# Electronic Supplementary Information
# For

# A seminaphthofluorescein-based fluorescent chemodosimeter for the highly selective detection of Cysteine

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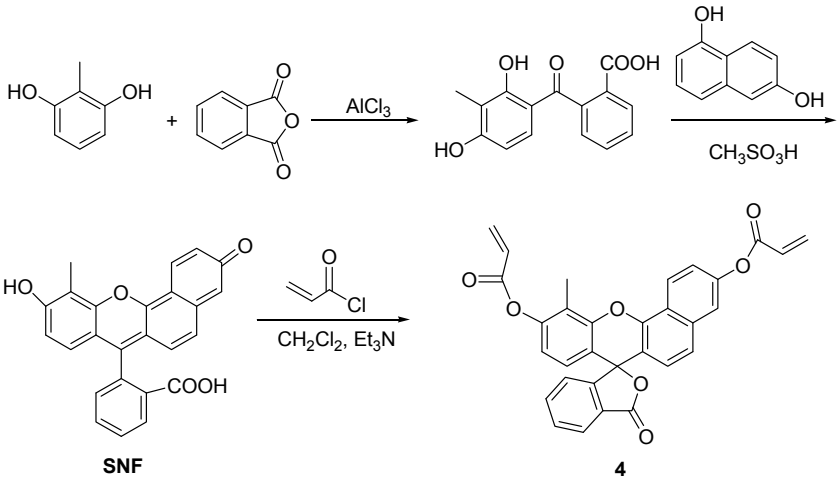
## Materials and instruments:

All chemicals were purchased from Sigma-Aldrich and Acros Organics and used without further purification. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer, using TMS as internal standard. ESI-HRMS (high resolution mass spectrometry) spectra were obtained on a Thermo Electron LTQ Orbitrap hybrid mass spectrometer. IR spectra were recorded on a Nicolet iS10 spectrometer using a diamond ATR attachment from Thermo Scientific Co. UV-visible spectra were collected on a Cary 50 UV-Vis spectrophotometer. Fluorescence spectra were collected on a Cary Eclipse (Varian, Inc.) fluorescence spectrophotometer. pH measurements were carried out with an Orion 410A pH meter. In all experiments enantiomerically pure natural amino acids were used except for Hcy which was used as the racemate.

## Synthetic procedure




**Scheme S1**. Synthesis of **4**

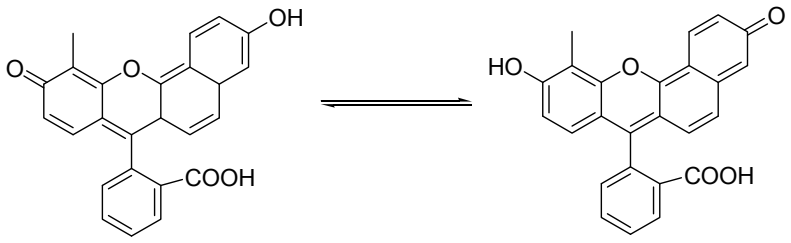
## Synthesis of Seminaphthofluorescein (SNF)

**SNF** was synthesized in two steps according to the method reported by Lippard *et al.* [1]. $^{1}$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 8.44 (d, 1H, *J*= 9.2 Hz ), 8.04 (d, 1H, *J* =

7.2 Hz), 7.74 (m, 2H), 7.30 (d, 1H, $J$ = 9.2 Hz), 7.26 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$= 9.2 Hz), 7.19 (d, 1H, $J$ = 7.6 Hz), 7.12 (s, 1H), 6.62 (m, 2H), 6.51 (d, 1H, $J$ = 8.8 Hz), 2.51 (s, 3H). ESI-FTMS $m/z$ = 395.0923 $[M-H]^-$, calc. 395.0919 for $C_{25}H_{15}O_5$.

## Synthesis of 4

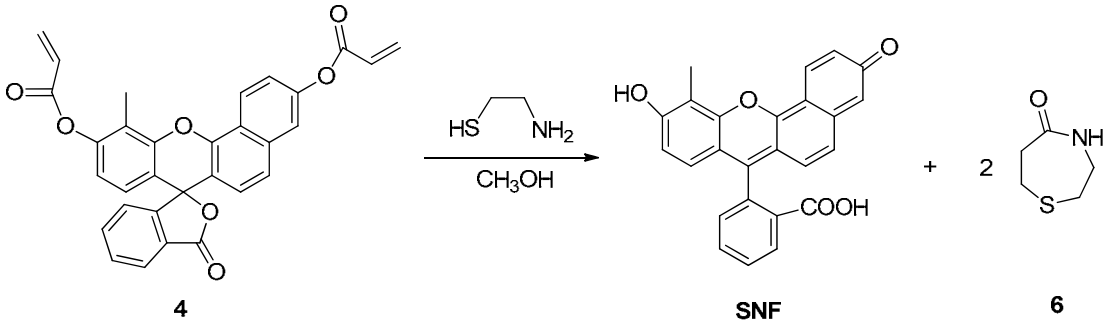
To a solution of SNF (120 mg, 0.303 mmol) and $Et_3N$ (2 equiv) in 10 mL of anhydrous $CH_2Cl_2$, acryloyl chloride (68.6 mg, 0.758 mmol, mixed with 5 mL of $CH_2Cl_2$) is added dropwise at 0 $^oC$. After stirring at this temperature for 90 min, the resulting mixture is allowed to warm to rt and stirred overnight. The mixture is diluted with $CH_2Cl_2$ (25 mL), washed with $H_2O$ (12 mL × 3) and dried over anhydrous $Na_2SO_4$. The solvent is removed by evaporation to afford a yellow solid. Purification by flash column chromatography (silica gel, $CHCl_3$/EtOAc 100:6) resulted in the isolation of **4** as a light yellow solid (99 mg, 65% yield). UV–Vis $\lambda_{max}$ (ε) 227 nm ($3.71 \times 10^4$ $M^{-1}$ $cm^{-1}$), 280 nm ($2.47 \times 10^4$ $M^{-1}$ $cm^{-1}$); IR (ATR, $cm^{-1}$), 1744, 1603, 1582, 1399, 1290, 1221, 1201, 1136, 1091, 978, 896, 819, 793, 746, 692. $^1H$ NMR ($CDCl_3$, 400MHz), δ (ppm): 8.58 (d, 1H, $J$ =9.2 Hz), 8.07 (d, 1H, $J$ = 8.0 Hz), 7.66 (m, 3H), 7.46 (m, 2H), 7.16 (d, 1H, $J$ = 6.4 Hz), 6.83 (m, 2H), 6.76 (d, 1H, $J$= 8.4 Hz), 6.70 (br s, 1H), 6.66 (br s, 1H), 6.41 (dd, 1H, $J_1$ =1.6 Hz, $J_2$ = 10.4 Hz), 6.37 (dd, 1H, $J_1$ =1.6 Hz, $J_2$ = 10.4 Hz), 6.08 (m, 2H), 2.51 (s, 3H). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ 169.52, 164.57, 164.05, 153.67, 150.31, 150.15, 149.84, 146.93, 135.38, 135.27, 133.49, 133.22, 130.09, 127.90, 127.49, 125.31, 124.83, 124.28, 123.96, 123.60, 122.09, 121.99, 119.45, 118.88, 118.15, 116.49, 112.42, 82.92, 9.87. ESI-FTMS $m/z$ = 505.1308 $[M+H]^+$, calc. 505.1287 for $C_{31}H_{21}O_7$.




naphthoxyquinone phenoxylnaphthoquinone

**Scheme S2**. The two tautomeric forms of the seminaphthofluorescein

**Preparation of 1,4-Thiazepan-5-one (6)**




**4** **SNF** **6**

To a 50 mL flask, **4** (50 mg, 0.099 mmol) and cysteamine (2.5 equiv) are combined in 20 mL of $CH_3OH$, and the mixture is stirred at rt for 2 h. After that, the solvents are dried *in vacuo* and the crude product is subjected to column chromatography (eluted with EtOAc) to afford 32 mg of SNF and 19.8 mg of **6** as off-white solid [2]. **6**: $^1$H NMR ($CDCl_3$, 400MHz), δ (ppm): 6.36 (s, 1H), 3.62 (m, 2H) 2.93 (t, 3H, $J$ = 5.2 Hz), 2.73 (m, 4H). $^{13}$C NMR ($CDCl_3$, 100 MHz), δ 177.51, 45.98, 40.99, 31.57, 24.69. ESI-FTMS *m/z* = 132.0478 $[M+H, 90\%]^+$, calc. 132.0483 for $C_5H_{10}NOS$; *m/z* =154.0297 $[M+Na, 100\%]^+$,154.0297, calc. 154.0303 for $C_5H_9NNaOS$.

## References

[1] C. J. Chang, J. Jaworskit, E. M. Nolan, M. Sheng and S. J. Lippard, *Proc. Natl. Acad. Sci. USA,* 2004, **101**, 1129-1134.

[2] P. Blondeau, R. Gauthier, C. Berse and D. Gravel, *Can. J. Chem.* 1971, **49**, 3866-3876.

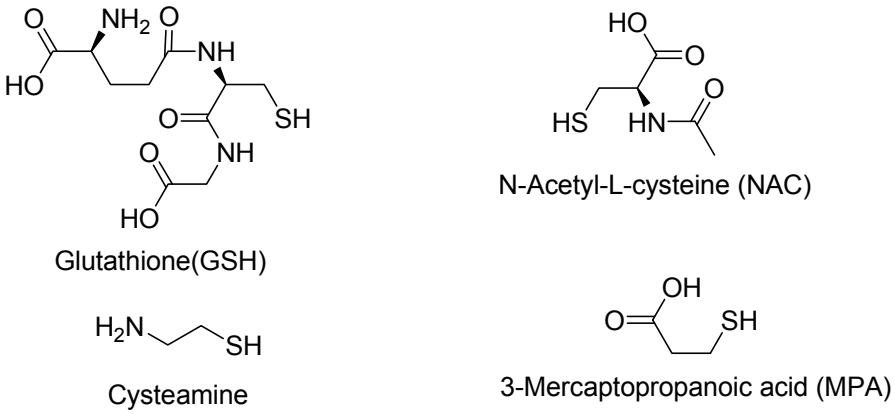



**Fig. S1**. Chemical structure of some thiol-containing compounds used in the experiments.

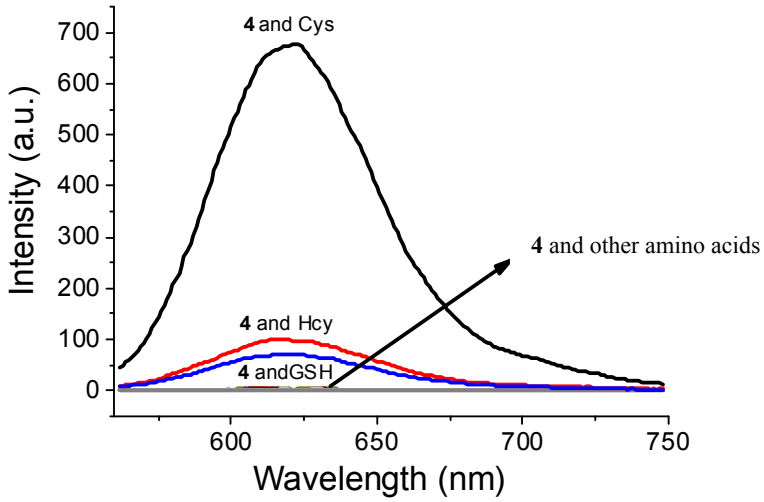



**Fig. S2**. Fluorescence spectra ($\lambda_{ex}$ = 550 nm) of **4** (10 μM) with the addition of different kinds of analytes (Cys, Hcy, GSH, leucine, proline, arginine, histidine, valine, methionine, threonine, glutamine, alanine, aspartic acid, norleucine, isoleucine, lysine, tryptophan, tyrosine, phenylalanine, cystine and homocystine) for 25 min in 1 mM CTAB buffered at pH 7.4 (Hepes buffer, 20 mM). Cys, 10 μM. Other analytes, 100 μM.

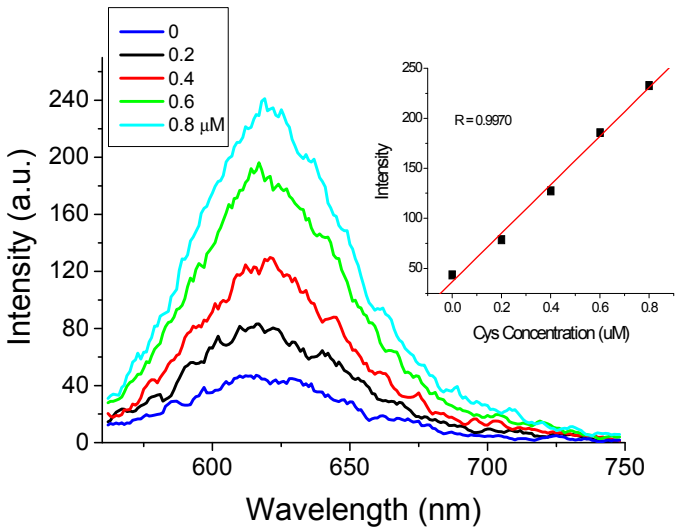



**Fig. S3**. Fluorescence spectra ($\lambda_{ex}$ = 550 nm) of **4** (10 μM) in the presence of Cys (0 – 0.8 μM) for 25 min in 1.0 mM CTAB media buffered at pH 7.4. The inset figure shows the plot of the fluorescence intensity at 621 nm as a function of Cys concentration. The high voltage of PMT was set at 700 v.

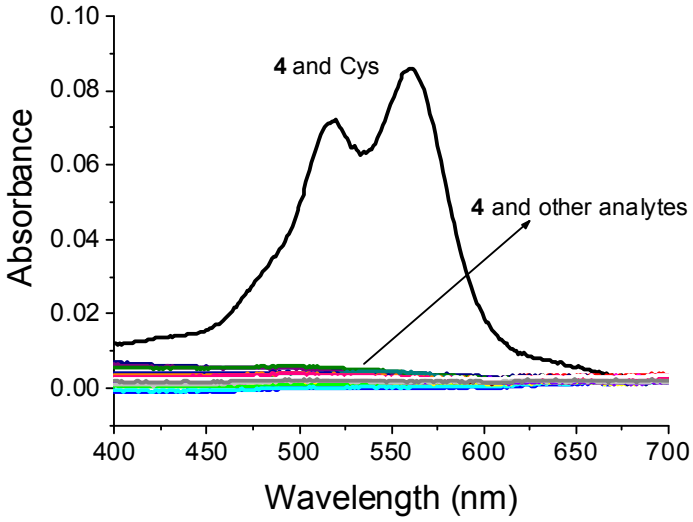



**Fig. S4.** Absorption spectra of **4** (10 μM) with the addition of 1 equiv of Cys, Hcy, GSH, leucine, proline, arginine, histidine, valine, methionine, threonine, glutamine, alanine, aspartic acid, norleucine, isoleucine, lysine, tryptophan, tyrosine, phenylalanine, cystine and homocystine for 25 min in 1 mM CTAB buffered at pH 7.4 (Hepes buffer, 20 mM). The absorption peak at 515 and 560 nm were assigned to the napthoxyquinone and phenoxynaphthoquinone mesomers tautomeric forms of SNF, respectively [1] (Scheme S2).

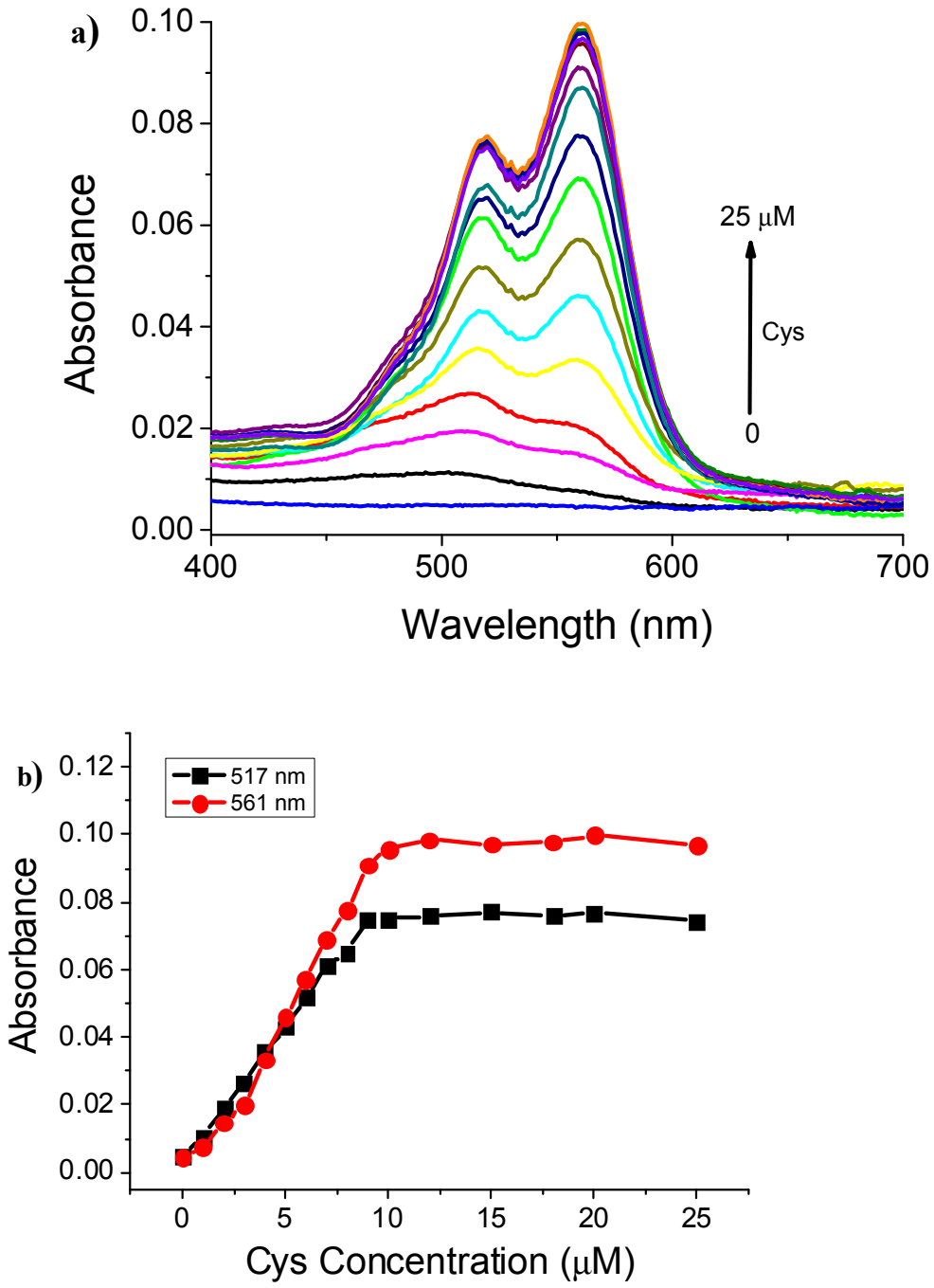



**Fig. S5**. (a) Absorption spectra of **4** (10 μM) with the addition of increasing concentrations of Cys in 1.0 mM CTAB media buffered at pH 7.4. Each spectrum was recorded 25 min after Cys added. (b) Absorbance at 517 nm (or 561 nm) as a function of Cys concentration.

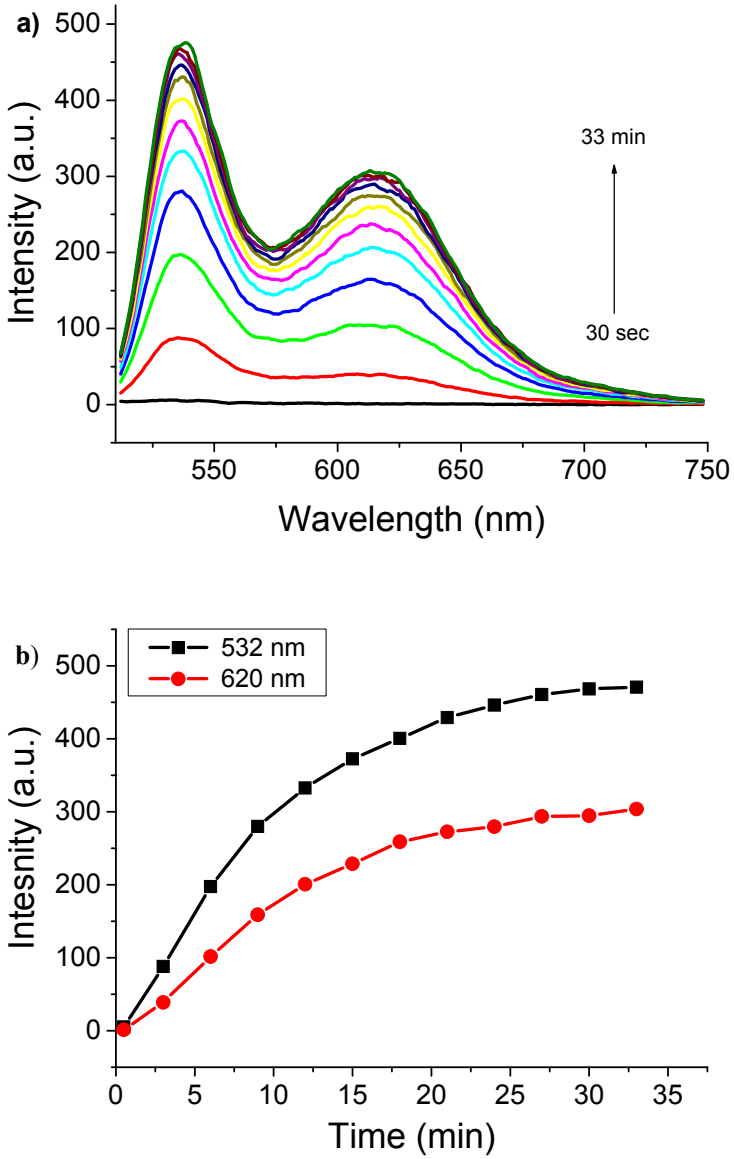



**Fig. S6**. (a) Time-dependent fluorescence spectra ($\lambda_{ex}$ = 500 nm) changes of **4** (10 μM) in the presence of 1 equiv of Cys in 1.0 mM CTAM media buffered at pH 7.4. (b) Intensity at 532 nm (or 621 nm) as a function of time.

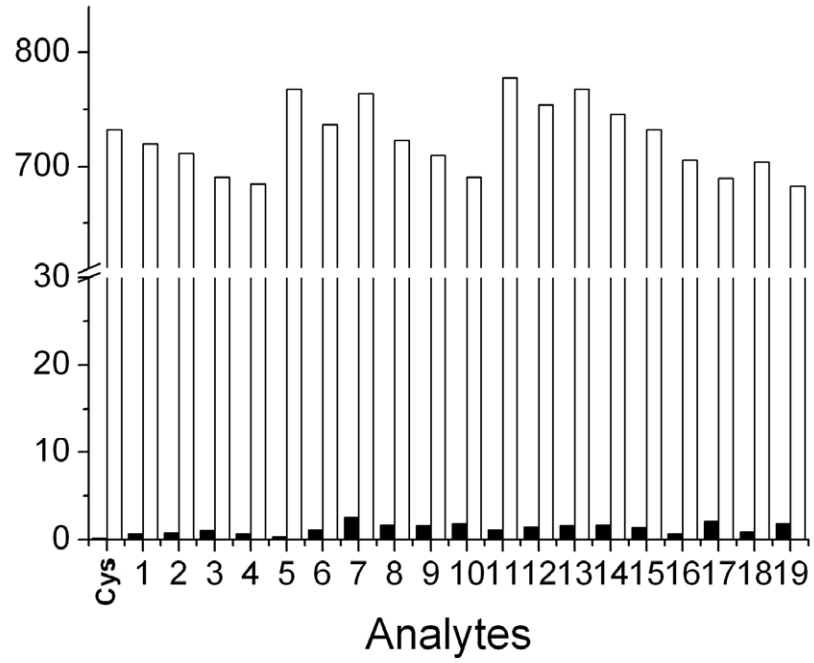



**Fig. S7**. Fluorescence response of **4** (10 μM) at 621 nm to various analytes (10 equiv). White bars represent the addition of other amino acids to the solution of **4**. Empty bars represent the subsequent addition of 1 equiv of Cys to the solution. Excitation was set at 562 nm. Reaction time, 25 min. From 1 to 17 are 10 equiv of amino acids. 1 = Leucine; 2 = Proline; 3 = Histidine; 4 = Arginine; 5 = Valine; 6 = Methionine; 7 = Threonine; 8 = Glutamine; 9 = Alanine; 10 = Aspartic acid; 11 = Isoleucine; 12 = Cystine; 13 = Homocystine; 14 = Lysine; 15 = Tyrosine; 16 = Tryptophan; 17 = Phenylalanine; and 18 and 19 are 1 equiv of Hcy and GSH, respectively.

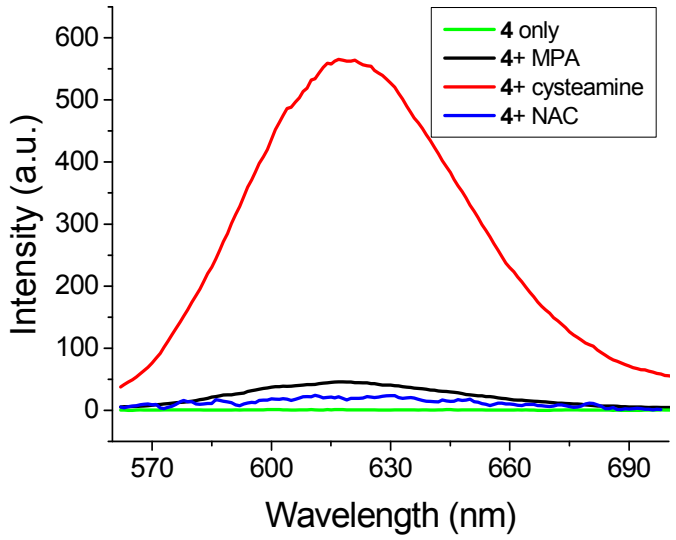



**Fig. S8**. Fluorescence spectra ($\lambda_{ex}$ = 550 nm) of **4** (10 μM) with the addition of other

thiol-containing compounds (4 equiv) in 1 mM CTAB buffered at pH 7.4 for 25 min.

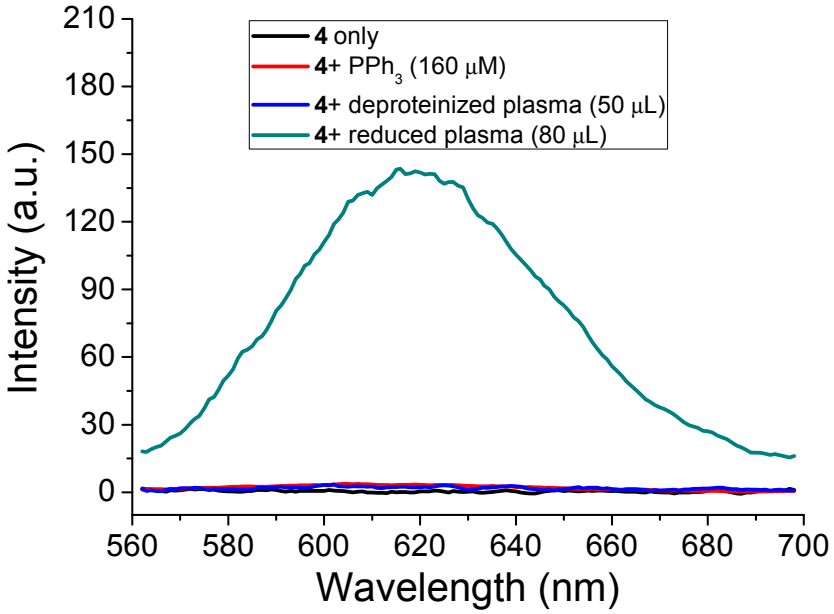



**Fig. S9**. Quantitative measurement of Cys content in human plasma sample. Fluorescence spectra ($\lambda_{ex}$ = 550 nm) of **4** (10 μM) in pH 7.4 Hepes buffer in the presence of 1.0 mM CTAB at different conditions for 25 min.

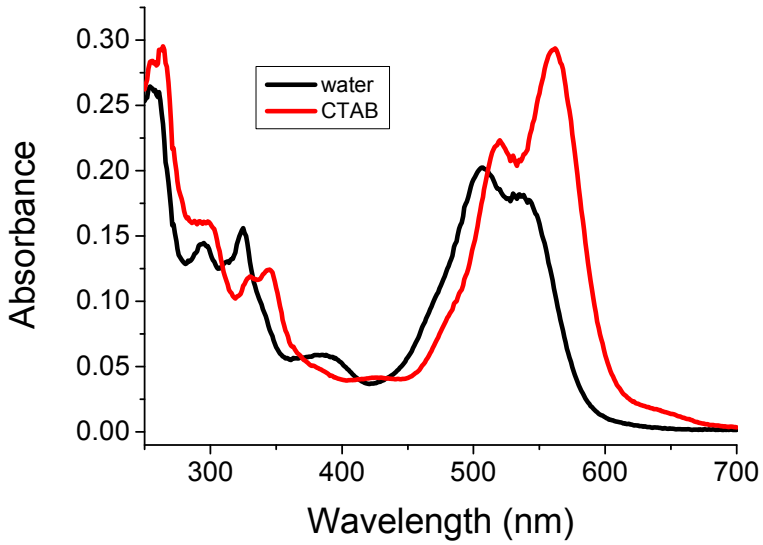



**Fig. S10**. Absorption spectra of SNF (10.0 μM) in water and CTAB (1.0 mM) buffered at pH 7.4 (Hepes buffer, 20 mM).

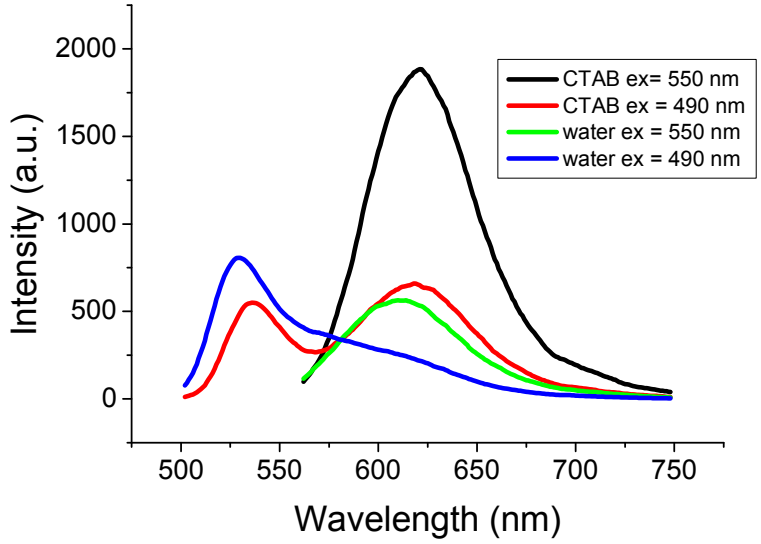



**Fig. S11**. Fluorescence spectra of SNF (10.0 μM) in water and CTAB (1.0 mM) buffered at pH 7.4 (Hepes buffer, 20 mM) at different excitation wavelengths.

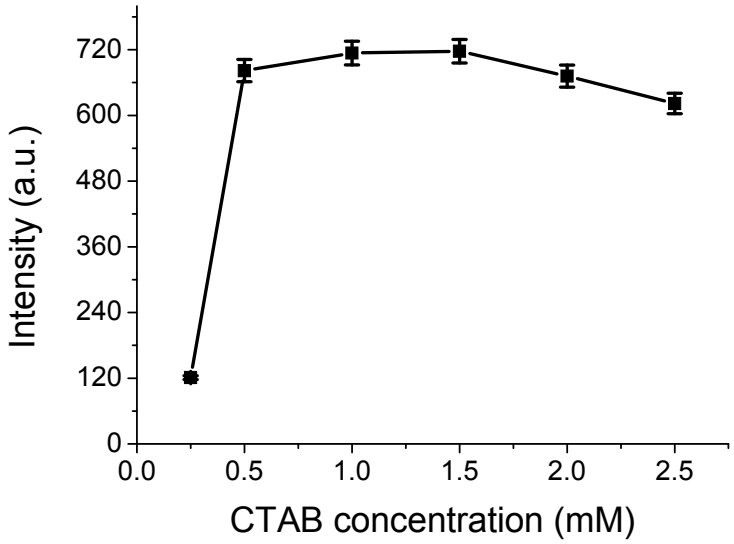



**Fig. S12**. Effect of CTAB concentration on the fluorescence intensity of **4** (10 μM) with Cys (10 μM) in pH 7.4 Hepes buffer. Reaction time, 25 min. $\lambda_{ex}$ /$\lambda_{em}$ = 562/621 nm.

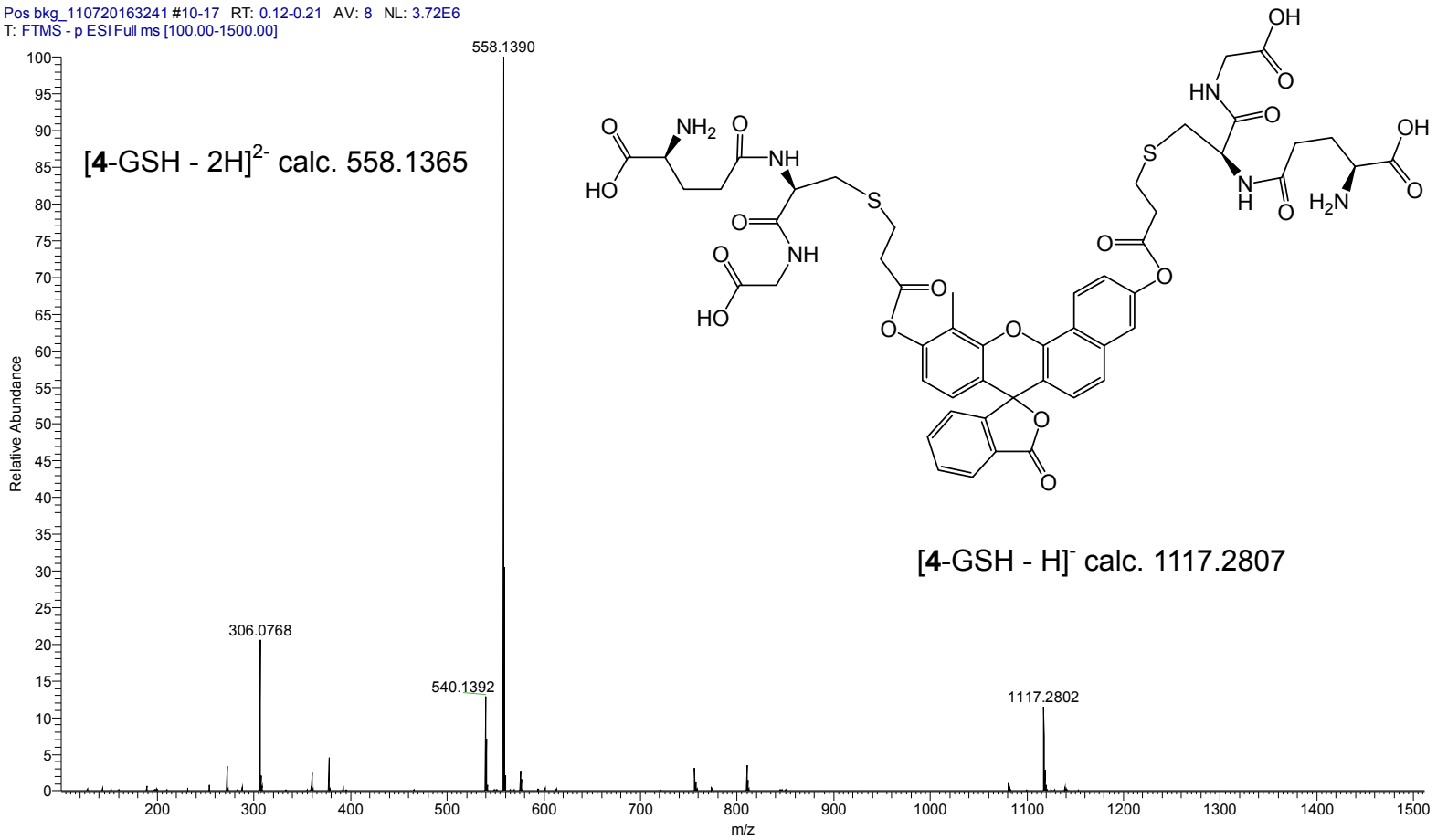



**Fig. S13**. HRMS of **4** with the addition of GSH. Briefly, GSH (9.0 mg) was dissolved in 0.5 mL $H_2O$, and then **4** (5.0 mg) dissolved in 4.0 mL $CH_3OH$ was added. The mixture was kept at rt for 2h and then diluted appropriately with $CH_3OH/H_2O$ (1:1, v/v) for the MS measurement.

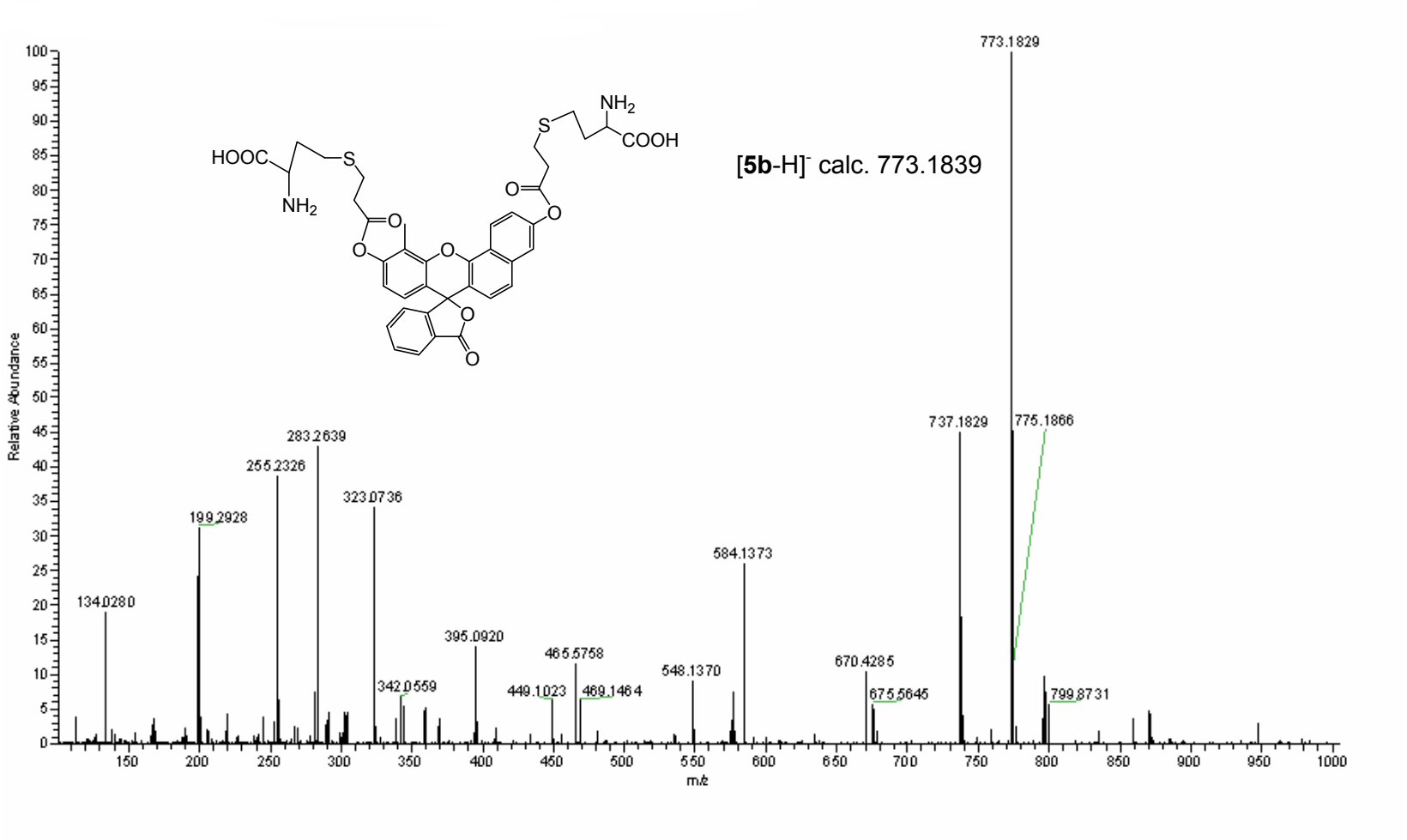



**Fig. S14**. HRMS of **4** with the addition of Hcy. Briefly, Hcy (4.0 mg) was dissolved in 0.5 mL $H_2O$, and then **4** (5.0 mg) dissolved in 4.0 mL $CH_3OH$ was added. The mixture was kept at rt for 3h and then diluted appropriately with $CH_3OH/H_2O$ (1:1, v/v) for the MS measurement.

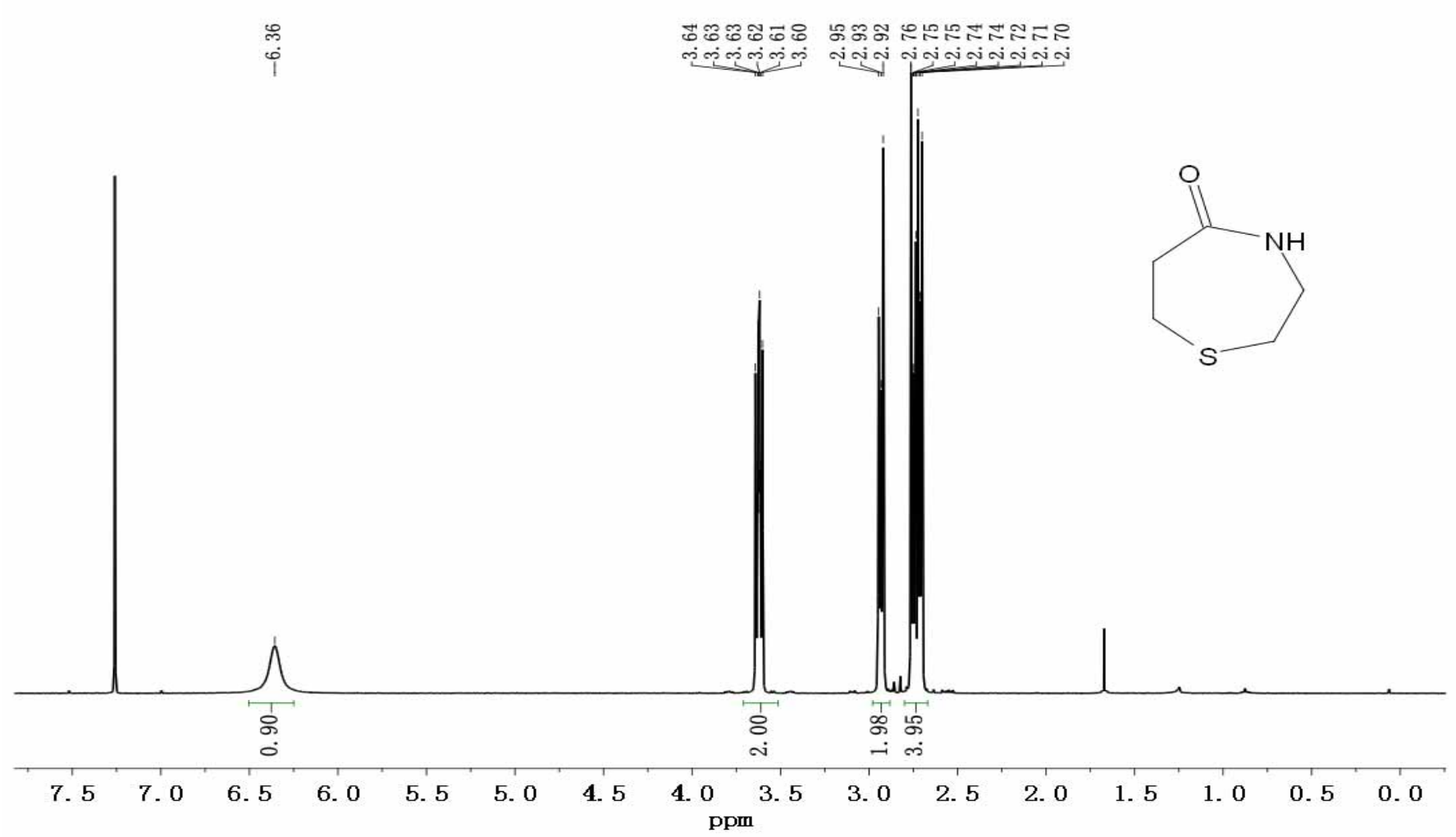



**Fig. S15**. $^{1}$H MNR (400 MHz) spectrum of **6** in $CDCl_3$.

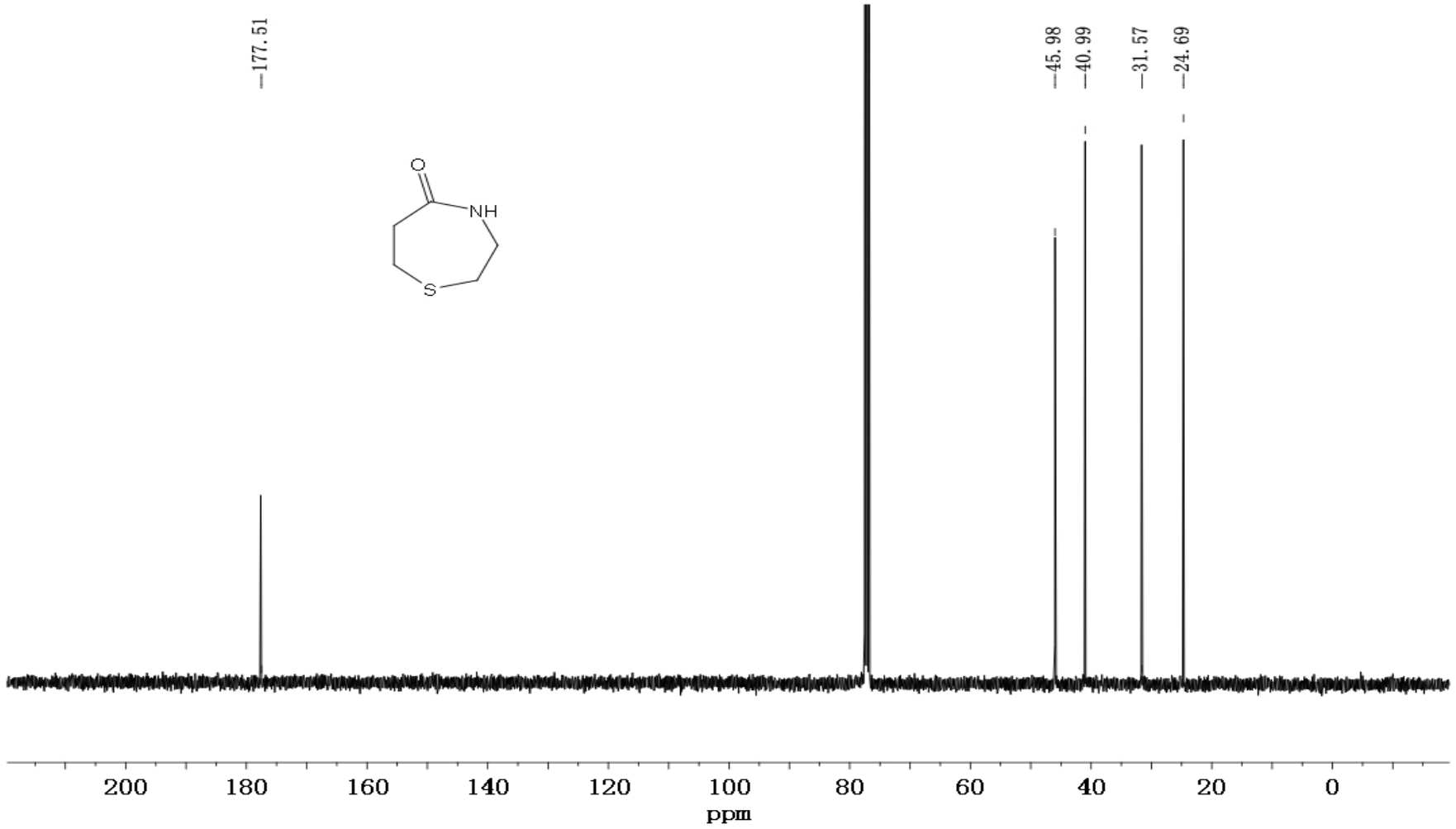



**Fig. S16**. $^{13}C$ MNR (100 MHz) spectrum of **6** in $CDCl_3$

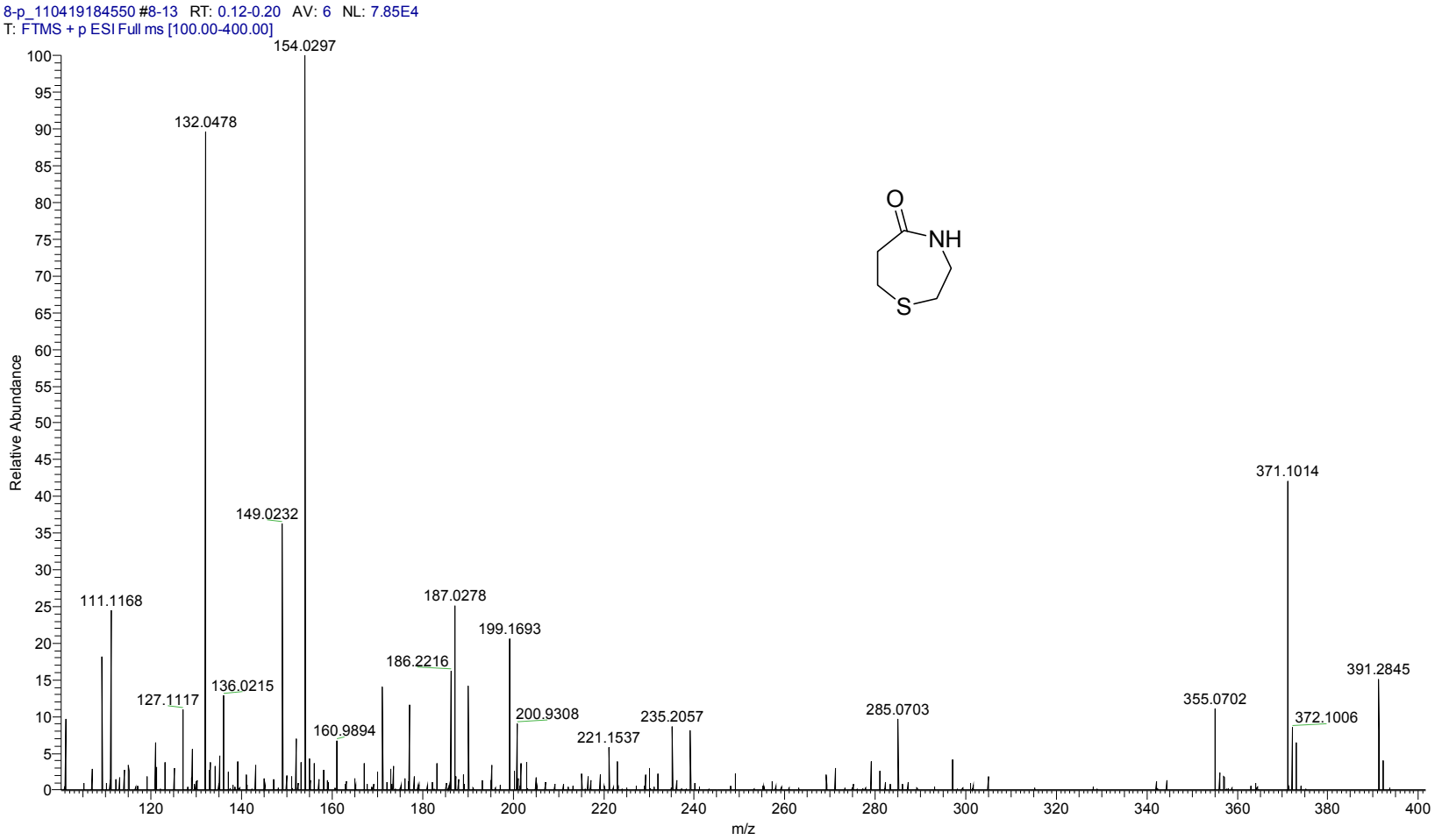



**Fig. S17**. HRMS of **6.**

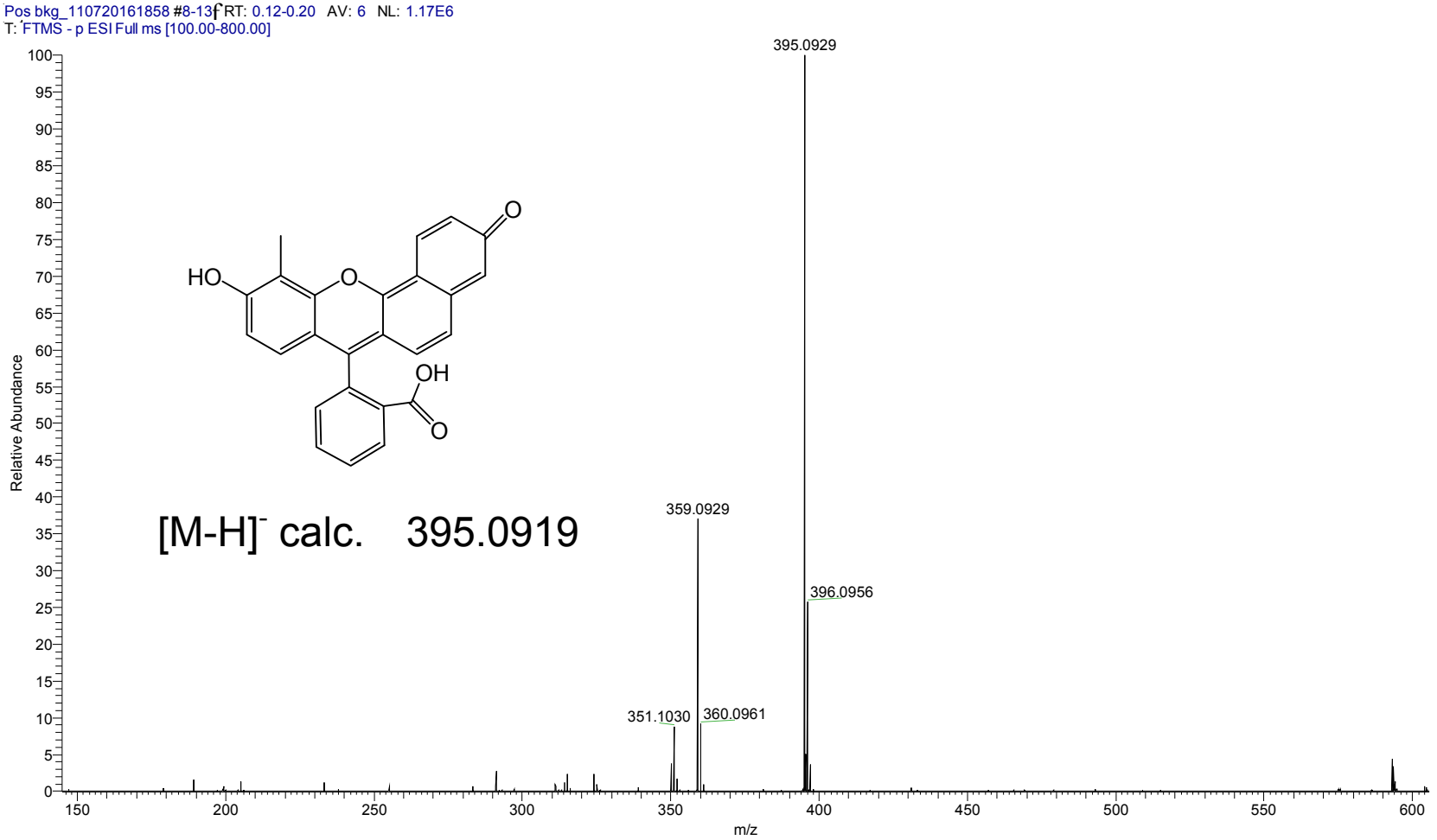



**Fig. S18**. HRMS of **SNF** obtained from the reaction of **4** with cysteamine

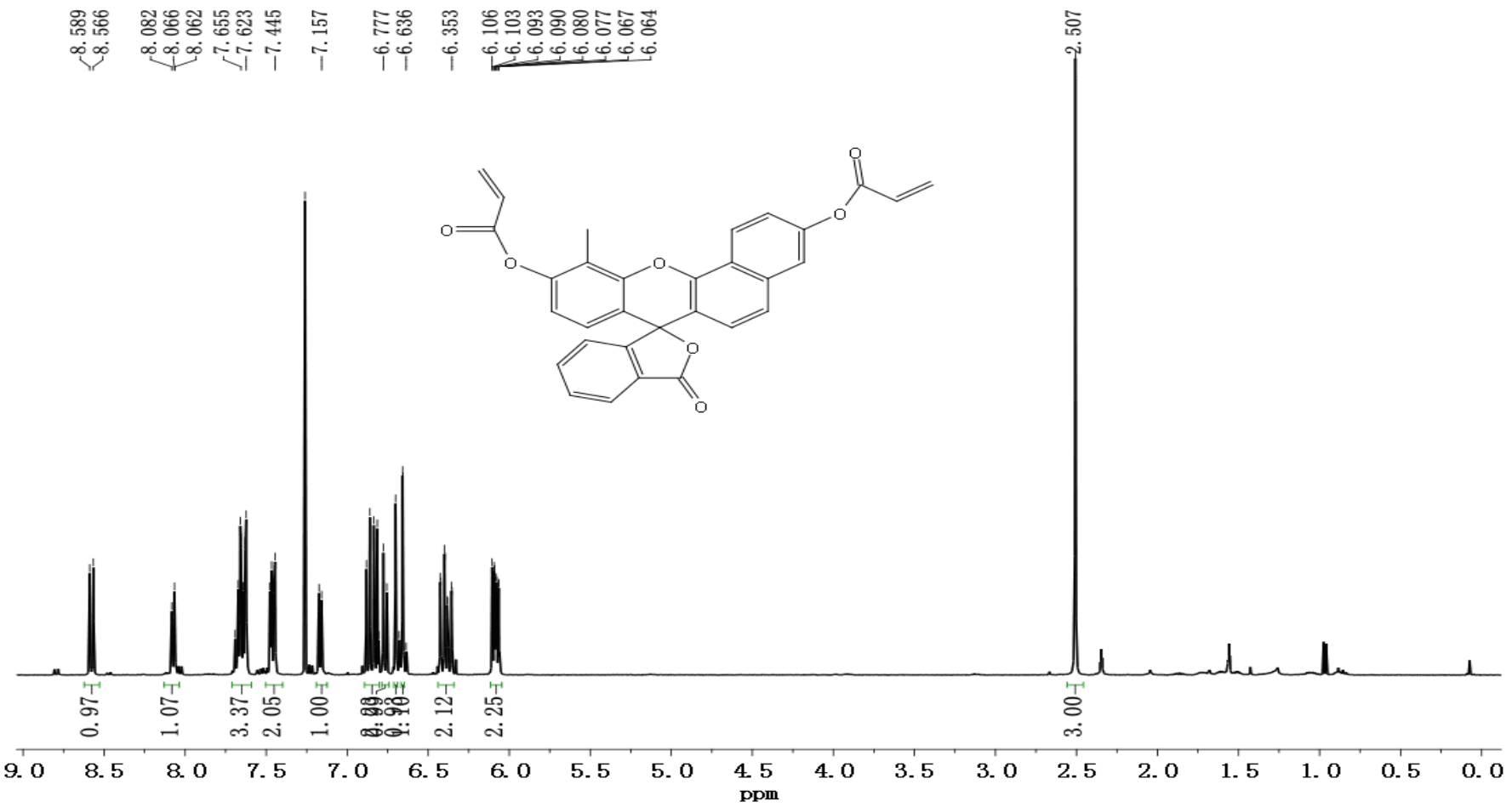



**Fig. S19**. $^{1}$H MNR (400 MHz) spectrum of **4** in $CDCl_3$

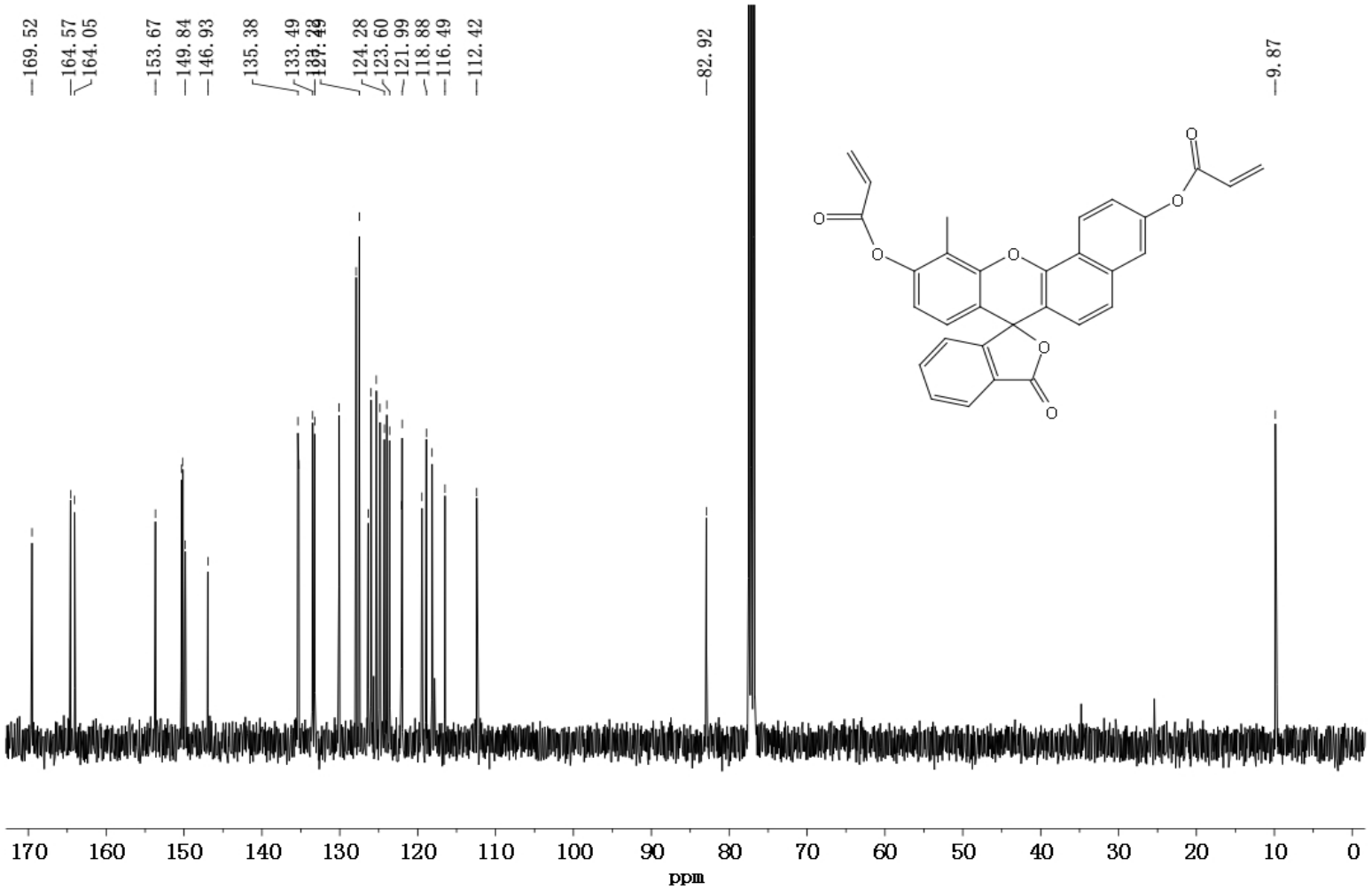



**Fig. S20**. $^{13}$C MNR (100 MHz) spectrum of probe **4** in $CDCl_3$

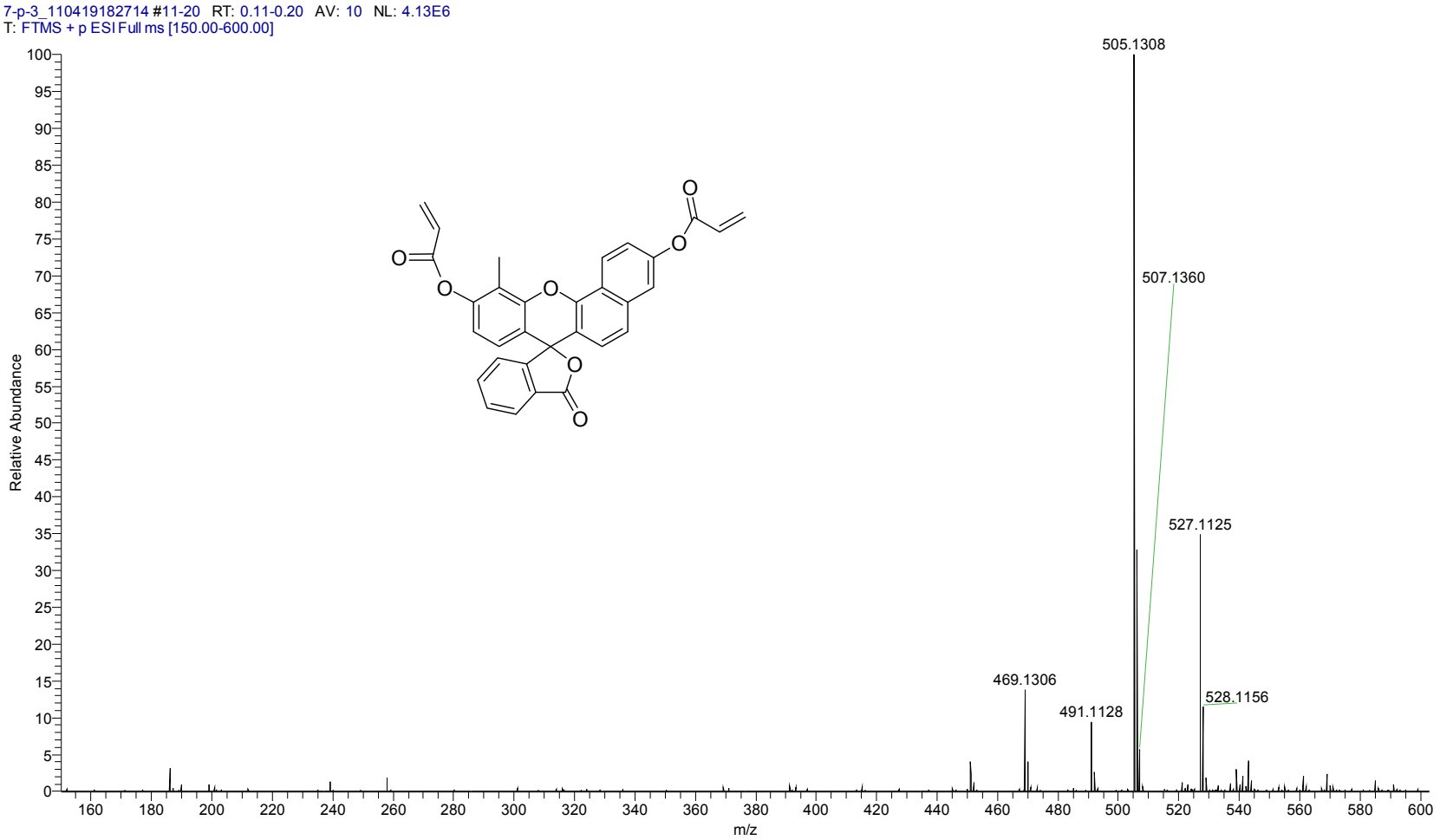



**Fig. S21**. HRMS of **4**

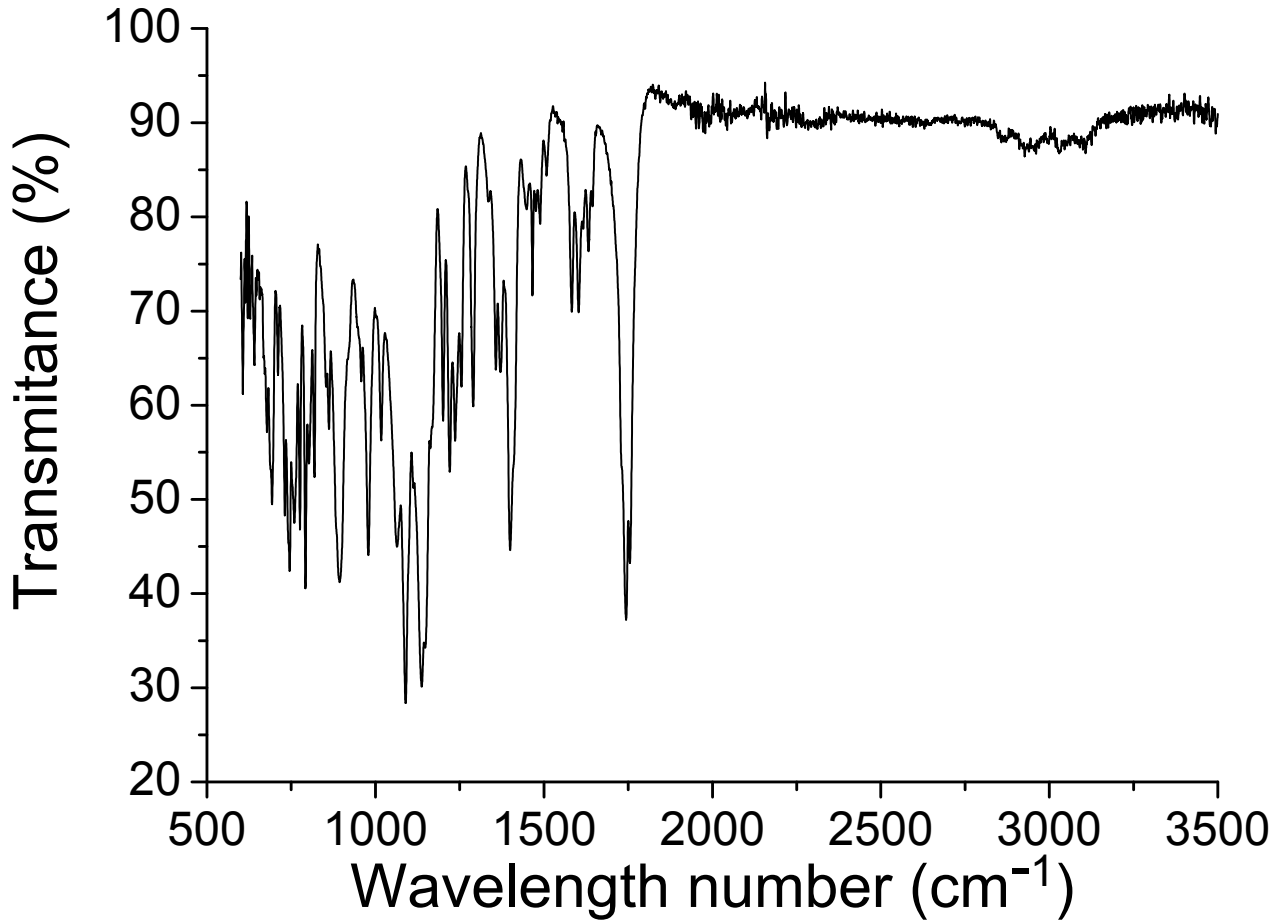



**Fig. S22.** IR Spectral of **4**

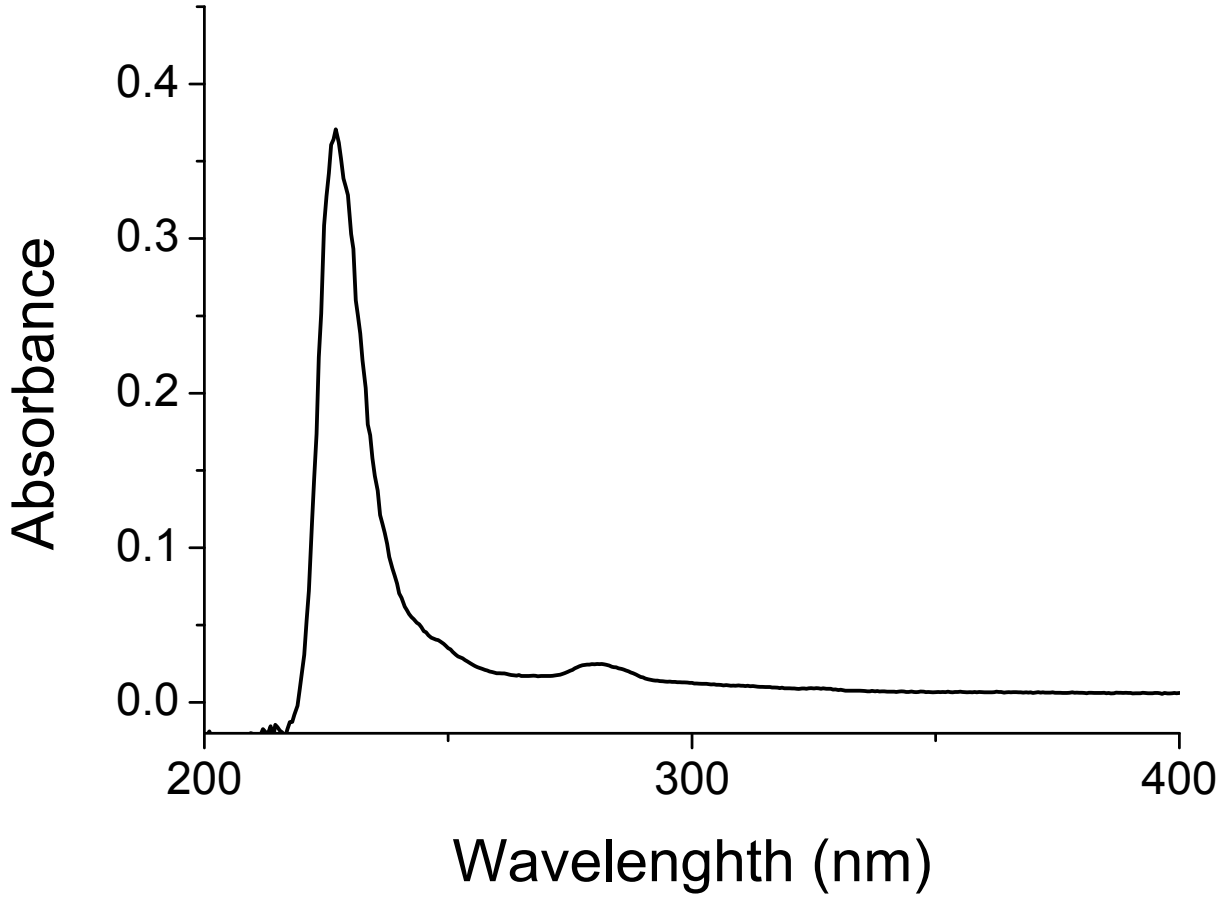



**Fig. S23.** Absorption spectra of **4** (10.0 μM) in water and ethanol (8:2, v/v) buffered at pH 7.4 (Hepes buffer, 20 mM).