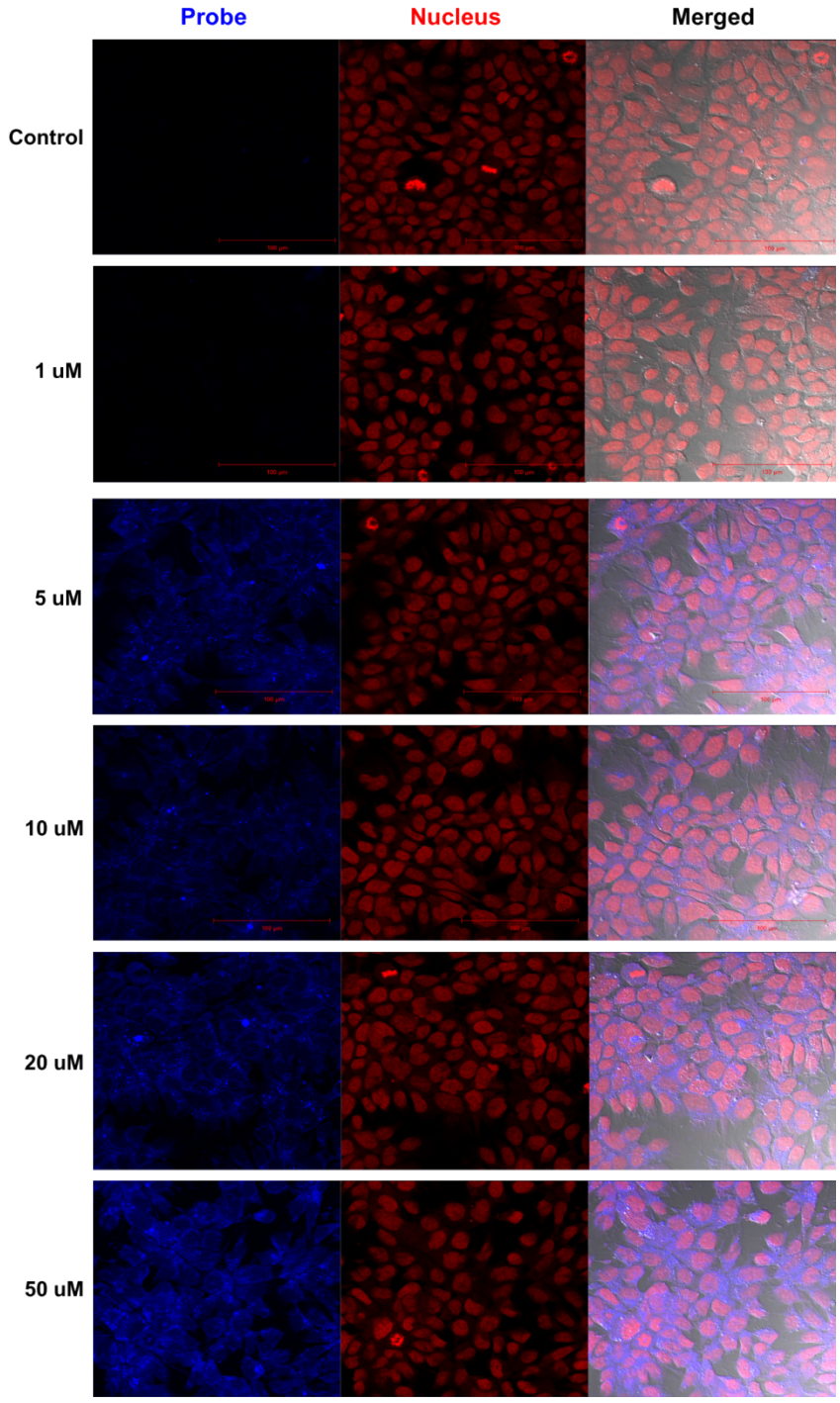


Fig. S10. Confocal fluorescence images of probe at various concentrations (0, 1, 5, 10, 20, 50 μM) used in treating living Hep3B cells. Scale bar: 100 μm

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