Supplementary Information

Selective Chiroptical Sensing of D/L-Cysteine Mixtures

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Contents:

1. General information

All purchased reagents were used without further purification. 2-Bromo-2'-nitroacetophenone (purity 98%) was purchased from VWR. L-Cysteine (purity ≥ 99%, $[\alpha]_D^{20}$ = +8.3 to +9.5º, c = 8 in 1 M HCl) and L-homocysteine (purity ≥ 95%, $[\alpha]_D^{25}$ = +25 to +29º, c = 1 in 1 M HCl) were purchased from Chem-Impex Int'l Inc. L-Serine (purity 99%, $[\alpha]_D^{20}$ = +13.7°, c = 10 in 1 M HCl) and L-cysteine methyl ester hydrochloride (purity 98%, $[\alpha]_D^{20}$ = −1.8º, c = 10 in methanol) 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.

The sensing reactions were carried out with six different 2-bromoacetophenones. For comparison of these chiroptical probes, CD spectra were collected with 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. For quantitative determinations of the enantiomeric composition of cysteine samples, CD measurements were taken in duplicate as an average of 3 runs each with a scanning speed of 50 nm/min. The UV spectra were taken in duplicate.

2. Probe comparison

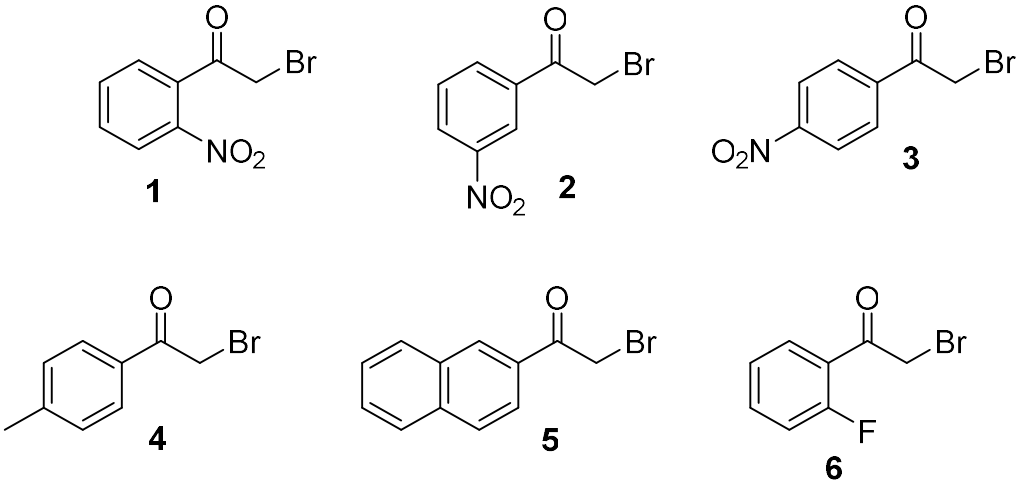


Figure 1. Structures of probes used in this study.

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. The procedure described above was repeated with probes **2-6**. Probe **1** showed the strongest CD signal upon cysteine binding in the buffered solutions and was used in all subsequent reactions.

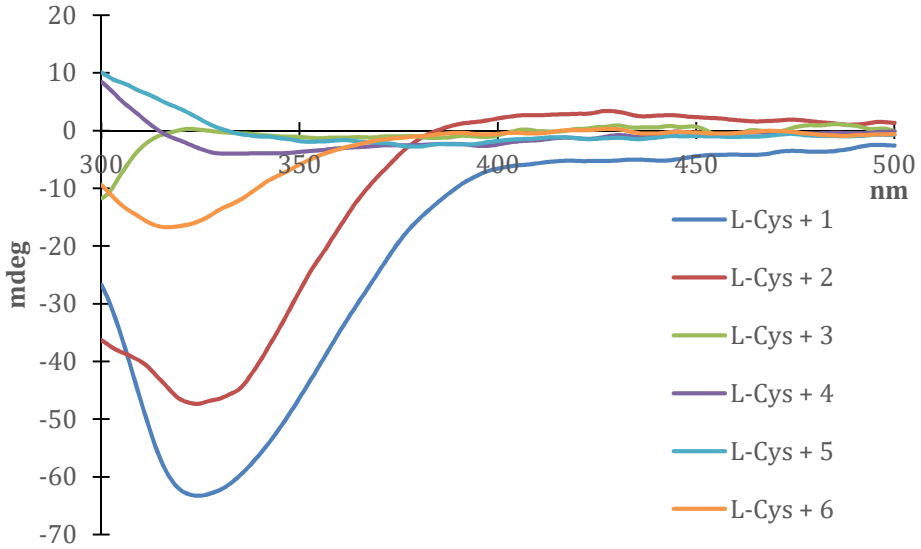


Figure 2. CD spectra of the reaction between L-cysteine and the different probes. CD measurements were taken at 0.28 mM.

3. Optimization studies

3.1. Initial testing of cysteine and homocysteine

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. The procedure described above was repeated with L-homocysteine. Probe **1** showed a strong CD signal upon cysteine binding whereas the reaction mixture with homocysteine remained CD silent above 300 nm.

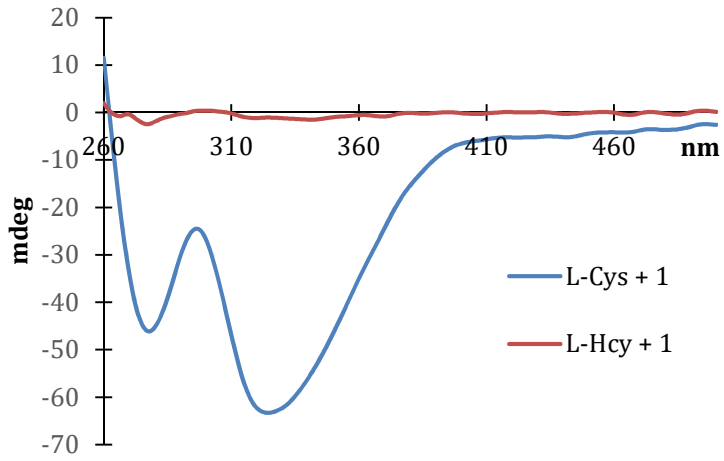


Figure 3. CD sensing results with cysteine and homocysteine. Measurements were taken at a concentration of 0.28 mM.

3.2. Base and buffer screening

A solution of D-cysteine (25.0 mM in water, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL) followed by 2 equivalents of K_2CO_3 (1.0 M, 20.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 2 hours. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. The procedure described above was repeated with Na_2CO_3, NaOH, KOH, $NaHCO_3$ and Cs_2CO_3 as base.

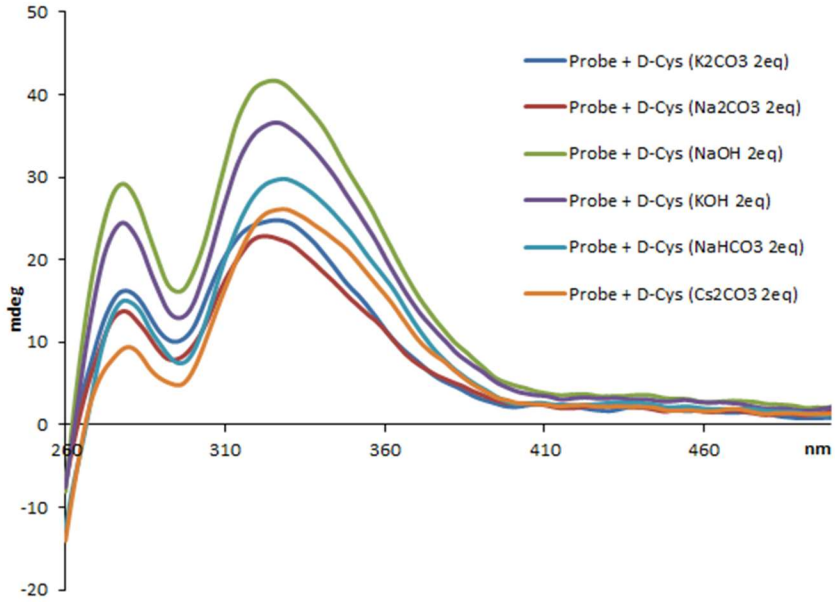


Figure 4. CD spectra of the reaction mixtures of the probe with D-cysteine in the presence of 2 equivalents of base. CD measurements were taken at a concentration of 0.28 mM.

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile. This procedure was repeated with potassium phosphate buffers at pH 6.0, 7.0 and 8.0. Probe **1** showed the strongest CD signal upon cysteine binding in the pH 8.5 buffer solution.

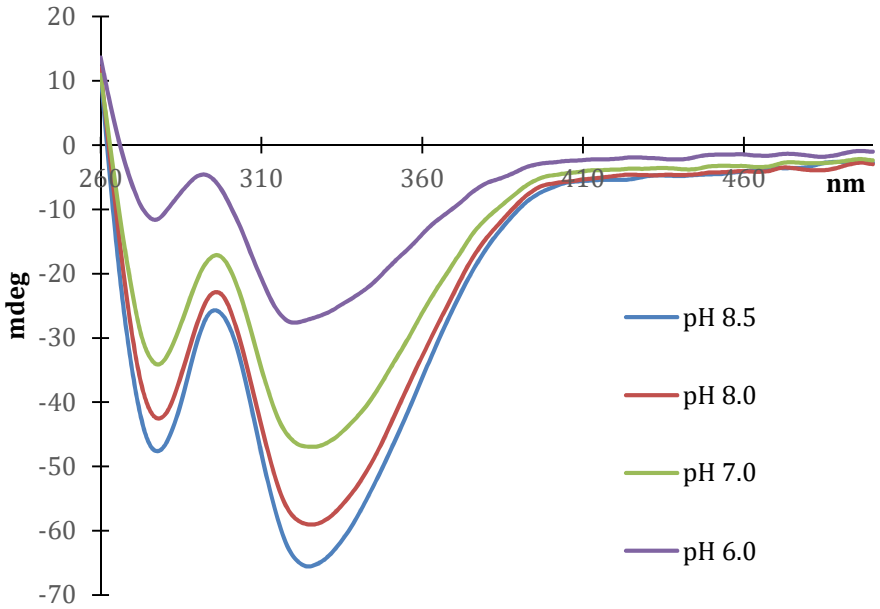


Figure 5. CD spectra of the reaction mixtures of the probe with L-cysteine in buffered solutions of varying pH. CD measurements were taken at 0.28 mM.

3.3. Reaction concentration

A stock solution of L-cysteine (12.50 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (12.5 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile to generate a 2.50 mM reaction solution and stirred for 50 minutes with heating at 70 °C. CD measurements were taken by diluting 60.0 µL aliquots with 2.0 mL of acetonitrile to generate a 2.50 mM reaction solution. This protocol was repeated with 6.25 mM, 2.50 mM and 1.25 mM cysteine and probe stock solutions to prepare 1.25 mM, 0.50 mM and 0.25 mM reaction solutions. These mixtures were stirred for 1 hour, 1.5 hours and 2.25 hours, respectively. CD measurements were taken by diluting 124.0 µL aliquots with 2.0 mL of acetonitrile, 342.0 µL aliquots with 2.0 mL of acetonitrile and 825.0 µL aliquots with 2.0 mL of acetonitrile, respectively.

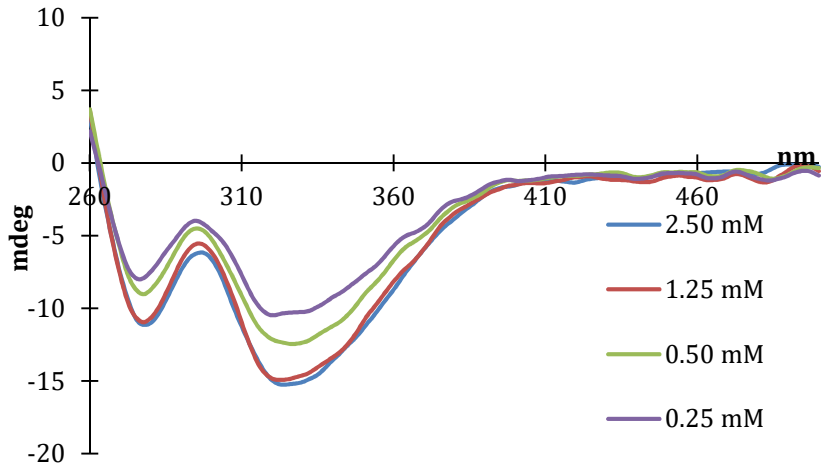


Figure 6. CD signals obtained with different reaction concentrations. CD measurements were taken at a concentration of 0.07 mM.

4. Mechanistic studies

4.1. Selectivity experiments

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 2 hours. CD measurements were taken by diluting 100 µL aliquots with 2.0 mL of acetonitrile. This procedure was repeated with L-homocysteine, L-alanine, L-serine, L-tyrosine and L-glutathione.

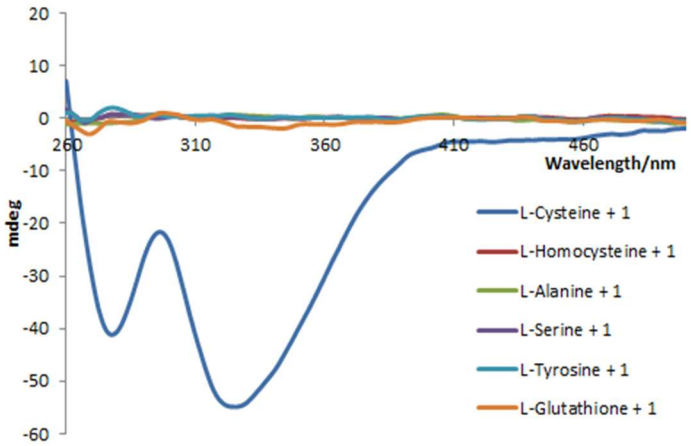


Figure 7. CD spectra of reaction mixtures of the probe with different substrates in potassium phosphate buffer (pH 8.0) after 2 hours of stirring. CD spectra were obtained at 0.24 mM.

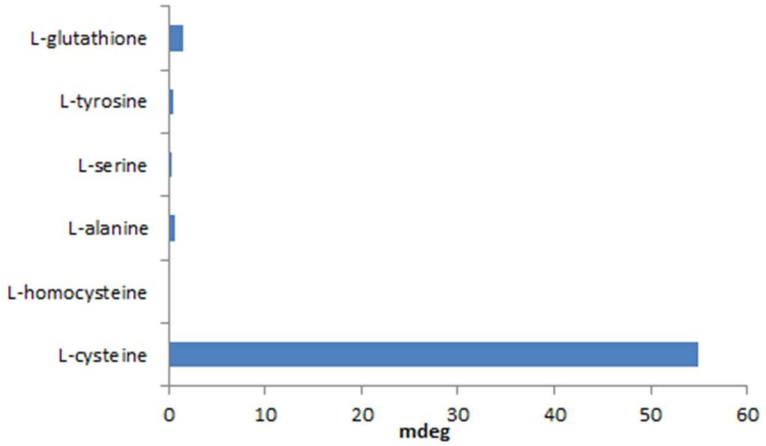


Figure 8. CD amplitude of the probe with different analytes at 326 nm. The CD spectra were obtained at 0.24 mM.

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken after diluting 120.0 µL aliquots of the reaction mixture with 2.0 mL of acetonitrile. The same procedure was repeated for the L-cysteine-glycine.

A stock solution of L-cysteine methyl ester hydrochloride (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M) with one equivalent of triethylamine was prepared. An aliquot of 400.0 µL of this stock solution was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken by diluting 120.0 µL aliquots with 2.0 mL of acetonitrile.

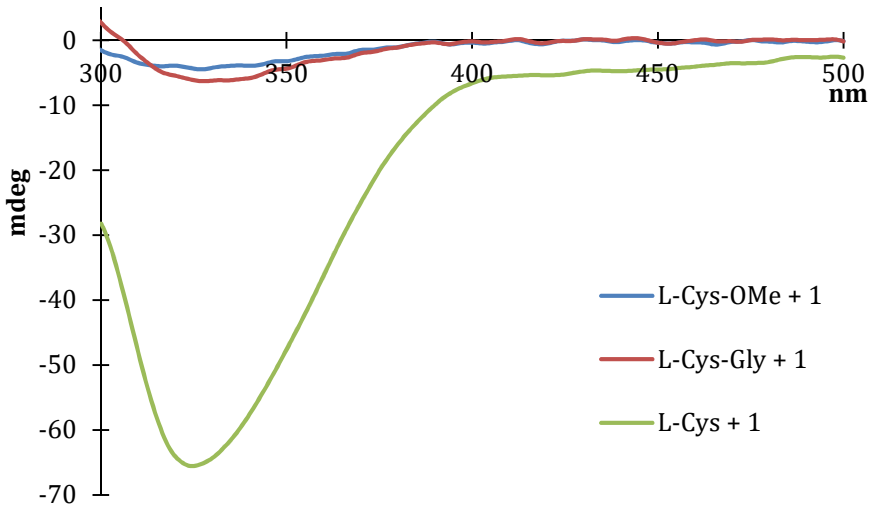


Figure 9. CD spectra of reaction mixtures of the probe with L-Cys, L-Cys-OMe and L-CysGly. The CD spectra were obtained at a concentration of 0.28 mM.

4.2. Competition study

A solution containing L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) and L-homocysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to **1** (25.0 mM in acetonitrile, 880.0 µL). The mixture was diluted to 6.0 mL with acetonitrile and stirred for 40 minutes. CD measurements were taken by diluting 180.0 µL aliquots with 2.0 mL of acetonitrile. This procedure was repeated for mixtures of L-cysteine and L-serine, L-alanine, L-tyrosine, and L-glutathione.

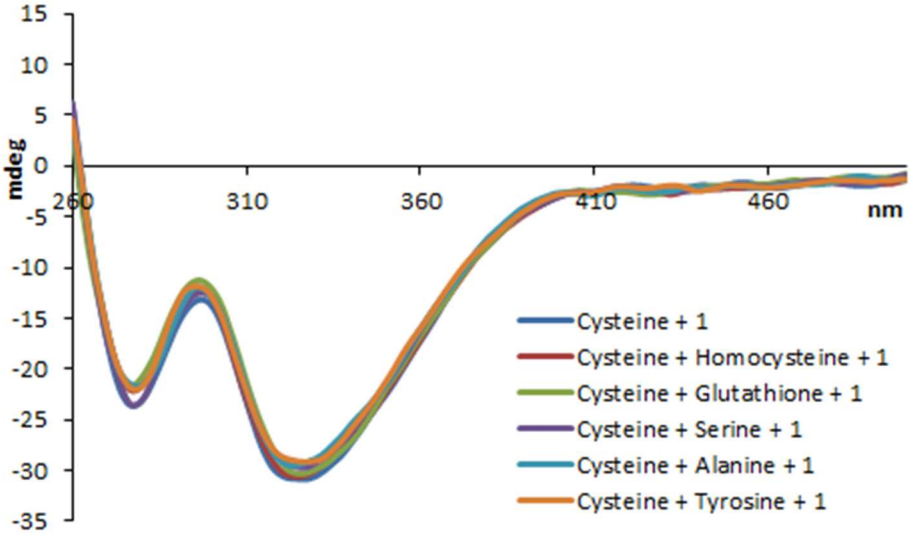


Figure 10. CD signals of mixtures of cysteine with other analytes in the presence of **1**. CD measurements were taken at a concentration of 0.14 mM.

4.3. Kinetic study

The reaction of L-cysteine with **1** was monitored using CD spectroscopy. A solution of L-cysteine (25.0 mM in pH 8.0 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred. CD measurements were taken at 10 minute intervals by diluting 120 µL aliquots with 2.0 mL of acetonitrile. The optimum CD signal was reached after 30 minutes of stirring.

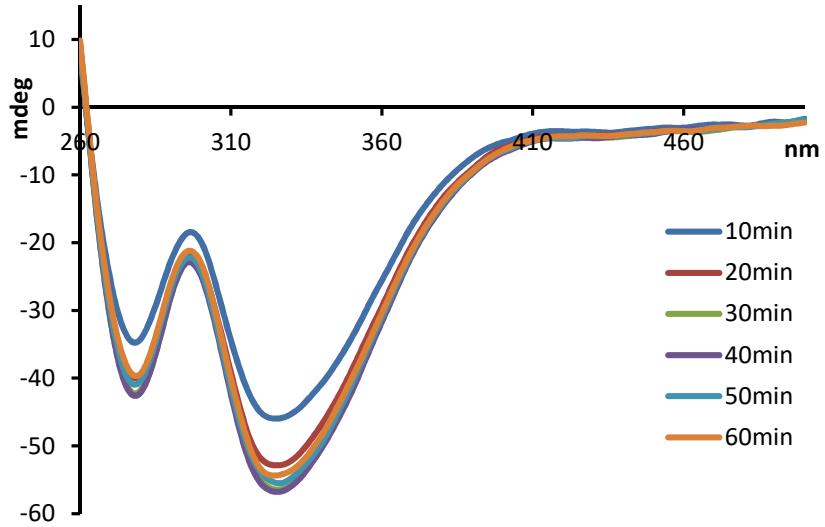
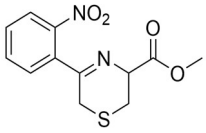


Figure 11. CD spectra of probe **1** with L-cysteine over different time intervals. CD measurements were taken at a concentration of 0.28 mM.

4.4. HRMS analysis

A solution containing L-cysteine methyl ester hydrochloride (25.0 mM in acetonitrile, 1.20 mL) was added to a solution of **1** (25.0 mM in acetonitrile, 400.0 µL) followed by 168.0 uL of triethylamine. The mixture was diluted to 2.0 mL with acetonitrile and stirred for 2 hours with heating at 70°C. A liquid-liquid extraction with water and dichloromethane was performed and the combined organic layers were concentrated under vacuum. HRMS (ESI-TOF) m/z: [M+Na]+ calculated for $C_{12}H_{11}N_2O_4S$ 303.0416, found 303.0411.



2

m/z = 280.0518

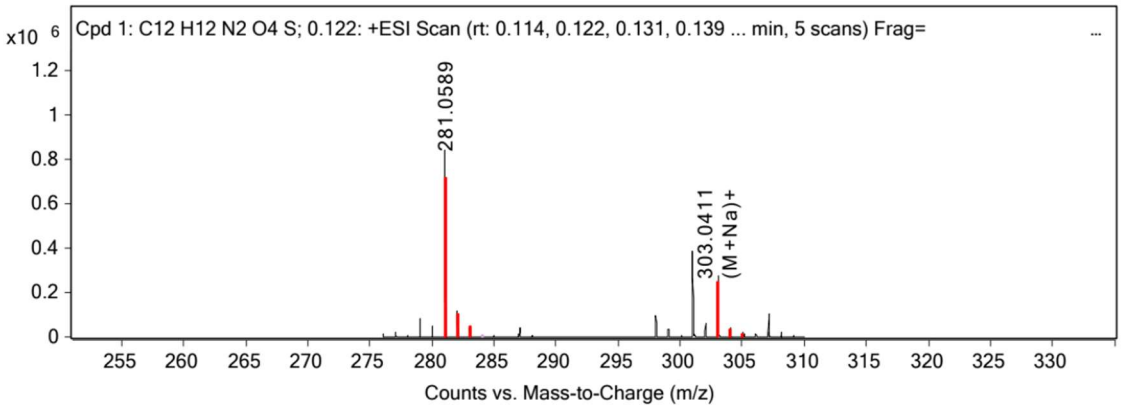
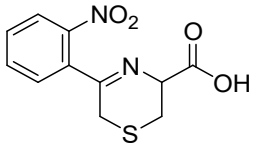


Figure 12. HRMS spectrum of the reaction between L-cysteine methyl ester hydrochloride and **1**.

A solution of L-cysteine (25.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) was added to a solution of **1** (25.0 mM in acetonitrile, 440.0 µL). The mixture was diluted to 2.0 mL with acetonitrile and stirred for 2 hours with heating at 70°C. The product was concentrated under vacuum. HRMS (ESI-TOF) m/z: [M+Na]+ calculated for $C_{11}H_{10}N_2O_4S$ 289.0259, found 289.0252.



m/z= 266.0361

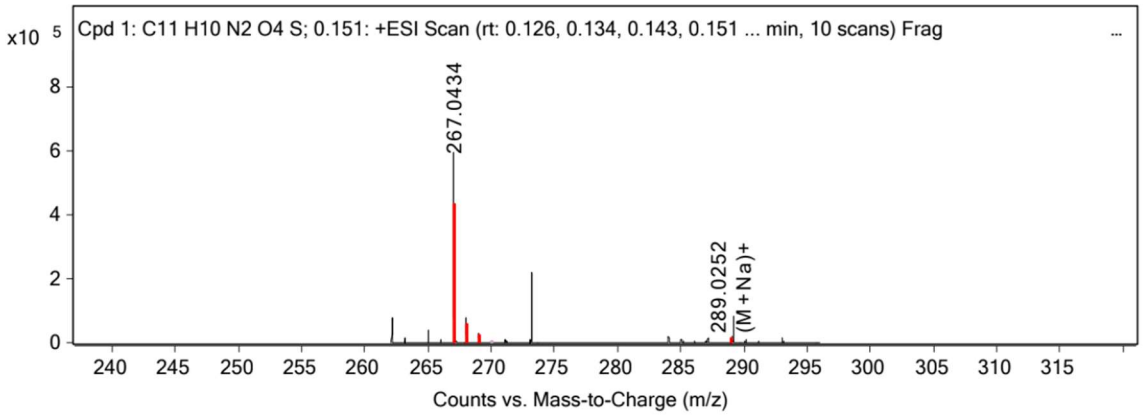


Figure 13. HRMS spectrum of the reaction between L-cysteine and **1**.

5. Quantitative UV and CD analysis

The change in the UV absorbance of **1** (10.0 mM) upon reaction with cysteine present in varying concentrations (0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mM) was investigated. Varying aliquots (400 µL, 320 µL, 240 µL, 160 µL, 80 µL and 0 µL) of a cysteine solution (50 mM, pH 8.5 potassium phosphate buffer, 0.25 M,) and 400 µL of a solution of **1** (50.0 mM in acetonitrile) were combined and diluted to 2.0 mL with acetonitrile. After 40 minutes, UV analysis was performed with 30.0 µL of the reaction mixtures after dilution with 6.0 mL of acetonitrile.

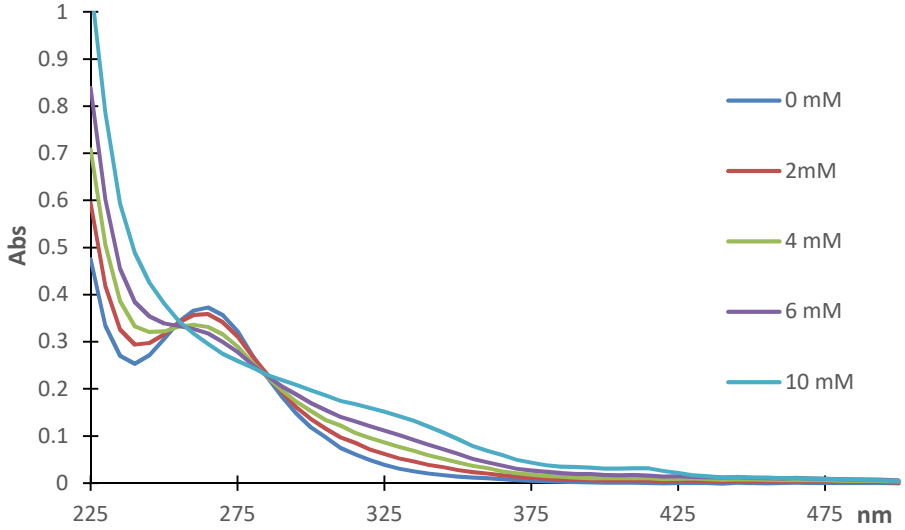


Figure 14. UV analysis of the reactions between probe **1** and increasing concentration of cysteine.

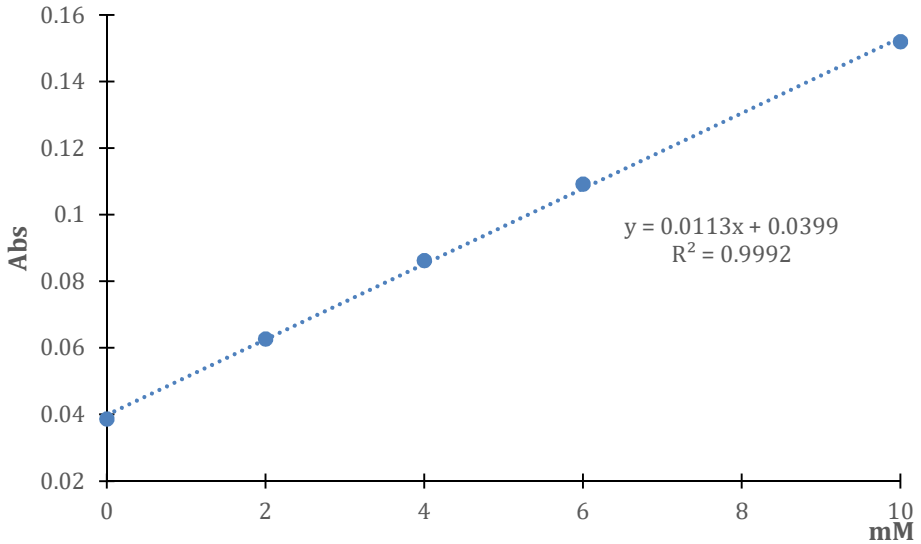


Figure 15. Plot of UV intensity at 325 nm versus the concentration of cysteine.

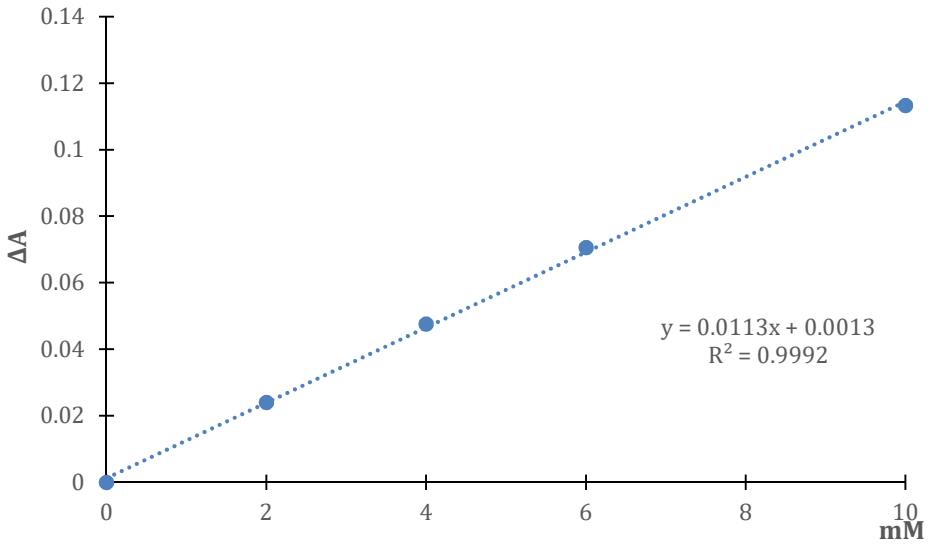


Figure 16. Plot of the difference between the UV intensity of the reaction product and the free probe at 325 nm versus the concentration of cysteine.

A calibration curve was constructed using samples containing cysteine with varying enantiomeric composition. A solution of cysteine (50.0 mM in pH 8.5 potassium phosphate buffer, 0.25 M, 400.0 µL) with varying *ee*'s (+100, +80, +60, +40, +20, 0, -20, -40, -60, -80, -100%) was added to a solution of **1** (50.0 mM in acetonitrile, 440.0 µL). The mixtures were diluted to 2.0 mL with acetonitrile and stirred for 40 minutes. An aliquot of 360.0 µL of the reaction mixture was then diluted with 6.0 mL of acetonitrile for CD analysis. The CD amplitudes at 326.0 nm were plotted against the enantiomeric excess of cysteine.

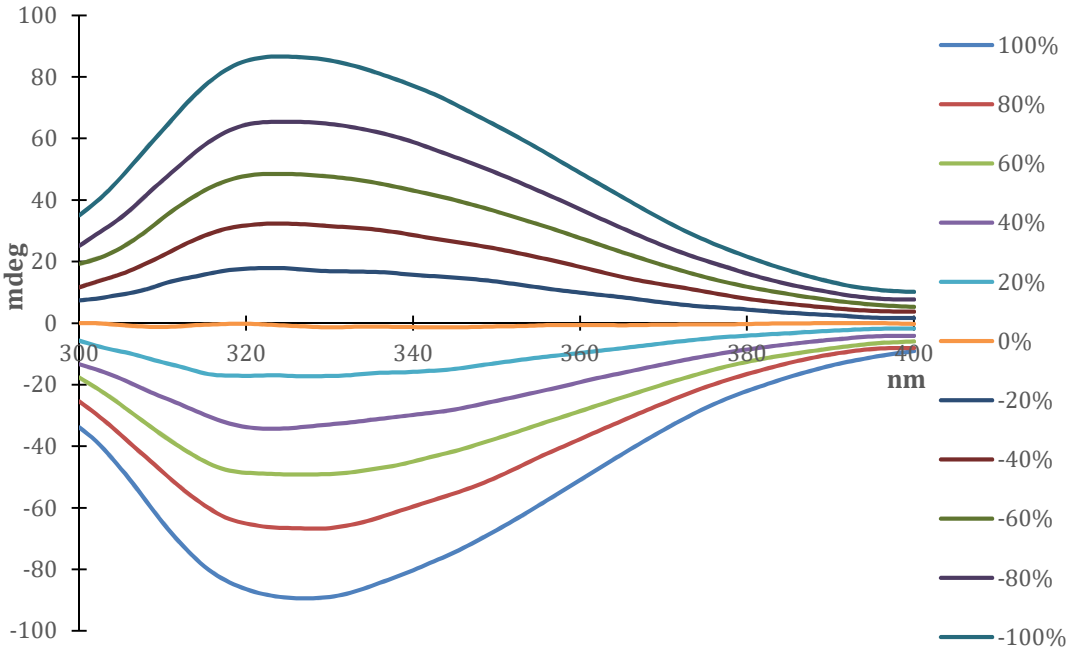


Figure 17. Chiroptical response of **1** to scalemic samples of cysteine. CD measurements were taken at 0.57 mM. The "–" sign indicates that D-cysteine was in excess.

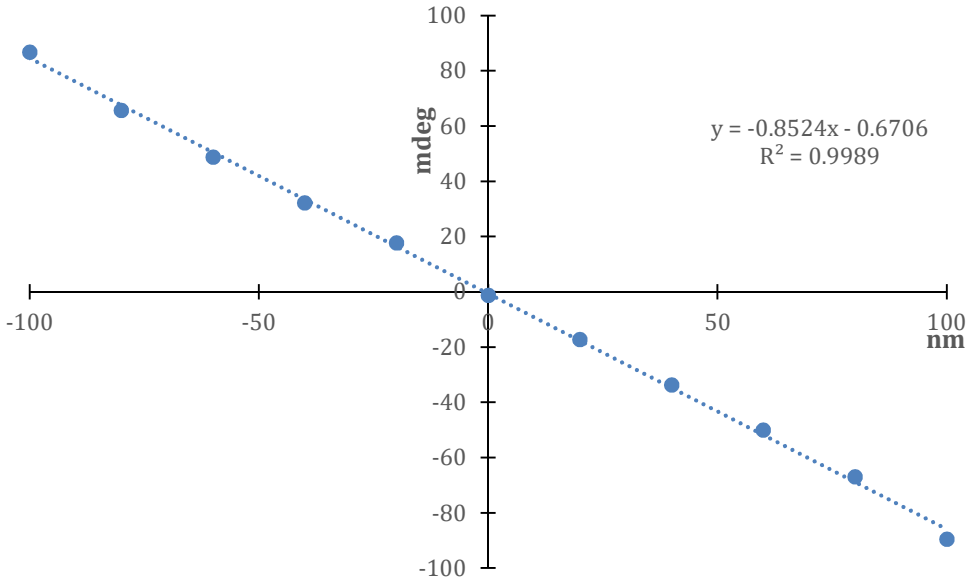


Figure 18. Plot of the CD amplitudes at 326 nm versus sample %*ee*.

6. Simultaneous determination of *er* and concentration

Eighteen scalemic samples of cysteine at varying concentrations and *er* in 400.0 µL of acetonitrile were prepared. They were subjected to simultaneous analysis of the concentration, enantiomeric ratio, and absolute configuration using **1**. First, each sample was added to a solution of **1** (50.0 mM in acetonitrile, 440.0 µL). The mixture was then diluted to 2.0 mL and stirred for 40 minutes. After stirring, 360.0 µL of the reaction mixture was diluted with 6.0 mL of acetonitrile for CD analysis and 30.0 µL of the reaction mixture was diluted with 6.0 mL of acetonitrile for UV analysis. UV spectra were obtained in duplicate as an average of three measurements as described above. The concentration of the samples was calculated using the intensities at 326 nm and the equation shown in Figure 14. CD spectra were obtained in duplicate as an average of three measurements with a scanning speed of 50 nm/min from 320 nm to 330 nm. The CD intensities were then normalized to the concentrations obtained from the UV analysis. The enantiomeric ratios were calculated using the intensities at 326 nm and the equation shown in Figure 17. The absolute configuration was determined from the sign of the Cotton effect.

Table 1. Chiroptical sensing of the absolute configuration, enantiomeric ratio (*er*) and concentration of 18 samples.

Sample number	Sample composition			Sensing results		
	Configuration	*er*	Concentration (mM)	Configuration	*er*	Concentration (mM)
1	L	40.0:60.0	9.0	L	40.0:60.0	9.0
2	D	82.0:18.0	6.0	D	78.8:21.2	5.9
3	L	2.0:98.0	5.0	L	3.9:96.1	4.8
4	L	35.0:65.0	5.5	L	34.3:65.7	5.4
5	D	70.0:30.0	4.0	D	72.8:27.2	3.5
6	D	80.0:20.0	4.5	D	82.1:17.9	4.2
7	L	16.0:84.0	8.0	L	18.3:81.7	7.9
8	D	73.0:27.0	6.5	D	71.1:28.9	6.4
9	D	92.0:8.0	7.0	D	93.6:6.4	6.6
10	L	44.0:56.0	3.5	L	41.8:58.2	2.8
11	L	39.0:61.0	8.5	L	39.3:60.7	8.4
12	D	72.0:28.0	9.5	D	68.7:31.3	9.3
13	L	41.0:59.0	5.0	L	39.7:60.3	4.8
14	L	24.0:76.0	10.0	L	26.3:73.7	10.3
15	D	90.0:10.0	9.0	D	85.9:14.1	9.2
16	D	60.0:40.0	4.5	D	59.5:40.5	4.4
17	L	16.0:84.0	5.0	L	13.7:86.3	5.1
18	L	44.0:56.0	7.5	L	44.1:55.9	7.3

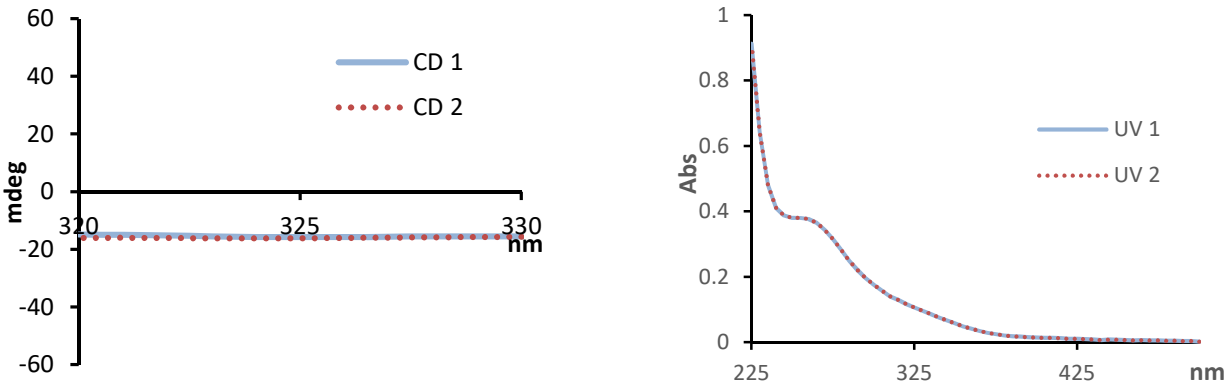


Figure 19. Quantitative CD and UV sensing of sample 1.

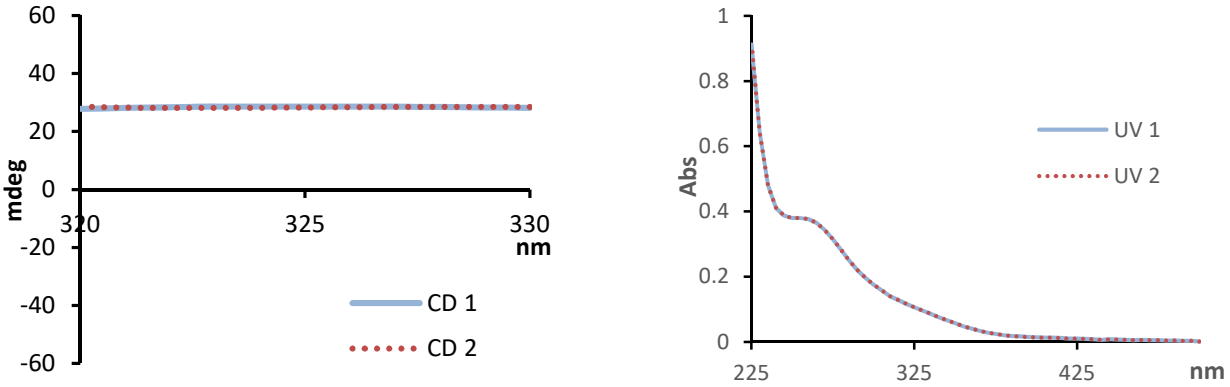


Figure 20. Quantitative CD and UV sensing of sample 2.

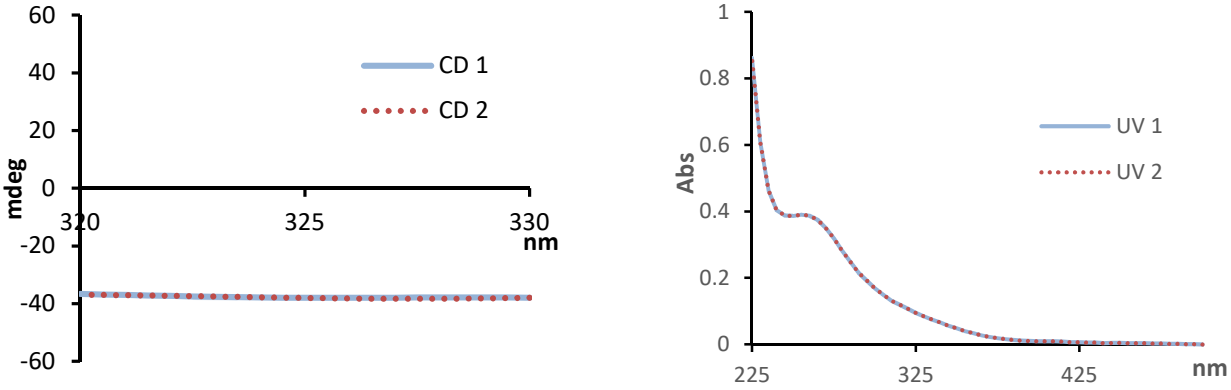


Figure 21. Quantitative CD and UV sensing of sample 3.

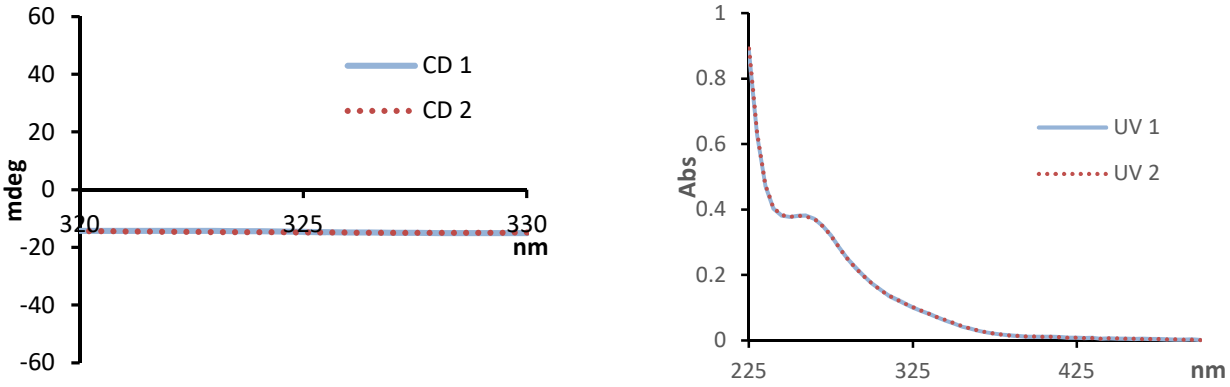


Figure 22. Quantitative CD and UV sensing of sample 4.

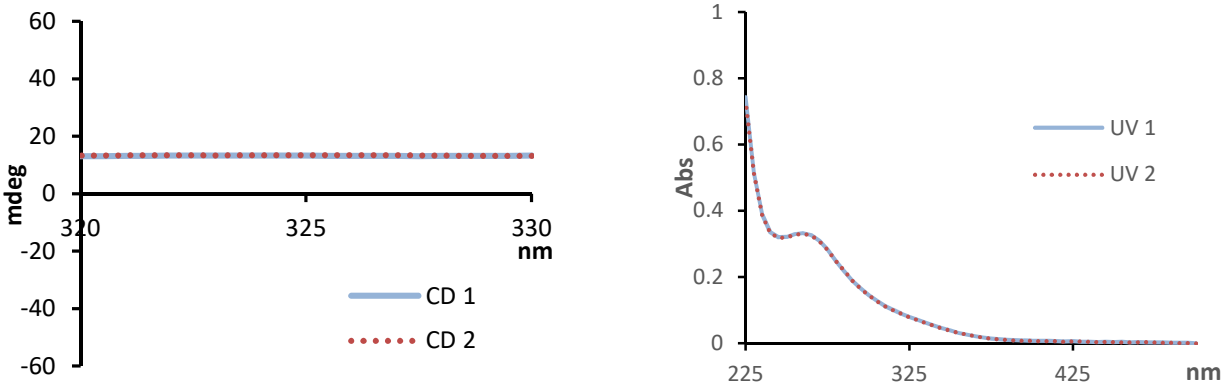


Figure 23. Quantitative CD and UV sensing of sample 5.

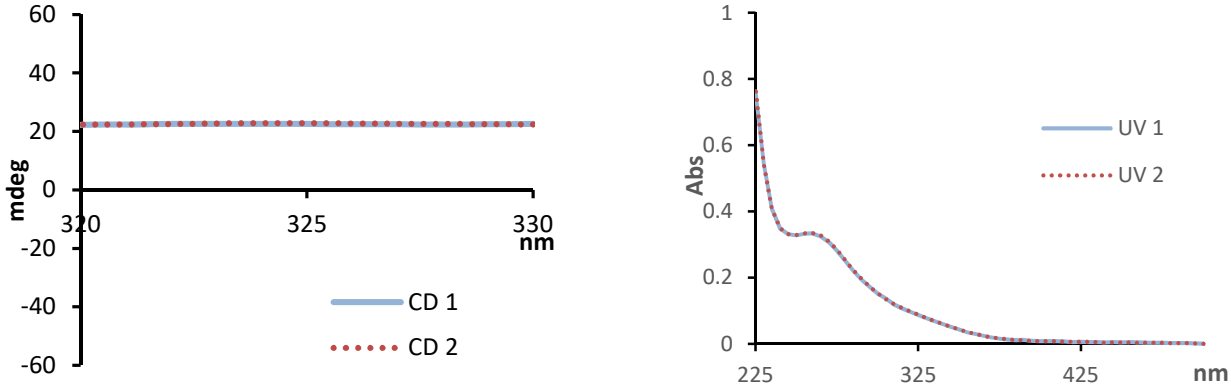


Figure 24. Quantitative CD and UV sensing of sample 6.

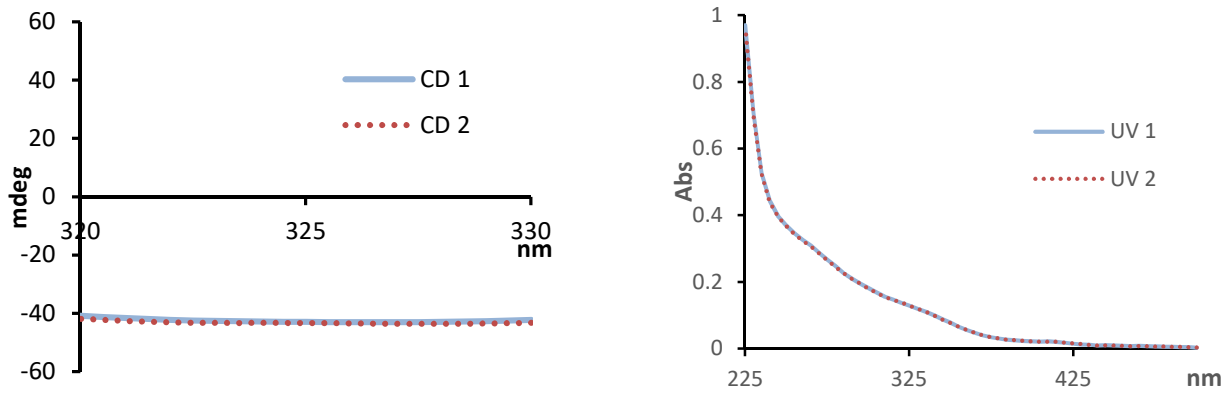


Figure 25. Quantitative CD and UV sensing of sample 7.

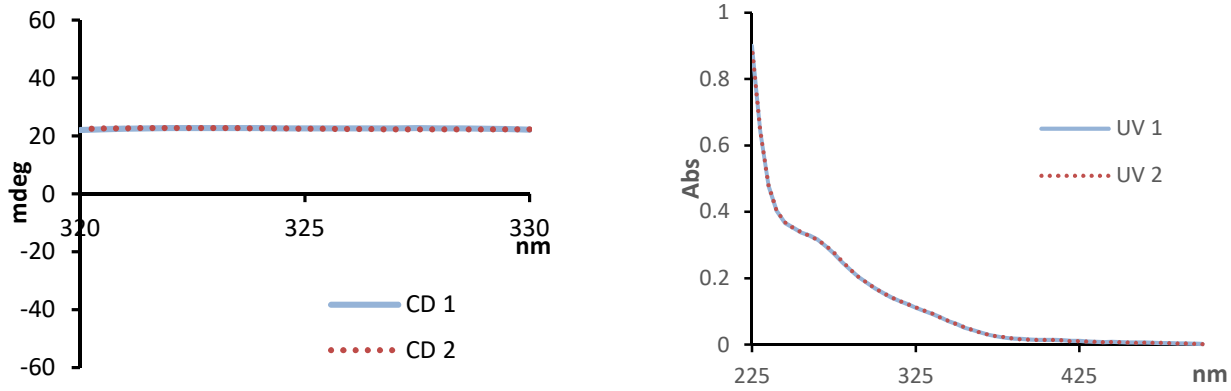


Figure 26. Quantitative CD and UV sensing of sample 8.

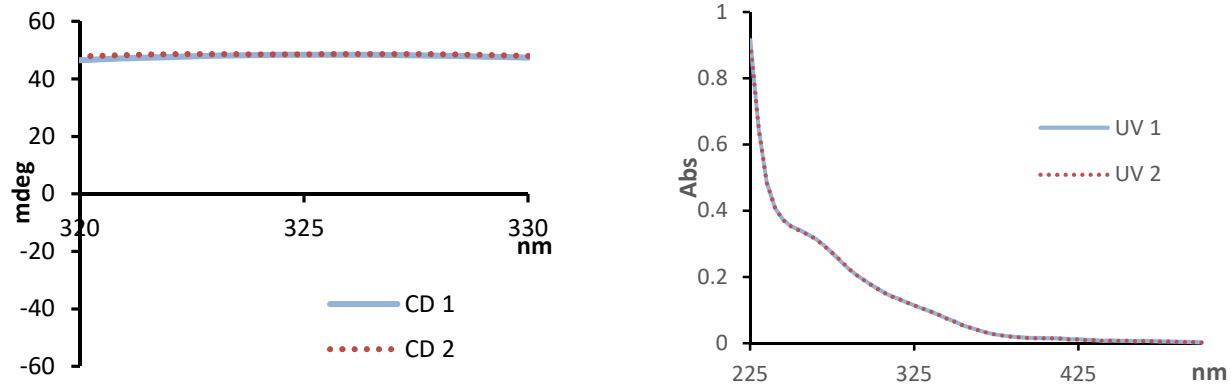


Figure 27. Quantitative CD and UV sensing of sample 9.

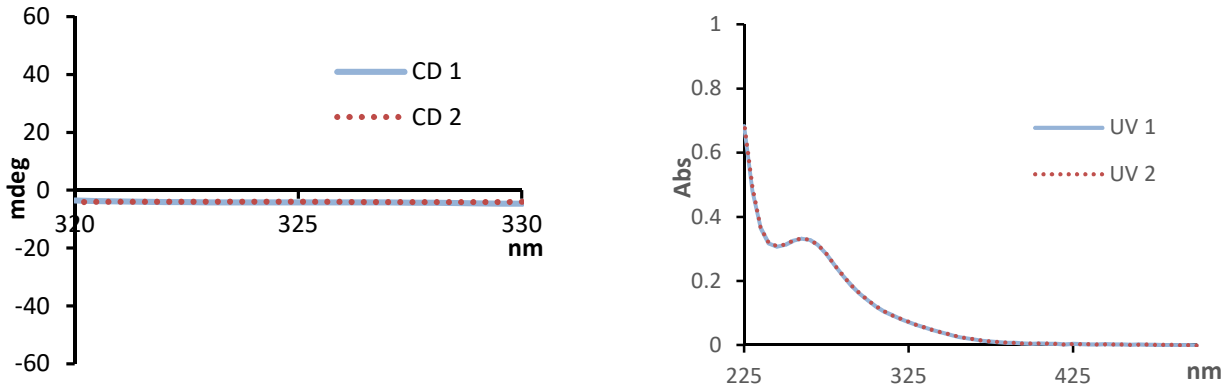


Figure 28. Quantitative CD and UV sensing of sample 10.

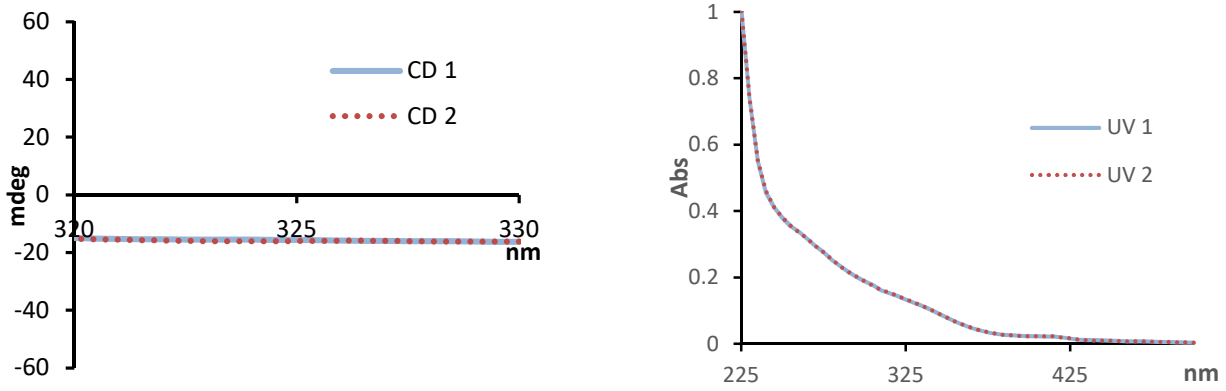


Figure 29. Quantitative CD and UV sensing of sample 11.

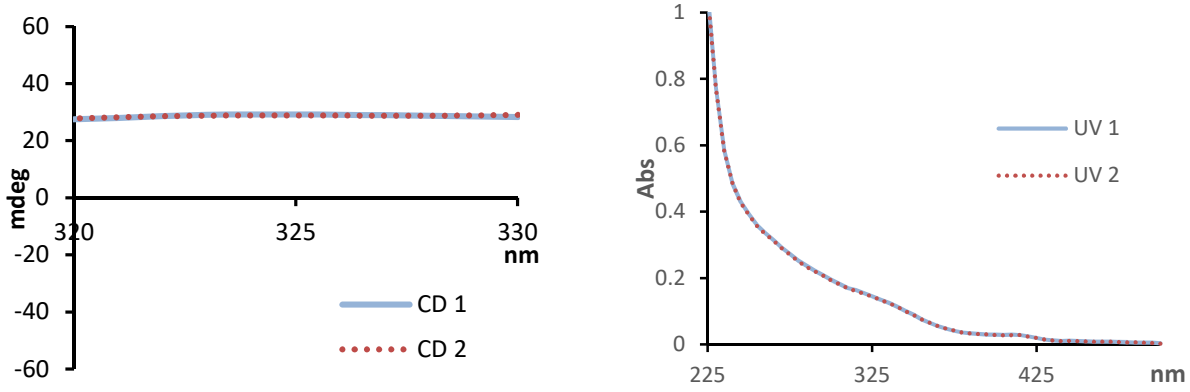


Figure 30. Quantitative CD and UV sensing of sample 12.

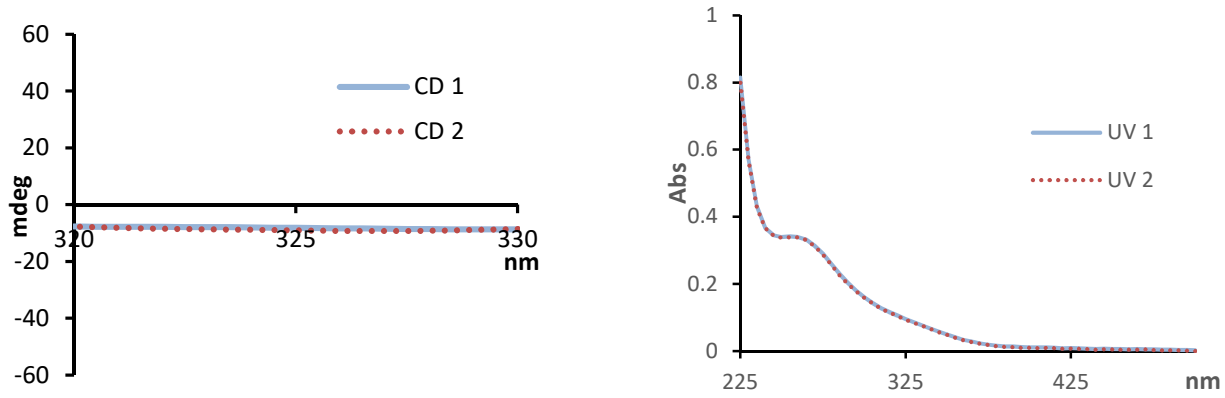


Figure 31. Quantitative CD and UV sensing of sample 13.

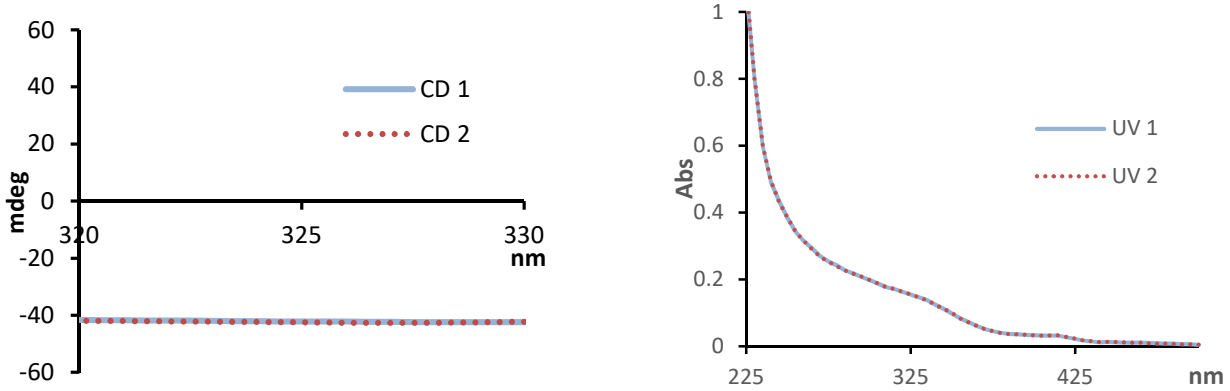


Figure 32. Quantitative CD and UV sensing of sample 14.

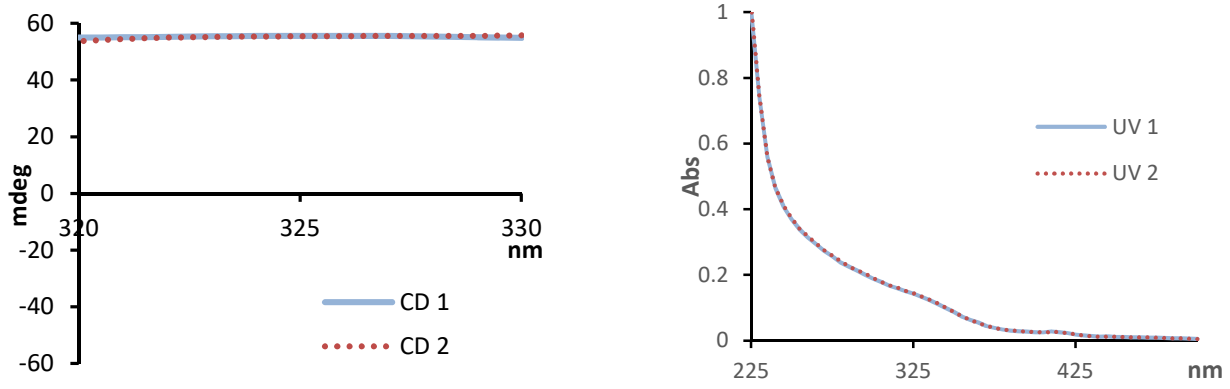


Figure 33. Quantitative CD and UV sensing of sample 15.

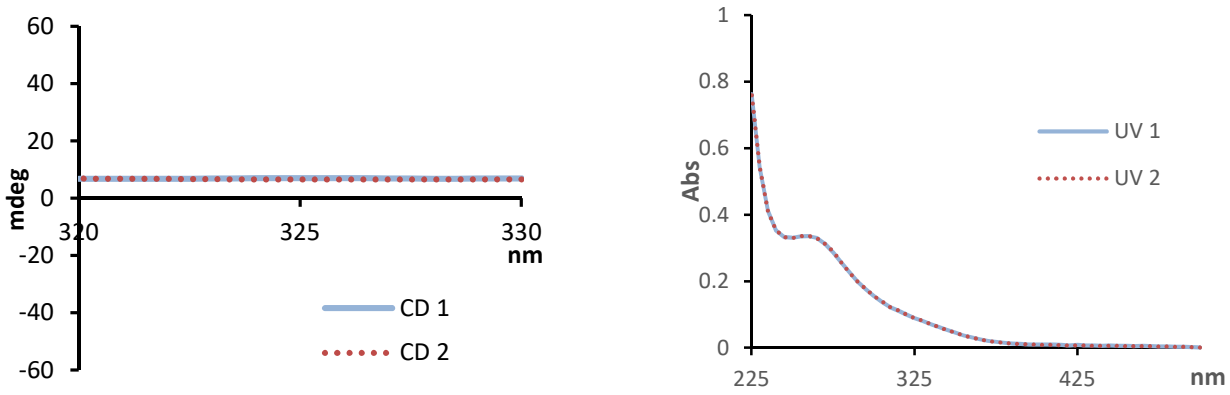


Figure 34. Quantitative CD and UV sensing of sample 16.

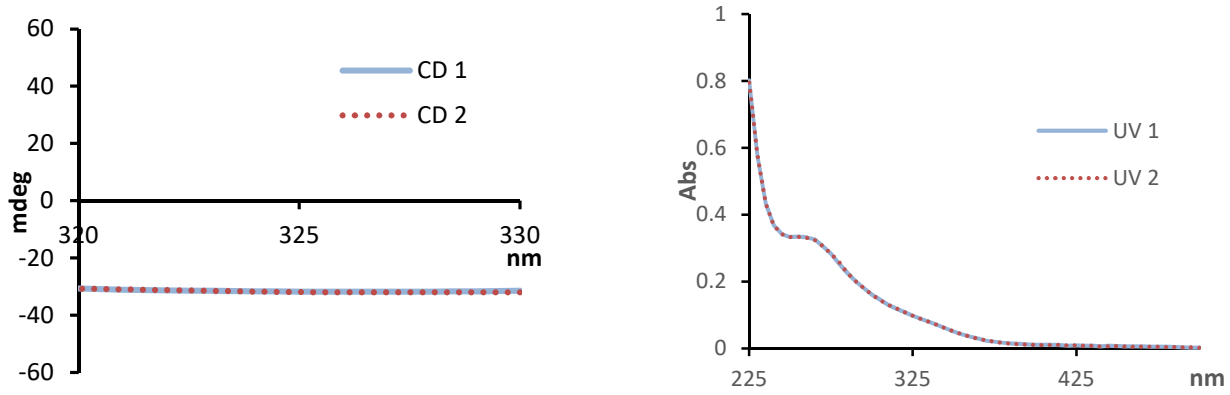


Figure 35. Quantitative CD and UV sensing of sample 17.

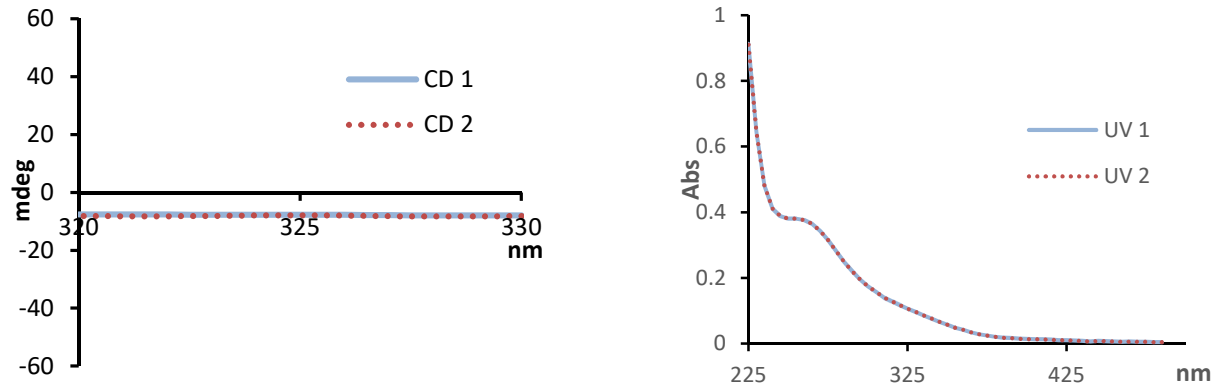


Figure 36. Quantitative CD and UV sensing of sample 18.