Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Fluorescent Detection of Glutathione S-Transferases in Low GSH Level Environment

Table of Contents

General Information **2**
Figure S1 ..... **3**
Figure S2 ..... **4**
Figure S3 ..... **4**
Figure S4 ..... **5**
Figure S5 ..... **5**
Cell culture **6**
MTT experiments **6**
Figure S6 ..... **6**
Fluorescent microscope imaging **6**
Synthesis of the probe **7**
^{1}H NMR and ^{13}C NMR spectrum **9**

General Information

Chemicals were purchased from commercial suppliers and used without purification. NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. Data for ^{1}H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a J value in Hertz (Hz); the number of protons (n) for a given resonance is indicated nH, and based on the spectral integration values. Analytical TLC was performed with 0.25 mm silica gel 60F plates with fluorescent indicator (254 nm or 365 nm). Flash column chromatography was performed using silica gel (particle size 200-300 mesh). HPLC was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an inline diode array UV-Vis detector. A reversed-phase C18 (Inertsil, 5 µm, 4.6 x 250 mm) column was used with a CH_3CN (B) / H_2O (A) gradient mobile phase containing 0.1% trifluoroacetic acid at a flow of 1 mL/min for the analysis. Absorption spectra were obtained on a Hitachi U3310 fluorometer. Fluorescence spectra were obtained on a Hitachi F4500 fluorometer. Kinetic experiments were performed in a Thermo Scientific Varioskan LUX 3020-265 microplate reader. Fetal Bovine Serum (FBS), PBS and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco. Glutathione S-transferase was purchased from Sigma Aldrich. A549 cells were purchased from Procell Life Science & Technology Co., Ltd. Fluorescent images of A549 cells were obtained with Nikon Eclipse Ti microscope.

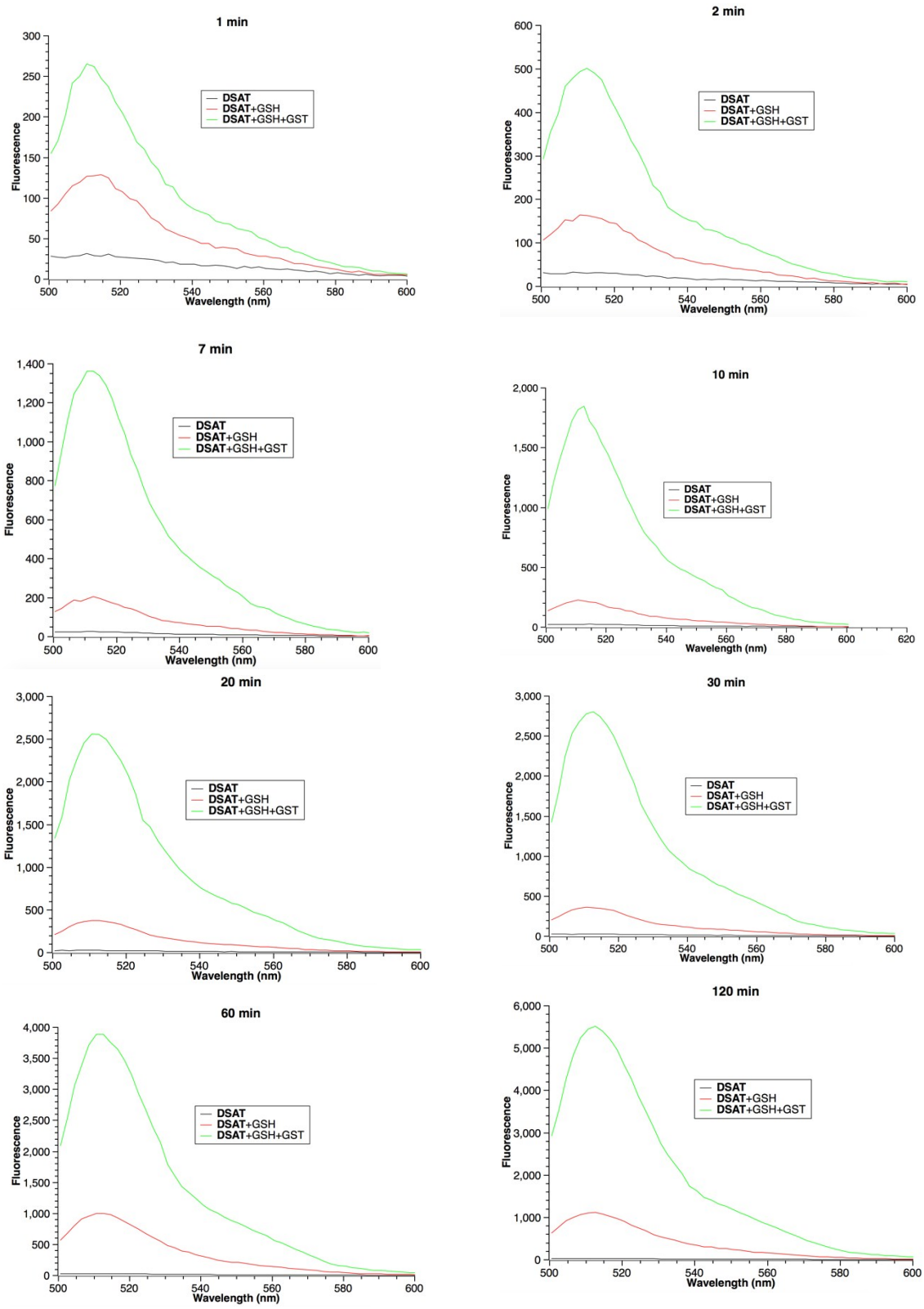


Figure S1 Fluorescent spectrum of **DSAT** (1 μM in PBS, pH = 7.4) in the absence and presence of GST (10 μg/mL) and GSH (800 μM) at different time points under 37 °C. Fluorescence data were collected with excitation at λ = 490 nm and emission at λ = 510 nm on fluorometer.

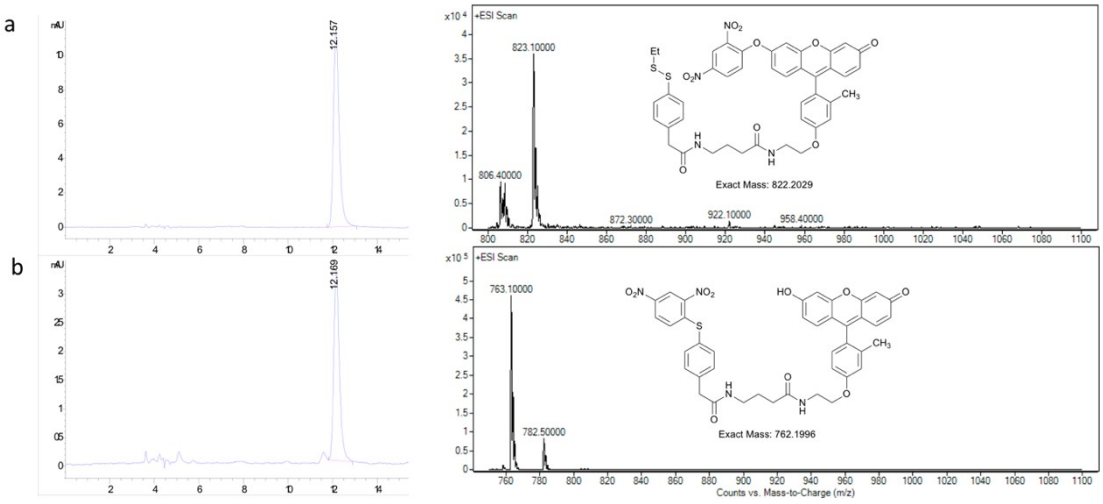


Figure S2 HPLC traces and LC-MS spectra of **DSAT** and the uncaged product.

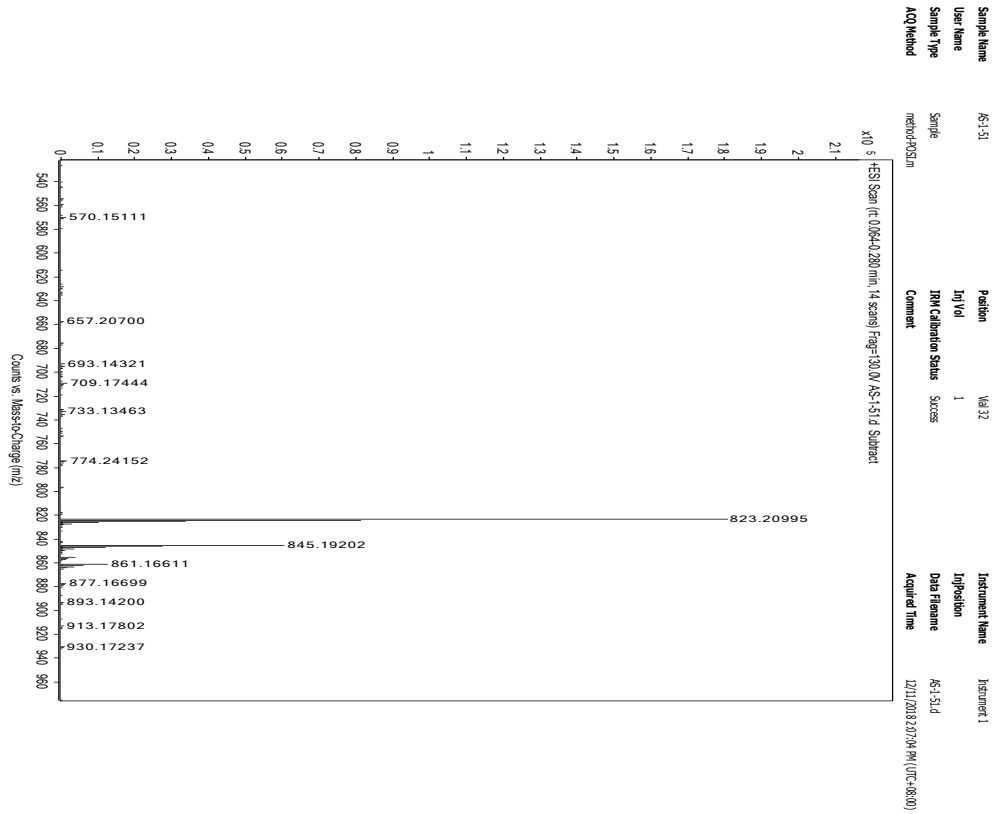


Figure S3 High resolution mass spectrum (HRMS) of **DSAT**.

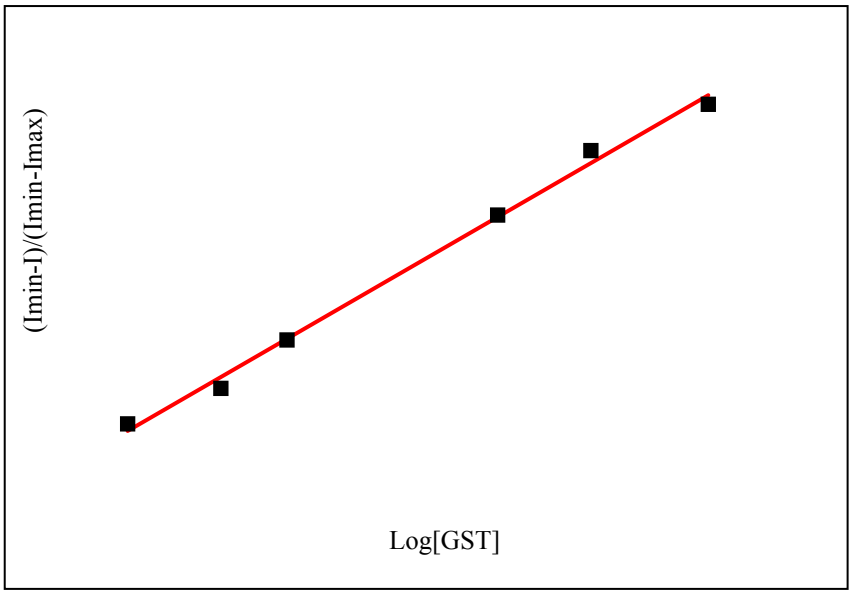


Figure S4 Normalized response of the fluorescence signal to various GST concentrations. A linear regression curve was then fitted to the normalized fluorescence intensity data, and the point at which the line crossed the ordinate axis was considered to be the detection limit (0.12 μg/mL).

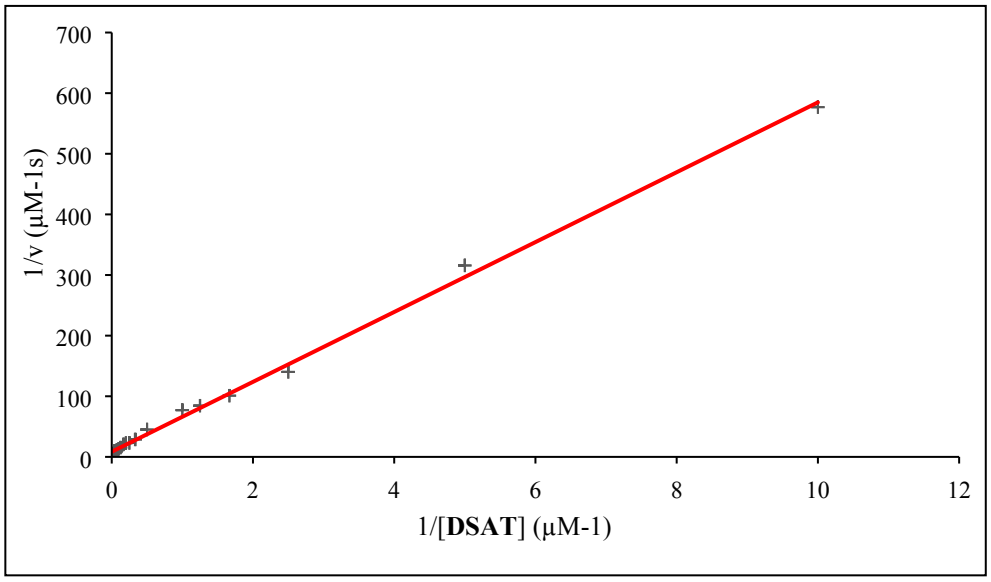


Figure S5 Lineweaver-Burk plot for the GST-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}$ [**DSAT**] / (K_m + [**DSAT**]), where V is the reaction rate, [**DSAT**] is the probe concentration (substrate), and K_m is the Michaelis constant. Conditions: 2 μg/mL GST, 0.8 mM GSH, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 50, and 100 μM of probe, $\lambda_{ex}/_{em}$ = 485/510 nm. Reaction at each concentration was repeated three times, and the error bars represent standard deviations. Points were fitted using a linear regression model (correlation coefficient R = 0.9976). V_{max} = 0.118 μM/s, K_m = 489.35 μM.

Cell culture

A549 cell lines were cultured in DMEM medium supplemented with 10% fetal calf serum (FBS) and penicillin/streptomycin mixture (penicillin 100 U ml^{-1}, streptomycin 100 μg ml^{-1}) at a concentration of 2 × 10^5/mL. The cells were cultured in a saturated humidity incubator at a temperature of 37 °C and 5% CO_2.

MTT experiments

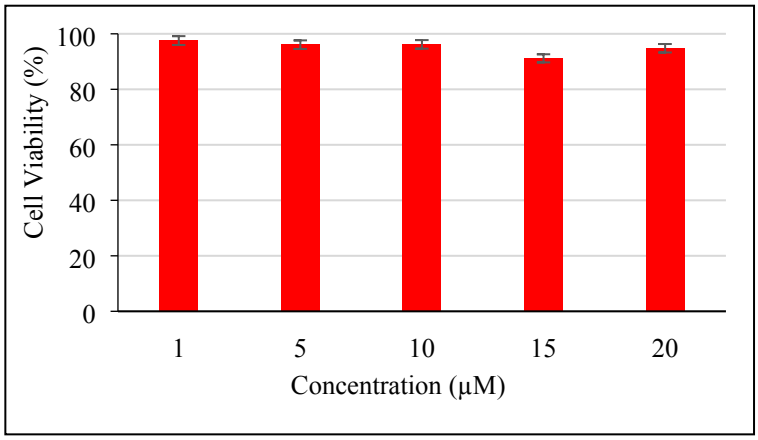


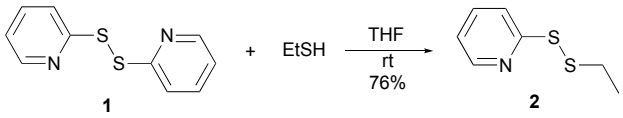
Figure S6 Effects of DSAT with varied concentrations (1, 5, 10, 15, 20 μM) on the viability of A549 cells. The viability of the cells without DSAT is defined as 100%. The results are the mean ± standard deviation of five separate measurements.

A549 cells were seeded into 96-well cell culture plates with a concentration of 5 × 10^3 cells/well. After incubation for 24 h, the medium was exchanged with a DMEM medium containing different concentrations of **DSAT** (0, 1, 5, 10, 15, 20 μM). The cells were incubated for 2 h and the medium was changed to fresh DMEM without probe. The absorbance was recorded on plate reader after incubation for another 24 h.

Fluorescent microscope imaging

A549 cells were seeded into culture dishes for laser confocal microscopy with a concentration of 5 × 10^3 cells/well. After incubation for 24 h, the cells was pre-treated with NMM, NEM, EA, GSH as indicated in figure 6 and 7 before incubation with **DSAT** solution (1 μM) in DMEM. Excessive DSAT was washed before obtaining the fluorescent images of A549 cells on Nikon Eclipse Ti microscope.

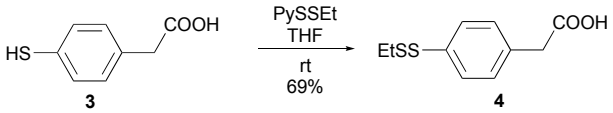
Synthesis of the probe



1 2

2-(Ethyldisulfaneyl)pyridine (2)

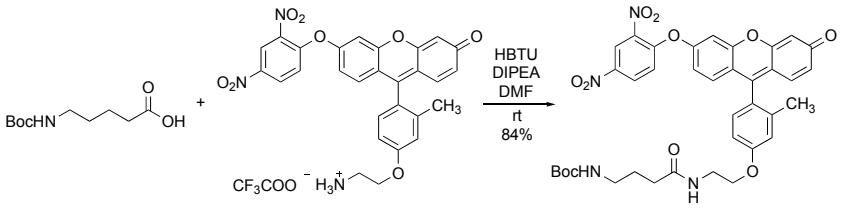
The solution of 1,2-di(pyridin-2-yl)disulfane (7.04 g, 32 mmol) in 30 mL THF was charged with Ar. Then EtSH (2.36 mL, 32 mmol) was added to the above solution and the reaction was allowed to stir overnight at room temperature. The solution was concentrated on rotaevaporator and the crude product was purified by column chromatography (4.16 g, 76%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.45 (d, *J* = 4.9 Hz, 1H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.64 (td, *J* = 7.7, 1.8 Hz, 1H), 7.07 (dd, *J* = 7.4, 4.9 Hz, 1H), 2.82 (q, *J* = 7.3 Hz, 2H), 1.33 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.51, 149.42, 136.85, 120.37, 119.37, 32.67, 14.10.



3 4

2-(4-(Ethylsulfinothioyl)phenyl)acetic acid (4)

2-(Ethyldisulfaneyl)pyridine (PySSEt) (1.47 g, 8.6 mmol) was added to a 2-(4-mercaptophenyl)acetic acid (1.31g, 5.9 mmol) solution in 15 mL THF and charged with Ar. The mixture was stirred for 5 h under room temperature before being diluted with water. The product was extracted with EtOAc and the organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (1.227 g, 69%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 3.62 (s, 2H), 2.74 (q, *J* = 7.3 Hz, 2H), 1.30 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.85, 136.84, 131.64, 129.93, 127.54, 40.47, 32.58, 14.09.

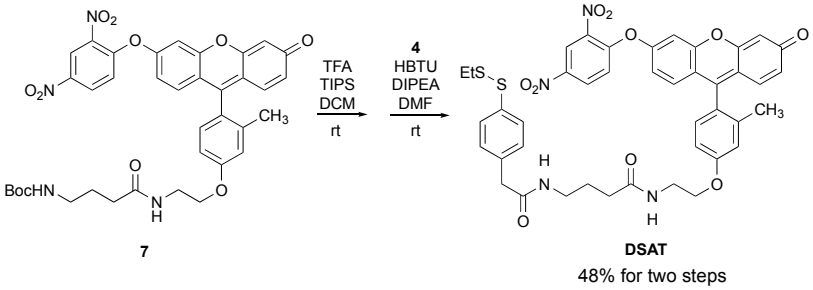


5 6 7

tert-Butyl (4-((2-(4-(6-(2,4-dinitrophenoxy)-3-oxo-3*H*-xanthen-9-yl)-3-methylphenoxy)ethyl)amino)-4-oxobutyl)carbamate (7)

To a solution of compound **5** (2.1 g, 10.3 mmol), HBTU (5.87 g, 15.5 mmol) and DIPEA (2.57 mL, 15.5 mmol) in anhydrous DMF (15 mL) was added the mixture of compound **6** (3.24 g, 5.2 mmol) and DIPEA (1.71 mL, 10.3 mmol) in 5 mL DMF. The mixture was diluted with EtOAc after the completion of the reaction monitored by TLC. The diluted solution was washed with water and the product was further extracted with EtOAc. The combined organic layer was washed with brine, dried with sodium sulfate and evaporated to give the crude product which

was purified by column chromatography (3.091 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (d, *J* = 2.8 Hz, 1H), 8.54 (dd, *J* = 9.1, 2.7 Hz, 1H), 8.11 (d, *J* = 5.8 Hz, 1H), 7.57 (d, *J* = 9.2 Hz, 1H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.21 (t, *J* = 9.8 Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.10 (s, 1H), 7.02 (d, *J* = 8.6 Hz, 1H), 6.96 (d, *J* = 9.8 Hz, 1H), 6.82 (s, 1H), 6.48 (d, *J* = 9.8 Hz, 1H), 6.23 (s, 1H), 4.08 (d, *J* = 6.0 Hz, 2H), 3.46 (t, *J* = 5.8 Hz, 2H), 2.90 (t, *J* = 6.7 Hz, 2H), 2.11 (t, *J* = 7.6 Hz, 2H), 2.04 (s, 3H), 1.61 (t, *J* = 7.4 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO) δ 184.29, 172.25, 159.30, 158.40, 158.04, 155.60, 153.25, 152.93, 147.86, 142.90, 140.38, 137.60, 130.76, 130.54, 130.31, 129.89, 123.84, 122.17, 122.08, 119.82, 117.94, 116.47, 116.19, 112.37, 107.13, 105.09, 77.42, 66.46, 54.89, 32.81, 28.25, 25.83, 19.48.

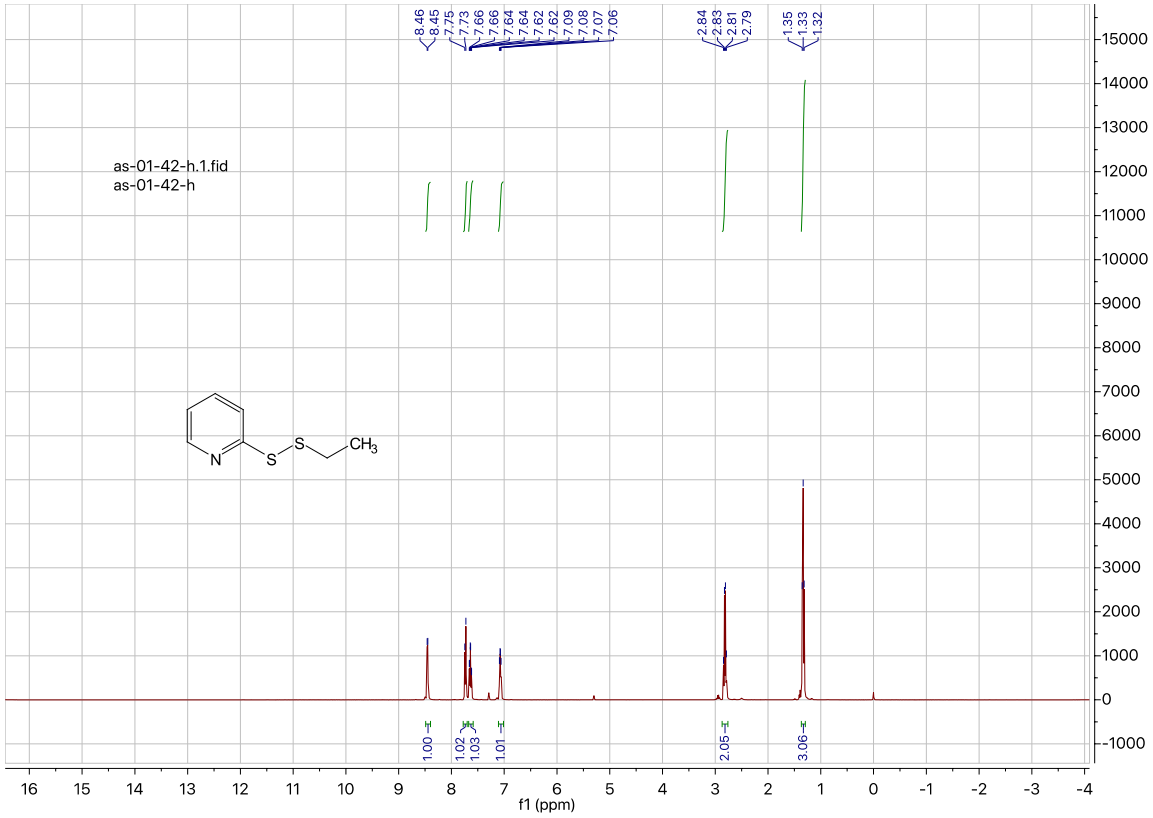


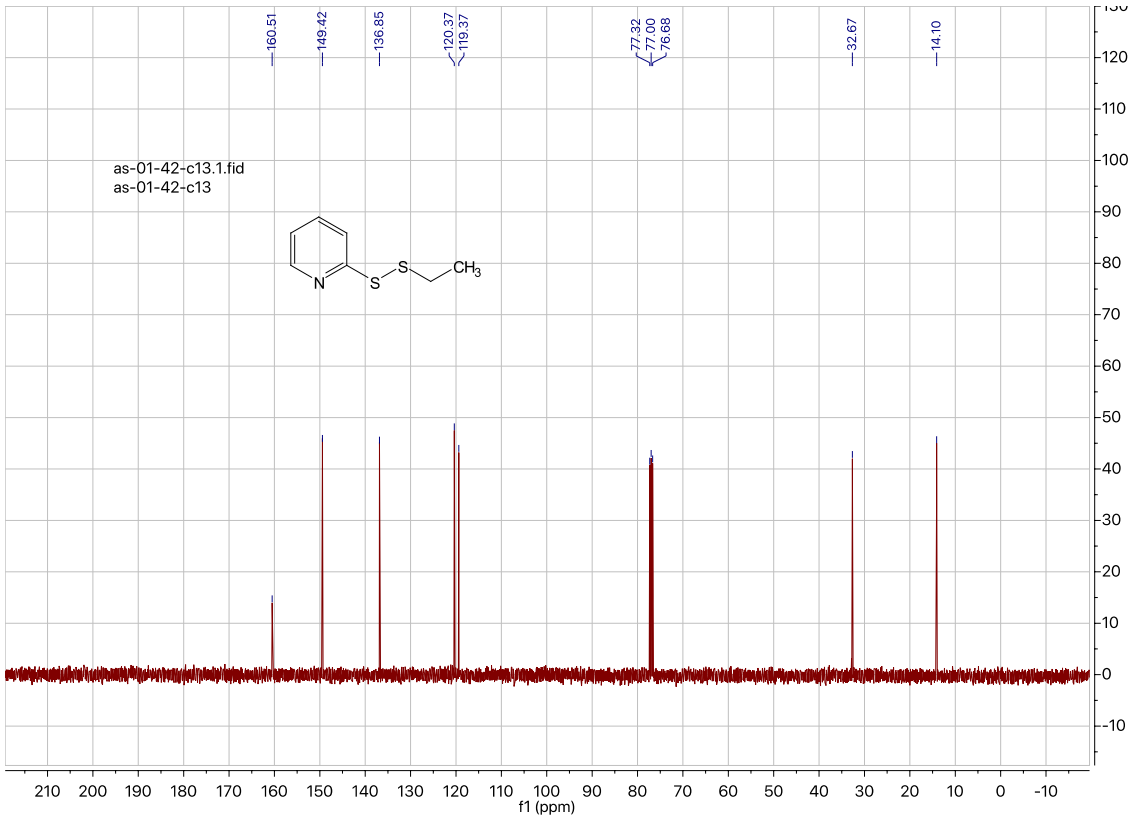
_N_-(2-(4-(6-(2,4-dinitrophenoxy)-3-oxo-3_H_-xanthen-9-yl)-3-methylphenoxy)ethyl)-4-(2-(4-(ethyldisulfaneyl)phenyl)acetamido)butanamide (DSAT)

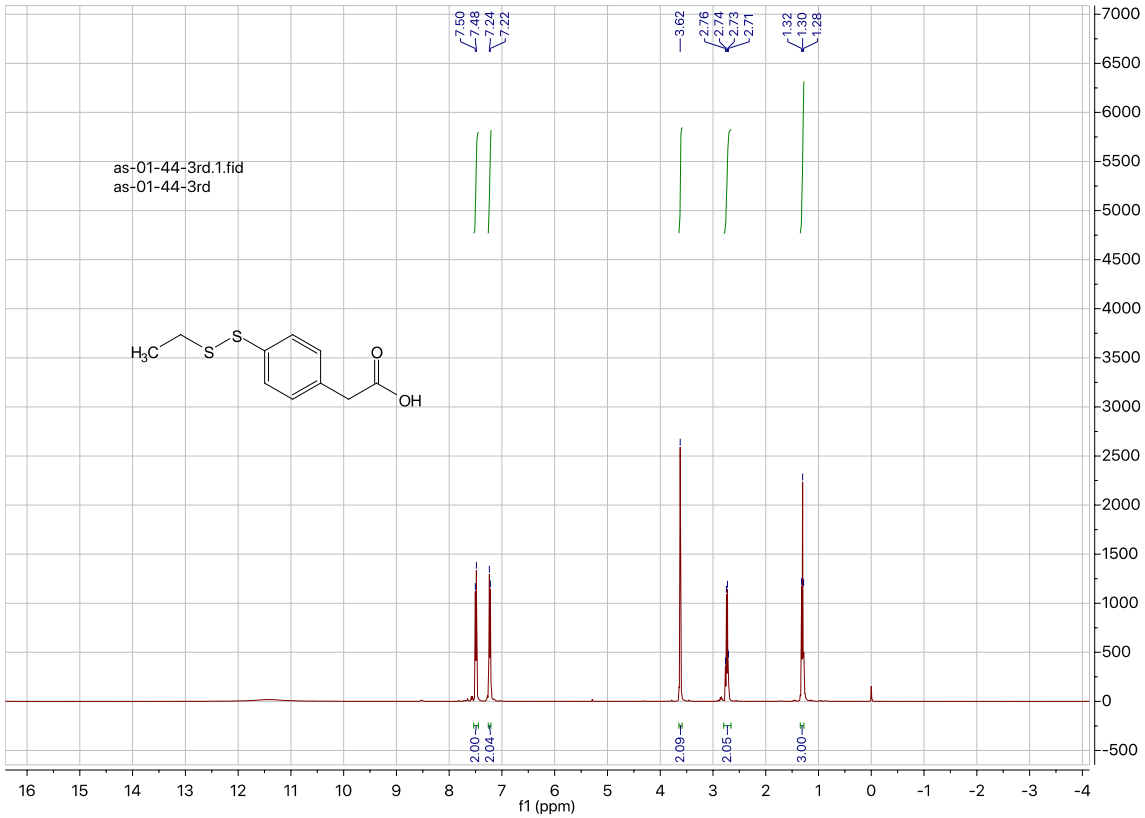
Compound **7** (2.39 g, 3.35 mmol) was dissolved in DCM. Then, 5 mL TFA and 1 mL TIPS were added and the mixture was stirred was concentrated on rotaevaporator after the completion of the reaction. The crude product was used in the next step withut further purification.

To a solution of compound **4** (0.766 g, 3.35 mmol), HBTU (2.55 g, 6.73 mmol) and DIPEA (2.78 mL, 16.77 mmol) in anhydrous DMF (10 mL) was added the above product in 5 mL DMF. The mixture was diluted with EtOAc after the completion of the reaction monitored by TLC. The diluted solution was washed with water and the product was further extracted with EtOAc. The combined organic layer was washed with brine, dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (1.326 g, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 – 8.89 (m, 1H), 8.54 (d, *J* = 9.2 Hz, 1H), 8.17 – 8.05 (m, 2H), 7.57 (d, *J* = 9.2 Hz, 1H), 7.50 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.26 (d, *J* = 7.7 Hz, 2H), 7.20 (t, *J* = 7.8 Hz, 2H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.09 (s, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 9.9 Hz, 1H), 6.48 (d, *J* = 9.8 Hz, 1H), 6.24 (s, 1H), 4.08 (d, *J* = 6.1 Hz, 2H), 3.47 (d, *J* = 5.9 Hz, 2H), 3.38 (s, 2H), 3.03 (t, *J* = 6.9 Hz, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.12 (d, *J* = 7.7 Hz, 2H), 2.02 (s, 3H), 1.65 (t, *J* = 7.5 Hz, 2H), 1.22 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 184.29, 172.13, 169.73, 159.26, 158.41, 158.06, 153.24, 152.86, 147.85, 142.92, 140.41, 137.59, 135.65, 134.48, 130.78, 130.52, 130.32, 130.27, 129.89, 127.41, 123.83, 122.16, 122.08, 119.81, 117.92, 116.44, 116.16, 112.38, 107.10, 105.08, 66.43, 54.90, 41.81, 38.38, 32.78, 31.93, 25.35, 19.46, 13.97. HRMS: Calculated for $C_{42}H_{38}N_4O_{10}S_2^+$ ([M+H]$^+$): 823.2108, Found 823.20995.

^{1}H NMR and ^{13}C NMR spectrum



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1.30
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2.09
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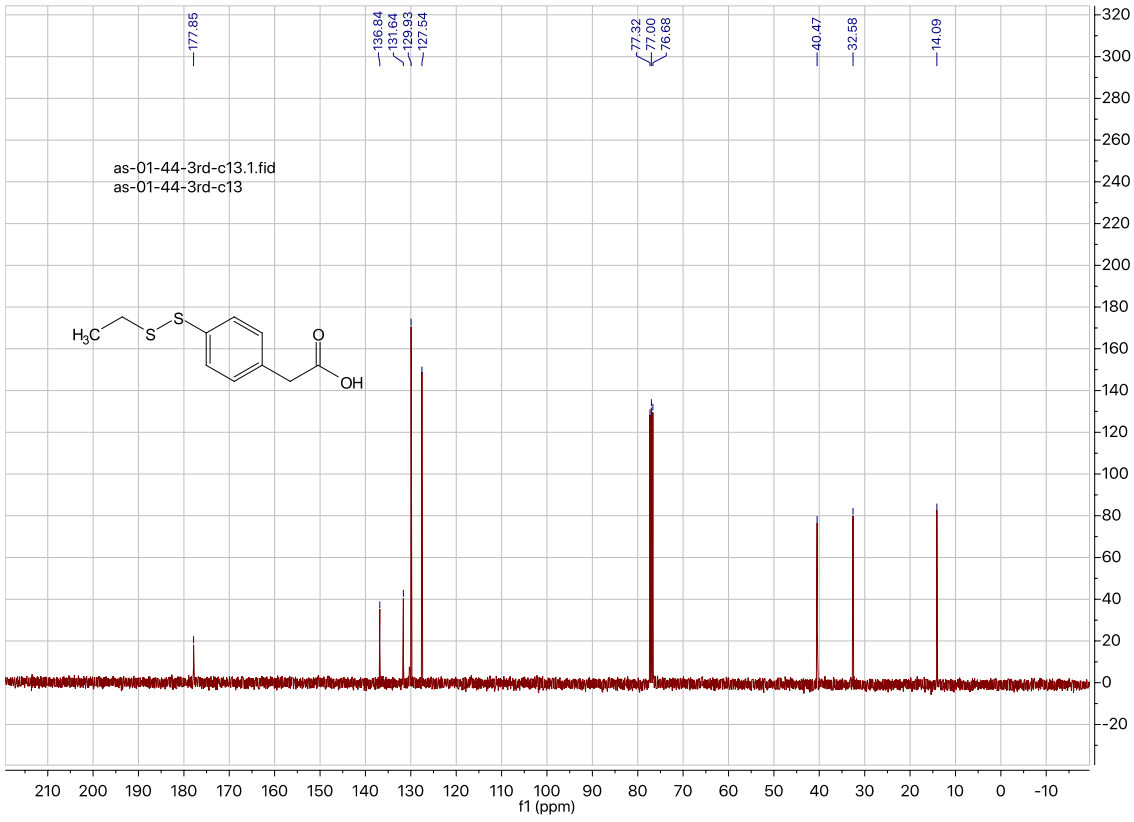
f1 (ppm)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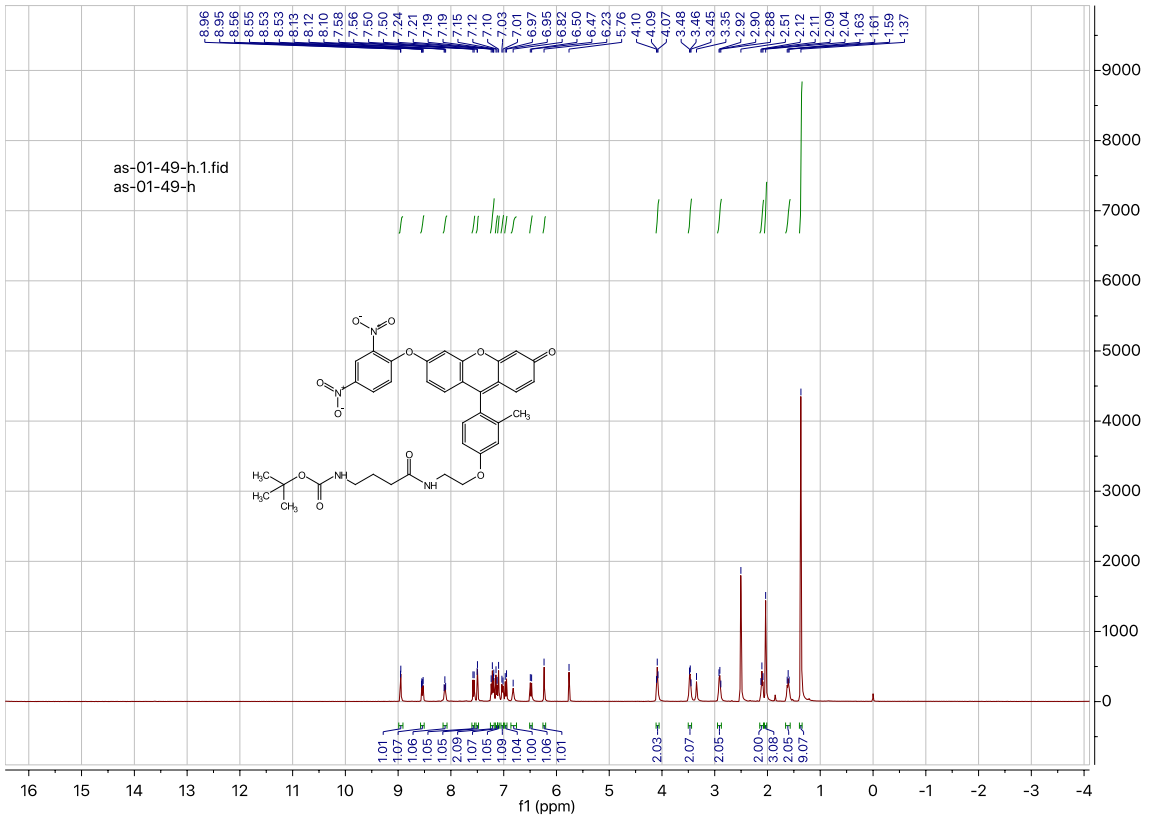
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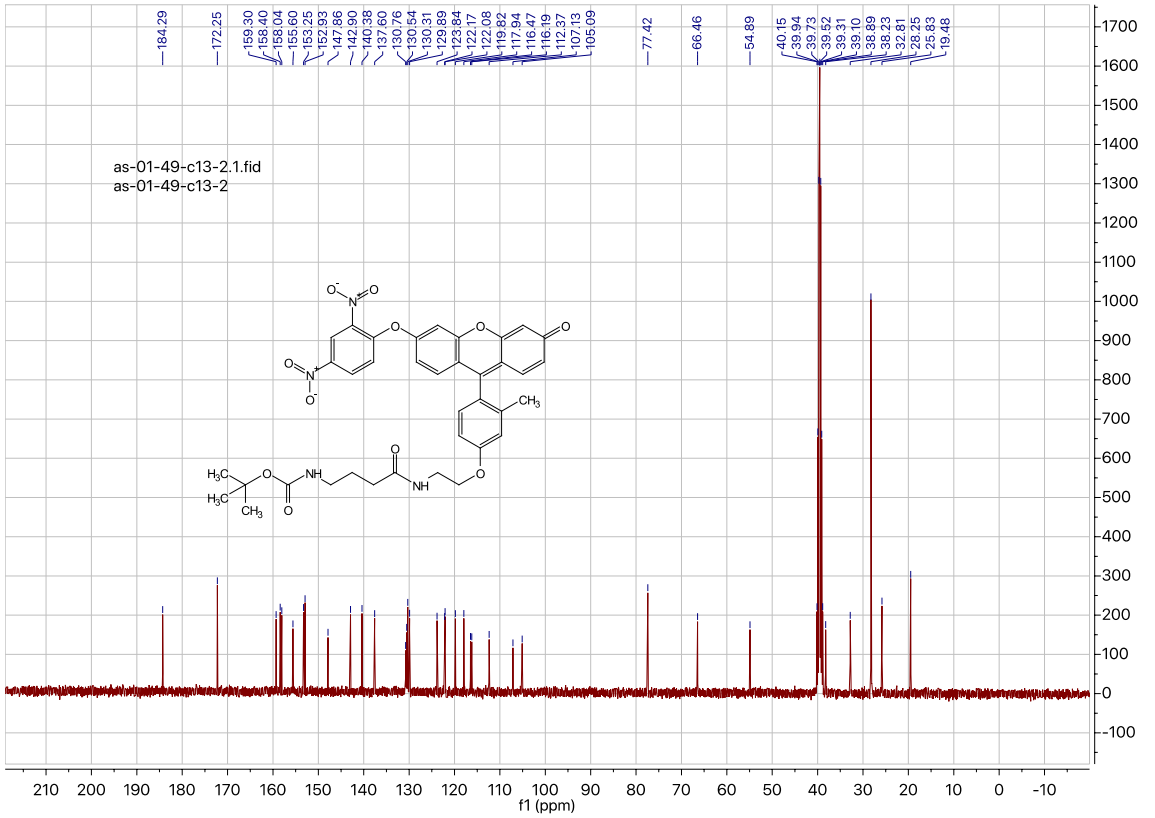
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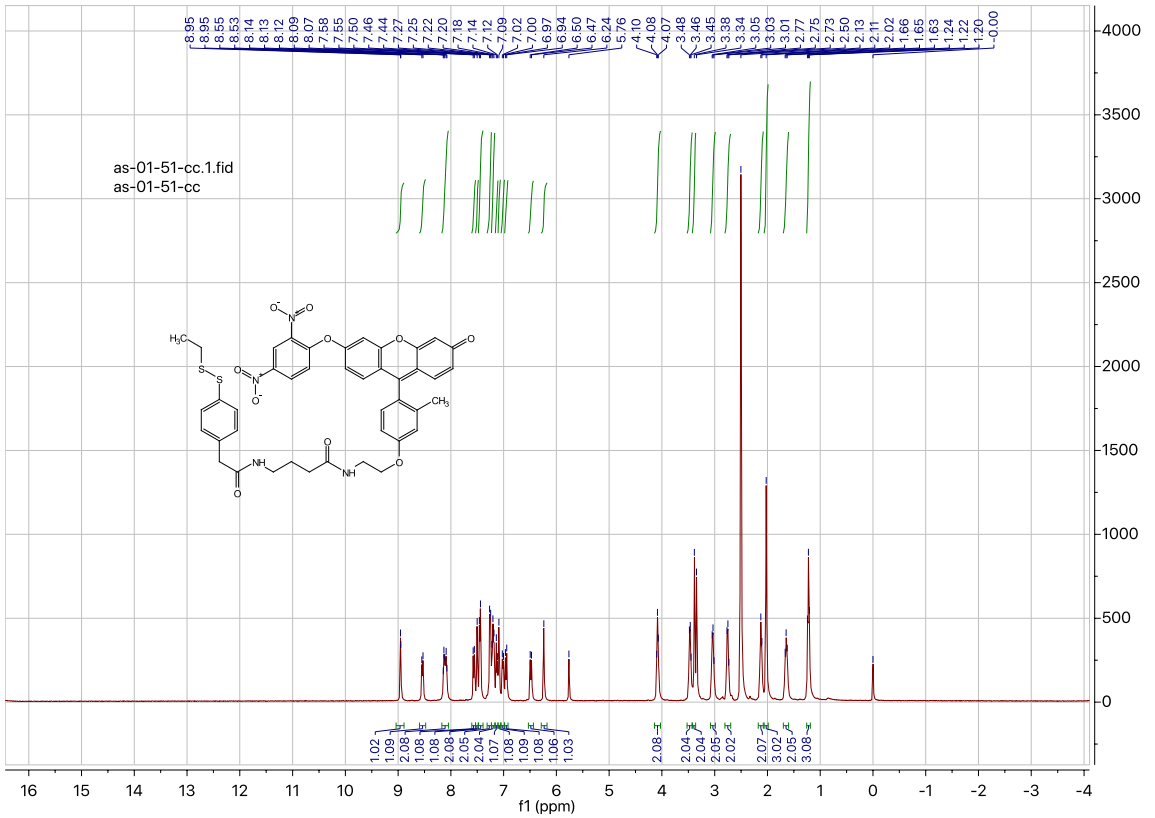
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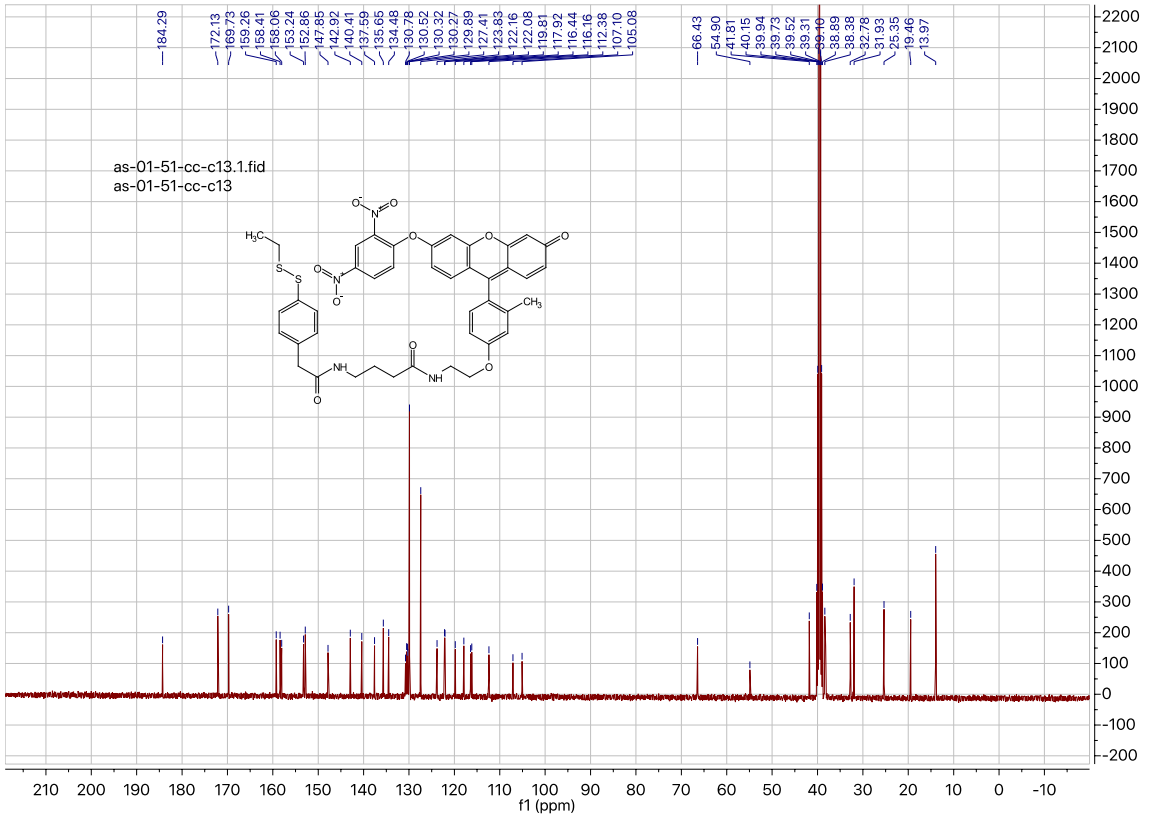
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