Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Bromo- and thiomaleimides as a new class of thiol-mediated fluorescence 'turn-on' reagents

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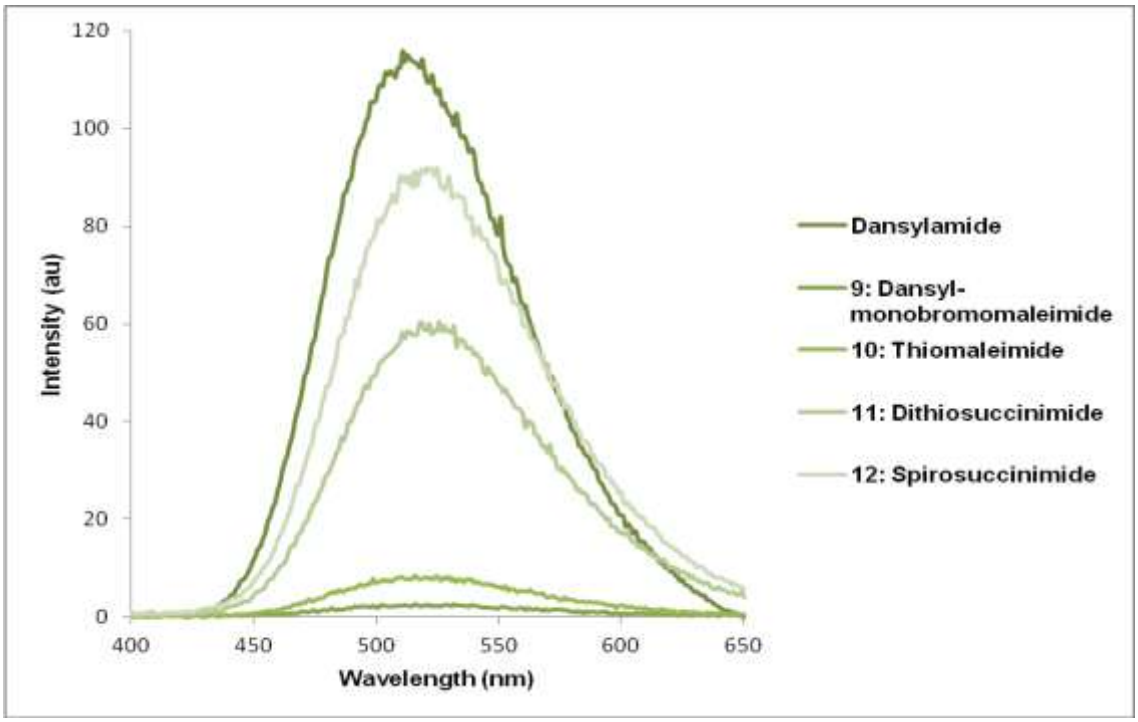
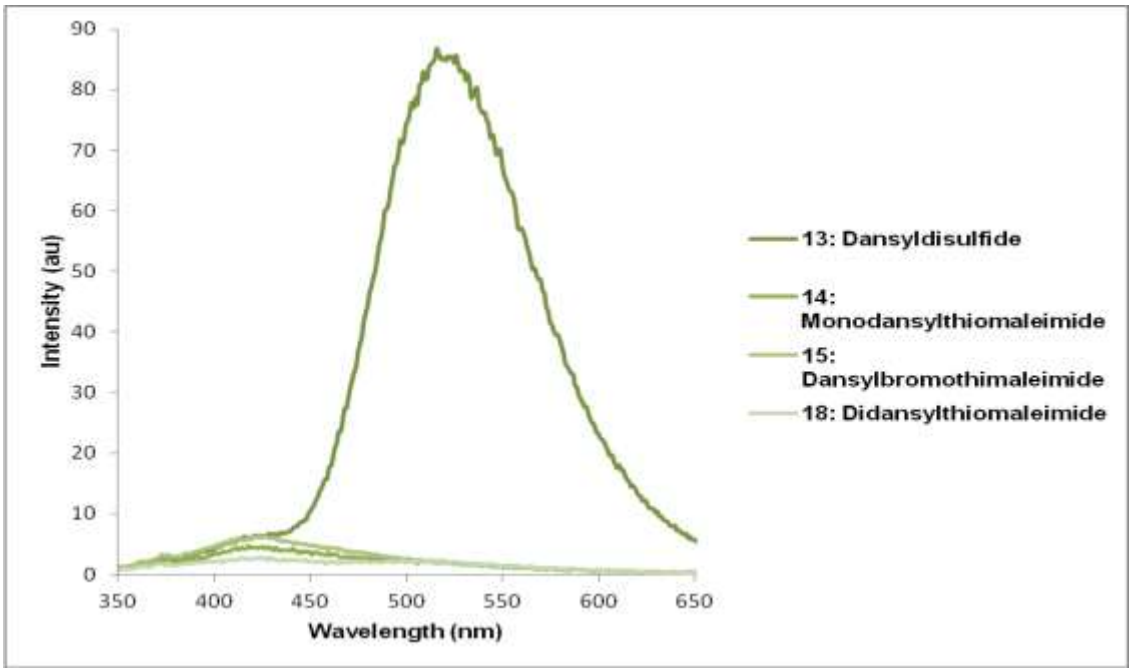
Supplementary Figure 1: Fluorescence Spectra of Dansylamide and Dansylmaleimide compounds 9-12



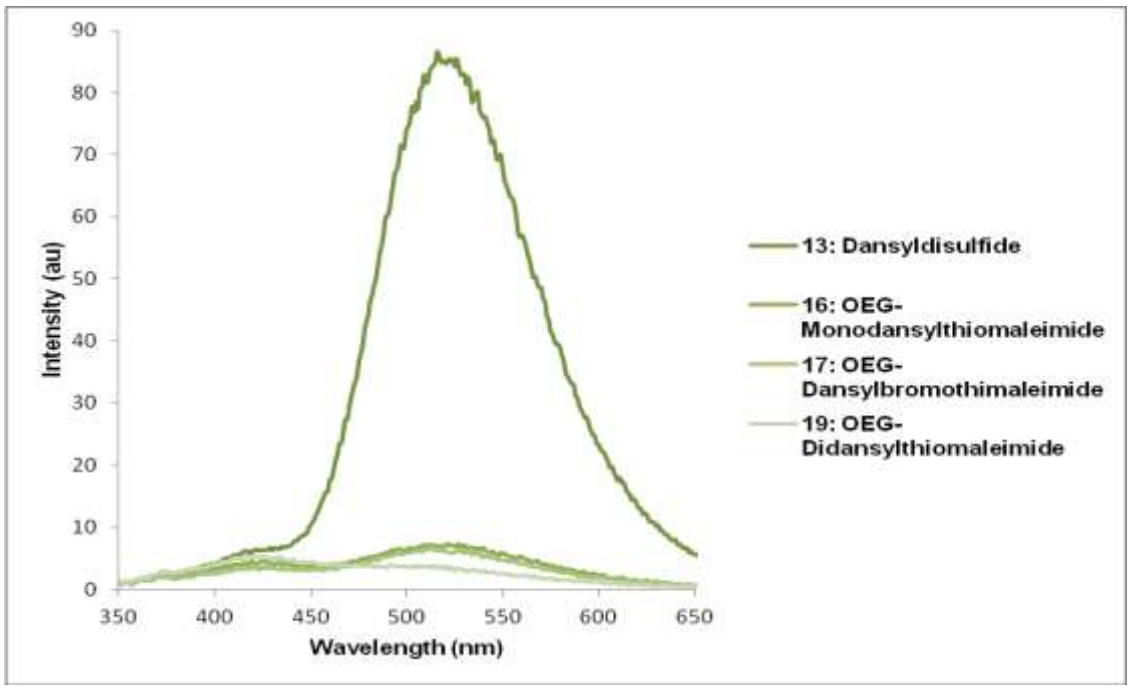
Fluorescence spectra of dansylamide (8 µM), dansyl-monobromomaleimide **9** (8 µM), thiomaleimide **10** (8 µM), dithiosuccinimide **11** (8 µM) and spirosuccinimide **12** (8 µM) in Acetonitrile at 25°C, λ_{exc} = 335 nm.

Supplementary Figure 2: Fluorescence Spectra of Dansyldisulfide 13 and Dansylmaleimide compounds 14, 15 and 18



Fluorescence spectra of dansyldisulfide **13** (5 µM) and dansylmaleimide compounds **14** (10 µM), **15** (10 µM) and **18** (5 µM) in acetonitrile at 25°C, λ_{exc} = 335 nm.

Supplementary Figure 3: Fluorescence Spectra of Dansyldisulfide 13 and Dansylmaleimide compounds 16, 17 and 19



Fluorescence spectra of dansyldisulfide **13** (5 µM) and dansylmaleimide compounds **16** (10 µM), **17** (10 µM) and **19** (5 µM) in acetonitrile at 25°C, λ_{exc} = 335 nm.

Supplementary Figure 4. Control images for cell imaging.

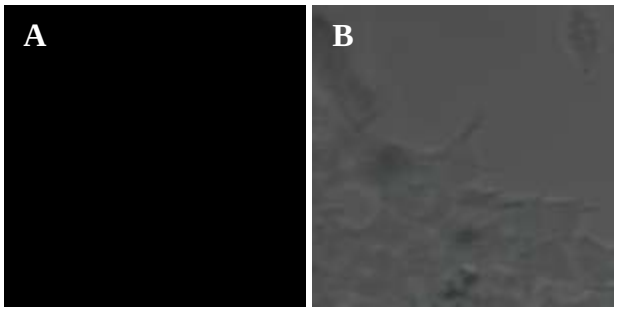


Figure 1.13 Confocal fluorescence images of HEK cells: (A) DMS:PBS Buffer pH 7 1:99 for 15 minutes at 37 °C; (B) Brightfield image of (A).

General Experimental

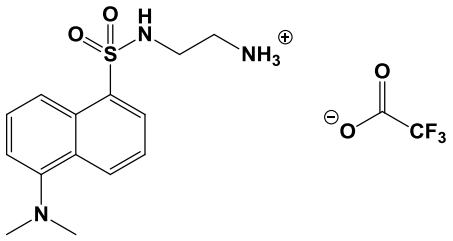
All reactions were carried out at atmospheric pressure, under argon, unless otherwise stated. Solvents and reagents were purchased from commercial suppliers and used without further purification unless stated. Normal phase silica gel, 40-63 μM, (BDH) and sand (VWR) were used for flash chromatography (FC). All reactions were monitored by TLC unless otherwise stated using TLC plates pre-coated with silica gel 60 F_{254} on aluminium (Merck KGaA). Detection was by UV (254 nm and 366nm) or by chemical stain (potassium permanganate or vanillin) and heat. LCMS was performed on a Waters Acquity uPLC and SQ detector, λ=254nm on C18 BEH 1.7 μM, 2.1 mm × 5.0 mm column, solvent; acetonitrile: H_2O, 98:2 – 2:98 over 30 minutes. HPLC purification was performed using reverse phase Varian ProStar system with a Model 210 solvent delivery mode and a Model 320 dual wave-length detector equipped with a Polaris C18 column (100 x 21.2 mm, 5 mm beads). Mass Spectroscopy; Electrospray (ES) was performed using a Waters LCT Premier XE, Chemical Ionisation (CI) and Electron Impact Ionisation (EI) was performed using a Thermo Finnigan MAT 900xp. Fluorescence measurements were performed using a Carey Eclipse Fluorescence Spectrophotometer. UV - visible measurements were performed using a Carey Win UV 100 Series Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz respectively on a Bruker Avance III 600, ^{1}H/^{13}C cryoprobe, z-gradients at ambient temperature, unless otherwise stated; all chemical shifts are reported as δ (ppm) and were referenced to the residual proton impurity of the deuterated solvent. For NMR experiments, $CDCl_3$ denotes deuterated (d^3) chloroform, DMSO denotes (d^6) dimethylsulfoxide, and CD_3OD denotes deuterated (d^4) methanol, CD_3CN denotes deuterated (d^3) acetonitrile. Deuterated solvents were chosen according to the position of the solvent peak in spectra and the solubility of the substrate. The multiplicity of the signal is indicated as s-singlet, d-doublet, t-triplet, q-quartet, qn-quintet, dd-double of doublets, dt-doublet of triplets, td-triplet of doublets, qd-quartet of doublets or m-mulitplet where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. All peaks should be taken as sharp unless otherwise described. Coupling constants are quoted in Hz to one decimal place. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode with frequencies given in reciprocal centimetres (cm^{-1}). Melting points were measured with a Gallenkamp apparatus and are uncorrected. Room temperature is defined as between 19-22°C. Optical rotation measurements were carried out using a PerkinElmer 343 polarimeter with a cell length of 10 cm. *In-vacuo* is used to describe solvent removal by

Büchi rotary evaporation between 17-80°C at approximately 10 mm Hg unless otherwise stated. The term 'de-gassed' refers to the process of removing O_2 from a solution by bubbling argon through the solution. Irradiations were carried out using a medium pressure 125W mercury lamp (Photochemical Reactors Ltd.) and a pyrex immersion well (Photochemical Reactors Ltd.) cooled by a condenser.

The cellular imaging studies were performed using HEK293 cells (Human Embryonic Kidney), immortalised eukaryotic cell lines derived from primary embryonic kidney cells transformed with sheared adenovirus type 5 DNA as previously described (Giblin et al, 1999).[1] Cells were grown and cultured in Minimum Essential Medium (MEM) containing Earle's salts and L-Glutamine (Invitrogen, UK) supplemented with 1% Penicillin-Streptomycin (from a stock of 1 mg/ ml streptomycin and 10,000 units/ml penicillin) and 10% Foetal Bovine Serum (Invitrogen, UK). Cells were grown in a T75 (75 cm^2) tissue culture flask (VWR, Merck House, UK) in a humidified atmosphere of 95% oxygen and 5% CO_2 and typically subcultured when 90% confluent. To passage cells, the culture medium was removed and the cells washed twice with Ca^{2+}, Mg^{2+} free Dulbeccos' phosphate buffered saline (Invitrogen, Paisley, UK). Cells were detached from the flask via incubation for 2 minutes at room temperature with 1 ml 0.25% Trypsin (Invitrogen, Paisley, UK) in Ca^{2+}, Mg^{2+} free Dulbeccos' phosphate buffered saline. To inhibit the tryptic activity, 9 mL of growth medium was added. Pelleted cells were re-suspended and used to seed a new T75 flask. During log phase growth, the doubling time of HEK293 cells was around 24-36 hours, and therefore it was necessary to subculture once weekly. Fresh culture medium was applied every 3 days. For microscopy, 1 mL of re-suspended cells were further diluted in 11 mL of medium, 2 mL of this suspension was seeded onto 25 mm coverslips and incubated for 48 hours at 37 °C, 95% oxygen and 5% CO_2 before experiments. The buffer used for making up the compound solutions was clear optiMEM solution from Gibco (11058).

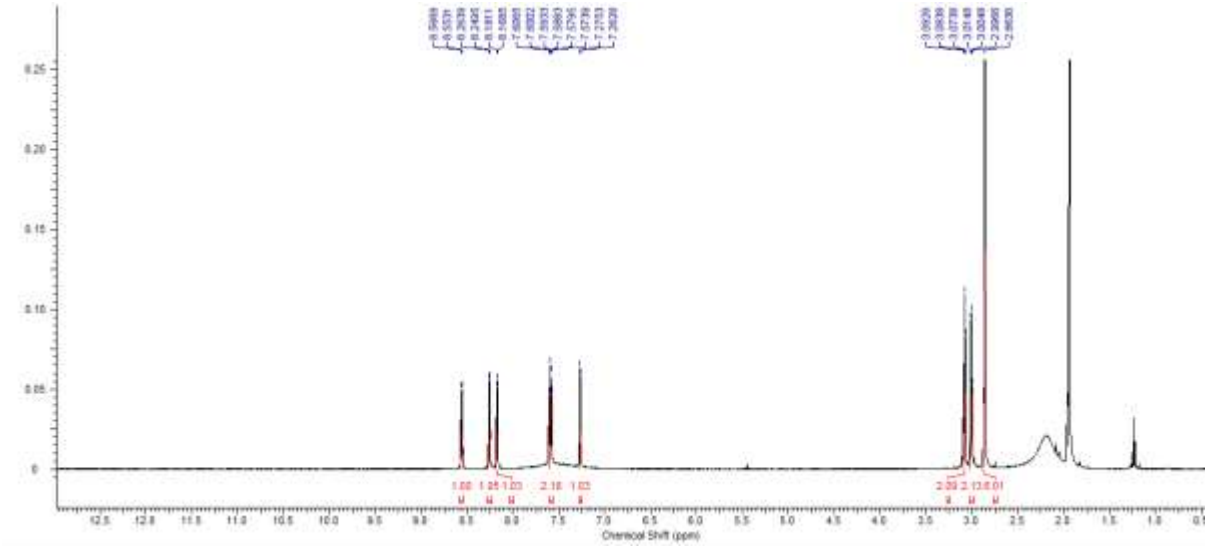
Cellular imaging studies were conducted using a Leica SP8 multiphoton confocal microscope with a DM6000 CS microscope stand, (The Centre for Cell & Molecular Dynamics University College London). The laser used for the cellular imaging studies was a Coherent Chameleon Vision II, two-photon excitation = 700 nm; emission = 490 - 550 nm using the Leica HyD PMT detectors, objective used: 25 x 0.95 water dipping.

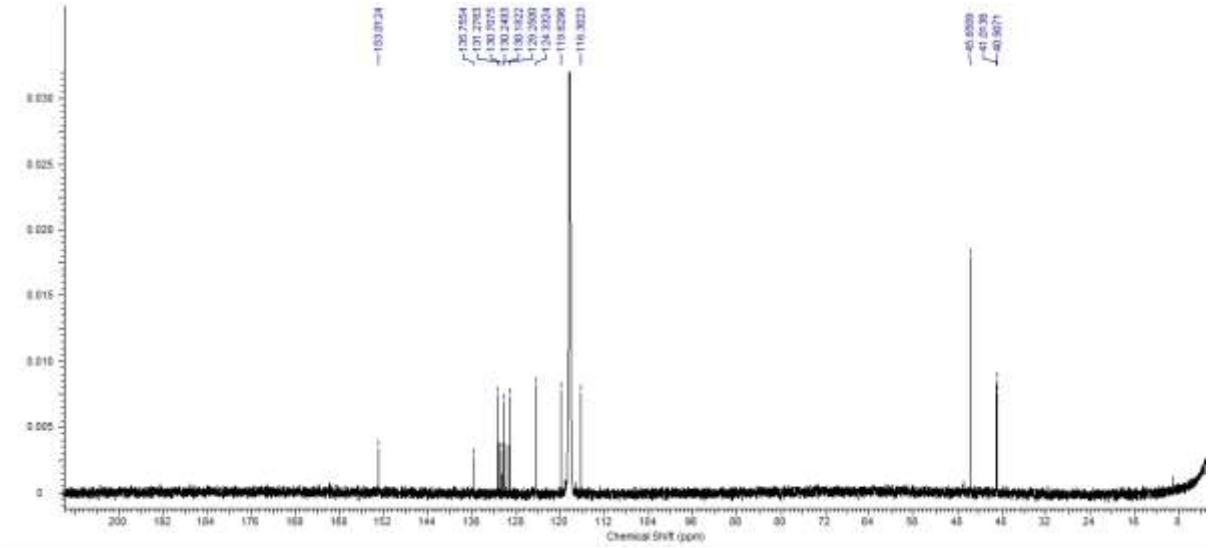
_N_-(2-aminoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 2,2,2-trifluoroacetate 8



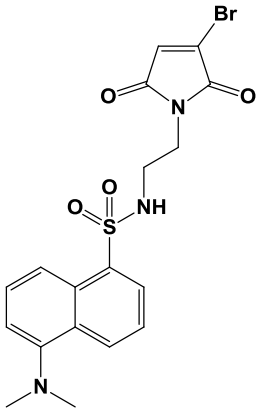
To a flame dried flask containing a solution of *tert*-butyl (2-aminoethyl)carbamate[2] (570 mg, 3.6 mmol) in dry dichloromethane (150 mL), at room temperature, was added a solution of dansyl chloride **7** (1.05 g, 3.92 mmol) and triethylamine (1.3 mL, 9.29 mmol) in dry dichloromethane (150 mL). The reaction mixture was stirred at room temperature for 4 hours. Then the solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 6:4) to afford a viscous green oil to which trifluoroacetic acid (40 mL) was added. The resulting grey solution was then stirred for 2 hours at room temperature and then the solvent was removed *in vacuo*. Addition of dichloromethane (100 mL) at 0 °C to the residue resulted in the precipitation of a solid after 2 hours which was washed with diethyl ether (50 mL) to afford the title compound as white solid (1.25 g, 3.10 mmol, 79%):

^{1}H NMR (600MHz, CD_3CN): δ = 8.56 (d, *J* = 8.5, 1H), 8.26 (d, *J* = 8.6, 1H), 8.17 (dd, *J* = 7.3 and 1.1, 1H), 7.60-7.57 (m, 2H), 7.27 (d, *J* = 7.5, 1H), 3.10-3.07 (m, 2H), 3.01-3.00 (m, 2H), 2.86 (s, 6H); ^{13}C NMR (150MHz, CD_3CN): δ = 153.0 (C), 135.8 (C), 131.3 (CH), 130.7 (C), 130.2 (C), 130.2 (CH), 129.3 (CH), 124.3 (CH), 119.8 (CH), 116.3 (CH), 45.7 (2 × CH_3), 41.0 (CH_2) 40.9 (CH_2); ^{19}F NMR (300MHz $CDCl_3$): 76.9; IR (neat): 3078 (br), 1660 (s) cm^{-1}; 76.89; LRMS (ES) 654 (100, $[M+Na]^+$), 293 (15, M), 292 (100, $[M-H]^+$), 255 (42); HRMS (ES) calcd for $C_{14}H_{19}N_3O_2S$ $[M-H]^+$: 292.1120, observed: 292.1121; m.p: 160-162°C; UV (Acetonitrile) ε_{335} = 4281 M^{-1} cm^{-1}.



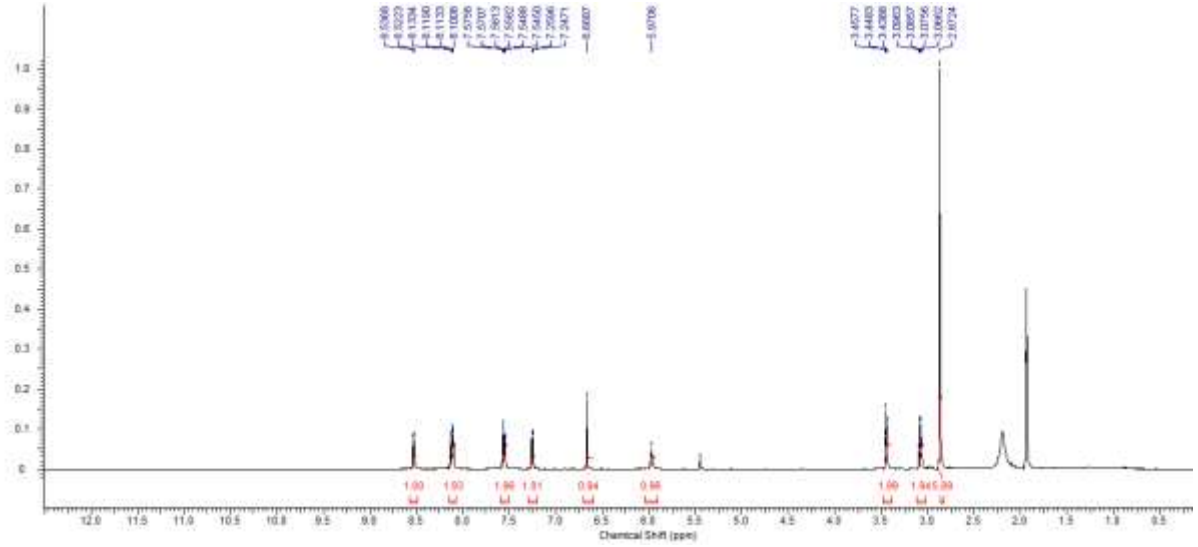


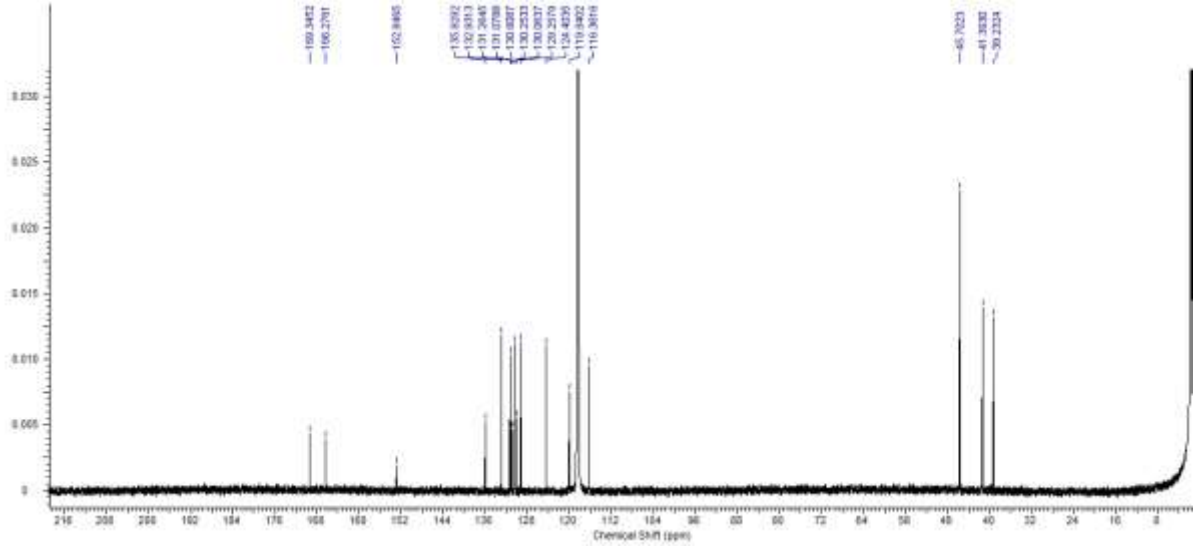
_N_–(2-(3-bromo-2,5-dioxo-2,5-dihydro-1_H_-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 9



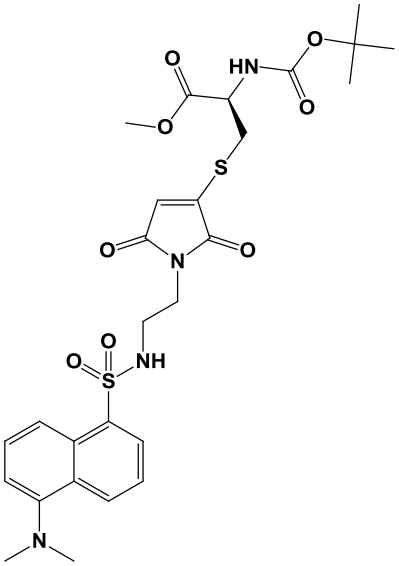
To a solution of dansyl-amine **8** (1.09 g, 2.68 mmol) in acetic acid (25 mL) was added bromomaleic anhydride (0.23 mL, 2.44 mmol). The resulting solution was then stirred at room temperature for 1.5 hours before it was refluxed at 170 °C for 2 hours. The solvent was removed *in vacuo* and the resultant brown gum was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 7:3) to afford a brown oil. Addition of ethyl acetate (50 mL) resulted in the precipitation of a brown solid which was washed with diethyl ether (20 mL) to afford the title compound as a brown powder (961 mg, 2.12 mmol, 79%):

^{1}H NMR (600MHz, CD_3CN): δ = 8.53 (d, *J* = 9.0, 1H), 8.13-8.10 (m, 2H), 7.58-7.55 (m, 2H), 7.26 (d, *J* = 7.8, 1H), 6.67 (s, 1H), 5.97 (br t, *J* = 6.1, 1H), 3.45 (t, *J* = 5.8, 2H), 3.09 (q, *J* = 6.0, 2H), 2.87 (s, 6H); ^{13}C NMR (150MHz, CD_3CN): δ = 169.3 (C), 166.3 (C), 152.8 (C), 135.9 (C), 132.9 (CH), 131.3 (CH), 131.1 (C), 130.6 (C), 130.3 (CH), 130.1 (C), 129.3 (CH), 124.4 (CH), 119.9 (CH), 116.4 (CH), 45.7 (2 × CH_3), 41.4 (CH_2), 39.2 (CH_2); IR (solid): 3326 (s), 1717 (s) cm^{-1}; LRMS (ES) 454 (12, [^{81}M+H]$^+$), 452 (7, [^{79}M+H]$^+$), 332 (18), 330 (31); HRMS (ES) calcd for $C_{18}H_{18}N_3O_4S^{79}Br$ [M+H]$^+$ 452.0280, Observed: 452.0292; m.p: 70-71°C; UV (Acetonitrile) ε_{335} = 4586 M^{-1} cm^{-1}.



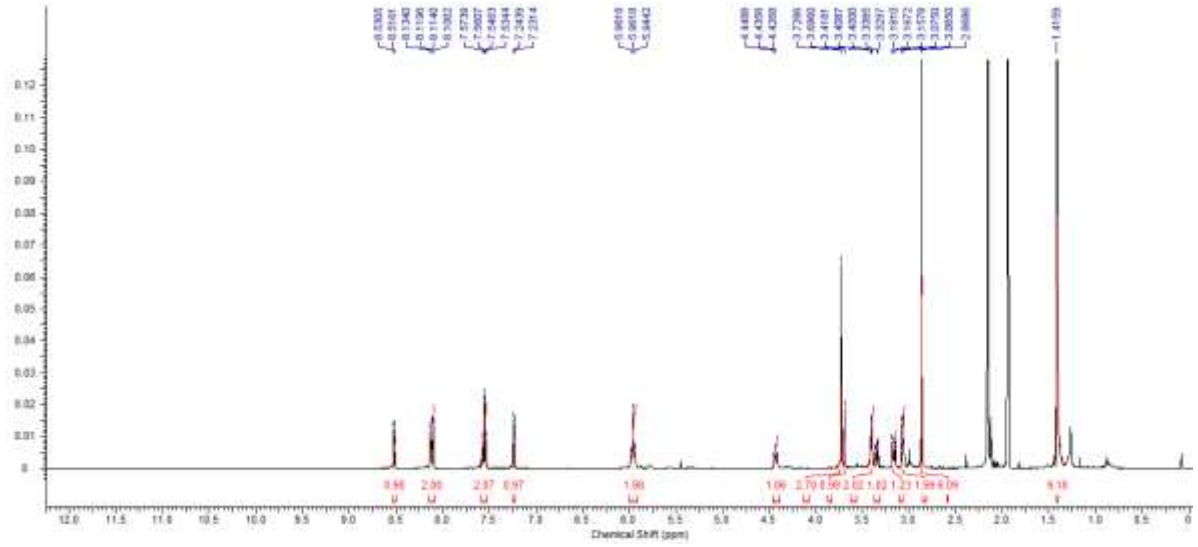


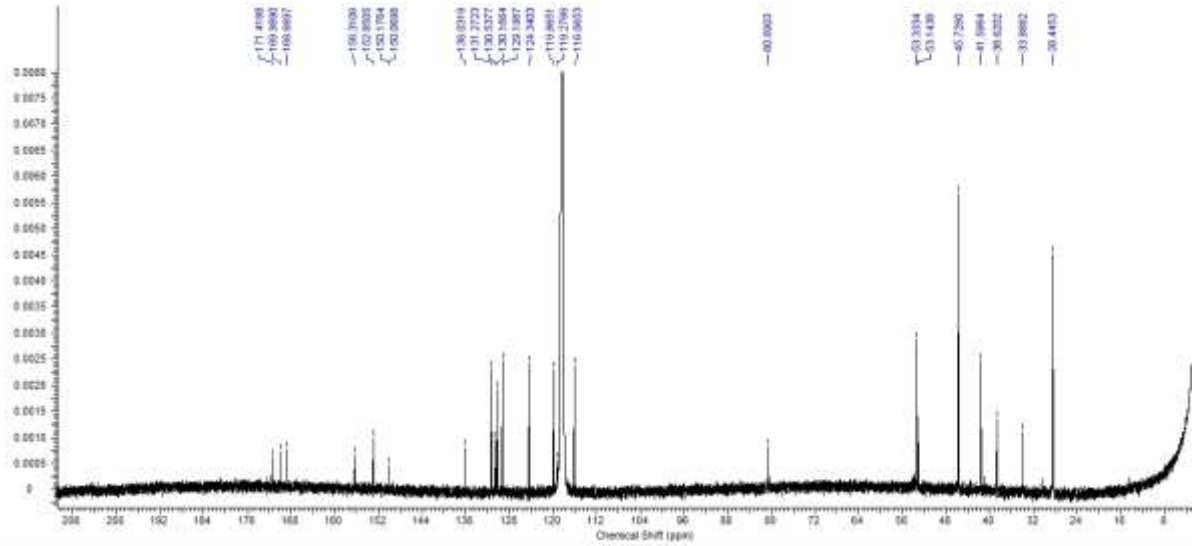
(*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-((1-(2-(5-(dimethylamino)naphthalene-1-sulfonamido)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)thio)propanoate 10



To a solution of dansyl-monobromomaleimide **9** (100 mg, 0.22 mmol) and sodium acetate (9 mg, 0.11 mmol) in methanol (200 mL), at room temperature was added a solution of *N*-Boc-Cys-OMe (22 µL, 0.11 mmol) in methanol (200 mL) over 3 hours. The solvent was then removed *in vacuo*, and the residue purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 6:4) to afford the title compound as a yellow oil (48 mg, 0.080 mmol, 73%):

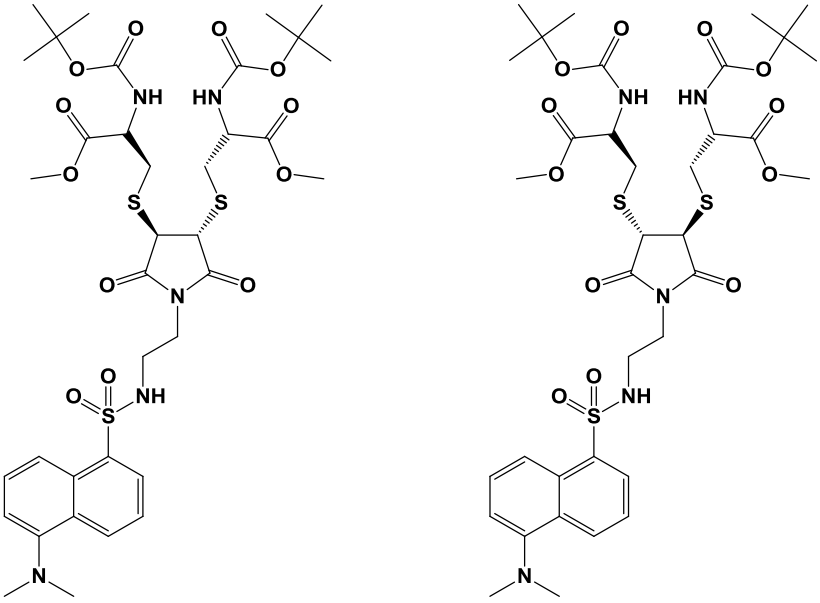
^{1}H NMR (600MHz, CD_3CN): δ = 8.53 (d, *J* = 8.6, 1H), 8.12-8.10 (m, 2H), 7.58-7.53 (m, 2H), 7.21 (d, *J* = 8.7, 1H), 5.98-5.94 (m, 2H), 4.45-4.41 (m, 1H,), 3.85 (s, 3H), 3.40 (q, *J* = 5.4, 2H), 3.35 (dd, *J* = 13.6 and 5.1, 1H), 3.16 (dd, *J* = 13.6 and 8.1, 1H), 3.07 (q, *J* = 5.8, 2H), 2.87 (s, 6H), 1.42 (s, 9H); ^{13}C NMR (150MHz, CD_3CN): δ = 171.4 (C), 170.0 (C), 168.7 (C), 156.3 (C), 152.8 (C), 150.2 (C), 150.1 (C), 136.0 (C), 131.3 (CH), 130.5 (C), 130.12 (CH), 129.2 (CH), 124.3 (CH), 119.9 (CH), 119.3 (CH), 116.1 (CH), 80.7 (C), 53.3 (2 × CH_3), 53.1 (CH), 45.7 (CH_3), 41.6 (CH_2), 38.6 (CH_2), 33.9 (CH_2), 28.5 (3 × CH_3); IR (neat): 3308 (br), 1703 (s) cm^{-1}; LRMS (EI) 606 (24, M), 506 (67), 170 (93); HRMS (EI) calcd for $C_{27}H_{34}N_4O_8S_2$: 606.1813, observed: 606.1810; $^{20}\alpha_D$: - 16.36° (c = 0.22, Methanol); UV (Acetonitrile) ε_{335} = 4939 M^{-1} cm^{-1}.





(*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(((3*R*,4*R*)-4-(((*R*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxypropyl)thio)-1-(2-(5-(dimethylamino)naphthalene-1-sulfonamido)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoate 11a

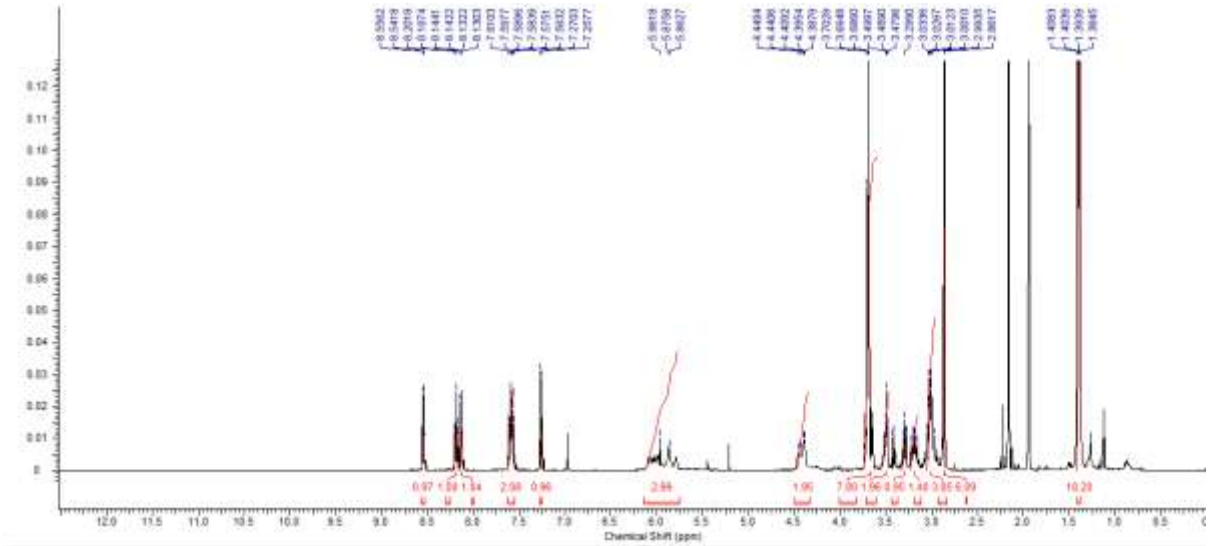
(*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(((3*S*,4*S*)-4-(((*R*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxypropyl)thio)-1-(2-(5-(dimethylamino)naphthalene-1-sulfonamido)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoate 11b

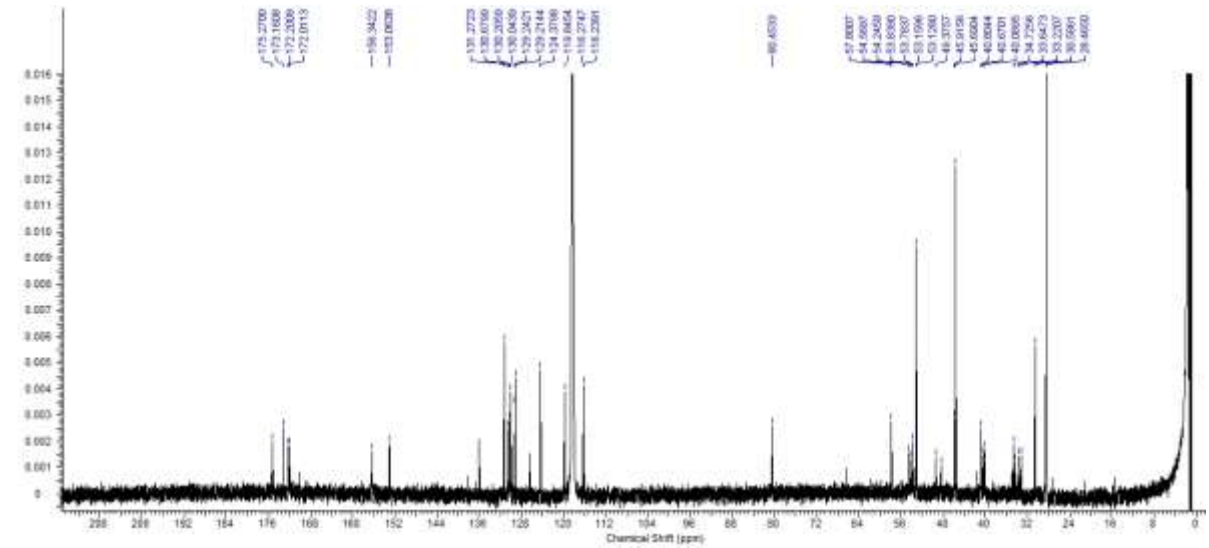


To a solution of cysteinethiomaleimide **10** (15 mg, 0.0247 mmol) and sodium acetate (2 mg, 0.0247 mmol) in methanol (100 mL), at room temperature, was added a solution of *N*-Boc-Cys-OMe (3.1 µL, 0.0247 mmol) in methanol (100 mL) over 1 hour. The solvent was removed *in vacuo* and the residue purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 6:4) to afford a brown gum, the title compounds, as an inseparable mixture of diasterioisomers (13 mg, 0.0157 mmol, 64%):

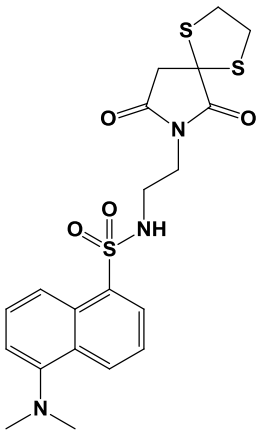
^{1}H NMR (600MHz, CD_3CN)*: δ = 8.56-8.51 (m, 1H 11a and 1H 11b), 8.20-8.17 (m, 1H 11a and 1H 11b), 8.14-8.10 (m, 1H 11a and 1H 11b), 7.63-7.53 (m, 2H 11a and 2H 11b), 7.28-7.23 (m, 1H 11a and 1H 11b), 6.08-5.78 (m, 3H 11a and 3H 11b), 4.46-4.39 (m, 2H 11a and 2H 11b), 3.73-3.64 (m, 8H 11a and 8H 11b), 3.53-3.48 (m, 2H 11a and 2H 11b), 3.32-3.28

(m, 1H 11a and 1H 11b), 3.23-3.16 (m, 1H 11a and 1H 11b), 3.05-2.98 (m, 4H 11a and 4H 11b), 2.86 (s, 6H 11a and 6H 11b), 1.41-1.38 (m, 18H 11a and 18H 11b); ^{13}C NMR (150MHz, CD_3CN): δ = 175.3 (2 × C), 175.1 (2 × C), 173.2 (4 × C), 172.2 (2 × C), 172.04 (2 × C), 156.4 (C), 156.3 (C), 153.1 (C), 153.0 (C), 131.3 (2 × CH), 130.71 (C), 130.68 (C), 130.20 (C), 130.19 (C), 130.0 (2 × CH), 129.24 (CH), 129.21 (CH), 124.4 (2 × CH), 119.85 (CH), 119.77 (CH), 116.3 (CH), 116.2 (CH), 80.5 (4 × C), 57.8 (4 × CH), 53.8 (4 × CH_3), 53.2 (4 × CH), 45.72 (2 × CH_3), 45.69 (2 × CH_3), 40.8 (CH_2), 40.7 (CH_2), 40.4 (CH_2), 40.3 (CH_2), 34.7 (CH_2), 34.4 (CH_2), 33.7 (CH_2), 33.2 (CH_2), 30.6 (6 × CH_3), 28.47 (6 × CH_3); IR (neat): 2967 (br), 1704 (s) cm^{-1}; LRMS (ES) 864 (100, $[M+Na]^+$), 686 (15); HRMS (ES) calcd for $C_{36}H_{50}N_5O_{12}S_3$ $[M+Na]^+$ 864.2594, observed: 864.2549; UV (Acetonitrile) ε_{335} = 4669 M^{-1} cm^{-1}.



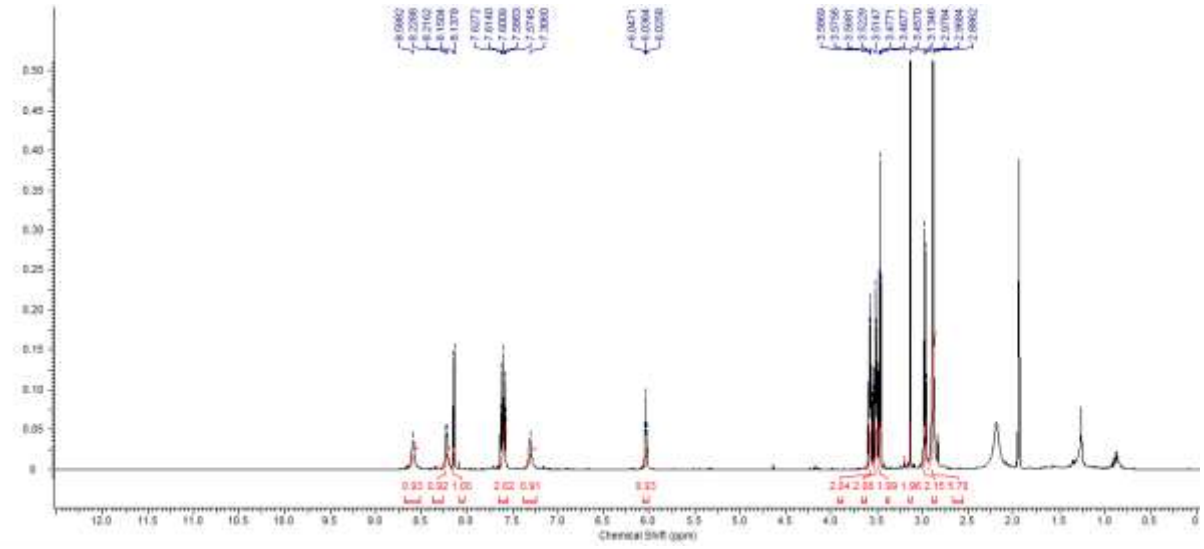


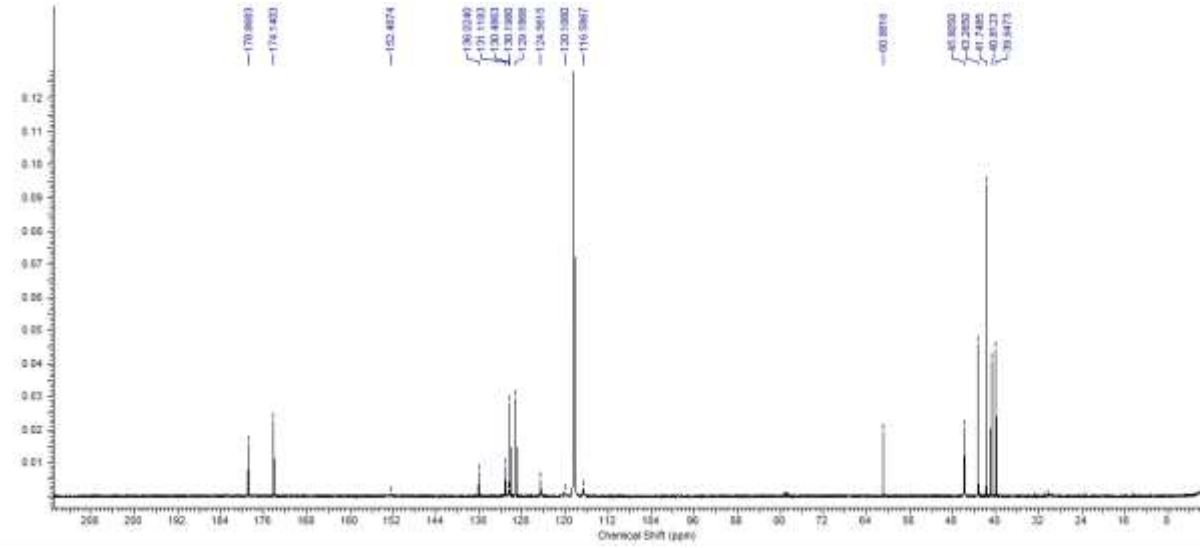
5-(dimethylamino)-*N*-(2-(6,8-dioxo-1,4-dithia-7-azaspiro[4.4]nonan-7-yl)ethyl)naphthalene-1-sulfonamide 12



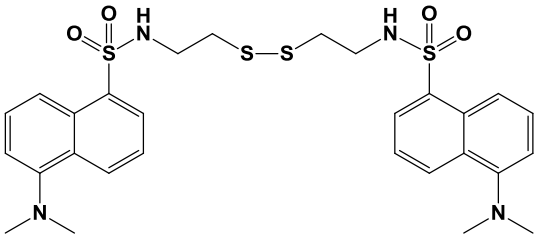
To a solution of dansyl-monobromomaleimide **9** (300mg, 0.67 mmol) and sodium acetate (131 mg, 1.34 mmol) in chloroform (35 mL) was added ethane-1,2-dithiol (28µL, 0.34 mmol). The reaction was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the residue purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 7:4) to afford the title compound as a yellow oil (133 mg, 0.29 mmol, 85%):

^{1}H NMR (600MHz, CD_3CN): δ = 8.59 (br s, 1H), 8.22 (br d, *J* = 7.2, 1H), 8.15-8.14 (m, 1H), 7.63-7.57 (m, 2H), 7.31 (br s, 1H), 6.04 (t, *J* = 6.4, 1H), 3.60-3.56 (m, 2H), 3.54-3.49 (m, 2H), 3.47 (t, *J* = 6.1, 2H), 3.13 (s, 2H), 2.97 (q, *J* = 6.3, 2H), 2.89 (s, 6H); ^{13}C NMR (150MHz, CD_3CN): δ = 178.9 (C), 174.1 (C), 152.5 (C), 136.0 (C), 131.1 (CH), 130.5 (C), 130.2 (C), 129.7 (CH), 129.2 (CH), 124.6 (CH), 120.1 (CH), 116.6 (CH), 60.9 (C), 45.8 (CH_2), 43.3 (2 × CH_2), 41.7 (2 × CH_3), 40.8 (CH_2), 39.9 (CH_2); IR (neat): 3293 (br), 1702 (s) cm^{-1}; LRMS (EI) 465 (100, M), 171 (80); HRMS (EI) calcd for $C_{20}H_{23}N_3O_4S_3$: 465.0851, observed: 465.0850; UV (Acetonitrile) ε_{335} = 3610 M^{-1} cm^{-1}.



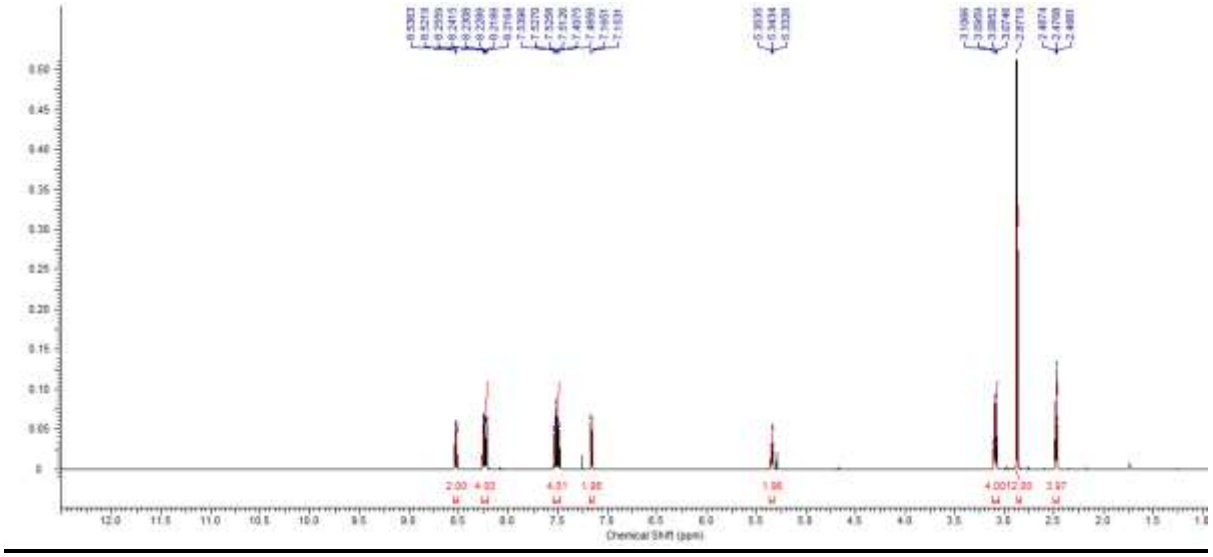


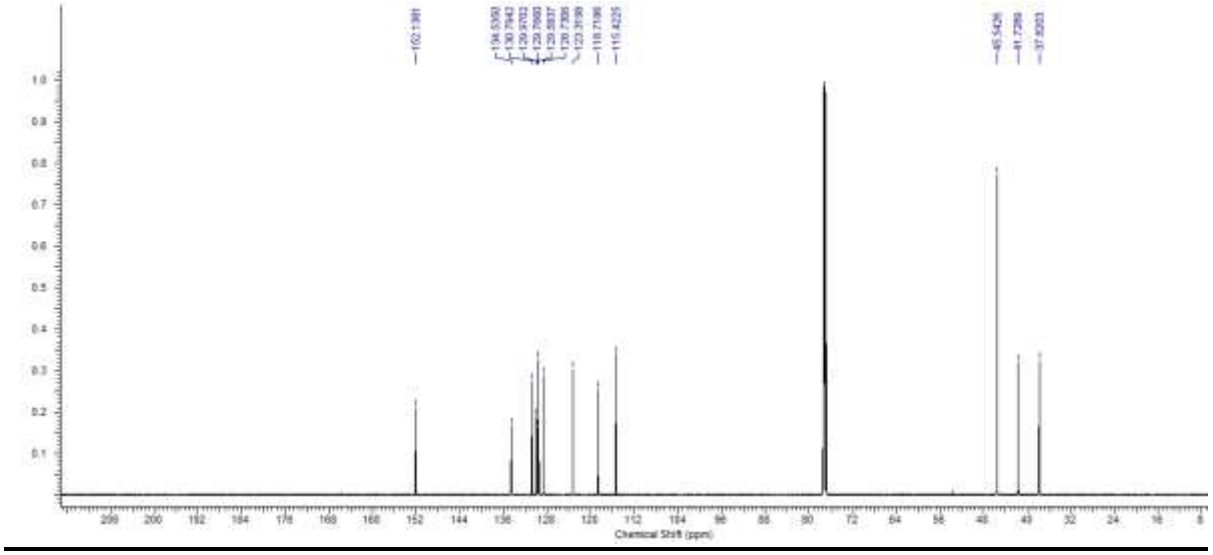
_N,N_'-(disulfanediylbis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide) 13



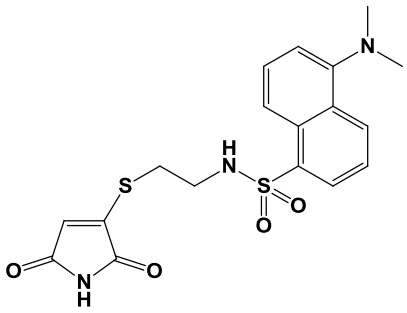
To a solution of cystamine hydrochloride (1.5 g, 6.7 mmol) in dichloromethane (30 mL) at room temperature was added triethylamine (5.07 mL, 37.0 mmol) followed by 4-dimethylaminopyridine (3.7 g, 37 mmol). The reaction mixture was stirred for 30 minutes. Dansyl chloride **7** (2.0 g, 7.4 mmol) was then added to the reaction mixture which was stirred for a further 16 hours. Then H_2O (50 mL) was added. The mixture was extracted into dichloromethane (3 x 40 mL) and the combined organic layers washed with brine (50 mL), dried (magnesium sulfate) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (dichloromethane: ethyl acetate, 9:1) to afford the title compound as a green gum (2.0 g, 3.23 mmol, 96%):

^{1}H NMR (600MHz, $CDCl_3$): δ = 8.53 (d, *J* = 8.5, 2H), 8.25 - 8.22 (m, 4H), 7.55 - 7.49 (m, 4H), 7.16 (d, *J* = 7.2, 2H), 5.43 (t, *J* = 6.2, 2H), 3.09 (q, *J* = 6.3, 4H), 2.87 (s, 12H), 2.48 (t, *J* = 6.4, 4H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 152.1 (2 × C), 134.5 (2 × C), 130.80 (2 × CH), 129.97 (2 × C), 129.8 (2 × CH), 129.6 (2 × C), 128.7 (2 × CH), 123.3 (2 × CH), 118.7 (2 × CH), 115.4 (2 × CH), 45.5 (4 × CH_3), 41.7 (2 × CH_2), 37.8 (2 × CH_2); IR (neat): 3287 (br), 1738 (s) (cm^{-1}) cm^{-1}; LRMS (ES) 641.1 (100, $[M+Na]^+$) 619 (31, M,); HRMS (ES) calcd for $C_{28}H_{34}N_4O_4S_4$ $[M+Na]^+$ 641.1361, observed 641.1345; UV (Acetonitrile) ε_{335} = 4458 M^{-1} cm^{-1}.



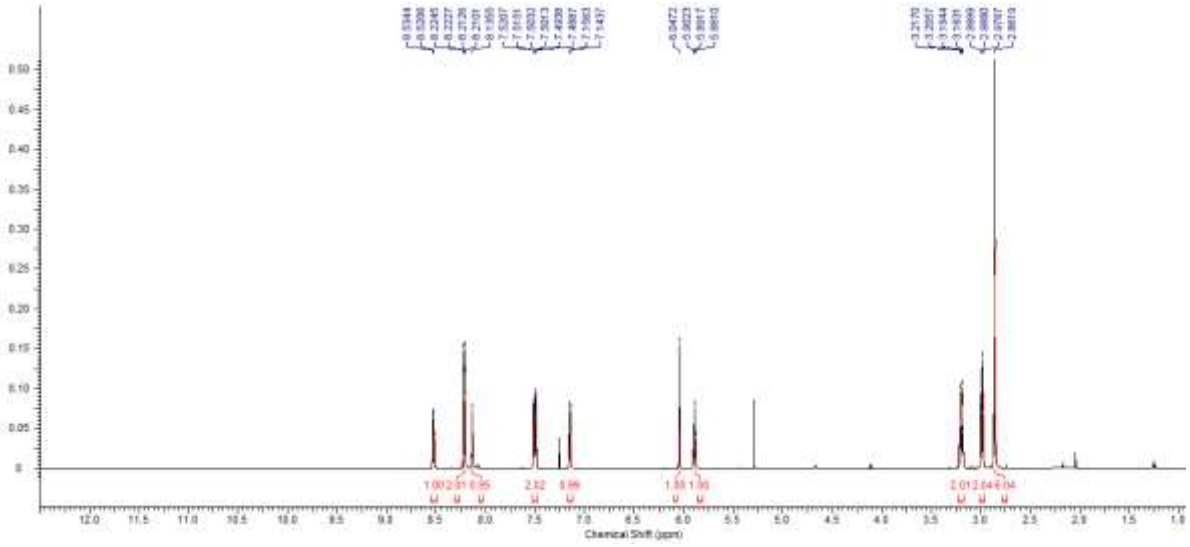


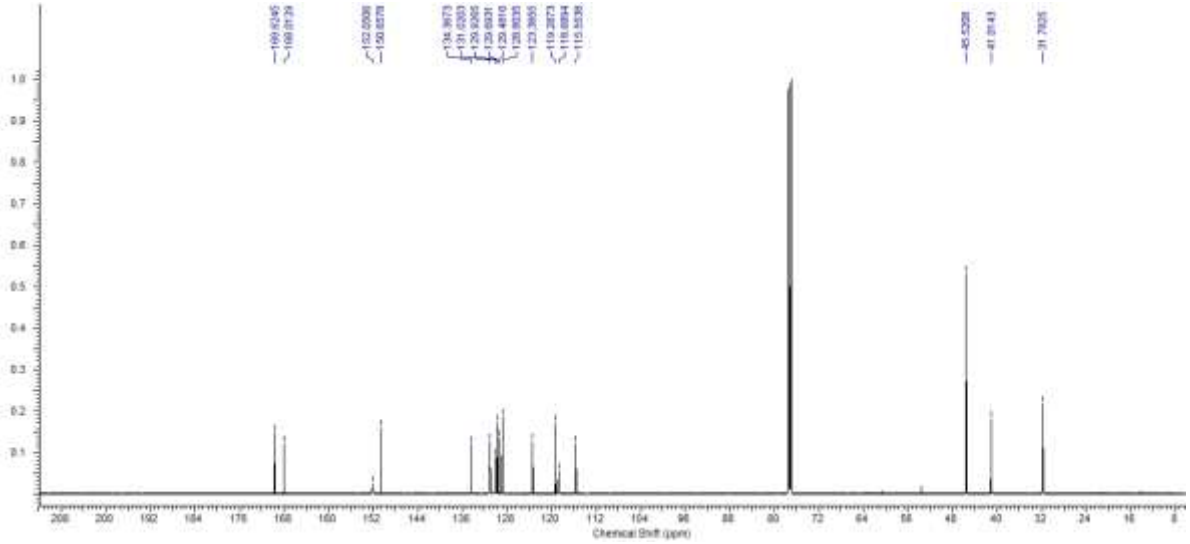
5-(dimethylamino)-*N*-(2-((2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)thio)ethyl)naphthalene-1-sulfonamide 14



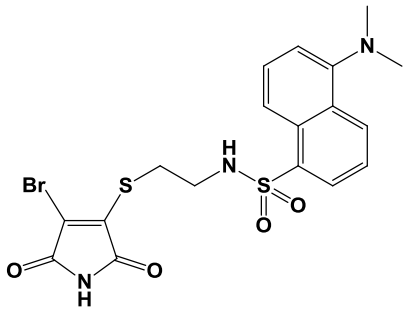
To a solution of dansyldisulfide **13** (100 mg, 0.16 mmol) in methanol (10 mL) at room temperature was added tris(2-carboxyethyl)phosphine hydrochloride (46 mg, 0.16 mmol). The reaction mixture was stirred for 3 hours. It was then added drop-wise to a solution of 3-bromo-1*H*-pyrrole-2,5-dione[xx] (56 mg, 0.32 mmol) in methanol (5 mL). The reaction mixture was stirred for 16 hours then sodium acetate (53 mg, 0.64 mmol) was added before all volatile material was removed *in vacuo*. Then H_2O (50 mL) was added. The reaction mixture was extracted into dichloromethane (3 x 40 mL) and the combined organic layers washed with brine (50 mL), dried (magnesium sulfate) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (dichloromethane: ethyl acetate, 7:3) to afford the title compound as a yellow gum (73 mg, 0.18 mmol, 56%):

^{1}H NMR (600MHz, $CDCl_3$): δ = 8.53 (d, *J* = 8.5, 1H), 8.22 (dd, *J* = 7.4 and 1.1, 2H), 8.14 (s, 1H), 7.52 - 7.49 (m, 2H), 7.15 (d, *J* = 7.5, 1H), 6.05 (s, 1H), 5.89 (t, *J* = 6.4, 1H), 3.20 (q, *J* = 6.7, 2H), 2.99 (t, *J* = 6.9, 2H), 2.87 (s, 6H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 169.6 (C), 168.0 (C), 152.1 (C), 150.7 (C), 134.4 (C), 131.0 (CH), 129.9 (C), 129.7 (CH), 129.5 (C), 128.8 (CH), 123.4 (CH), 119.3 (CH), 118.7 (CH), 115.6 (CH) 45.5 (2 × CH_3), 41.0 (CH_2), 31.8 (CH_2); IR (neat): 3278 (br) cm^{-1}, 1720 (s); LRMS (ES) 404 (100, M-H$^+$), 344 (91); HRMS (ES) calcd for $C_{18}H_{19}N_3O_4S_2$ $[M-H]^+$ 404.0739, observed 404.0733; UV (Acetonitrile) ε_{335} = 3628 M^{-1} cm^{-1}.



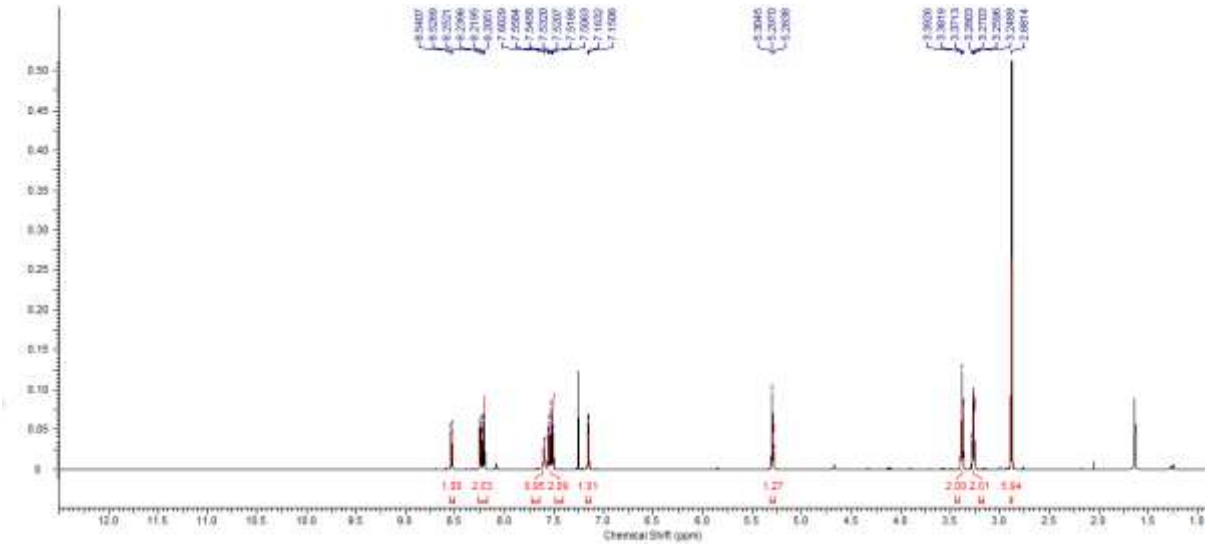


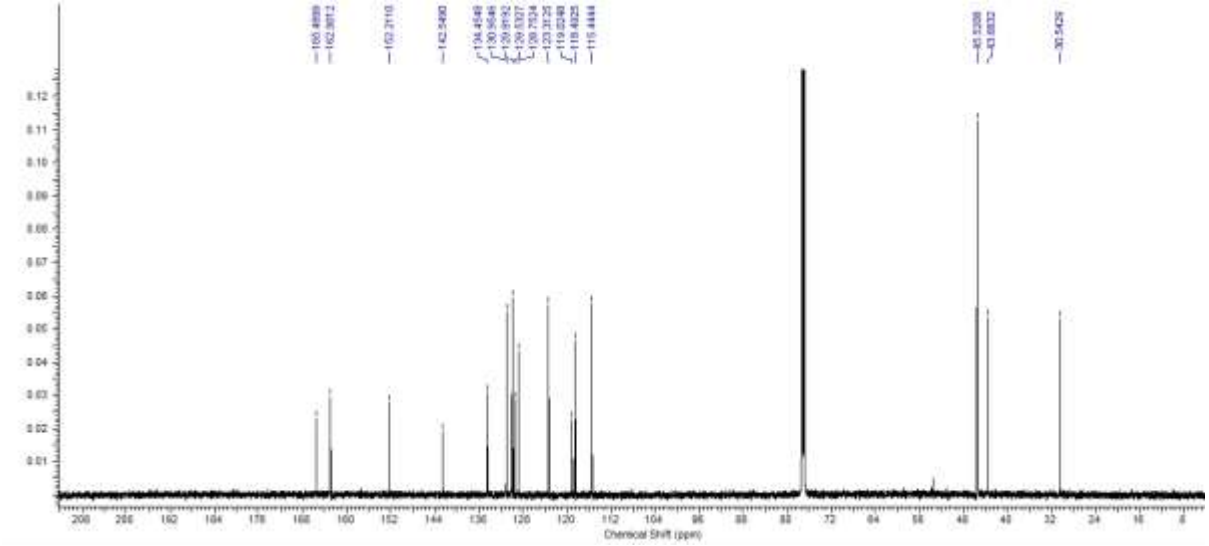
_N_-(2-((4-bromo-2,5-dioxo-2,5-dihydro-1_H_-pyrrol-3-yl)thio)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 15



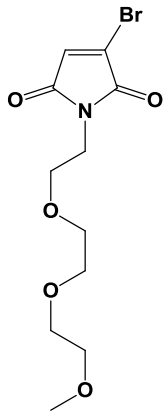
To a solution of dansyldisulfide **13** (48 mg, 0.08 mmol) in methanol (10 mL) at room temperature was added tris(2-carboxyethyl)phosphine hydrochloride (23 mg, 0.08 mmol). The reaction mixture was stirred for 3 hours. It was then added drop-wise to a solution of 3,4-dibromo-1*H*-pyrrole-2,5-dione (41 mg, 0.16 mmol) in methanol (5 mL). The reaction mixture was stirred for 16 hours then sodium acetate (27 mg, 0.32 mmol) was added before all volatile material was removed *in vacuo*. H_2O (50 mL) was added and the reaction mixture was extracted into dichloromethane (3 x 40 mL) and the combined organic layers washed with brine (50 mL), dried (magnesium sulfate) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (dichloromethane: ethyl acetate, 8:2) to afford the title compound as a yellow gum (17 mg, 0.035 mmol, 22%):

^{1}H NMR (600MHz, $CDCl_3$): δ = 8.53 (d, *J* = 8.52, 1H), 8.25 - 8.21 (m, 2H), 7.56 - 7.51 (br s, 1H) 7.53 (m, 2H), 7.16 (d, *J* = 7.4, 1H), 5.30 (t, *J* = 6.3, 1H), 3.38 (t, *J* = 6.3, 2H), 3.26 (q, *J* = 6.3, 2H), 2.88 (s, 6H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 165.5 (C), 162.9 (C), 152.2 (C), 142.5 (C), 134.4 (C), 131.0 (CH), 129.94 (C), 129.92 (CH), 129.5 (C), 128.7 (CH), 123.3 (CH), 119.0 (C), 118.5 (CH), 115.4 (CH) 45.5 (2 × CH_3), 43.7 (CH_2), 30.5 (CH_2); IR (neat) 3295 (br), 1776 (s), 1726 (s) cm^{-1}; LRMS: (ES) 486 (100, $[^{81}M+H]^+$), 483 (91, $[^{79}M+H]^+$), 471 (37), 469 (37); HRMS (ES) calcd for $C_{18}H_{18}N_3O_4S_2Br$ $[M+H]^+$ 484.0000, observed 483.9982; UV (Acetonitrile) ε_{335} = 3512 M^{-1} cm^{-1}.



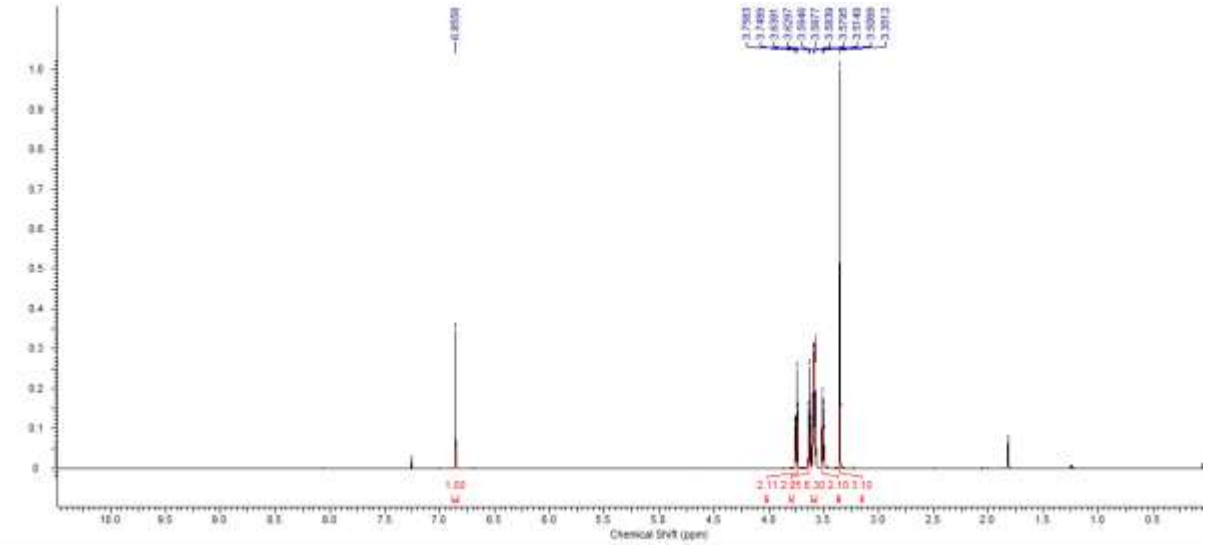


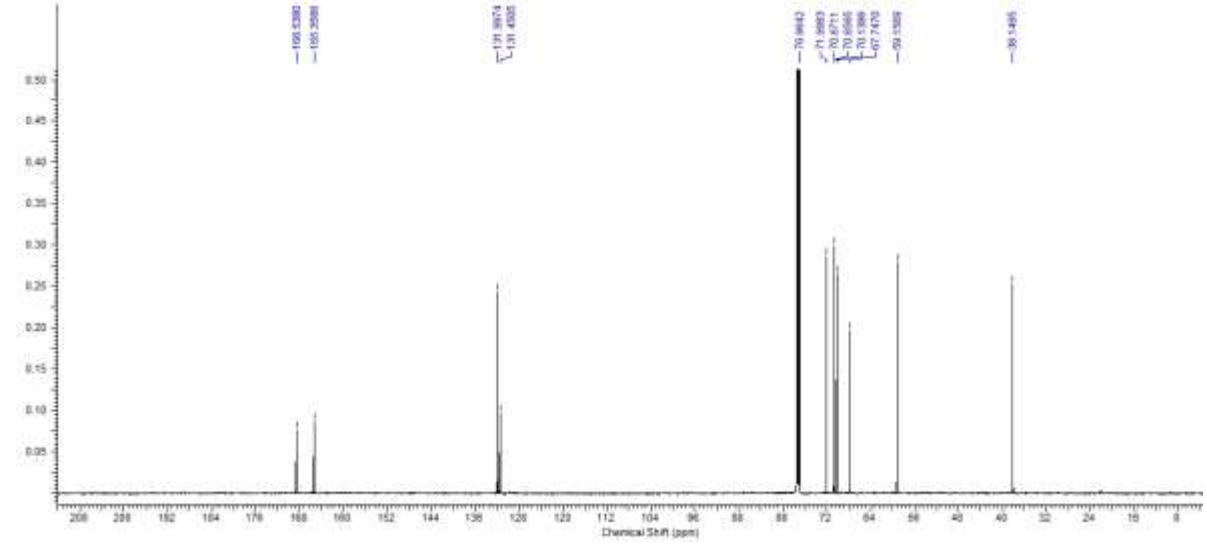
3-Bromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione 22



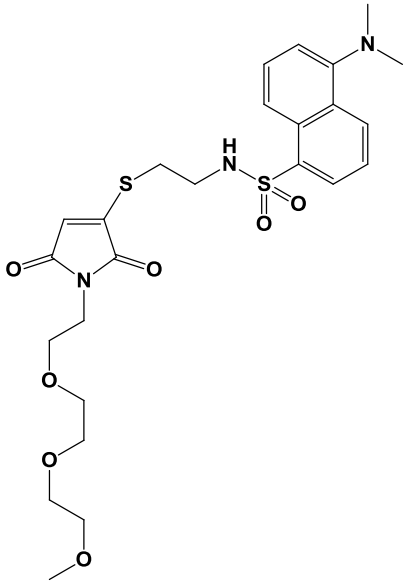
A suspension of 2-(2-(2-Methoxyethoxy)ethoxy)ethanamine[3] (100 mg, 0.61 mmol) and bromomaleic anhydride (0.52 mL, 0.56 mmol) in acetic acid (30 mL) was refluxed at 170 °C for 90 minutes. The solution was allowed to cool to room temperature. All volatile material was removed *in-vacuo*, residual acetic acid was removed by azeotroping with toluene (3 x 10 mL). Then H_2O (50 mL) was added and mixture was extracted into dichloromethane (3 x 40 mL) and the combined organic layers washed with brine (50 mL), dried (magnesium sulfate) and concentrated *in-vacuo*. The residue was purified by flash chromatography on silica gel (chloroform: ethyl acetate, 9:1) to afford the title compound as a yellow oil (156 mg, 0.48 mmol, 86%):

^{1}H NMR ($CDCl_3$): δ = 6.86 (s, 1H), 3.75 (t, *J* = 5.6, 2H), 3.63 (t, *J* = 5.6, 2H), 3.61 - 3.57 (m, 6H), 3.57 - 3.50 (m, 2H), 3.35 (s, 3H); ^{13}C NMR ($CDCl_3$): δ = 168.5 (C), 165.4 (C), 131.8 (CH), 131.5 (C), 72.0 (CH_2), 70.7 (CH_2), 70.2 (CH_2), 70.1 (CH_2), 70.0 (CH_2), 59.2 (CH_3), 38.2 (CH_2); IR (neat): 2873 (s), 1712 (s) cm^{-1}; LRMS (CI) 324 (100, [^{81}M]), 322 (100, [^{79}M]), 204 (69), 202 (67); HRMS (CI) calcd for $C_{11}H_{17}NO_5Br$ $[M+H]^+$: 322.0290, observed: 322.0302.





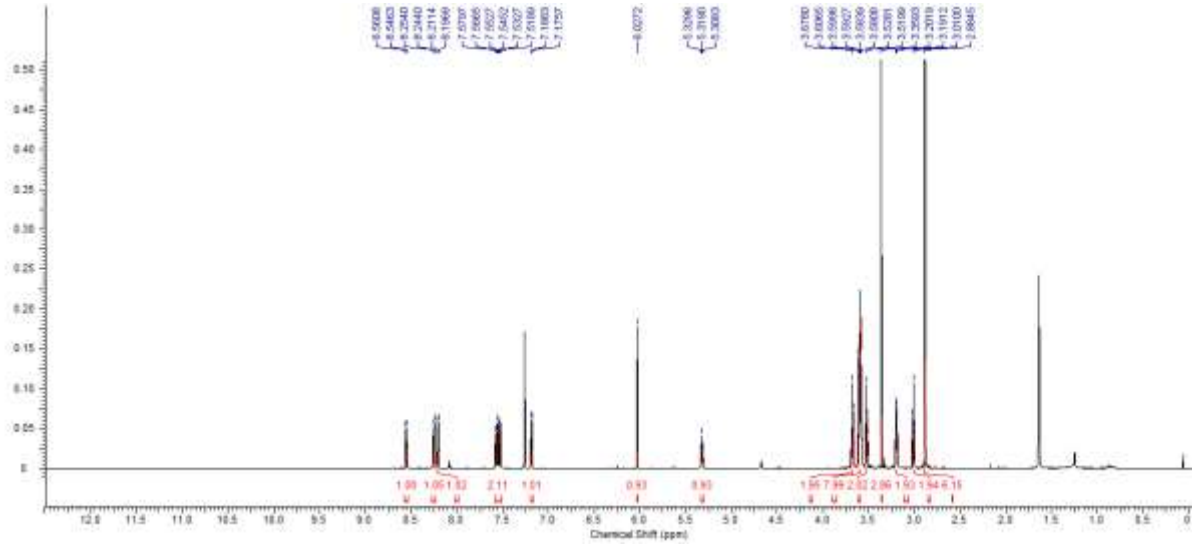
5-(dimethylamino)-*N*-(2-((1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)thio)ethyl)naphthalene-1-sulfonamide 16

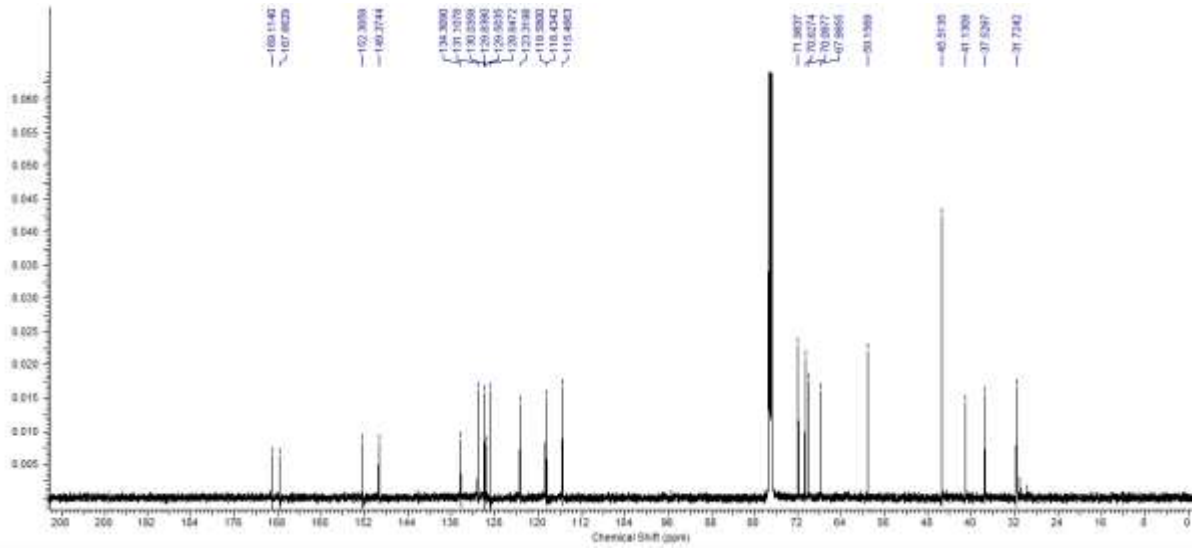


To a de-gassed solution of dansyldisulfide **13** (105 mg, 0.17 mmol) in methanol (10 mL) at room temperature was added (2-carboxyethyl)phosphine hydrochloride (49 mg, 0.17 mmol). The reaction mixture was stirred for 15 minutes. It was then added drop-wise to a de-gassed solution of 3-Bromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione **22** (105 mg, 0.33 mmol) and potassium acetate (162 mg, 1.65 mmol) in methanol (5 mL). The reaction mixture was stirred for 30 minutes. All volatile material was removed *in vacuo*. The residue was purified by flash chromatography (chloroform: methanol, 98:2) to afford the title compound as a yellow gum (100 mg, 0.18 mmol, 55%):

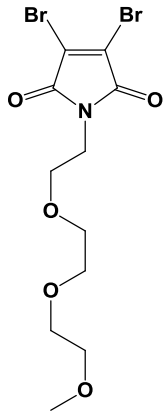
^{1}H NMR (600MHz, $CDCl_3$): δ = 8.55 (d, *J* = 8.52, 1H), 8.25 - 8.20 (m, 2H), 7.58 - 7.52 (m, 2H), 7.18 (d, *J* = 7.4, 1H), 6.03 (s, 1H), 5.32 (t, *J* = 6.4, 1H), 3.68 (t, *J* = 5.7, 2H), 3.62 – 3.52 (m, 10H), 3.36 (s, 3H), 3.20 (q, *J* = 6.7, 2H), 3.01 (t, *J* = 6.8, 2H), 2.88 (s, 6H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 169.1 (C), 167.7 (C), 152.3 (C), 149.4 (C), 134.3 (C), 131.1 (CH), 130.0 (C), 129.8 (CH), 129.5 (C), 128.8 (CH), 123.3 (CH), 118.6 (CH), 118.4 (CH), 115.4 (CH) 72.0 (CH_2), 70.7 (CH_2), 70.6 (CH_2) 70.1 (CH_2), 70.0 (CH_2), 67.9 (CH_2), 59.2 (CH_3), 45.5 (2 × CH_3), 41.1 (CH_2), 37.5 (CH_2) 31.7 (CH_2); IR (neat): 3294 (br), 2873 (s), 1698 (br)

cm^{-1}; LRMS (ES) 550 (5, $[M-H]^+$), 283 (63), 255 (100); HRMS (ES) calcd for $C_{25}H_{33}N_3O_7S_2$ $[M-H]^+$ 550.1682, observed: 550.1703; UV (Acetonitrile) $\varepsilon_{335} = 1347\ M^{-1}\ cm^{-1}$.



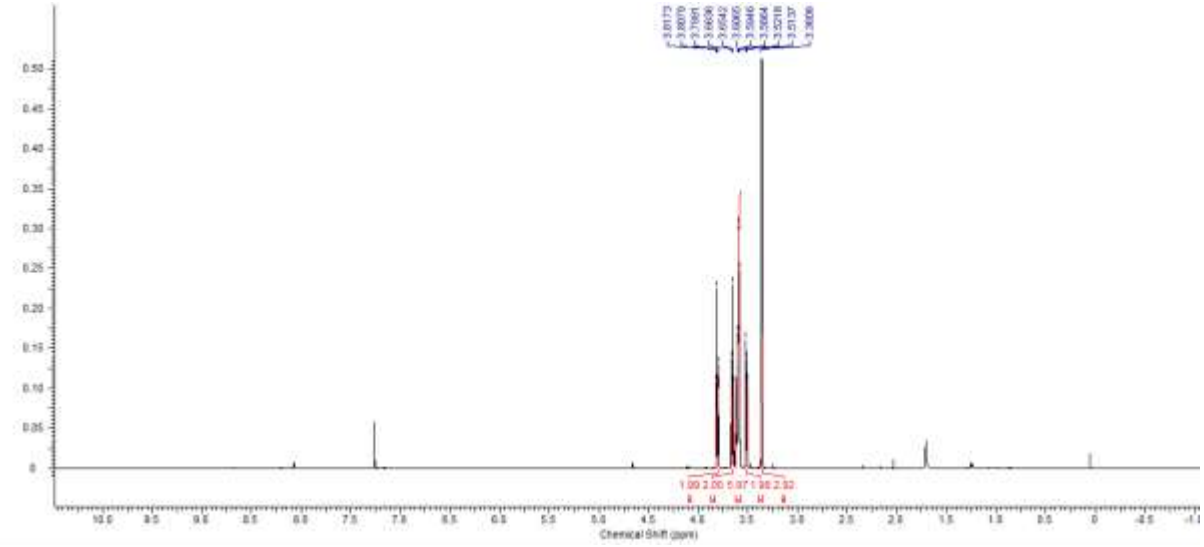


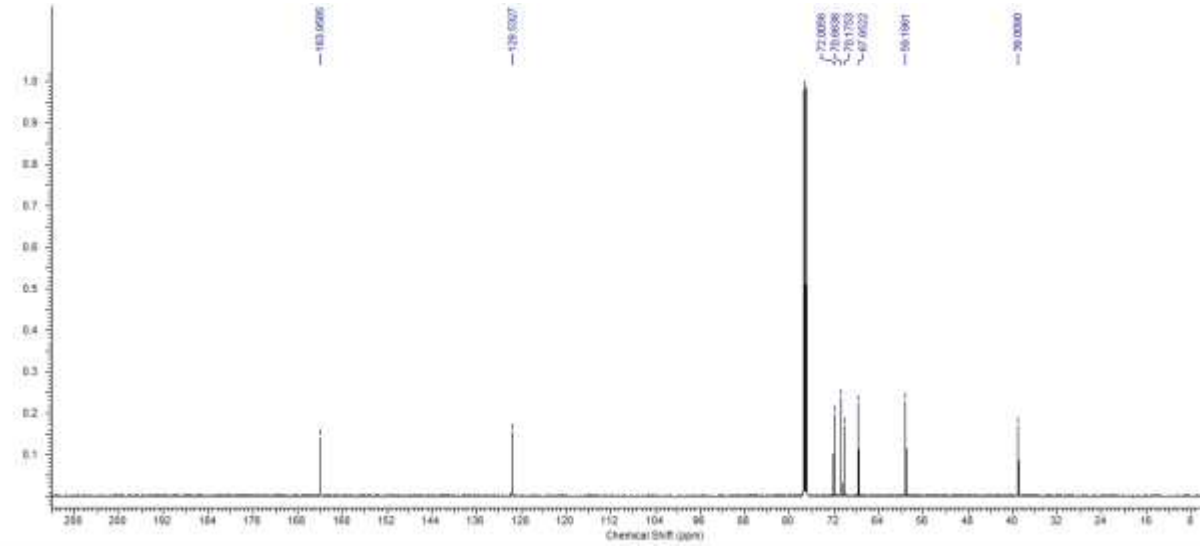
3,4-Dibromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione 23



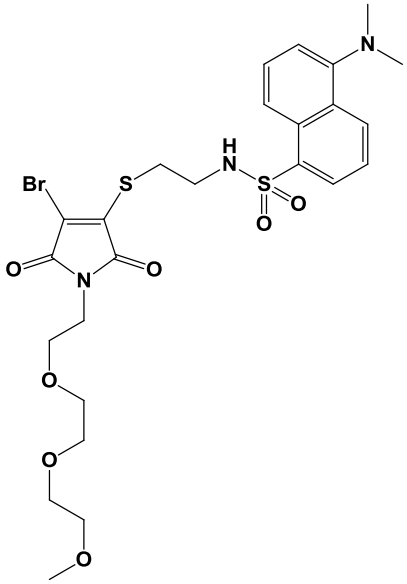
To a solution of triphenylphosphine (511 mg, 1.95 mmol) in distilled tetrahydrofuran (5 mL) at -78 °C was added diisopropyl azodicarboxylate (0.38 mL, 1.95 mmol) drop-wise. The reaction mixture was stirred for 5 minutes at -78 °C. Triethylenglycol monomethylether (0.25 mL, 1.56 mmol) in distilled tetrahydrofuran (5 mL) was then added drop-wise and the reaction mixture stirred for 5 minutes at -78 °C. Neopentenyl alcohol (121 mg, 1.37 mmol) in distilled tetrahydrofuran (2 mL) was added and the reaction mixture stirred at -78 °C for 5 minutes. Then dibromomaleimide (497 mg, 1.95 mmol) was added and the reaction mixture was stirred at -78 °C for 10 minutes before it was allowed to warm to room temperature and stirred for 20 hours. All volatile material was removed *in-vacuo* and the residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 7:3) to afford the title compound as a yellow oil (400 mg, 1.00 mmol, 64%):

^{1}H NMR ($CDCl_3$): δ = 3.81 (t, *J*, = 5.6, 2H), 3.65 (t, *J* = 5.6, 2H), 3.62 - 3.58 (m, 6H), 3.52 - 3.51 (m, 2H), 3.35 (s, 3H); ^{13}C NMR ($CDCl_3$): δ = 164.0 (2 × C), 129.5 (2 × C), 72.0 (CH_2), 70.71 (CH_2), 70.66 (CH_2), 70.2 (CH_2), 67.7 (CH_2), 59.2 (CH_3) 39.0 (CH_2); IR (neat): 2872 (s), 1720 (s) cm^{-1}; LRMS (CI) 404 (47, [$^{81+81}$M+H]$^{+}$), 402 (100, [$^{81+79}$M+H]$^{+}$), 400 (49, [$^{79+79}$M+H]$^{+}$), 284 (284), 282 (58), 280 (31); HRMS (CI) calcd for $C_{11}H_{16}NO_5Br_2$ [M+H]$^{+}$: 399.9395, observed: 399.9406.





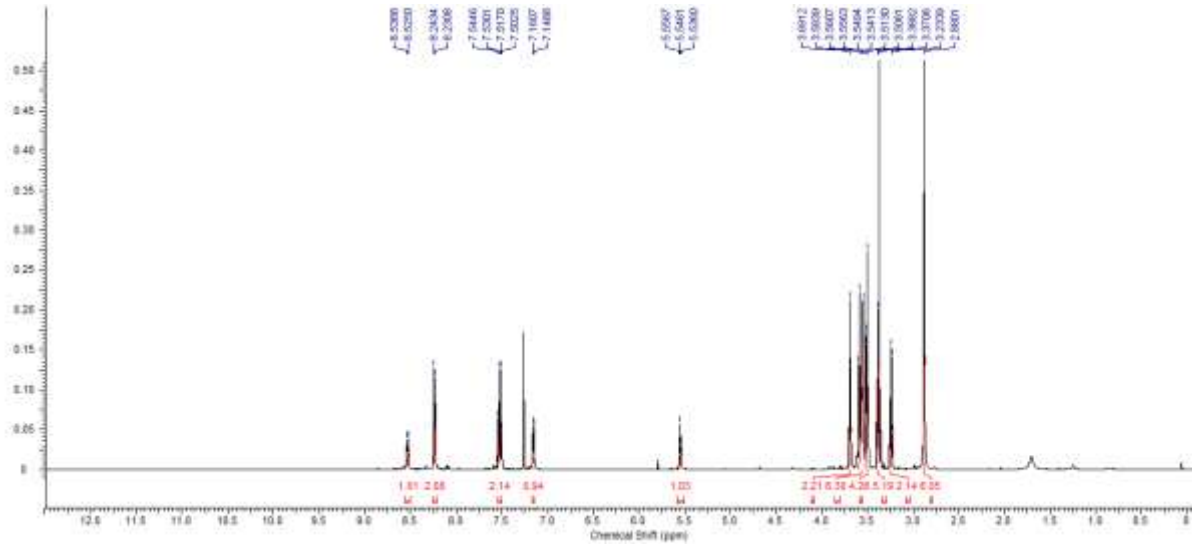
_N_-(2-((4-bromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1_H_-pyrrol-3-yl)thio)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 17

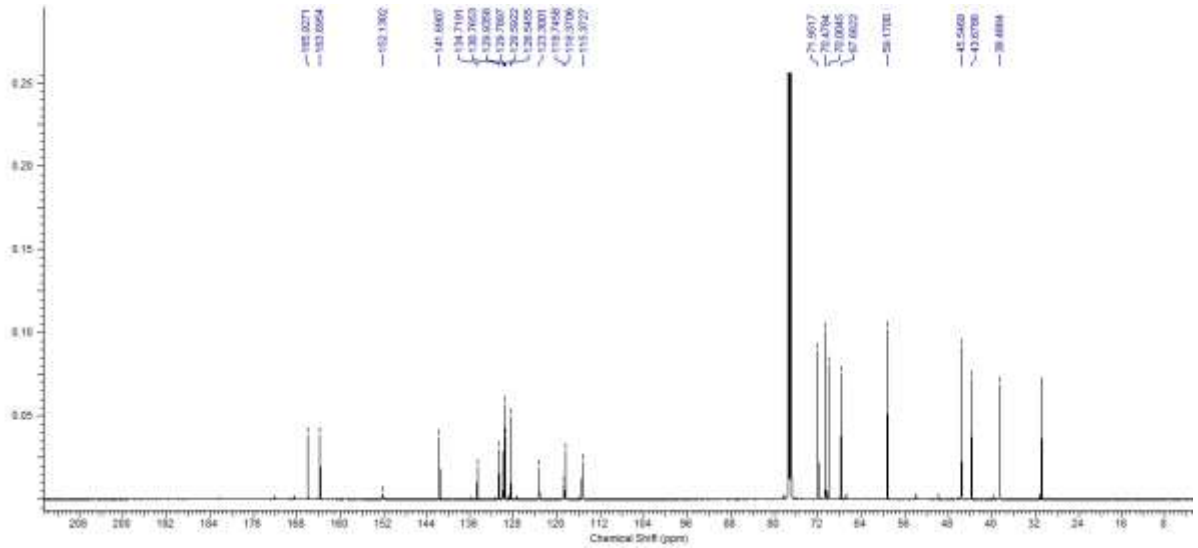


To a de-gassed solution of dansyldisulfide **13** (25 mg, 0.04 mmol) in methanol (10 mL) at room temperature was added tris(2-carboxyethyl)phosphine hydrochloride (12 mg, 0.04 mmol). The reaction mixture was stirred for 15 minutes at room temperature. It was then added drop-wise to a de-gassed solution of 3,4-dibromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione **23** (32 mg, 0.08 mmol) and potassium acetate (24 mg, 0.24 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 30 minutes. All volatile material was removed *in vacuo*, and the residue purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, gradient elution from 1:0 to 0:1) to afford the title compound as a yellow gum (25 mg, 0.04 mmol, 50%):

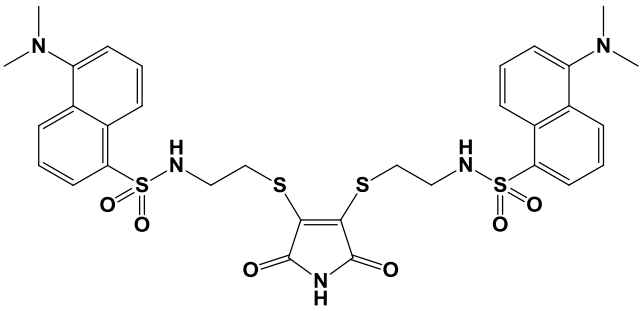
^{1}H NMR (600MHz, $CDCl_3$): δ = 8.53 (d, *J* = 8.2, 1H), 8.24 - 8.23 (m, 2H), 7.54 - 7.50 (m, 2H), 7.15 (d, *J* = 7.4, 1H), 5.55 (t, *J* = 6.2, 1H), 3.69 (t, *J* = 5.6, 2H), 3.59-3.49 (m, 10H), 3.40-3.35 (m, 5H), 3.24 (q, *J* = 5.4, 2H), 2.88 (s, 6H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 166.0 (C), 163.7 (C), 152.1 (C), 141.7 (C), 134.7 (C), 130.8 (CH), 120.0 (C), 129.8 (CH), 129.6 (C), 128.6 (CH), 123.3 (CH), 118.8 (CH), 118.4 (C), 115.4 (CH), 72.0 (CH_2), 70.51(CH_2), 70.48 (CH_2), 70.0 (CH_2), 67.7 (CH_2), 59.2 (CH_3), 45.6 (2 × CH_3), 43.7 (CH_2), 38.5 (CH_2),

30.8 (CH_2); IR (neat): 2925 (br), 1713 (s) cm^{-1}; LRMS (ES) 654 (100, $[^{81}M+Na]^+$), 652 (98, $[^{79}M+Na]^+$), 632 (48, ^{81}M), 630 (45, ^{79}M); HRMS (ES) calcd for $C_{25}H_{32}N_3O_7S_2Br$ $[M+H]^+$ 630.0943, observed: 630.0910; UV (Acetonitrile) $\varepsilon_{335} = 2117\ M^{-1}\ cm^{-1}$.



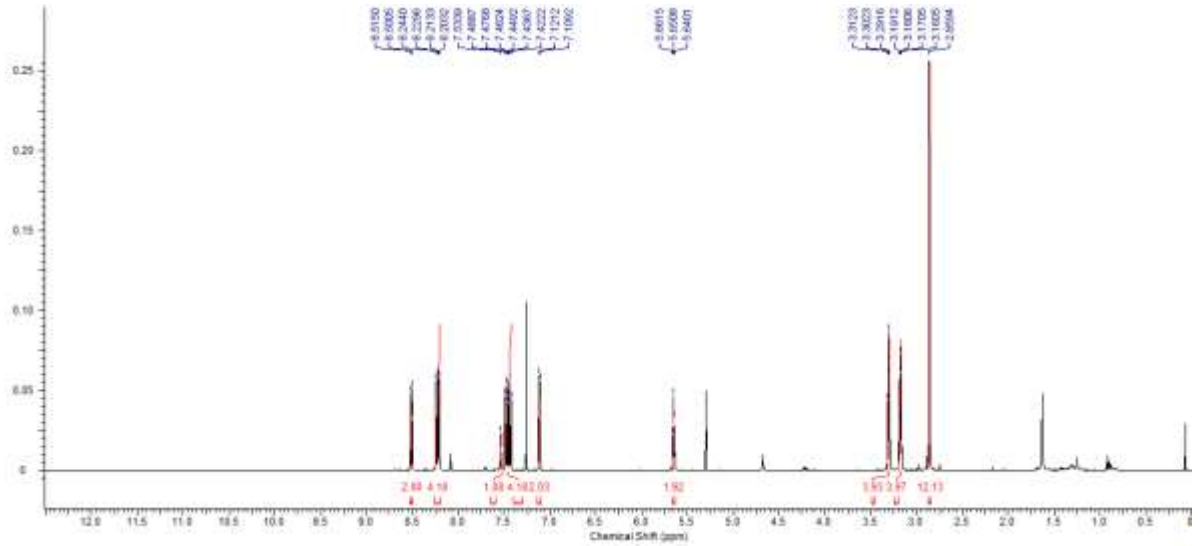


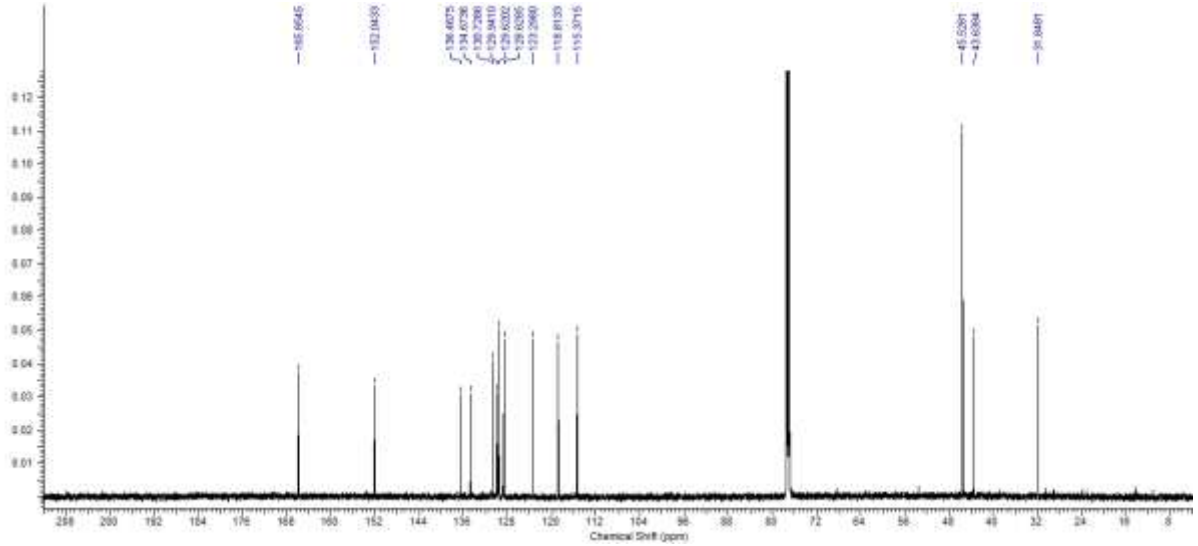
_N_,_N_'-(((2,5-dioxo-2,5-dihydro-1_H_-pyrrole-3,4-diyl)bis(sulfanediyl))bis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide) 18



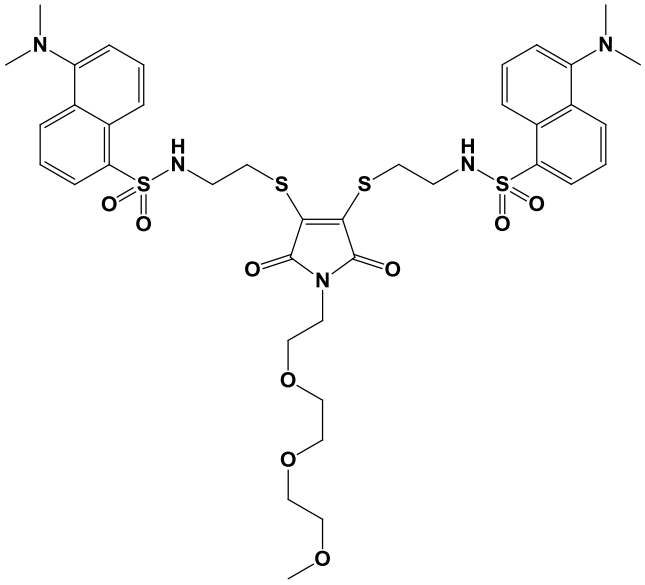
To a solution of dansyldisulfide **13** (100 mg, 0.16 mmol) in methanol (10 mL) at room temperature was added tris(2-carboxyethyl)phosphine hydrochloride (46 mg, 0.16 mmol). The reaction mixture was stirred for 3 hours. Then 3,4-Dibromo-1*H*-pyrrole-2,5-dione (36mg, 0.14 mmol) in methanol (5 mL) was then added to the reaction mixture which was stirred for 16 hours. Then sodium acetate (53 mg, 0.64 mmol) was added to the reaction mixture before all volatile material was removed *in vacuo*. H_2O (50 mL) was added and the reaction mixture was extracted into dichloromethane (3 x 40 mL) and the combined organic layers washed with brine (50 mL), dried (magnesium sulfate) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (dichloromethane: ethyl acetate, 8:2) to afford the title compound as a bright yellow gum (40 mg, 0.056 mmol, 40%):

^{1}H NMR (600MHz, $CDCl_3$): δ = 8.50 (d, *J* = 8.5, 2H), 8.24 – 8.19 (m, 4H), 7.79 (br s, 1H) 7.48 – 7.41 (m, 4H), 7.10 (d, *J* = 7.4, 2H), 5.75 (t, *J* = 6.3, 2H), 3.29 (t, *J* = 6.1, 4H), 3.17 (q, *J* = 6.1, 4H), 2.85 (s, 12H); ^{13}C NMR (150MHz, $CDCl_3$): δ = 166.1 (C), 152.0 (C), 136.4 (2 × C), 134.7 (2 × C), 130.7 (2 × CH), 129.93 (2 × C), 129.85 (2 × CH), 129.67 (2 × C), 128.6 (2 × CH), 123.3 (2 × CH), 118.8 (2 × CH), 115.4 (2 × CH), 45.5 (4 × CH_3), 43.7 (2 × CH_2), 31.8 (2 × CH_2); IR (neat): 3288 (br), 1720 (s) cm^{-1}; LRMS (ES) 736.1 (100, $[M+Na]^+$), 714 (36, M); HRMS (ES) calcd for $C_{32}H_{35}N_5O_6S_4$ $[M+Na]^+$: 736.1368, observed 736.1390; UV (Acetonitrile) ε_{335} = 4752 M^{-1} cm^{-1}.





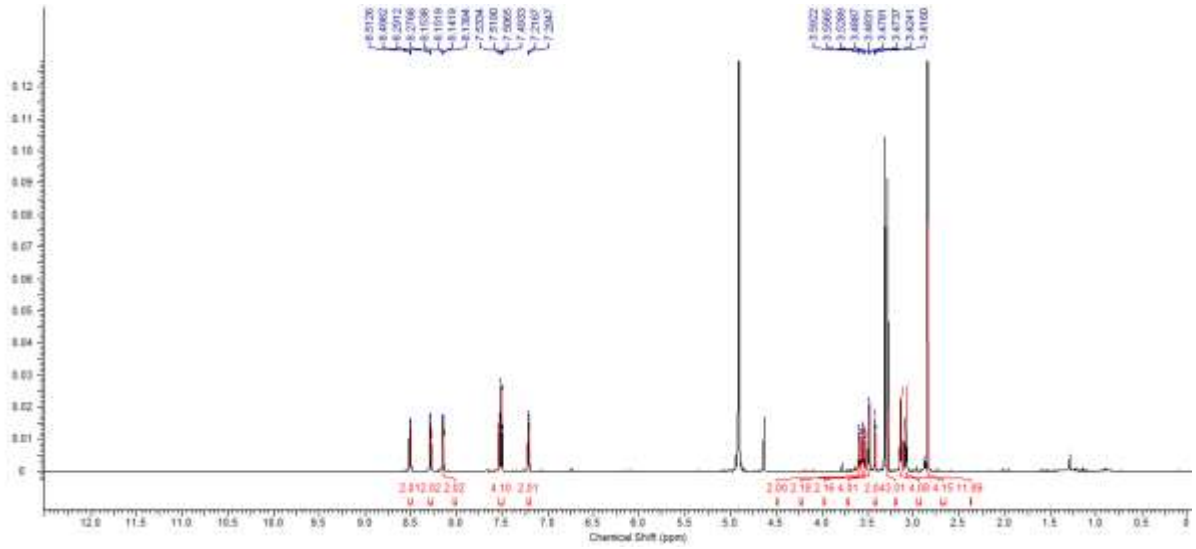
_N,N_'-(((1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1_H_-pyrrole-3,4-diyl)bis(sulfanediyl))bis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide) 19

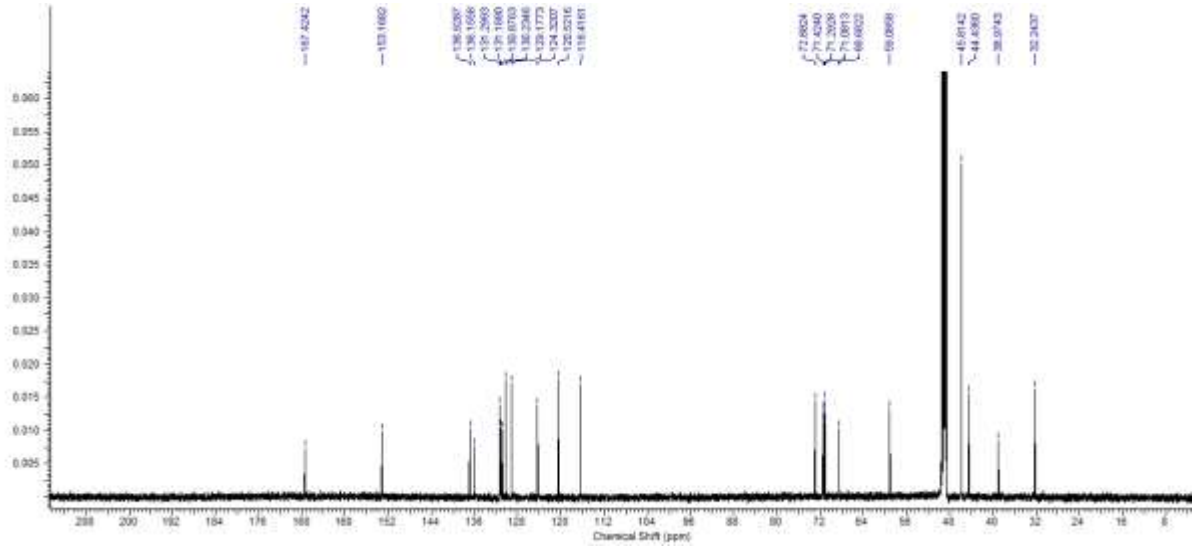


To a de-gassed solution of dansyldisulfide **13** (50 mg, 0.08 mmol) and potassium acetate (32 mg, 0.32 mmol) in methanol (10 mL) at room temperature was added tris(2-carboxyethyl)phosphine hydrochloride (23 mg, 0.08 mmol). The reaction mixture was stirred for 15 minutes. A de-gassed solution of 3,4-dibromo-1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-1*H*-pyrrole-2,5-dione **23** (32 mg, 0.08 mmol) in methanol (5 mL) was then added to the reaction mixture which was stirred for 30 minutes. All volatile material was removed *in vacuo* and the residue was purified by flash chromatography (chloroform: methanol, 96:4) to afford the title compound as a bright yellow gum (22 mg, 0.026, 32%):

^{1}H NMR (600MHz, MeOD): δ = 8.51 (d, *J* = 8.5, 2H), 8.28 (d, *J* = 8.6, 2H), 8.15 (dd, *J* = 6.1 and 1.1, 2H), 7.51 (q, *J* = 8.0, 4H), 7.21 (d, *J* = 7.4, 2H), 3.60 – 3.41 (m, 12H), 3.30 (s, 3H), 3.15 - 3.08 (m, 8H)), 2.85 (s, 12H); ^{13}C NMR (150MHz, MeOD): δ = 167.4 (2 × C), 153.2 (2 × C), 136.9 (2 × C), 136.2 (2 × C), 131.3 (2 × CH), 131.2 (2 × C), 130.9 (2 × C), 130.2 (2 × CH), 129.2 (2 × CH), 124.3 (2 × CH), 120.5 (2 × CH), 116.4 (2 × CH), 72.9 (CH_2), 71.4 (CH_2), 71.3 (CH_2), 71.1 (CH_2), 68.7 (CH_2), 59.1 (CH_3) 45.8 (4 × CH_3), 44.4 (2 × CH_2), 39.0

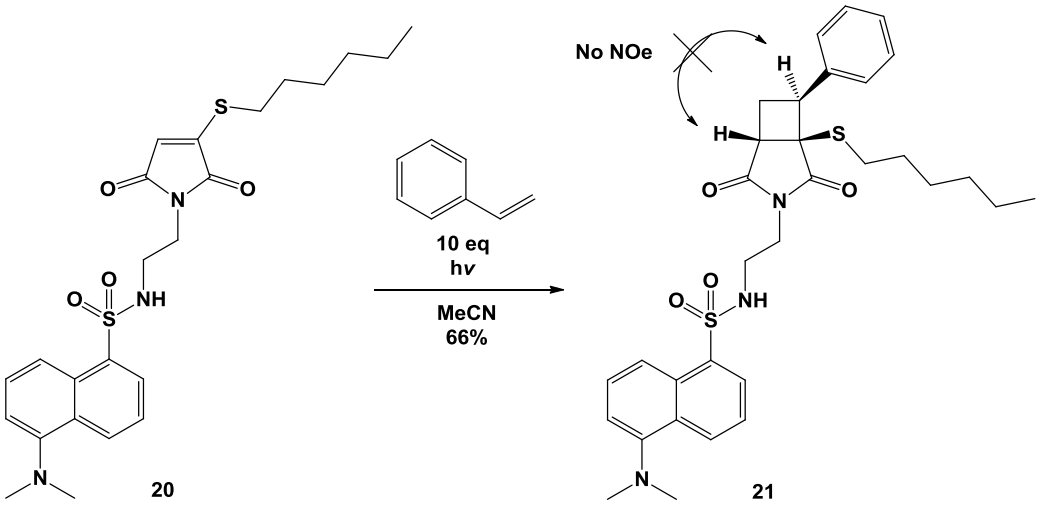
(CH_2), 32.2 (4 × CH_2); IR (neat): 3323 (br), 1652 (br) cm^{-1}; LRMS (ES) 882 (100, $[M+Na]^+$), 860.3 (50, M^+); HRMS (ES) calcd for $C_{39}H_{49}N_5O_9S_4$ $[M+Na]^+$: 882.2311, observed 882.2294; UV (Acetonitrile) ε_{335} = 3940 M^{-1} cm^{-1}.





Photocycloadditon Reactions with Thiomaleimides

We were also intrigued by other possible reactions that would lead to removal of the dansylmaleimide double bond and subsequently turn-on fluorescence. Tedaldi and co-workers recently reported that thiomaleimides undergo highly efficient [2+2] photocycloadditions with olefins to form cyclobutanes.[4] There would potentially be broad application for turn-on reporter molecules in photochemical bioconjugation and surface modification.[5] We thus were prompted to conduct a [2+2] cycloaddition with an *N*-dansylmaleimide expecting that the PET quenching mechanism would be disrupted and the resultant cyclobutane would be fluorescent. *N*-Dansylthiomaleimide **20**, **Scheme 1.0**, synthesised by addition of hexanethiol to dansyl-bromomaleimide **9**, underwent cycoladdition with styrene to produce cyclobutane **21** in a reaction similar to that with the analagous *N*-H thiomaleimide.[4]



Scheme 1.0

Fluorescence Spectra of Dansylmaleimide compounds 20 and 21

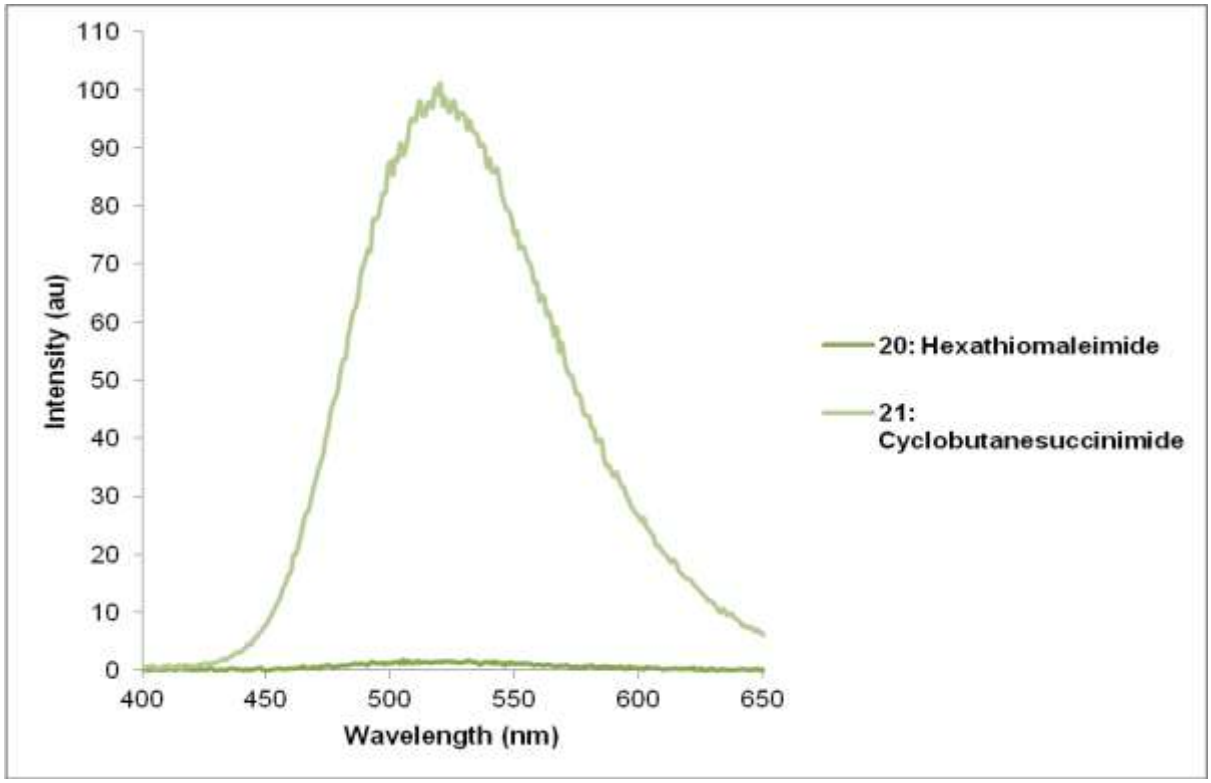


Figure 1.3 Fluorescence spectra of hexathiomaleimide **20** (8 µM) and cyclobutanesuccinimide **21** (8 µM) in Acetonitrile at 25°C, λ_{exc} = 335 nm.

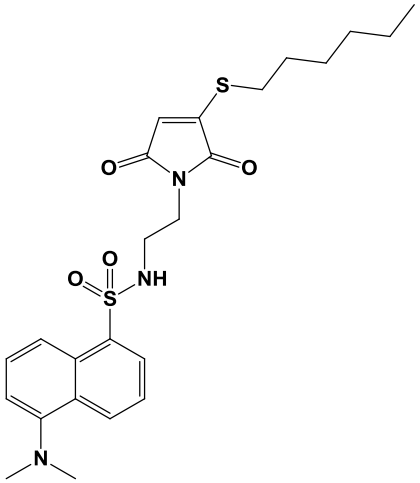
Fluorescence Quantum Yields of Dansylmaleimide compounds 20 and 21

Compound	ϵ_{max} [a] (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_{fl} [b]
20	7434	514	0.009
21	4523	516	0.32

[a] Measured at 335 nm 25°C. [b] Quantum yields were calculated according to the method shown in ref[6]. [c] Value taken from ref[7].

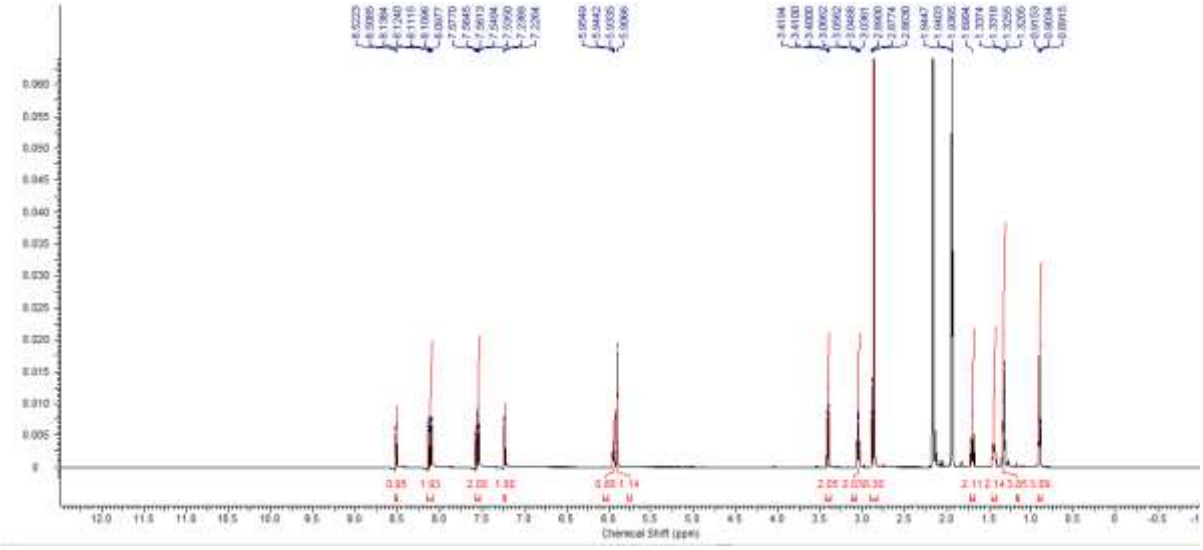
Table 1.0 Molar Absorption Coefficients and Fluorescence Quantum Yields in Acetonitrile at λ_{exc} = 335 nm.

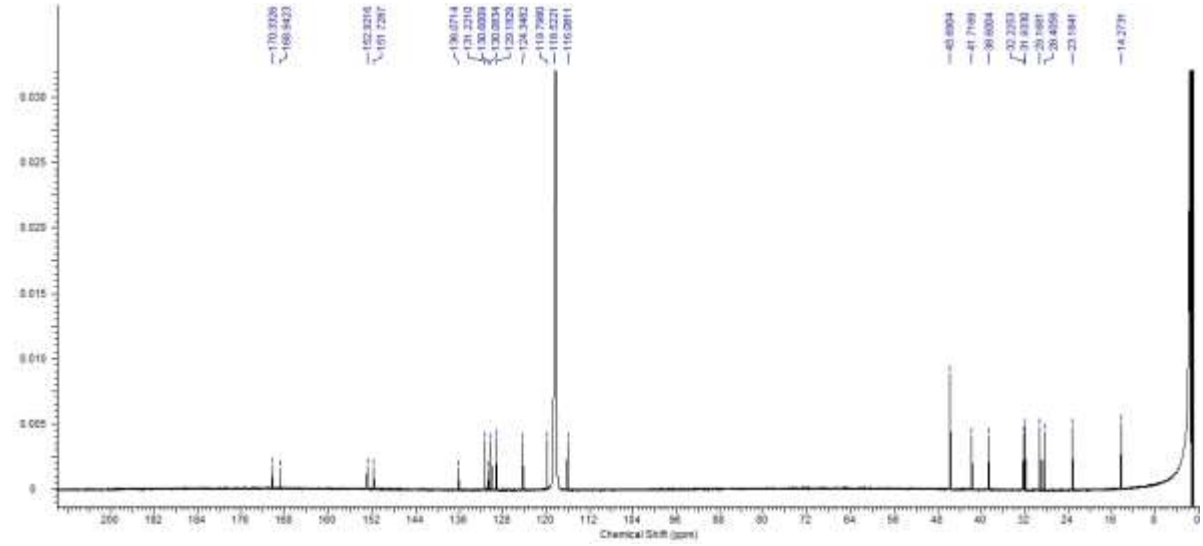
5-(dimethylamino)-*N*-(2-(3-(hexylthio)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl)naphthalene-1-sulfonamide 20



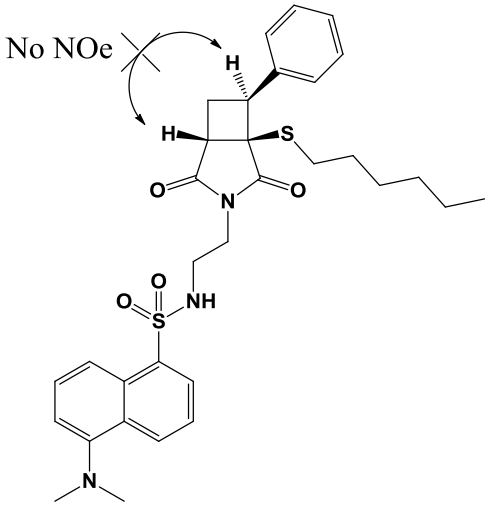
To a solution of dansyl-monobromomaleimide **9** (200 mg, 0.44 mmol) and sodium acetate (18 mg, 0.22 mmol) in methanol (200 mL), at room temperature was added a solution of hexane thiol (31 µL, 0.22 mmol) in methanol (100 mL) over 3 hours. The solvent was then removed *in vacuo*, and the residue purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 4:1) to afford the title compound as a yellow oil (49 mg, 0.10 mmol, 46%):

^{1}H NMR (600MHz, CD_3CN): δ = 8.52 (d, *J* = 8.5, 1H), 8.14-8.10 (m, 2H), 7.58-7.54 (m, 2H), 7.23 (d, *J* = 7.6, 1H), 5.94 (br t, *J* = 6.2, 1H), 5.91 (s, 1H), 3.41 (t, *J* = 5.9, 2H), 3.05 (q, *J* = 6.0, 2H), 2.89-2.86 (m, 8H), 1.70 (qn, *J* = 7.5, 2H), 1.44 (br qn, *J* = 7.5, 2H), 1.34-1.31 (m, 4H), 0.90 (t, *J* = 3.5, 3H); ^{13}C NMR (125MHz, CD_3CN): δ = 170.3 (C), 168.9 (C), 152.9 (C), 151.7 (C), 136.1 (C), 131.2 (CH), 130.6 (C), 130.14 (C), 130.08 (CH), 129.2 (CH), 124.4 (CH), 119.8 (CH), 118.5 (CH), 116.1 (CH), 45.7 (2 × CH_3), 41.7 (CH_2), 38.6 (CH_2), 32.2 (CH_2), 31.9 (CH_2), 29.2 (CH_2), 28.4 (CH_2), 23.2 (CH_2), 14.3 (CH_3); IR (neat): 3294 (br), 1700 (s) cm^{-1}; LRMS (ES) 489 (10, M), 488 (27, $[M-H]^+$), 339 (32), 325 (45); HRMS (ES) calcd for $C_{24}H_{31}N_3O_4S_2$ $[M-H]^+$ 488.1678, observed: 488.1674: UV (Acetonitrile) ε_{335} = 7437 M^{-1} cm^{-1}.



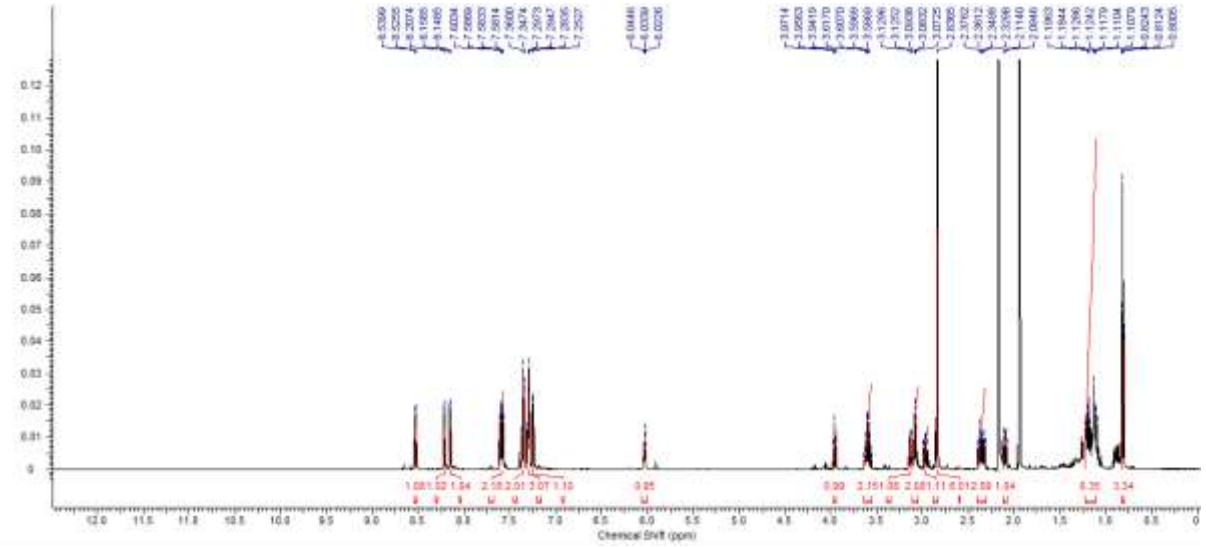


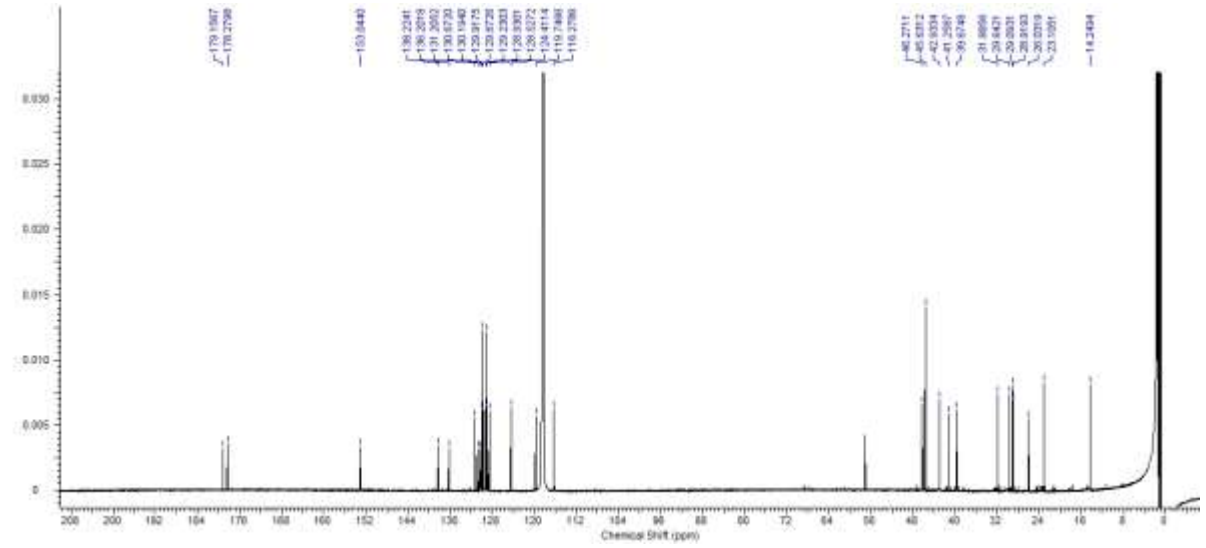
5-(dimethylamino)-*N*-(2-((1*R*,5*S*,7*S*)-1-(hexylthio)-2,4-dioxo-7-phenyl-3-azabicyclo[3.2.0]heptan-3-yl)ethyl)naphthalene-1-sulfonamide 21



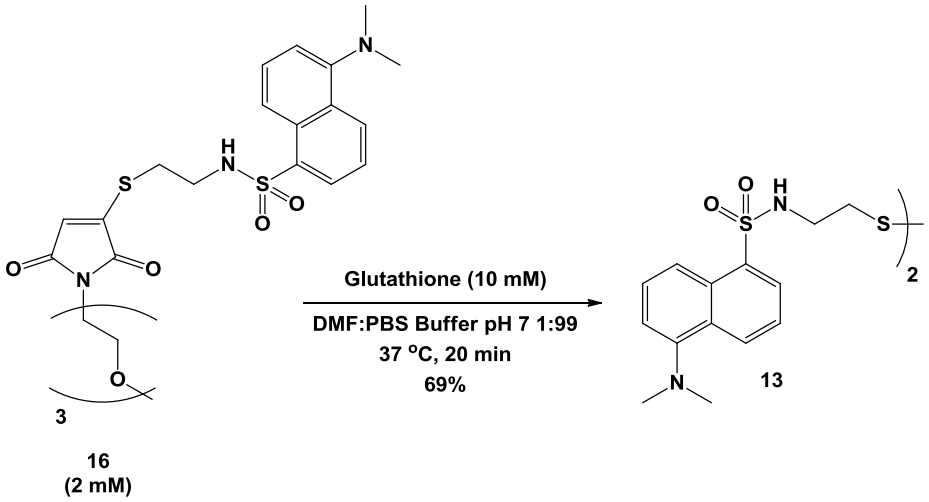
To a de-gassed solution of 5-(dimethylamino)-N-(2-(3-(hexylthio)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)naphthalene-1-sulfonamide **20** (5 mg, 0.0102 mmol) in acetonitrile (2 mL), at room temperature, was added styrene (0.23 mL, 0.20 mmol). The resulting solution was irradiated in pyrex glassware for 15 minutes. The solvent was removed *in-vacuo* and the residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate, 7:3) to afford the title compound as a green gum (4 mg, 0.00674 mmol, 66%):

^{1}H NMR (600MHz, CD_3CN): δ = 8.53 (d, *J* = 8.6, 1H), 8.21 (d, *J* = 8.6, 1H), 8.15 (dd, *J* = 7.3 and 1.1, 1H) 7.62-7.57 (m, 2H), 7.36 (t, *J* = 7.3, 2H) 7.32-7.28 (m, 3H), 7.25 (d, *J* = 7.6, 1H), 6.03 (t, *J* = 6.6, 1H), 3.96 (t, *J* = 8.9, 1H), 3.64-3.55 (m, 2H), 3.14 (ddd, *J* = 11.1, 3.2 and 1.2, 1H), 3.10-3.06 (m, 2H), 2.99-2.94 (m, 1H) 2.84 (s, 6H), 2.40-2.32 (m, 2H), 2.13-2.08 (m, 1H), 1.27-1.08 (m, 8H), 0.81 (t, *J* = 7.2, 3H); ^{13}C NMR (125MHz, CD_3CN): δ = 179.2 (C), 178.3 (C), 153.0 (C), 138.2 (C), 136.2 (C), 131.2 (CH), 130.7 (C), 130.2 (C), 129.9 (CH), 129.7 (CH), 129.2 (CH), 128.9 (CH), 128.5 (CH), 124.4 (CH), 119.7 (CH), 116.3 (CH), 57.1 (C), 46.3 (CH), 45.6 (3 × CH_3), 42.9 (CH), 41.6 (CH_2), 39.7 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 14.3 (CH_3); IR (neat): 3310 (br), 1697 (s) cm^{-1}; LRMS (ES) 593 (31, M), 592 (100, M-H$^+$), 283 (21), 255 (50); HRMS (ES) calcd for $C_{32}H_{39}N_3O_4S_2$ [M-H]$^+$ 592.2394, observed: 592.2353; UV (Acetonitrile) ε_{335} = 4523 M^{-1} cm^{-1}.





Confirmation of the cleavable nature of dansylthiomaleimide 16 with glutathione under approximately physiological conditions:

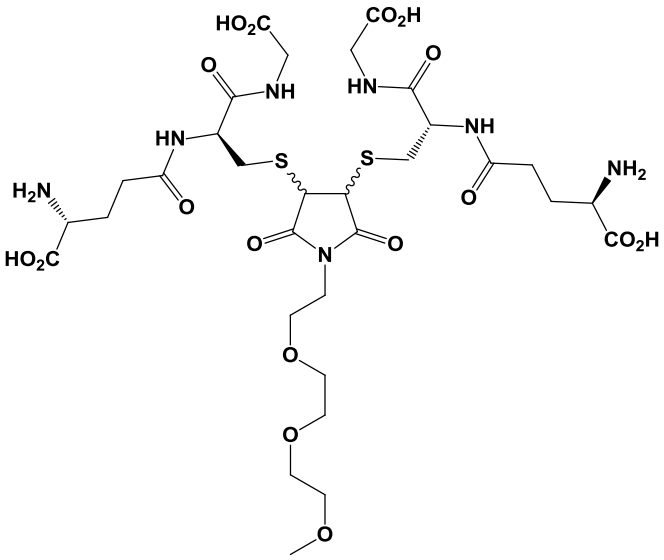


To a solution of dansylthiomaleimide **16** (9 mg, 0.016 mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) : dimethylformamide, 1:1.3 (7 mL) at 37°C was added a solution of glutathione (25 mg, 0.082 mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) (1 mL). The reaction mixture was stirred for 20 minutes. The mixture was then extracted into dichloromethane (3 x 20 mL) and the combined organic layers washed with brine (20 mL), dried (magnesium sulfate) and concentrated *in vacuo* to afford dansyldisulfide **13** as a dark yellow gum (7 mg, 0.011 mmol, 69%):

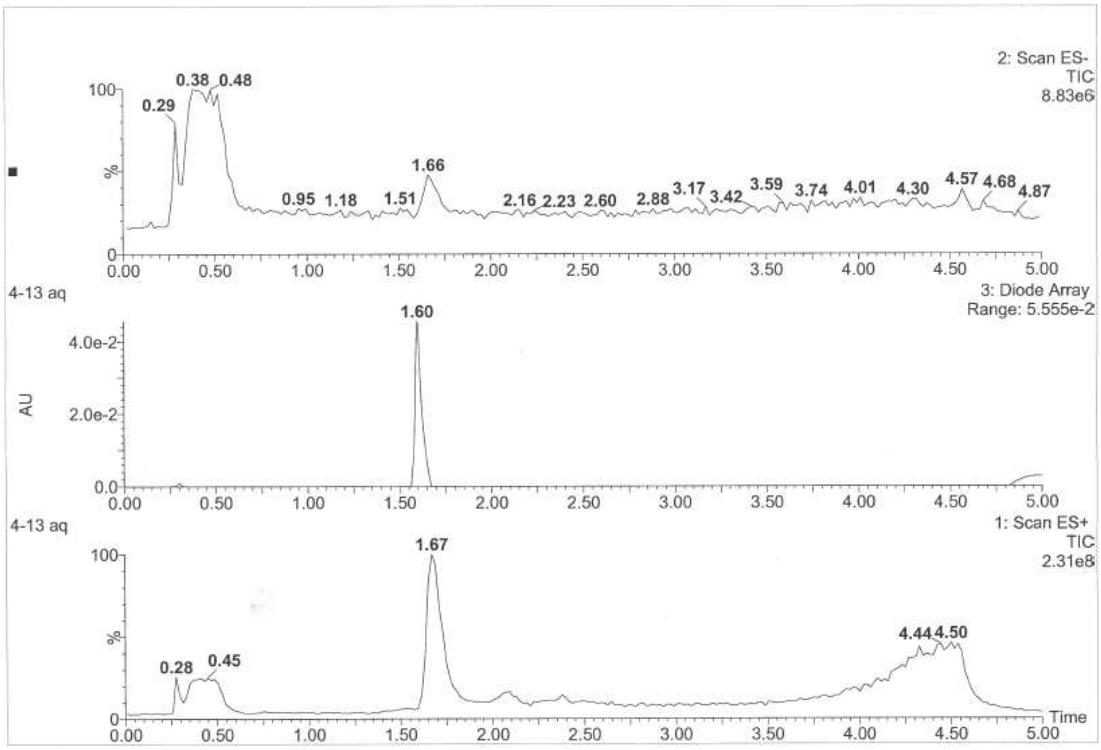
Data for dansyldisulfide **13** matched that obtained previously.

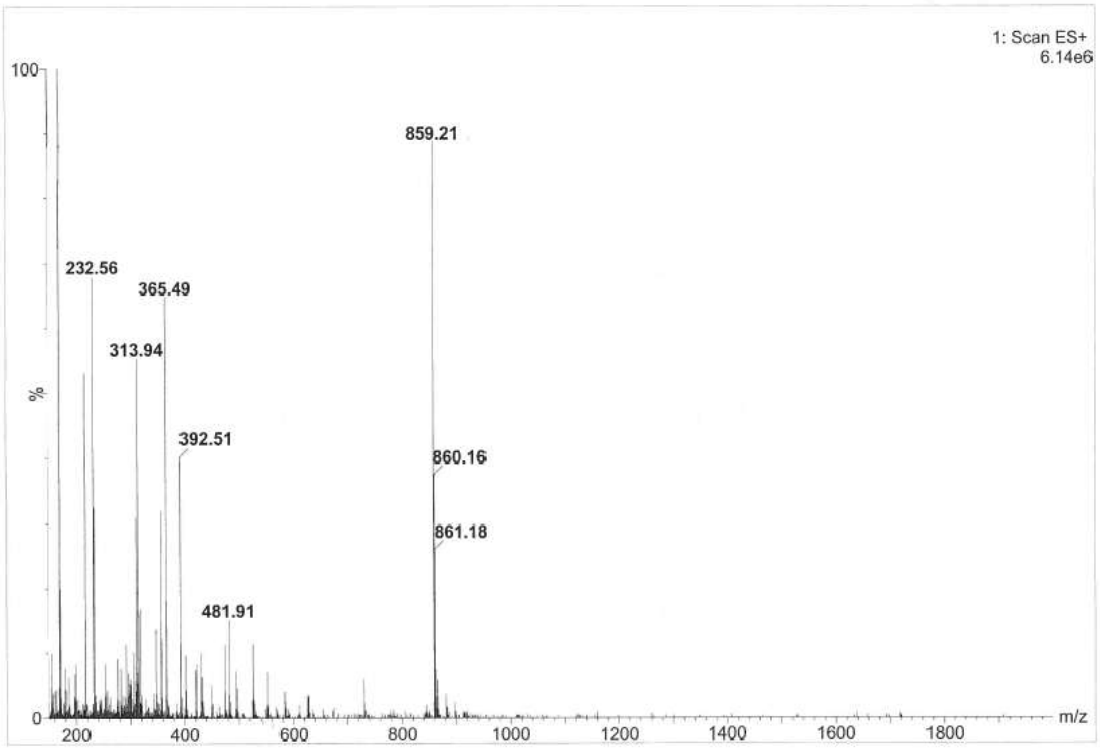
LCMS analysis of the colourless aqueous phase indicated the presence of glutathione-maleimide **22**.

(2*R*,2'*R*)-5,5'-(((2*S*,2'*S*)-((1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,5-dioxopyrrolidine-3,4-diyl)bis(sulfanediyl))bis(1-((caboxymethyl)amino)-1-oxopropane-3,2-diyl))bis(azanediyl))bis(2-amino-5-oxopentanoic acid) 22



LCMS (ES) 859 (88, $[M+4H]^+$)





Fluorescence Spectra of the Initial and Final Reaction mixtures for the reaction of dansylthiomaleimide 16 with glutathione under approximately physiological conditions

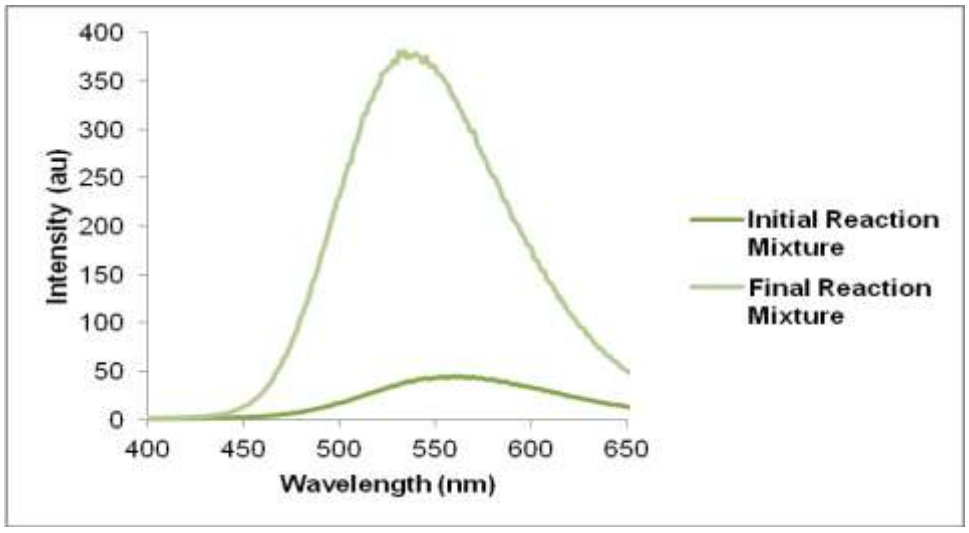
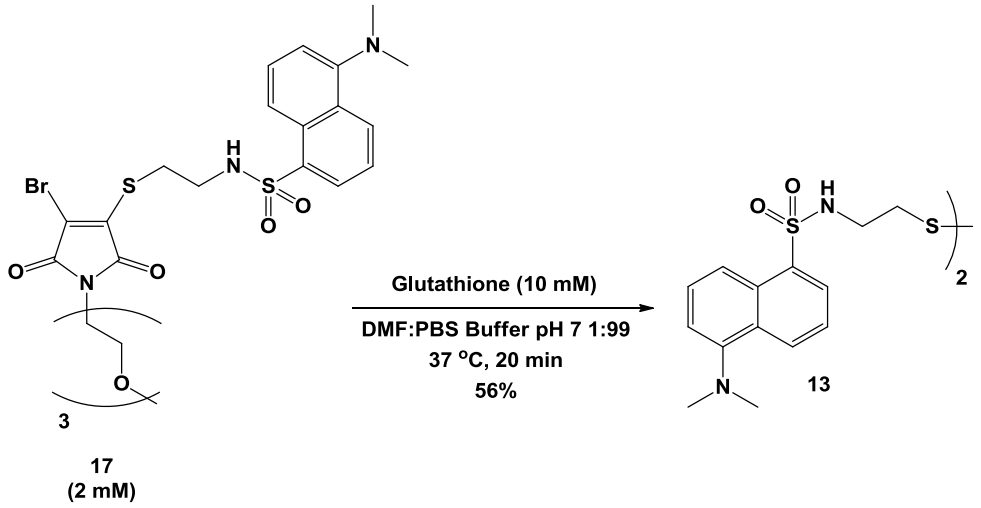


Figure 1.4 Fluorescence Spectra of Initial and Final Reaction mixture, λ_{exc} = 335 nm.

Reaction mixture	Concentration (mM)	Maximum intensity (au)	λ_{em} (nm)	Fold increase in fluorescence (536 nm)
Initial	0.11	45	560	-
Final	0.11	374	536	10

Table 1.1

Confirmation of the cleavable nature of dansylbromothiomaleimide 17 with glutathione under approximately physiological conditions:

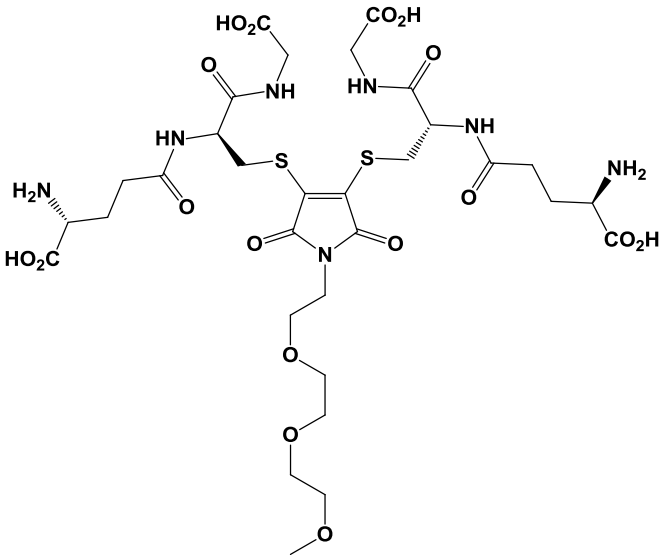


To a solution of dansylbromothiomaleimide **17** (5.8 mg, 9.2×10^{-3} mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) : dimethylformamide, 1:1.4 (4 mL) at 37°C was added a solution of glutathione (14 mg, 0.046 mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) (0.6 mL). The reaction mixture was stirred for 20 minutes. The mixture was then extracted into dichloromethane (3 x 10 mL) and the combined organic layers washed with brine (10 mL), dried (magnesium sulfate) and concentrated *in vacuo* to afford dansyldisulfide **13** as a dark yellow gum (3.2 mg, 5.2×10^{-3} mmol, 57%):

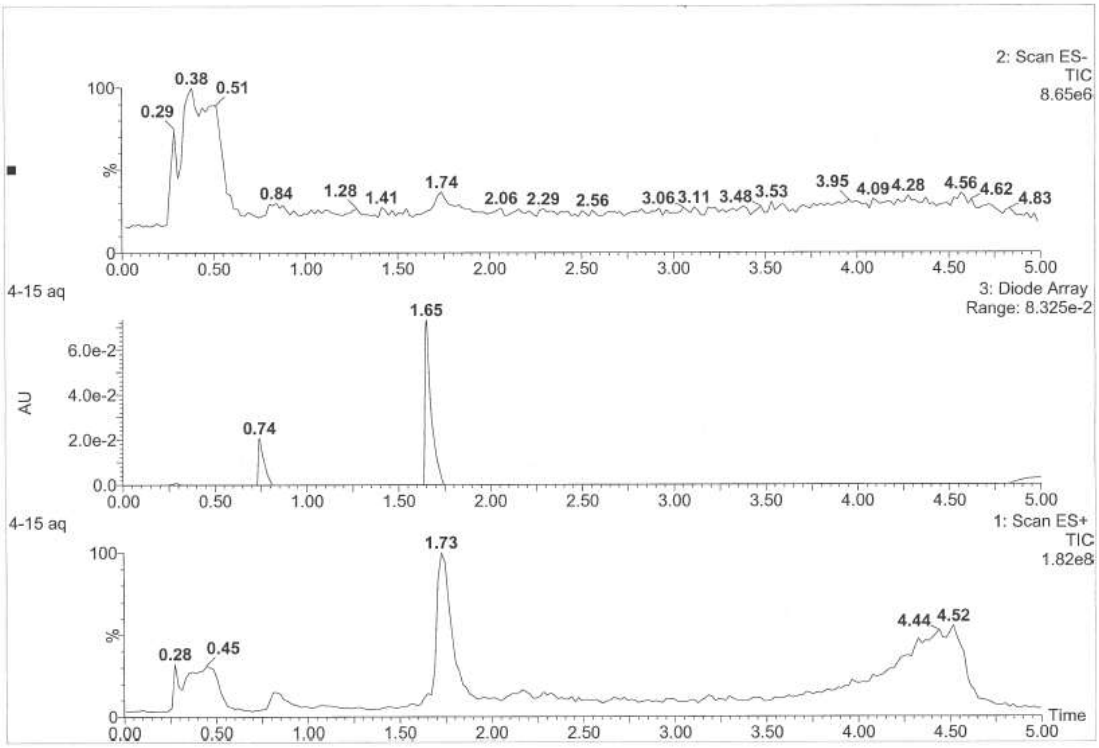
Data for dansyldisulfide **13** matched that obtained previously.

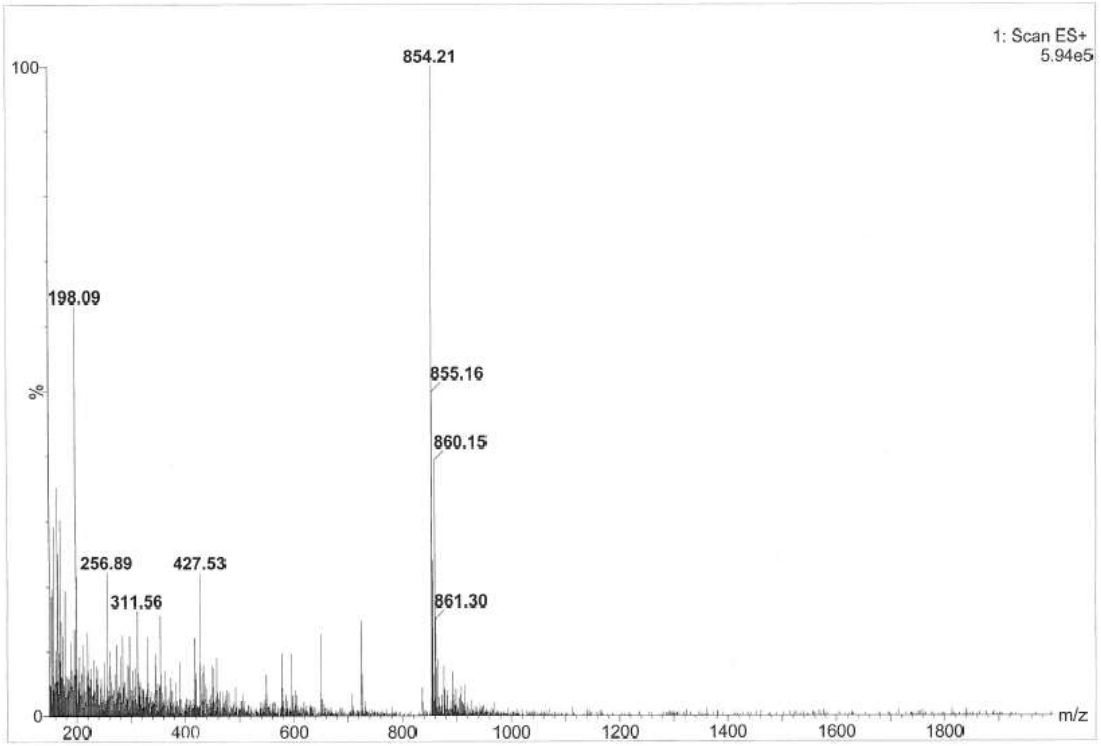
LCMS analysis of the yellow aqueous phase indicated the presence of glutathione-maleimide **23**.

(2*R*,2'*R*)-5,5'-(((2S,2'*S*)-((1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(sulfanediyl)bis(1-((carboxymethyl)amino)-1-oxopropane-3,2-diyl))bis(azanediyl))bis(2-amino-5-oxypentanoic acid) 23



LCMS (ES) 854 (100, $[M+H]^+$)


2: Scan ES-
TIC
8.65e6
0.29
0.38
0.51
0.84
1.28
1.41
1.74
2.06
2.29
2.56
3.06
3.11
3.48
3.53
3.95
4.09
4.28
4.56
4.62
4.83
4-15 aq
3: Diode Array
Range: 8.325e-2
AU
0.74
1.65
4-15 aq
1: Scan ES+
TIC
1.82e8
0.28
0.45
1.73
4.44
4.52
Time


1: Scan ES+
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854.21
198.09
855.16
860.15
256.89
427.53
311.56
861.30
m/z

Fluorescence Spectra of the Initial and Final Reaction mixtures for the reaction of dansylbromothiomaleimide 17 with glutathione under approximately physiological conditions

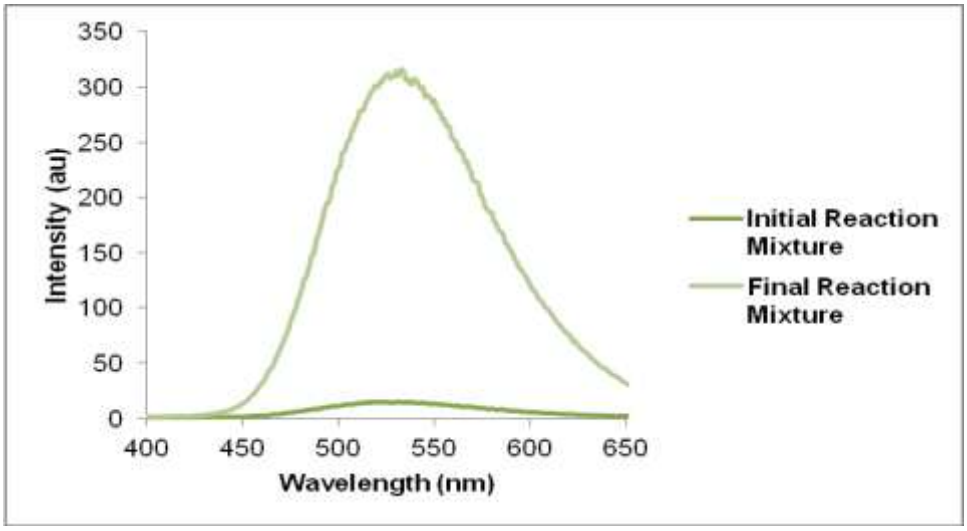
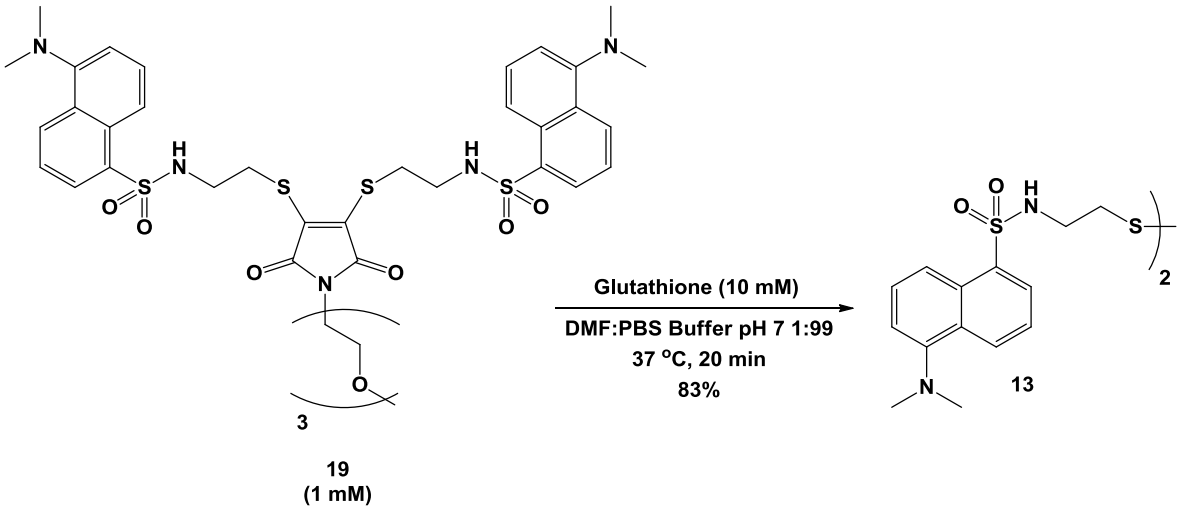


Figure 1.5 Fluorescence Spectra of Initial and Final Reaction mixture, λ_{exc} = 335 nm.

Reaction mixture	Concentration (mM)	Maximum intensity (au)	λ_{em} (nm)	Fold increase in fluorescence (536 nm)
Initial	0.077	15	532	-
Final	0.077	307	536	21

Table 1.2

Confirmation of the cleavable nature of didansylthiomaleimide 19 with glutathione under approximately physiological conditions:



To a solution of didansylthiomaleimide **19** (8.6 mg, 0.01 mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) : dimethylformamide, 1:1.25 (9 mL) at 37°C was added a solution of glutathione (30.7 mg, 0.1 mmol) in aqueous buffer (150 mM NaCl, 100 mM NaH_2PO_4, pH 7.4) (1 mL). The reaction mixture was stirred for 20 minutes. The mixture was then extracted into dichloromethane (3 x 20 mL) and the combined organic layers washed with brine (20 mL), dried (magnesium sulfate) and concentrated *in vacuo* to afford dansyldisulfide **13** as a dark yellow gum (7.1 mg, 8.3×10^{-3} mmol, 83%):

Data for dansyldisulfide **13** matched that obtained previously.

LCMS analysis of the yellow aqueous phase indicated the presence of glutathione-maleimide **23**.

Fluorescence Spectra of the Initial and Final Reaction mixtures for the reaction of didansylthiomaleimide 19 with glutathione under approximately physiological conditions

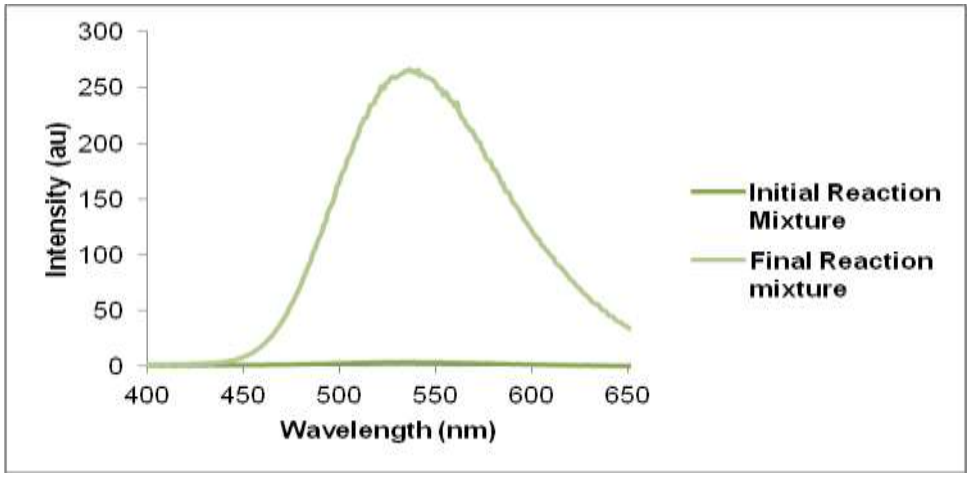


Figure 1.6 Fluorescence Spectra of Initial and Final Reaction mixture, λ_{exc} = 335 nm.

Reaction mixture	Concentration (mM)	Maximum intensity (au)	λ_{em} (nm)	Fold increase in fluorescence (536 nm)
Initial	0.05	4	533	-
Final	0.05	266	536	72

Table 1.3

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