Supporting information

Differences in heterocycle basicity distinguish homocysteine from cysteine using aldehyde-bearing fluorophores

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Materials

All chemicals were purchased from Sigma Aldrich and used as received. Ultrapure water obtained from a Milli-Q direct water purification system was used to prepare all aqueous solutions. All spectroscopic measurements were carried out in DMSO:buffer (1:99) solutions. **1** and **2** were synthesized from the procedure reported in the literature.[1]

Methods

^{1}H-NMR and ^{13}C-NMR were recorded on a ARX-400 Advance Bruker spectrometer. All chemical shifts (δ) are reported in ppm relative to DMSO-d_6 (2.50 ppm, ^{1}H; 39.52 ppm, ^{13}C) unless otherwise indicated. ESI-HRMS (high resolution mass spectrometry) were recorded at the PSU Bioanalytical Mass Spectrometry Facility on a ThermoElectron LTQ-Orbitrap high resolution mass spectrometer.

Spectrophotometric measurement

Fluorescence spectra were obtained on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) with slit widths set at 5 nm for both excitation and emission, respectively. Fluorescence spectra were corrected for the wavelength dependent response of the R928 photomultiplier tube with the help of a manufacturer generated correction file.

pK_a measurements

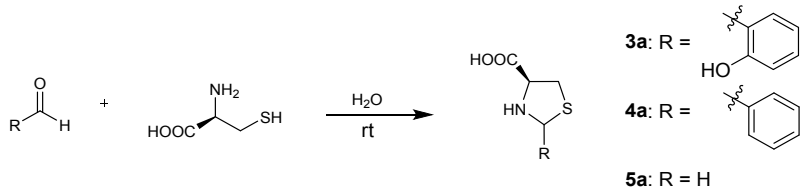
p*K*a values of the amine of 2-substituted thiazolidines/thiazinanes-4-carboxylic acids, **4a**, **4b**, **5a** and **5b** were obtained by the titration of their aqueous solutions with 0.01M NaOH. In the case of **3a** and **3b** 0.1 M NaOH was used. The pH measurements were conducted using an Orion 410A pH meter, which was standardized with Scientific Products potassium hydrogen phthalate pH 4.00 buffer, BDH pH 7.00 and Scientific Products boric acid/potassium hydroxide pH 10.00 buffer. All pH titrations were done in triplicates and their average is reported.

Deproteinization of plasma

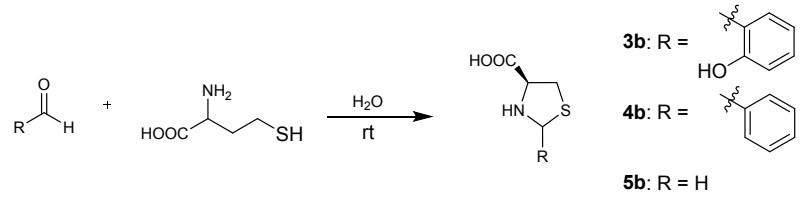
Deproteinization of plasma was carried out by reconstituting the lyophilized bovine plasma with ultrapure water to 1/3 of the reconstitution volume. Two equivalents of acetonitrile (2/3 of the reconstitution volume) were added. This solution was vortexed for 10 min. after letting the solution stand for 10 minutes, it was centrifuged at 4000 RPM for 30 minutes to pellet the proteins. The decanted supernatant was aliquoted into sample vials and re-lyophilized. The vials were then sealed and stored in a refrigerator (4 ºC).

Synthesis of compounds 3a, 3b, 4a, 4b, 5a and 5b

Scheme S1. Synthetic route of **3a**, **4a** and **5a**



Scheme S2. Synthetic route of **3b**, **4b** and **5b**



(2*S*,4*S*) and (2*R*,4*S*)-2-(2-hydroxy phenyl) thiazolidine-4-carboxylic acid (3a)

L-cysteine (1.67 mmol) and salicylaldehyde (1.67 mmol) in 5 mL of distilled H_2O are stirred at RT for 2 h. The precipitates are filtered, washed with iced cold H_2O and dried under vacuum to yield **3a** as a white solid (307 mg, 83%).^{1}H-NMR (400 MHz, DMSO-d_6); δ (ppm): 9.29 (m, 1H), 7.06 (m, 1H), 6.80 (m, 1H), 5.84 (s, 1H), 5.65 (s,1H), 4.21 (t, J = 4.6 Hz 1H), 3.85 (m, 1H), 3.18 (m, 1H), 3.03 (m, 1H). ^{13}CNMR (101 MHz, DMSO-d_6); δ (ppm): 172.88, 172.40, 155.13, 154.54, 128.99, 128.06, 127.85, 127.56, 126.04, 124.17, 118.99, 118.70, 116.62, 115.03, 67.61, 65.56, 65.13, 64.73, 38.10, 37.01. EI-MS, *m/z* 226.05602 $[M+H]^+$, calculated for $C_{10}H_{11}NO_3S$, *m/z* 225.04596.

(2*S*,4*R*), (2*S*,4*S*), (2*R*,4*R*) and (2*R*,4*S*)-2-(2-hydroxyphenyl)-1,3-thiazinane-4-carboxylic acid (3b)

DL-homocysteine (1.88 mmol) and salicylaldehyde (1.88 mmol) in 6 mL of distilled water and 0.25 mL of ethanol are stirred at RT for two days. The resultant precipitate is filtered, washed with iced cold H_2O and dried under vacuum to yield **3b** as a white solid. (322 mg, 82%). ^{1}H-NMR (400 MHz, DMSO-d_6); δ (ppm): 7.31 (m, 1H), 7.11 (m, 1H), 6.80 (m, 2H), 3.43 (s, 1H), 3.57 (d, J = 3.60, 1H), 3.19 (t, J = 11.64 Hz, 1H), 2.86 (m, 1H), 2.05 (m, 1H), 1.49 (m, 1H). ^{13}CNMR (101 MHz, DMSO-d_6); δ (ppm): 173.48, 154.12, 128.71, 127.81, 126.42, 118.96, 115.63, 59.45, 59.26, 28.77, 27.93. EI-MS, *m/z* 240.07182 $[M+H]^+$, calculated for $C_{11}H_{13}NO_3S$, *m/z* 239.06161.

(2*S*,4*S*) and (2*R*,4*S*)-2-phenylthiazolidine-4-carboxylic acid (4a)

Compound **4a** was prepared according to the procedure described for **3a** but using benzaldehyde instead of salicylaldehyde. Compound **4a** was obtained as white solid (488 mg, 99%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 7.4 (m, 5H) 5.76 (s, 1H), 4.24 (q, J = 4.53 Hz, 1H), 3.28 (m, 1H), 3.15 (m, 1H) . ^{13}CNMR (101 MHz, DMSO-d_6) δ (ppm) 172.95, 172.19, 141.18, 139.91, 128.31, 127.25, 127.57,126.91, 71.73 71.06, 6540, 64.85, 38.39,37.94. EI-MS, *m/z* 210.06017 $[M+H]^+$, calculated for $C_{10}H_{11}NO_2S$, *m/z* 209.05105.

(2*S*,4*R*), (2*S*,4*S*), (2*R*,4*R*) and (2*R*,4*S*)-2-phenyl-1,3-thiazinane-4-carboxylic acid (4b)

Compound **4b** was prepared according to the procedure described for **3b** but using benzaldehyde instead of salicylaldehyde in 6 mL of distilled H_2O and stirred at RT for three days. Compound **4b** was obtained as white solid (273 mg, 65%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 7.35 (m, 5H), 5.27 (s, 1H), 3.62 (dd, J = 11.99, 2.65 Hz, 1H), 3.23 (dt, J = 2.64, 13.80 Hz, 1H), 2.91 (td, J =13.55, 3.43 Hz, 1H), 2.07 (qd , J = 13.5, 2.58, 3.58 Hz, 1H), 1.52 (dq, J = 3.48, 3.21, 11.71 Hz, 1H). ^{13}CNMR (101 MHz, DMSO-d_6) δ (ppm) 173.50, 140.76, 128.32, 127.79, 126.53, 64.06, 59.19, 28.87, 27.72. EI-MS, *m/z* 224.07707 $[M+H]^+$, calculated for $C_{11}H_{13}NO_2S$, *m/z* 223.06670.

(*S*)-thiazolidine-4-carboxylic acid (5a)

Compound **5a** was prepared according to the procedure described for **3a** but using formaldehyde instead of salicylaldehyde. Compound **5a** was obtained as a white solid. (152 mg, 68.5 %). ^{1}H-NMR (400 MHz, D_2O) δ (ppm) 4.43 (m,2H), 4.36 (d, J = 10.2 Hz, 1H), 3.43 (m, 1H), 3.33 (m, 1H). ^{13}CNMR (101 MHz, D_2O) δ (ppm) 171.87, 63.89, 48.55, 32.91. EI-MS, *m/z* 134.0252 $[M+H]^+$, calculated for $C_4H_7NO_2S$, *m/z* 133.01975.

(*R*) and (*S*)-1,3-thiazinane-4-carboxylic acid (5b)

DL-homocysteine (1.67 mmol) and formaldehyde (1.67 mmol) in 6 mL of distilled water were stirred at RT for 2 days. The precipitates were filtered. Ethanol was added to the filtrate and left the solution standing overnight. After one day, transparent shiny crystal were obtained. Crystals were washed with iced cold ethanol and dried under vacuum to yield **5b**. (135 mg, 55%) ^{1}H-NMR (400 MHz, D_2O) δ (ppm) 4.26 (dd, J = 32.0, 13.2 Hz, 2H), 3.68 (dd, J = 11.9, 2.4 Hz 1H), 2.96 (m, 1H), 2.85 (m, 1H), 2.53 (dd, J = 14.8, 3.2 Hz, 1H), 2.04 (qd, J= 1H). ^{13}CNMR (101 MHZ, D_2O) δ (ppm) 173.27, 59.42, 45. 12, 28.10, 26.00. EI-MS, *m/z* 148.04422 $[M+H]^+$, calculated for $C_5H_9NO_2S$, *m/z* 147.03540.

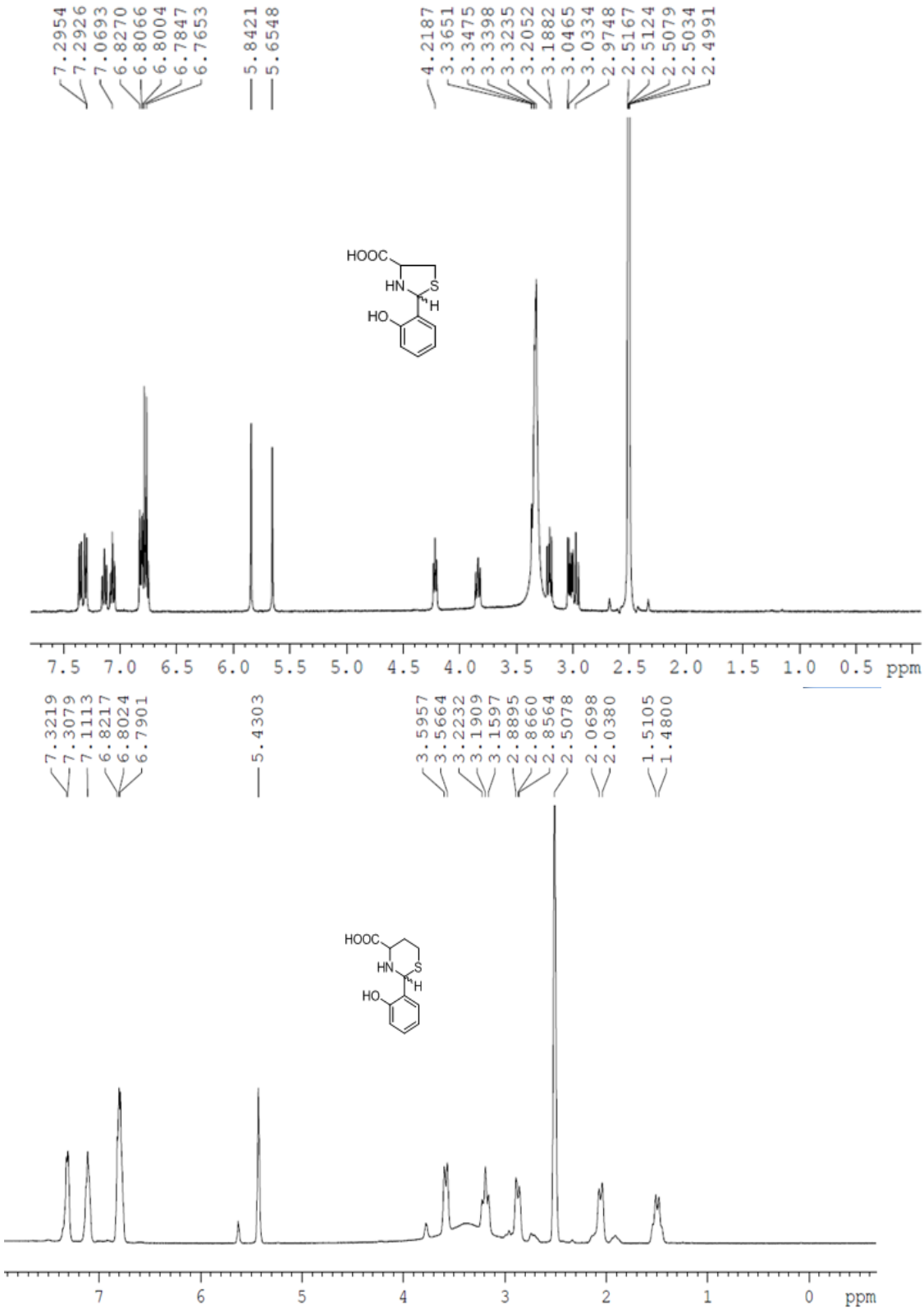


Figure S1. [1]HNMR spectrum of **3a** (top) and **3b** (bottom)

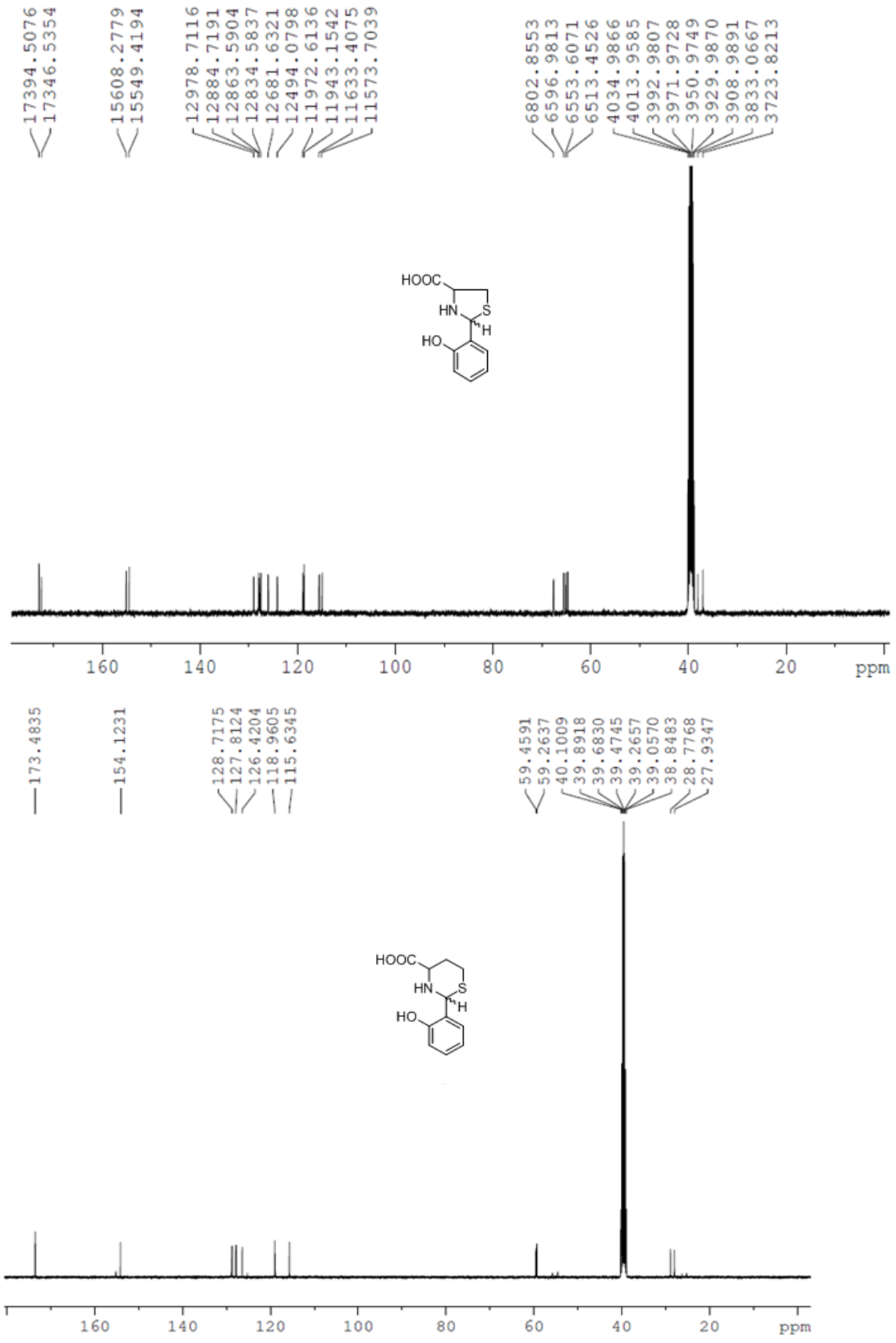


Figure S2. ^{13}CNMR spectrum of **3a** (top) and **3b** (bottom)

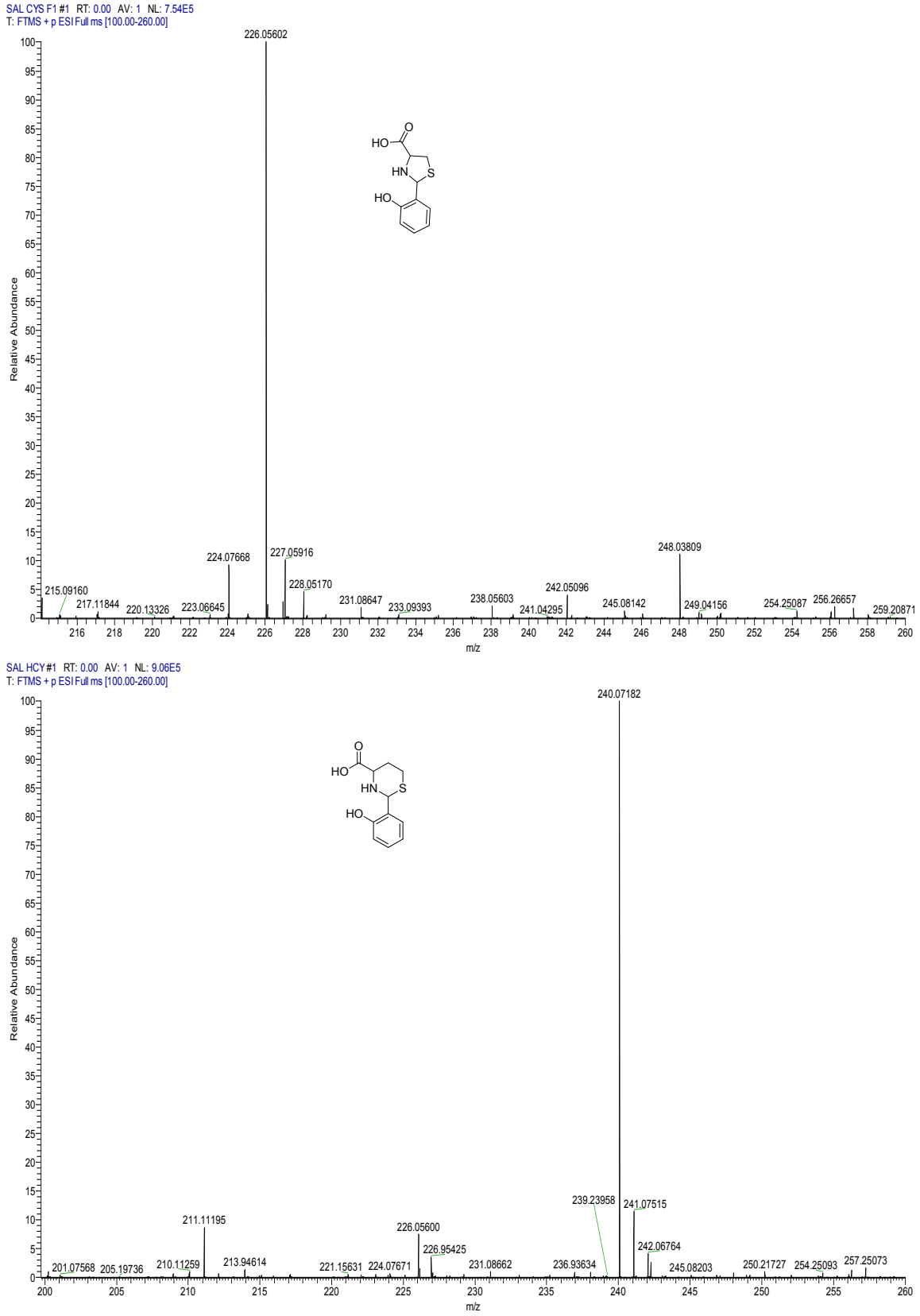


Figure S3. Mass spectrum of **3a** (top) and **3b** (bottom)

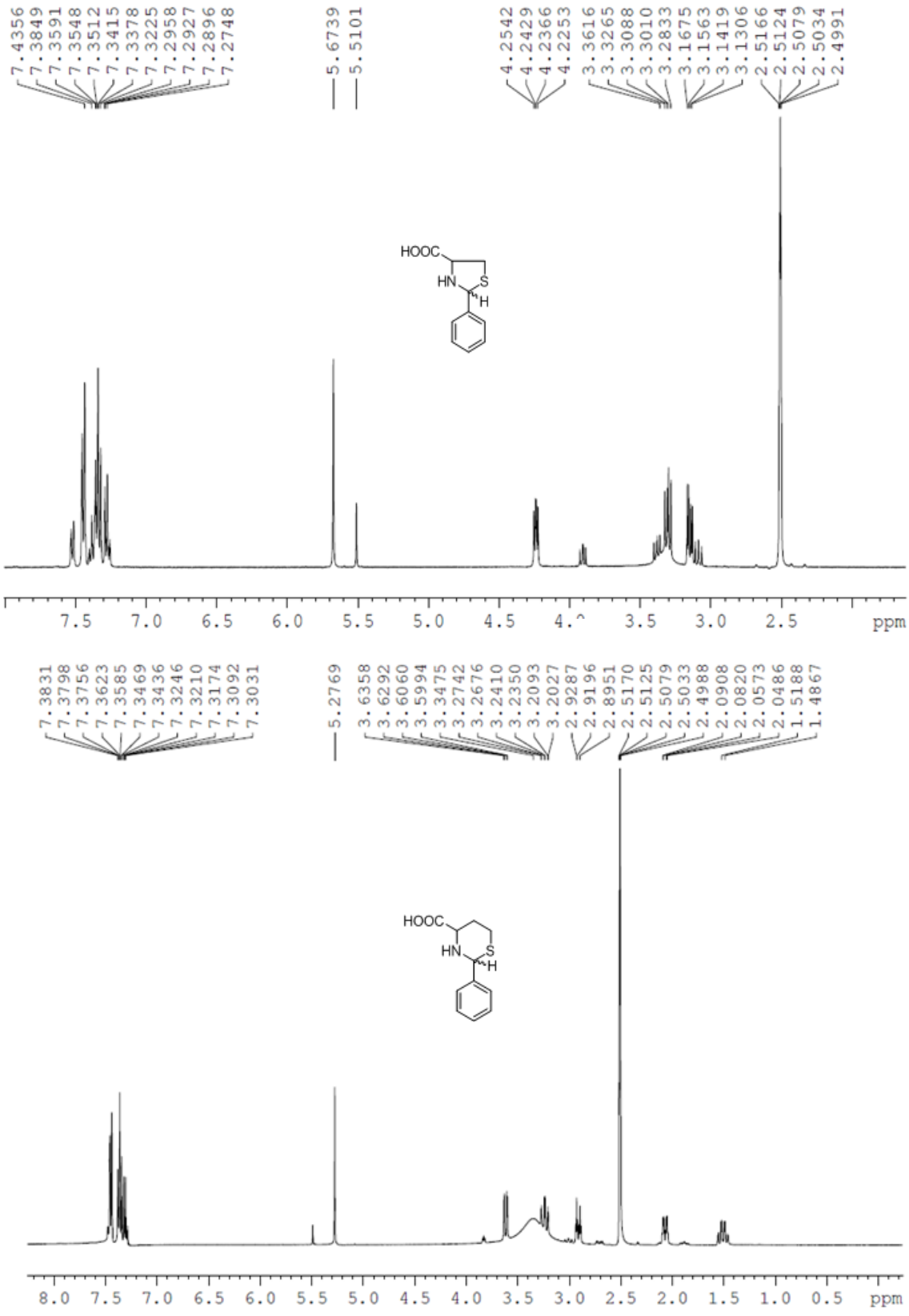


Figure S4. [1]HNMR spectrum of **4a** (top) and **4b** (bottom)

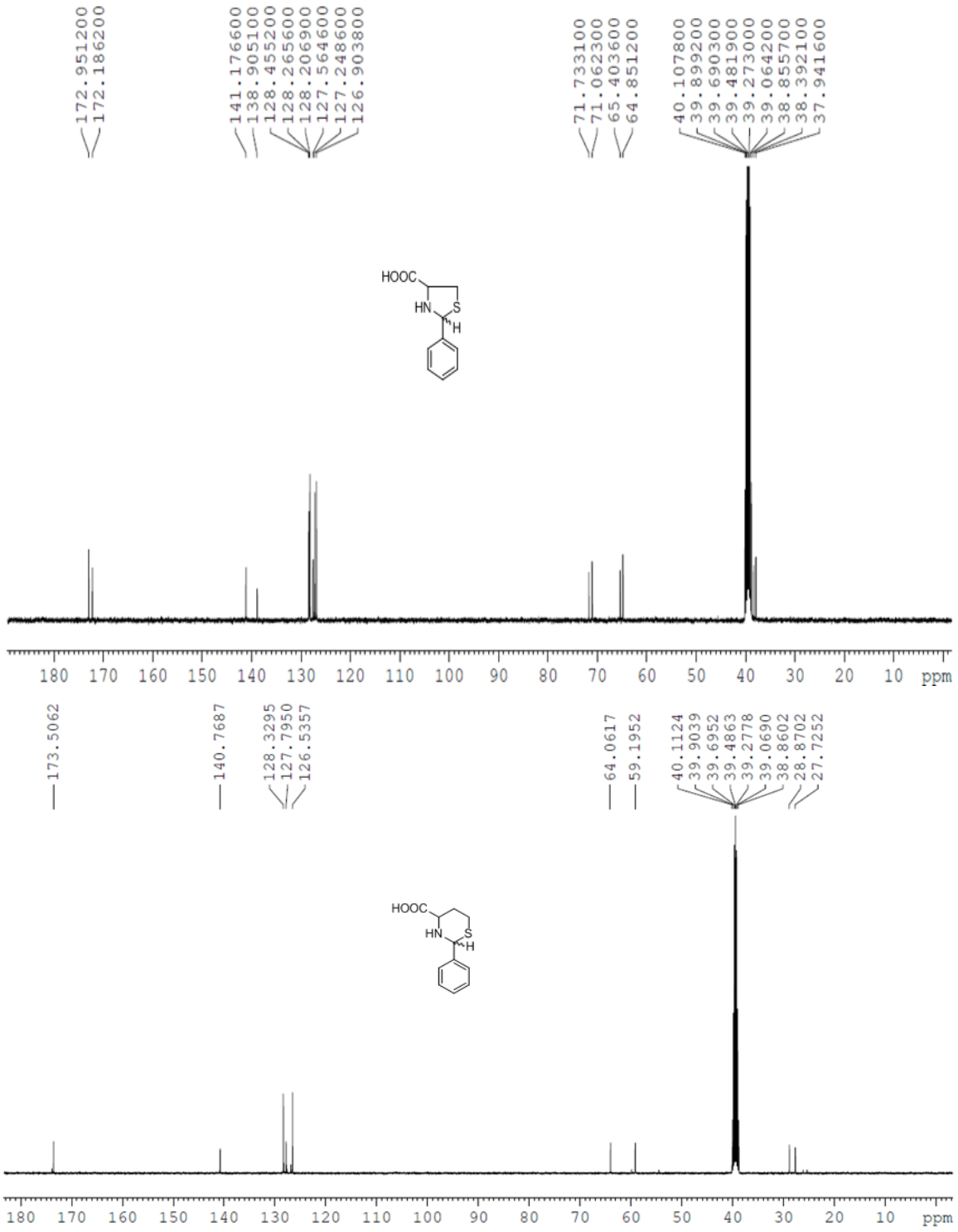


Figure S5. ^{13}CNMR spectrum of **4a** (top) and **4b** (bottom)

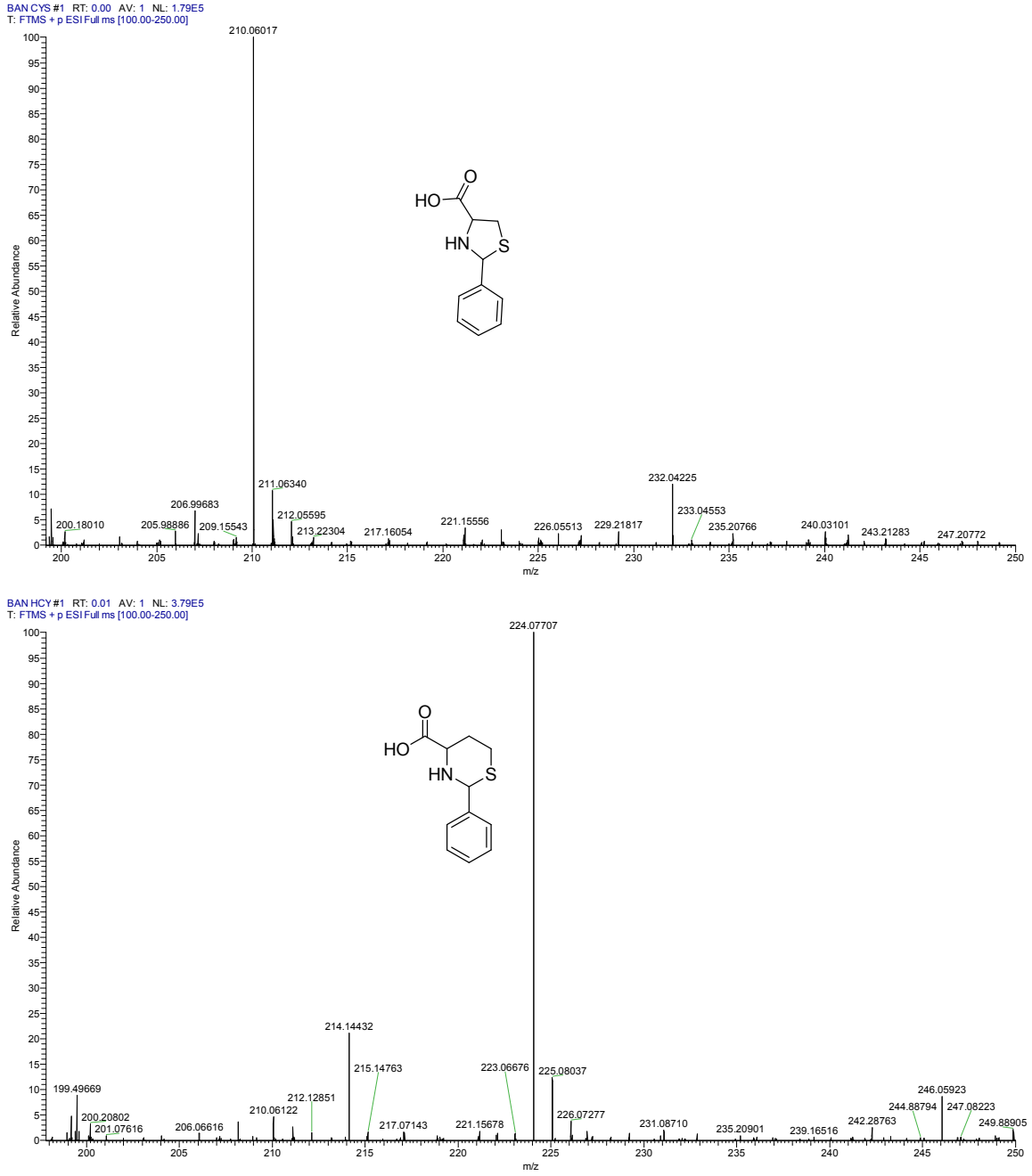


Figure S6. Mass spectrum of **4a** (top) and **4b** (bottom)

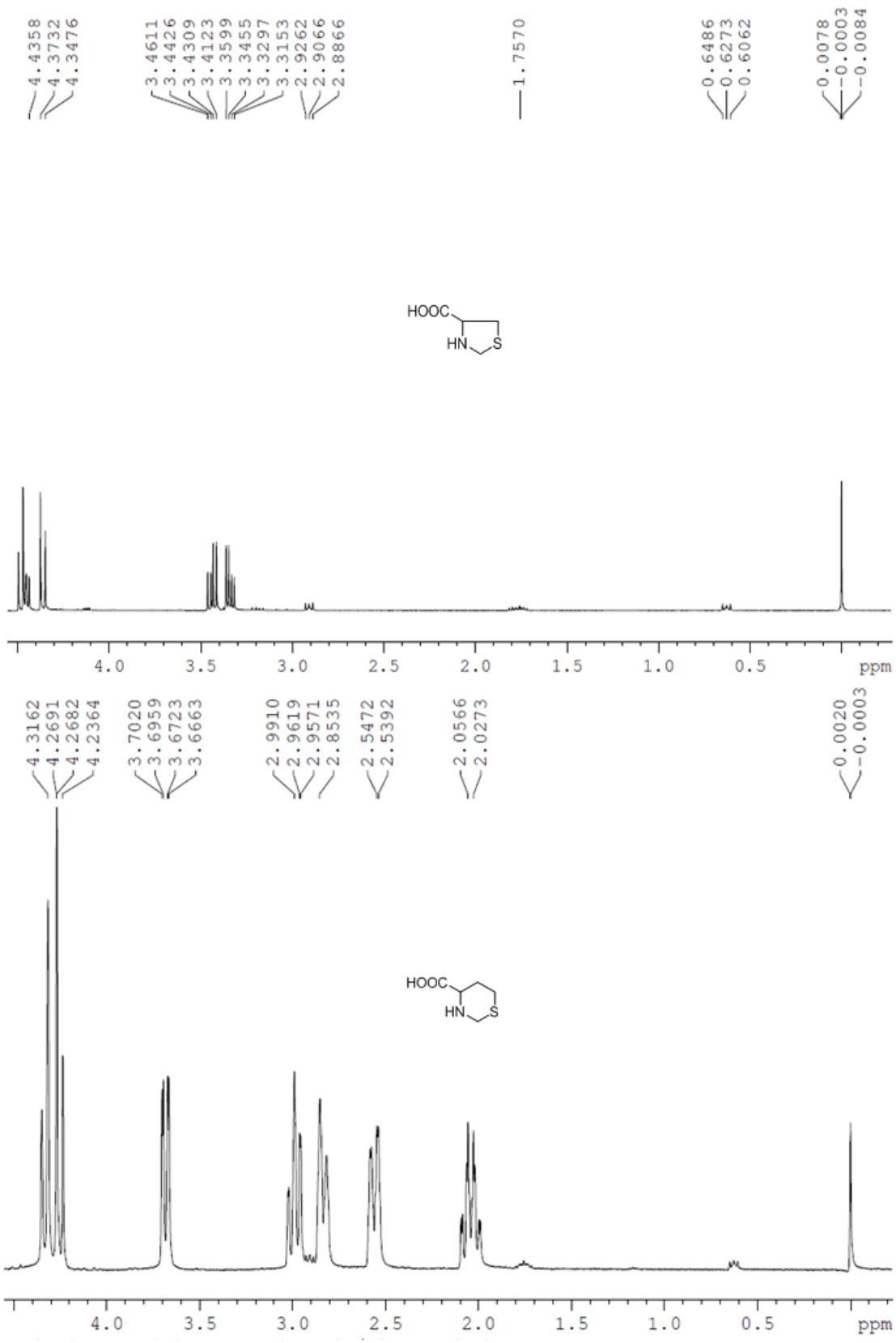


Figure S7. [1]HNMR spectrum of **5a** (top) and **5b** (bottom)

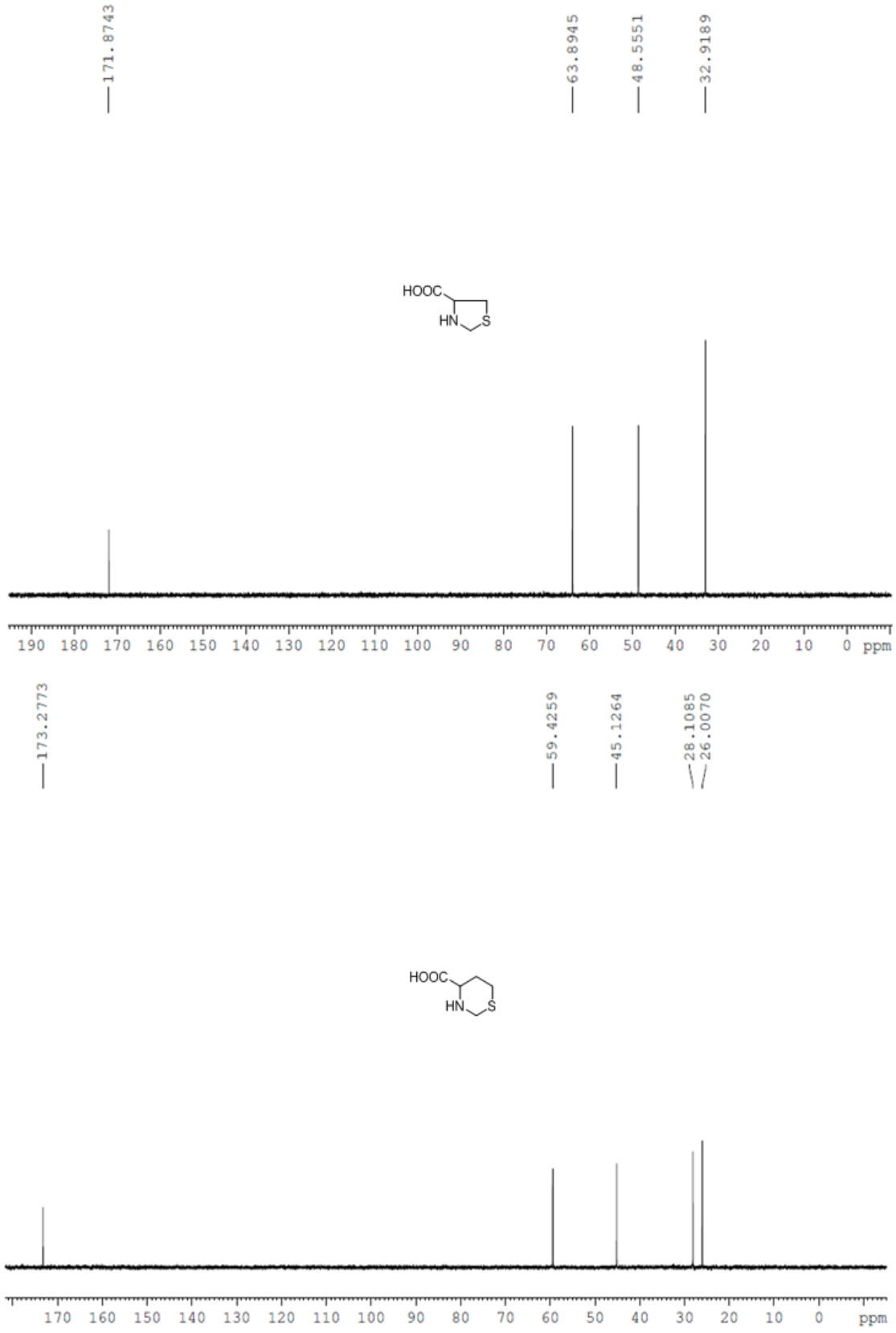


Figure S8. ^{13}CNMR spectrum of **5a** (top) and **5b** (bottom)

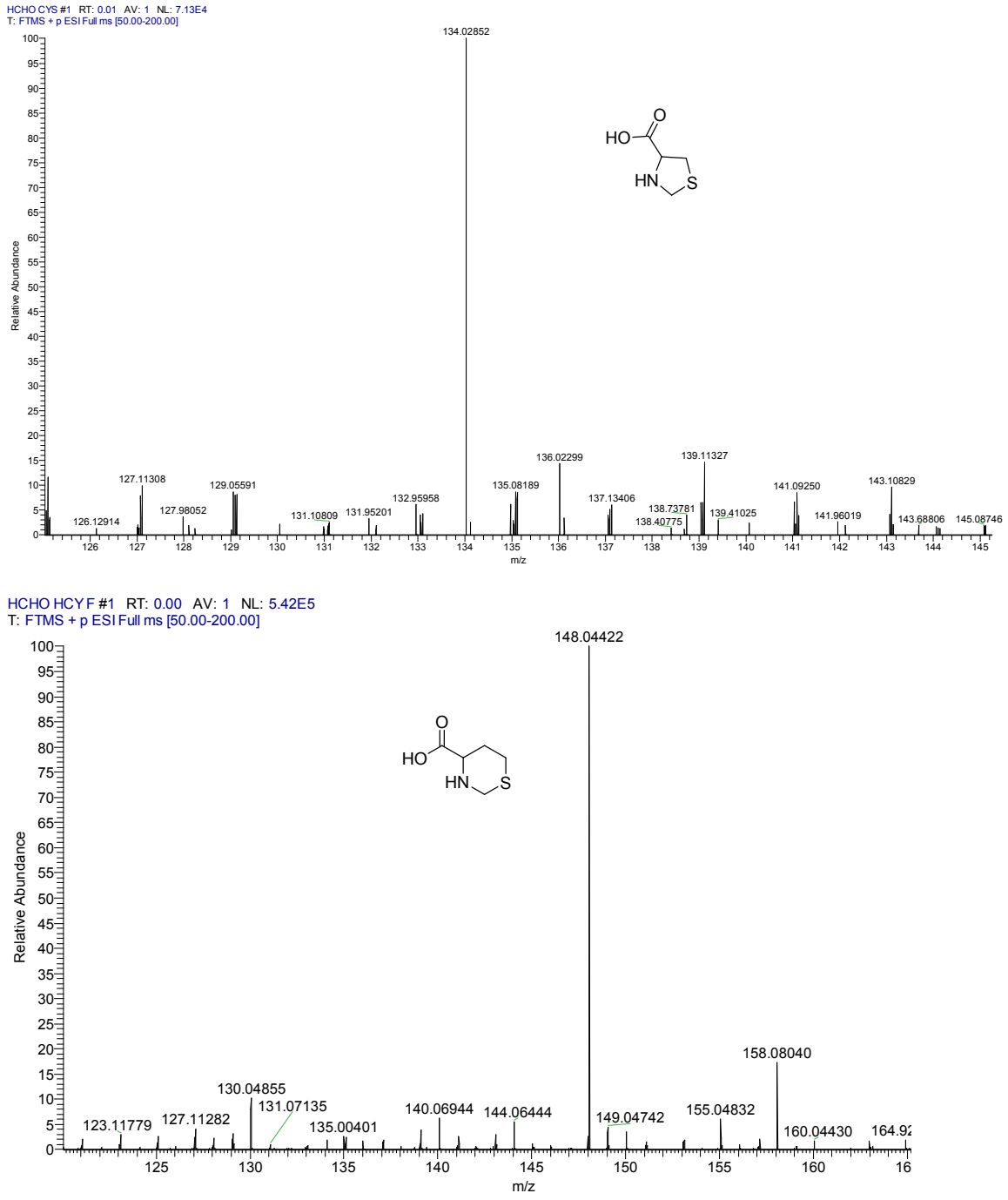


Figure S9. Mass spectrum of **5a** (top) and **5b** (bottom)

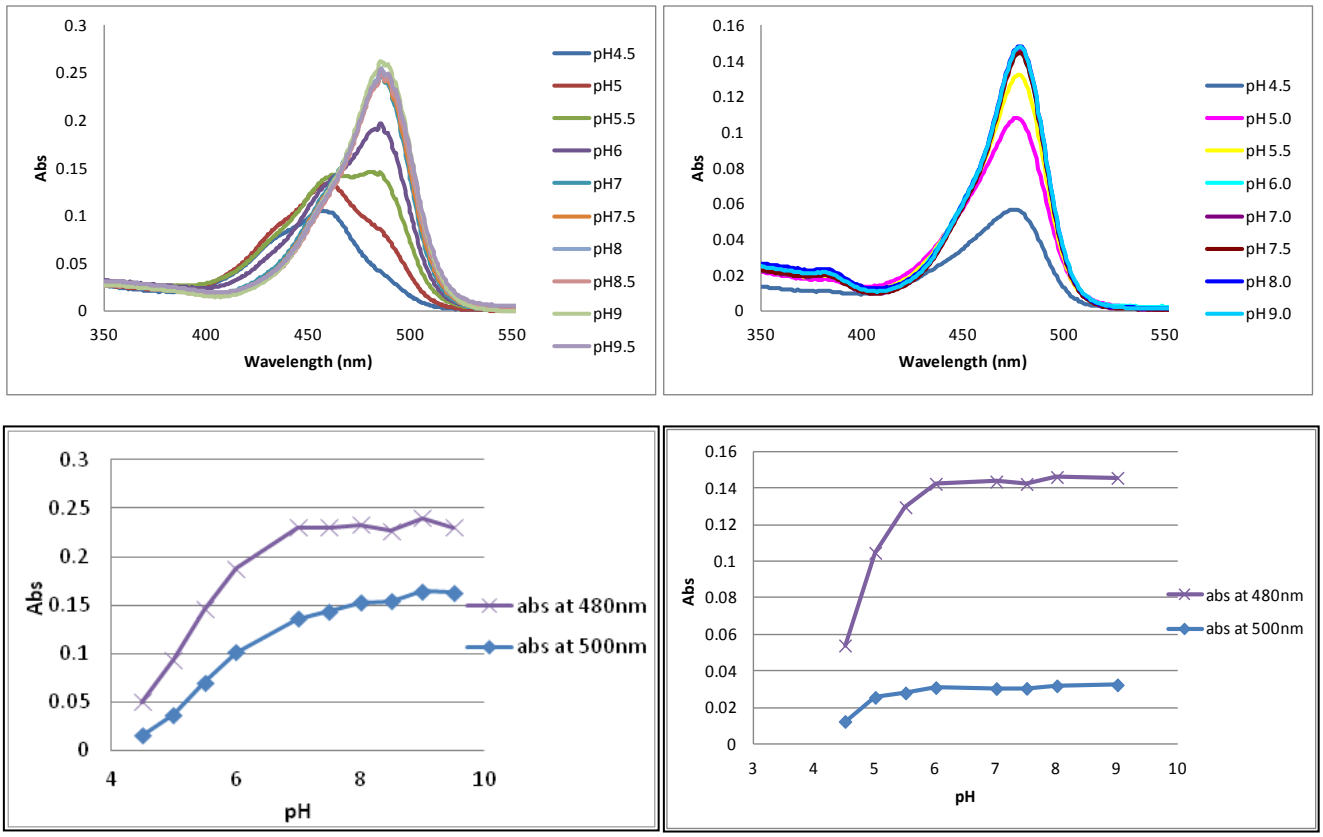


Figure S10. Absorbance spectra (top) and absorbance as a function of pH (bottom) of **1** (left) and **2** (right), (4.0×10^{-6} M) in 0.1M phosphate and carbonate buffer at pH 4.5-9.0, λ_{abs} at 480 nm and 500 nm

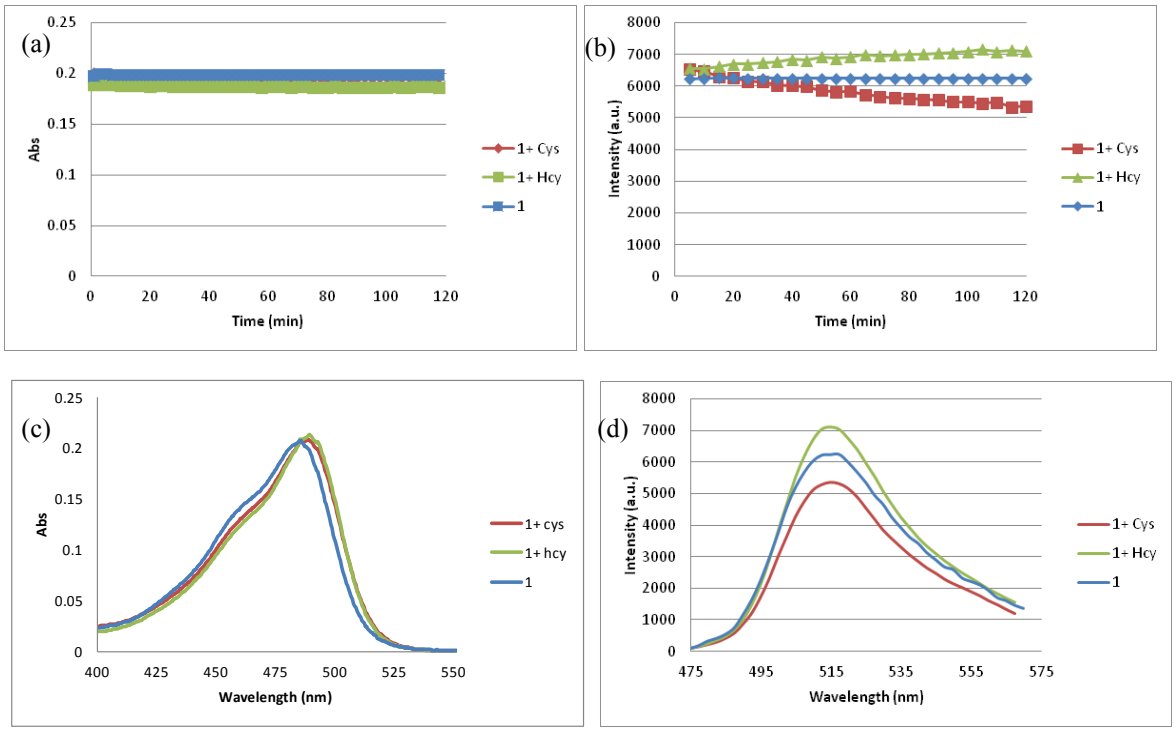


Figure S11. Optical sensing behavior of **1** towards Cys and Hcy at pH 6.0, λ_{abs} and λ_{ex} = **480 nm** - (a,b) Time dependent absorbance (left) and fluorescence (right) spectral changes of **1** with Cys and Hcy, λ_{abs} and λ_{ex} = **480 nm**. (c,d) Time course absorbance (left) and fluorescence (right) spectral changes of **1** with Cys and Hcy, λ_{ex} = 480 nm. Solutions are composed of 4 μM of **1** with 1 mM of analyte in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 ºC, λ_{em} = 515nm.

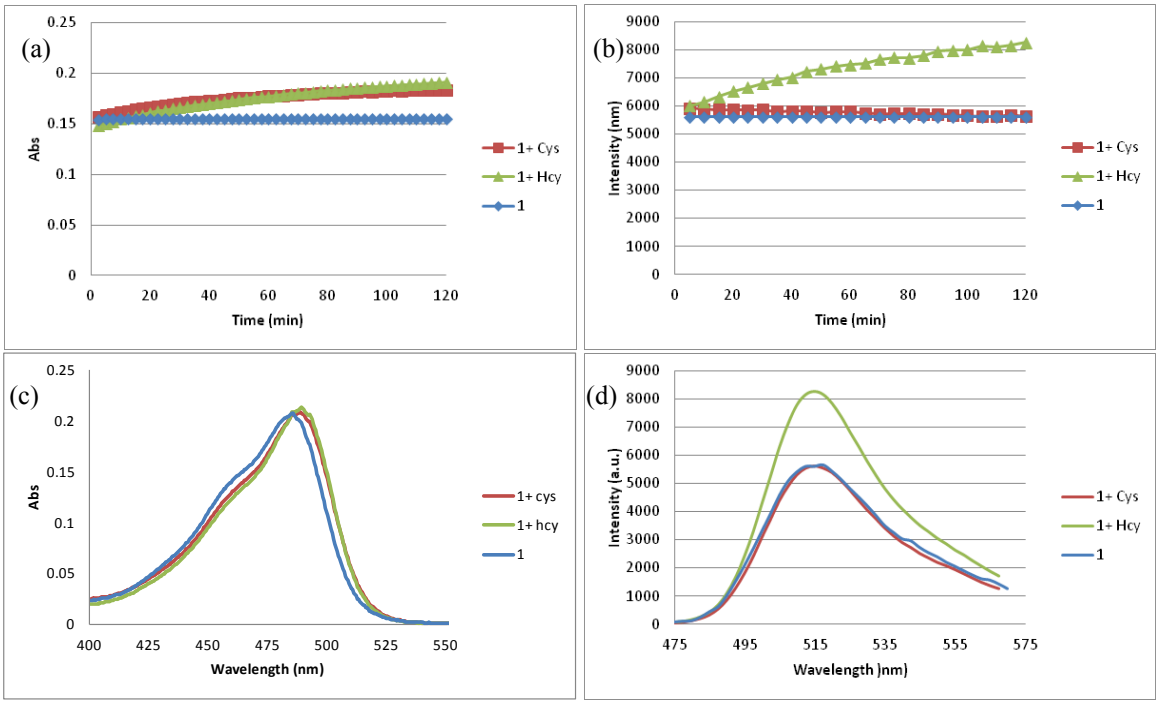


Figure S12. Optical sensing behavior of **1** towards Cys and Hcy at pH 6.0, λ_{abs} and λ_{ex} = **495 nm** - (a,b) Time dependent absorbance (left) and fluorescence (right) spectral changes of **1** with Cys and Hcy, λ_{abs} and λ_{ex} = **495 nm**. (c,d) Time course absorbance (left) and fluorescence (right) spectral changes of **1** with Cys and Hcy, λ_{ex} = 495 nm. Solutions are composed of 4 μM of **1** with 1 mM of analyte in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 ºC, λ_{em} = 515 nm.

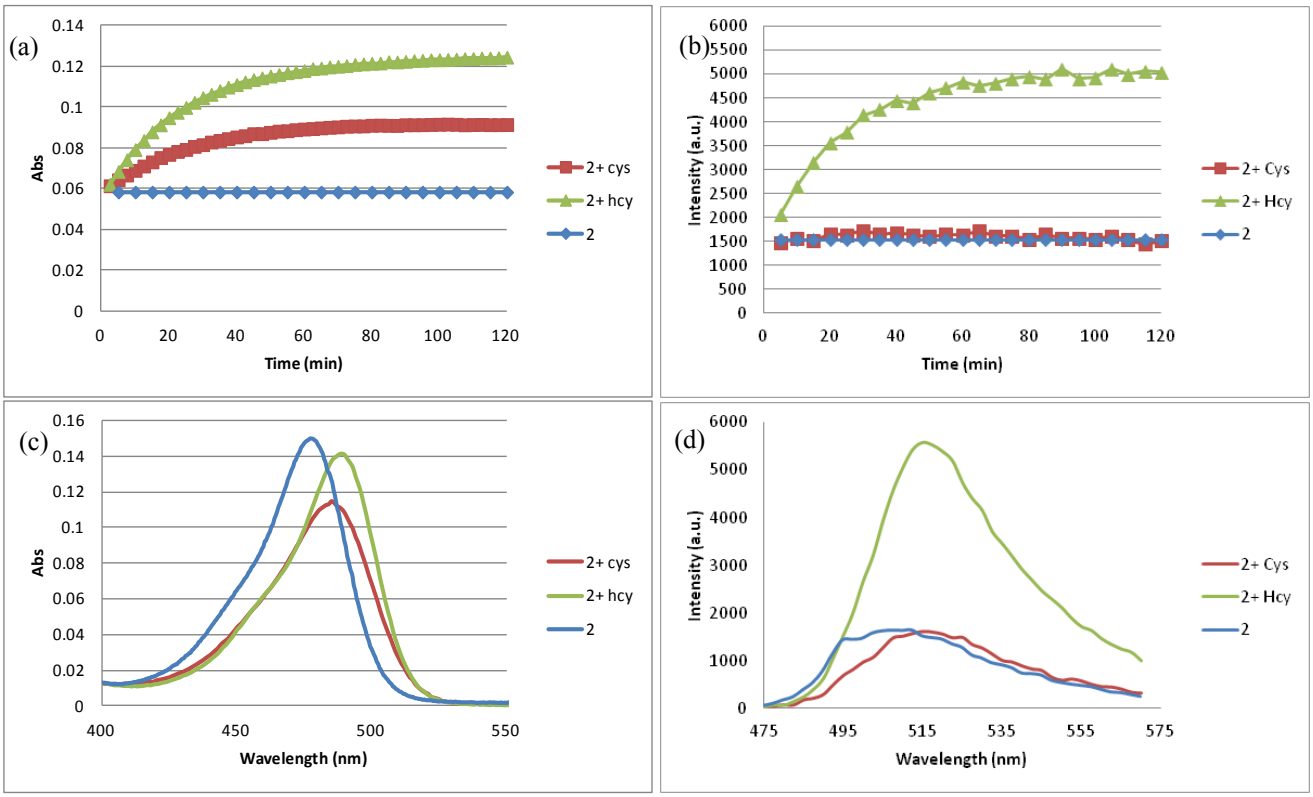


Figure S13. Optical sensing behavior of **2** towards Cys and Hcy at pH 6.0 - (a,b) Time dependent absorbance (left) and fluorescence (right) spectral changes of **2** with Cys and Hcy, λ_{abs} and λ_{ex} = 495 nm. (c,d) Time course absorbance (left) and fluorescence (right) spectral changes of **2** with Cys and Hcy, λ_{ex} = 495 nm. Solutions are composed of 4 μM of **2** with 1 mM of analyte in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 ºC, λ_{em} = 515 nm.

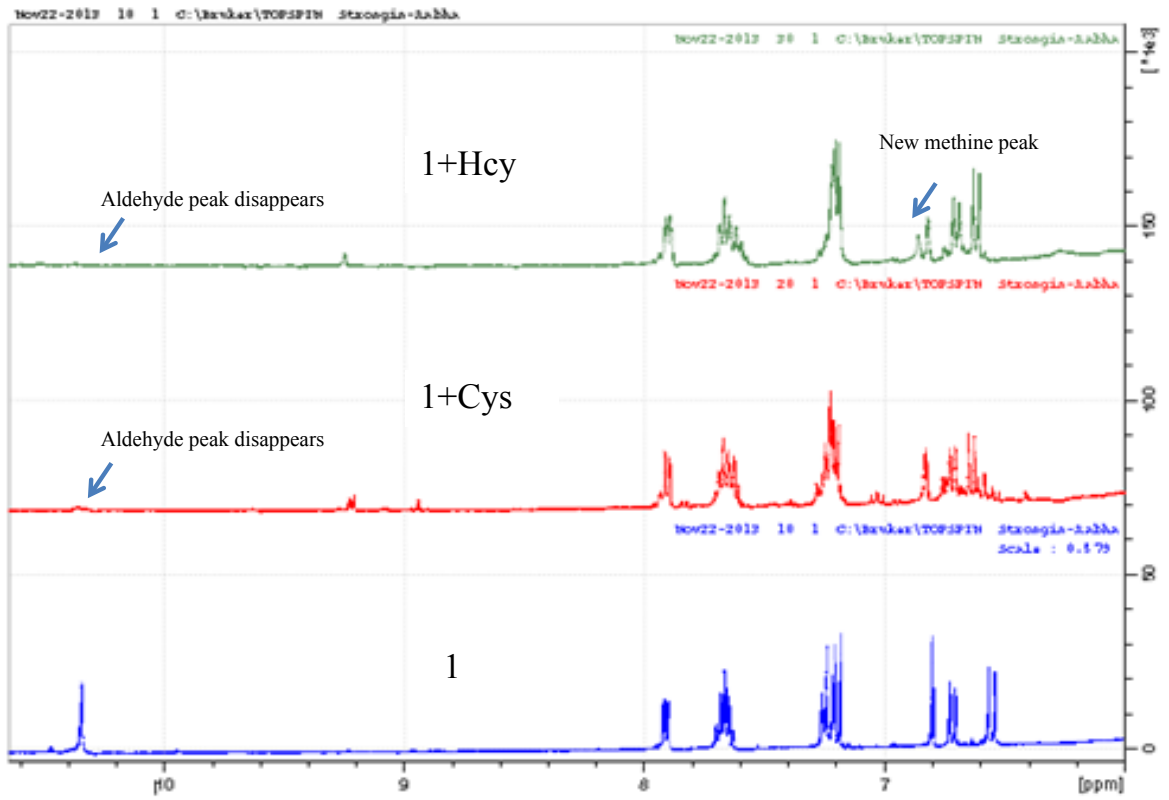


Figure S14. H[1] NMR of **1**,**1** in the presence of Hcy (3 equiv) and **1** in presence of Cys (3 equiv) at pH 6.

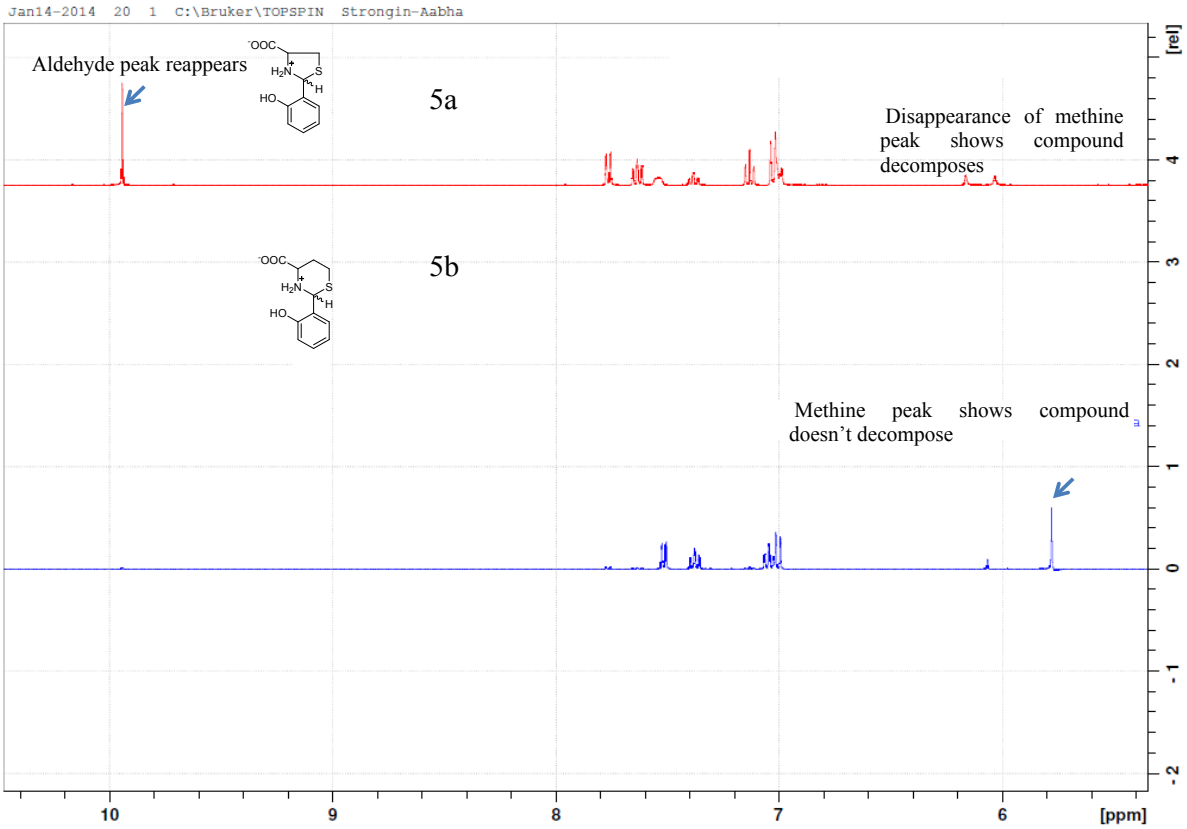


Figure S15. [1]H NMR of salicylaldehyde-Cys derived thiazolidines **3a** and salicylaldehyde-Hcy derived thiazinanes **3b** in D_2O:H_2O.

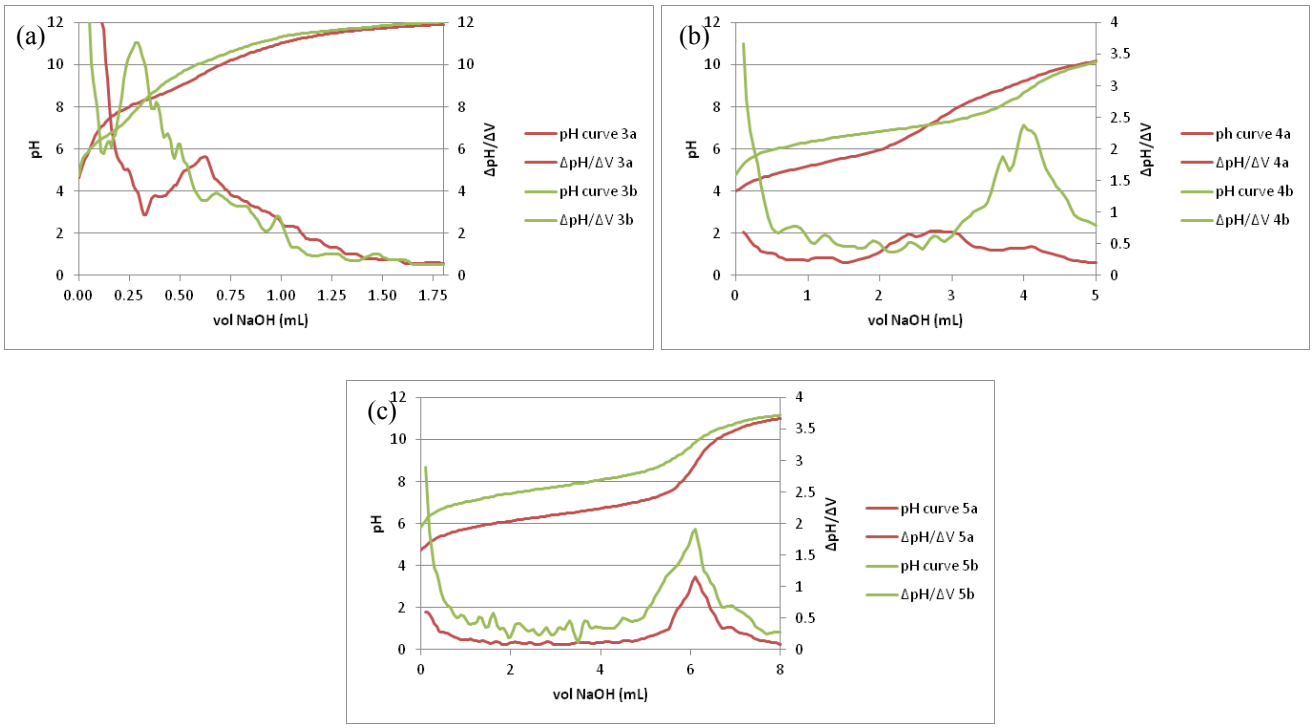


Figure S16. Average of triplicates of pH titration curve and derivatives **3a**, **3b**, **4a**, **4b**, **5a** and **5b** obtained from pH titration- (a) salicylaldehyde-Cys derived thiazolidine **3a** and salicylaldehyde -Hcy derived thiazinane **3b**, (b) benzaldehyde-Cys derived thiazolidine **4a** and benzaldehyde -Hcy derived thiazinane **4b**, (c) HCHO-Cys derived thiazolidine **5a** and HCHO-Hcy derived thiazinane **5b**. For (a) 0.1 M NaOH was used and for (b and c) 0.01 M of NaOH was used.

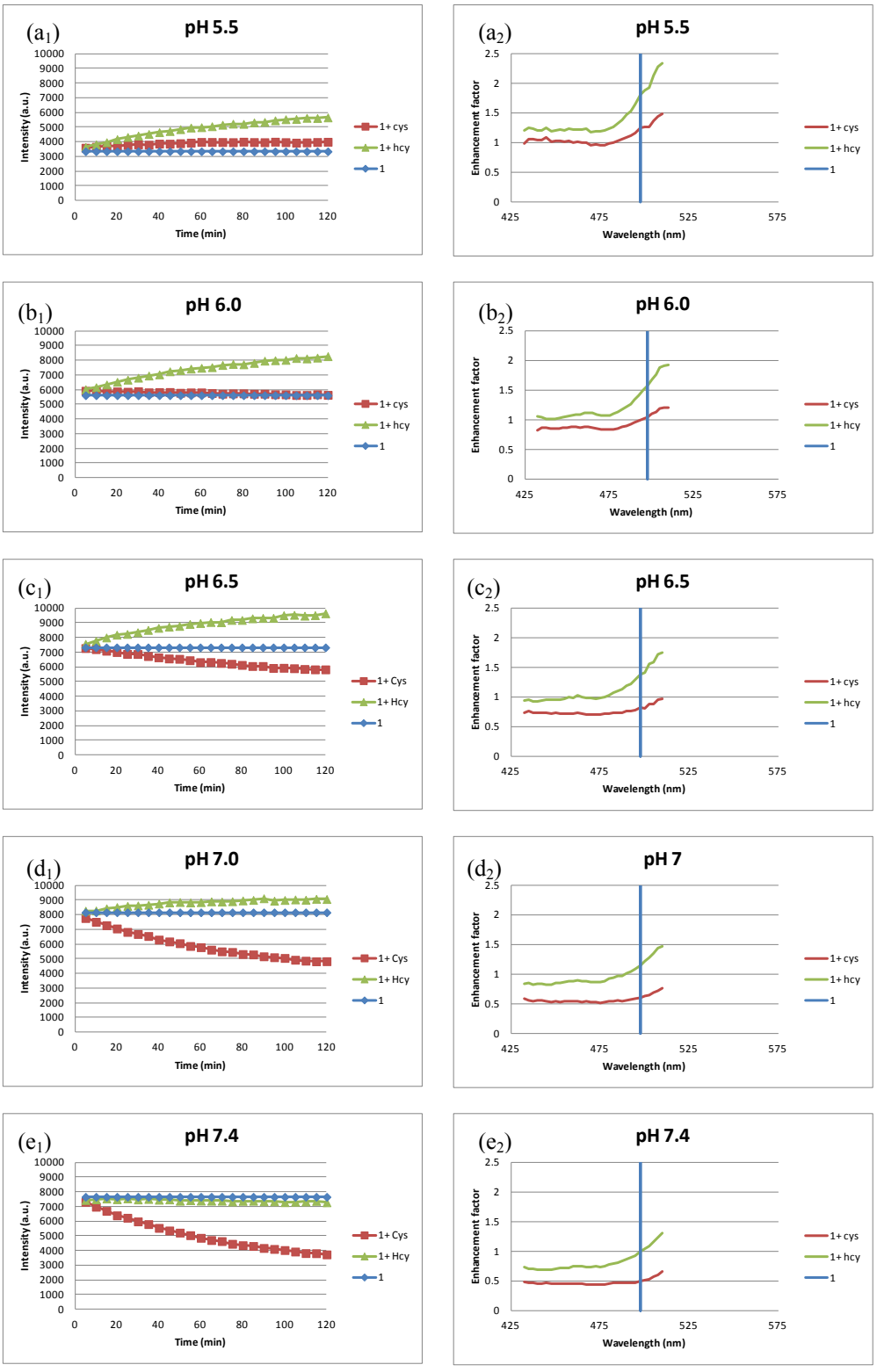


Figure S17. Time dependent fluorescence spectral changes (left) and enhancement factor (right) of fluorescence of **1** with Cys and Hcy, (a_1 and a_2) pH 5.5, (b_1 and b_2) pH 6.0, ($c_1$and c_2) pH 6.5, ($d_1$and d_2) pH 7.0, ($e_1$and e_2) pH 7.4. Solutions are composed of 4 μM of **1** with 1 mM analyte in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 ºC, λ_{ex} = 495 nm and λ_{em} = 515 nm. For Time dependent fluorescence spectral changes, λ_{ex} =495 and λ_{em} =515. Enhancement factor (EF) is the response of **1**+ analyte divided by 1. When EF < 1 quenching is observed, while when EF > 1 enhancement is observed. The vertical line in the enhancement factor plots is the experimentally determined optimal excitation wavelength of 495 nm. At pH 6.0 (b2), excitation at 495 results in enhancement for Hcy but no enhancement for Cys in response to **1**.

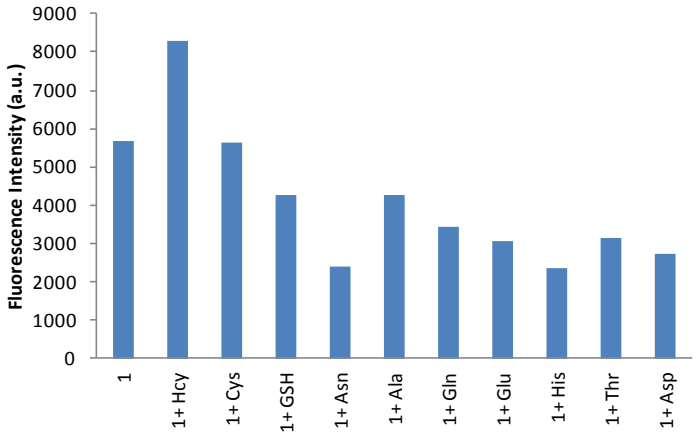


Figure S18. Optical sensing behavior of **1** towards Cys and Hcy as compared to other biologically relevant species. Fluorescence response of **1** upon addition of different analytes (Cys, Hcy, GSH,Asn, Ala, Gln, Thr, Asp, Arg, His, Glu, 1 mM). All Solutions are composed of 4 μM of **1** with 1 mM analyte in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 ºC, λ_{ex} = 495nm λ_{em} = 515 nm.

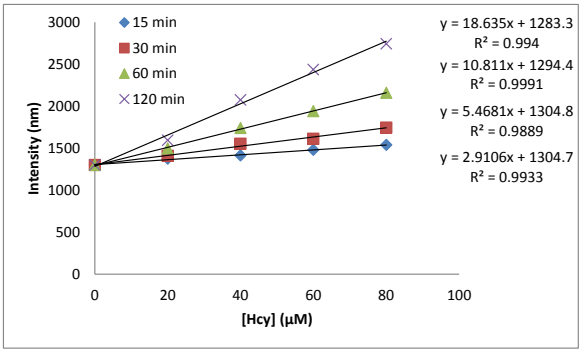


Figure S19. Spectral response of **2** towards increasing levels of Hcy in phosphate buffer (100 mM, pH 6.0). Solutions are composed of 4 μM of **2** with 0-80 μM of Hcy in phosphate buffer (100 mM, pH 6.0):DMSO 99:1at 20 ^{0}C, λ_{ex} = 500 nm and λ_{em} = 515 nm.

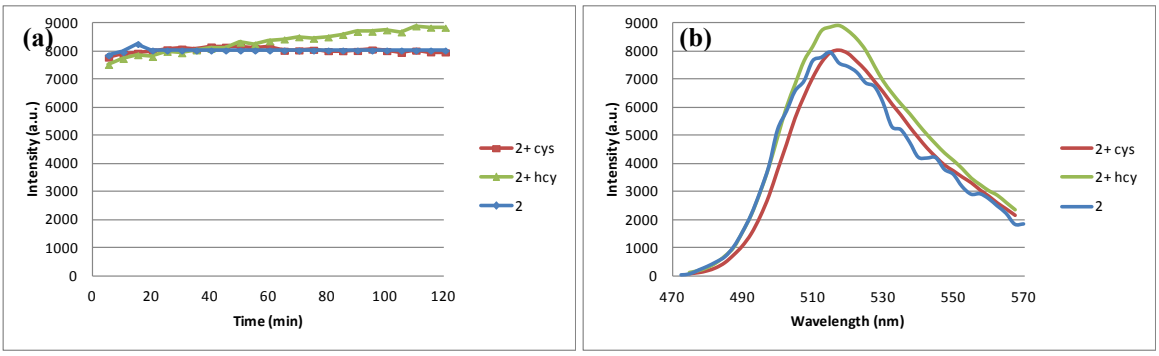


Figure S20. Optical sensing behavior of **2** towards Cys and Hcy at their biological concentrations at pH 6.0 - (a) Time dependent fluorescence spectral changes of **2** with Cys and Hcy. (b) fluorescence spectra of **2** with Cys and Hcy after two hours. Solutions are composed of 30 μM of **2** with 250 μM of Cys and 15 μM of Hcy in phosphate buffer (100 mM, pH 6.0):DMSO 99:1 at 20 °C, λ_{ex} = 500 nm and λ_{em} = 515 nm.

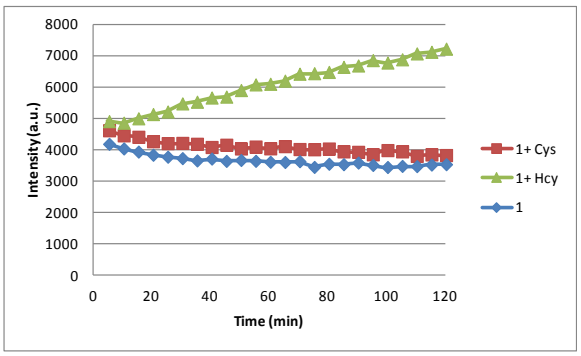


Figure S21. Optical sensing behavior of **2** towards Cys and Hcy in deproteinized plasma at pH 6.0. Time dependent fluorescence spectral changes of **2** with Cys and Hcy. Solutions are composed of 25 μM of **2** with 250 μM of Cys and 100 μM of Hcy in phosphate buffer (100 mM, pH 6.0):DMSO 99:1at 20 °C, λ_{ex} = 495 nm and λ_{em} = 515 nm.

Reference

1. Wang, W. H.; Rusin, O.; Xu, X. Y.; Kim, K. K.; Escobedo, J. O.; Fakayode, S. O.; Fletcher, K. A.; Lowry, M.; Schowalter, C. M.; Lawrence, C. M.; Fronczek, F. R.; Warner, I. M.; Strongin, R. M., Detection of homocysteine and cysteine. *J. Am. Chem. Soc*. **2005,** *127* (45), 15949-15958.