Supporting Information

Host-guest type multiple site fluorescent probe for GSH detection in living organisms

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Materials and Instruments

All amino acids used in this work were purchased from Sigma-Aldrich. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. Hitachi F−7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Hitachi U-3900 UV-Vis spectrophotometer was employed to measure UV-Vis spectra. Shanhai Huamei Experiment Instrument Plants provided a PO-120 quartz cuvette (10 mm). Cell images were obtained by confocal laser scanning microscope of Nikon A1R systems. Tissue sections imaging experiments were performed with a Leica DMI8 fluorescence microscope. Microtome Cryostat Microm HM525NX was used to obtain the tissue slice. Male 5-week-old BALB/c-nu mice were purchased from SPF (Beijing) Biotechnology Co., Ltd. This study was performed in strict accordance with the Chinese guidelines for the care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee of Scientific Research in Shanxi University (Taiyuan, China).

General spectral measurements

Stock solution of GSH547 (2 mM) was prepared in DMSO. Stock solutions of various amino acids and other analytes were prepared by direct dissolution in deionized water. All chemicals used were of analytical grade.

The detection experiments were measured in PBS/DMSO (1/1, v/v, pH 7.4, 10 mM). The procedure was as follows: into the aqueous solution containing 5 μM **GSH547**, an analyte sample was added. The process was monitored by fluorescence or UV-Vis spectrometer.

Cell culture and imaging

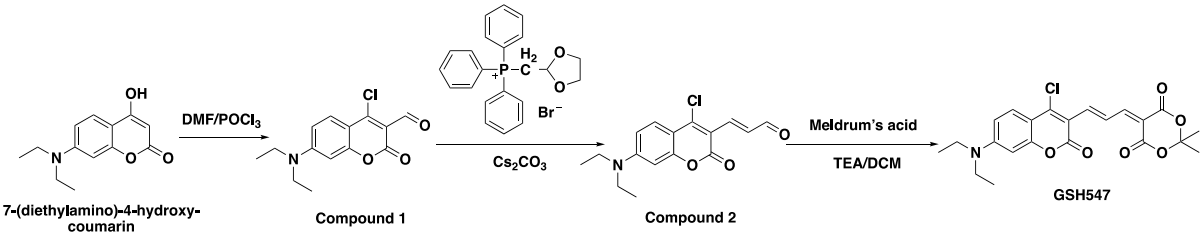
HeLa cells were grown in 1640 medium supplemented with 12% FBS and 1% antibiotics at 37 °C in humidified environment of 5% CO_2. Cells were plated on a 6-well plate with slides and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times. HeLa cells were pre-incubated in PBS with or without N-ethyl maleimide (NEM, a generally used thiols scavenger, 1 mM) for 30 min which were further labelled with **GSH547**. Cells were washed with PBS 3 times before fluorescent imaging.

Ex Vivo Mouse Liver and Tumor Imaging

Male 5-week-old BALB/c-nu mouse was sacrificed and the liver was harvested immediately. The 10 mm fresh slices were obtained. The slices were incubated directly with **GSH547** (50 μM) or pre-incubated with NEM which were then incubated with

GSH547 after washing with PBS. Then, the fluorescence images were taken by the fluorescence microscope.

Synthesis



Scheme S1. Synthesis of probe **GSH547**.

Synthesis of compound 1: Compound **1** was synthesized according to the procedures reported in previous works.[24] ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.33 (s, 1H), 7.87 (d, J = 9.3 Hz, 1H), 6.71 (d, J = 9.4 Hz, 1H), 6.46 (s, 1H), 3.51 (q, J = 7.0 Hz, 4H), 1.28 (t, J = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.07, 159.99, 156.47, 154.08, 153.62, 129.32, 111.08, 110.57, 107.76, 96.67, 45.37, 12.46. HR-MS [compound **1** + H]$^+$: m/z Calcd 280.0729, found 280.0735. Crystal data for $C_{14}H_{14}ClNO_3$: crystal size: 0.3 X 0.10 X 0.10, monoclinic, space group $P2_1/c$, a = 10.281(4)Å, b = 13.526(5) Å, c = 9.721(3) Å, α = 90°, β = 107.048(5)°, γ = 90°, V = 1292.4(8) Å^3, Z = 4, T= 150.0 K, 2301 reflections measured, 1451 unique (R_{int} = 0.0504) R_1 = 0.0914, wR_2 = 0.2277, Goof = 1.03.

Synthesis of compound 2: Compound **1** (5.58 g, 20.00 mmol), 1,3-dioxolan-2-yl)methyl-triphenylphosphonium bromide (9.42 g, 22.00 mmol) and cesium carbonate (7.17 g, 22.00 mmol) were added to a round bottom flask containing 50 mL DMF. The mixture was stirred at room temperature for 24 h which was then poured into hydrochloric acid solution (2 M, 90 mL) slowly and stirred for 0.5 h. Then, 200 mL deionized water was added to precipitate the crude product which was then separated by chromatography column with dichloromethane/petroleum ether (1/5, v/v) as eluent to obtain compound **2** as a brown-yellow solid (2.72 g) with a yield of 44.6%. A small amount of solid was dissolved in ethyl acetate and evaporated at room temperature to obtain crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (d, J = 7.7 Hz, 1H), 7.77 (s, 1H), 7.74 (d, J = 6.2 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 6.72 (dd, J = 9.2, 2.5 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 3.47 (q, J = 7.2 Hz, 4H), 1.26 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.77, 158.19, 155.00, 152.62, 150.84, 143.51, 132.33, 128.26, 111.84, 110.17, 107.74, 96.61, 45.21, 12.46. HR-MS [compound **2** + H]$^+$: m/z Calcd 306.0890, found 306.0891. Crystal data for $C_{16}H_{16}ClNO_3$: crystal size: 0.2 X 0.13 X

0.12, monoclinic, space group $P2_1/n$, a = 11.5427(2) Å, b = 16.8415(3) Å, c = 14.7424(3) Å, α = 90°, β = 91.8980(10)°, γ = 90°, V = 2864.30(9) Å^3, Z=4, T = 150.0 K, 50905 reflections measured, 5856 unique (R_{int} = 0.0433) R_1 = 0.0330, wR_2 = 0.0830, Goof = 1.032.

Synthesis of probe GSH547: Compound **2** (0.61 g, 2.00 mmol) and Meldrum's acid (0.58 g, 4.00 mmol) were dissolved in 40 mL dichloromethane. Triethylamine (3 drops) was added and the mixture was stirred at room temperature for 5 h. After that, the reaction was quenched with saturated brine and extracted with dichloromethane. The organic phase was collected and distilled under reduced pressure. The crude product was purified by chromatography column with dichloromethane as eluent to obtain GSH547 as a dark blue solid (0.60 g) with a yield of 69.6%. A small amount of solid was dissolved in ethyl acetate and evaporated at room temperature to obtain crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (dd, *J* = 14.7, 12.5 Hz, 1H), 8.23 (d, *J* = 12.4 Hz, 1H), 7.92 (d, *J* = 14.9 Hz, 1H), 7.75 (d, *J* = 9.4 Hz, 1H), 6.91 (dd, *J* = 9.4, 2.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 3.53 (q, *J* = 7.0 Hz, 4H), 1.69 (s, 6H), 1.16 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.76, 160.52, 158.65, 157.98, 155.37, 153.79, 151.14, 146.24, 128.79, 128.21, 112.34, 111.76, 111.39, 108.02, 104.78, 96.93, 45.16, 27.67, 12.92. HR-MS [**GSH547** + H]$^+$: m/z Calcd 432.1212, found 432.1208. Crystal data for $C_{22}H_{22}ClNO_6$: crystal size: 0.2 X 0.15 X 0.12, monoclinic, space group $P2_1/c$, a = 9.6535(3) Å, b = 14.5524(4) Å, c = 14.1991(5) Å, α = 90°, β = 0.901(1)°, γ = 90°, V = 1994.47(11) Å^3, Z = 4, T = 150(2) K, 4089 reflections measured, 3224 unique (R_{int} = 0.0581) R_1 = 0.0508, wR_2 = 0.0345, Goof = 1.081.

General spectral measurements and bioimaging

The detailed description is introduced in the ESI. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shanxi University and approved by the Animal Ethics Committee of the Radiation Protection Institute of Drug Safety Evaluation Center in China (Production license: SYXK (Jin) 2018-0005).

Figure S1. Time dependent fluorescence intensities changes at 547 nm and 640 nm upon addition of 200 μM GSH to 5 μM **GSH547** in the detection system. $l_{ex\ 547}$ = 440 nm, slit 2.5/5 nm, 700 v; $l_{ex\ 650}$ = 560 nm, slit 5/5 nm, 700 v.

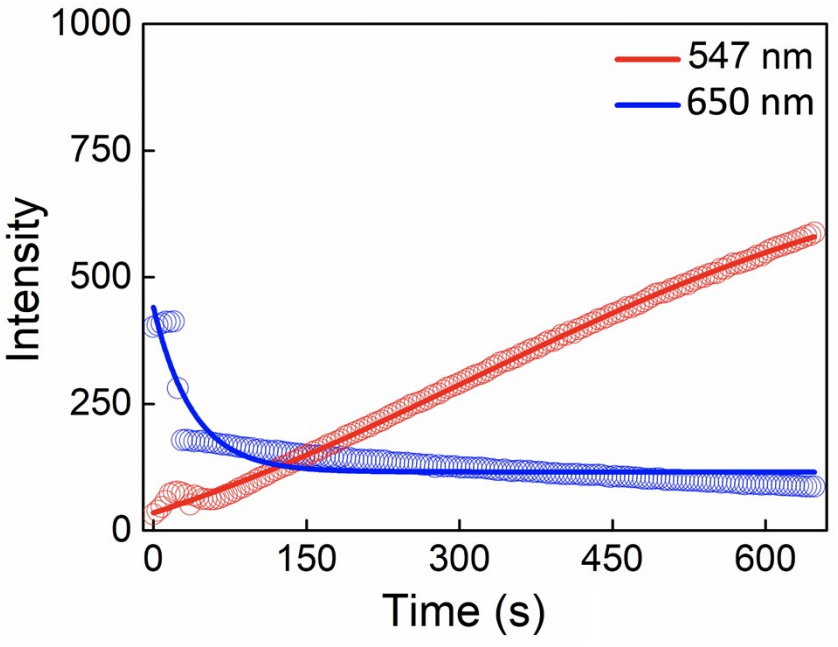


Figure S2. ^{1}H-^{1}H COSY of **GSH547** in DMSO-d_6.

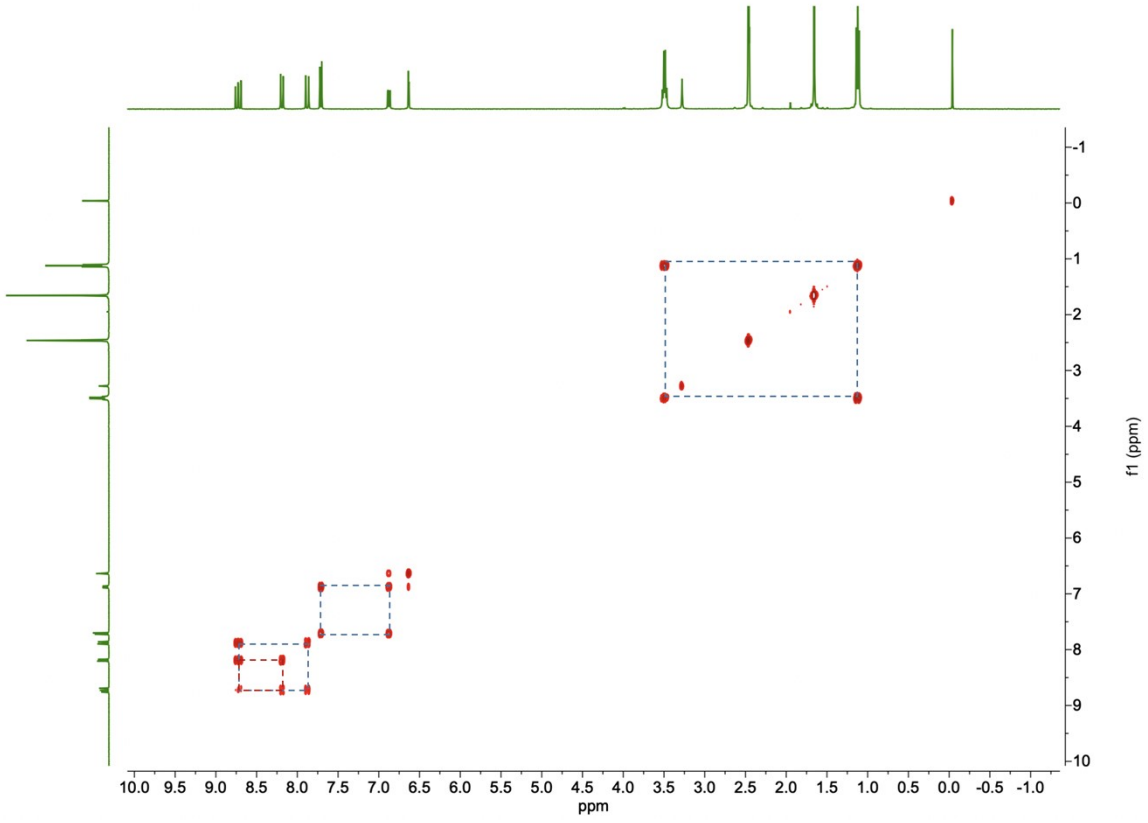


Figure S3. Signals assignment of **GSH547** defined by the analyzation of ^{1}H-^{1}H COSY.

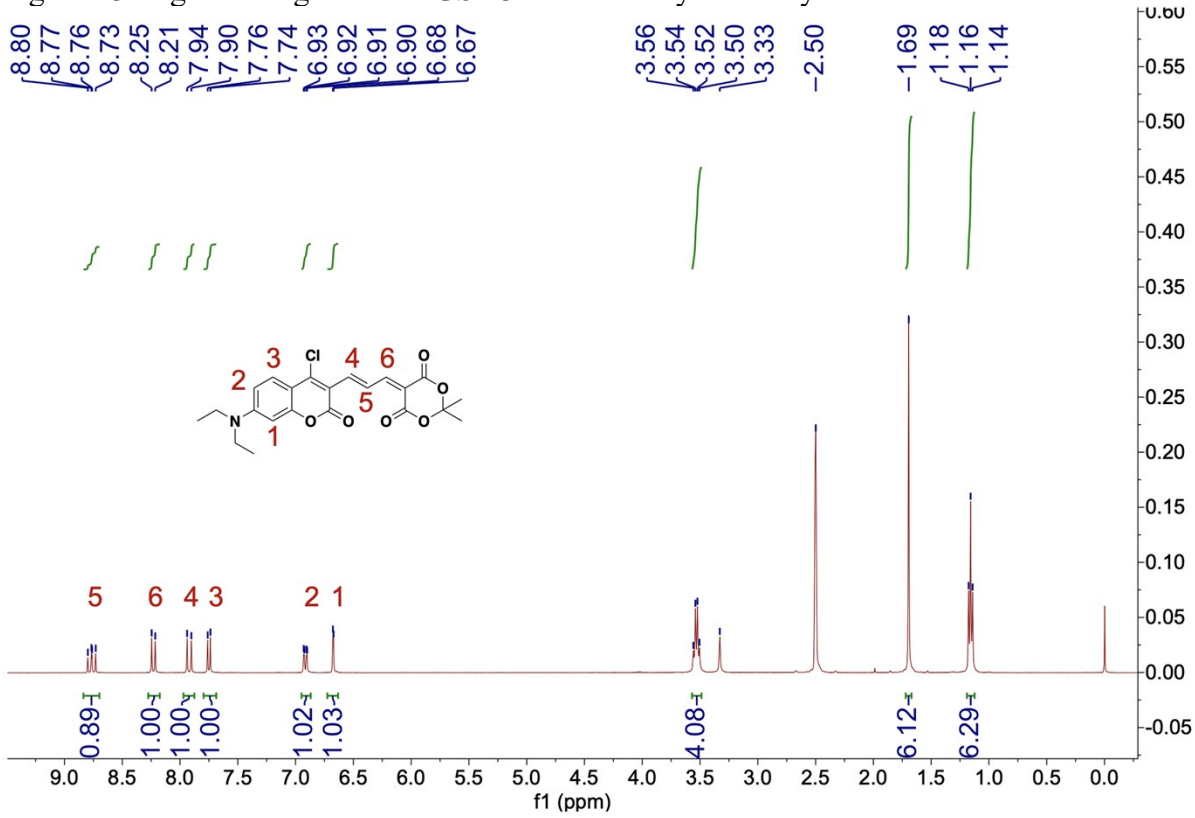


Figure S4. ^{1}H NMR spectrum of the reaction system upon addition of GSH to **GSH547** in DMSO.

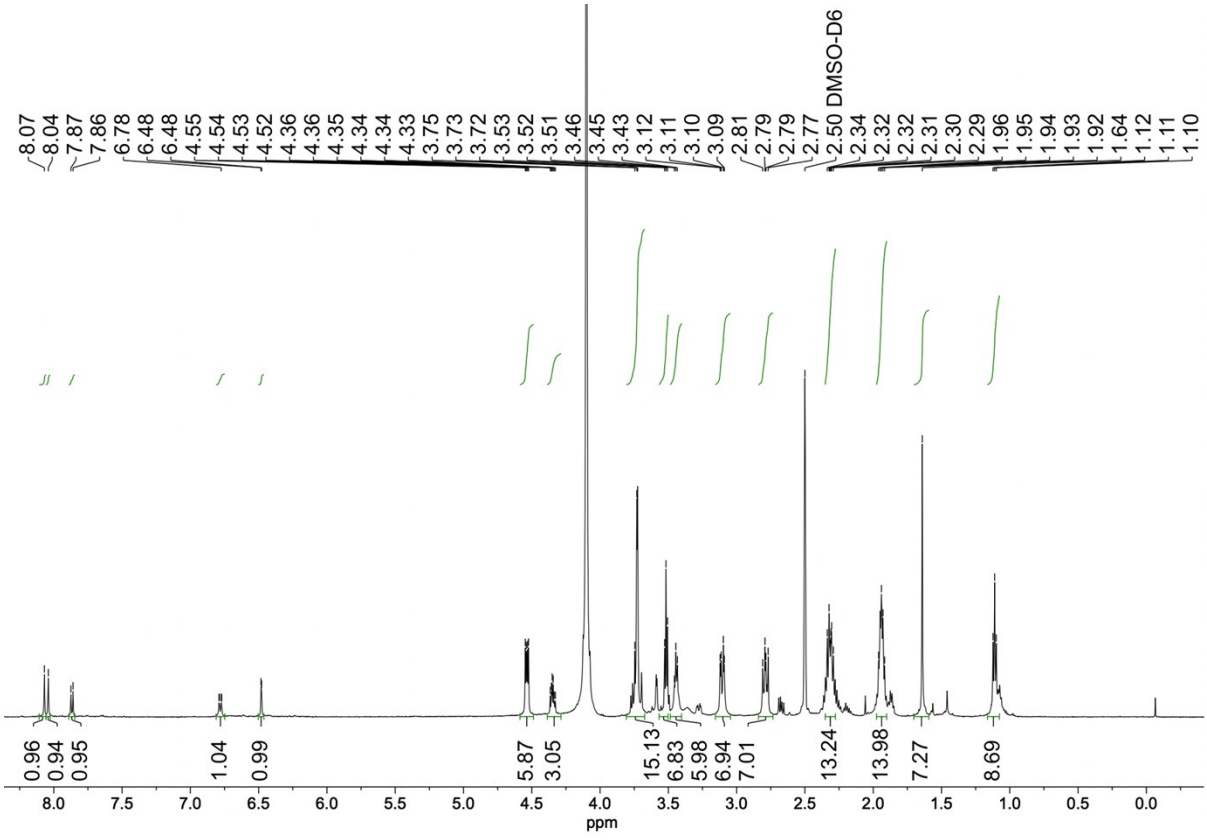


Figure S5. ^{1}H NMR spectra comparation of **GSH547** in DMSO with or without GSH addition.

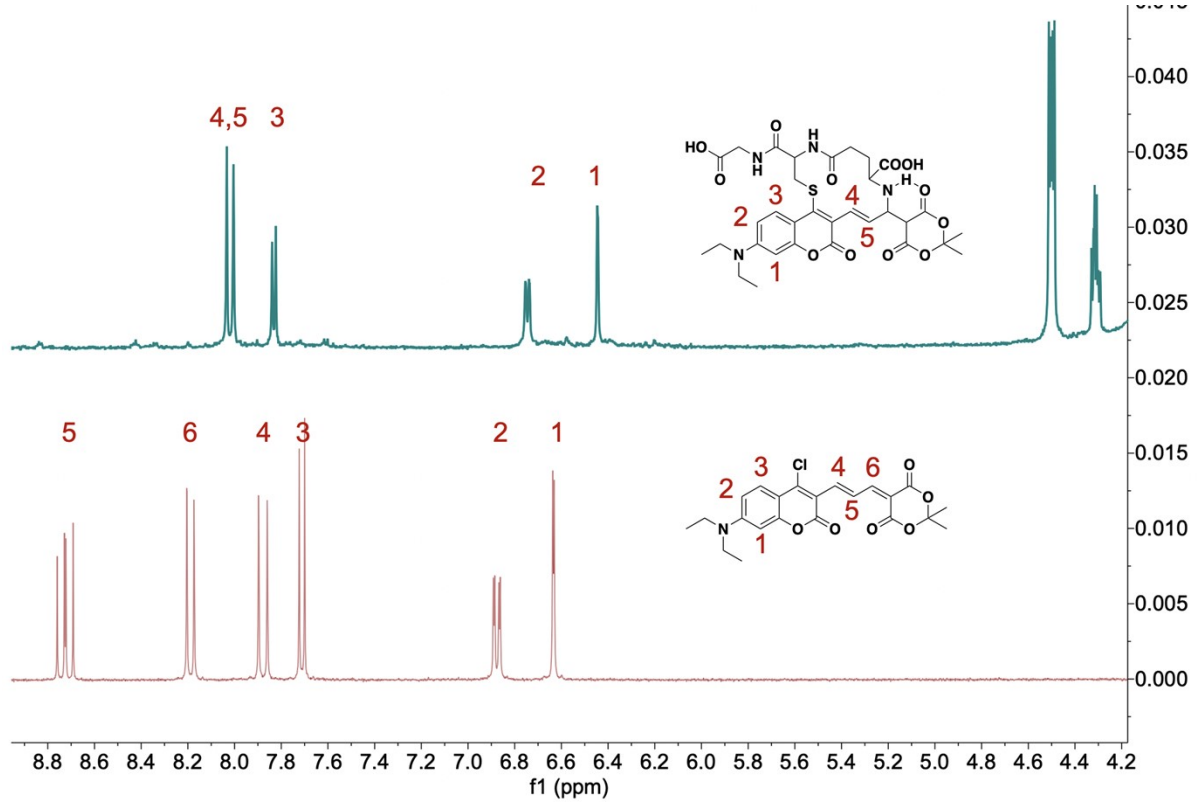


Figure S6. LC-MS of **GSH547** upon addition of GSH.

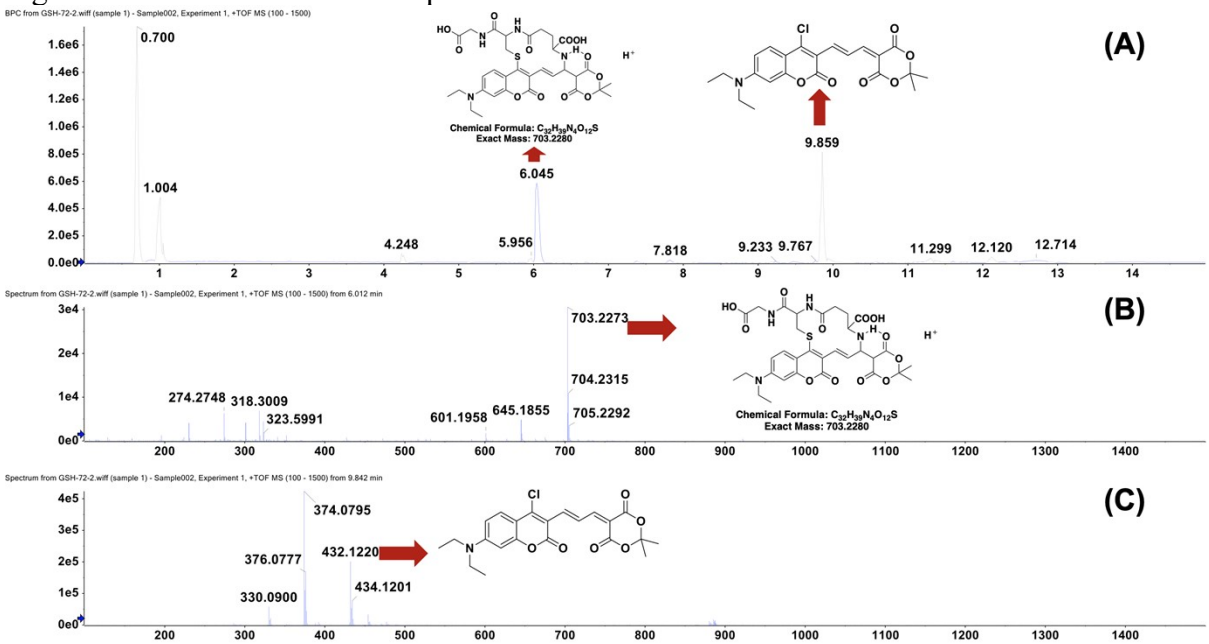
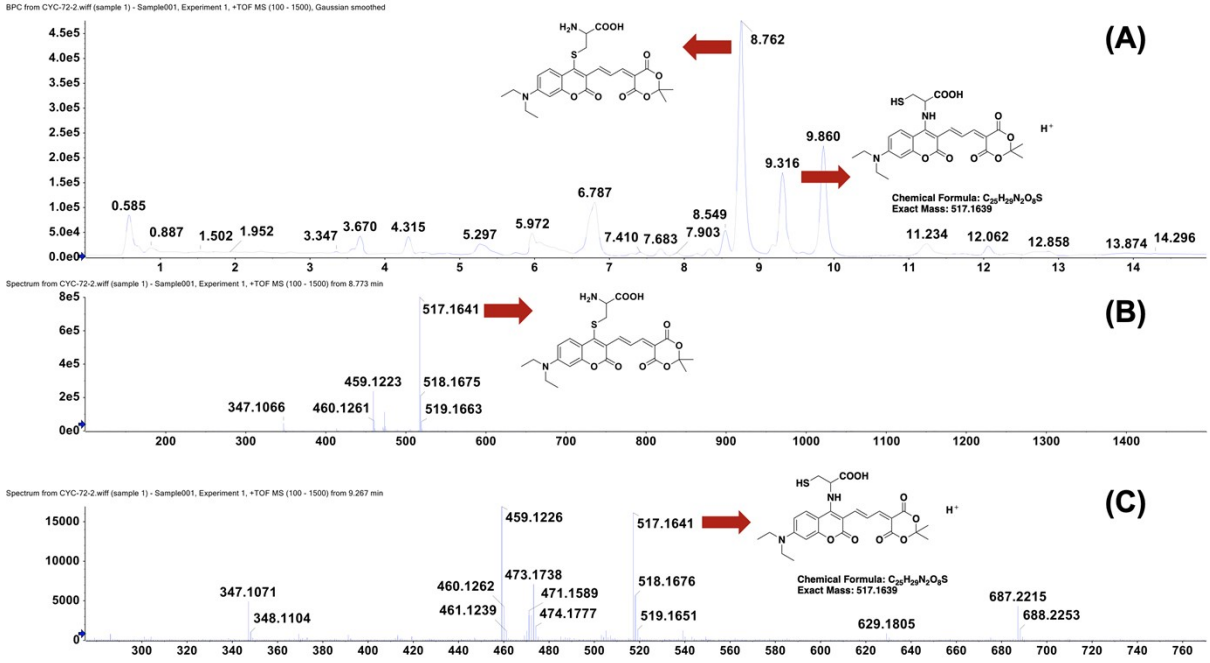
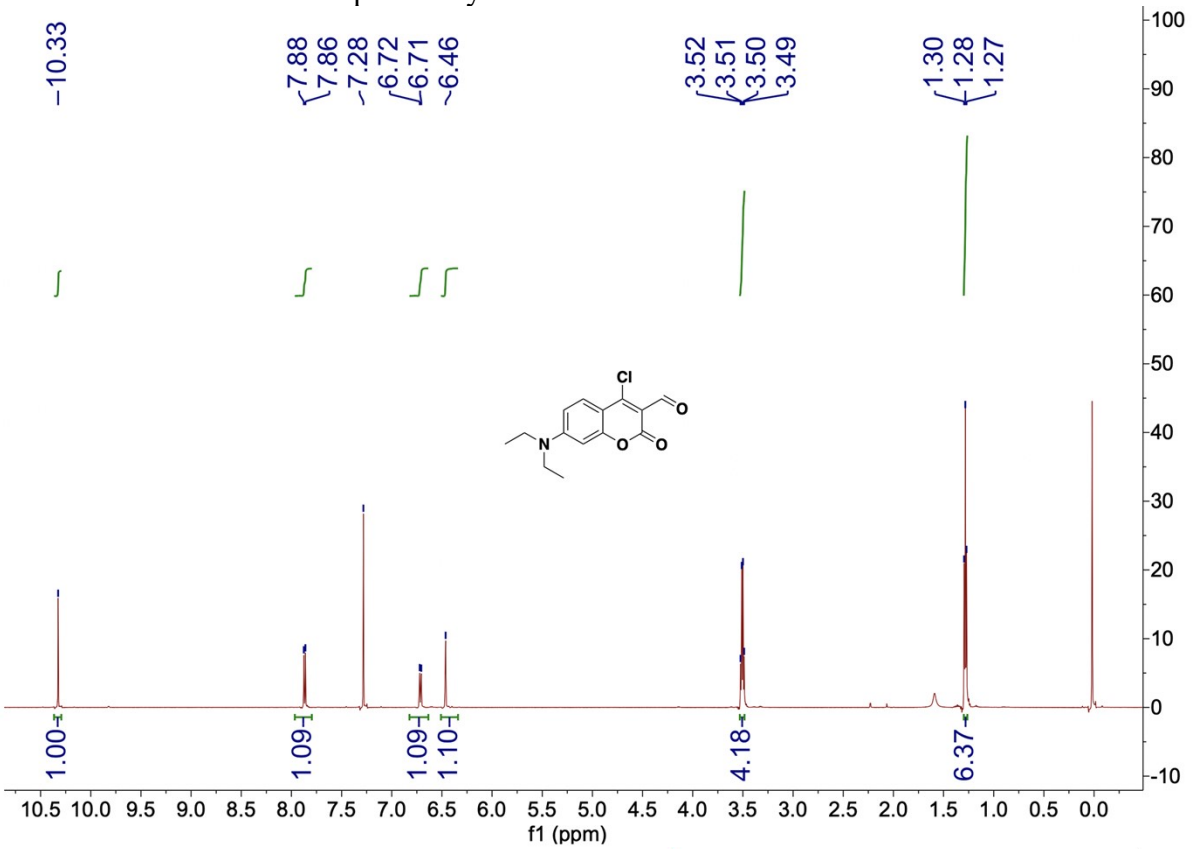
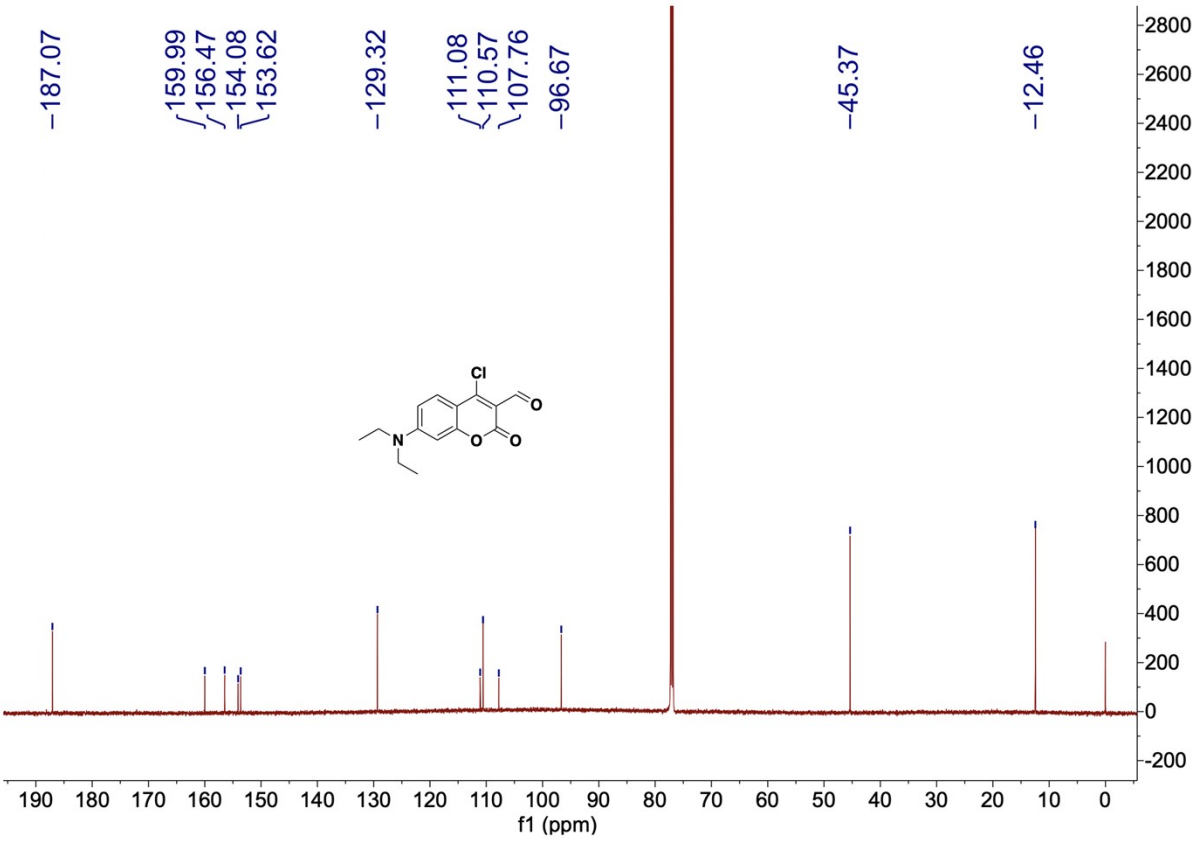


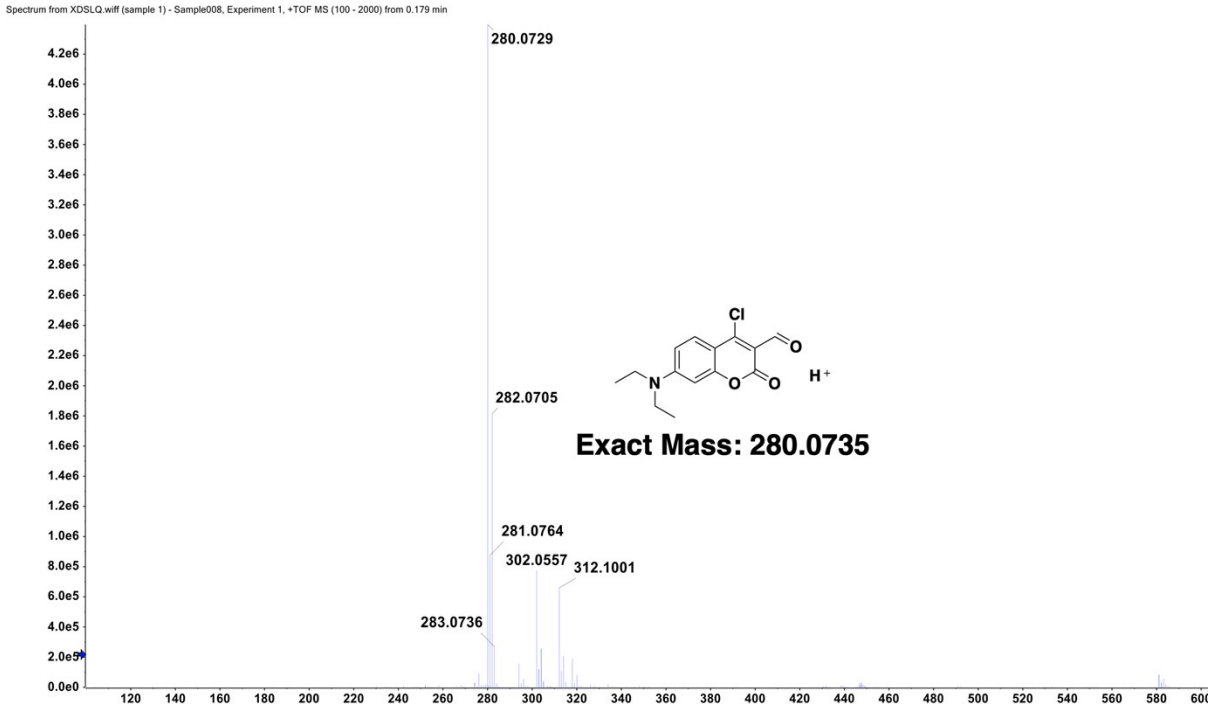
Figure S7. LC-MS of **GSH547** upon addition of Cys.

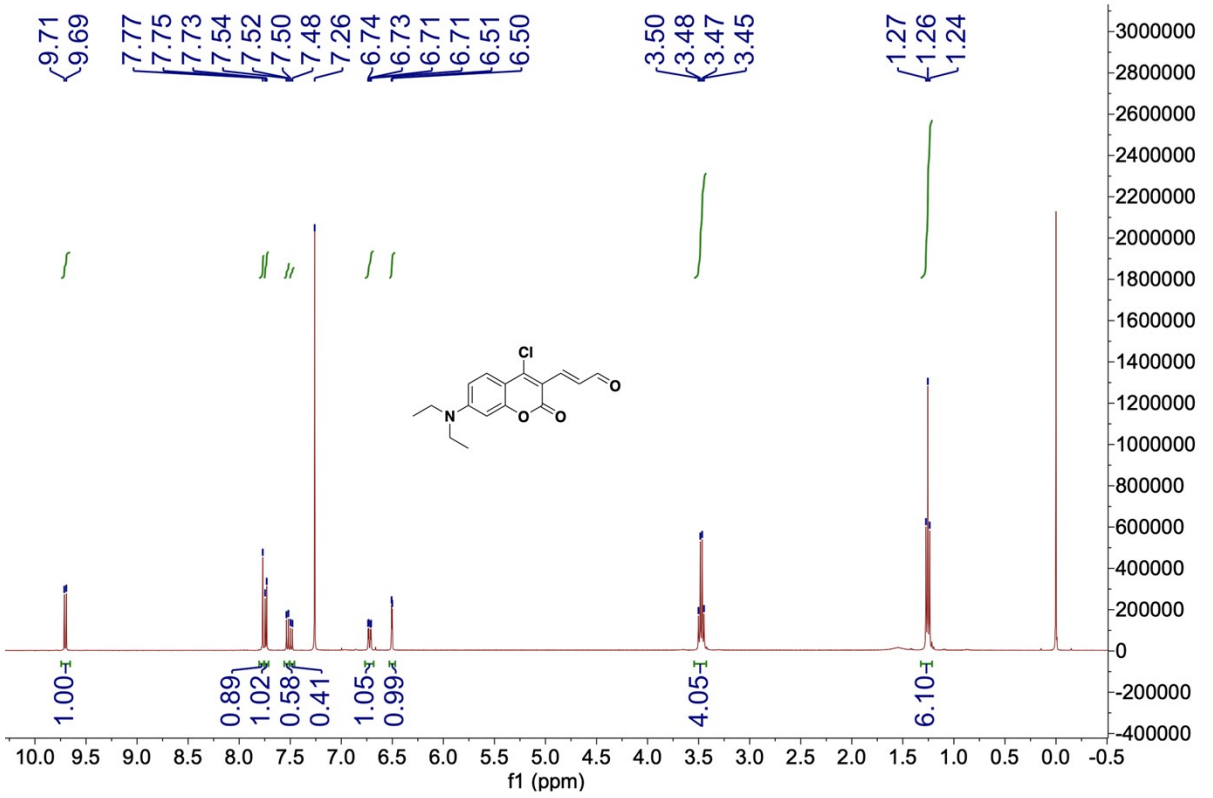


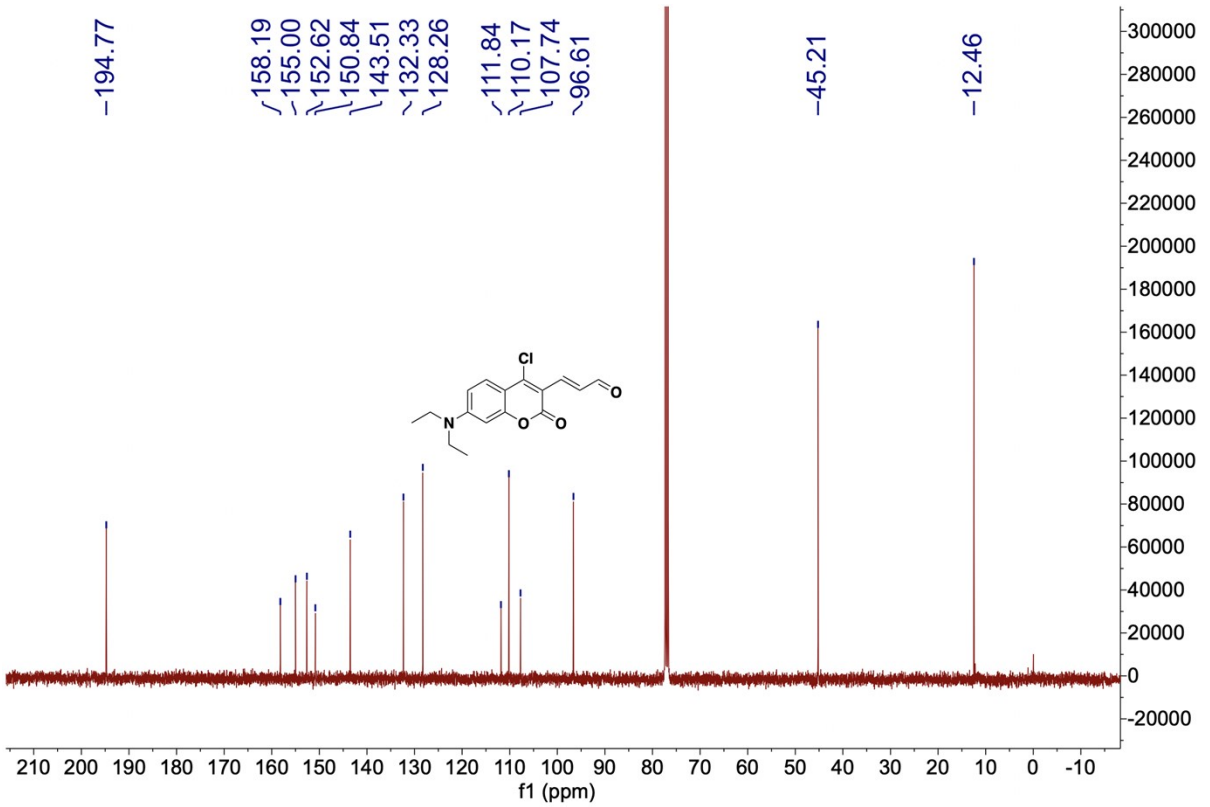
Characterization of the compounds synthesized in this work.

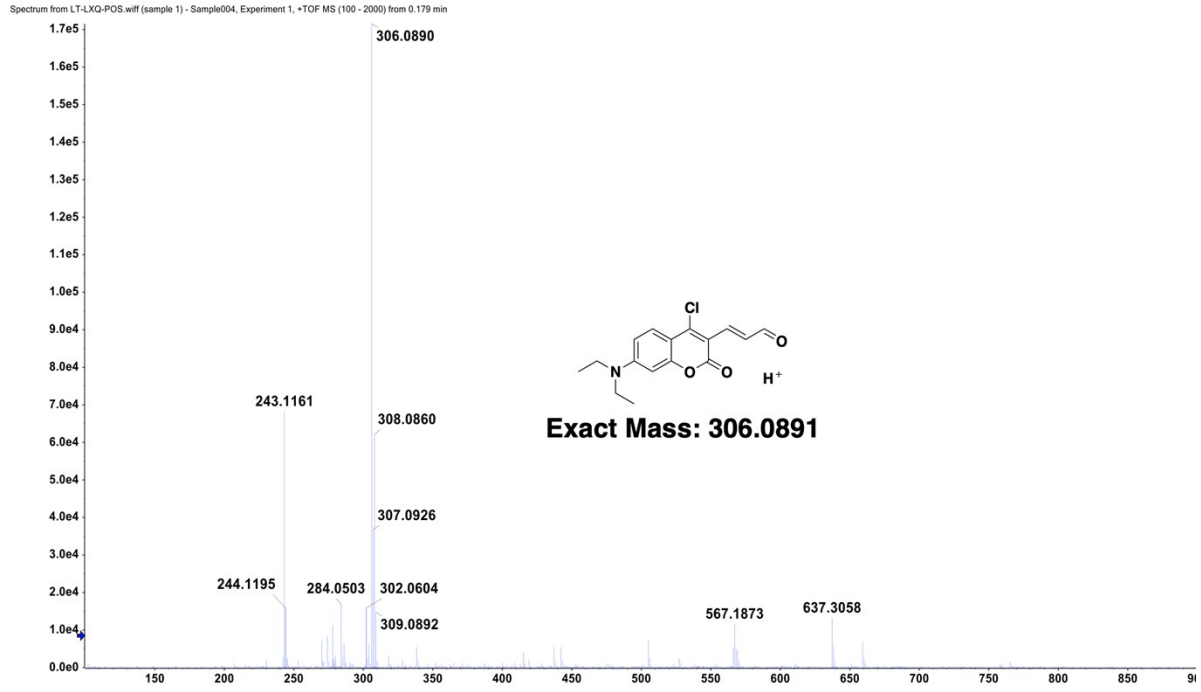


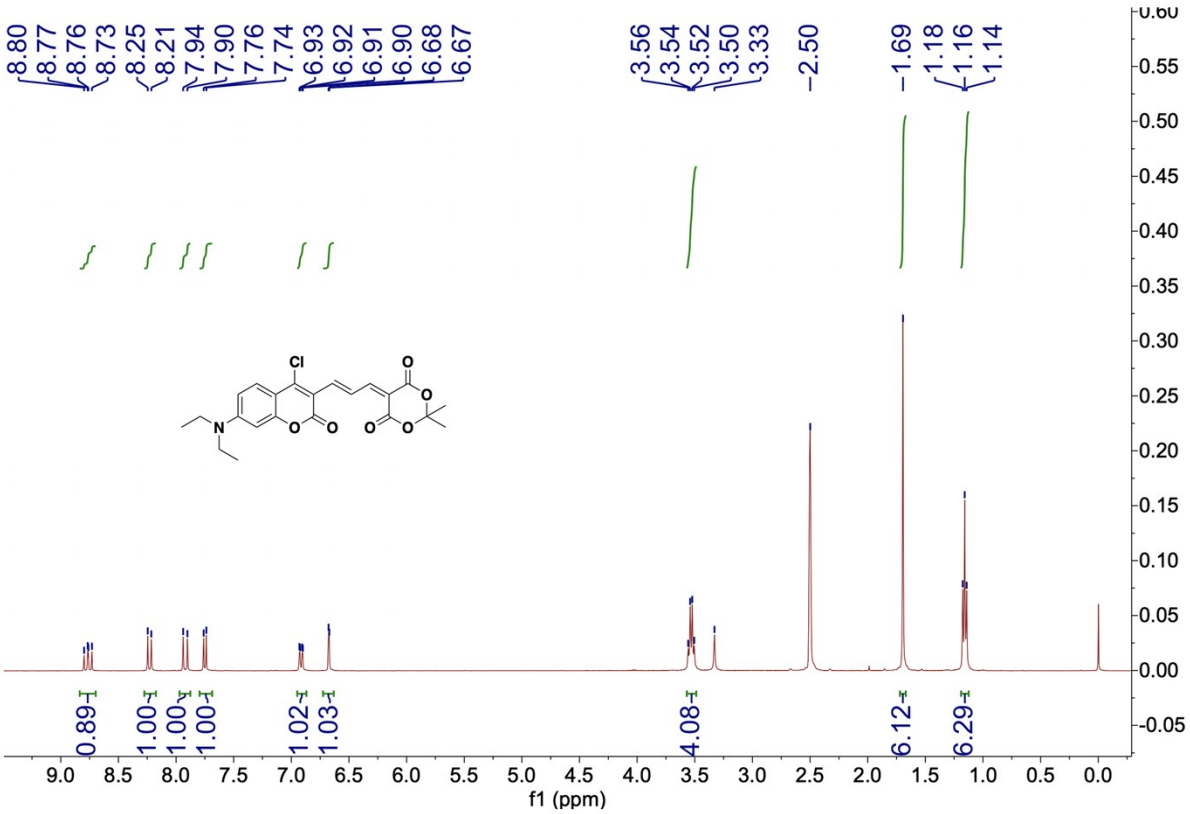


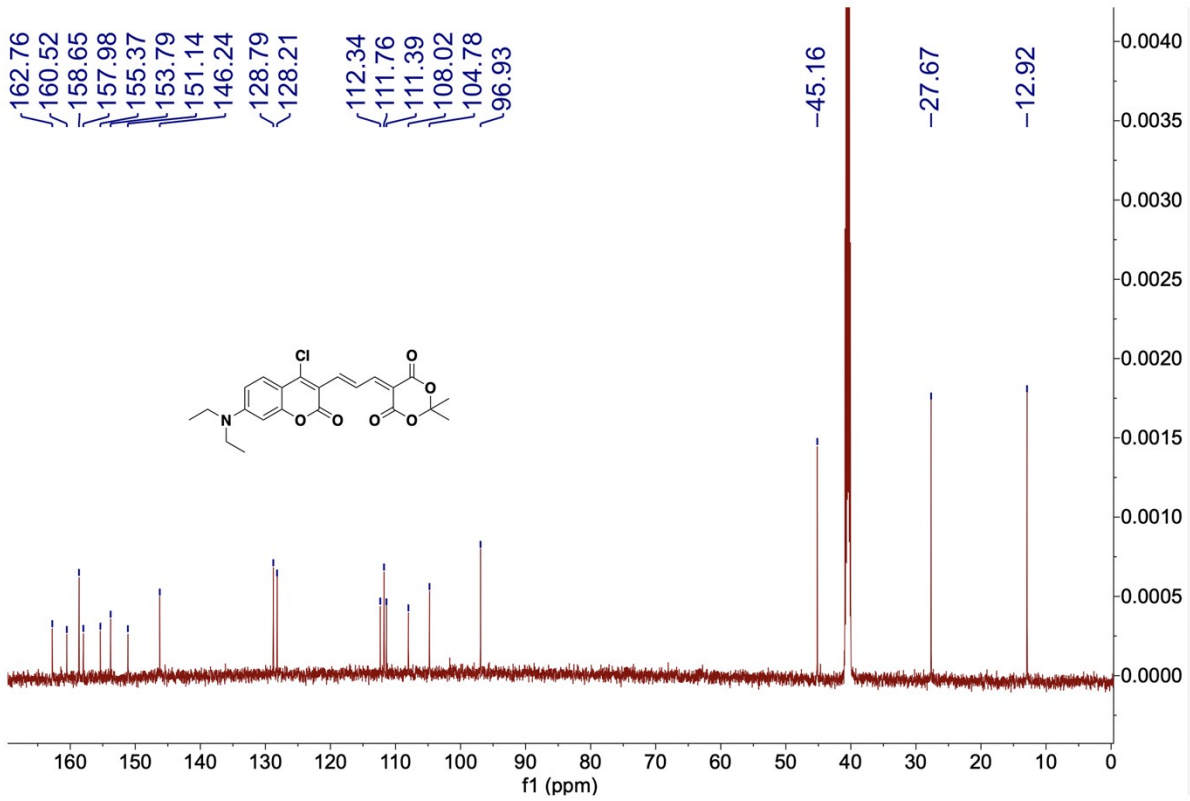

Spectrum from XDSLQ.wiff (sample 1) - Sample008, Experiment 1, +TOF MS (100 - 2000) from 0.179 min
280.0729
282.0705
281.0764
302.0557
312.1001
283.0736
Cl
O
N
O
O
H+
Exact Mass: 280.0735


9.71
9.69
7.77
7.75
7.73
7.54
7.52
7.50
7.48
7.26
6.74
6.73
6.71
6.71
6.51
6.50
3.50
3.48
3.47
3.45
1.27
1.26
1.24
Cl
O
N
O
O
1.00
0.89
1.02
0.58
0.41
1.05
0.99
4.05
6.10
f1 (ppm)

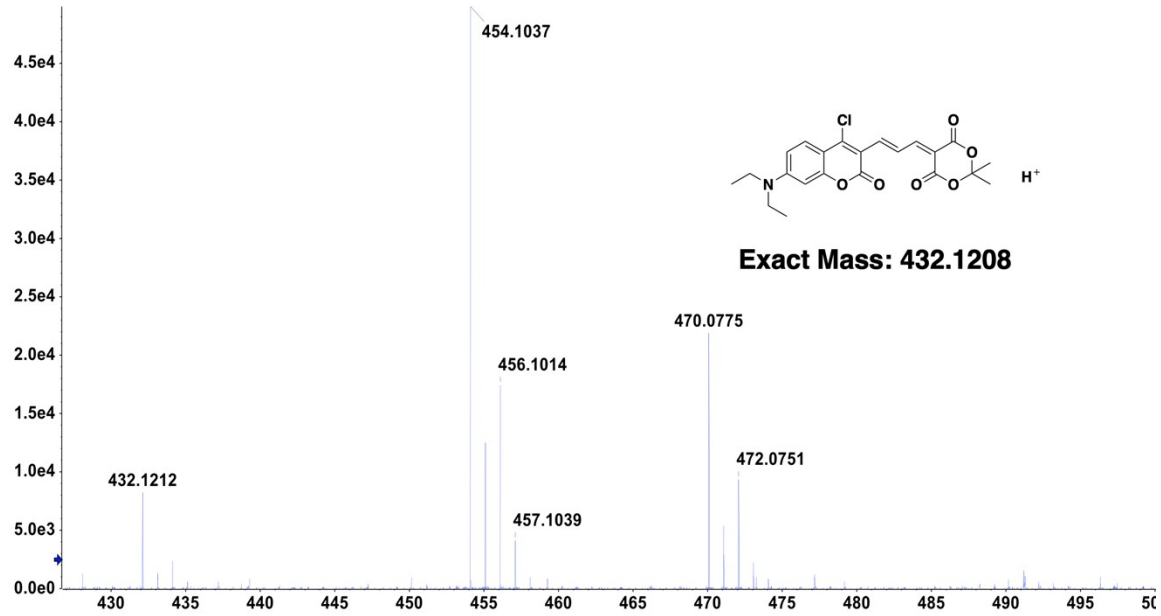

194.77
158.19
155.00
152.62
150.84
143.51
132.33
128.26
111.84
110.17
107.74
96.61
45.21
12.46
Cl
O
N
O
O
f1 (ppm)


Spectrum from LT-LXQ-POS.wiff (sample 1) - Sample004, Experiment 1, +TOF MS (100 - 2000) from 0.179 min
306.0890
243.1161
308.0860
307.0926
244.1195
284.0503
302.0604
309.0892
567.1873
637.3058
Cl
O
N
O
O
H+
Exact Mass: 306.0891


8.80
8.77
8.76
8.73
8.25
8.21
7.94
7.90
7.76
7.74
6.93
6.92
6.91
6.90
6.68
6.67
3.56
3.54
3.52
3.50
3.33
2.50
1.69
1.18
1.16
1.14
Cl
O
O
O
O
O
O
O
N
0.89
1.00
1.00
1.00
1.02
1.03
4.08
6.12
6.29
f1 (ppm)


162.76
160.52
158.65
157.98
155.37
153.79
151.14
146.24
128.79
128.21
112.34
111.76
111.39
108.02
104.78
96.93
45.16
27.67
12.92
Cl
O
O
O
O
O
O
O
N
f1 (ppm)

Spectrum from LT-MSS.wiff (sample 1) - Sample001, Experiment 1, +TOF MS (100 - 2000) from 0.218 min



Exact Mass: 432.1208