SUPPORTING INFORMATION

Extremely Selective and Fluorescent Detection of Cysteine Or Superoxide: with Aliphatic Ester Hydrolysis

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Experimental Section

General Remarks. All reagents used herein were used as received from commercial suppliers (Aldrich, Acros, and Junsei companies). ^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance 400 MHz spectrometer. TMS was used as an internal standard. ^{1}H and ^{13}C NMR spectral signals were calibrated internally by the respective protio impurity or carbon resonance of the NMR spectroscopic solvent, *e.g.*, DMSO-d_6. ESI-mass spectrometry was performed on a VG AUTOSPEC ULTIMA by the research support staff at KAIST. This instrument possesses a trisector double focusing magnetic sector analyzer and was operated at a resolution of 80,000. Absorption spectra were measured using a JASCO V–530 UV/Vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer.

Synthesis of probe 1. In a 100 mL round bottom flask, Fluorescein (0.50 g, 1.5 mmol) and vinylacetic acid (0.25 mL, 3.0 mmol) were dissolved up in 30 mL of tetrahydrofuran. Then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.69 mg, 3.61 mmol) and 4-dimethylaminopyridine (DMAP) (0.367 mg, 3.01 mmol) were added at room temperature. The reaction mixture was allowed to react at room temperature overnight; after this time, the solvent was removed under pressure. The obtained crude material was subjected to column chromatography and purified by using hexane: ethyl acetate (8:2) as an eluent. Probe **1** was obtained as a white solid (0.47 g. 67 % yield).

^{1}H NMR spectroscopy: (400 MHz, $CDCl_3$): 8.10 (d, $^{3}J_{(H,H)}$ = 7.24 Hz, 1H), 7.69-7.60 (2H, m), 7.22-7.12 (5H, m), 6.84 (d, $^{3}J_{(H,H)}$ = 1.52 Hz, 4H), 6.03 (d, $^{3}J_{(H,H)}$ = 15.6 Hz, 2H), 1.95 (dd, $^{4}J_{(H,H)}/^{3}J_{(H,H)}$ = 1.64, 6.92 Hz, 6H).

^{13}C NMR spectroscopy: (100 MHz, $CDCl_3$): 169.11, 164.10, 152.87, 152.20, 151.57, 147.93, 135.30, 130.06, 128.86, 126.13, 125.15, 124.07, 121.59, 117.83, 116.30, 110.41, 81.76, 18.27.

ESI-MS: **Fig. S3** probe-**1** $[M+Na]^+$ = 491.1107 (calc.), 491.1101(exp.)

ESI-MS: **Fig. S4** of probe **1 + L-cys** $[M+H]^+$ = 333.068 (calc.), 333.075 (exp.**) [Fluorescein]**

Analysis of Cys in living cells. A human neuroblastoma cell line, SH-SY5Y, was grown in a 1:1 mixture of Eagle's Minimum Essential Medium, and Ham's F12 Medium supplemented with 10 % FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and incubated in a humidified 5 % CO_2 incubator at 37° C. Cells were seeded in a 24-well plate in ~80 % confluence for one day before the experiments. Cells were incubated with probe **1** (1~20 μM) or vehicle (DMSO) for 1 h, washed with PBS. For the *N*-ethyl maleimide (NEM) treated samples, cells were pre-incubated with NEM (0.25 mM) for 30 min. Cells were washed with PBS before fluorescence imaging. Fluorescence images of the cells were obtained by an epifluorescence microscope (Olympus, JP IX-71). The excitation source was a 490 nm laser and all images were taken under the same experimental conditions to minimize variations in fluorescence intensity.

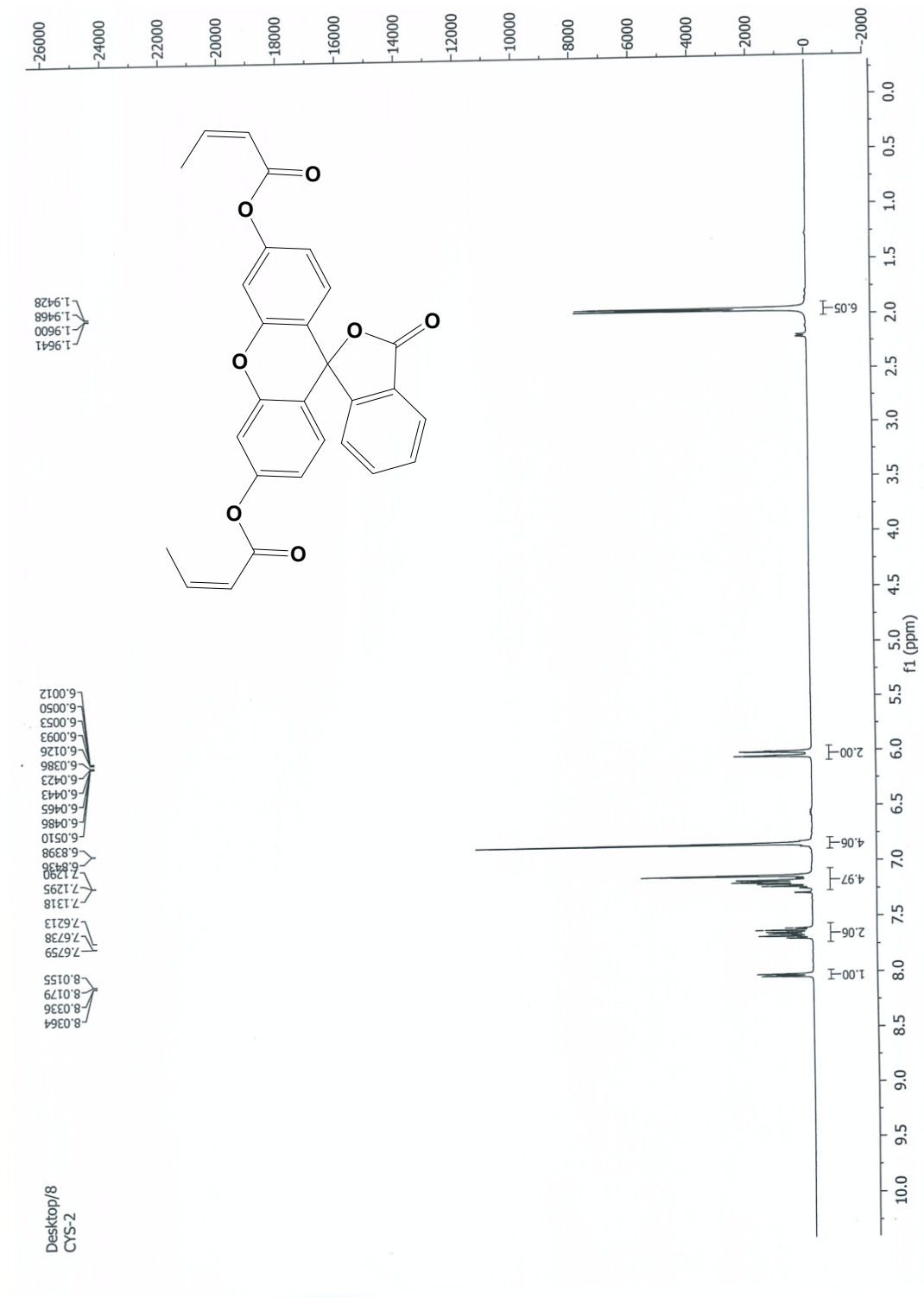

Fig. S1. ^{1}H NMR spectrum of Probe **1.**

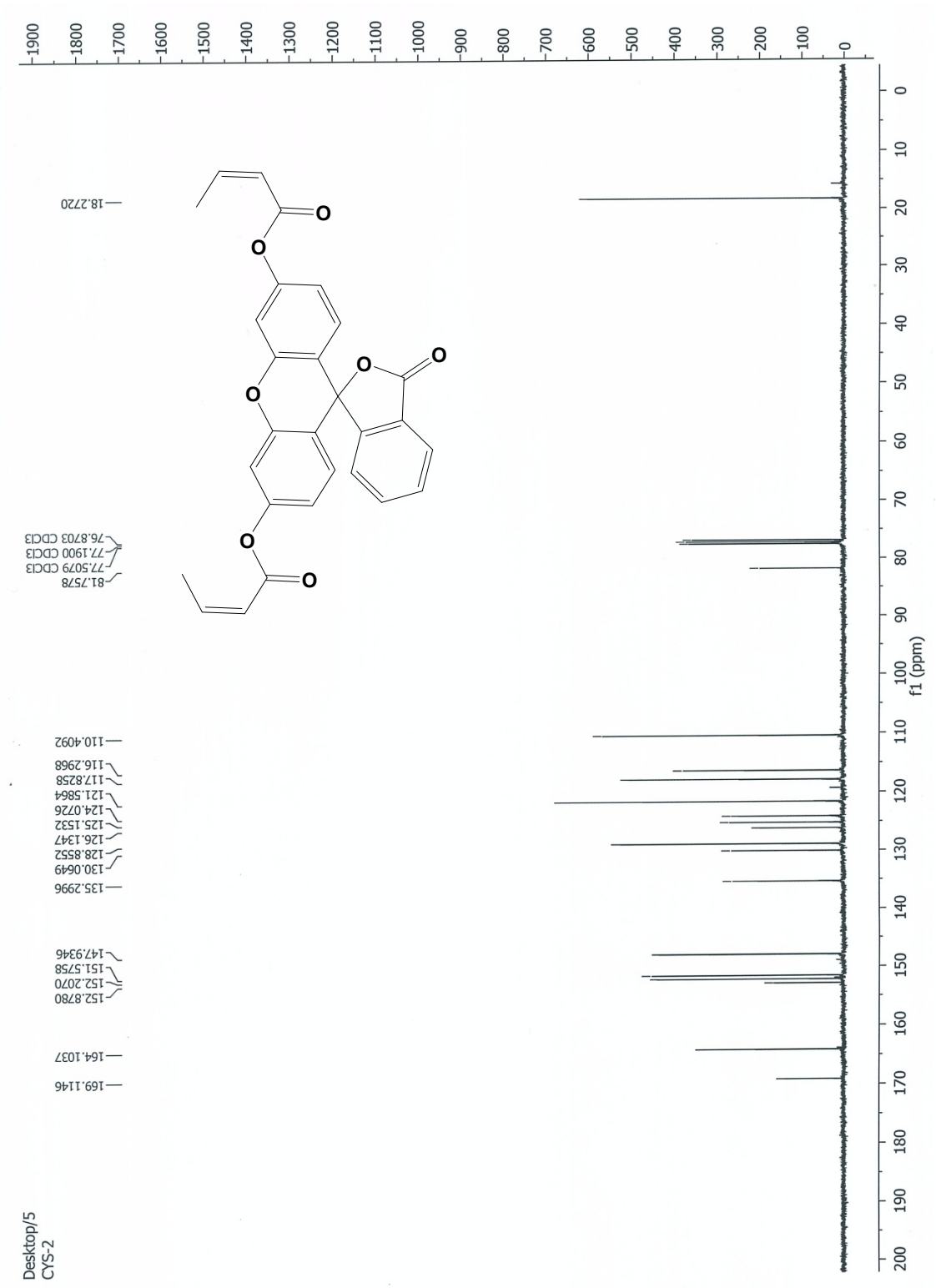

Fig. S2a. ^{13}C NMR spectrum of Probe **1.**

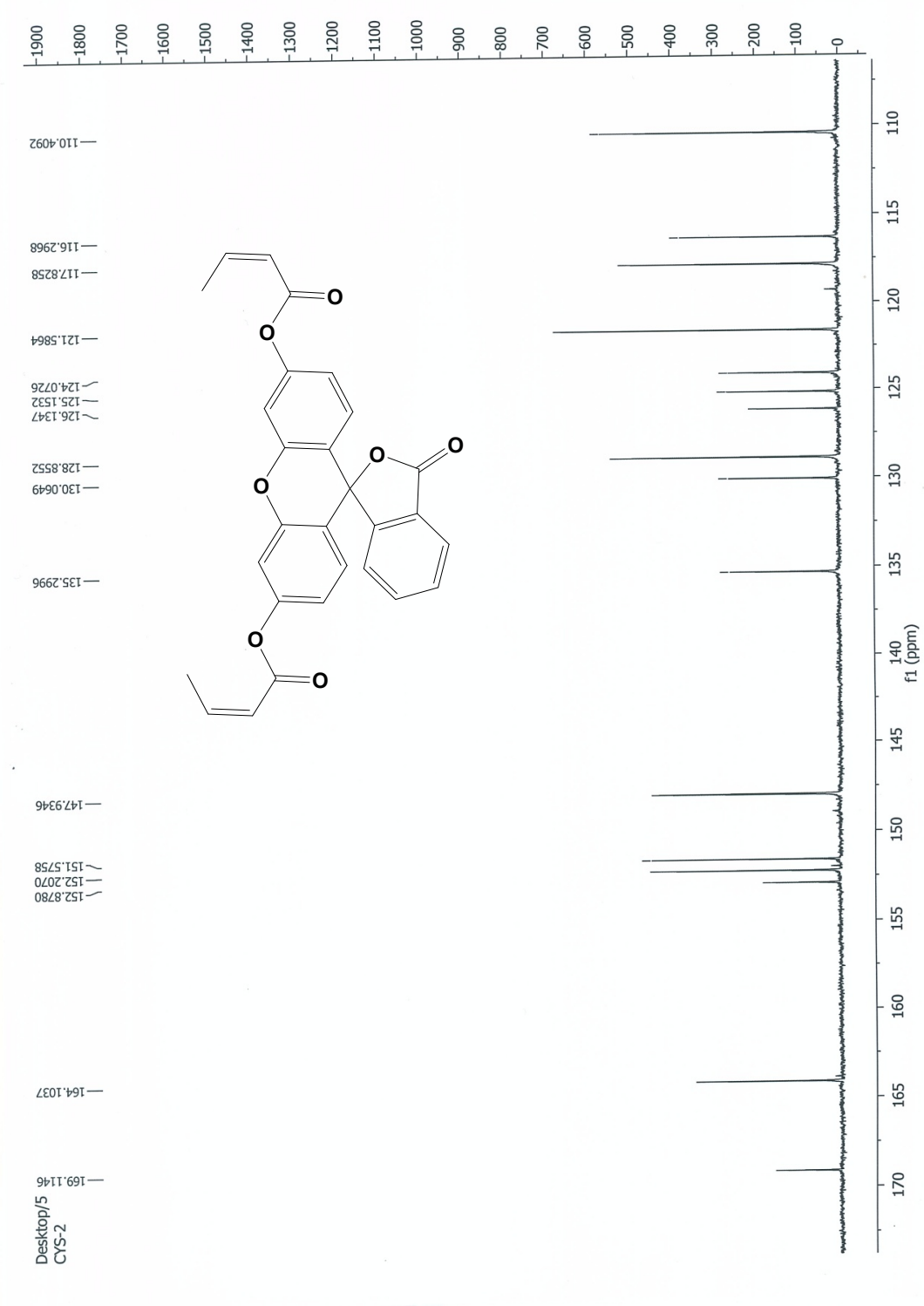

Fig. S2b. ^{13}C NMR spectrum of Probe **1** (EXPANDED VIEW).

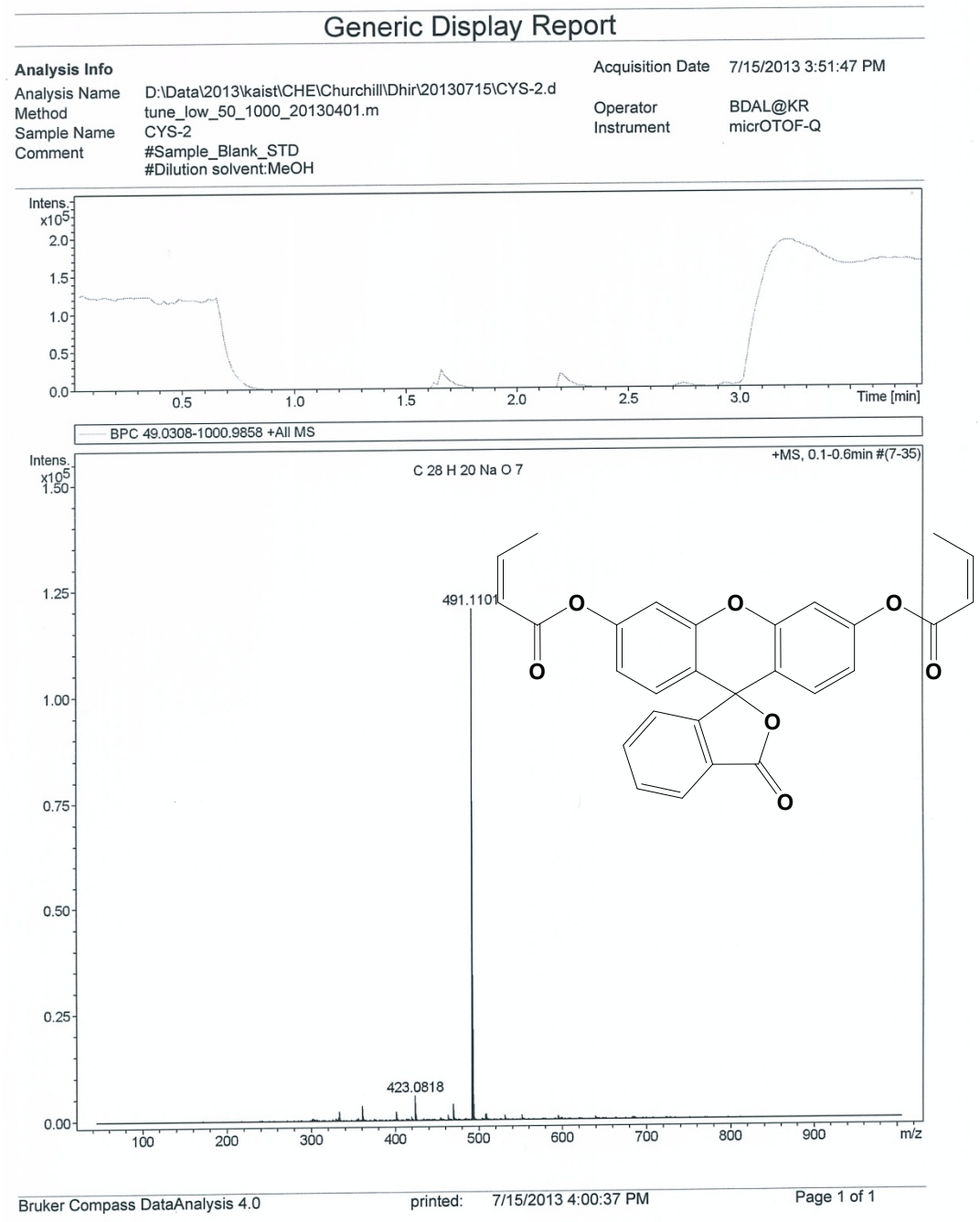

Fig. S3. ESI–mass spectrum of Probe **1.**

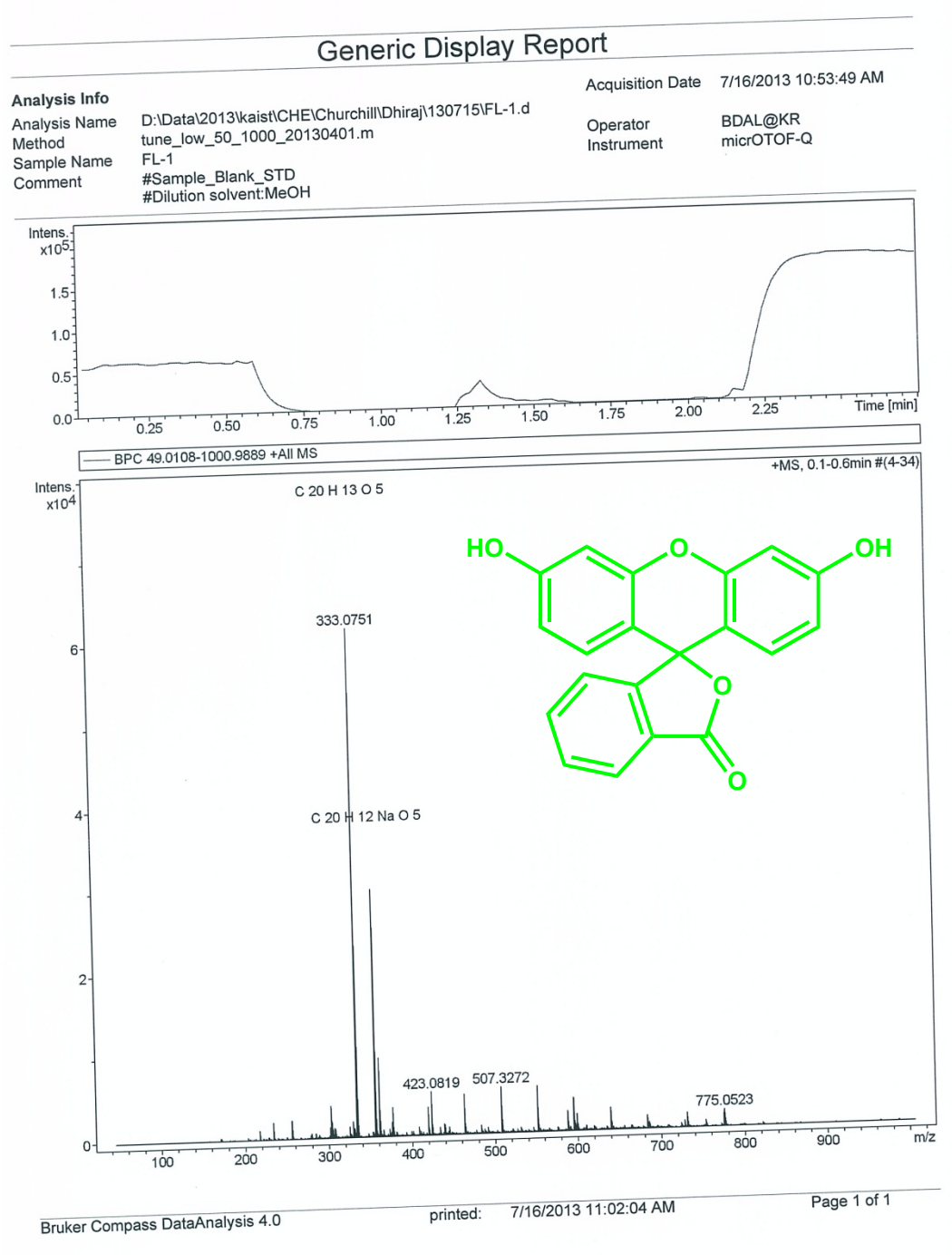

Fig. S4. ESI–mass spectrum of Probe **1** + dissolved cysteine. This data aided in identifying fluorescein as a final product.

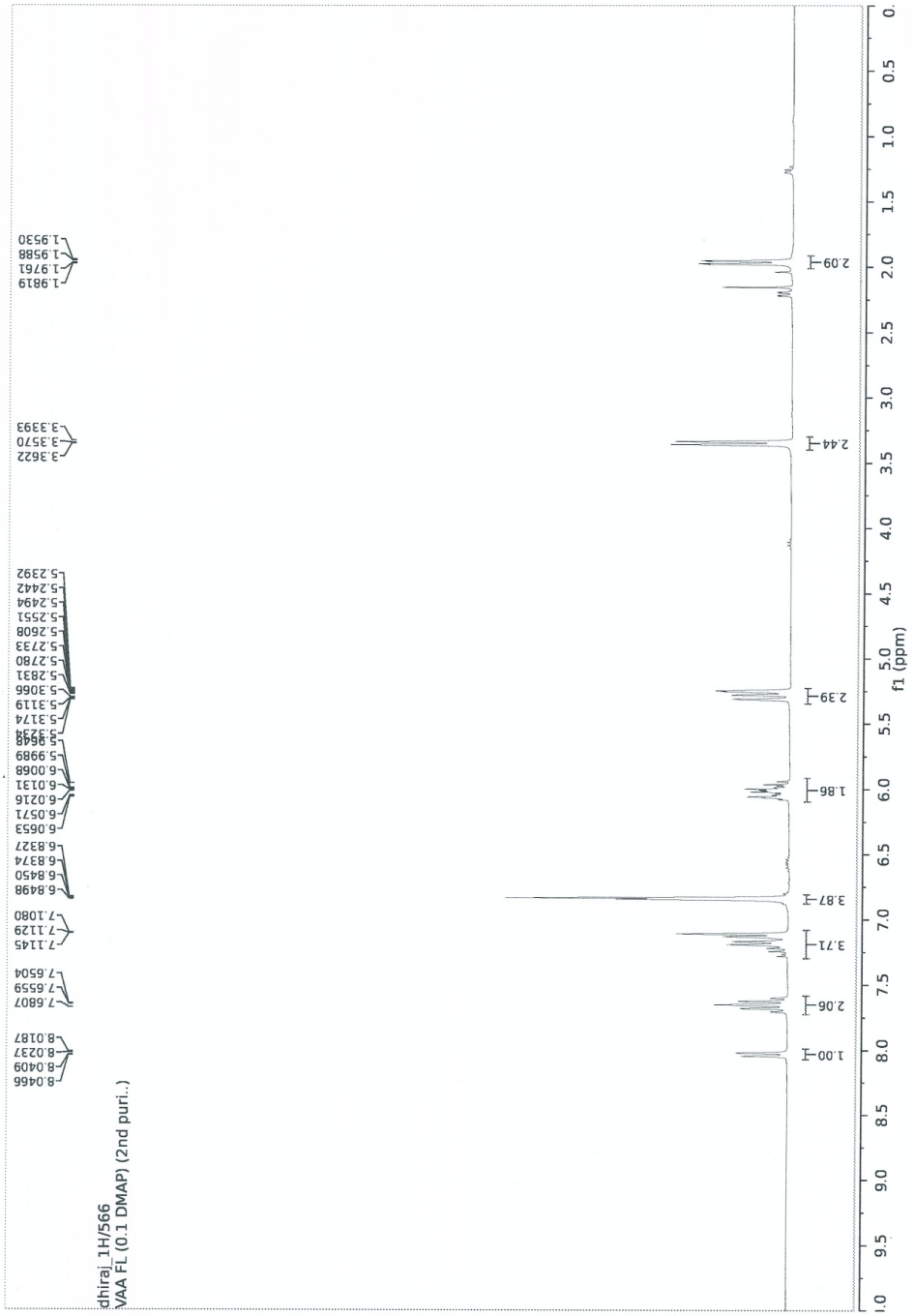

Fig. S5. ^{1}H NMR spectrum of probe **1** and probe **1a** with 0.2 equiv of DMAP (4-dimethylaminopyridine)**.**

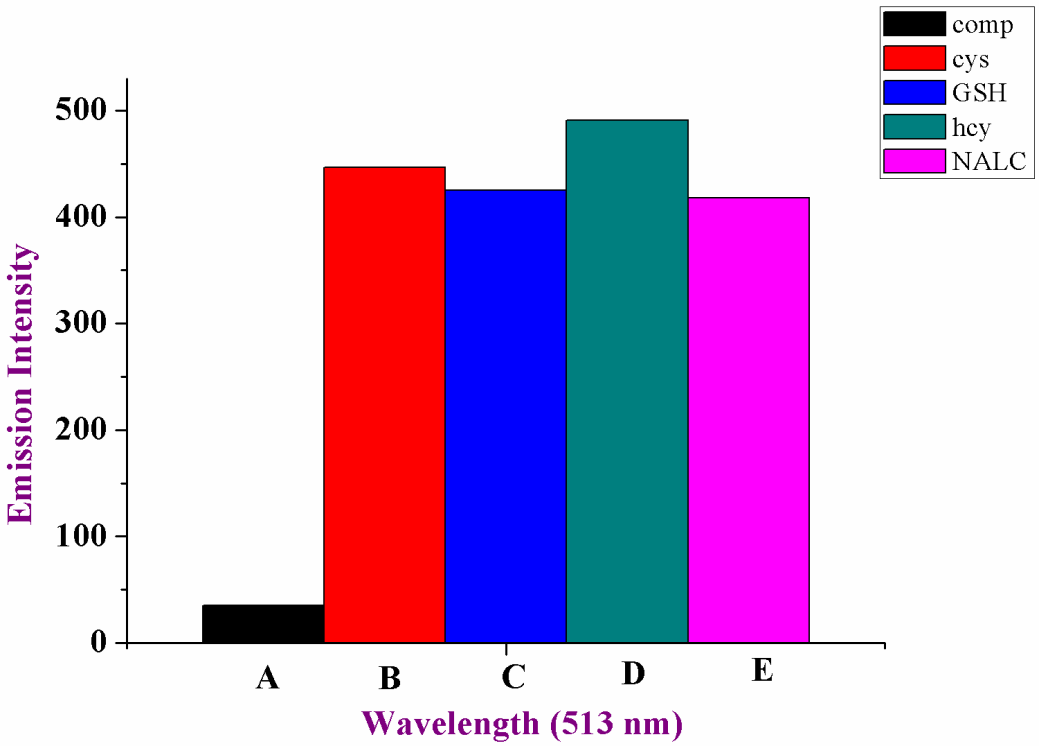

Fig. S6. Relative fluorescence intensity of compound **1** (4.0×10^{-6} M, buffered H_2O:DMSO 80:20; pH 7.4 PBS) with *Cys* (~10 equiv) with other amino acids (from left to right: A–compound, B–comp + *cys*, C–comp + *cys* + GSH, D–comp + *cys* + *GSH*, E–comp + *cys* + *N*–acetyl–*L*–*Cys* (~10 equiv). λ_{exci} = 490 nm. Slit width = 1.5.

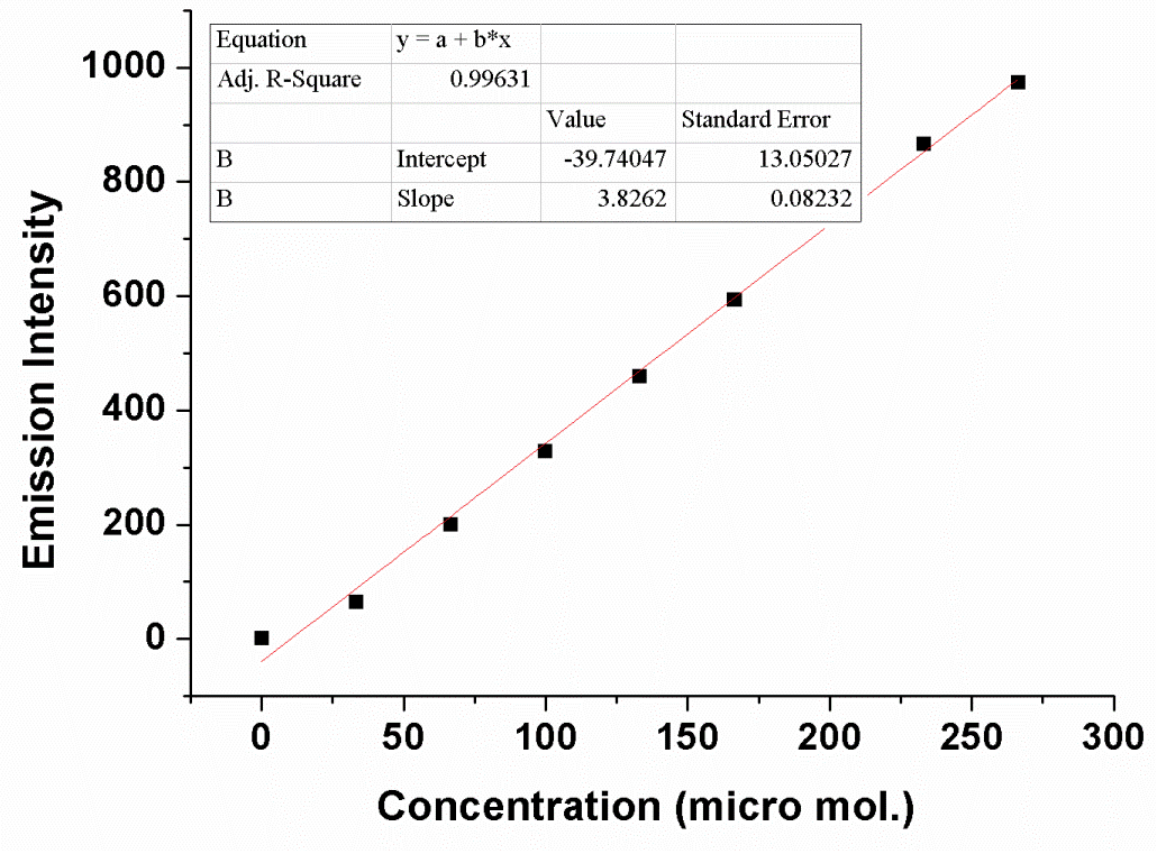

Fig. S7. Detection limit determinations for cysteine calculated from the linear fit (33.3 – 266 μM in water).

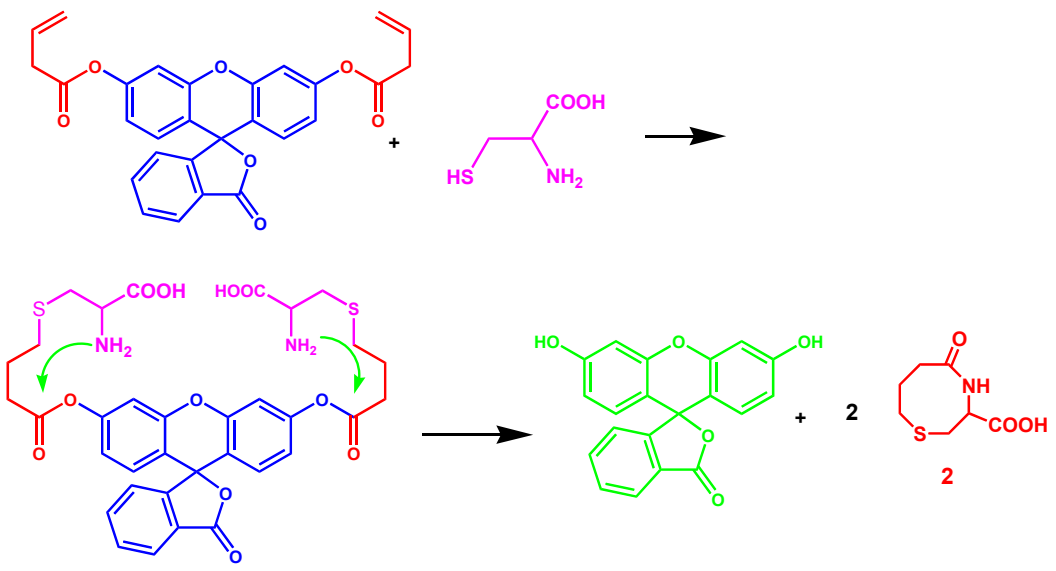

Fig. S8. Proposed sensing mechanism with probe **1a.**

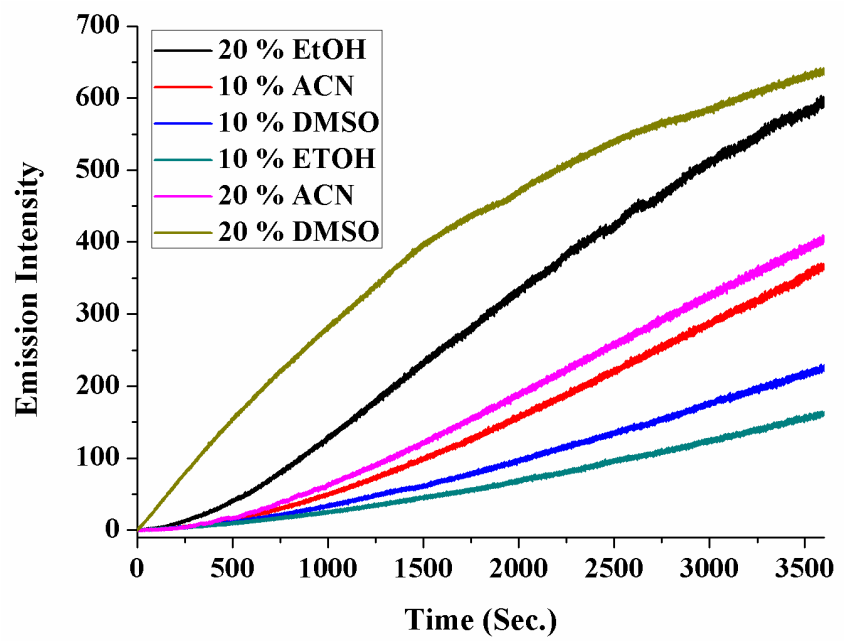

Fig. S9. Time–dependent emission spectra of probe (2.0×10^{-6} M) with Cys (200 μM in water) λ_{exci} = 490 nm) slit width Ex. & Em. = 1.5 nm.

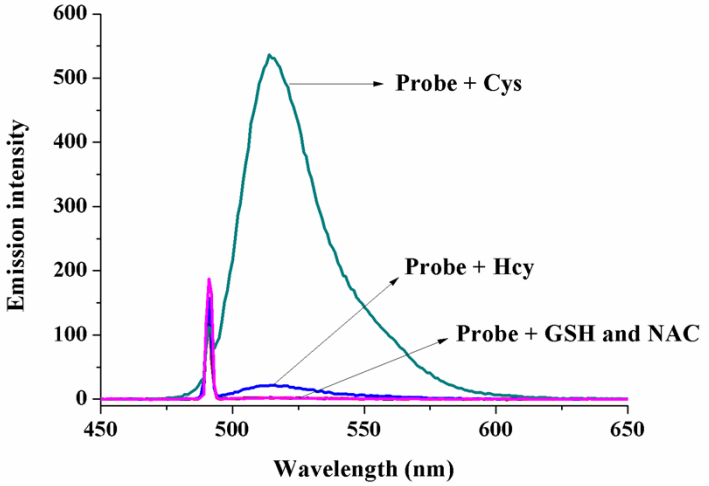

Fig. S10. Emission spectra of probe (4.0×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4 PBS) with amino acids *L–Cys*, *Hcy*, *N*–acetyl–*L–Cys* and GSH (66 μM in water) incubated for 1 h at RT. Slit width for Ex. & Em. = 1.5 nm.

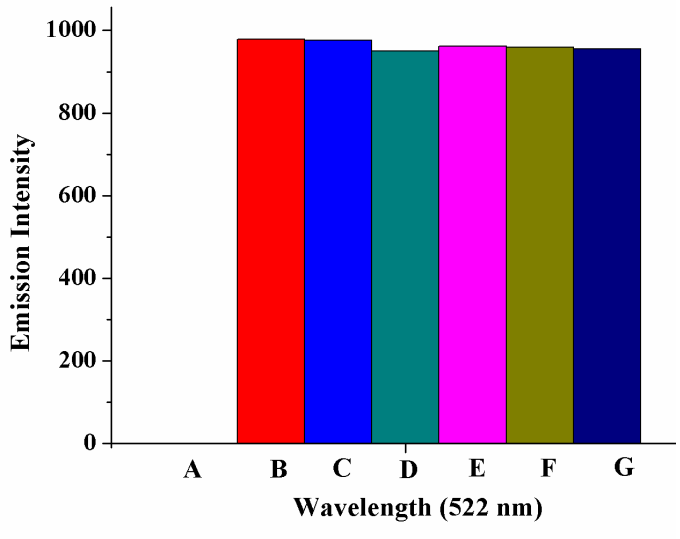

Fig. S11. Relative fluorescence intensity of probe-**1** (4×10^{-6} M, H_2O: DMSO 80:20;) with KO_2 and the presence of other interfering ROS (A) comp, (B) KO_2, (C) KO_2 + NaOCl, (D) KO_2 + H_2O_2. (E) KO_2 + ·OH, (F) KO_2 + ^{t}BuOOH, (G) KO_2 + ·O^{t}Bu, (~10 equiv) incubated for 30 min at RT. λ_{exci} = 490 nm.

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