Supporting Information

Chiroptical Sensing of Homocysteine

F. Yushra Thanzeel, Lily S. Zandi and Christian Wolf*

Department of Chemistry, Georgetown University, Washington, DC 20057, USA
cw27@georgetown.edu

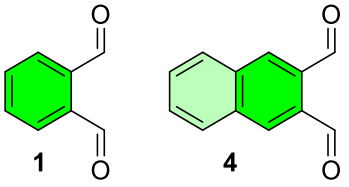
Contents:

1. General information

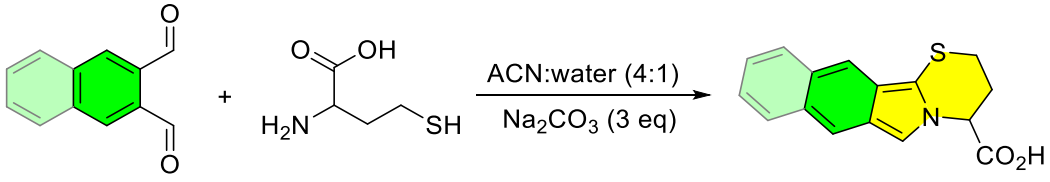
All purchased reagents were used without further purification. 2,3-Naphthalenedialdehyde was purchased from TCI America (purity ≥99%). Phthalaldehyde (purity 98%) was purchased from Ark Pharm, Inc. L-Homocysteine (purity ≥ 95%, $[\alpha]_D^{25}$ = +25 to +29º, c = 1 in 1 M HCl) and L-cysteine (purity ≥ 99%, $[\alpha]_D^{20}$ = +8.3 to +9.5º, c = 8 in 1 M HCl) were purchased from Chem-Impex Int'l Inc. D/L-Homocysteine (purity ≥95%), L-serine (purity 99%, $[\alpha]_D^{20}$ = +13.7°, c = 10 in 1 M HCl) and L-methionine (purity ≥ 98%, $[\alpha]_D^{20}$ = +23.40, c = 5 in 6 M HCl) were purchased from Sigma Aldrich. D-Cysteine (purity 98%, $[\alpha]_D^{20}$ = −7.9º, c = 5 in 5 M HCl) and L-Glutathione (purity 98%, $[\alpha]_D^{20}$ = −13.5º, c = 2 in H_2O) were purchased from Combi Blocks Inc. L-Alanine (purity ≥ 99%, $[\alpha]_D^{25}$ = –14.5° ±1.5°, c = 10 % in 6 M HCl) and L-Tyrosine (purity ≥ 99%, $[\alpha]_D^{25}$ = –10.0° ± -1.5°, c = 5% in 1 M HCl) were purchased from Novabiochem. L-Cystine (purity 98%, $[\alpha]_D^{20}$ = –222.5º (c = 1 in 1 M HCl) was purchased from TCI America.

Sensing reactions were performed with probes **1** and **4** as described below. The CD spectra were collected with a standard sensitivity of 100 mdeg, a data pitch of 0.5 nm, a bandwidth of 1 nm, in a continuous scanning mode with a scanning speed of 500 nm/min and a response of 1 s, using a quartz cuvette (1 cm path length). The data were baseline corrected and smoothed using a binomial equation. UV spectra were collected with an average scanning time of 0.0125 s, a data interval of 5.00 nm and a scan rate of 400 nm/s. TRIZMA buffer (250.0 mM) was prepared using TRIZMA HCl and TRIZMA base in distilled water. The pH was adjusted using 5 M HCl. Potassium phosphate buffer (250.0 mM) was prepared using K_2HPO_4 and KH_2PO_4 in distilled water. The pH was adjusted to 8.0 using 5 M HCl and 5 M NaOH. Sodium borate buffer (250.0 mM) was prepared using boric acid and NaOH in distilled water. The pH was adjusted to 8.5 using 5 M HCl and 5 M NaOH. Carbonate-bicarbonate buffer (250.0 mM) was prepared using $NaHCO_3$ and Na_2CO_3 in distilled water. The pH was adjusted to 10.0 using 5 M HCl and 5 M NaOH.

Figure 1. Structures of probes used in the study.



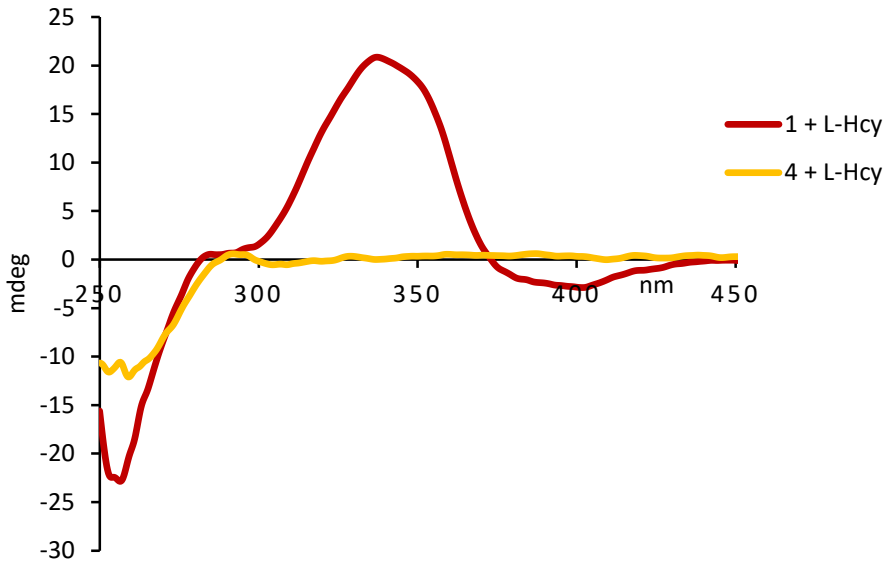
2. Probe comparison



Scheme 1. Reaction of probe **1** and **4** with homocysteine.

To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 3 equivalents of Na_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. The reaction mixture was stirred for 2 hours. CD analysis was performed after diluting 300.0 µL aliquots with 2.0 mL of acetonitrile. This procedure was repeated with probe **4** and CD analysis was performed after diluting 70.0 µL aliquot with 2.0 mL of acetonitrile.

Figure 2. CD spectra of the reaction between probe **1** or **4** with L-homocysteine.



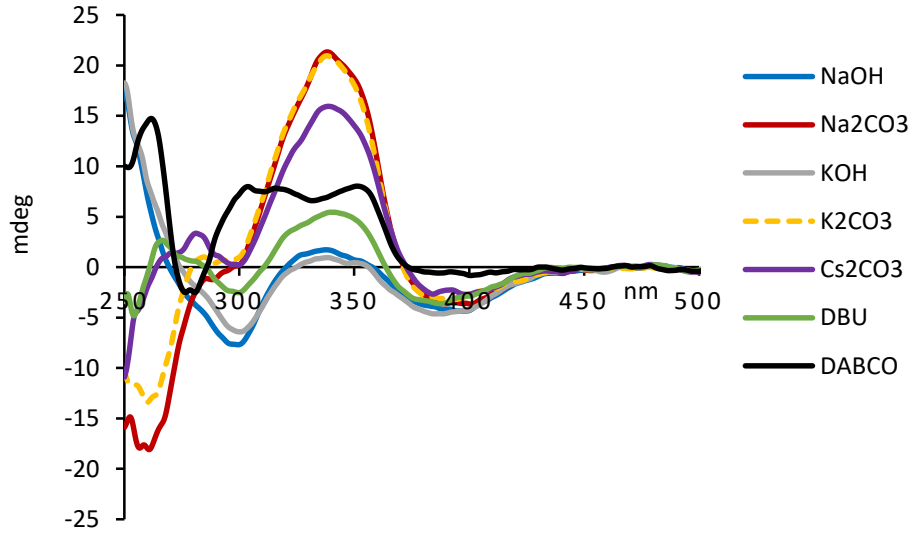
CD measurements were taken at 0.74 mM with probe **1** and at 0.17 mM with probe **4**.

3. Optimization studies

3.1. Base screening

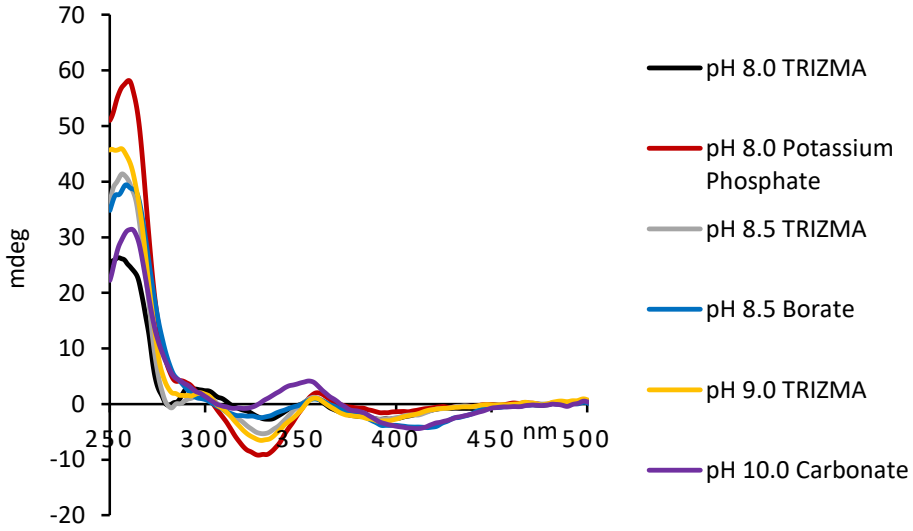
To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of Na_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. The reaction mixture was stirred for 2 hours. The above procedure was repeated with K_2CO_3, NaOH, KOH, Cs_2CO_3, DBU, DABCO and with several buffer systems: TRIZMA (pH 8.0, 8.5 and 9.0, 250.0 mM), potassium phosphate (pH 8.0, 250.0 mM), sodium borate buffer (pH 8.5, 250.0 mM) and $NaHCO_3$/Na_2CO_3 (pH 10.0, 250.0 mM). The highest CD intensities above 300 nm were observed in the presence of K_2CO_3 and Na_2CO_3.

Figure 3. CD spectra of probe **1** upon binding of L-homocysteine in the presence of different bases. CD measurements were taken by diluting 300.0 µL aliquots with 2.0 mL of acetonitrile.



CD measurements were taken at 0.74 mM.

Figure 4. CD spectra of probe **1** upon binding of L-homocysteine using different buffers. CD measurements were taken by diluting 280.0 µL aliquots with 2.0 mL of acetonitrile.

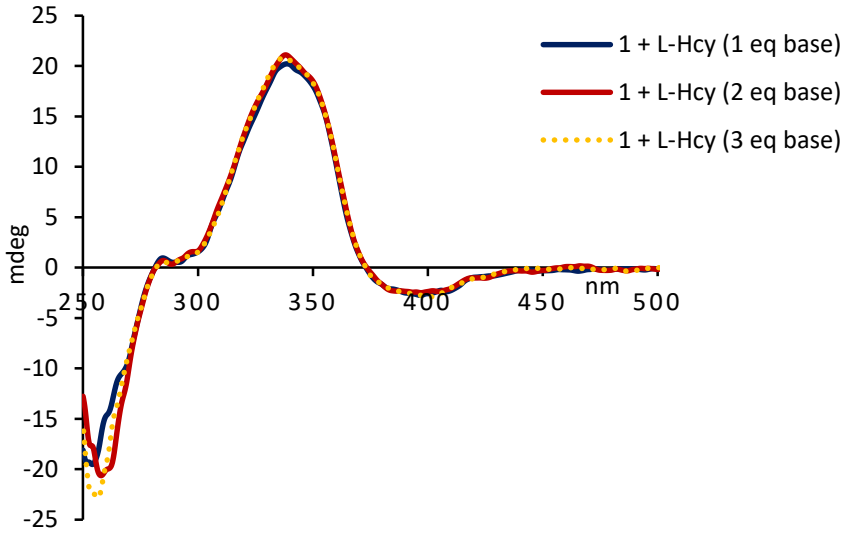


CD measurements were taken at 0.61 mM.

3.2. Base equivalents

To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 1 equivalent of Na_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. The reaction mixture was stirred for 2 hours. The above procedure was repeated with 2 and 3 equivalents Na_2CO_3. The amount of base in the reaction mixture had no effect on the CD signal. CD measurements were taken by diluting 300.0 µL aliquots with 2.0 mL of acetonitrile

Figure 5. CD spectra of probe **1** with L-homocysteine with different equivalents of Na_2CO_3 base.

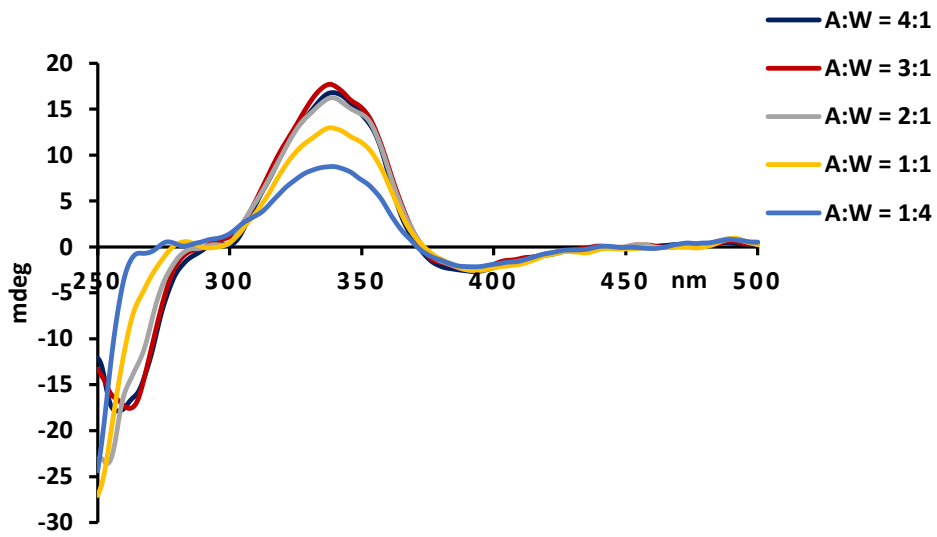


CD measurements were taken at 0.74 mM.

3.3. Solvent screening

To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile and water to obtain different ratios of water: acetonitrile (A:W). The reaction mixture was stirred for 2 hours. The highest CD intensities were observed when the acetonitrile: water ratio is between 2:1 and 4:1. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile.

Figure 6. CD spectra of probe **1** upon binding of L-homocysteine under different ratios of acetonitrile: water (A:W).



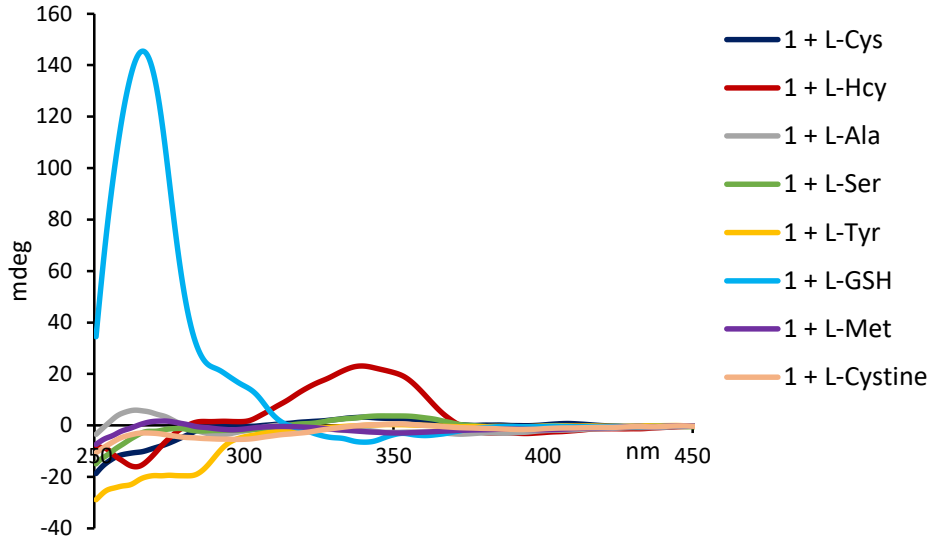
CD measurements were taken at 0.28 mM.

4. Mechanistic studies

4.1. Selectivity experiments

To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. The reaction mixture was stirred for 2 hours. This procedure was repeated with L-cysteine, L-alanine, L-serine, L-tyrosine, L-glutathione, L-methionine and L-cystine. Three equivalents of K_2CO_3 were used to ensure the L-cystine stock solution was homogeneous. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. The CD responses (Figure 7) and color of the reaction solutions after 2 hours (Figure 9) are shown below.

Figure 7. CD spectra of **1** in the presence of other amino acids and biothiols.



CD measurements were taken at 0.28 mM.

Figure 8. CD responses of probe **1** at 334 nm with other competing analytes.

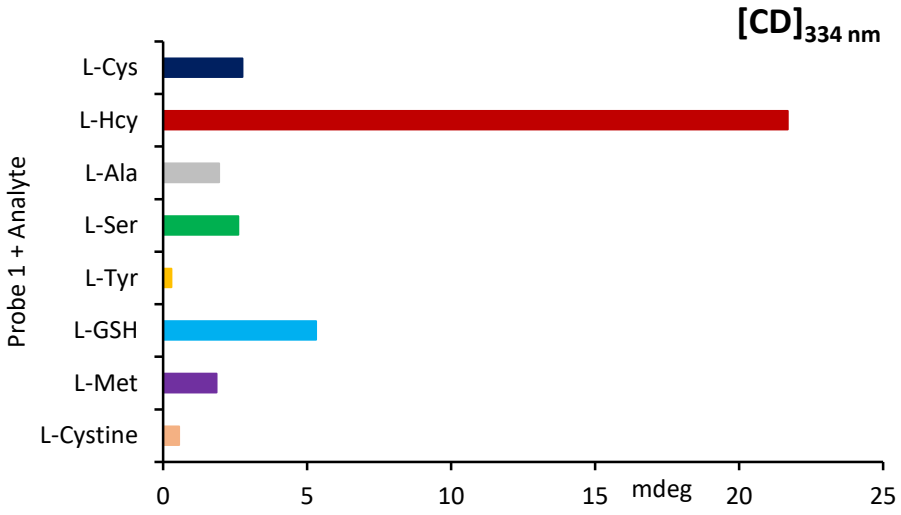
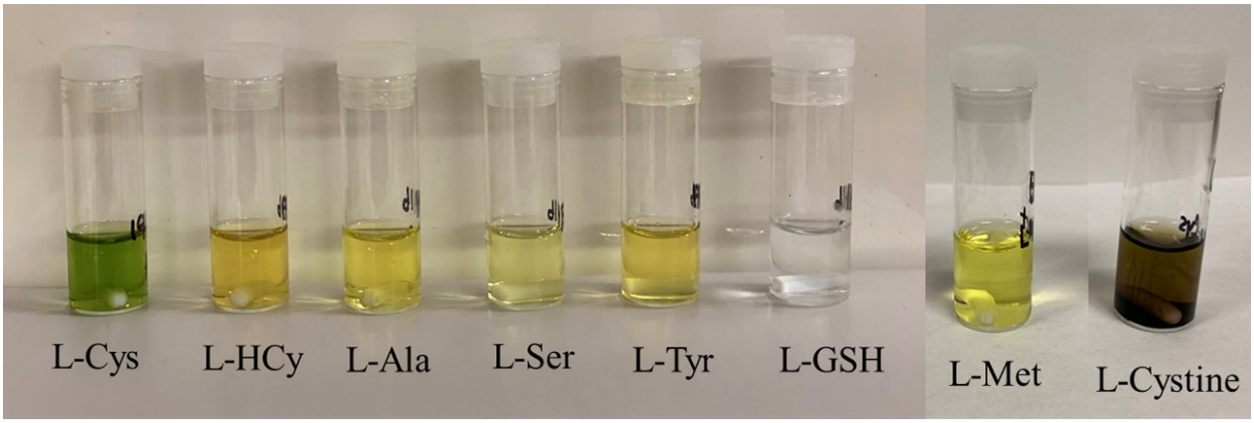


Figure 9. Naked eye detection using probe **1**.



4.2. Kinetic study & CD signal stability

The capture of L-homocysteine in water by probe **1** in the presence of K_2CO_3 was studied using CD spectroscopy. To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. The reaction was complete in 15 minutes and the CD signal was stable for at least 2 hours.

Figure 10. CD spectra at different time intervals.

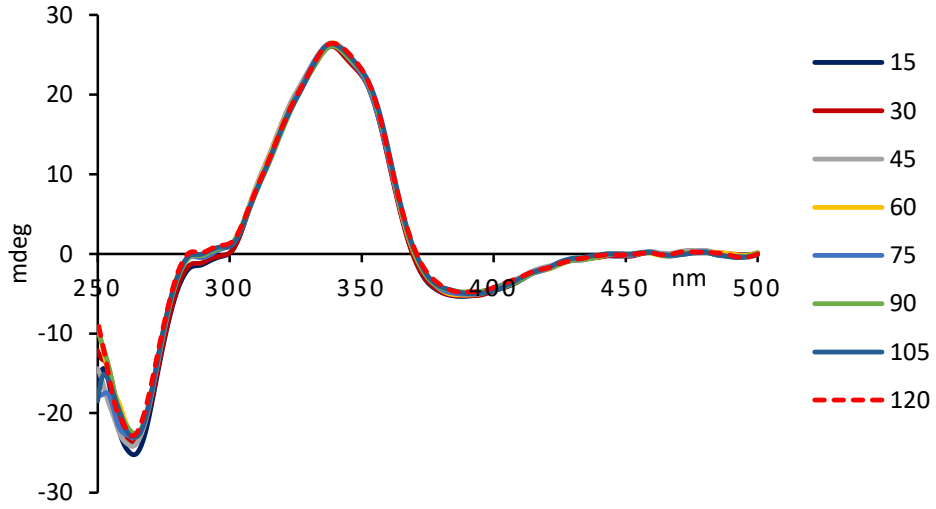
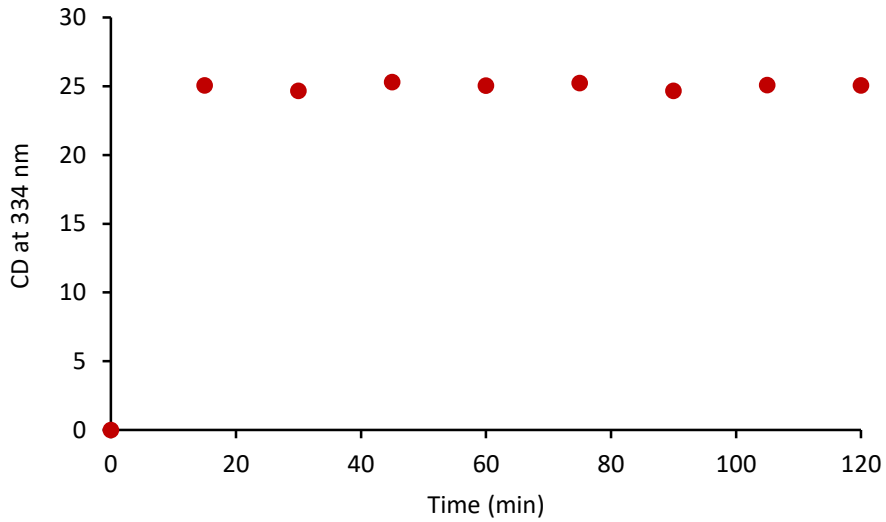
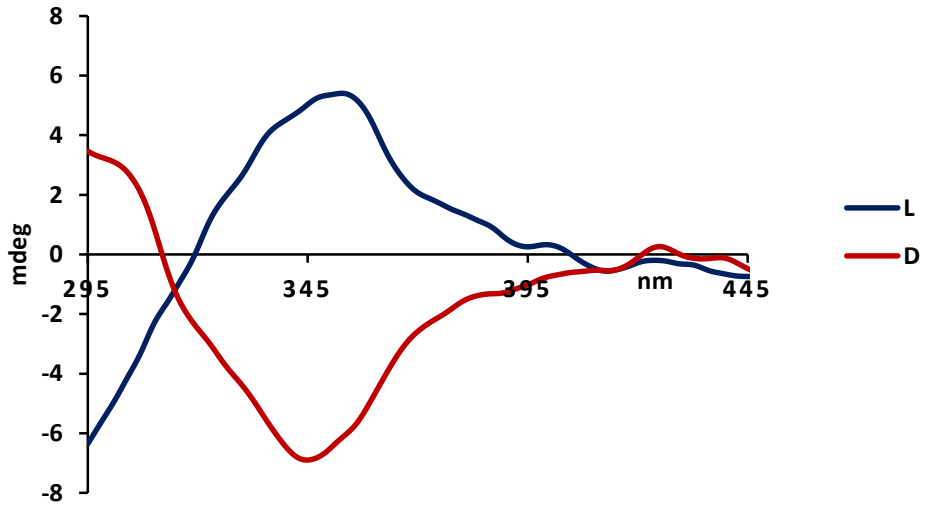


Figure 11. CD intensity at 334 nm at different time intervals.



To a solution of probe **1** (25.0 mM in acetonitrile, 880.0 µL) was added L-cysteine (50.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 200.0 µL) and the mixture was diluted with water (200.0 µL) and acetonitrile (720.0 µL). After 2 hours, CD measurements were taken by diluting 240.0 µL aliquots with 2.0 mL of acetonitrile. The above procedure was repeated with D-cysteine. The results show that the formation of a CD active species with cysteine is considerably slower compared to homocysteine.

Figure 12. CD sensing of cysteine enantiomers with **1** after 2 hours.



4.3. MS analysis

The reaction of L-homocysteine and probe **1** was studied using ESI-MS spectroscopy. To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of NaOH, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. After 2 hours, the reaction mixture was acidified with formic acid (1.0 M, 20.0 µL) and diluted to 4.0 mL using acetonitrile:water (1:1). A 10.0 µL aliquot of this mixture was diluted with 2.0 mL of water for ESI-MS analysis and infused at a flow rate of 0.1 mL/min using a syringe pump. The voltages of the capillary and needle were set at 55 and 5000 V, respectively. The above procedure was repeated with L-cysteine.

Figure 13. ESI-MS spectrum of the reaction between L-homocysteine and probe **1** (positive ion mode).

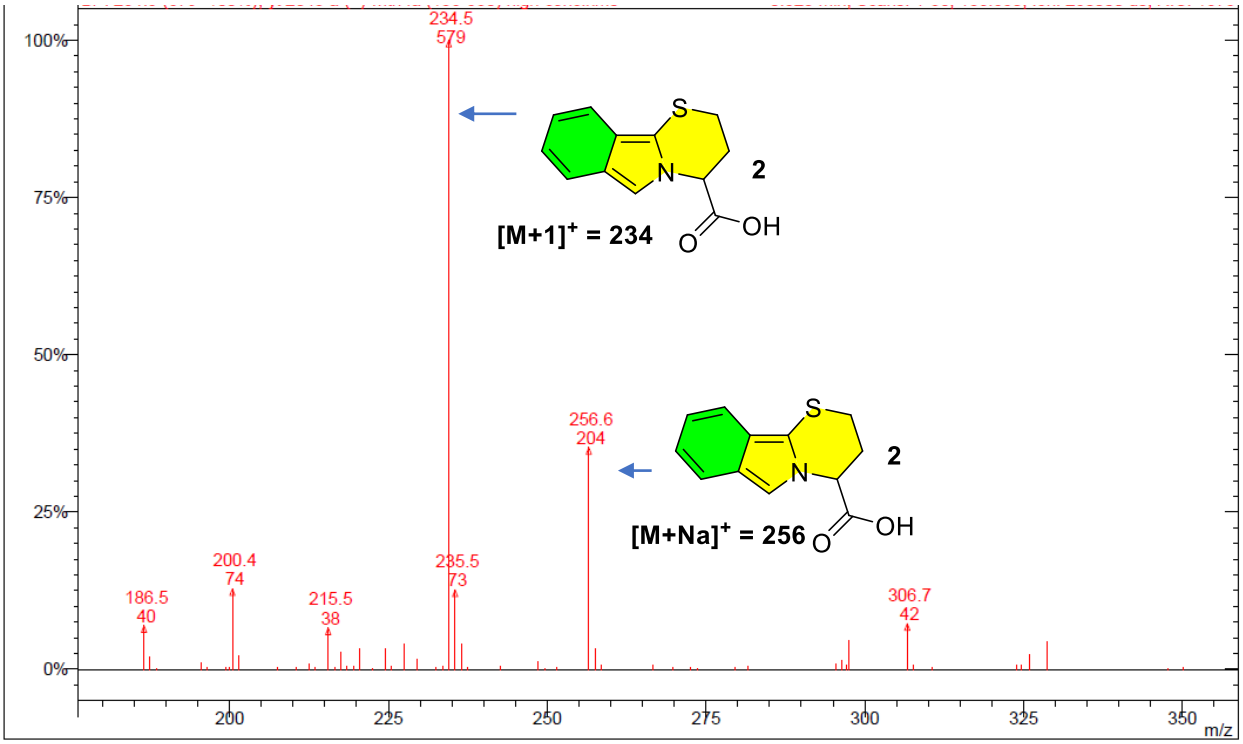
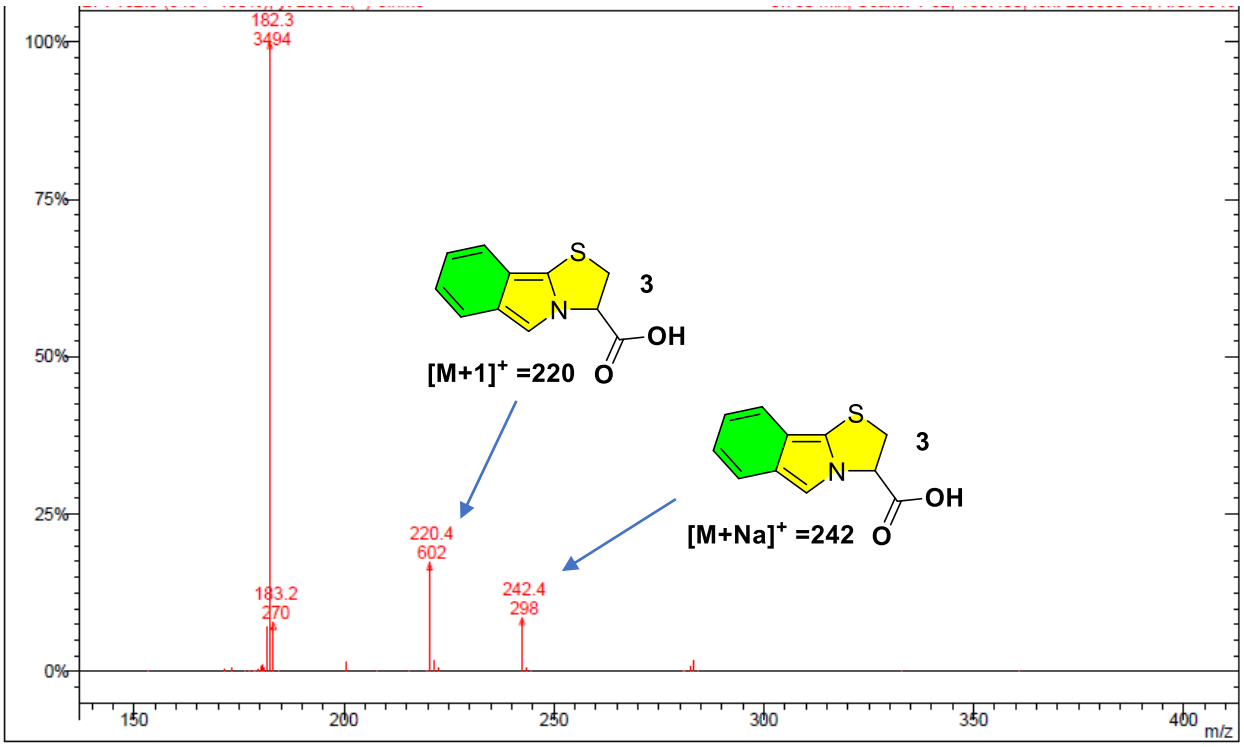


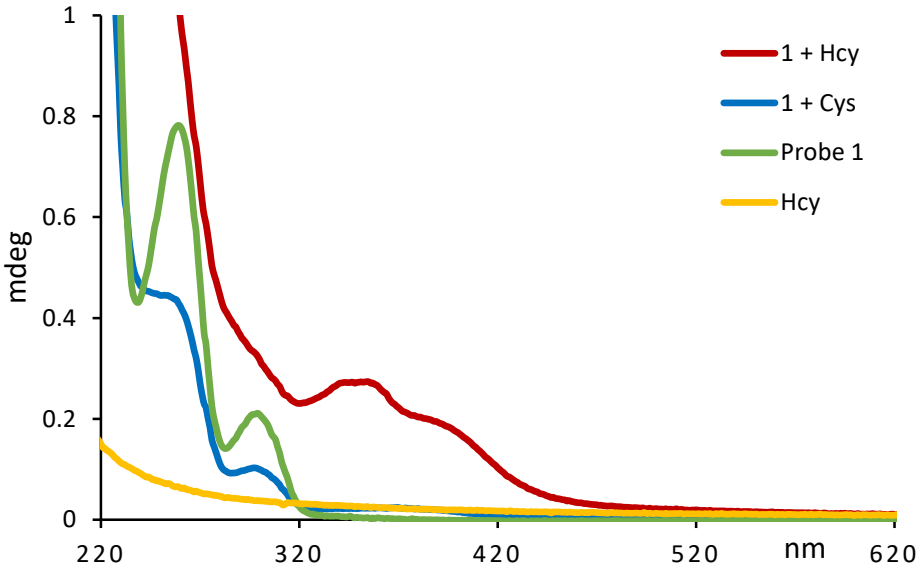
Figure 14. ESI-MS spectrum of the reaction between L-cysteine and probe **1** (positive ion mode).



4.4. UV analysis

The reactions of L-homocysteine and L-cysteine with probe **1** was studied using UV spectroscopy. To a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) was added L-homocysteine (25.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 400.0 µL) and the mixture was diluted to 2.0 mL with acetonitrile. UV measurements were taken by diluting 40.0 µL aliquots with 2.0 mL of acetonitrile.

Figure 15. UV analysis of the reactions between probe **1** and biothiols.



UV measurements were taken at 0.10 mM.

The change in the UV absorbance of probe **1** upon varying concentrations of homocysteine was studied. To a solution of probe **1** (25.0 mM in acetonitrile, 880.0 µL, 10.0 mM) was added homocysteine (25.0 mM aqueous solution containing 2 equivalents of K_2CO_3) in varying concentrations (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 mM) and the mixture was diluted to 2.0 mL with acetonitrile:water (4:1). The reactions were stirred overnight, and UV measurements were taken by diluting 50.0 µL aliquots with 2.0 mL of acetonitrile.

Figure 16. UV analysis of the reactions between probe **1** and increasing concentration of homocysteine

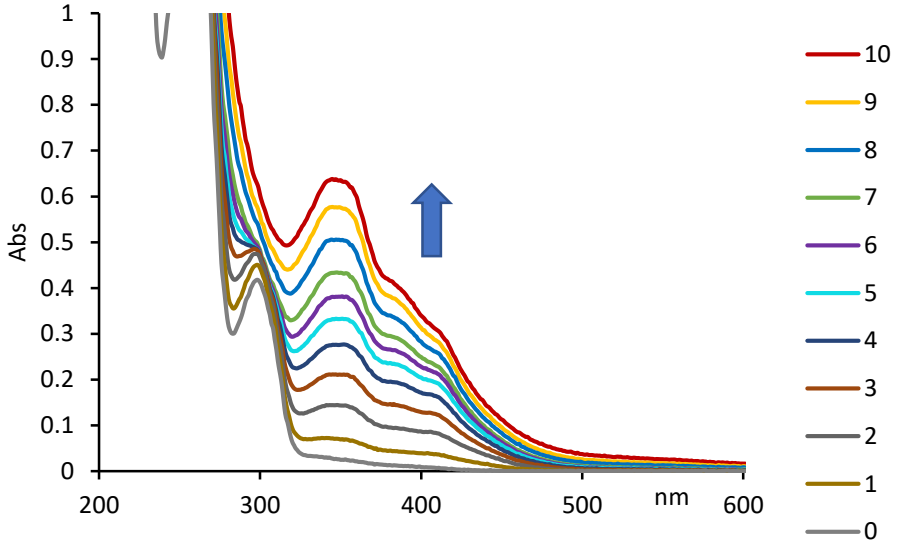
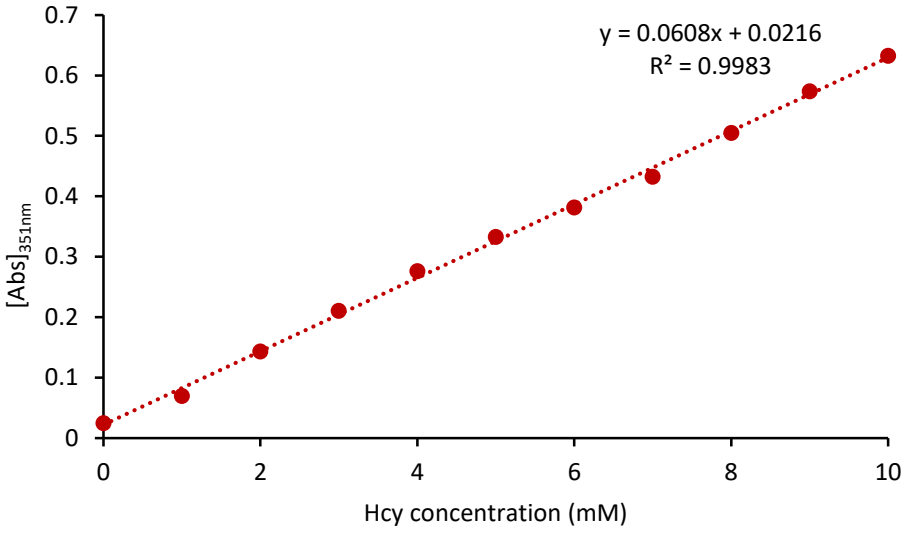
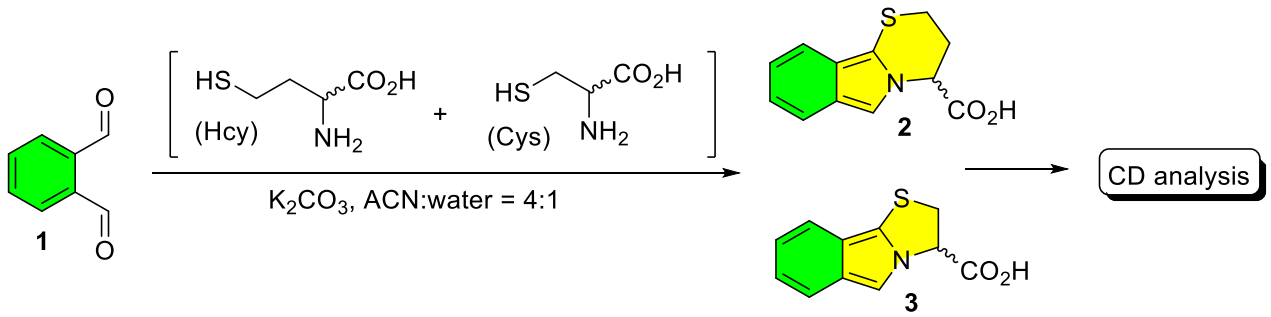


Figure 17. Plot of UV intensity at 351 nm versus the concentration of homocysteine.



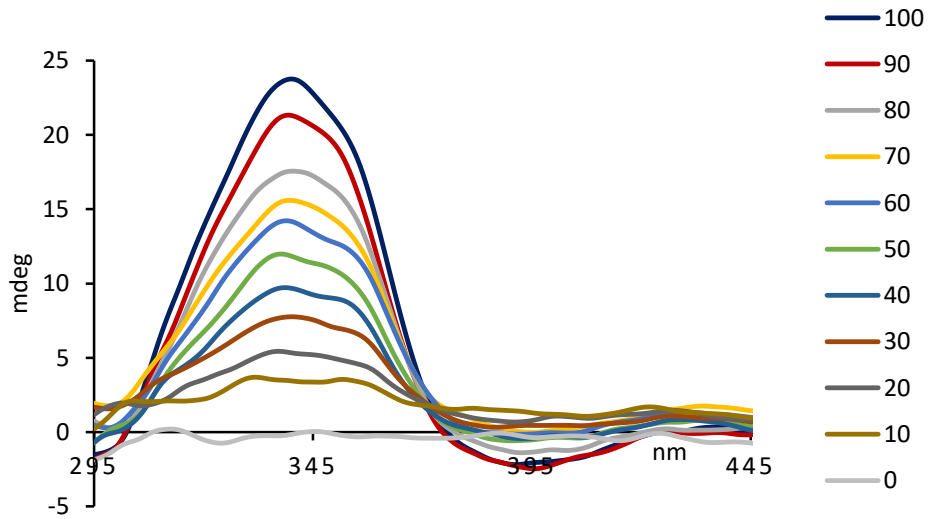
5. Quantification of enantiomeric excess of homocysteine in the presence of cysteine



Scheme 2. Quantification of enantiomeric excess of homocysteine in the presence of cysteine using probe **1**.

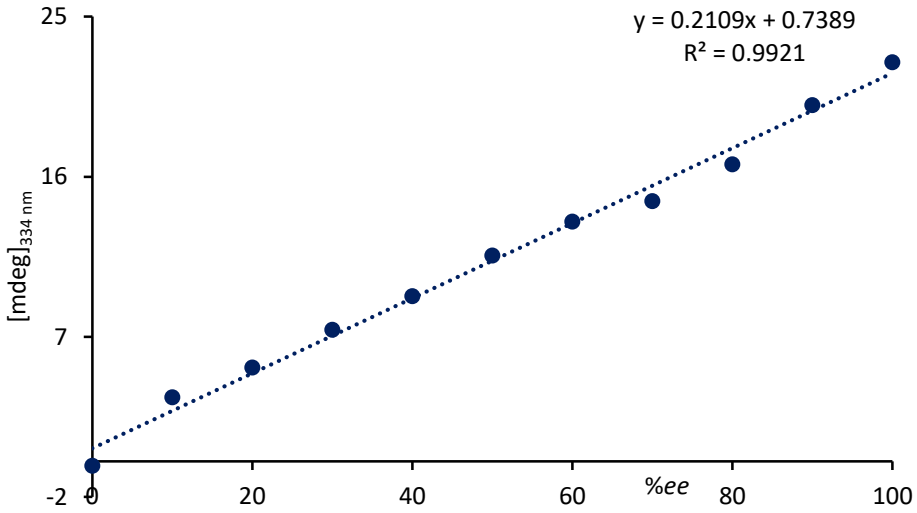
A calibration curve was constructed using samples containing homocysteine with varying enantiomeric composition. To a solution of probe **1** (25.0 mM in acetonitrile, 880.0 µL) was added homocysteine (50.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 200.0 µL) with varying *ee*'s (+100, +90, +80, +70, +60, +50, +40, +30, +20, +10, 0%) and the mixture was diluted with water (200.0 µL) and acetonitrile (720.0 µL). After 15 minutes, CD measurements were taken by diluting 200.0 µL aliquots with 2.0 mL of acetonitrile. The CD amplitudes at 334.0 nm were plotted against the enantiomeric excess of homocysteine.

Figure 18. Chiroptical response of probe **1** to scalemic samples of homocysteine.



CD measurements were taken at 0.50 mM.

Figure 19. Plot of the CD amplitudes at 334 versus sample *ee*.



Ten scalemic samples of homocysteine (50.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 200.0 µL) at varying enantiomeric ratios were prepared in the presence of L-cysteine (50.0 mM aqueous solution containing 2 equivalents of K_2CO_3, 200.0 µL) and the mixture was added to a solution of probe **1** (25.0 mM in acetonitrile, 880.0 µL) and diluted to 2.0 mL using acetonitrile. After 15 minutes, CD measurements were taken by diluting 200.0 µL aliquots with 2.0 mL of acetonitrile. The CD responses at 334 nm were used to determine the %*ee* of homocysteine in the presence of cysteine.

Table 1. Sensing of the %*ee* of L-homocysteine in the presence of cysteine.

Entry	**Sample composition (%)**	**Sensing results (%)**
1	95.0	98.8
2	85.0	87.0
3	75.0	79.0
4	65.0	71.5
5	55.0	52.2
6	45.0	42.6
7	35.0	38.3
8	25.0	23.1
9	15.0	18.0
10	5.0	5.2