Electronic Supplementary Information

Turn-on NIR fluorescence and colorimetric cyanine probe for monitoring thiol content in serum and glutathione reductase assisted glutathione redox process

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General method for measurements of photophysical properties

UV/Vis spectra were recorded on Perkin Elmer Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer. 1 cm cells were used for absorption and emission titration. For UV/Vis and fluorescence titrations stock solution of **DNBSCy** probe were prepared (c = 2000 μM) in 10 mM PBS buffer (pH = 7.4). The solutions of guest cations and amino acids were prepared in buffer solution in the order of 10^{-3} M. Working solutions of the probe and metal ions were prepared from the stock solutions. Excitation was carried out at 600 nm excitation wavelength and 10 nm emission slit widths.

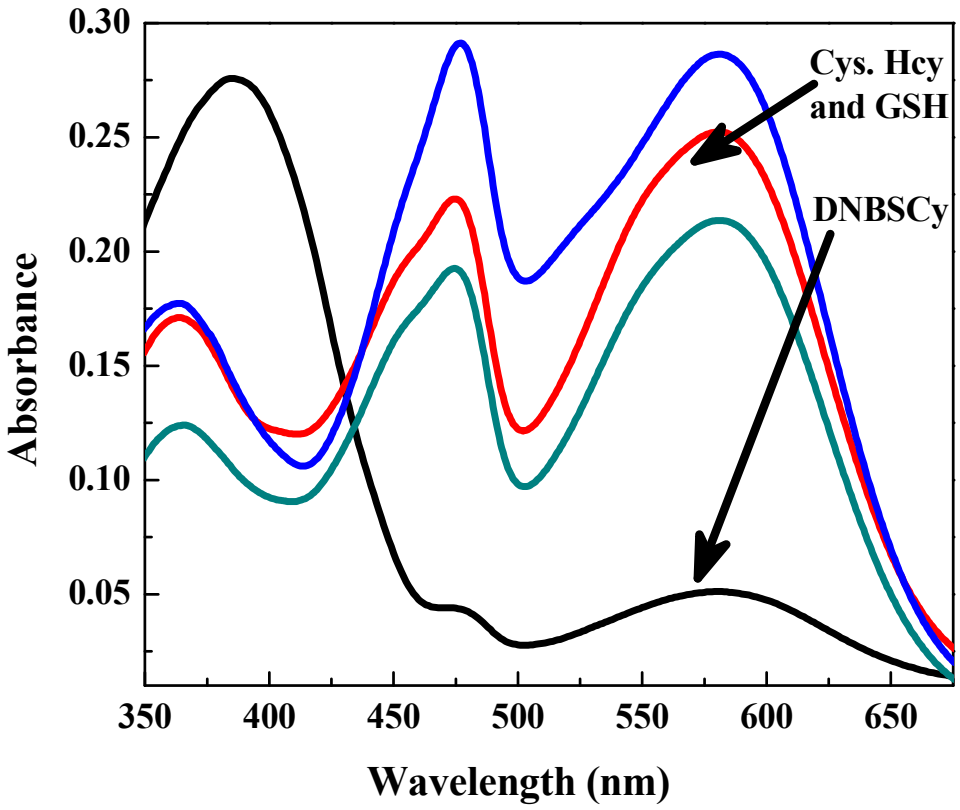


Fig. S1 UV/Vis absorption spectra of **DNBSCy** (10.0 μM) and on addition of 10.0 equivalents of Cys, Hcy and GSH in 10 mM PBS buffer medium (pH = 7.4).

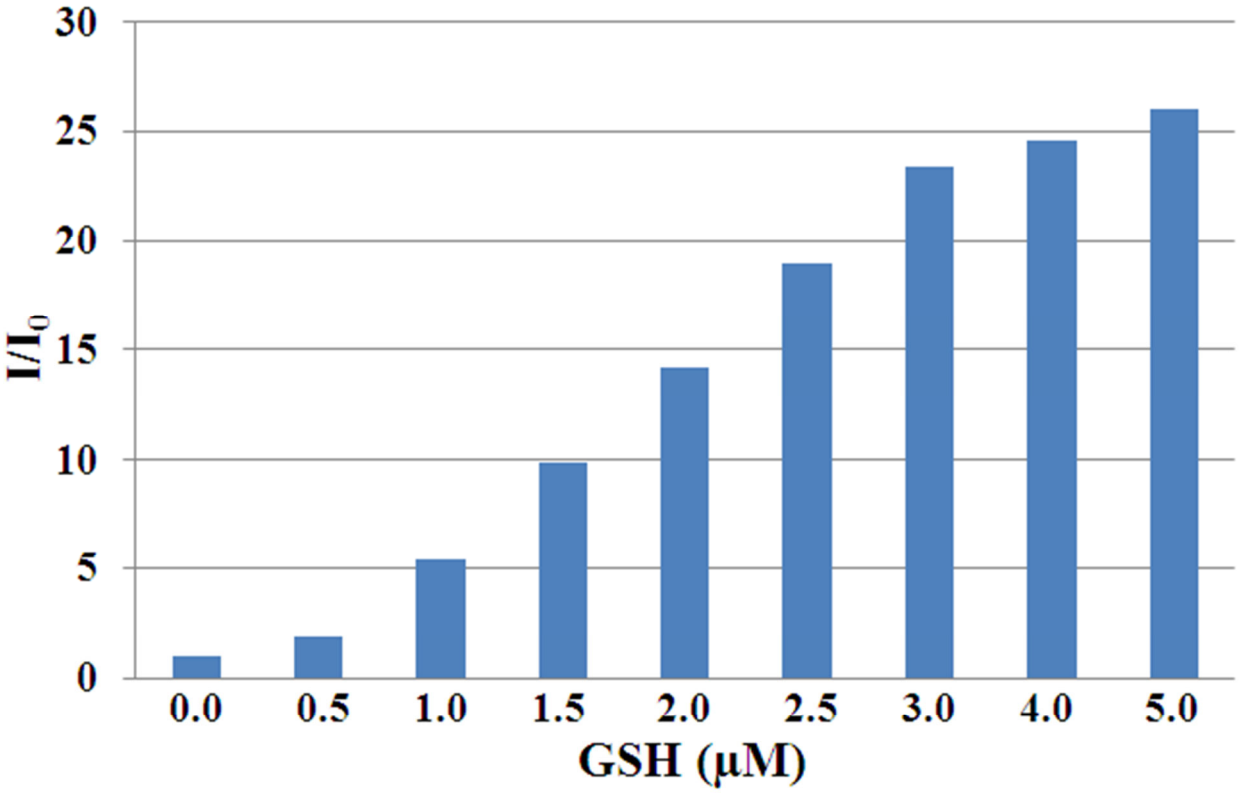


Fig. S2 Relative fluorescence response of **DNBSCy** (10.0 μM) on addition different concentration of GSH in 10 mM PBS buffer medium (pH = 7.4).

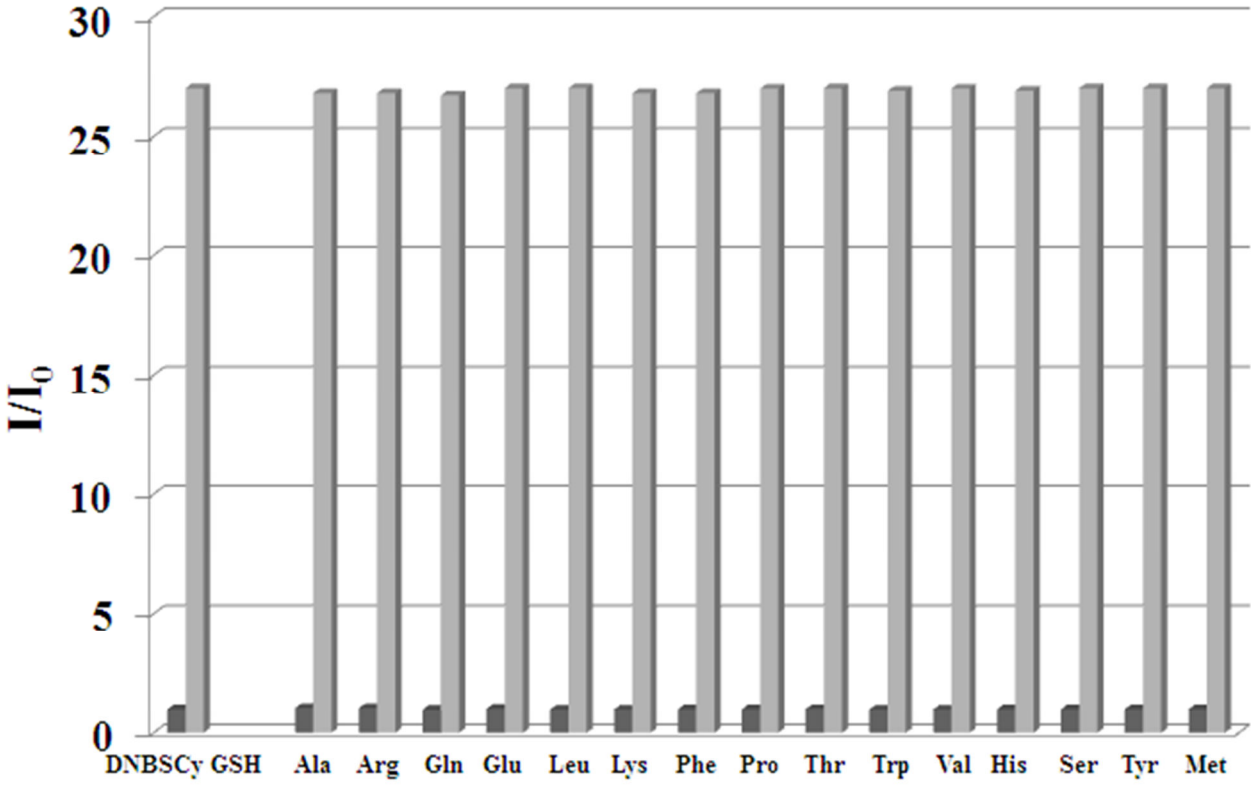


Fig. S3 Relative fluorescence intensities of **DNBSCy** with GSH in the presence of various other amino acids. Dark grey bar: **DNBSCy** (10.0 μM) with 10 equiv. of amino acid stated. Light grey bar: 10.0 μM of **DNBSCy** and 5 equiv. of GSH with 10 eqv. of amino acid stated. (E_λ = 695 nm). The responses of the **DNBSCy** to GSH, in the absence of amino acids, is included as controls, dark grey bar, no amino acid added, light grey bar, 10.0 μM of **DNBSCy** with 5 eqv. of GSH in 10 mM PBS buffer medium (pH = 7.4).

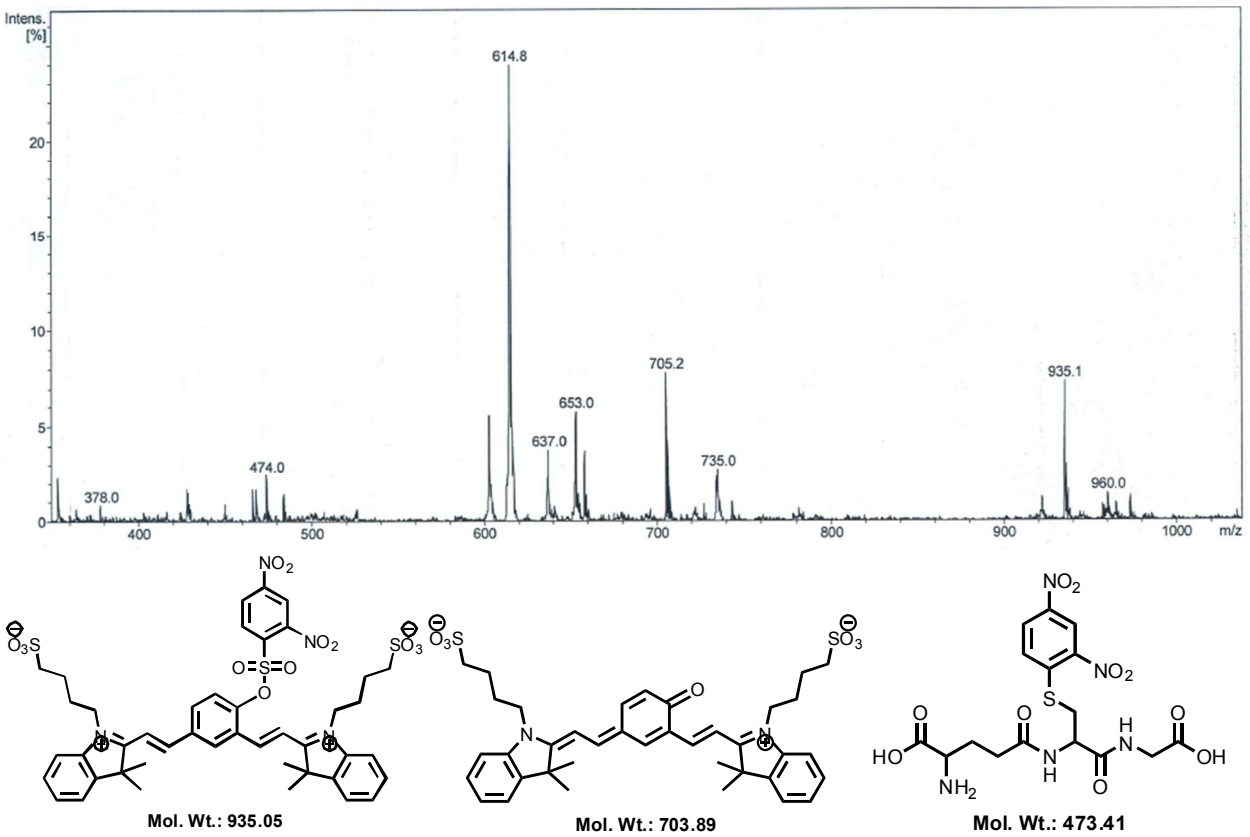


Fig. S4 ESI mass spectra (positive ion mode) for the reaction of 10.0 μM **DNBSCy** with GSH (10.0 μM) in water. Mass peak observed at 935.1 was attributed to unreacted **DNBSCy**, peak at m/z = 705.2, (calculated for $[C_{38}H_{43}N_2O_7S_2^- + H]^+$) corresponding to this cyanine dye (**Cy-quinone**); peaks at 614.8 ($[M + 2H]^+$) , 637.0 ($[M + H+ Na]^+$) and 653 ($[M + H+ K]^+$) were attributed to the oxidized form of GSH (GS–SG). Peak at m/z = 474.0 corresponding to the side product γ-glutamyl-S-(2,4-dinitrophenyl)cysteinylglycine (calculated for $C_{16}H_{19}N_5O_{10}S$).

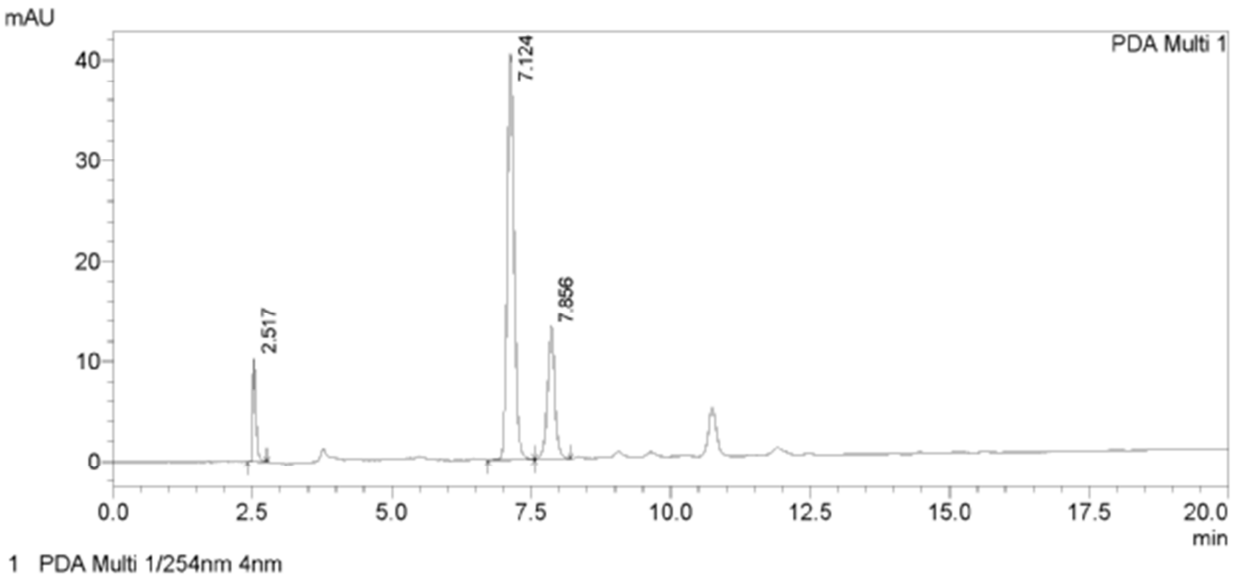


Fig. S5 RP-HPLC (grad. 20-100% ACN in water, 20 min.) of the reaction mixture of **DNBSCy** and GSH in 10 mM PBS buffer medium (pH = 7.4).

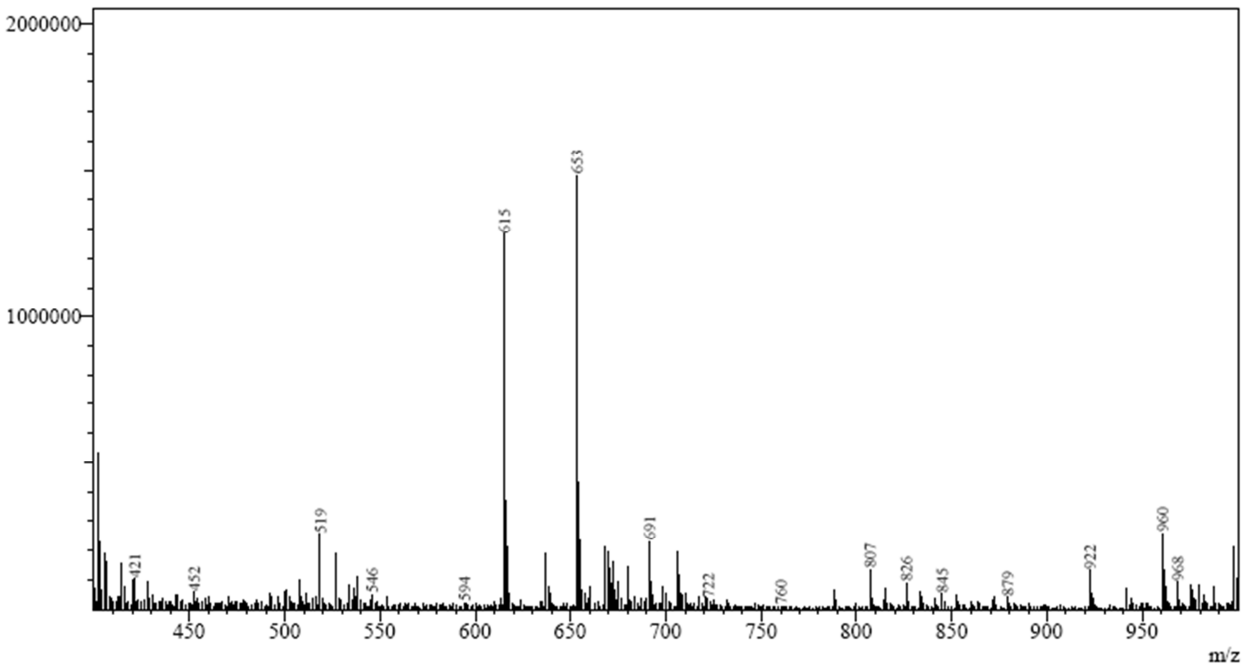


Fig. S6 ESI mass spectra (positive ion mode) of the eluant fraction collected at 2.517 min in RP-HPLC (Fig. S5). Mass peak observed at 615 ($[M + 2H]^+$) and 653 ($[M + H+ K]^+$) were attributed to the oxidized form of GSH (GS–SG).

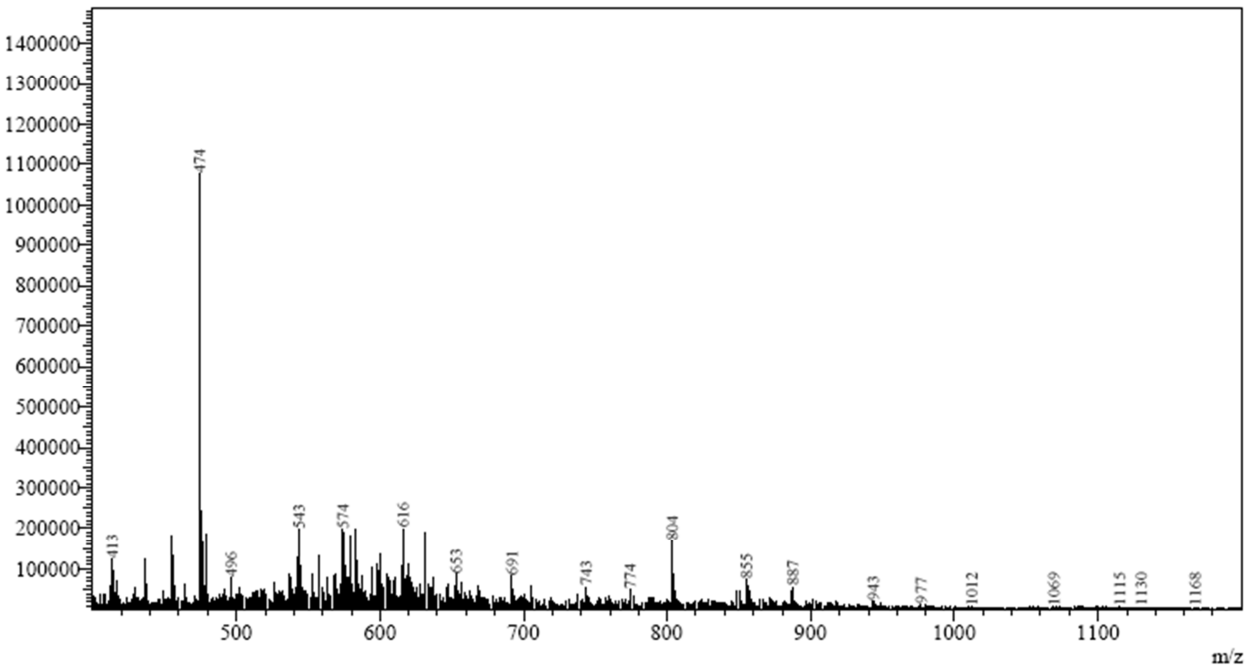


Fig. S7 ESI mass spectra (positive ion mode) of the eluant fraction collected at 7.124 min in RP-HPLC (Fig. S5). Mass peak at m/z = 474.0 corresponding to the side product γ-glutamyl-S-(2,4-dinitrophenyl)cysteinylglycine (calculated for $C_{16}H_{19}N_5O_{10}S$).

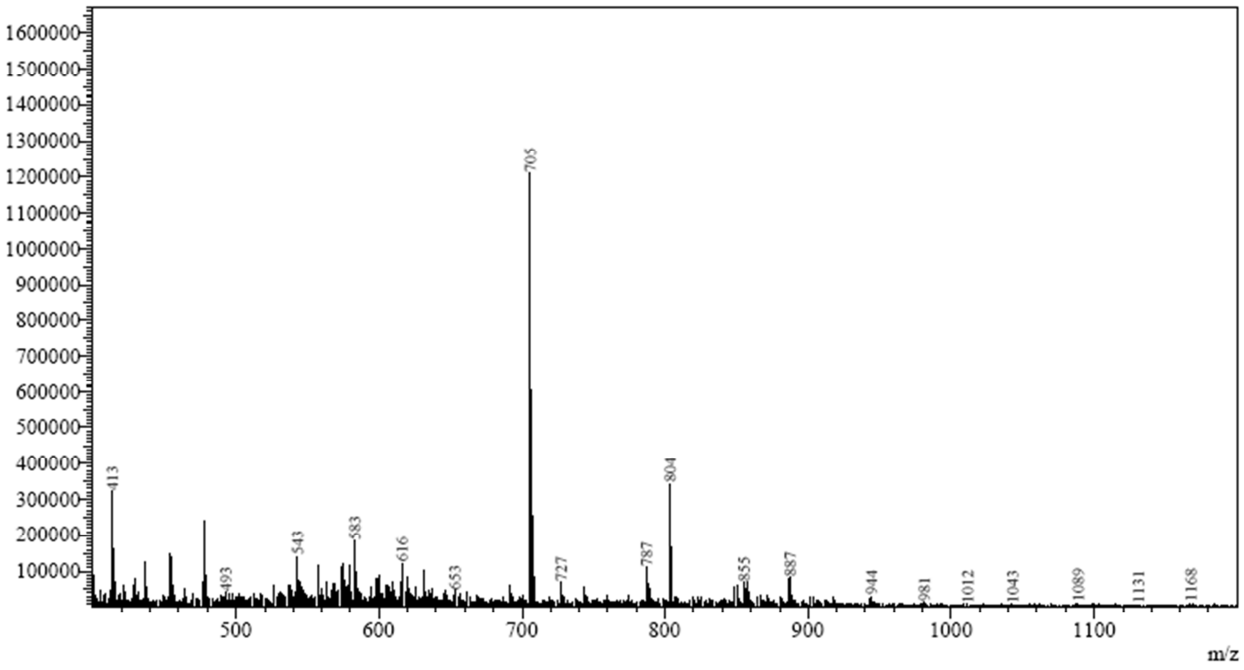


Fig. S8 ESI mass spectra (positive ion mode) of the eluant fraction collected at 7.856 min in RP-HPLC (Fig. S5). Mass peak at m/z = 705, (calculated for $[C_{38}H_{43}N_2O_7S_2^- + H]^+$) corresponding to this cyanine dye (**Cy-quinone**).

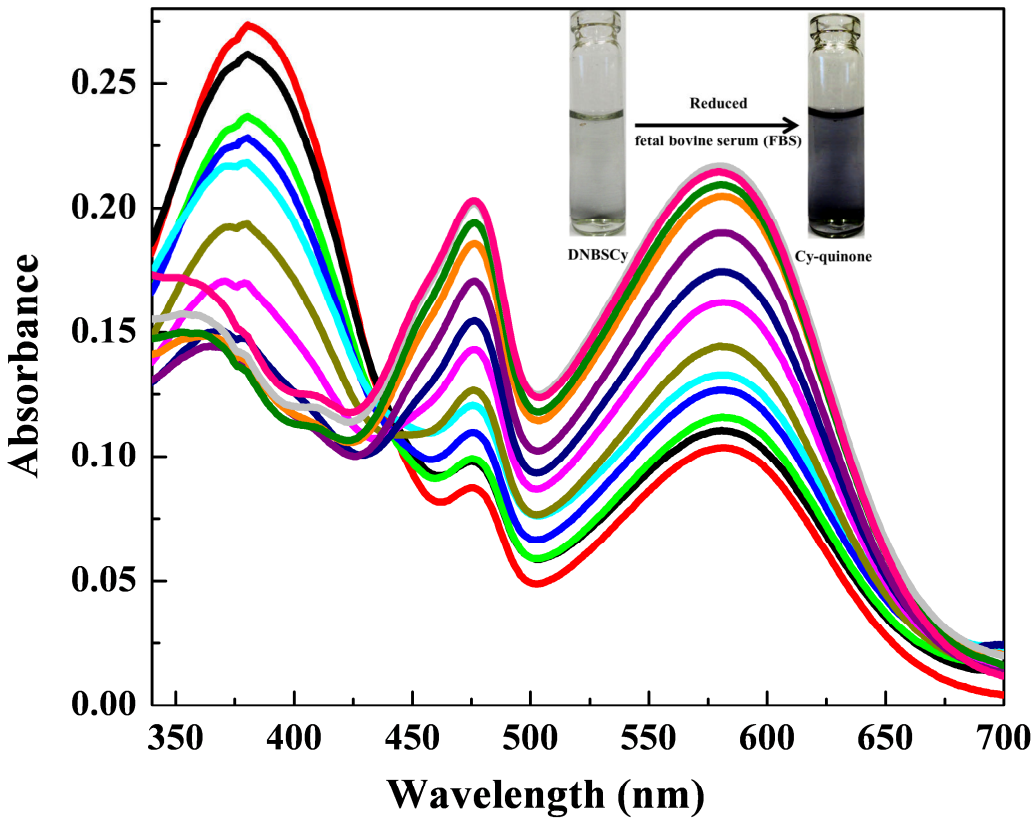


Fig. S9 Absorption spectra of probe **DNBSCy** (10.0 μM) upon addition of various amounts (0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 μL) of reduced fetal bovine serum solution to the 10 mM PBS buffer solution (pH 7.4).

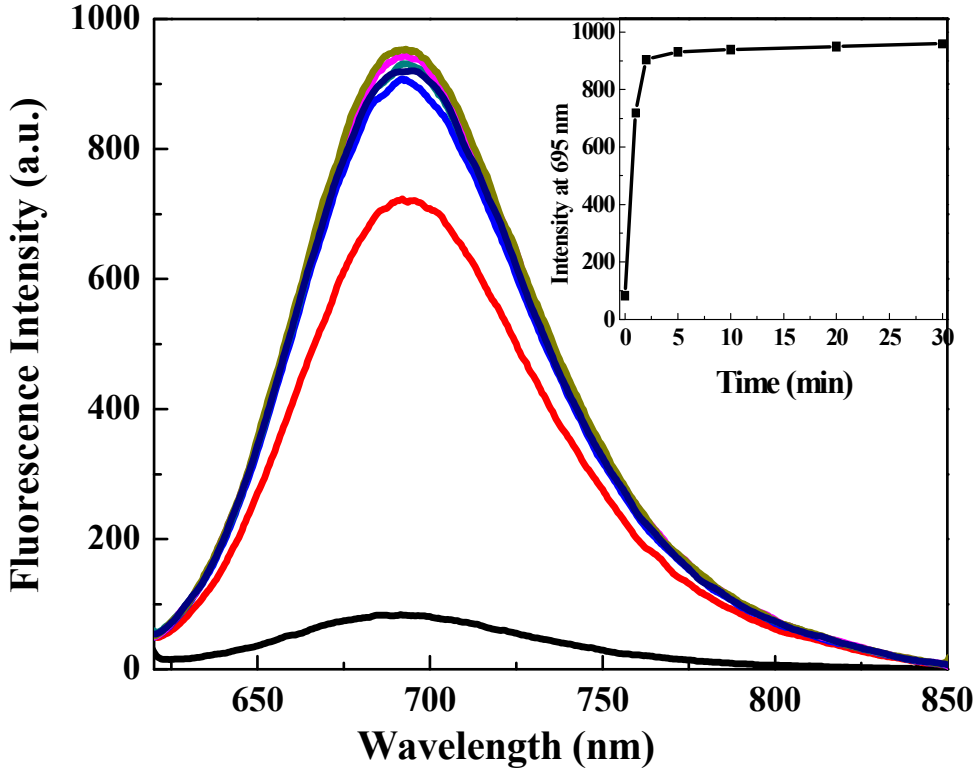


Fig. S10 Fluorescence spectra of probe **DNBSCy** (10.0 μM) in response to enzymatic conversion of GSSG (2 mM) to free GSH using glutathione reductase (1 unit/mL) and 0.4 mM of NADPH in the10 mM PBS buffer solution (pH 7.4).

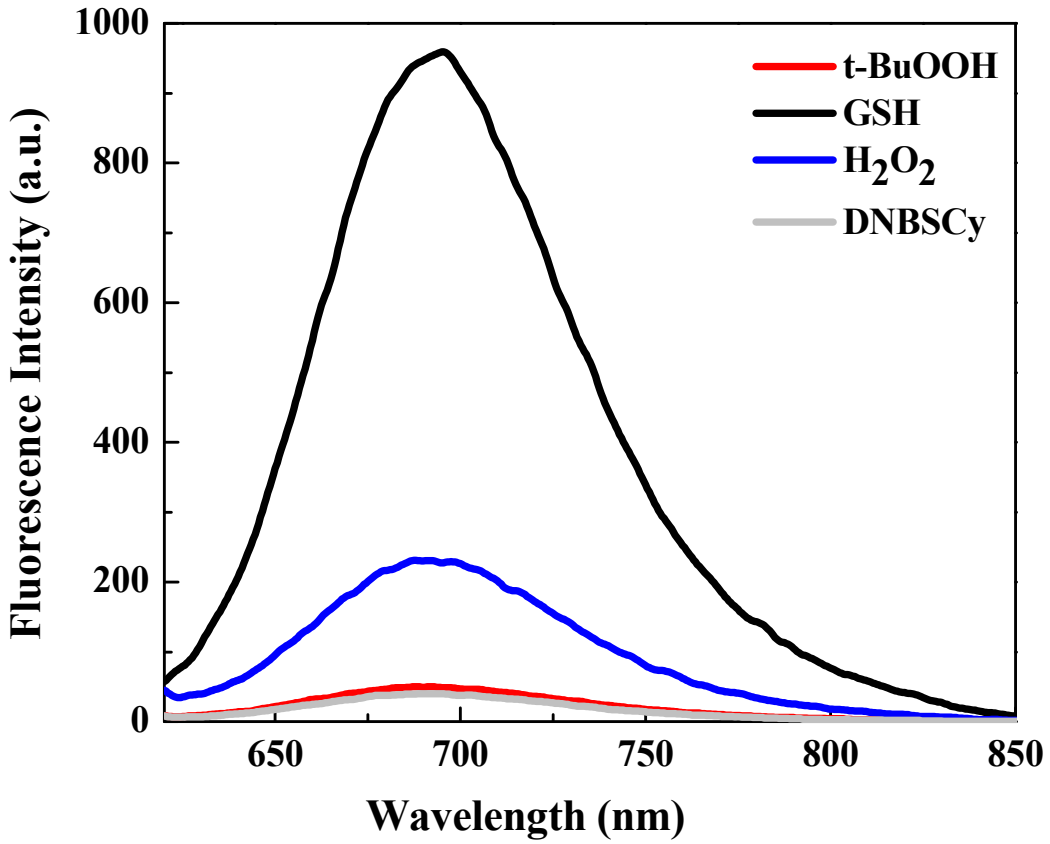


Fig. S11 Fluorescence spectra of probe **DNBSCy** (10.0 μM) in presence of 1.0 equivalent of GSH, t-BuOOH and H_2O_2 in the10 mM PBS buffer solution (pH 7.4).

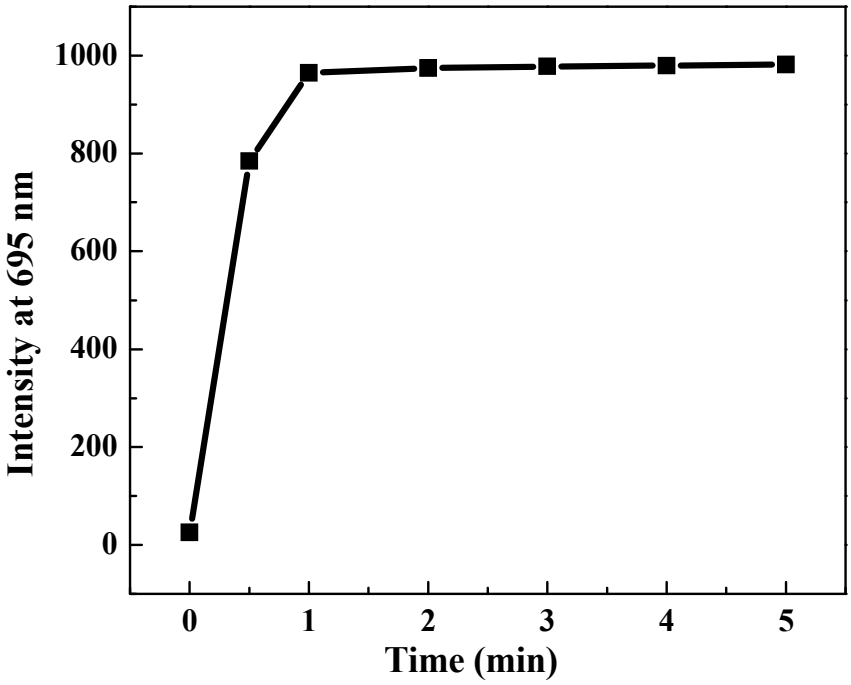


Fig. S12 Emission intensity response of the probe **DNBSCy** (10.0 μM) upon addition of 2 mM of GSH in the10 mM PBS buffer solution (pH 7.4).

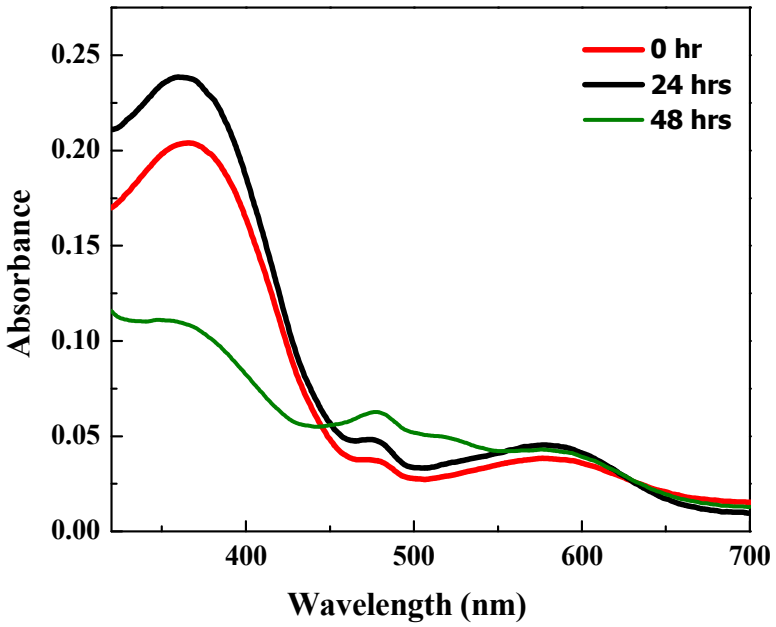


Fig. S13 Absorption spectra of probe **DNBSCy** (10.0 μM) in water.

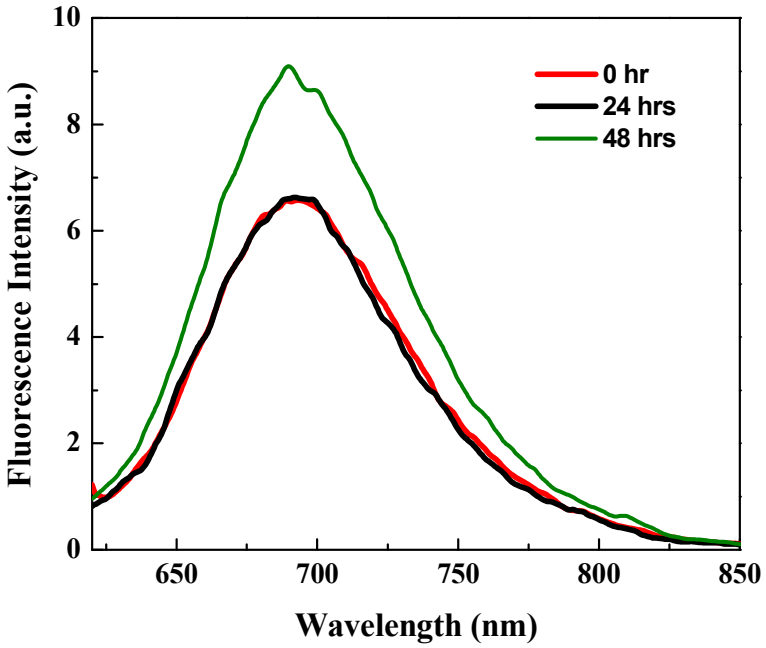
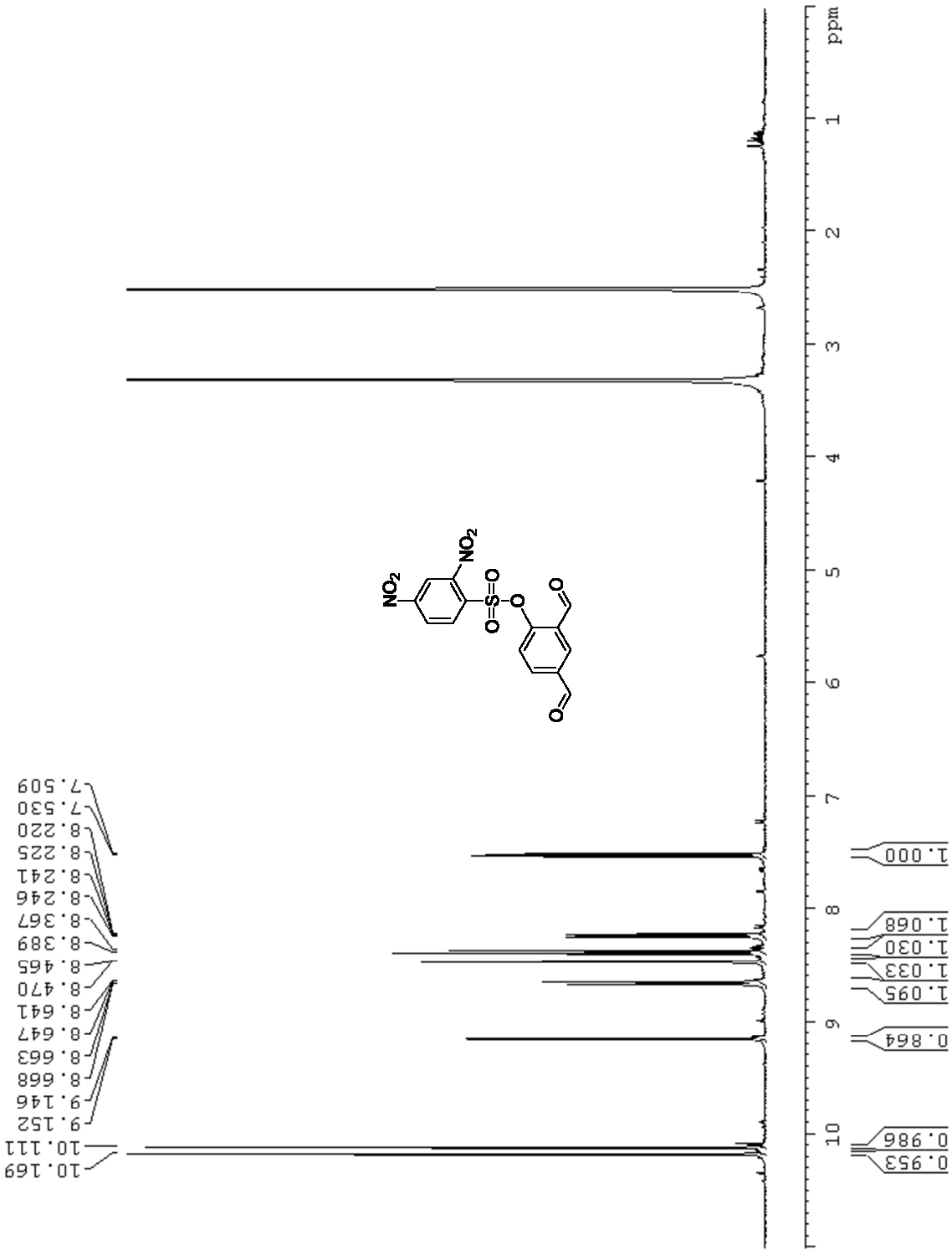
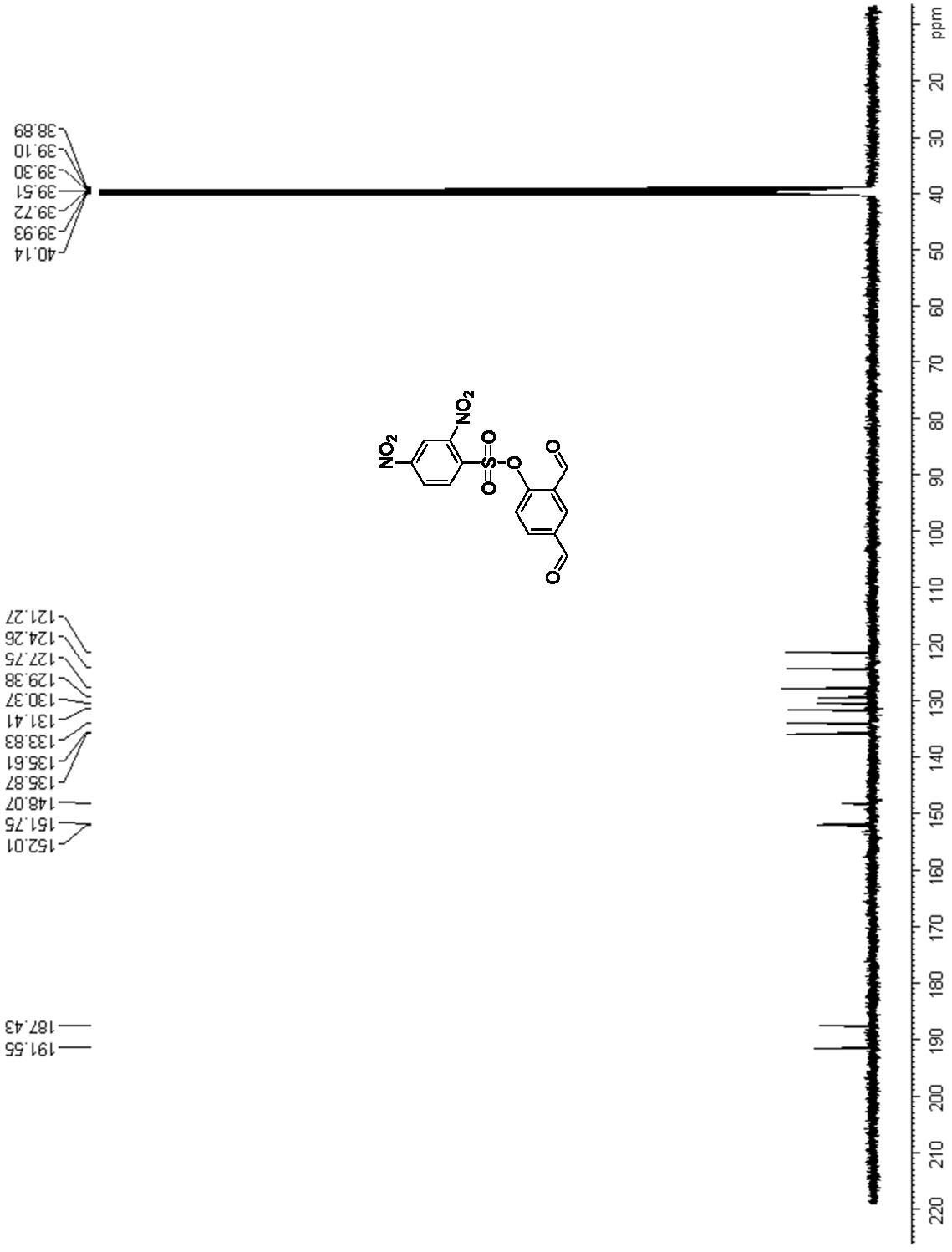


Fig. S14 Emission spectra of probe **DNBSCy** (10.0 μM) in water.

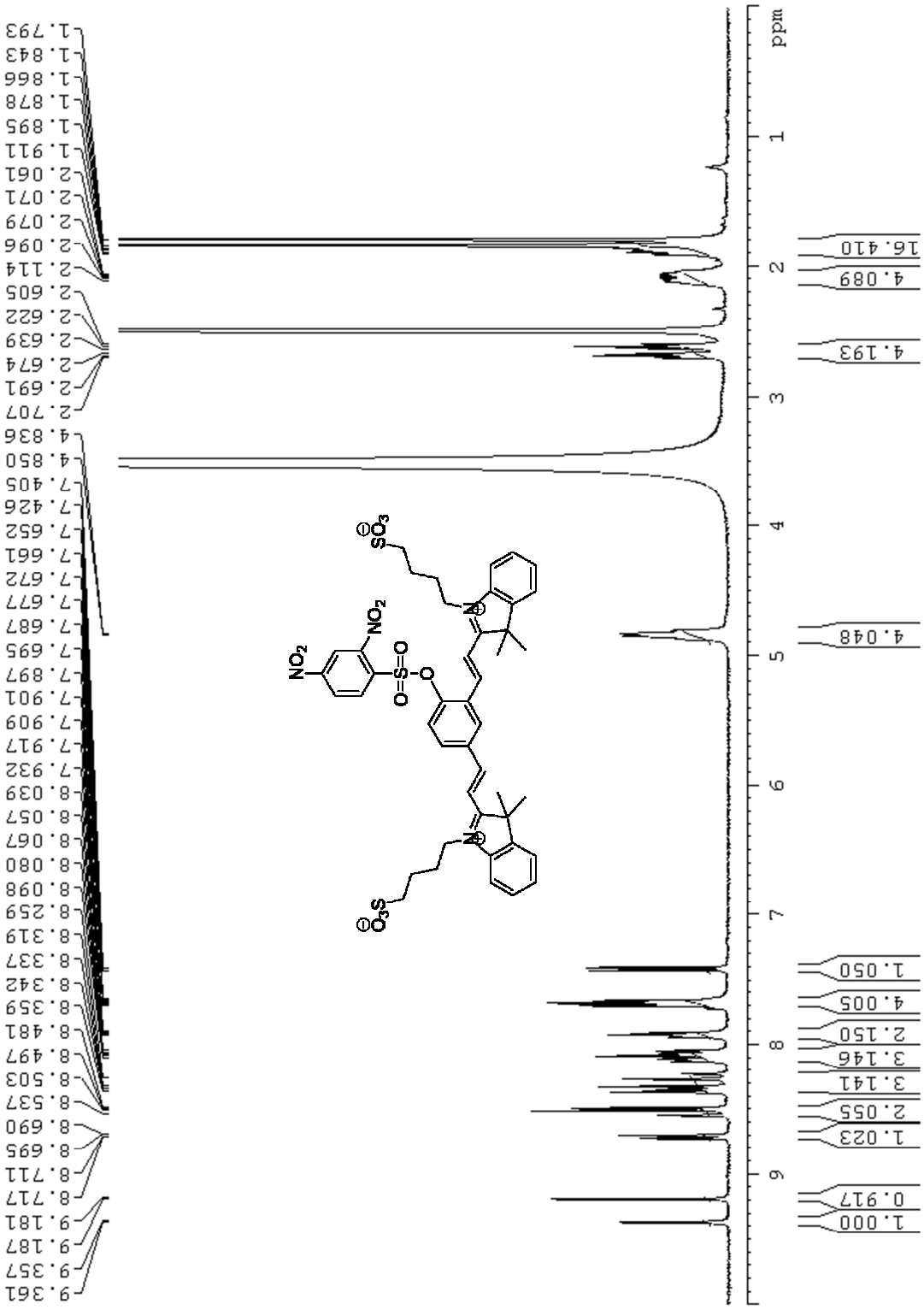
^{1}H NMR spectrum of 2,4-dinitrophenyl 2,4-diformylphenyl sulphate



^{13}C NMR spectrum of 2,4-dinitrophenyl 2,4-diformylphenyl sulphate



^{1}H NMR spectrum of dinitrobenzenesulphonyl-cyanine (DNBSCy) probe



^{13}C NMR spectra of dinitrobenzenesulphonyl-cyanine (DNBSCy) probe