Electronic Supplementary Material (ESI) for Analyst.

A water soluble glucopyranosyl conjugate as selective and reactive probe for cysteine in buffer and its application to living cells

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S01. Synthesis and characterization of P_2: **P_2** derivative was synthesized by using a literature reported procedure.[1] [1] (a) Bolletta, F.; Fabbri, D.; Lombardo, M.; Prodi, L.; Trombini, C.; Zaccheroni, N. *Organometallics* **1996**, *15*, 2415-2417.; (b) Drillaud, N.; Estelle, B.-L.; Pezron, I.; Len, C.| *J. Org. Chem.* **2012**, *77*, 9553−9561.

P_2 was obtained as a yellow solid (85 %). **^{1}HNMR (400 MHz, $CDCl_3$, δ ppm)**: 1.9 (t, ^{1}J=2.2 Hz and ^{2}J = 2.2 Hz, 1H), 2.8 (s, 6H), 3.8 (d, J= 7.4, 2H), 4.62 (t, ^{1}J = 9.8 Hz and ^{2}J = 8.2 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.50-7.58 (m, 2H), 8.23-8.29 (m, 2H), 8.53 (d, J = 8.4 Hz, 1H).

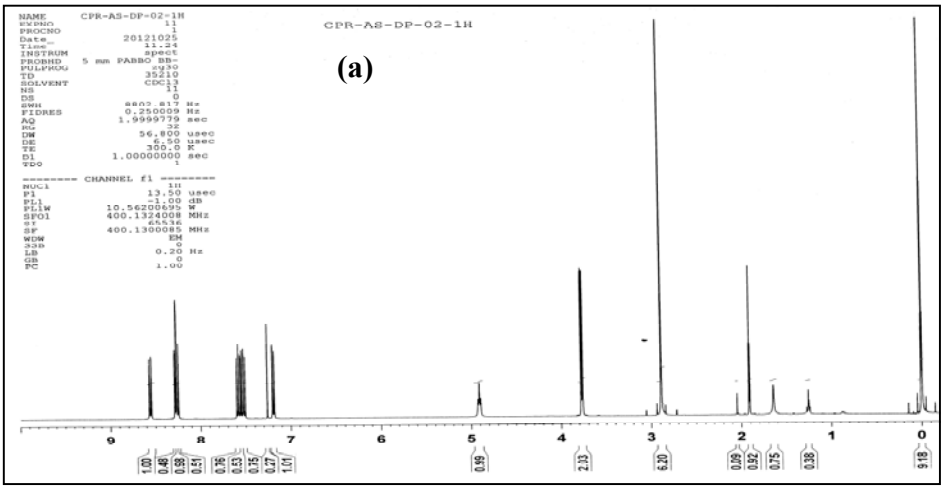


Fig. S01: (a) ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **P_2**.

S02. Synthesis and characterization of P_4: This was synthesized by using a literature reported procedure.[2] [2] Nicolas, D.; Estelle, B.-L.; Isabelle, P.; Christophe, L. *J. Org. Chem.*, **2012**, *77*, 9553-9561.

The yield is 69 % as white crystals. **^{1}HNMR (400 MHz, $CDCl_3$, δ ppm)**: 2.0 (S, 3H), 2.08 (S, 3H), 2.12 (S, 3H), 2.16 (S, 3H), 3.8-3.9 (m, 1H), 4.14 (d, J= 6.4 Hz, 1H), 4.28-4.3 (dd, ^{1}J= 2.2 Hz and ^{2}J= 2.2 Hz 1H), 4.65 (d, J=7.6 Hz, 1H), 4.95 (t, ^{1}J= 7.2 Hz and ^{2}J = 6.8 Hz,1H), 5.15 (t, ^{1}J= 7.8 Hz and ^{2}J= 5.6 Hz, 1H) 5.25 (t, J= 8.2 Hz and ^{2}J= 6.4 Hz 1H).

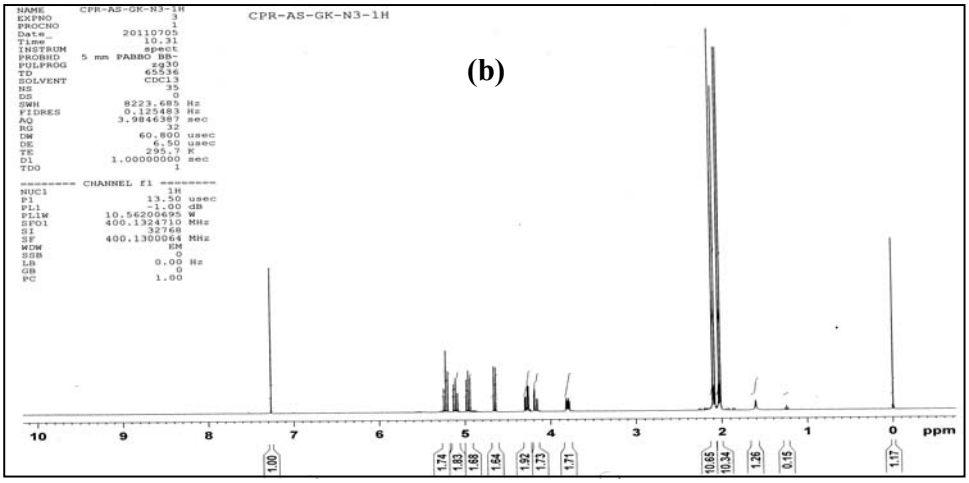
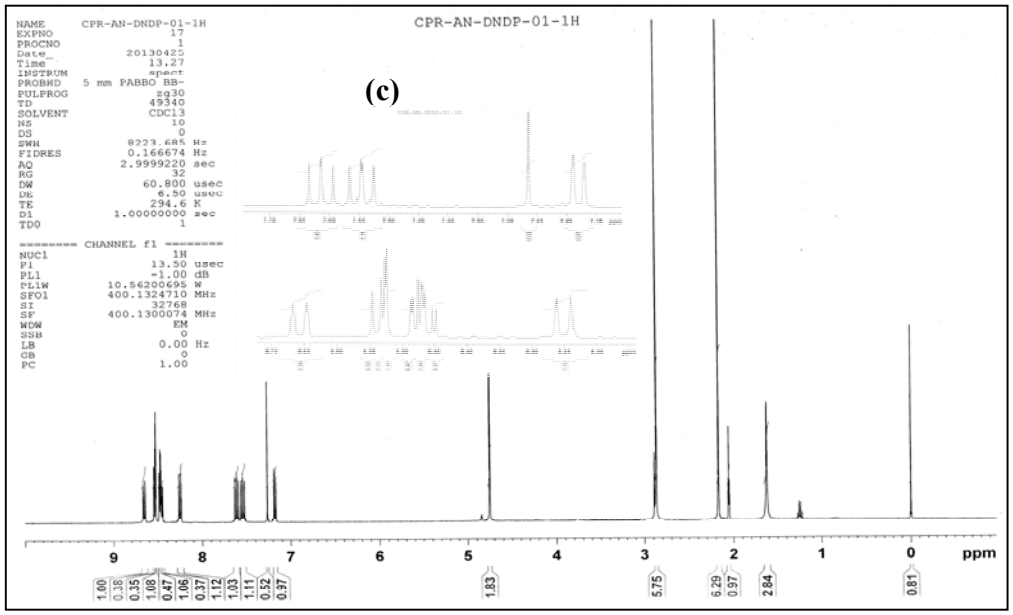
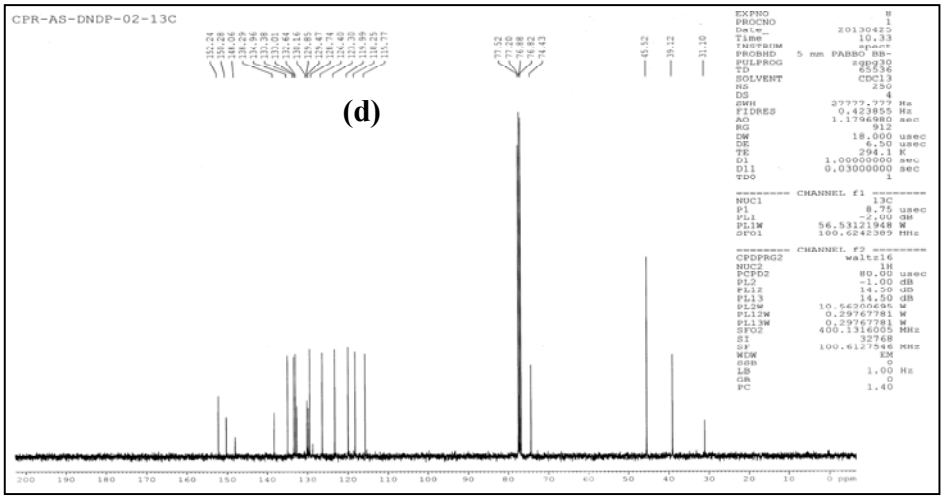


Fig. S02. (b) ^{1}H NMR ($CDCl_3$, 400 MHz) for **P_4.**

S03. Characterization of C_1





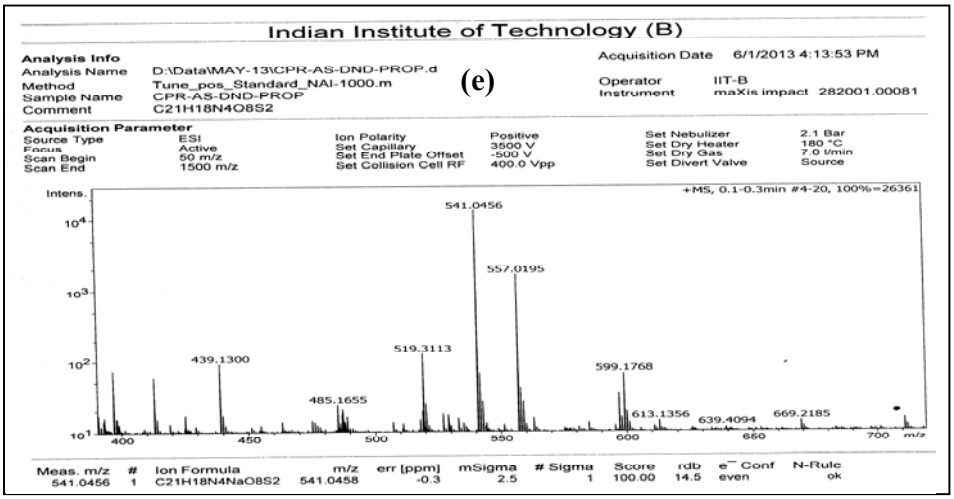


Fig. S03: (c) ^{1}H NMR ($CDCl_3$, 400 MHz) (d) ^{13}C NMR ($CDCl_3$, 400 MHz) (e) HRMS for $\mathbf{C_1}$.

S04. Characterization of P_5

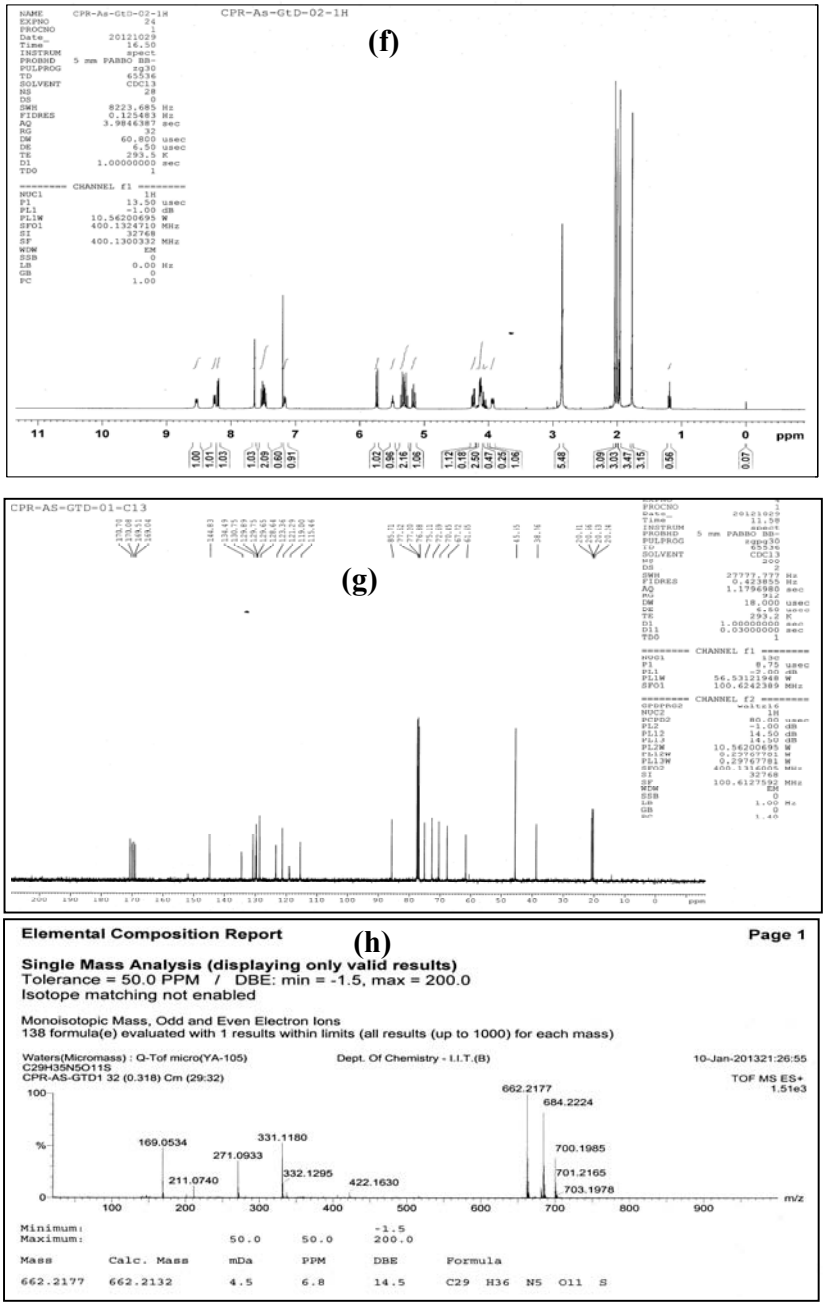
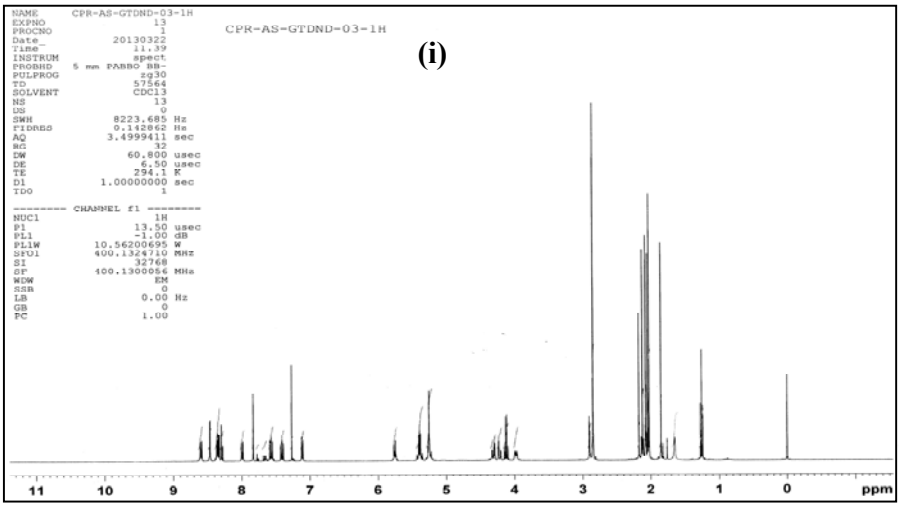
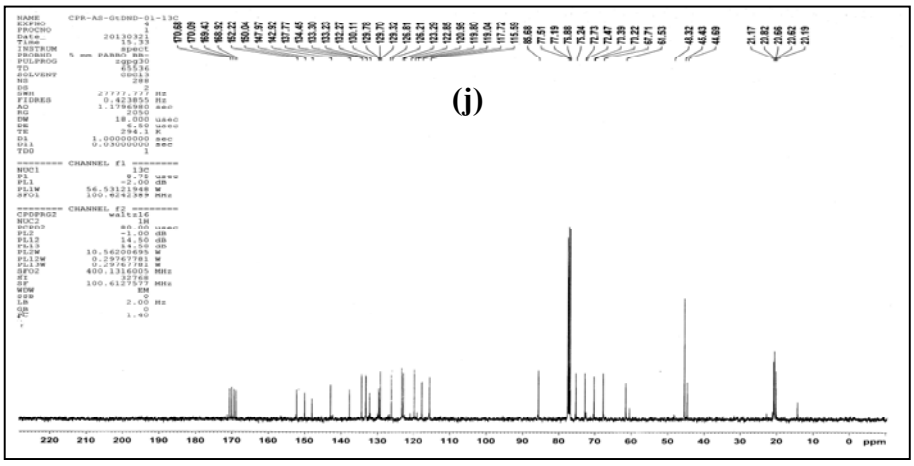


Fig. S04. (f) ^{1}H NMR ($CDCl_3$, 400 MHz) (g) ^{13}C NMR ($CDCl_3$, 400 MHz) (h) HRMS for **P_5.**

S05. Characterization of P_6





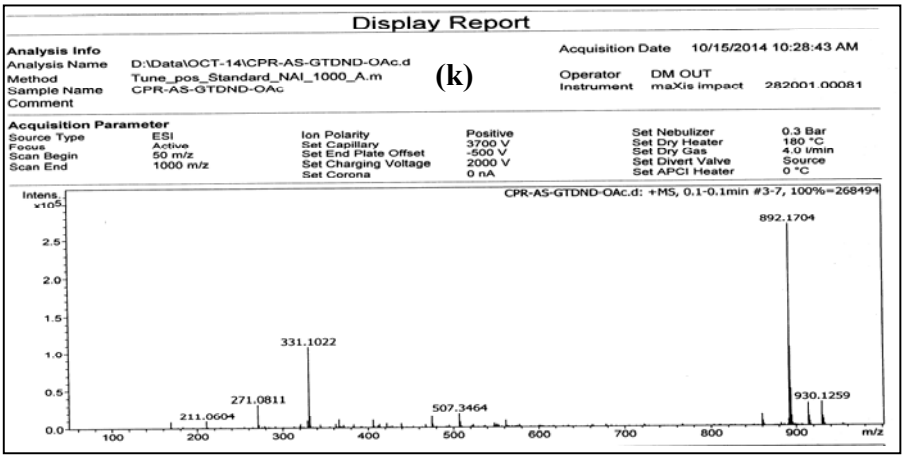


Fig. S05. (i) ^{1}H NMR ($CDCl_3$, 400 MHz) (j) ^{13}C NMR ($CDCl_3$, 400 MHz) (k) ESI MS for **P_6**.

S06. Characterization of L

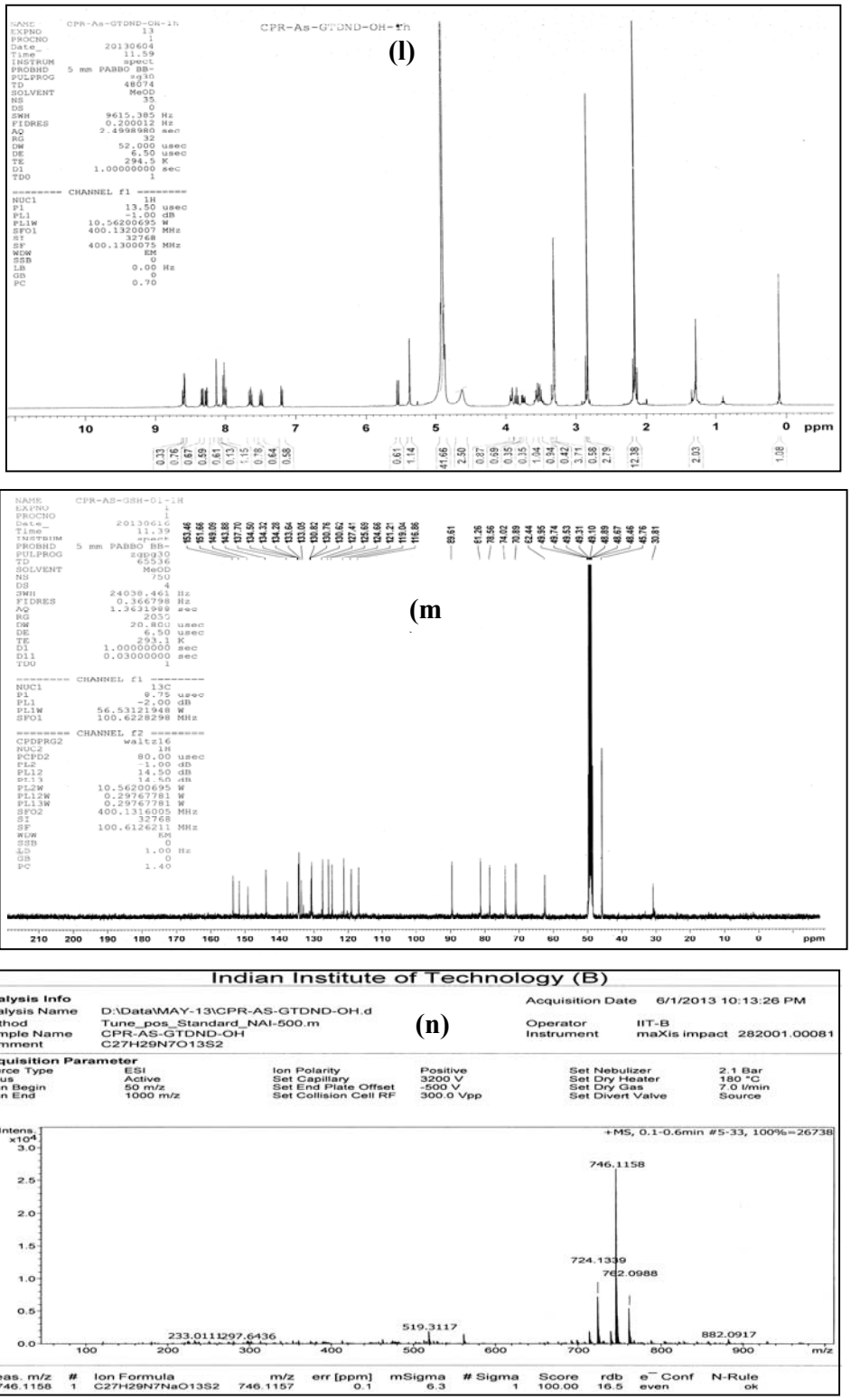
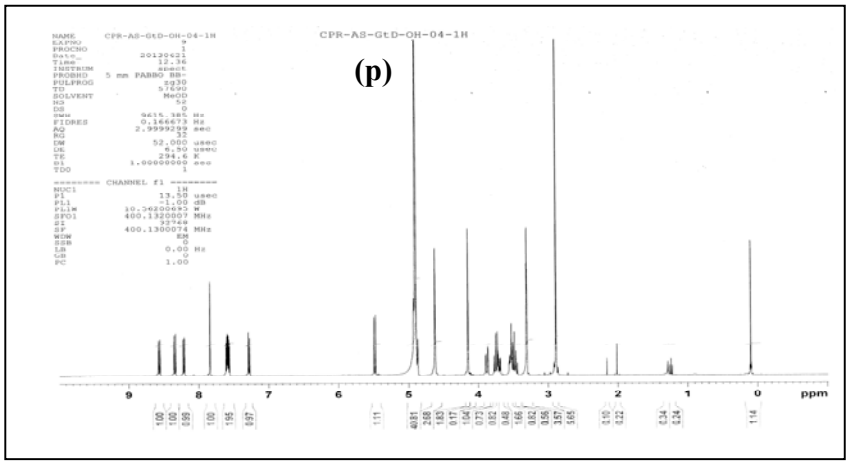
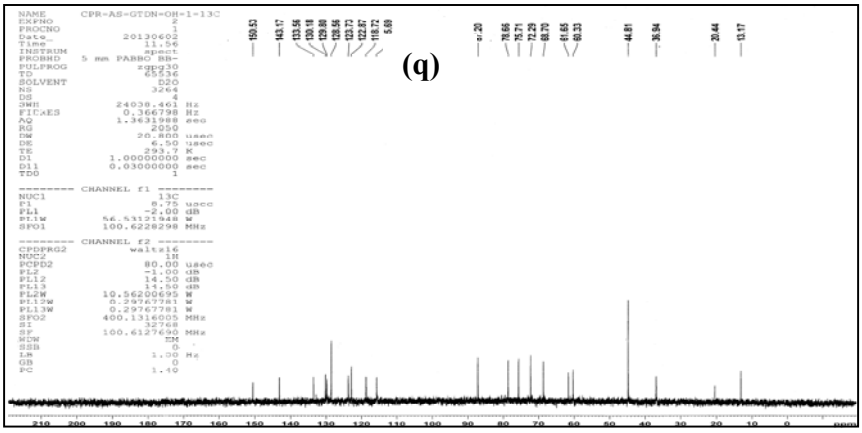


Fig. S06: (l) ^{1}H NMR (CD3OD, 400 MHz) (m) ^{13}C NMR (CD3OD, 400 MHz) (o) HRMS for **L**.

S07. Characterization of L_1





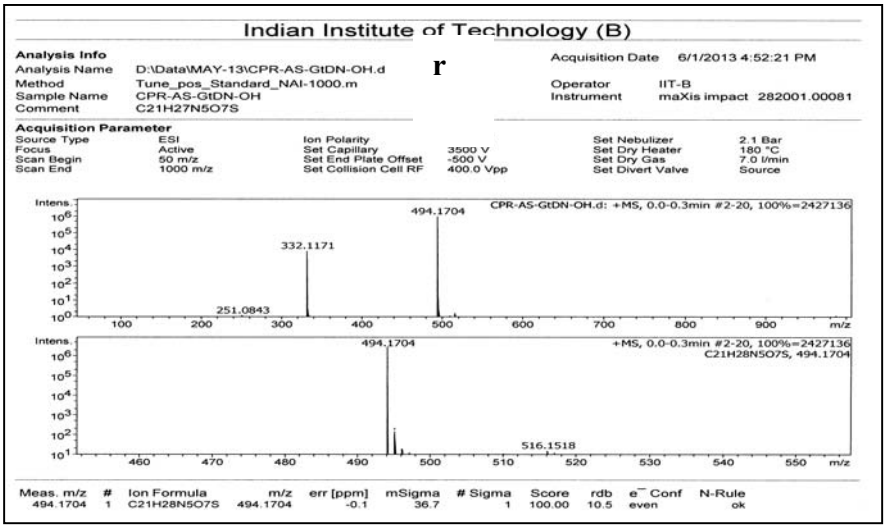


Fig. S07: (p) ^{1}H NMR (CD_3OD, 400 MHz) (q) ^{13}C NMR (D_2O, 400 MHz) (r) HRMS for $\mathbf{L_1}$.

S08. Fluorescence studies of L with amino acids

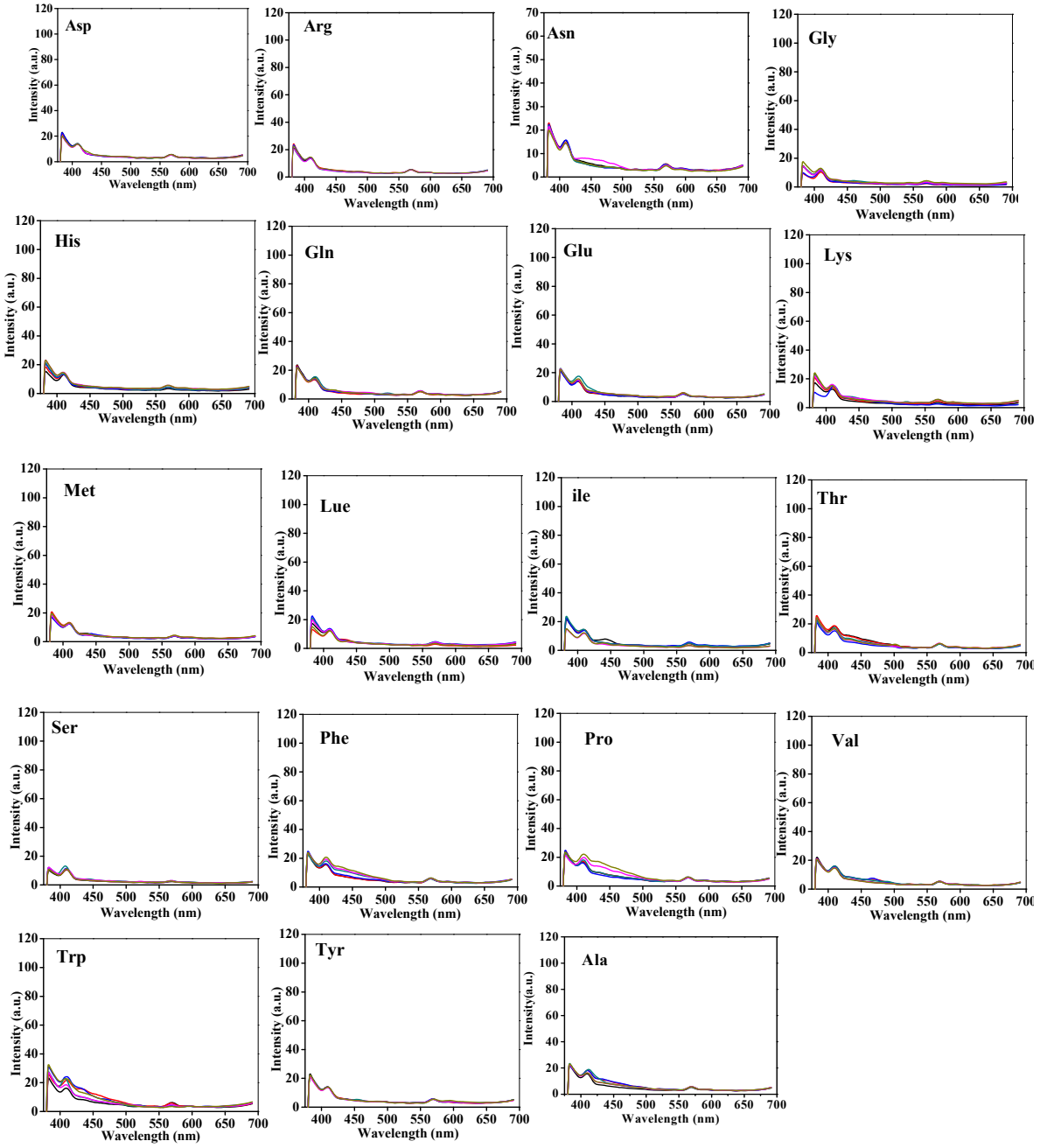


Fig. S08 Fluorescence spectra obtained for the titration of **L** [5 μM, λ_{ex} = 360 nm] with different amino acids in HEPES buffer pH at 7.4.

S09 Determination of Limit of Detection (LOD) of Cys by L

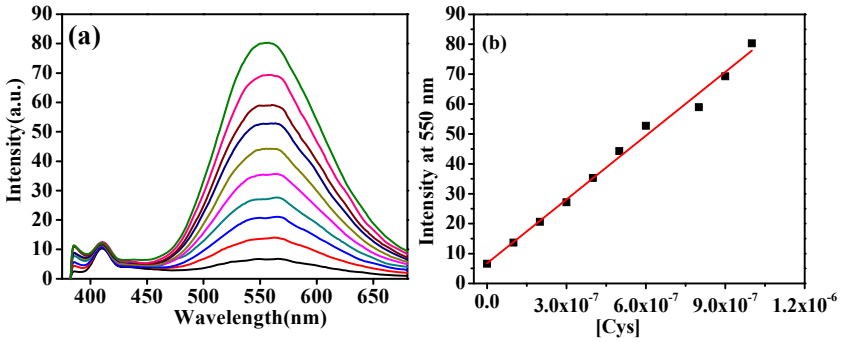
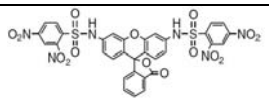
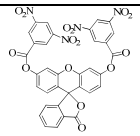
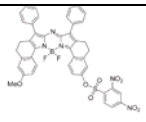
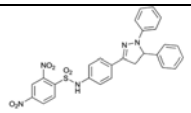
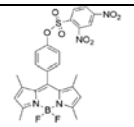
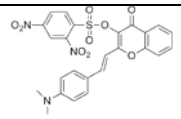
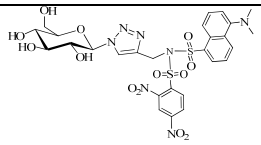


Fig. S09 (a) Fluorescence spectral traces of **L** during titration with Cys to determine LOD. (b) The linear dynamic fluorescence response for the titration of **L** with Cys to determine the detection limit (LOD). The LOD was derived by using the formula 3σ/k where σ = standard deviation of the blank (10 blank samples) and k = is the slope of linear calibration curve.

S10. Comparison of the detection limits of recently developed fluorescent probes for Cys in the literature.

Probe	Detection medium	Detection Limit (M)	Reference
	Tris-HCl buffer	100×10^{-6}	*Bioorg. Med. Chem. Lett*. **2008**, *18*, 2246
	HEPES Buffer:DMSO (20:80)	2.13×10^{-5}	*Org. Lett.*, **2013**, *15* , 3630–3633
	$CH_3CN:H_2O$: DMSO (79:20:1)	5×10^{-7}	*Org. Biomol. Chem.*, **2012**, *10*, 1966
	$CH_3CN:H_2O$	4.19×10^{-7}	*Analyst*, **2013**, *138*, 7169–7174

	CH_3OH/H_2O (4:1)	4×10^{-7}	*Org. Biomol. Chem.*, **2011**, *9*, 3844
	HEPES buffer: CH_3CN (70:30)	7×10^{-8}	*RSC Adv.*, **2013**, *3*, 11543–11546
	HEPES buffer (100 %)	2.5×10^{-7}	Present work

S11. Histogram for the competitive amino acid titrations of [L] with amino acids

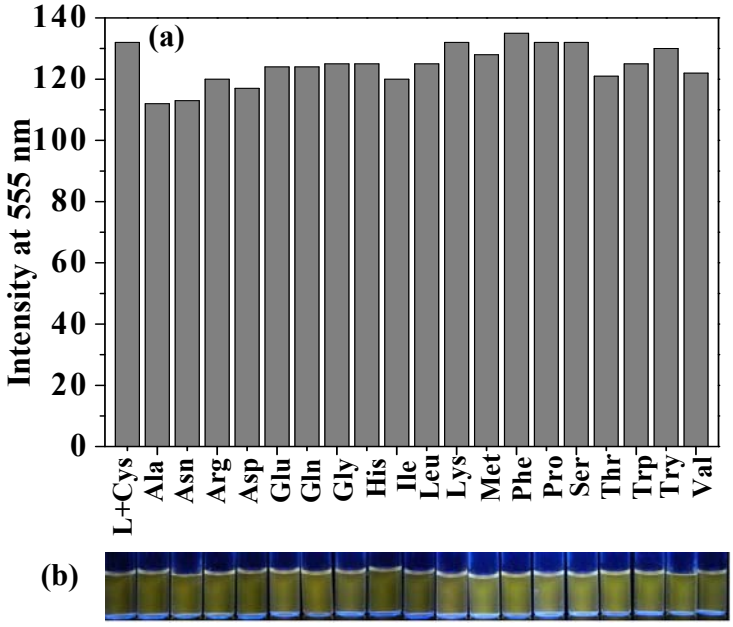


Fig. S11. Fluorescence spectra obtained for the competitive titration of **L** (5μM) with Cys in presence of different amino acids (200μM). (a) Histogram showing the fluorescence response of **L** at 550 nm band when titrated with different amino acids. (b) Visual fluorescent color change of {**L**+Amino acids) +Cys} with different amino acids under 365 nm UV-light.

S12. Fluorescence spectra for the titration of [L] with different molecular weight thiols

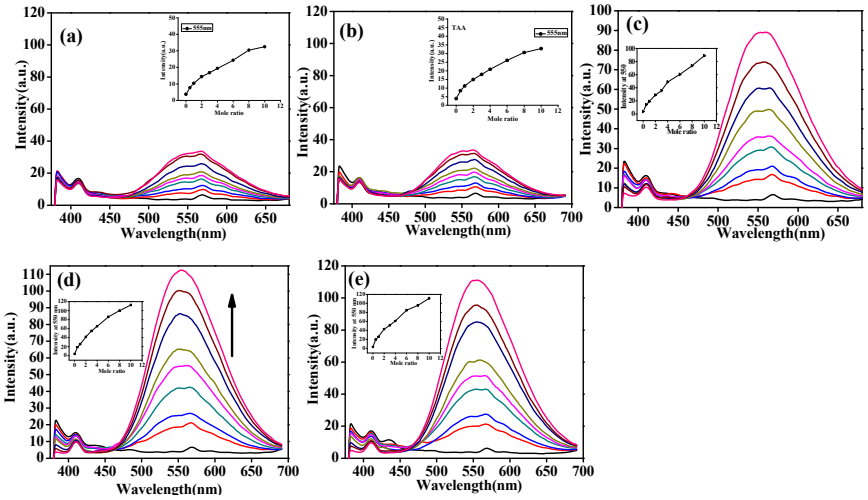


Fig. S12: Fluorescence spectra obtained for the titration of **L** [5 μM, λ_{ex} = 360 nm] with molecular weight thiols in *HEPES buffer* at pH 7.4; (a) MPA, (b) TAA, (c) Hcy, (d) DTT_{red} and (e) GSH_{red}.

S13. Fluorescence spectra for the titration of [L] with oxidized state of BSA, HSA and GSH

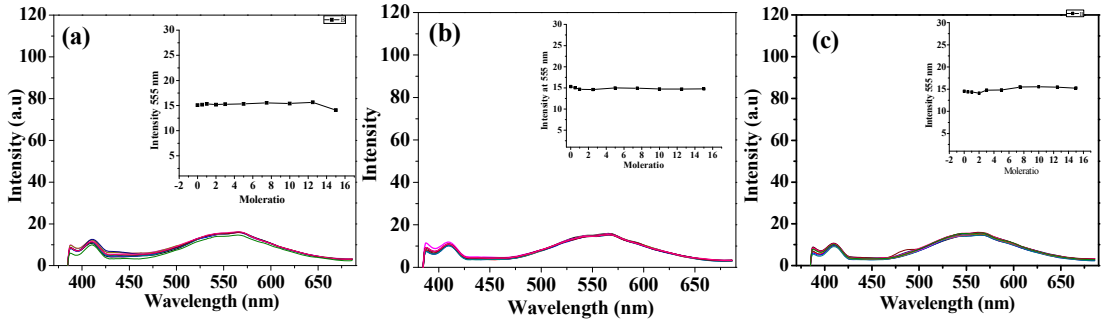


Fig. S13. Fluorescence spectra obtained for the titration of **L** (5 μM, λ_{ex} = 360 nm) in HEPES buffer at pH 7.4 with oxidized state of (a) BSA, (b)HSA and (c) GSH_{OX}

S14. Fluorescence spectra for the titration of [L] with reduced state of BSA, HSA and GSH

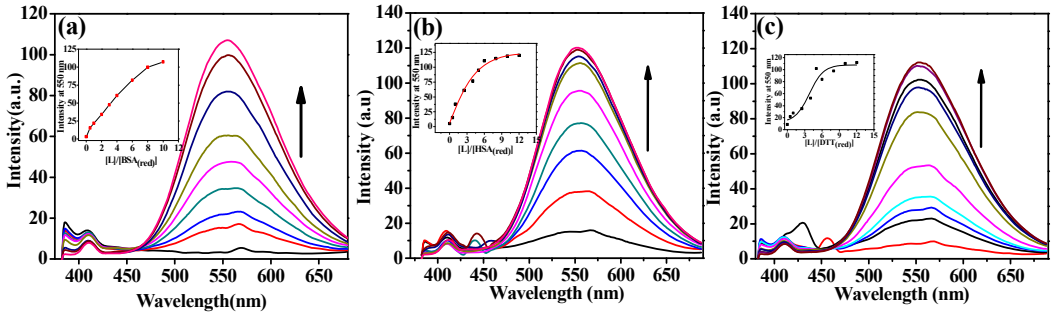


Fig. S14: Fluorescence spectra obtained for the titration of **L** (5 μM, λ_{ex} = 360 nm) in HEPES buffer at pH 7.4 with reduced state of (a) BSA, (b)HSA and (c) GSH.

S15. Absorption spectra obtained during the titration [L] with different amino acids

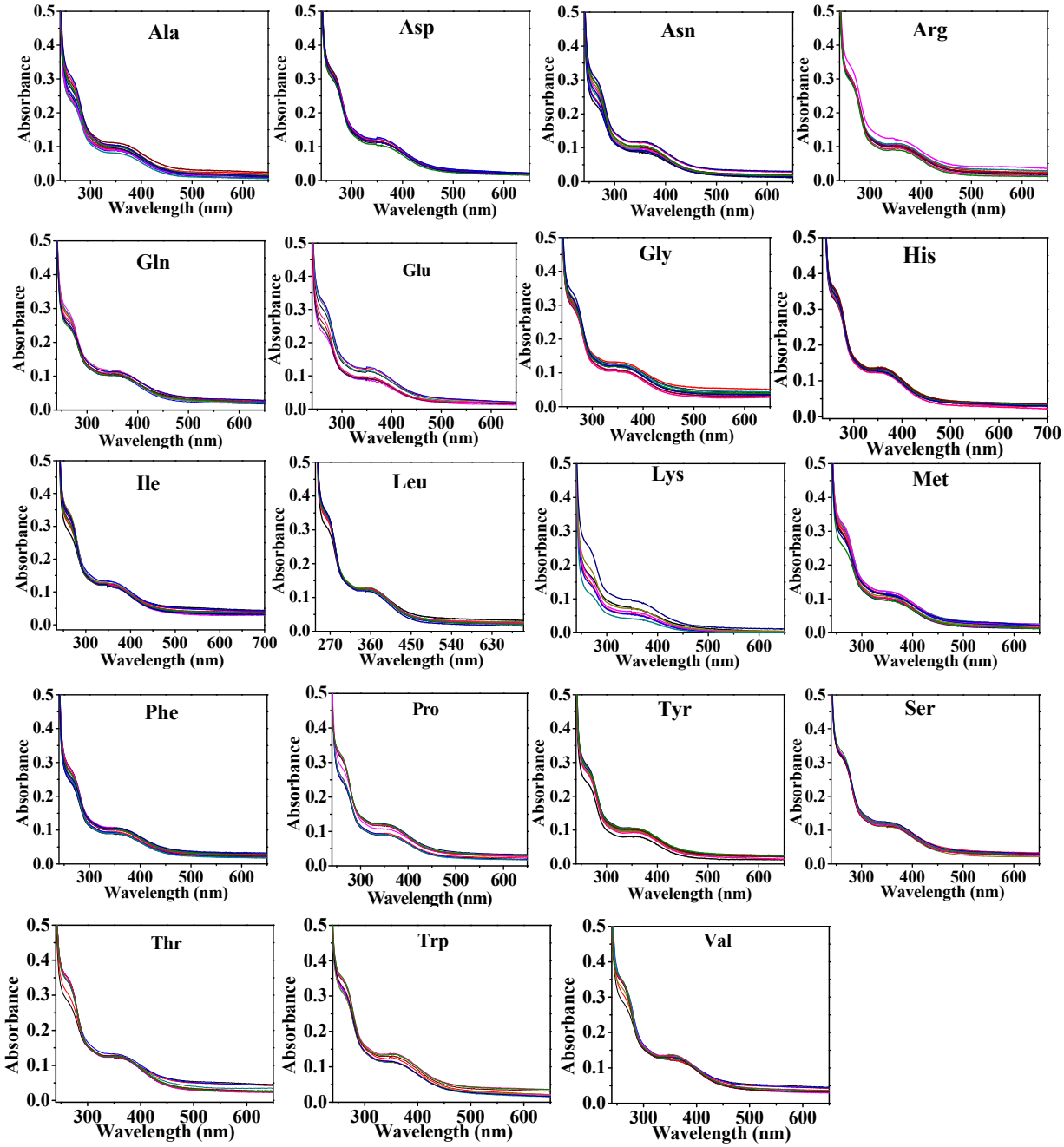


Fig. S15. UV-Visible spectral traces obtained during the titration of **L** (10μM) with different amino acids in HEPES buffer at pH 7.4.

S16. **Absorption spectra for the titration of [L] with oxidized forms of GSH**

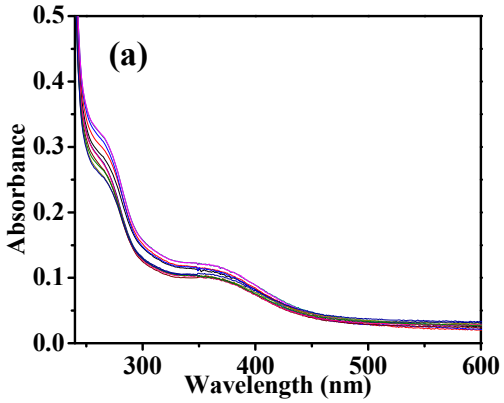


Fig. S16. UV-Visible spectral traces obtained during the titration of **L** (10μM) with GSH_{ox} in HEPES buffer at pH 7.4.

S17. **ESI-MS spectra obtained during the titration of [L] with Cys**

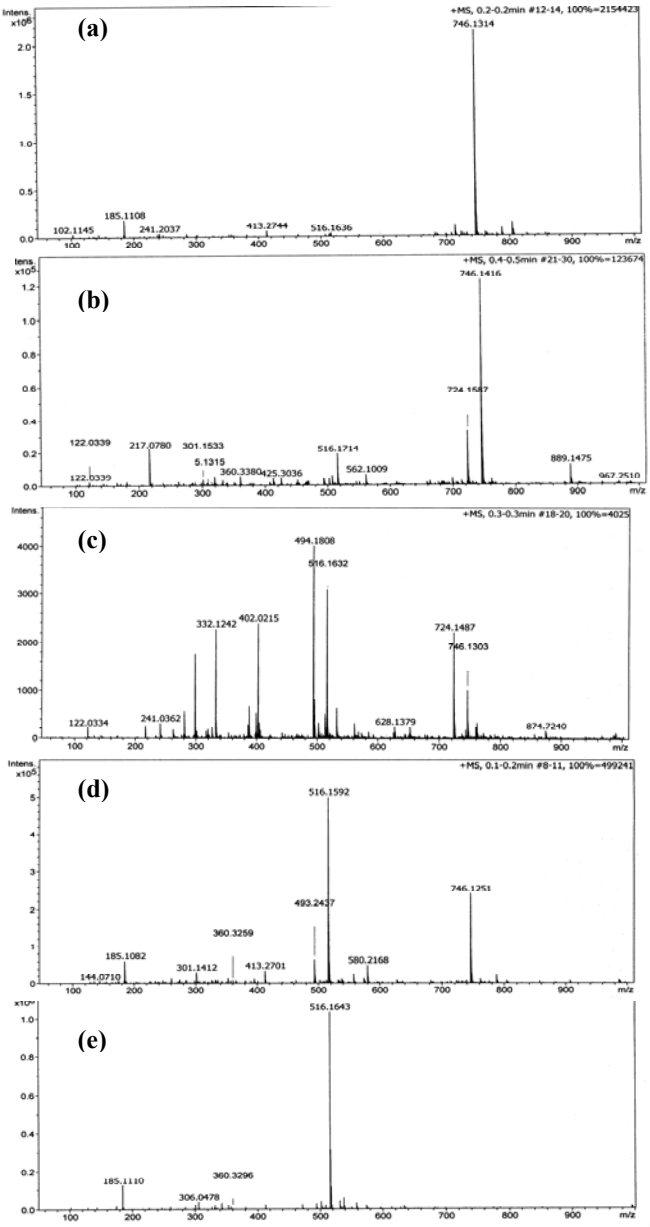


Fig. S17. ESI-MS spectral titration of **L** with Cys in CD_3OD-D_2O (1:1): (a) [**L**] followed by 'n' equivalents of Cys, where, (a) n=0, (b) n= 1, (c) n = 2.5, (d) n = 5 and (e) n = 10.