Electronic Supplementary Material (ESI) for RSC Advances

SUPPORTING INFORMATION

Rapid and selective detection of Cys in living neuronal cells utilizing a novel Fluorescein with chloropropionate–ester functionalities

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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.*, $CDCl_3$. 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: 3-Chloropropionyl chloride (0.65 mL, 3.0 equiv.) in $CHCl_3$ (10 ml) was added dropwise over a period of 5 min to an ice-cold stirred solution of the Fluorescein (0.50 g) and 1 N NaOH (4 mL) in chloroform (20 mL). The mixture was stirred at RT for 24 h and then evaporated to dryness. The resulting oily residue was subjected to column chromatography (CH_2Cl_2) to obtain probe as a white solid. (0.208 gm, yield – 27 %)

^{1}H NMR spectroscopy: (400 MHz, $CDCl_3$): 8.01 (d, $^3J_{(H,H)}$ = 7.12 Hz, 1H), 7.68-7.59 (2H, m), 7.15 (d, $^3J_{(H,H)}$ = 7.12 Hz, 1H), 7.10 6.81 (S, 4H), 3.84 (t, $^3J_{(H,H)}$ = 6.48 Hz, 4H), 3.04 (t, $^3J_{(H,H)}$ = 6.48 Hz, 4H).

^{13}C NMR spectroscopy: (100 MHz, $CDCl_3$): 169.05, 168.30, 152.87, 151.75, 151.53, 135.31, 130.10, 129.02, 126.04, 125.26, 123.01, 117.65, 116.77, 110.34, 81.48, 38.66, 37.65.

ESI-MS: **Fig. S3** probe-**1** $[M+Na]^+$ = 535.0327 (calc.), 535.0327 (exp.)

ESI-MS: **Fig. S4** of probe **1** + **L-cys** $[M+H]^+$ = 333.076 (calc.), 333.073 (exp.**) [Fluorescein]**

Analysis of Cys in living cells: We performed assays with SH–SY5Y cells (Fig. 5). Human neuroblastoma (cell line SH–SY5Y) were 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 (37° C). Cells were seeded in a 24–well plate in ~80 % confluence for one day before fluorescence microscopy experiments were performed. Cells were incubated with probe **1** (1 μM) or vehicle (DMSO) for 10 minutes, and then washed with PBS. For the *N*–ethyl maleimide (NEM) for the samples treated with cells were pre–incubated with NEM (1 mM) for 30 min. Cells were then washed with PBS before fluorescence images were acquired. Fluorescence images of the cells were obtained by an epifluorescence microscope (Olympus, JP IX–71). The excitation source was a 490 nm laser; all images were taken under the same experimental conditions to minimize variations in fluorescence intensity. As a result of these SH–SY5Y cell assays, the probe was found to be cell–permeable and selective for the detection of intracellular Cys through the NEM experimental technique.

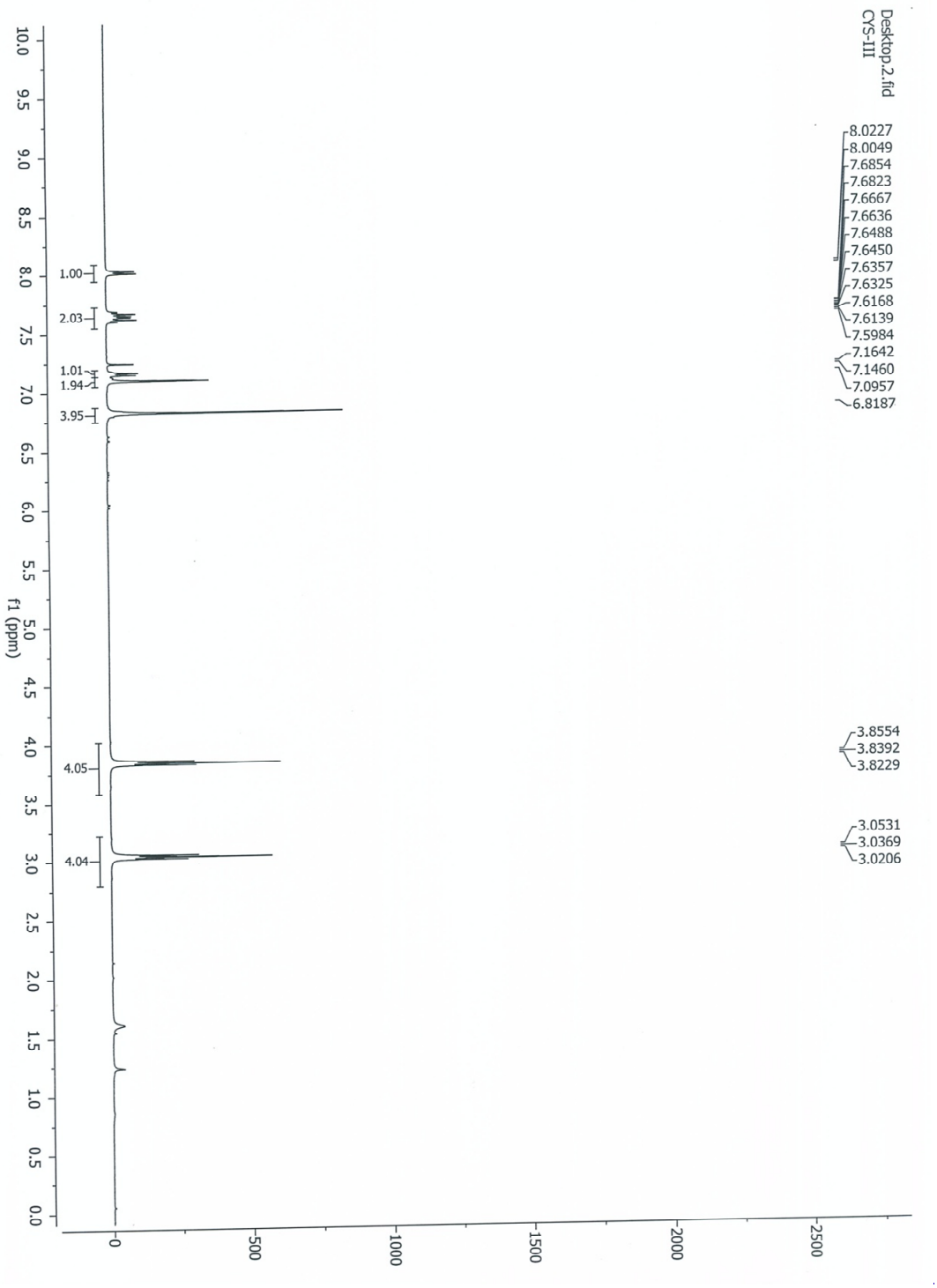

Fig. S1. ^{1}H NMR spectrum of Probe **1.**

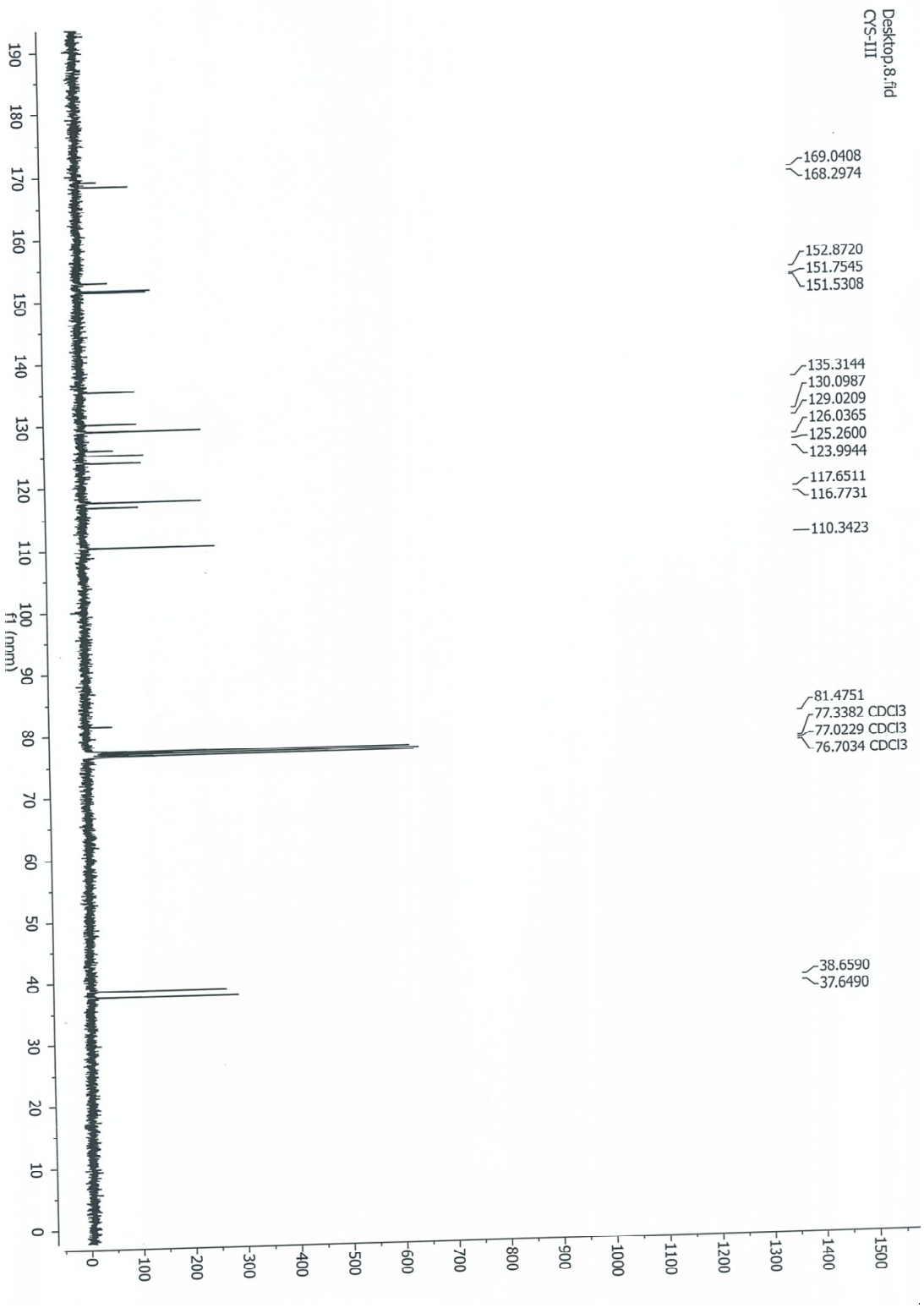

Fig. S2. ^{13}C NMR spectrum of Probe **1.**

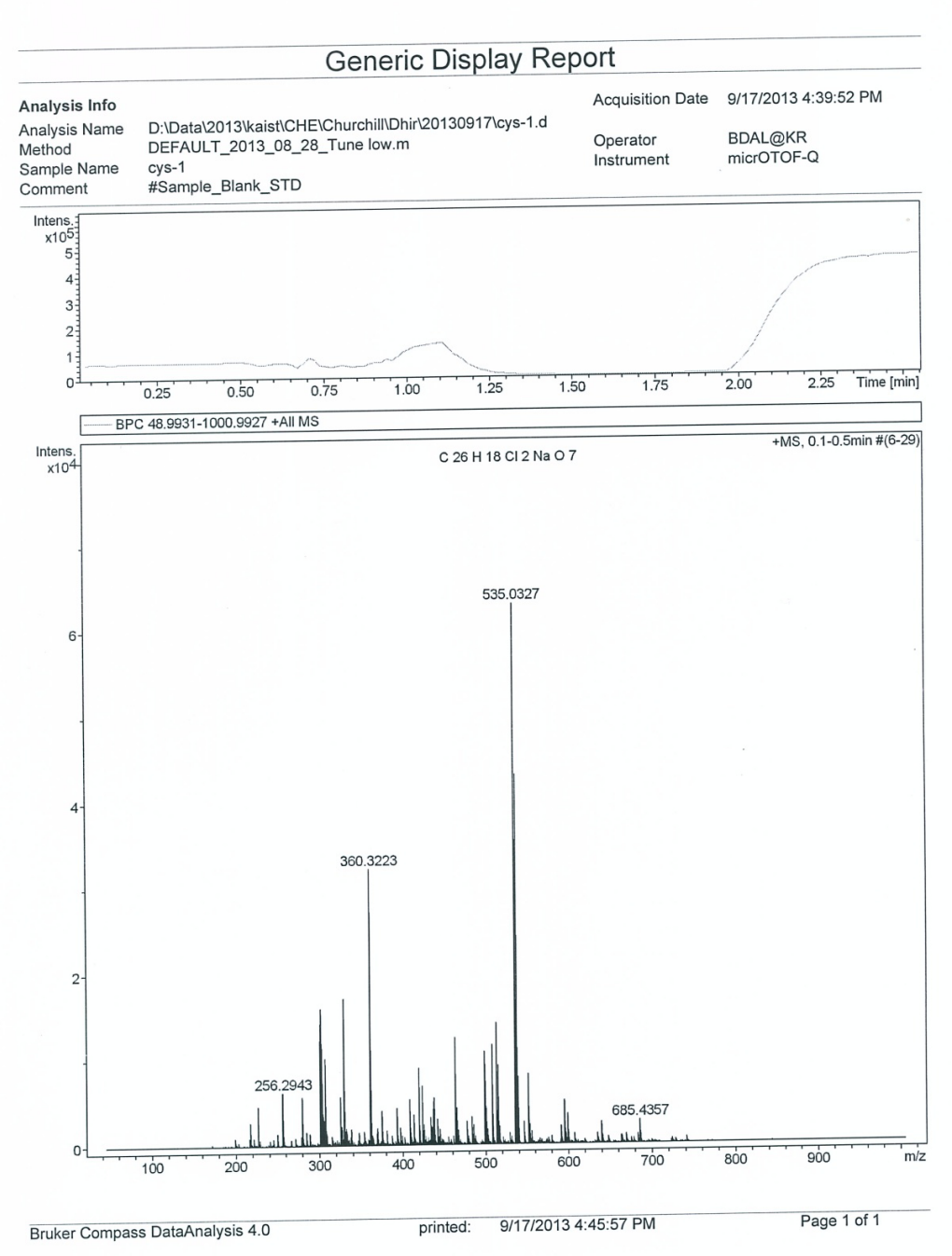

Fig. S3. ESI–mass spectrum of Probe **1.**

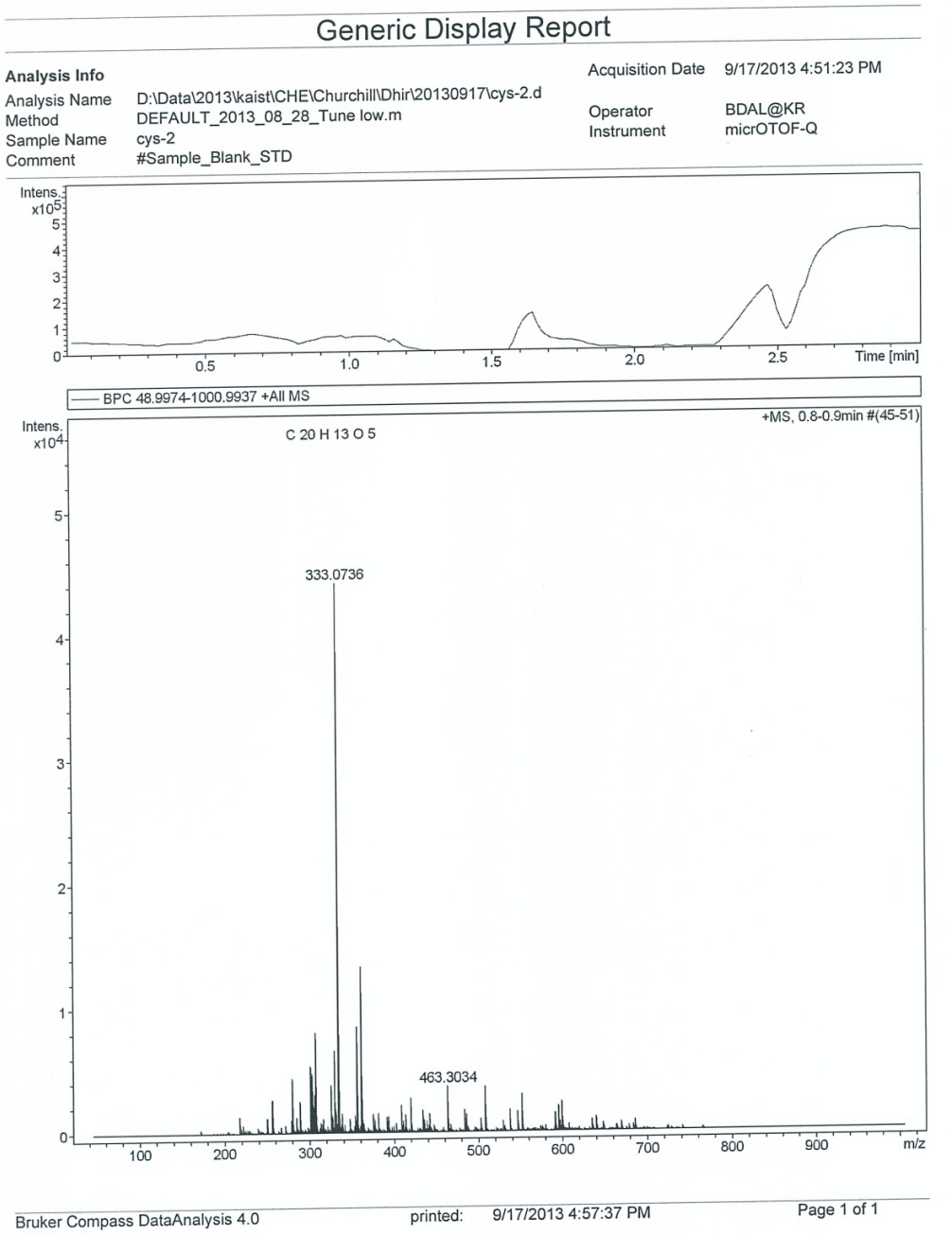

Fig. S4. ESI–mass spectrum of Probe **1** + cysteine showing fluorescein as a final product.

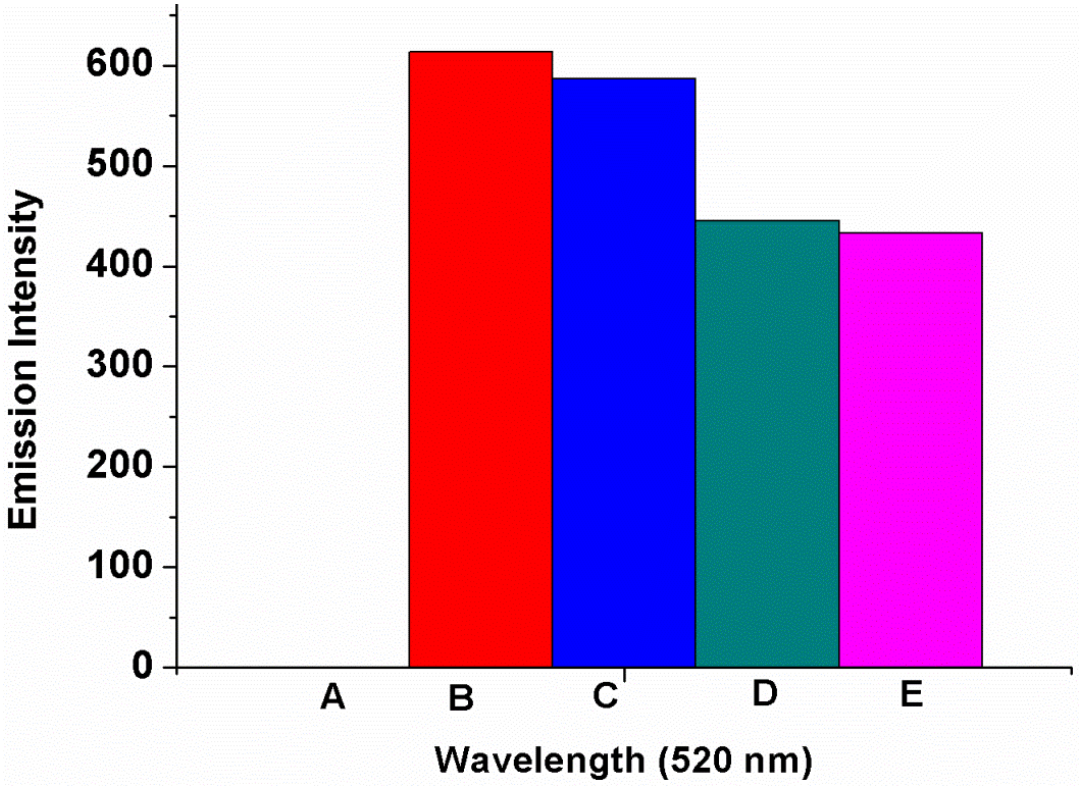

Fig. S5. Competition study results: Relative fluorescence intensity of compound **1** (4.0×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4 PBS) with *Cys* (~10 equiv) with other amino acids 10 equiv. (from left to right: A – compound, B–comp + *cys*, C – comp + *cys* + Hcy, D – comp + *cys* + *GSH*, E – comp + *cys* + *N*–acetyl–*L*–*Cys* (~10 equiv). λ_{exci} = 490 nm. Slit width = 1.5 nm.

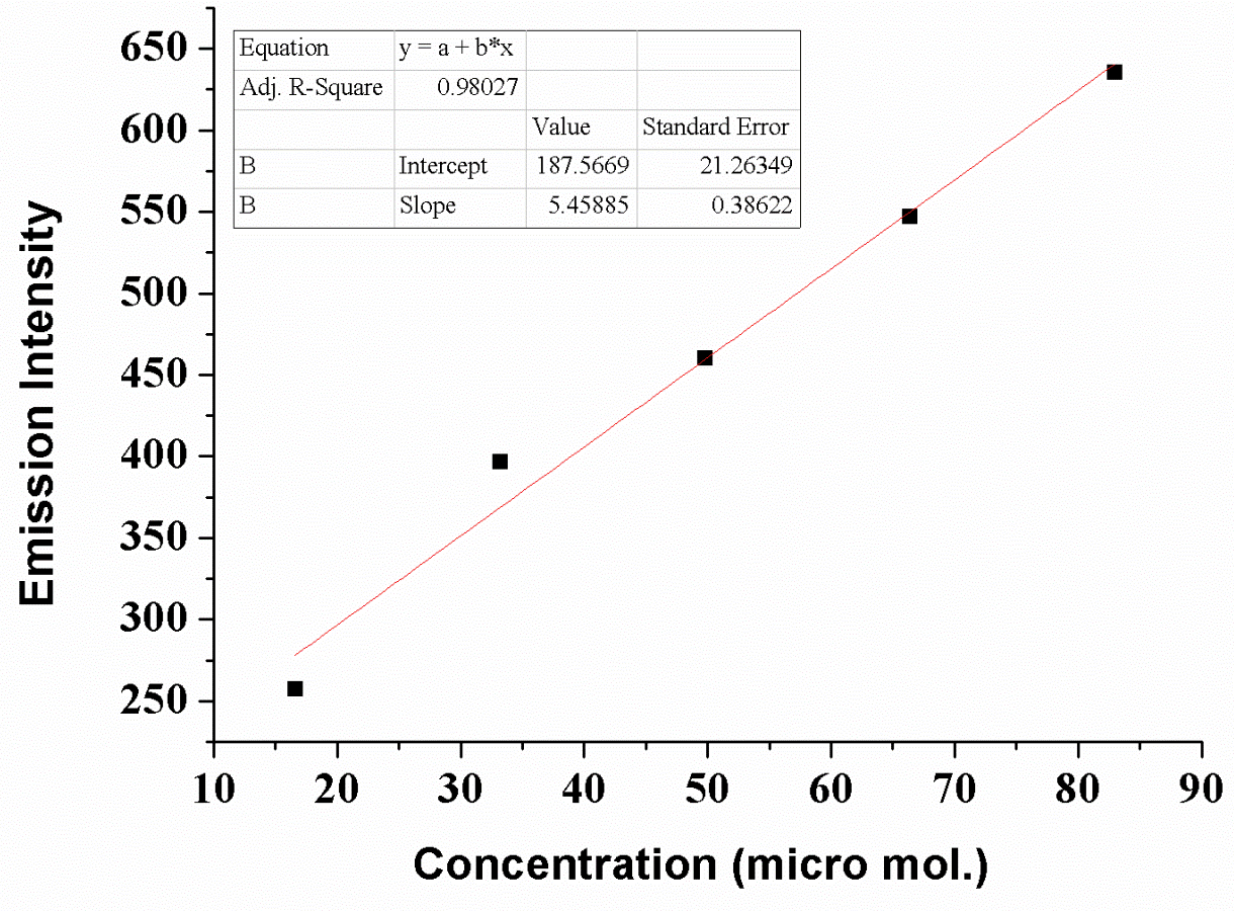

Fig. S6. (C) Emission spectra change of **Probe 1** as a function of concentration Cys (16.6 – 166.5 μM) in mixture solution of (4.0×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4 PBS) . Each spectrum was recorded at 10 min after the addition of Cys to **probe 1**.

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