Supplementary Information

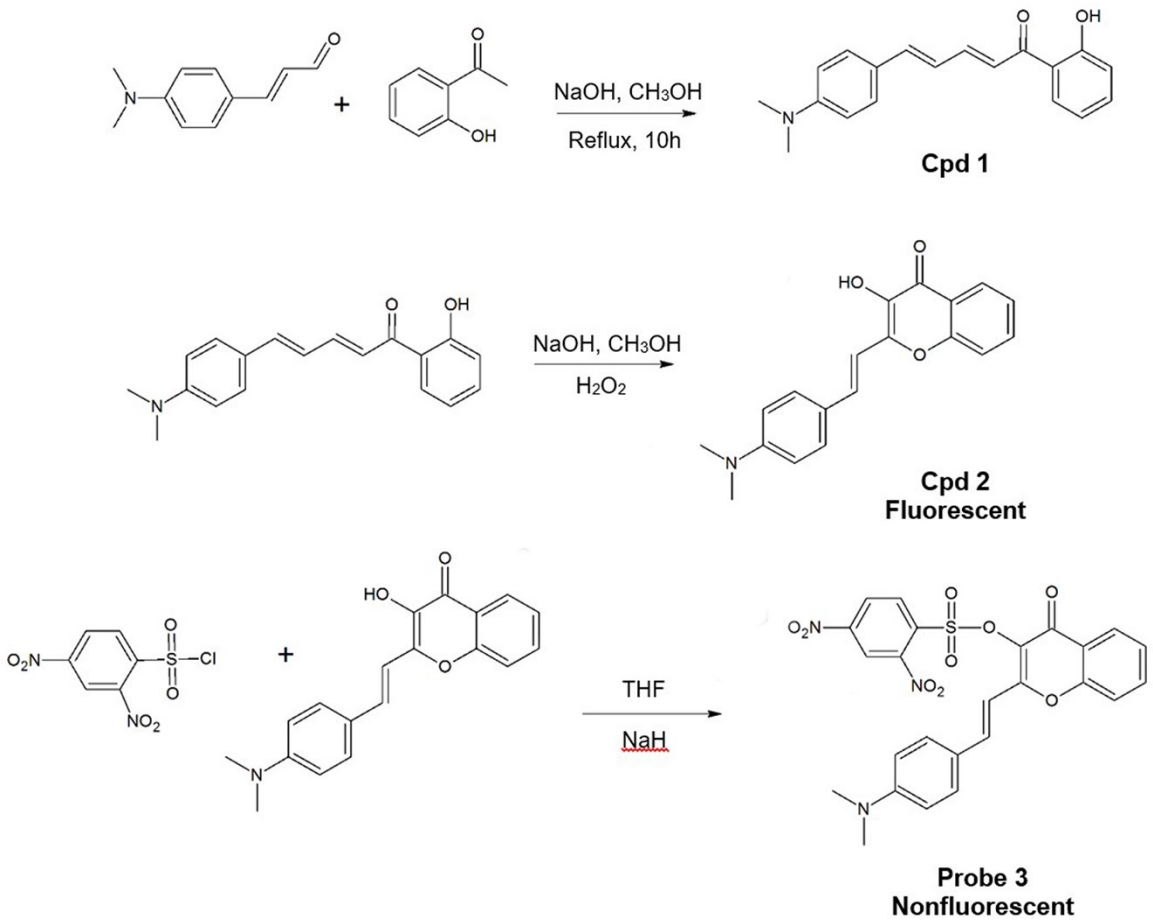
Using a 3-Hydroxyflavone Derivative as a Fluorescent Probe for the Indirect Determination of Aminothiols Separated by Ion-Pair HPLC

Lina Mikaliunaite[a], David B. Green[a]*

[a]Department of Chemistry, Pepperdine University, Malibu, CA, 90263, United States
*Corresponding author. Tel.: +1 310 506 4355. E-mail: david.green@pepperdine.edu

Synthesis of the aminothiol-selective reagent, 2-(4'-dimethylaminocinnamyl)flav-3-yl 2,4-dinitrobenzenesulfonate (probe 3)

The 3-step synthesis of the selective reagent for the detection of the aminothiols, 2-(4'-dimethylaminocinnamyl)flav-3-yl 2,4-dinitrobenzenesulfonate (**probe 3**), was adapted from the literature[1]. (2*E*,4*E*)-5-(4-(dimethylamino)phenyl)-1-phenylpenta-2,4-dien-1-one (**cpd 1**) is made by heating 2-hydroxyacetophenone with 4-(dimethylamino)cinnamaldehyde.



Scheme S1. Synthesis of the non-fluorescent probe 3.

The chalcone (**cpd 1)** is oxidized to the highly fluorescent 4'-(dimethylamino)cinnamyl-3-hydroxyflavone (**cpd 2**) with hydrogen peroxide under basic conditions. Finally, **cpd 2** is quickly converted to **probe 3** (2-(4'-dimethylaminocinnamyl)flav-3-yl 2,4-dinitrobenzenesulfonate) by reaction with 2,4- dinitrobenzenesulfonyl chloride.

Synthesis of (2E,4E)-5-(4-(dimethylamino)phenyl)-1-phenylpenta-2,4-dien-1-one (cpd 1)

A mixture of 4-(dimethylamino)cinnamaldehyde (0.364 g, 2.08 mmol), 2'-hydroxyacetophenone (0.270 g, 1.98 mmol) and NaOH (0.224 g, 5.60 mmol) in methanol (20 mL) was heated at reflux with stirring for 10 h. The mixture was cooled over ice, followed by the

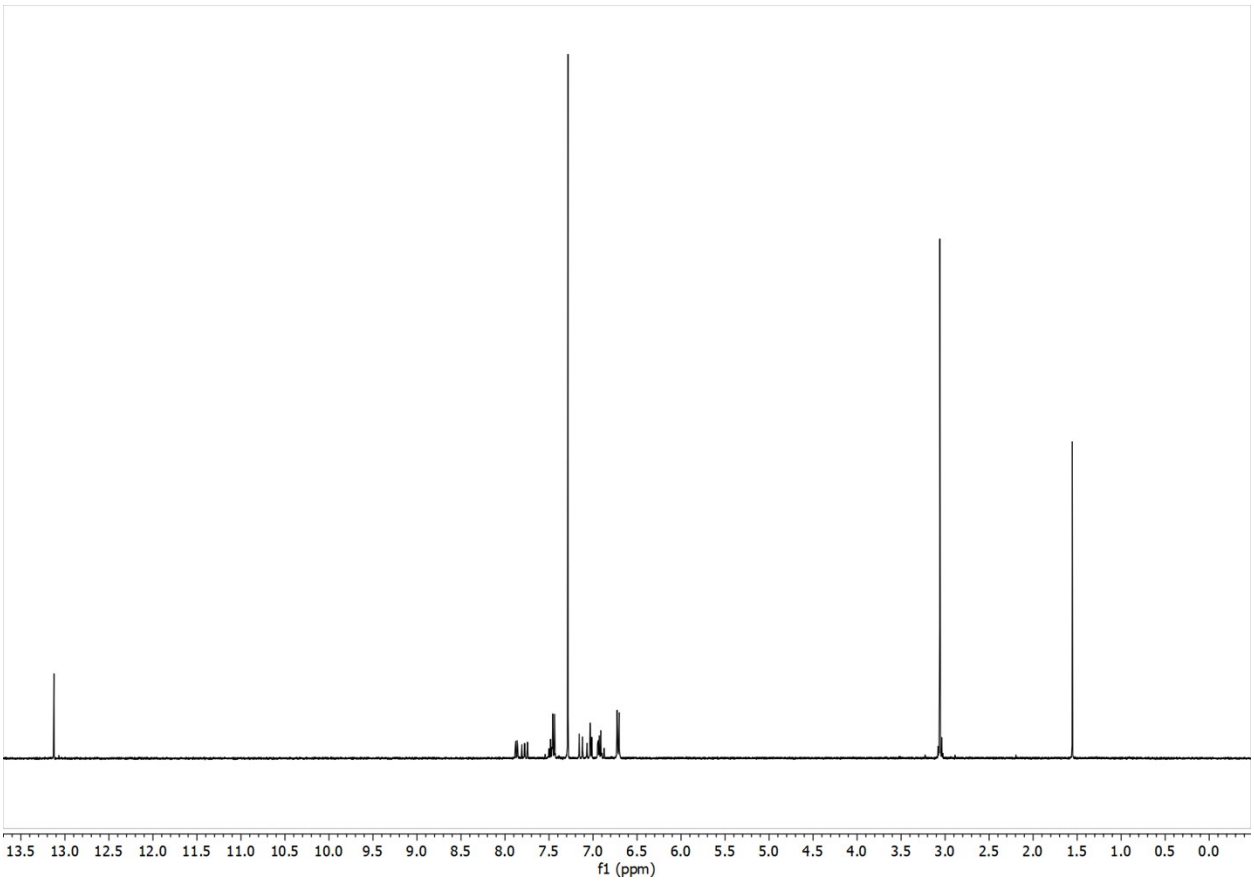
slow addition of ice water (50 mL). The pH of the resulting suspension was adjusted to 2.0 with HCl to dissolve any remaining 4-(dimethylamino)cinnamaldehyde. The dark purple solid was collected and washed with ice-cold 10% $NaHCO_3$ (0.290 g, 50% yield). The structure was verified by NMR spectroscopy (Figure S1) the spectrum of which identical to reference 1 (ESI).

Synthesis of 4'-(dimethylamino)cinnamyl-3-hydroxyflavone (cpd 2)

A mixture of **cpd 1** (0.293 g, 1.00 mmol) in 20 mL methanol and 2 mL 20% NaOH was cooled in ice bath. In 100 μL increments over 5 min, 1 mL of 30% H_2O_2 was added to the mixture, and the solution was heated at reflux with stirring for 3 h. Upon cooling to room temperature, the orange solid was collected and washed with cold methanol (0.187 g, 61% yield). The structure was verified by NMR spectroscopy (Figure S2).

Synthesis of 2-(4'-dimethylaminocinnamyl)flav-3-yl 2,4-dinitrobenzenesulfonate (probe 3)

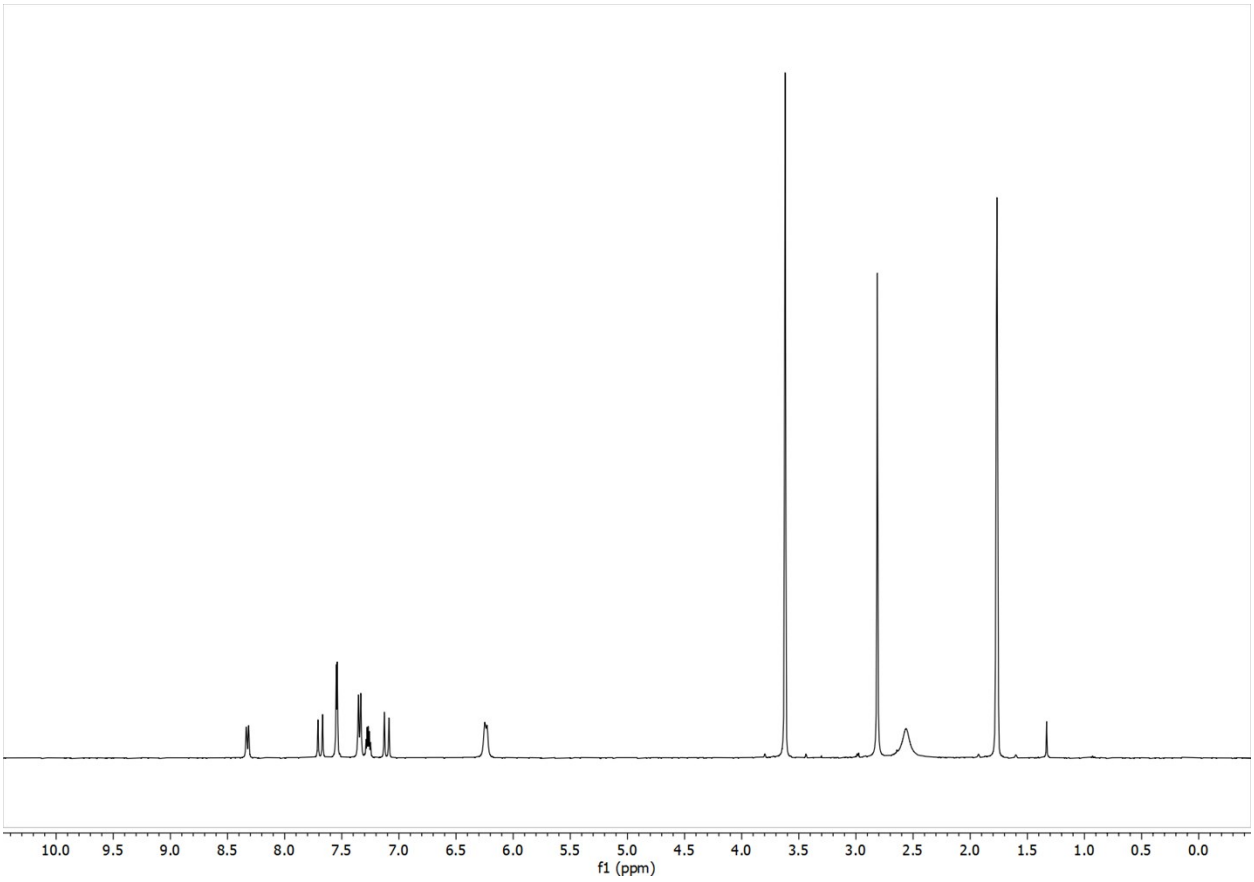
A suspension of **cpd 2** (0.309 g, 1.01 mmol), 2,4-dinitrobenzenesulfonyl chloride (0.352 g, 1.32 mmol) and NaH (57 mg, 2.4 mmol) in 10 mL THF was stirred under argon for 2 h and the reaction subsequently quenched with 50 mL of water. The solvent was evaporated *in vacuo* and the solid redissolved in CH_2Cl_2. **Probe 3** was purified by column chromatography (SiO_2) with 100% CH_2Cl_2 (0.261 g, 48% yield). The CH_2Cl_2 was evaporated *in vacuo*. The NMR spectrum in CD_3CN (Fig. S3) showed no resonances except for those of **probe 3** and residual NMR solvent protons. Further, a solution of **probe 3** (8 μM probe in 50 mM phosphate buffer (pH 7.5), 6 mM OSA, 7.5 mM CTAB, and 20% ACN) showed negligible fluorescence (long dash in Fig. S4), indicating the absence of any residual **cpd 2**.



SI Figure 1. ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.12 (s, 1H, ArO-*H*), 7.87 (dd, *J* = 8.1, 1.7 Hz, 1H, Ar-*H*), 7.78 (dd, *J* = 14.5, 11.2 Hz, 1H, Ar-*H*), 7.53 – 7.41 (m, 3H, Ar-*H*), 7.14 (d, *J* = 14.5 Hz, 1H, Ar-*H*), 7.09 – 6.99 (m, 2H, =C-*H*), 6.97 – 6.92 (m, 1H, =C-*H*), 6.92 – 6.85 (m, 1H, =C-*H*), 6.71 (d, *J* = 8.9 Hz, 2H, Ar-*H*), 3.06 (s, 6H, N-(CH_3)$_2$).

2.2.2 Synthesis of 4'-(dimethylamino)cinnamyl-3-hydroxyflavone (Cpd 2)



SI Figure S2. ^{1}H NMR spectrum of Cpd 2 in THF-d_8.

^{1}H NMR (400 MHz, THF) δ 8.33 (d, *J* = 8.0 Hz, 1H, Ar-*H*), 7.73 – 7.64 (m, 1H, Ar-*H*), 7.54 (d, *J* = 3.8 Hz, 2H, Ar-*H*), 7.34 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.27 (dq, *J* = 8.0, 4.4, 4.0 Hz, 1H, Ar-*H*), 7.11 (d, *J* = 16.1 Hz, 1H, =C-*H*), 6.24 (d, *J* = 8.3 Hz, 2H, =C-*H*), 2.81 (s, 6H N-(C*H*$_3$)$_2$), 2.56 (s, 1H, O-*H*).

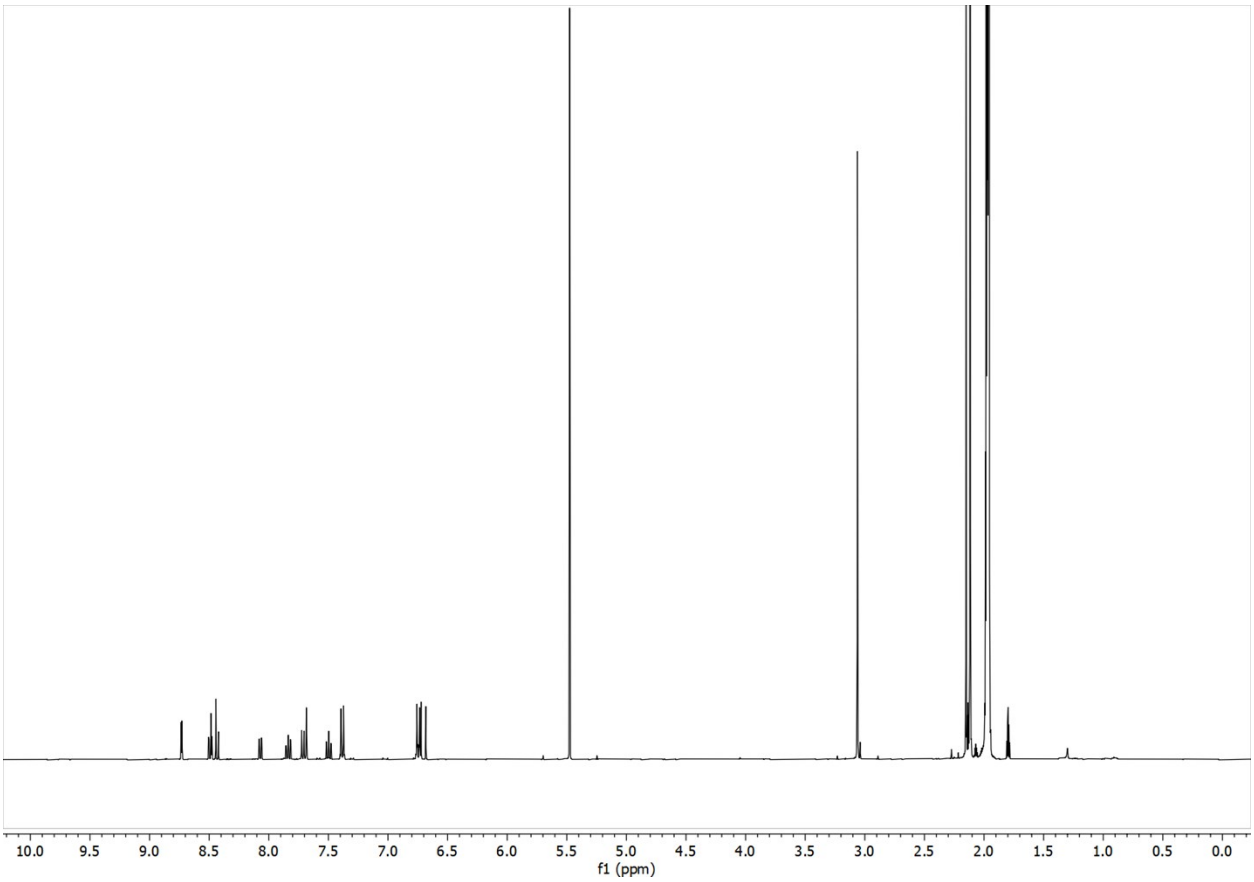


Figure S3. ^{1}H NMR spectrum of Probe 3 in CD_3CN.

^{1}H NMR (400 MHz, Acetonitrile) δ 8.73 (dd, *J* = 2.2, 0.4 Hz, 1H, Ar-*H*), 8.49 (dd, *J* = 8.7, 2.2 Hz, 1H, Ar-*H*), 8.43 (dd, *J* = 8.7, 0.4 Hz, 1H, Ar-*H*), 8.07 (ddd, *J* = 8.0, 1.7, 0.5 Hz, 1H, Ar-*H*), 7.84 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H, Ar-*H*), 7.75 – 7.66 (m, 2H, Ar-*H*), 7.50 (ddd, *J* = 8.1, 7.2, 1.1 Hz, 1H, Ar-*H*), 7.43 – 7.34 (m, 2H, Ar-*H*), 6.78 – 6.70 (m, 2H, =C-*H*), 3.06 (s, 6H, N-(C*H*$_3$)$_2$).

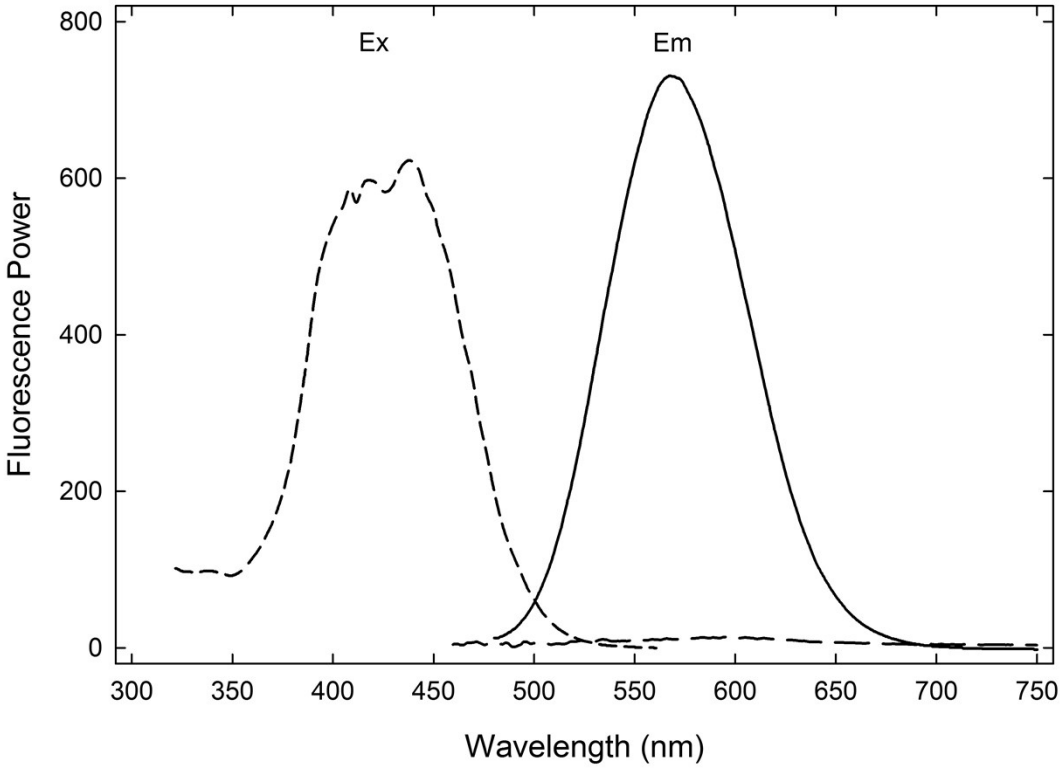


Figure S4. Excitation (Ex, short dash line) and emission (Em, solid line) spectra of cpd 2, and the emission spectrum of probe 3 (long dash line).

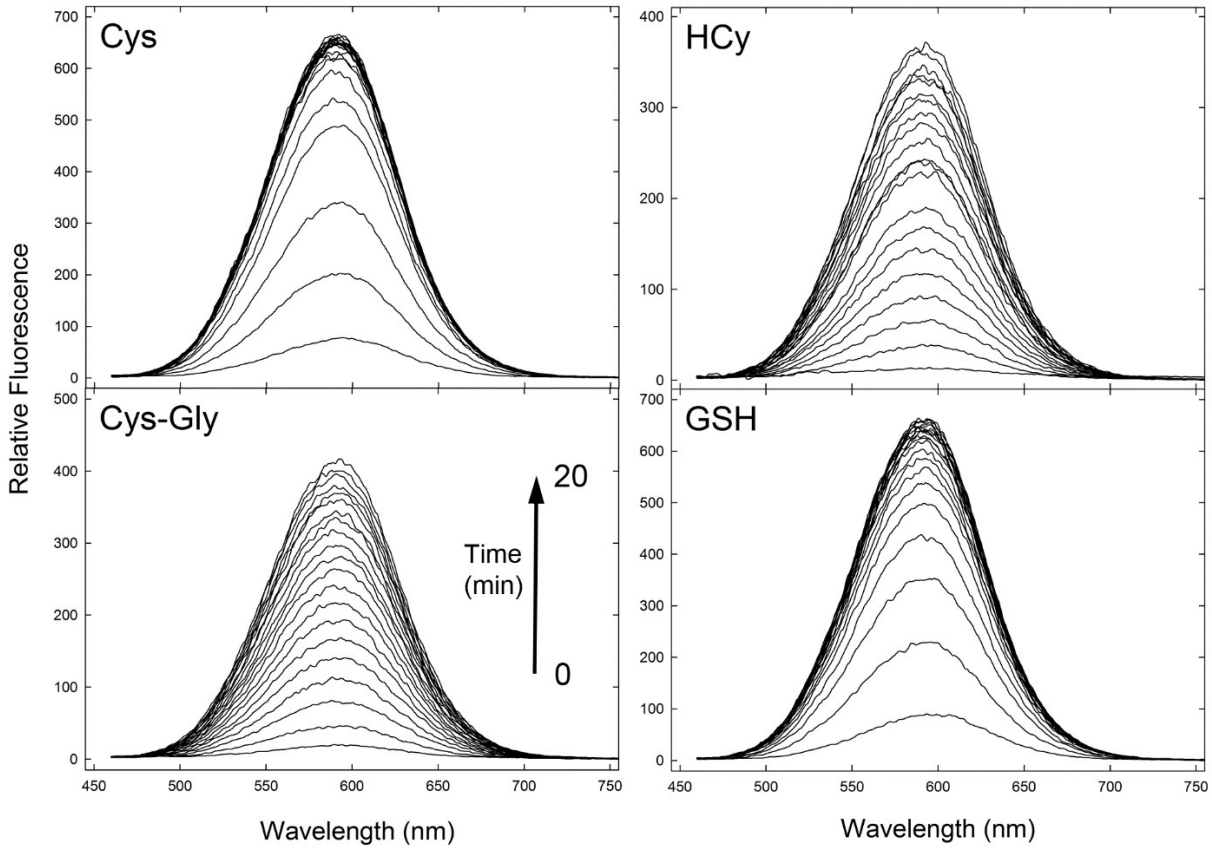


Figure S5. Reaction progress of 35 μM aminothiols and 40 μM probe 3 monitored by the fluorescence of the produced cpd 2 (ex. 420 nm) over 20 minutes of reaction time.

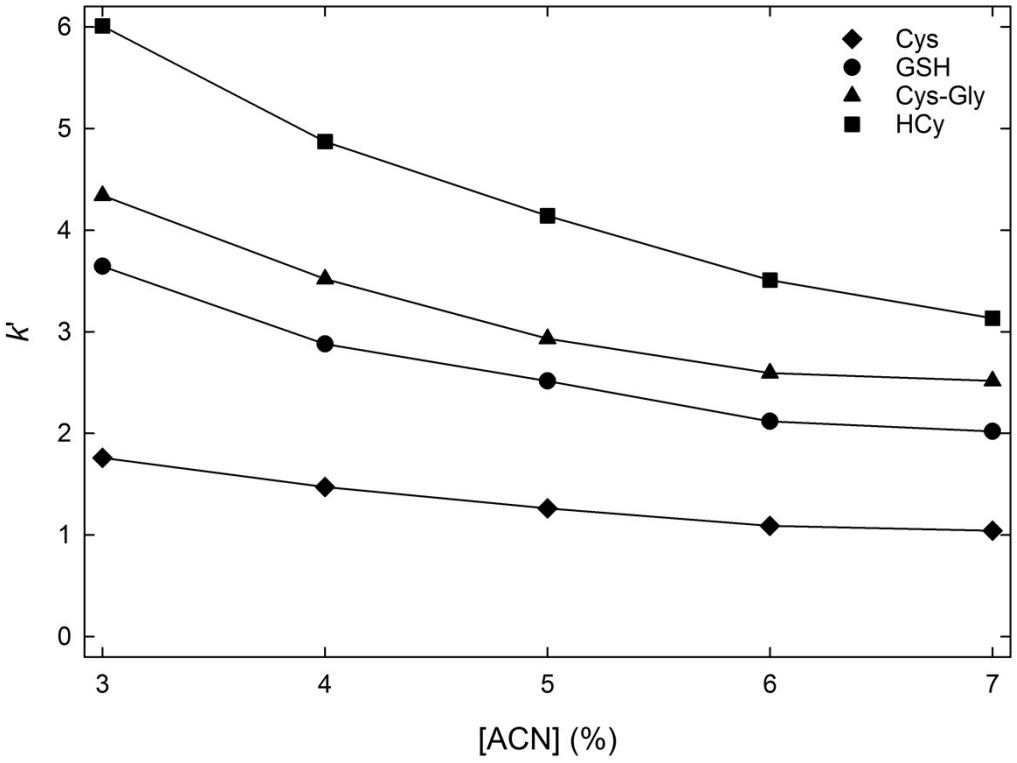


Figure S6. The observed changes in the retention factors, *k'*, for the aminothiols when changing the concentration of ACN from 3% to 7% in the chromatographic solvent.

References

1. S. Chen, P. Hou, B. Zhou, X. Song, J. Wu, H. Zhang and J. W. Foley, *RSC Advances*, 2013, **3**, 11543-11546.