Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Michael addition-elimination-cyclization based turn-on fluorescence (MADELCY TOF) probes for cellular cysteine imaging and estimation of blood serum cysteine and aminoacylase-1

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Section A: General Information

All the syntheses were performed in oven-dried glassware and under an inert atmosphere of nitrogen or argon. The chemicals were purchased from Sigma-Aldrich, Thermo-scientific, Hi-media, Spectrochem, Avra, and Thomas Baker and were used without further purification. The aminoacylase 1 (ACY-1) enzyme and human blood serum were purchased from Sigma-Aldrich. Anhydrous dichloromethane (DCM) and *N*,*N*-diisopropylethylamine (DIPEA) were prepared by distilling them over calcium hydride (CaH_2). The progress of the reactions was monitored by thin-layer chromatography (TLC) using 0.25 mm Merck pre-coated (60 F_{254}) plates, and UV light (254 nm or 365 nm) was employed for visualizing UV-active spots on the TLC. Synthesized compounds were purified using column chromatography on silica gel (100-200 mesh). Synthesis of fluorescent compounds and their purifications by column chromatography was performed in the dark by wrapping the reaction apparatus and the columns with aluminum foil.

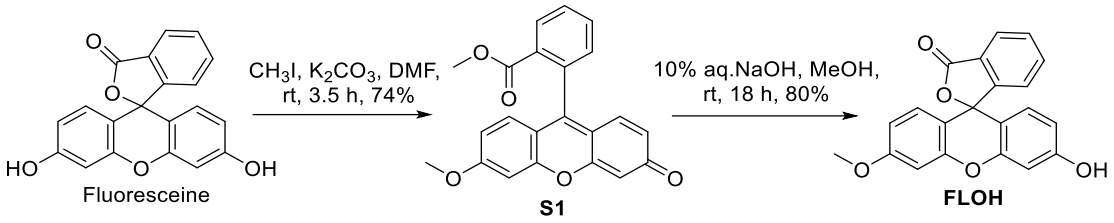
The ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400, 500, and 700 MHz spectrometers using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) were reported in ppm relative to the residual solvent signal. High-resolution mass spectra (HRMS) were recorded on Bruker micro-TOF mass spectrometer. The High-Performance Liquid Chromatography (HPLC) studies were performed on Agilent Technologies (1260 Infinity II) instrument using reverse-phase analytical column, C18-Zorbax ODS (5 μm, 4.6 × 250 mm). The mobile phase was acetonitrile-water containing 0.1% trifluoroacetic acid (TFA); solvent elution profile employed: for 0-5 min elution with 100% water followed by gradual increase to 100% acetonitrile over 30 min. The flow rate was 1 mL/min, and the UV absorbance was monitored at 215 nm wavelength unless specified. For semi-preparative HPLC, C18-Zorbax ODS (5 μm, 9.4 × 250 mm) column was used using a similar gradient solvent system (without 0.1% TFA) as employed for analytical HPLC with a 3 mL/min flow rate. HPLC grade acetonitrile (ACN) and TFA were purchased from Sigma-Aldrich. Type-1 water (resistivity 18.2 MΩ.cm at 25°C) from the Merck-Millipore system was used for HPLC and buffer preparations. The aqueous samples were lyophilized on a Labconco freeze dryer. UV-Vis spectra were recorded on Agilent Carry 3000 UV-Vis spectrophotometer using quartz cuvette (450 μL) of 5 mm path length. Fluorescence measurements were recorded on Flurolog-3 from HORIBA Jobin Yvon using a quartz cuvette (1 mL) of 10 mm path length.

Probe stock solutions were prepared in ACN. The stock solutions of various analytes such as GSH, amino acids (Cys, Hcy, Lys, Trp, Ser, Pro, Val, Phe, Met, Leu, Ile, His, Gly, Glu, Gln, Asp, Asn, Ala, and Thr) and salts (NaCl, $CaCl_2$, KI, $CdCl_2$, Na_2CO_3, $FeCl_2$, $FeCl_3$, $MgCl_2$, $ZnCl_2$, Na_2SO_4, $NaHCO_3$, NaOAc, $NaSO_3H$) were prepared in type 1 water.

HeLa cells were cultured in a Galaxy 170 S, New Brunswick, Eppendorf CO_2 incubator maintaining a temperature of 37 °C and CO_2 level of 5%. Cell culture consumables such DMEM, FBS, penicillin-streptomycin, and trypsin-EDTA were purchased from Invitrogen and Thermofisher scientific. Confocal imaging dishes (Catalog no. 100350) were purchased from SPL Life Sciences. Cells were imaged under Zeiss LSM 780 inverted confocal microscope using a 60× oil objective and 488 nm excitation wavelength. The absorbance in the cell viability assay was measured using SpectraMaxi3X multimode reader (Molecular Devices).

Section B: Synthesis of probes 1-7

Scheme S1a. Synthesis of FLOH



3'-hydroxy-6'-methoxy-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (**FLOH**)[1]

To a stirred solution of fluoresceine (5.0 gm, 15.05 mmoL) in DMF (30 mL) was added K_2CO_3 (4.27 gm, 30.08 mmoL) and methyl iodide (1.82 mL, 30.08 mmoL) at room temperature under N_2 atmosphere. After 3.5 h, the reaction mixture was diluted with water (25 mL) and filtered. The resultant residue was washed with water, dried in the air, and purified by column chromatography (DCM/MeOH 50:1) using silica gel to give orange-colored solid **S1** (3.99 gm, 74%, R_f 0.35 in 100% EtOAc).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.76 (td, *J* = 7.5, 1.3 Hz, 1H), 7.69 (td, *J* = 7.7, 1.3 Hz, 1H), 7.33 (dd, *J* = 7.5, 1.0 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.88 (dd, *J* = 18.3, 9.3 Hz, 2H), 6.76 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.56 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 3.93 (s, 3H), 3.65 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 185.73, 165.62, 164.07, 158.98, 154.31, 150.20, 134.64, 132.70, 131.14, 130.58, 130.34, 130.22, 129.92, 129.67, 128.85, 117.60, 114.84, 113.44, 105.78, 100.35, 77.30, 77.05, 76.79, 55.99, 52.41, 1.02.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 132.70, 131.14, 130.58, 130.22, 129.92, 129.67, 128.85, 113.44, 105.78, 100.34, 55.99.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{22}H_{17}O_5$ $[M+H]^+$ 361.1076; found 361.1071.

To a stirred solution of **S1** (1.2 gm, 3.33 mmoL) in MeOH (30 mL) was added 10% aqueous NaOH solution (4 mL) at room temperature under N_2 atmosphere. After 18 h, the reaction mixture was concentrated in *vacuo* on a rotary evaporator. The resultant crude mixture was diluted with water (50 mL) and acidified to pH ~2 with 1N HCl (~25 mL). The precipitated yellow solid was filtered and dried in the air. The crude solid was purified by column chromatography (DCM/MeOH 20:1) on silica gel to give FLOH as yellow solid (80%, 0.92 gm, R_f 0.4 in DCM/MeOH 20:1).

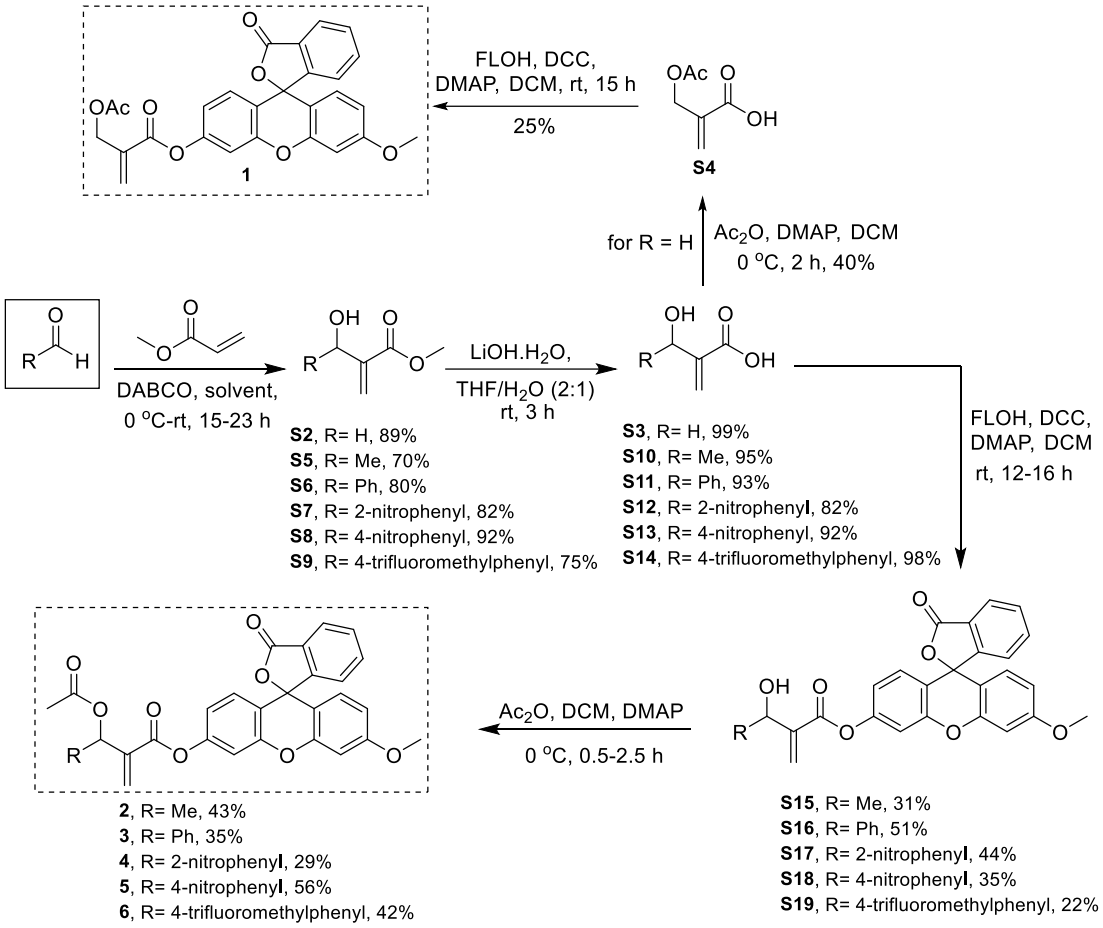
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.01 (d, *J* = 7.3 Hz, 1H), 7.80 (t, *J* = 7.4 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.73 – 6.63 (m, 3H), 6.58 (d, *J* = 1.4 Hz, 2H), 3.82 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 169.12, 161.48, 160.02, 152.33, 152.22, 136.12, 130.61, 129.54, 129.40, 126.49, 125.12, 124.46, 113.26, 112.35, 111.45, 109.89, 102.67, 101.24, 40.63, 40.42, 40.21, 40.00, 39.79, 39.58, 39.38.

^{13}C NMR DEPT-135 (100 MHz, DMSO-d_6) δ 136.11, 130.60, 129.54, 129.41, 125.12, 124.46, 113.26, 112.35, 102.67, 101.24, 56.14.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{21}H_{14}O_5$ $[M+H]^+$ 347.0919; found 347.0914.

Scheme S1b. Synthesis of probes **1-6**



Methyl 2-(hydroxymethyl)acrylate (S2)[2]

To a stirred solution of formalin (37% in water, 0.34 mL, 4.17 mmoL) in 1,4-dioxane/water (1:1, 10 mL) was added DABCO (0.47 gm, 4.16 mmoL) followed by the addition of methyl acrylate (1.13 mL, 12.48 mmoL) dropwise at room temperature. After 15 h, the reaction mixture was diluted with EtOAc (30 mL) and water (25 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 3). All the organic layers were combined and washed with brine solution (30 mL), dried (Na_2SO_4), filtered, and concentrated in *vacuo* on a rotary evaporator to yield crude product that was purified by column chromatography (hexane/EtOAc 3:1) on silica gel to give **S2** as clear liquid (0.43 gm, 89%, R_f 0.6 in hexane/EtOAc 3:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.28 (s, 1H), 5.87 (d, *J* = 1.3 Hz, 1H), 4.35 (s, 2H), 3.81 (s, 3H), 2.39 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 166.79, 139.29, 125.90, 62.52, 51.94.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 125.91, 51.94, 51.94.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_5H_8O_3$ $[M+H]^+$ 117.0552; found 117.0555.

Methyl 3-hydroxy-2-methylenebutanoate (S5)[2]

Following the procedure as described for **S2**, acetaldehyde (0.60 mL, 10.08 mmoL), DABCO (1.13 gm, 10.08 mmoL) and methyl acrylate (2.80 mL, 30.98 mmoL) afforded **S5** as colourless liquid (0.92 gm, 70%, R_f 0.25 in hexane/EtOAc 3:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 18 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (s, 1H), 5.85 (s, 1H), 4.64 (p, *J* = 6.1 Hz, 1H), 3.80 (s, 3H), 2.73 (d, *J* = 5.6 Hz, 1H), 1.40 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 167.09, 143.46, 124.18, 67.12, 51.89, 22.05.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 124.19, 67.12, 51.90, 22.05.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_6H_{10}O_3$ $[M+Na]^+$ 153.0528; found 153.0522.

Methyl 2-(hydroxy(phenyl)methyl)acrylate (S6)

To a stirred solution of benzaldehyde (0.83 gm, 7.84 mmoL) in MeOH (5 mL) was added DABCO (0.877 gm, 7.84 mmoL) followed by the addition of methyl acrylate (4.97 mL, 54.87 mmoL) dropwise at 0°C under N_2 atmosphere. The reaction was allowed to warm to room temperature. After 23 h, the reaction mixture was concentrated in *vacuo* on a rotary evaporator, and the resultant crude mixture was diluted with water (50 mL) and EtOAc (30 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (30 mL × 2). All the organic layers were combined and washed with brine solution (40 mL), dried (Na_2SO_4), filtered, and concentrated in *vacuo* on a rotary evaporator to yield a crude product that was purified by column chromatography (hexane: EtOAc 3:1) on silica gel to give **S6** as white viscous semi-solid (1.21 gm, 80%, R_f 0.33 in hexane/EtOAc 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (dt, *J* = 15.0, 7.3 Hz, 4H), 7.31 (t, *J* = 7.0 Hz, 1H), 6.36 (s, 1H), 5.86 (s, 1H), 5.59 (d, *J* = 5.2 Hz, 1H), 3.75 (s, 3H), 3.09 (d, *J* = 5.6 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 166.79, 141.97, 141.27, 128.45, 127.85, 126.59, 126.16, 73.29, 51.97.
^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 128.45, 127.85, 126.59, 126.16, 73.29, 51.97.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{11}H_{12}O_3[M+H]^+$ 215.0684; found 215.0679.

Methyl 2-(hydroxy(2-nitrophenyl)methyl)acrylate (S7)

Following the procedure as described for **S6**, 2-nitrobenzaldehyde (0.75 gm, 4.96 mmoL), DABCO (0.556 gm, 4.96 mmoL) and methyl acrylate (3.21 mL, 34.85 mmoL) afforded **S7** as colourless liquid (82%, 0.97 gm, R_f 0.42 in hexane/EtOAc 3:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 18 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.79 – 7.75 (m, 1H), 7.67 (td, *J* = 7.8, 1.0 Hz, 1H), 7.53 – 7.46 (m, 1H), 6.39 (s, 1H), 6.23 (d, *J* = 4.6 Hz, 1H), 5.75 (s, 1H), 3.76 (s, 3H), 3.46 (d, *J* = 4.8 Hz, 1H), 1.66 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 166.45, 148.37, 140.72, 136.09, 133.49, 128.91, 128.73, 126.52, 124.61, 67.74, 52.18.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 133.49, 128.91, 128.73, 126.52, 124.61, 67.74.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{11}H_{11}NO_5$ $[M+Na]^+$ 260.0535; found 260.0536.

Methyl 2-(hydroxy(4-nitrophenyl)methyl)acrylate (S8)

Following the procedure as described for **S6**, 4-nitrobenzaldehyde (1.0 gm, 6.62 mmoL), DABCO (0.74 gm, 6.62 mmoL) and methyl acrylate (1.50 mL, 16.49 mmoL) afforded **S8** as white solid (1.44 gm, 92%, R_f 0.6 in hexane/EtOAc 3:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 13 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.5 Hz, 2H), 6.42 (s, 1H), 5.90 (s, 1H), 5.65 (d, *J* = 6.2 Hz, 1H), 3.77 (s, 2H), 3.34 (s, *J* = 6.2 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 166.40, 148.56, 147.48, 140.94, 127.33, 127.30, 123.64, 72.79, 52.23.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 127.33, 127.30, 123.64, 72.79, 52.24.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{11}H_{11}NO_5$ $[M+Na]^+$ 260.0535; found 260.0529.

Methyl 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)acrylate (S9)

Following the procedure as described for **S6**, 4-(trifluoromethyl)benzaldehyde (0.30 gm, 1.72 mmoL), DABCO (0.19 gm, 1.72 mmoL) and methyl acrylate (1.10 mL, 12.14 mmoL) afforded **S9** as colourless liquid (0.334 gm, 75%, R_f 0.33 in hexane/EtOAc 3:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 16 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 2H), 6.39 (s, 1H), 5.87 (s, 1H), 5.62 (d, *J* = 5.7 Hz, 1H), 3.76 (s, 3H), 3.27 (d, *J* = 6.0 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.58, 145.25, 141.36, 126.84, 125.43, 125.40, 125.37, 125.34, 123.01, 72.92, 52.12.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 126.87, 126.84, 125.43, 125.40, 125.37, 125.34, 72.92.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{12}H_{11}F_3O_3$ $[M+H]^+$ 261.0739; found 261.0733.

2-(hydroxymethyl)acrylic acid (S3)[3]

To a stirred solution of **S2** (0.20 gm, 1.72 mmoL) in THF/water (2:1, 15 mL) was added $LiOH.H_2O$ (0.115 gm, 2.74 mmoL) at room temperature. After 3 h, the reaction mixture was concentrated *in vacuo* on a rotary evaporator to remove THF. The resultant aqueous solution was diluted with water (10 mL), and the pH was adjusted to ~1 by adding dilute HCl (1 N) followed by extraction with EtOAc (15 mL × 3). All the organic layers were combined and washed with brine solution (25 mL), dried (Na_2SO_4), filtered, and concentrated in *vacuo* on a rotary evaporator to give analytically pure **S3** as a clear liquid (0.17 gm, 99%, R_f 0.6 in 100% EtOAc).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.43 (s, 1H), 5.99 (s, 1H), 4.38 (s, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.88, 138.65, 128.33, 62.11.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 128.34, 62.11.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_4H_6O_3$ $[M+H]^+$ 103.0395; found 103.0397.

3-hydroxy-2-methylenebutanoic acid (S10)[3]

Following the procedure as described for **S3**, **S5** (0.53 gm, 4.07 mmoL) and $LiOH.H_2O$ (0.30 gm, 7.16 mmoL) afforded analytically pure **S10** as colourless liquid (0.45 gm, 95%, R_f 0.33 in hexane/EtOAc 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (s, 1H), 5.97 (s, 1H), 4.68 (q, *J* = 6.4 Hz, 1H), 1.44 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.97, 142.79, 126.55, 66.93, 21.97.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 126.55, 66.93, 21.96.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_5H_8O_3$ $[M+H]^+$ 117.0552; found 117.0546.

2-(hydroxy(phenyl)methyl)acrylic acid (S11)

Following the procedure as described for **S3**, **S6** (0.20 gm, 1.06 mmoL) and $LiOH.H_2O$ (0.071 gm, 1.69 mmoL) afforded analytically pure **S11** as colourless liquid (0.17 gm, 93%, R_f 0.28 in hexane/EtOAc 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.35 (m, 4H), 7.32 (dd, *J* = 7.9, 5.2 Hz, 1H), 6.51 (s, 1H), 5.98 (s, 1H), 5.60 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 140.91, 128.56, 128.54, 128.04, 126.64, 72.88.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 128.57, 128.54, 128.04, 126.64, 72.88.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{10}H_{10}O_3$ $[M+Na]^+$ 201.0528; found 201.0522.

2-(hydroxy(2-nitrophenyl)methyl)acrylic acid (S12)

Following the procedure as described for **S3**, **S7** (0.30 gm, 1.27 mmoL) and $LiOH.H_2O$ (0.09 gm, 2.15 mmoL) afforded analytically pure **S12** as white solid (0.231 gm, 82%, R_f 0.33 in hexane/EtOAc 3:1).

^{1}H NMR (500 MHz, DMSO) δ 7.90 (d, *J* = 8.7 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.63 (d, *J* = 7.0 Hz, 1H), 7.53 (t, *J* = 7.1 Hz, 1H), 6.19 (s, 1H), 6.04 (s, 1H), 5.72 (s, 1H), 3.35 (s, 1H).

^{13}C NMR (125 MHz, DMSO) δ 167.19, 148.77, 144.11, 137.75, 133.50, 129.35, 128.95, 124.59, 124.44, 65.71.

^{13}C NMR DEPT-135 (125 MHz, DMSO) δ 133.50, 129.35, 128.95, 124.59, 124.44, 65.71.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{10}H_9NO_5$ $[M+H]^+$ 224.0559; found 224.0553.

2-(hydroxy(4-nitrophenyl)methyl)acrylic acid (S13)

Following the procedure as described for **S3**, **S8** (1.70 gm, 7.17 mmoL) and $LiOH.H_2O$ (0.51 gm, 12.17 mmoL) afforded analytically pure **S13** as dark yellow solid (1.471 gm, 92%, R_f 0.6 in hexane/EtOAc 1:3).

^{1}H NMR (500 MHz, CD_3OD) δ 8.21 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 6.40 (t, *J* = 1.2 Hz, 1H), 6.09 (t, *J* = 1.5 Hz, 1H), 5.69 (s, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ 167.33, 150.19, 147.25, 143.25, 127.74, 124.42,

^{13}C NMR_DEPT-135 (126 MHz, CD_3OD) δ 127.74, 124.42, 122.89, 70.81, 122.89, 70.81.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{10}H_9NO_5$ $[M+Na]^+$ 246.0378; found 246.0345.

2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)acrylic acid (S14)

Following the procedure as described for **S3**, **S9** (0.17 gm, 0.65 mmoL) and $LiOH.H_2O$ (0.05 gm, 1.19 mmoL) afforded analytically pure **S14** as white solid (0.16 gm, 98%, R_f 0.34 in Hexane/EtOAc 2.5:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 6.54 (s, 1H), 6.00 (s, 1H), 5.63 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.69, 144.85, 140.77, 129.30, 126.93, 125.51, 125.49, 125.46, 125.43, 72.38.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 129.30, 126.92, 125.51, 125.48, 125.45, 125.42, 72.38.

HRMS (QTOF MS ESI+) *m/z* calculated $[M+Na]^+$ = (269.0400) found $[M+H]^+$ = (269.0400).

2-(acetoxymethyl)acrylic acid (S4)[1]

To a stirred solution of **S3** (0.12 gm, 1.13 mmoL) and DMAP (27.5 mg, 0.23 mmoL) in anhydrous DCM (8 mL) was added a solution of acetic anhydride (0.18 mL, 1.92 mmoL in) in DCM (1mL) dropwise at 0 °C under Ar atmosphere in dark. After 2 h, the reaction mixture was concentrated in *vacuo* on a rotary evaporator and diluted with water (10 mL) and DCM (10 mL). The aqueous layer was separated and extracted with DCM (15 mL × 3). All the organic layers were combined and washed with brine solution (25 mL), dried (Na_2SO_4), filtered, and concentrated in *vacuo* on a rotary evaporator to give the crude product that was purified by column chromatography (hexane/EtOAc 3:1) on silica gel to give **S4** as white solid (65 mg, 40%, R_f 0.45 in hexane/EtOAc 3:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.53 (s, 1H), 6.01 (s, 1H), 4.84 (s, 2H), 2.14 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.48, 170.01, 134.72, 130.05, 62.39, 20.85.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 130.05, 62.12, 20.86.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_6H_8O_4$ $[M+H]^+$ 145.0501; found 145.0495.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(acetoxymethyl)acrylate (1)[4]

To a stirred solution of **S4** (37.0 mg, 0.26 mmoL) in anhydrous DCM (5 mL) was added *N,N'*-dicyclohexaneylcarbodiimide (DCC, 52.0 mg, 0.26 mmoL), DMAP (3.0 mg, 0.026 mmoL) and FLOH (88.0 mg, 0.26 mmoL) at room temperature in dark under N_2 atmosphere. After 15 h, the precipitated solid was filtered and washed with DCM (5 mL). The filtrate was concentrated in *vacuo* on a rotary evaporator. The resultant crude mixture was redissolved in ACN (5 mL) and kept at 0 ºC for 30 min. The precipitated urea by-product was filtered, and the filtrate was concentrated in *vacuo* on a rotary evaporator to give the crude product that was purified by column chromatography (hexane/EtOAc 3:1) on silica gel to give **1** as white solid (30.0 mg, 25%, R_f 0.5 in hexane/EtOAc 3:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.5 Hz, 1H), 7.68 (td, *J* = 7.4, 1.0 Hz, 1H), 7.63 (dd, *J* = 10.8, 4.1 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.13 (s, 1H), 6.83 (d, *J* = 1.1 Hz, 2H), 6.78 (d, *J* = 2.5 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 6.64 (d, *J* = 2.5 Hz, 1H), 6.62 (s, 1H), 6.07 (s, 1H), 4.92 (s, 2H), 3.85 (s, 3H), 2.14 (s, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 170.37, 163.15, 161.51, 153.05, 152.25, 151.93, 151.67, 135.14, 134.69, 129.90, 129.16, 129.03, 126.51, 125.15, 124.01, 117.30, 117.03, 112.02, 110.91, 110.30, 109.30, 100.89, 84.68, 82.38, 62.28, 55.62, 20.86.

^{13}C NMR DEPT-135 (175 MHz, $CDCl_3$) δ 135.14, 129.89, 129.16, 129.03, 127.06, 125.15, 124.01, 117.30, 112.02, 110.31, 100.89, 62.28, 55.62, 20.86.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{27}H_{20}O_8$ $[M+Na]^+$ 495.1056; found 495.1050.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 3-hydroxy-2-methylenebutanoate (S15)

Following the procedure as described for **1**, **S10** (0.10 gm, 0.86 mmoL), DCC (0.18 gm, 0.86 mmoL), DMAP (10.5 mg, 0.09 mmoL) and FLOH (0.298 gm, 0.86 mmoL) afforded **S15** as white solid (0.12 gm, 31%, R_f 0.32 in DCM/MeOH 20:1) after column chromatography (DCM/MeOH 20:1) on silica gel. The reaction time was 15 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 1H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.14 (s, 1H), 6.86 (s, 2H), 6.81 (d, *J* = 2.4 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.66 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.53 (s, 1H), 6.12 (s, 1H), 4.76 (q, *J* = 5.8 Hz, 1H), 3.87 (s, 3H), 1.49 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.29, 164.50, 161.52, 153.05, 152.25, 151.95, 151.71, 143.01, 135.15, 129.90, 129.15, 129.03, 126.50, 126.48, 125.15, 124.00, 117.41, 117.04, 112.01, 110.91, 110.40, 100.91, 82.40, 66.97, 55.60, 22.26.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 135.15, 129.90, 129.15, 129.03, 126.48, 125.15, 124.00, 117.41, 112.01, 110.39, 100.91, 66.97, 55.61.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{26}H_{20}O_7$ $[M+H]^+$ 445.1287; found 445.1282.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(hydroxy(phenyl)methyl)acrylate (S16)

Following the procedure as described for **1**, **S11** (0.10 gm, 0.56 mmoL), DCC (0.12 gm, 0.56 mmoL), DMAP (7.0 mg, 0.056 mmoL) and FLOH (0.19 gm, 0.56 mmoL) afforded **S16** as white solid (0.15 gm, 51%, R_f 0.4 in hexane/EtOAc 1:1). after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 15 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 1H), 7.66 (dt, *J* = 22.5, 7.2 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.02 (dd, *J* = 12.3, 2.0 Hz, 1H), 6.83 – 6.77 (m, 2H), 6.74 – 6.67 (m, 2H), 6.67 – 6.62 (m, 2H), 6.17 (s, 1H), 5.71 (s, 1H), 3.86 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.28, 164.17, 161.49, 153.03, 152.22, 151.85, 151.57, 141.54, 141.03, 135.13, 129.88, 129.09, 129.02, 128.65, 128.19, 127.97, 126.74, 126.47, 125.13, 123.97, 117.30, 116.99, 112.00, 110.86, 110.34, 100.87, 82.38, 73.02, 55.61.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 135.13, 129.88, 129.09, 129.02, 128.65, 128.19, 127.97, 126.75, 125.13, 123.97, 117.31, 112.00, 110.31, 100.87, 73.02, 55.61.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{31}H_{22}O_7$ $[M+H]^+$ 507.1444; found 507.1438.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(hydroxy(2-nitrophenyl)methyl)acrylate (S17)

Following the procedure as described for **1**, **S11** (0.13 gm, 0.56 mmoL), DCC (0.115 gm, 0.56 mmoL), DMAP (7.0 mg, 0.056 mmoL) and FLOH (0.19 gm, 0.56 mmoL) afforded **S17** as white solid (44%, 0.14 gm, R_f 0.27 in hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 12 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 13.2, 7.9 Hz, 2H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.68 (ddd, *J* = 23.4, 16.3, 7.5 Hz, 3H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.05 (dd, *J* = 7.7, 2.2 Hz, 1H), 6.83 – 6.78 (m, 2H), 6.73 (ddd, *J* = 11.9, 5.9, 2.8 Hz, 2H), 6.67 (s, 1H), 6.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.32 (s, 1H), 6.02 (s, 1H), 3.86 (s, 3H), 3.39 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.30, 163.93, 161.50, 153.04, 152.21, 151.87, 151.52, 148.31, 140.25, 136.02, 135.15, 133.73, 129.89, 129.12, 129.01, 128.90, 128.72, 126.46, 125.13, 124.85, 123.98, 117.22, 117.08, 112.02, 110.86, 110.28, 100.89, 82.37, 67.65, 55.62.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{31}H_{21}NO_9$ $[M+H]^+$ 552.1295; found 552.1289.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(hydroxy(4-nitrophenyl)methyl)acrylate (S18)

Following the procedure as described for **1**, **S13** (0.14 gm, 0.62 mmoL), DCC (0.128 gm, 0.62 mmoL), DMAP (8.00 mg, 0.06 mmoL) and FLOH (0.22 gm, 0.62 mmoL) afforded **S18** as white solid (35%, 77.0 mg, R_f 0.56 in hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 20 h.

^{1}H NMR (500 MHz, CD_3Cl) δ 8.25 (d, *J* = 8.5 Hz, 2H), 8.05 (d, *J* = 7.5 Hz, 1H), 7.69 (s, 1H), 7.65 (d, *J* = 8.8 Hz, 3H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.03 (s, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.79 (s, 1H), 6.74 (d, *J* = 13.2 Hz, 2H), 6.71 (s, 2H), 6.67 – 6.64 (m, 1H), 6.20 (s, 1H), 5.79 (s, 1H), 3.86 (s, 3H), 3.09 (d, *J* = 5.4 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.26, 163.84, 161.52, 152.93, 152.16, 151.90, 151.31, 148.19, 147.64, 140.65, 135.17, 129.93, 129.22, 129.10, 129.01, 127.52, 126.45, 125.17, 123.94, 117.27, 117.15, 117.13, 112.08, 110.78, 110.23, 100.87, 82.32, 72.28, 55.61.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{31}H_{21}NO_9$ $[M+H]^+$ 552.1295; found 552.1289.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(hydroxy(4(trifluoromethyl)phenyl)methyl)acrylate (S19)

Following the procedure as described for **1**, **S14** (0.10 gm, 0.41 mmoL), DCC (0.08 gm, 0. 41 mmoL), DMAP (4.9 mg, 0.04 mmoL) and FLOH (0.14 gm, 0.41 mmoL) afforded **S19** as white solid (22%, 50.0 mg, R_f 0.5 in Hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 15 h.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.5 Hz, 1H), 7.68 (dd, *J* = 19.5, 7.7 Hz, 4H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.04 (dd, *J* = 7.4, 2.1 Hz, 1H), 6.81 (dd, *J* = 16.6, 5.4 Hz, 2H), 6.76 – 6.70 (m, 2H), 6.69 – 6.63 (m, 2H), 6.17 (s, 1H), 5.75 (d, *J* = 4.4 Hz, 1H), 3.86 (s, 3H), 2.96 (d, *J* = 5.2 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.26, 163.99, 161.52, 152.98, 152.19, 151.90, 151.42, 144.94, 141.00, 135.15, 129.91, 129.18, 129.02, 128.70, 127.02, 126.47, 125.61, 125.58, 125.54, 125.50, 125.16, 123.96, 117.23, 117.21, 117.19, 112.05, 110.83, 110.29, 110.27, 100.88, 82.33, 72.51, 55.61.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 135.16, 129.91, 129.19, 129.02, 128.70, 127.02, 125.61, 125.58, 125.54, 125.50, 125.16, 123.96, 117.23, 112.05, 110.29, 100.88, 72.51, 55.61.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{32}H_{21}F_3O_7$ $[M+H]^+$ 575.1318; found 575.1312.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 3-hydroxy-2-methylenebutanoate (2)

To a cooled (0 °C) and stirred solution of **S15** (0.05 gm, 0.11 mmoL) in anhydrous DCM (3 mL) was added DMAP (3.0 mg, 0.02 mmoL) followed by the addition of solution of Ac_2O (12.8 µL, 0.14 mmoL) in DCM (1mL) dropwise in dark under Ar atmosphere. After 2 h, the reaction mixture was concentrated in *vacuo* on a rotary evaporator, and the resultant

crude was diluted with water (10 mL) and DCM (10 mL). The aqueous layer was separated and extracted with DCM (5 mL × 3). All the organic layers were combined and washed with brine solution (25 mL), dried (Na_2SO_4), filtered, and concentrated in *vacuo* on a rotary evaporator to give a crude product that was purified by column chromatography (hexane/EtOAc 3:1) on silica gel to give **2** as a white solid (23.0 mg, 43%, R_f 0.45 in hexane/EtOAc 2.5:1).

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.7 Hz, 1H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 5.3 Hz, 1H), 6.85 (s, 2H), 6.80 (s, 1H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.66 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.57 (s, 1H), 6.07 (s, 1H), 5.81 (q, *J* = 6.4 Hz, 1H), 3.87 (s, 3H), 2.13 (s, 3H), 1.51 (dd, *J* = 6.4, 2.2 Hz, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 169.82, 169.31, 163.26, 161.49, 153.06, 152.25, 151.92, 151.74, 140.40, 135.15, 129.89, 129.15, 129.04, 127.16, 126.49, 125.14, 124.02, 117.35, 116.95, 112.00, 110.90, 110.34, 100.87, 82.41, 77.21, 77.03, 76.85, 68.06, 55.62, 21.18, 20.13

^{13}C NMR DEPT-135 (175 MHz, $CDCl_3$) δ 135.15, 129.88, 129.15, 129.04, 127.16, 125.14, 124.02, 117.35, 112.00, 110.34, 100.87, 77.23, 68.06, 55.62, 21.18, 20.13

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{28}H_{22}O_8$ $[M+H]^+$ 487.1393; found 487.1392.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(acetoxy(phenyl)methyl)acrylate (3)

Following the procedure as described for **2**, **S16** (0.05 gm, 0.10 mmoL), DMAP (14.0 mg, 0.11 mmoL), Ac_2O (90.0 µL, 1.14 mmoL) afforded **3** as white solid (23.0 mg, 35%, R_f 0.6 in hexane/EtOAc 3:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 0.5 h.

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.7 Hz, 1H), 7.68 (ddd, *J* = 7.6, 2.2, 1.1 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.45 (s, 1H), 7.44 (s, 1H), 7.39 (td, *J* = 7.2, 1.4 Hz, 2H), 7.36 (dd, *J* = 7.1, 1.6 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 7.01 (dd, *J* = 10.6, 2.3 Hz, 1H), 6.80 – 6.77 (m, 3H), 6.72 – 6.63 (m, 5H), 6.10 (d, *J* = 0.7 Hz, 1H), 3.86 (d, *J* = 1.3 Hz, 3H), 2.17 (d, *J* = 2.0 Hz, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 182.60, 169.43, 161.47, 152.22, 151.56, 139.06, 137.46, 135.12, 129.86, 129.10, 129.02, 128.65, 128.63, 127.76, 127.74, 125.12, 123.99, 112.00, 110.85, 100.84, 73.09, 55.61, 21.13.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{33}H_{24}O_8$ $[M+Na]^+$ 571.1369; found 571.1372.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(acetoxy(2-nitrophenyl)methyl)acrylate (4)

Following the procedure as described for **2**, **S17** (0.08 gm, 0.15 mmoL), DMAP (3.5 mg, 0.03 mmoL), Ac_2O (16.4 µL, 0.17 mmoL) afforded **4** as white solid (29%, 25.0 mg, R_f 0.37 in Hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 2.5 h.

^{13}C NMR (175 MHz, $CDCl_3$) δ 169.31, 169.12, 162.82, 161.49, 153.07, 152.23, 151.88, 151.61, 147.94, 138.03, 135.16, 133.63, 133.12, 130.50, 129.88, 129.43, 129.15, 129.03, 128.62, 126.45, 125.33, 125.13, 124.01, 117.23, 117.05, 112.03, 110.86, 110.28, 100.86, 82.37, 68.85, 55.62, 20.82.

^{13}C NMR DEPT-135 (175 MHz, $CDCl_3$) δ 135.16, 133.63, 130.50, 129.88, 129.43, 129.15, 129.02, 128.62, 125.33, 125.13, 124.01, 117.23, 112.03, 110.28, 100.86, 77.23, 68.85, 55.62, 20.82.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{33}H_{23}NO_{10}$ $[M+Na]^+$ 616.1220; found 616.1214.

Synthesis of 3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(acetoxy(4-nitrophenyl)methyl)acrylate (5)

Following the procedure as described for **2**, **S18** (0.20 gm, 0.34 mmoL), DMAP (9.0 mg, 0.07 mmoL), Ac_2O (57.0 µL, 0.60 mmoL) afforded **5** as white solid (56%, 0.12 gm, R_f 0.7 in hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 2 h.

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 8.6, 3.6 Hz, 2H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.71 – 7.67 (m, 1H), 7.64 (dd, *J* = 8.7, 1.8 Hz, 3H), 7.16 (dd, *J* = 7.5, 2.1 Hz, 1H), 7.07 – 6.96 (m, 1H), 6.81 (dd, *J* = 8.5, 3.6 Hz, 2H), 6.78 (dd, *J* = 5.9, 2.4 Hz, 1H), 6.76 – 6.69 (m, 3H), 6.65 (d, *J* = 8.8 Hz, 1H), 6.22 (s, 1H), 3.86 (s, 3H), 2.20 (s, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 169.23, 169.22, 169.15, 162.55, 161.49, 152.94, 152.91, 152.15, 151.30, 147.89, 144.68, 137.99, 135.16, 129.92, 129.24, 129.05, 129.03, 128.53, 126.46, 126.44, 125.16, 123.96, 123.86, 117.24, 117.07, 112.08, 110.77, 110.16, 100.82, 82.27, 72.09, 55.62, 21.00.

^{13}C NMR DEPT-135 (175 MHz, $CDCl_3$) δ 135.16, 129.92, 129.24, 129.05, 129.03, 128.53, 125.15, 123.96, 123.94, 123.86, 117.08, 117.06, 112.07, 110.16, 100.82, 72.09, 55.62, 21.00.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{33}H_{23}NO_{10}$ $[M+H]^+$ 594.1400; found 594.1395.

3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(acetoxy(4-(trifluoromethyl)phenyl)methyl)acrylate (6)

Following the procedure as described for **2**, **S19** (0.04 gm, 0.07 mmoL), DMAP (2.0 mg, 0.014 mmoL), Ac_2O (7.9 µL, 0.084 mmoL) afforded **6** as white solid (42%, 18.0 mg, R_f 0.56 in hexane/EtOAc 1:1) after column chromatography (hexane/EtOAc 3:1) on silica gel. The reaction time was 2 h.

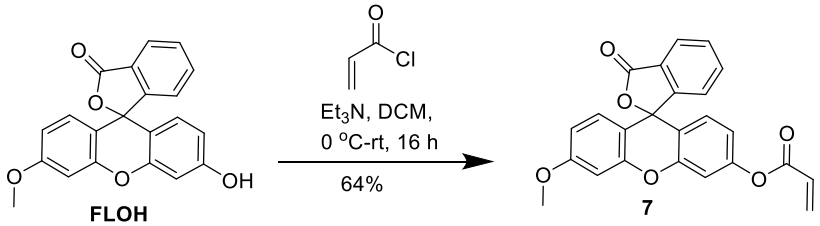
^{1}H NMR (700 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.67 – 7.62 (m, 3H), 7.58 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.02 (s, 1H), 6.83 – 6.77 (m, 3H), 6.72 (d, *J* = 6.1 Hz, 1H), 6.71 (s, 2H), 6.65 (d, *J* = 8.8 Hz, 1H), 6.16 (s, 1H), 3.86 (s, 3H), 2.18 (s, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 169.25, 162.72, 161.50, 153.00, 152.97, 152.19, 151.88, 151.87, 151.42, 141.52, 138.44, 135.15, 129.90, 129.19, 129.03, 128.64, 128.01, 126.48, 126.46, 125.66, 125.64, 125.62, 125.60, 125.15, 123.97, 117.15, 117.14, 112.05, 110.82, 110.21, 100.84, 82.31, 72.40, 55.62, 21.03

^{13}C NMR DEPT-135 (175 MHz, $CDCl_3$) δ 135.14, 129.90, 129.19, 129.03, 128.64, 128.01, 125.66, 125.64, 125.62, 125.60, 125.15, 123.96, 117.15, 112.04, 110.20, 100.84, 77.23, 72.40, 55.62, 21.03.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{34}H_{23}F_3O_8$ $[M+H]^+$ 617.1423; found 617.1418.

Scheme S1c. Synthesis of probe **7**



3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl acrylate (7)[5]

To the cooled (0 °C) and stirred solution of FLOH (0.17 gm, 0.05 mmoL) in anhydrous DCM (8 mL) was added acryloyl chloride (0.23 gm, 2.50 mmoL) dropwise under N_2 atmosphere, resulting in a turbid yellow solution. After the complete addition of acryloyl chloride, triethylamine (0.25 gm, 2.50 mmoL) was added dropwise, during which time the reaction mixture turned into a clear orange-colored solution. The reaction was allowed to warm to room temperature. After 16 h, the reaction mixture was concentrated in *vacuo* on a rotary evaporator to give the crude mixture that was purified by column chromatography (hexane/DCM 3:7) using silica gel to give **7** as white solid compound (0.13 gm, 64%, R_f 0.5 in hexane/EtOAc 3:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 1H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.16 (s, 1H), 6.85 (s, 2H), 6.81 (d, *J* = 2.2 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.68 – 6.62 (m, 2H), 6.35 (dd, *J* = 17.3, 10.5 Hz, 1H), 6.08 (d, *J* = 10.5 Hz, 1H), 3.87 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.29, 163.97, 161.50, 153.06, 152.29, 151.92, 151.84, 135.12, 133.25, 129.86, 129.08, 129.03, 127.56, 126.54, 125.12, 124.03, 117.38, 116.84, 111.98, 110.95, 110.28, 100.89, 82.46, 55.60.

^{13}C NMR DEPT-135 (125 MHz, $CDCl_3$) δ 135.13, 133.27, 129.87, 129.08, 129.03, 127.55, 125.12, 124.03, 117.38, 111.98, 110.29, 100.88, 55.61.

HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{24}H_{16}O_6$ $[M+H]^+$ 401.1025; found 401.1029.

Section C: Figures and Tables

Figure S1. Stacked HPLC chromatograms of probes **1**-**6** after semi-preparative HPLC. The samples for the HPLC purification were prepared in sodium phosphate buffer (10 mM, pH 7, containing 30% ACN). The purified probes were characterized by HRMS and NMR. The UV absorbance was monitored at 215 nm, and the retention times are mentioned along with the peaks.

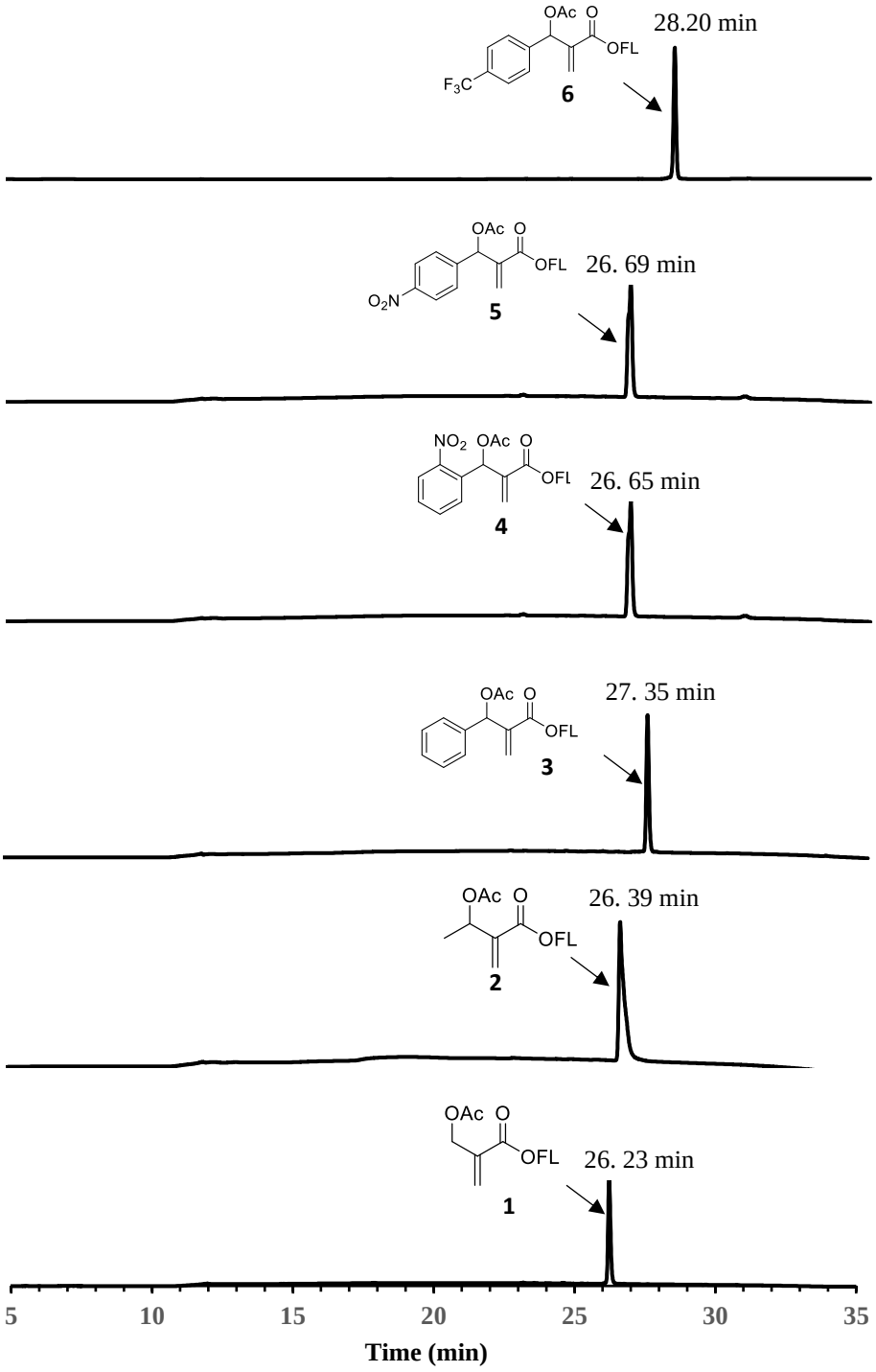
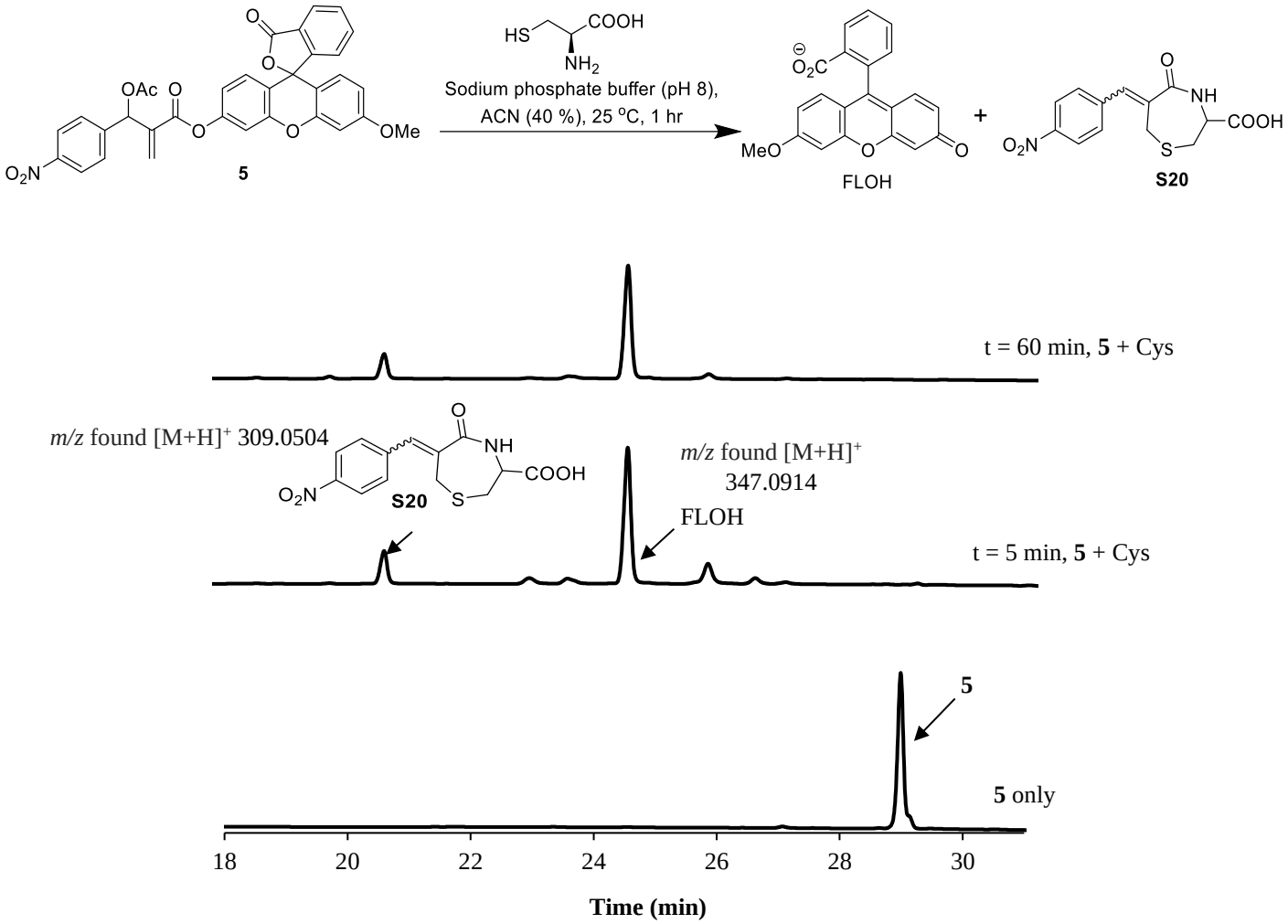


Figure S2. HPLC studies for monitoring the reaction of probe **5** with Cys and the characterization of 7-membered cyclic adduct **S20**. To a solution of sodium phosphate buffer (pH 8, 291.6 μL, 50 mM), ACN (192.5 μL) and Cys (4.94 μL, 100 mM in water) was added **5** (4.94 μL, 100 mM in ACN) at room temperature. The reaction mixture was incubated at room temperature, and aliquots (7 μL) were periodically injected into HPLC to monitor the progress of the reaction. Peaks were collected manually and characterized by HRMS. The UV absorbance was monitored at 215 nm.



Isolation and characterization of 7-membered cyclic adduct (S20).

To a stirred solution of sodium phosphate buffer (50 mM, pH 7, 21.38 mL), ACN (12 mL) and Cys (4.2 mg, 0.04 mmoL) was added a solution of **5** (21.0 mg, 0.04 mmoL) in ACN (2 mL) at room temperature. The color of the reaction mixture turned yellow in 10 min. After 8 h, aliquots (7 μL) of the reaction mixture were withdrawn and injected into HPLC to ensure the completion of the reaction. The reaction mixture was concentrated in *vacuo* on a rotary evaporator to remove ACN. The resultant aqueous solution was frozen at -80 °C and lyophilized overnight. MeOH (50 mL) was added to extract organic compounds from the sodium phosphate salts to the resultant lyophilized sample. The clear supernatant was concentrated in *vacuo* on a rotary evaporator, and the resultant crude product was purified using column chromatography (DCM/Methanol 10:1) using silica gel to give **S20** as white solid (5.0 mg, 46 %).

^{1}H NMR (700 MHz, CD_3OD) δ 8.29 (d, *J* = 8.6 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H), 7.21 (s, 1H), 4.29 (d, *J* = 9.8 Hz, 1H), 3.71 (d, *J* = 15.3 Hz, 1H), 3.45 (dd, *J* = 28.8, 14.5 Hz, 2H), 2.96 – 2.88 (m, 1H).

^{13}C NMR (175 MHz, CD_3OD) δ 172.76, 147.26, 141.59, 140.03, 132.51, 130.21, 128.88, 123.31, 59.29, 34.55, 26.49.
HRMS (QTOF MS ESI+) *m/z* calcd. for $C_{13}H_{12}N_2O_5S$ $[M+H]^+$ 309.0545; found 309.0540.

Figure S3. Spectral properties of FLOH (**a**) UV-Vis and (**b**) Fluorescence. λ_{ex} = 475 nm and slit width = 1.3/ 1.3 nm. The sample was prepared by adding FLOH (10 µL, 1 mM in ACN) to the solution of sodium phosphate buffer (10 mM, pH 7, 700 µL) and ACN (290 µL), followed by recording the spectra of the resultant solution.

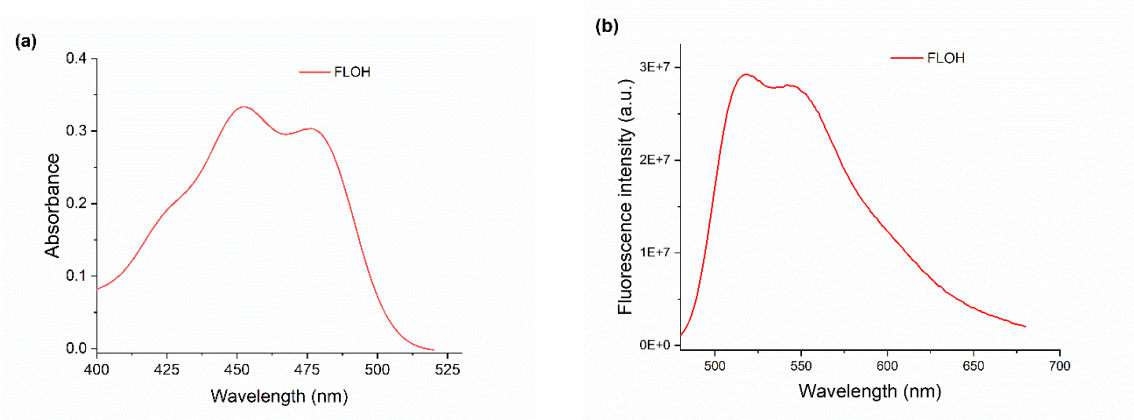


Figure S4. Pseudo-first-order kinetics of the reaction between **5** (10 µM) and 100 µM of (**a**) Cys, (**b**) Hcy, and (**c**) GSH. The reactions were carried out at room temperature in sodium phosphate buffer (pH 7, 10 mM) containing 30% ACN and (**d**) The overlay of plots **a-c**. λ_{ex}= 475 nm, λ_{em}= 519 nm, slit width 1.3/1.3 nm. Each data point is an average of three recordings, and the error bars correspond to standard deviation values.

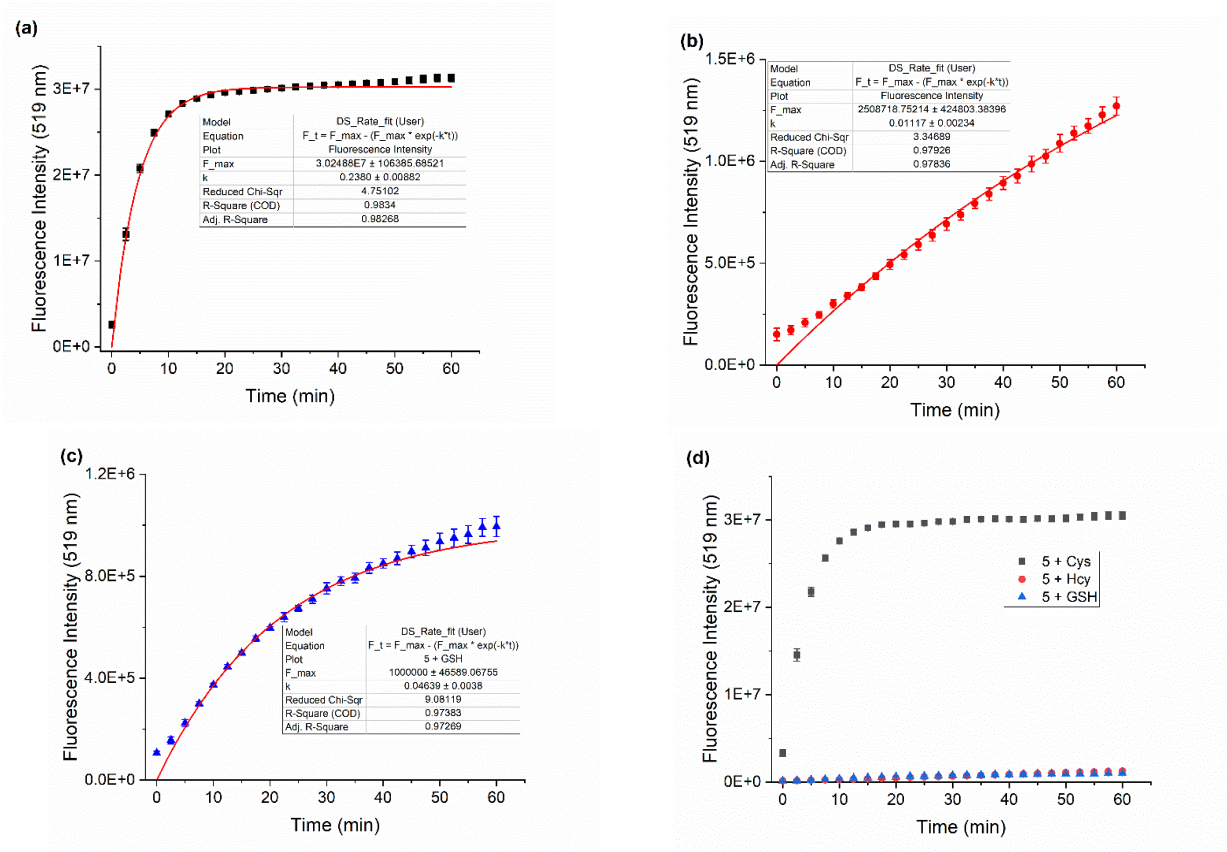
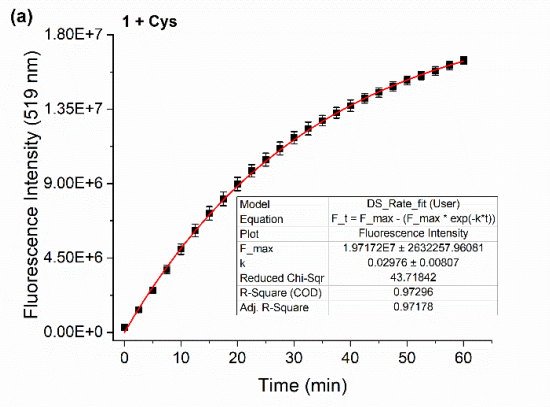
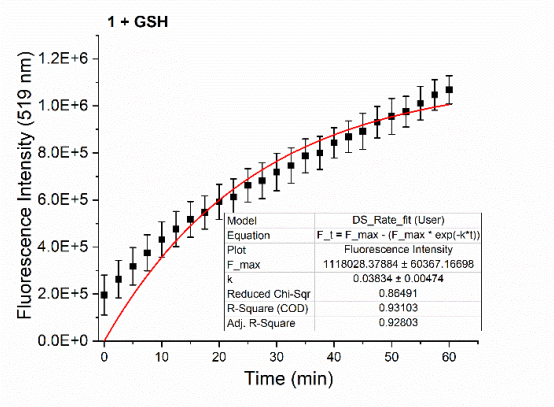
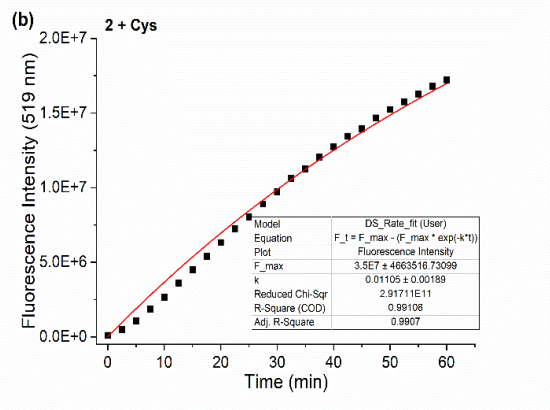
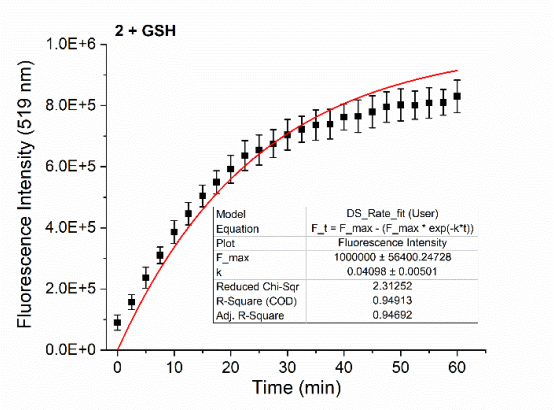


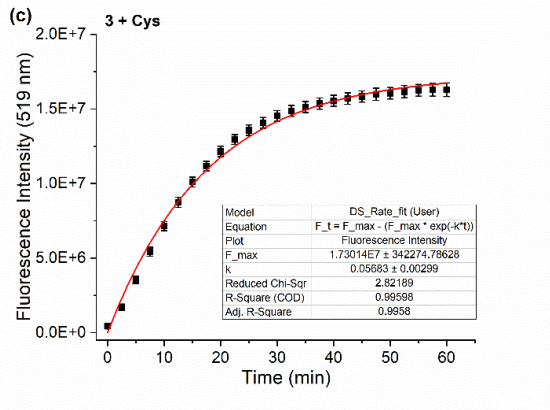
Figure S5. Pseudo first-order kinetics of the reaction between 10 μM of probes (**a**) **1**, (**b**) **2**, (**c**) **3**, (**d**) **4** and (**e**) **6** and 100 μM of Cys (left panels) or GSH (right panels). The reactions were carried out at room temperature in sodium phosphate buffer (pH 7, 10 mM) containing 30% ACN. λ_{ex}= 475 nm, λ_{em}= 519 nm, slit width 1.3/1.3 nm. Each data point is an average of three recordings, and the error bars correspond to standard deviation values.

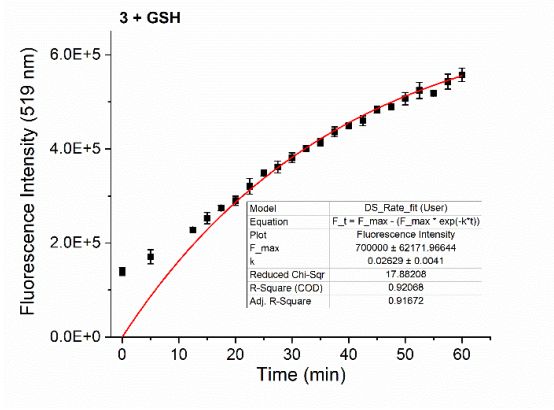


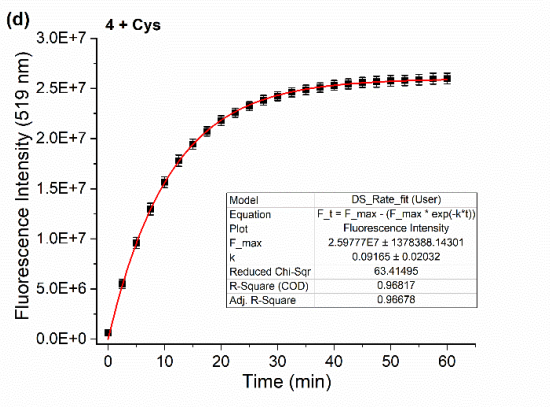


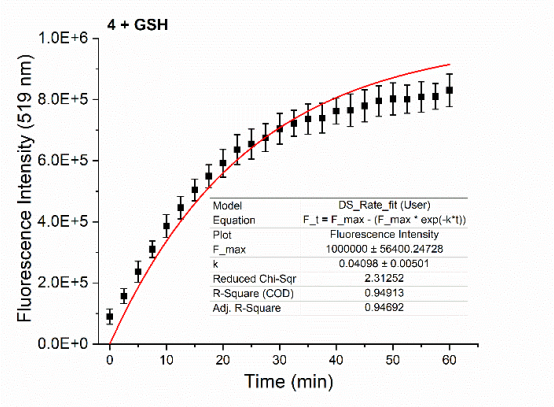












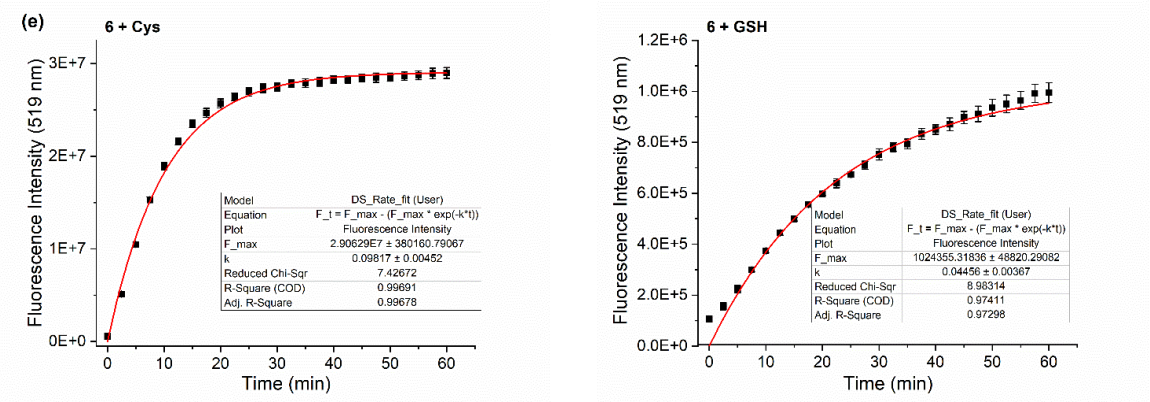


Figure S6. Effect of pH (5-10) on Cys-triggered FLOH release for probes (**a**) **1**, (**b**) **2**, (**c**) **3**, (**d**) **4**, (**e**) **5** and (**f**) **6**. The samples were prepared by incubating each probe (10 μM) in solutions containing 10 mM sodium phosphate buffer of varying pH (5-10) and ACN (7:3) with Cys (100 μM) or without. Subsequently, the fluorescence intensity corresponding to FLOH at the 60 min time poin (λ_{ex}= 475 nm, λ_{em}= 519 nm, slit width 1.3/1.3 nm) was recorded. Each data point is an average of three recordings, and the error bars correspond to standard deviation values. The inset in each plot represents the magnified version of the data obtained at pH 5.

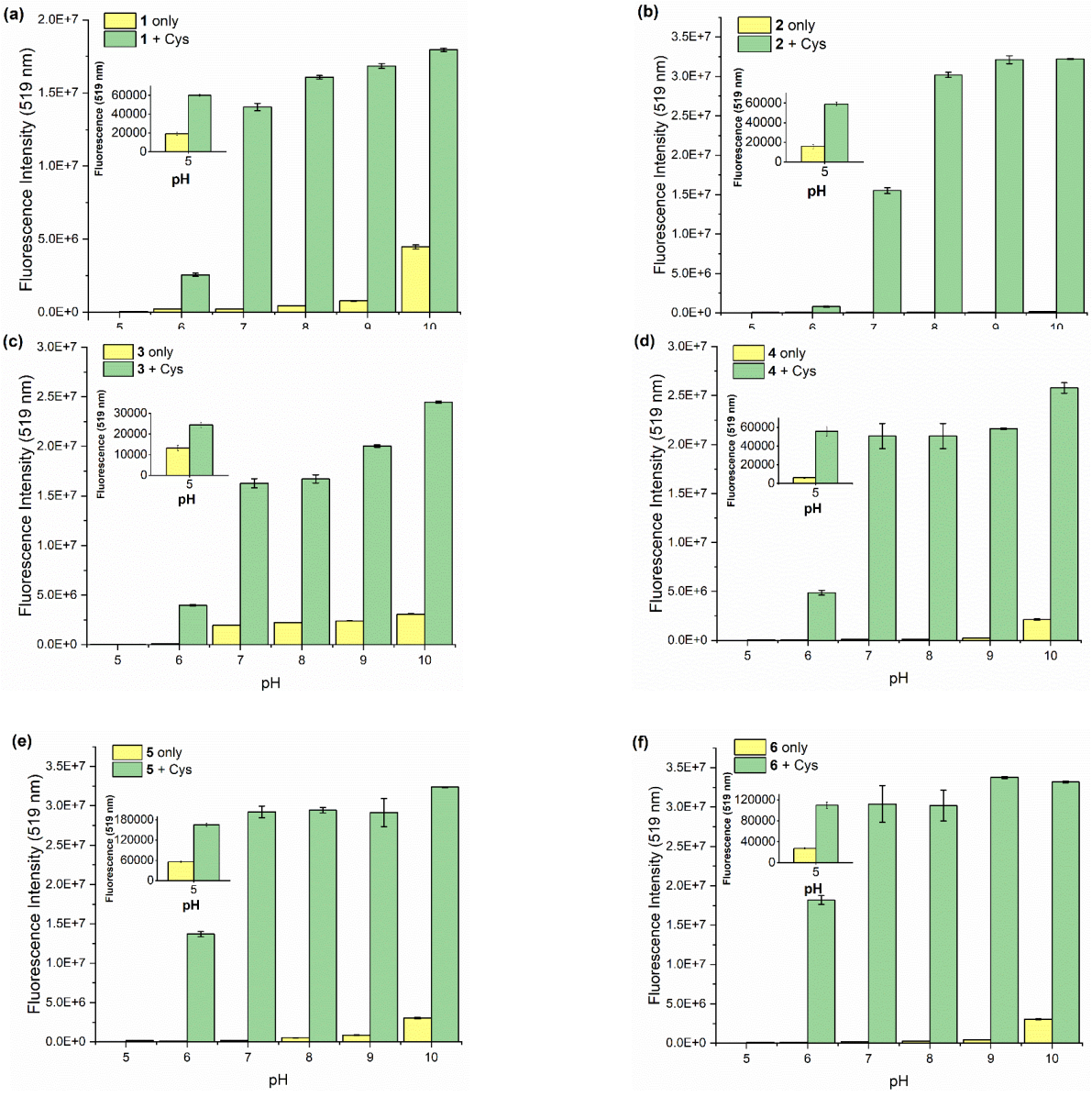
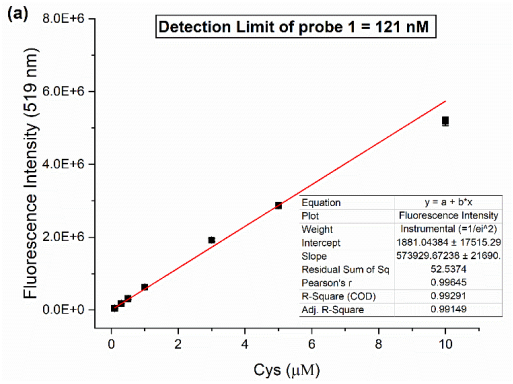
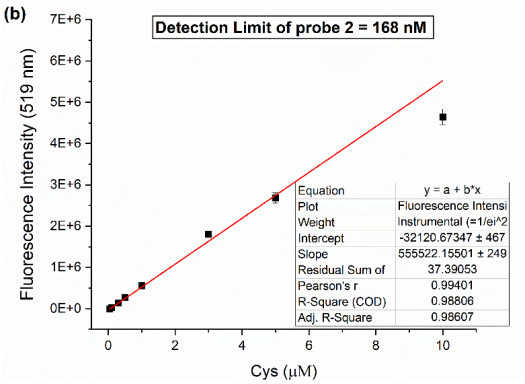
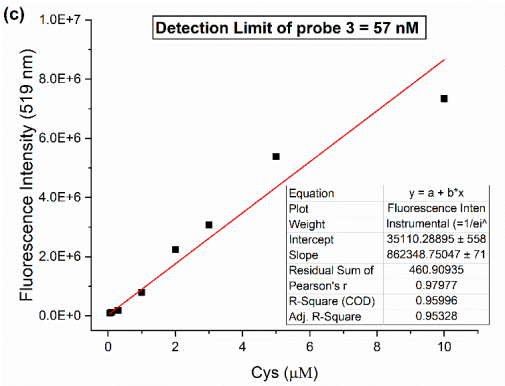
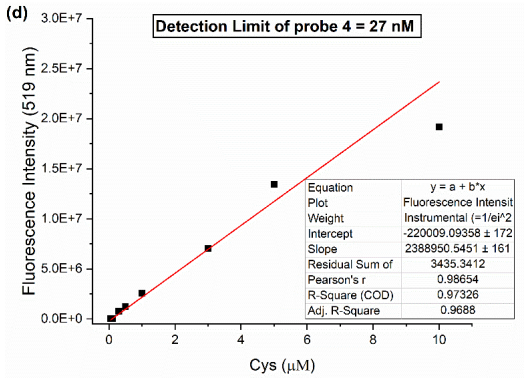


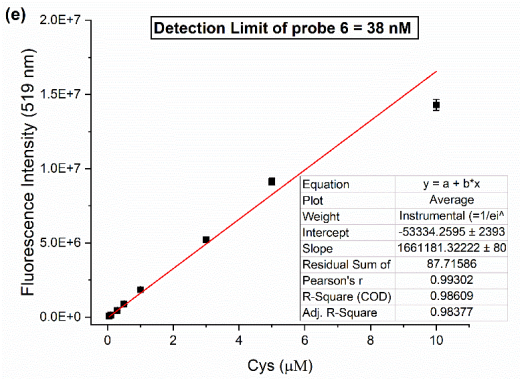
Figure S7. The detection limit of probes (**a**) **1**, (**b**) **2**, (**c**) **3**, (**d**) **4**, (**e**) **6** and (**f**) **7** for Cys. The fluorescence emission of each probe (10 μM) at 519 nm upon its incubation with Cys demonstrated a linear relationship with Cys concentrations (0 to 10 μM). The detection limits were calculated using the formula 3σ/k, σ is the standard deviation of blank, and k is the slope (see section D for more details). The analysis was carried out in sodium phosphate buffer (10 mM, pH 7): CH_3CN (7:3) at 60 min time point. λ_{ex}= 475 nm, λ_{em}= 519 nm; slits = 1.3/1.3 nm. Each data point is an average of three recordings, and the error bars correspond to standard deviation values**.**











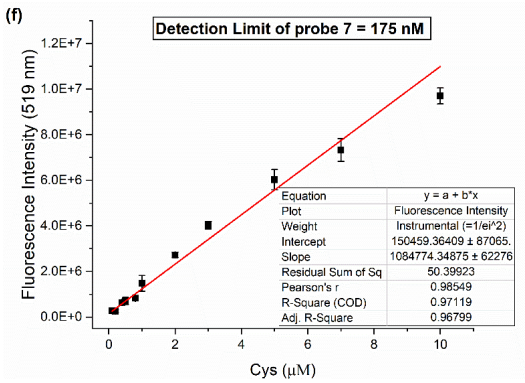


Figure S8. Fluorescence imaging of Cys in HeLa cells with probes **1-6** using confocal microscopy. Cells only (1st panel from left, top), cells incubated with different concentrations of **5** (10, 20, and 40 μM respectively) for 40 min, (2nd to 4th panels, respectively from left, top), cells preincubated with Triton-X 100 (0.17 mM) for 5 min before incubating with **5** (20 μM) for 40 min (5th panel from left, top) and cells incubated with probes **4, 6, 3, 2** and **1** (10 μM), respectively for 40 min (1st to 5th panels, respectively from left, bottom) and with **1** (20 μM) for 40 min (6th panel from left, bottom). The top images of each panel represent fluorescence images, and the bottom represents the merged image of fluorescence and differential interference contrast (DIC) images. Fluorescence emission was collected at 495−540 nm (excited at 488 nm). Scale bar= 50 μm.

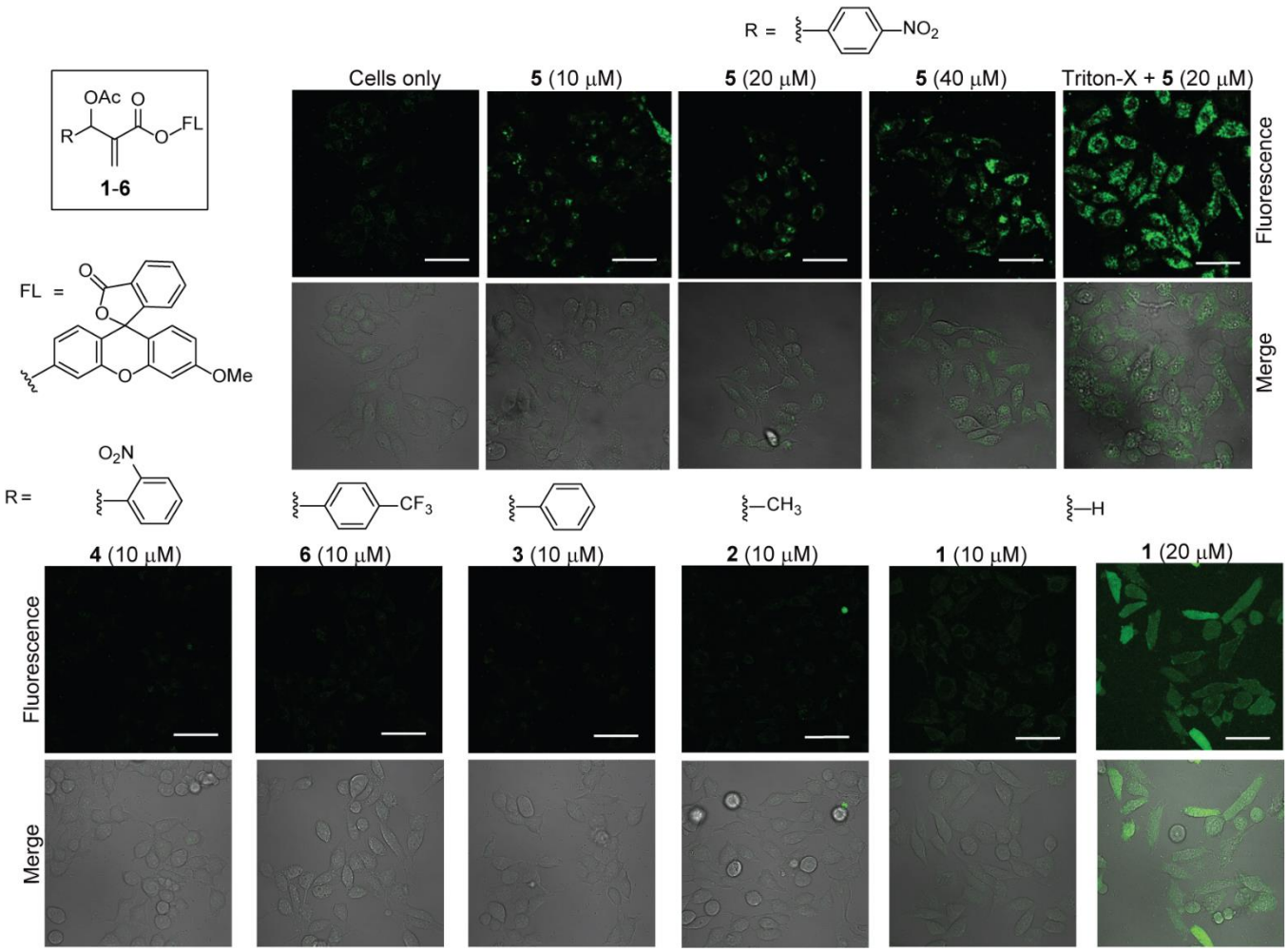


Figure S9. Cell viability studies on the HeLa cell line with probes **1-6** using MTT assay. All the probes demonstrated no cytotoxicity over the course of 12 h at concentrations employed in the imaging experiments (10 and/or 20 μM). Each data point is an average of three recordings, and the error bars correspond to standard deviation values.

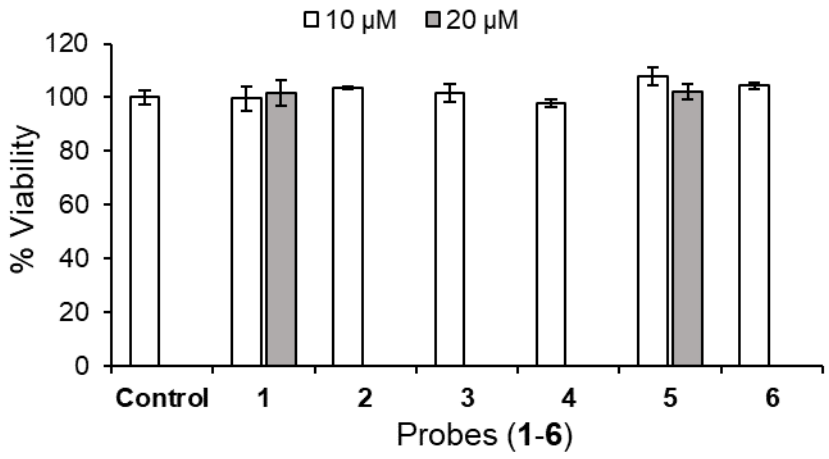
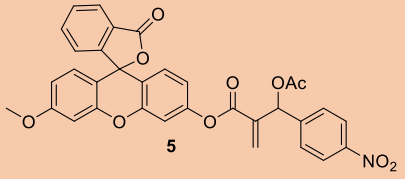
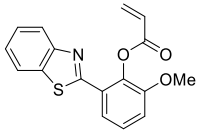
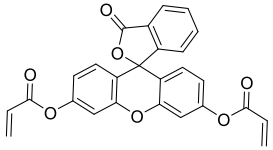
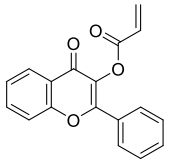
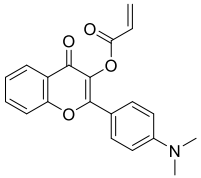
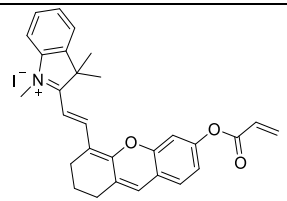
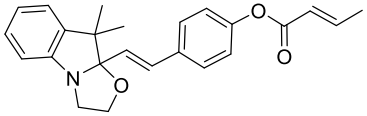
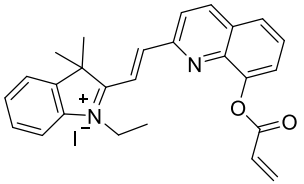
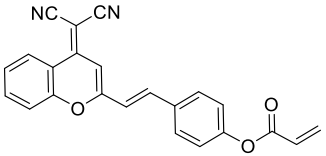
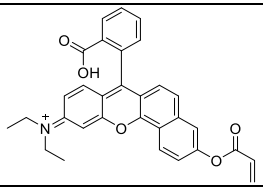
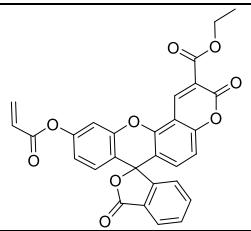
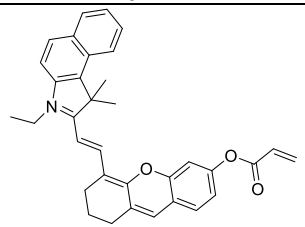
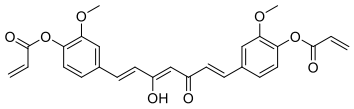
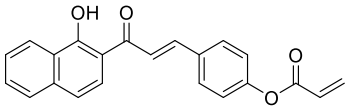
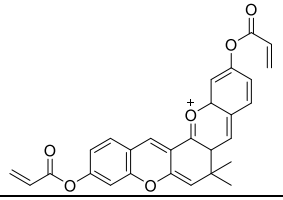
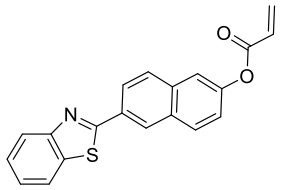
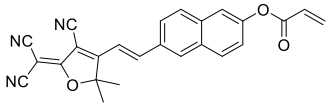
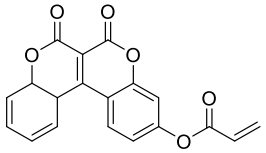
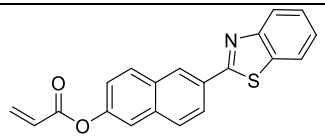
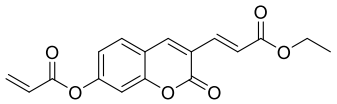
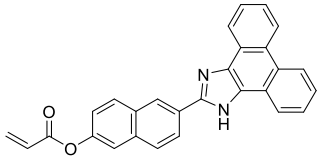
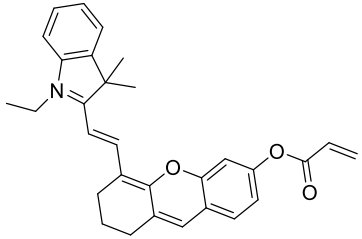


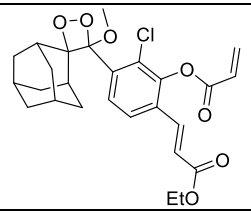
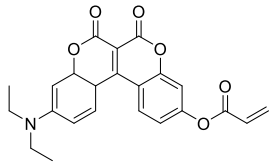
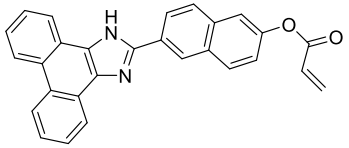
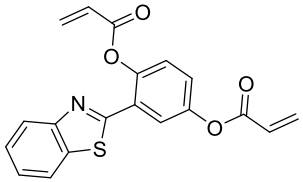
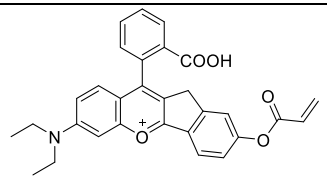
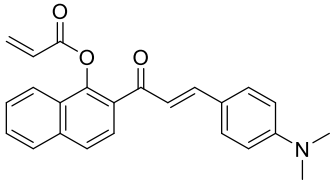
Table S1. A summary of the sensitivity of Michel addition-cyclization probes for Cys and ACY-1 detection reported in the literature, as compared to our most sensitive MADELCY TOF probe (row 1).

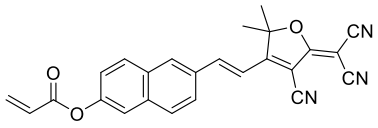
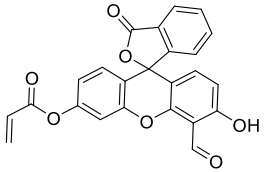
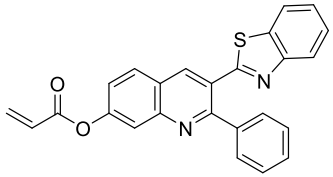
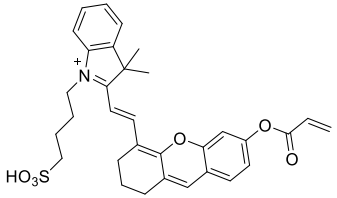
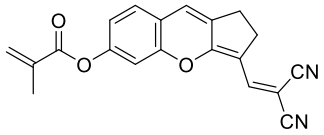
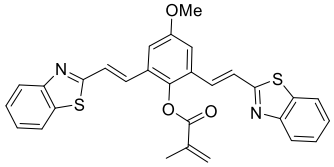
Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
1		**8.2 nM**	**9.5 pM**	***THIS WORK***
2		110 nM	-	*Angew. Chem. Int. Ed.* 2011, **50**, 10690 –10693
3		121 nM	-	*Chem. Commun.*, 2012, **48**, 8341–8343
4		1 μM	-	*ACS Appl. Mater. Interfaces* 2014, **6**, 4402−4407
5		200 nM	-	*ACS Appl. Mater. Interfaces* 2014, **6**, 17543−17550

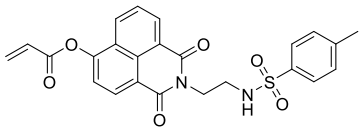
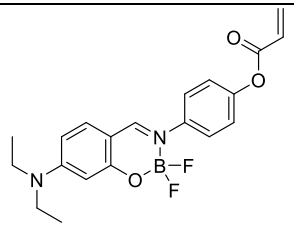
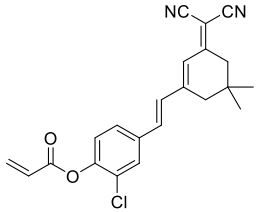
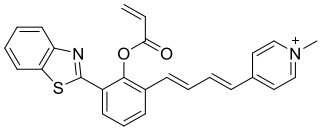
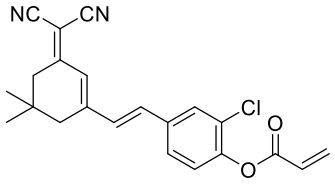
Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
6		160 nM	-	*Anal. Chem.* 2015, 87, **9**, 4856–4863
7		5 μM	-	*Anal. Chem.* 2016, **88**, 7178−7182
8		38.6 nM	-	*Anal. Methods*, 2021, **13**, 1965
9		200 nM	-	*ACS Sens.* 2016, **1**, 7, 882–887
10		76 nM	-	*Anal. Methods*, 2017, **9**, 1891–1896

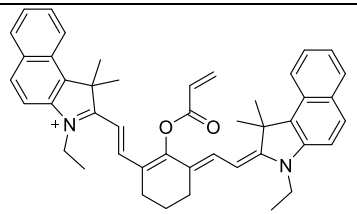
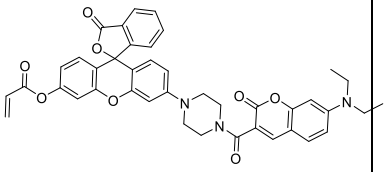
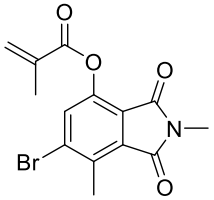
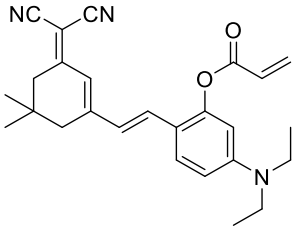
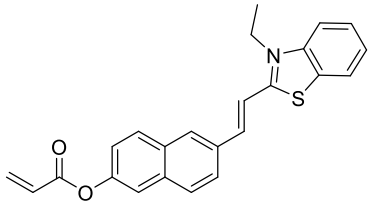
Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
11		84 nM	-	*Anal. Chem.* 2017, 89, **3**, 1937–1944
12		14.5 nM	-	*ACS Appl. Mater. Interfaces* 2015, **7**, 27968−27975
13		190 nM	-	*Ind. Eng. Chem. Res.* 2017, **56**, 27, 7650–7655
14		91 nM	-	*Biosensors and Bioelectronics* 2017, **92**, 583–588
15		0.8 nM*	-	*Chem. Sci.*, 2018, **9**, 5461–5466
16		180 nM	-	*New J. Chem.*, 2018, **42**, 18109-18116

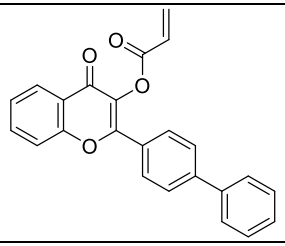
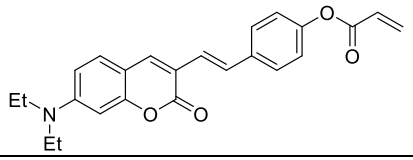
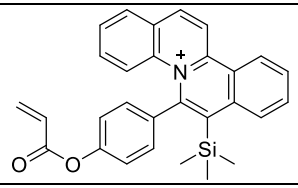
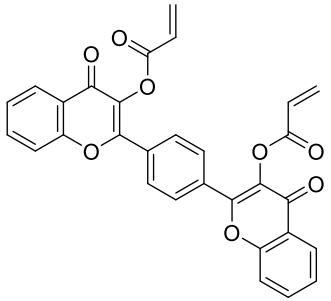
Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
17		7.22 μM	-	*Analyst*, 2018, **143**, 5779-5784
18		388 nM	-	*J. Mater. Chem. B*, 2017, **5**, 5892--5897
19		14.8 nM	-	*RSC Adv.*, 2018, **8**, 37410-34416
20		930 nM	-	*Analyst*, 2019, **144**, 2320–2326
21		388 nM	-	*Anal. Methods*, 2019, **11**, 4323–4327
22		210 nM	-	*Chem. Commun.*, 2019, **55**, 9685-9688

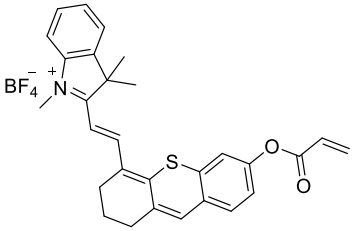
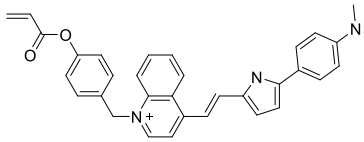
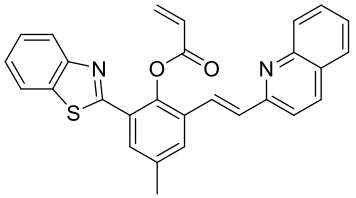
Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
23		75 nM	-	*ACS Sens.* 2019, **4**, 87−92
24		411 nM	-	*Anal. Chem.* 2018, **90**, 10, 6138–6143
25		388 nM	-	*Anal. Methods*, 2019, **11**, 4323
26		32.9 nM	-	*New J. Chem.*, 2019, **43**, 13463
27		24.1 nM	-	*J. Mater. Chem. B*, 2019, **7**, 3970
28		55 nM	-	*Anal. Methods*, 2019, **11**, 2513

Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
29		260 nM	-	*Anal. Methods*, 2019, **11**, 1312
30		61 nM	-	*Chem. Commun.*, 2019, **55**, 11762-11765
31		47 ppb (132.6 nM)	-	*Anal. Methods*, 2019, **11**, 1248
32		21.1 nM	-	*New J. Chem.*, 2019, **43**, 3725
33		1.06 μM	-	*New J. Chem.*, 2019, **43**, 72
34		500 nM	-	*Front. Chem.*, 2019, **7**, 32, 1-8.

Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
35		1.80 μM	-	*Anal. Chem.* 2019, **91**, 5513−5516
36		95.1 nM	-	*Anal. Methods,* 2019, **11**, 2579
37		173 nM	-	*New J. Chem.*, 2020, **44,** 15432-15438
38		102 nM	-	*Anal. Chem.* 2020, **92**, 20, 14236–14243
39		173 nM	-	*New J. Chem.*, 2020, **44**, 15432-15438

Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
40		330 nM	-	*Chem. Commun.*, 2021, **57**, 4811–4814
41		200 nM	-	*New J. Chem.*, 2019, *43*, 14763-14771
42		45 nM	-	*Analyst*, 2021, **146**, 2212
43		1.29 μM	-	*Org. Biomol. Chem*., 2021, **19**, 873
44		74 nM	-	*Analyst*, 2021, **146**, 4642–4648

Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
45		18.5 nM	-	*J. Mater. Chem. B*, 2021, **9**, 8263
46		Yes (ND[#])	Yes (ND[#])	*Anal. Chem.* 2016, **88**, 12161
47		180 nM	-	*RSC Adv.*, 2021, **11**, 33294
48		130 nM	-	*Chem. Commun.*, 2021, **57**, 8198

Sr. No.	Structure	Detection limit of the target analyte		Reference
		Cys	ACY-1	
49		16 nM	-	*J. Mater. Chem. B*, 2020, **8**, 2269 - 2274
50		20.8 nM	-	*Anal. Chem.* 2020, **92**, 3, 2802–2808
51		1.72 nM	-	*New J. Chem.*, 2021, **45**, 19073-19081

[*]The enhanced sensitivity of this probe is attributed to the presence of two Michael acceptor moieties in one molecule. [#]Not determined

Table S2. Comparison of the analytical properties of MADELCY TOF probes (**1-6**) towards Cys.

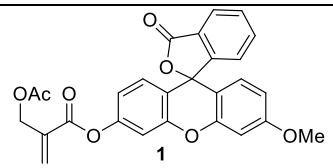
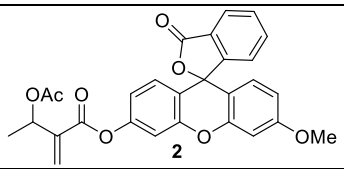
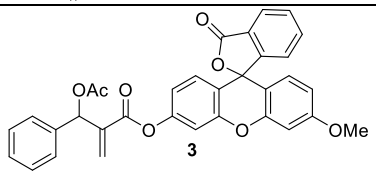
Probe	[a]$t_{1/2}$ (min)	[a]Detection limits (nM)	[a,b]F_t/F_o
1	23.3	121	62
2	62.7	168	216
3	12.2	57	8
4	7.6	27	156
5	2.9	8.2	238
6	7.1	38	153

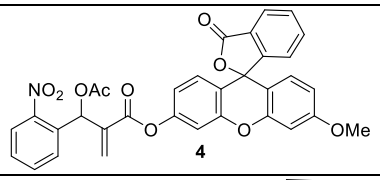
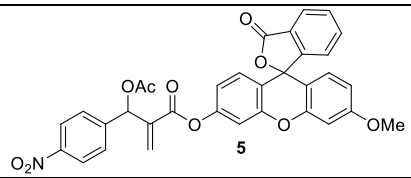
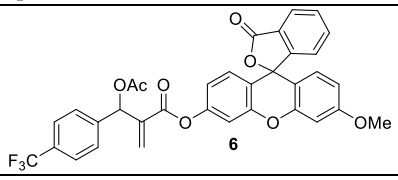
[a]Please see Section D: General procedures for experimental details. The data was acquired using 10 μM of each probe in sodium phosphate buffer (pH 7) and 100 μM of Cys. [b]F_t and F_o are the fluorescence intensity at 519 nm corresponding to FLOH release at a 60 min time point obtained in solutions containing 10 μM of each probe in sodium phosphate buffer and ACN (7:3) at pH 7 in the presence of Cys (100 μM) and in its absence.

Table S3. Comparison of sensitivity of MADELCY TOF probe **5** with the commercially available Cys detection kits.

Sr. No.	Catalog No.	Commercial source	Detection limits	Link	Working principle
1	***Probe 5***	***Our synthesized probe***	***8.2 nM***	***This work***	***Fluorescence***
2	ab211099	Abcam	10 μM	https://www.abcam.com/cysteine-assay-kit-fluorometric-ab211099.html#:~:text=The%20assay%20can%20detect%20as,in%20a%20variety%20of%20samples.	Fluorescence
3	BN00788	Assay Genie	10 μM	https://www.assaygenie.com/cysteine-assay-kit-fluorometric-bn00788/	Fluorescence
4	MAK255	Sigma-Aldrich	10 μM	https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/165/429/mak255bul.pdf	Fluorescence
5	LS-K568-100	LSBio	10 μM	https://www.lsbio.com/assaykitmanuals/K568_Datasheet.pdf	Fluorescence

Table S4. Calculated LogP and cLogP values of probes **1-6**

Compound structure	Chemical formula	Molecular weight	LogP*	cLogP value*
	$C_{27}H_{20}O_8$	472.45	4.04	3.61
	$C_{28}H_{22}O_8$	486.48	4.36	3.92
	$C_{33}H_{24}O_8$	548.55	5.75	5.11

 **4**	$C_{33}H_{23}NO_{10}$	593.54	ND[#]	4.78
 **5**	$C_{33}H_{23}NO_{10}$	593.54	ND[#]	4.86
 **6**	$C_{34}H_{23}F_3O_8$	616.55	6.68	5.60

[*]LogP and cLogP values were calculated using software ChemDraw 19.1. [#]Not determined

Section D: General procedures

Cell culture and imaging experiments (Figures 6 and S7):
Adherent HeLa cells were cultured in DMEM medium supplemented with 10% FBS (fetal bovine serum) and antibiotics (100 units/mL penicillin-streptomycin) in cell culture flasks under standard culture conditions (37 ^oC and 5% CO_2). All the cells treatment solutions were made in DMEM w/o FBS and antibiotics. For cell imaging experiments, 200 μL of cell suspension was seeded at a cell density of 3.5×10^4 in a 35 mm confocal petri-dish. After 14-16 h of incubation under standard culture conditions, the culture media was removed, and the cells were washed with DMEM (200 μL × 2) followed by the treatment with DMEM (200 μL) containing probes **1**-**6** at desired concentrations in 10% ACN. After 40 min of incubation under standard culture conditions, media was removed, and the cells were washed with DMEM (200 μL × 2) to remove excess reagents. Finally, cells were imaged in fluoroBrite media under a confocal laser scanning microscope using an excitation wavelength of 488 nm. For quenching experiments, before the probe treatment, cells were pre-incubated with Cys quenching reagents, iodoacetamide (100 μM), and H_2O_2 (1 mM) in DMEM w/o FBS for 20 min and 30 min respectively followed by DMEM washings (200 μL × 3). In the fluorescence regeneration experiment, after treatment with quenching reagents, cells were washed with DMEM and incubated with exogenous Cys or NAC (0.5 mM) in DMEM for 30 min followed by the probe treatment and imaged under similar conditions as described above. For the permeabilization experiment[6], cells were treated with Triton-X 100 (0.17 mM) in DMEM. After 5 min of incubation at room temperature, cells were washed with DMEM (200 μL × 2) and treated with a solution of probe **5** in DMEM containing 10% ACN. The cells were washed and imaged using a confocal microscope.

Cell viability assays (Figure S9)
The toxicity of probes (**1**-**6**) towards HeLa cells was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay. The cells were seeded in a 96-well plate at a density of 3×10^3 cells per well. After an incubation of 15-16 h at 37^{o}C in 5% CO_2 in a humidified environment, cells were treated with different probes (**1**-**6,** 10 and/or 20 μM) in triplicates. After 12 h of incubation, the probe-containing media was replaced with the fresh media containing MTT at a final concentration of 0.45 mg/mL. After a 4 h incubation, the MTT solution was removed and the formazan crystals were dissolved in 100 μL DMSO followed by an agitation of the multi-well plate for 15-20 min. The absorbance of each well was measured using a microplate reader at a wavelength of 570 nm.
To assess the cell viability, the following equation was used:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Selectivity and cross-reactivity studies of 5 with Cys and other amino acids, cations, and anions (**Figure 3)**
Probe **5** (10 μL of 1 mM solution in ACN) was added to a solution of sodium phosphate buffer (690 μL, 10 mM, pH 7) and ACN (290 μL) containing Cys (10 μL of 10 mM solution in water) and other amino acids (10 μL of 10 mM solution in water) or cations/anions (10 μL of 100 mM solution in water). The reaction mixture was mixed by pipetting up and down for a few seconds and incubated for 1 h at room temperature, followed by recording the fluorescence response of **5** at 519 nm. A similar procedure was followed for the control experiments without Cys, 10 μL of water was added in place of Cys (10 μL of 10 mM solution in water).

Kinetics of FLOH release upon treatment of probes 1-7 with different thiols (Cys, Hcy, and GSH) (Figures 2c, S4 and S5)

Probes **1**-**6** (10 μL, 1 mM in ACN, 1eq) was added to a solution of sodium phosphate buffer (690 μL, 10 mM, pH 7), ACN (290 μL), and Cys or Hcy or GSH (10 μL, 10 mM solution in water, 10 eq). The reaction was mixed and the fluorescence response of probes **1**-**6** at 519 nm corresponding to the released FLOH was recorded with time for 60 min at room temperature. The pseudo-first-order rate constants (*k*) were determined by fitting the changes in the fluorescence intensity (F_t) to the pseudo-first-order rate equation 1b. The half-lives ($t_{1/2}$) of the reactions were calculated by employing the equation: $t_{1/2} = 0.693/k$

Pseudo-first-order integrated rate law equation:

$$ln\frac{(F_{max} - F_t)}{F_{max}} = -kt \qquad --- \text{ eq. 1a}$$

Rearranging eq. 1a for a nonlinear curve fitting,

$$F_t = F_{max} - \left(F_{max} \times e^{-kt}\right) \qquad --- \text{ eq. 1b}$$

where,

F_{max} = The maximum fluorescence intensity value obtained at 60 min

F_t = Fluorescence intensity at time *t*

k = Pseudo first-order rate constant

Effect of pH on the Cys-triggered FLOH release of probes 1-6 (**Figure S6)**

Probes **1**-**6** (10 μL of 1 mM solution in ACN) was added to solutions containing 690 μL of 10 mM sodium phosphate buffer of different pH values (5, 6, 7, 8, 9 and 10), ACN (290 μL) and Cys (10 μL of 10 mM solution in water). The reaction mixture was mixed by pipetting up and down for a few seconds and incubated for 1 h at room temperature, followed by recording the fluorescence response of **1**-**6** at 519 nm. A similar procedure was followed for the control experiments without Cys, 10 μL of water was added in place of Cys (10 μL of 10 mM solution in water).

Calculation of detection limits[7] of probes 1-7.

1) For Cys[7-8] (Figures 4 and S7)

The detection limit of probe **5** for Cys was estimated from the fluorescence titration spectra of probe **5** generated against Cys concentrations. The standard deviation of the blank (σ) was calculated by eq. 1. Briefly, a solution of **5** (10 μL, 1 mM solution in ACN) was incubated (without Cys) in a solution of sodium phosphate buffer (700 μL, pH 7, 10 mM) and ACN (290 mL) for 60 min, followed by recording the fluorescence response at 519 nm ten times (*N* =10).

$$\sigma = \sqrt{\frac{\sum(Fo - \overline{Fo})^2}{N-1}} \quad --- \text{ eq. 1}$$

where *N*, F_o and $\overline{Fo}$ are the number of replicates for the measurement, fluorescence intensity, and mean fluorescence intensity of the blank solution of probe **5**, respectively. Under similar conditions, in the presence of Cys (0-6 μM), a linear response (R^2 = 0.99508) of **5** at 519 nm was obtained against Cys concentrations (Figure 4b inset). The slope (*k*) was calculated from a linear graph generated, and the detection limit was calculated using eq. 2,

$$\text{Detection limit} = 3\frac{\sigma}{k} \quad \text{----- eq. 2}$$

where, σ = standard deviation of the blank sample and *k* = slope.

Similar protocol, as described above, was followed for calculating the detection limit of probe **1**-**4**, **6** and **7** for Cys that gave a value of 121, 168, 57, 27, 38 and 175 nM, respectively (Figure S7).

2) For ACY-1 (Figure 5c)

The general procedure involves recording the fluorescence response of **5** towards Cys generated in the catalytic reaction (NAC to Cys) with increasing concentrations of ACY-1 and at a fixed concentration of NAC. The detection limit was calculated from the linear calibration plot generated between the fluorescence response of **5** at 519 nm and ACY-1. Briefly, different concentrations of ACY-1 were incubated in 120 μL of sodium phosphate buffer (10 mM, pH 7)

containing NAC (1 mM). After 30 min, the reaction mixture was quenched with 1 mL ACN and centrifuged at 15000 g for 15 min. The supernatant was carefully transferred to another vial and dried in a vacuum concentrator at 30 °C. The dried sample was dissolved in sodium phosphate buffer (700 μL, 10 mM, pH 7) and ACN (290 μL), followed by the addition of **5** (10 μL, 1 mM in solution ACN). After 60 min, the fluorescence response of **5** at 519 nm was recorded, which gave a linear graph (R^2 = 0.9959) between the fluorescence response of **5** and ACY-1 concentrations (0-10 nM). Slope (*k*) was calculated from the resultant liner plot. Under similar conditions, the standard deviation of the blank without ACY-1, was calculated using eq. 1 (*N* = 10), and the detection limit was calculated by using eq. 2 as described above.

Quantification of Cys in human blood serum by standard addition method (Figure 4c)[7, 9]

Human blood serum (200 μL) was thawed at room temperature. To this was added tris(2-carboxyethyl)phosphine, TCEP (30 μL, 233 mM solution in water), and the resulting mixture was vortex-mixed thoroughly. After 60 min of incubation at room temperature, the proteins of the resultant reduced serum were precipitated by adding ACN (1 mL) and the resultant turbid solution was centrifuged at 15000 g for 15 min at room temperature. The supernatant was carefully transferred to another centrifuge vial and dried thoroughly in a vacuum concentrator at room temperature. The dried sample was mixed with 1 mL of sodium phosphate buffer (pH 7, 10 mM) containing 4 mM EDTA. The resultant mixture was vortexed for 5 min, and centrifuged again at 15000 g for 5 min. The supernatant corresponding to the final reduced serum for Cys estimation analysis was carefully transferred to another vial. For fluorescence analysis, **5** (10 μL, 1 mM solution in ACN) was added to solutions containing 10 μL of reduced serum, sodium phosphate buffer (680 μL, 10 mM, pH 7), ACN (290 μL), and 10 μL of Cys stock solutions in water to achieve a final concentration of 0-2 μM in a final volume of 1000 μL. The mixture was incubated for 60 min at room temperature, followed by recording the fluorescence response of **5** at 519 nm at various concentrations of Cys that gave a linear graph (R^2 = 0.99824) between the fluorescence response and Cys concentration. The slope (*m*) and intercept (*c*) are calculated from the linear graph generated using the equation, y = mx + c, and the unknown Cys concentration in the blood serum was calculated using the equation[7]: $[Cys]_{unknown} = -\frac{c}{m}$. Recoveries of added cysteine to serum samples were calculated using equation[7]: Recovery (%) = $([Cys]_{spiked\ sample} - [Cys]_{unspiked\ sample})/[Cys]_{added} \times 100$ and were in general ≥ 96%. Three independent experiments (n = 3) were performed for calculating the standard deviations associated with each analysis.

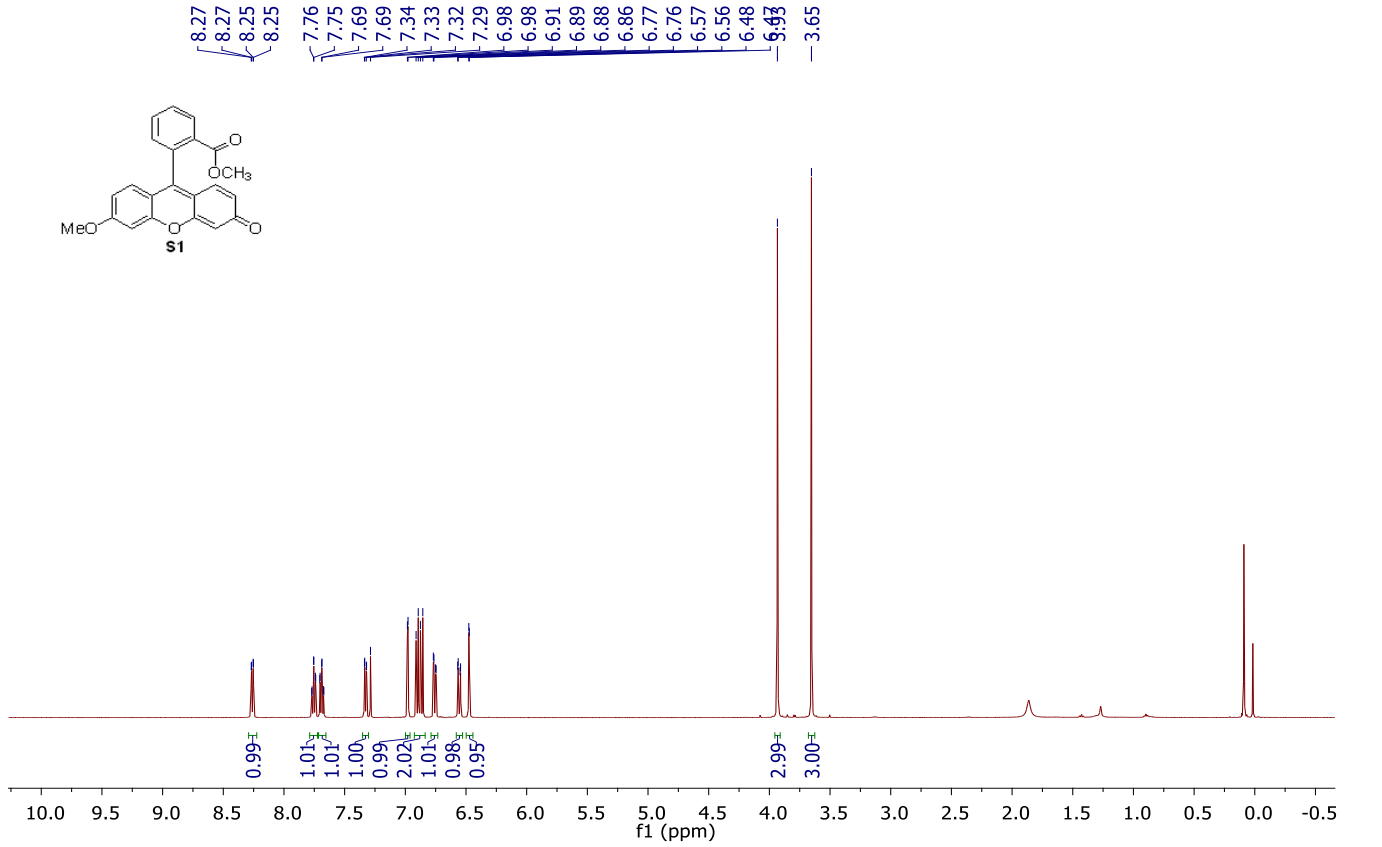
Quantification of ACY-1 in human blood serum by calibration curve method (Figure 5d)[7]

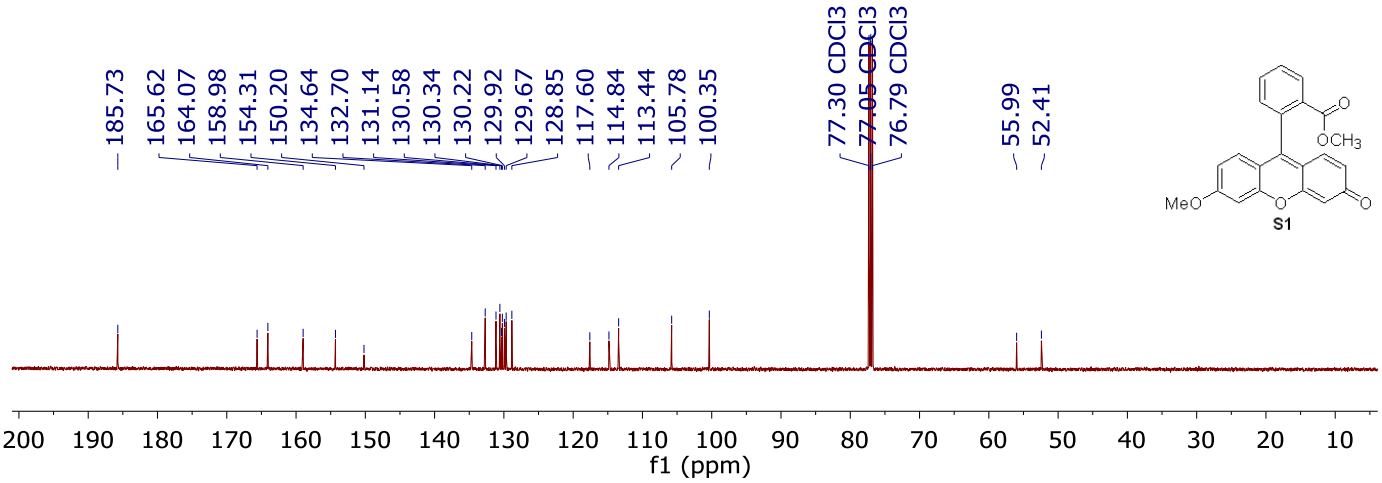
The calibration plot for estimating ACY-1 was generated in 10% diluted human serum. Briefly, to a 100 μL of freshly prepared 10% human blood serum in sodium phosphate buffer (10 mM, pH 7) was added NAC (10 μL, 10 mM in water) and 10 μL of ACY-1 stock solutions in 10 mM sodium phosphate buffer pH 7 to achieve a final concentration of 0 – 200 nM in a final volume of 120 μL. The reaction mixture was mixed carefully by pipetting up and down for a few seconds and incubated at 37 °C. After 30 min, the reaction was quenched by adding 1 mL of ACN and centrifuged at 15000 g for 15 min to settle down the precipitated proteins. The supernatant was transferred to another vial and dried in a vacuum concentrator at 30 °C. For fluorescence analysis, the dried sample was mixed with sodium phosphate buffer (690 μL, 10 mM, pH 7), ACN (290 μL), and **5** (10 μL, 1 mM solution in ACN). After 60 min of incubation, the fluorescence signal of **5** at 519 nm was recorded for various concentrations of ACY1 that gave a linear (R^2 = 0.9959) graph between the fluorescence response and ACY-1 concentration (0-10 nM). The background signal corresponding to serum Cys was subtracted from each data point to nullify its contribution. Test samples were prepared by spiking two known ACY-1 concentrations (0.7 and 1.4 nM) in the serum. Fluorescence signal at 519 nm was recorded for these samples following the above protocol to estimate ACY-1 concentrations using the calibration plot. Recoveries of ACY-1 added to serum samples were ≥ 99% and calculated using equation[7]: Recovery (%) = $[ACY1]_{found}/[ACY1]_{spiked} \times 100$. Three independent experiments (n = 3) were performed for calculating the standard deviations associated with each analysis.

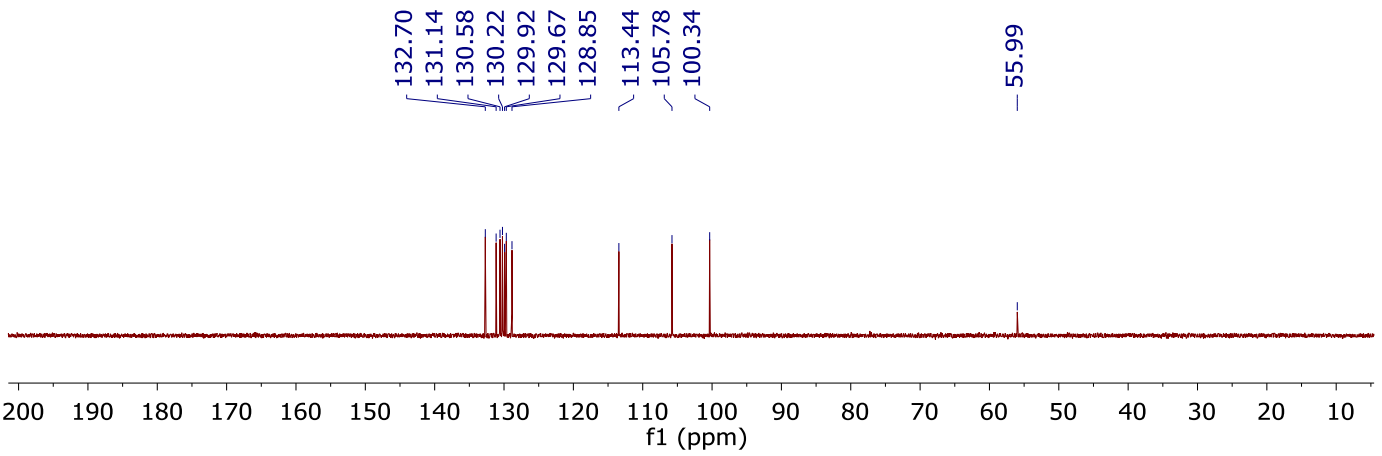
General procedure for the enzymatic (ACY-1) studies (Figure 5b)

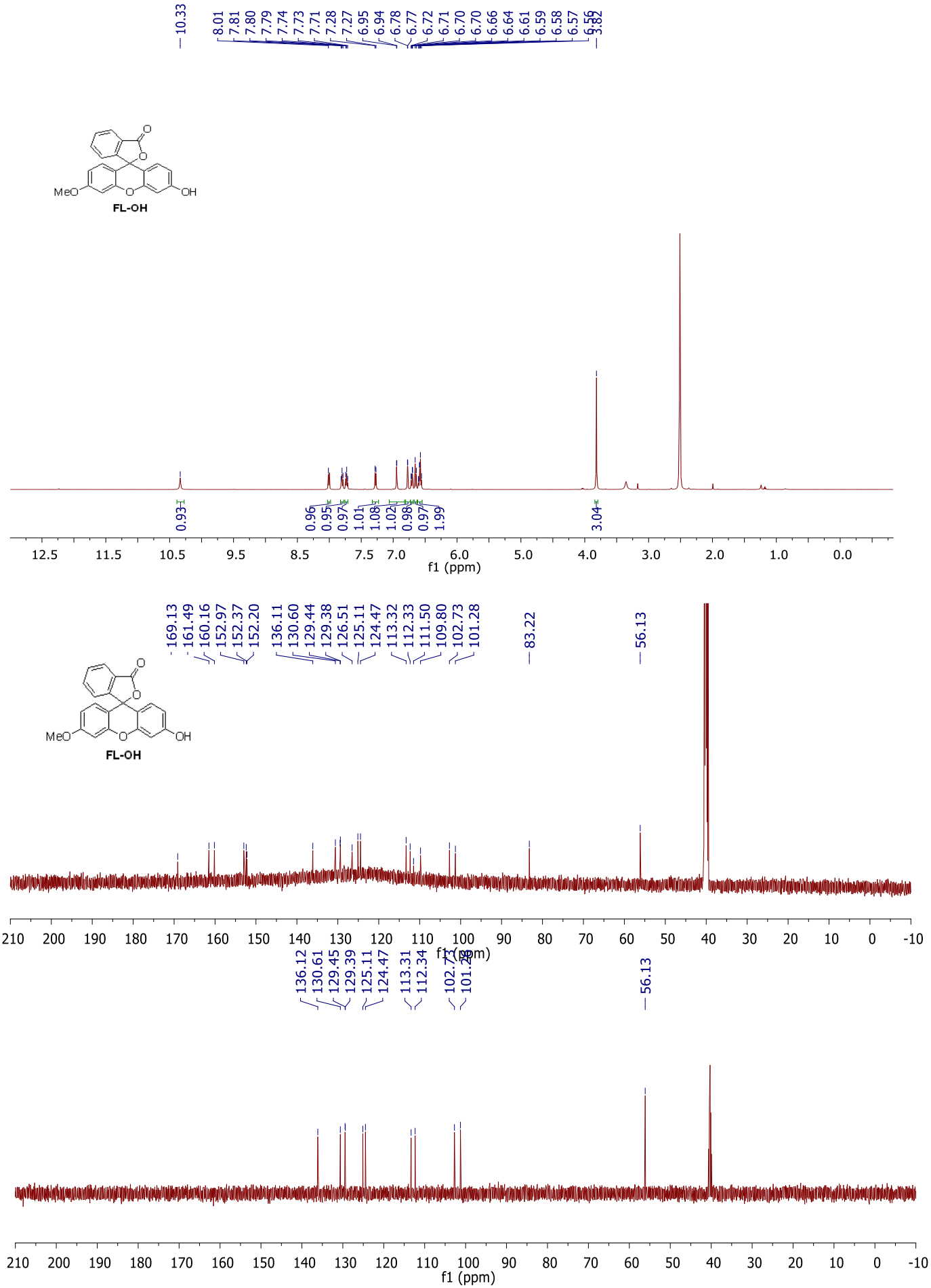
For enzymatic assays, 200 units of ACY-1 (60.6 μL, 11.63 mM in sodium phosphate buffer pH 7, 10 mM) was added to a solution containing sodium phosphate buffer (pH 7, 630 μL, 10 mM) and NAC (10 μL, 10 mM solution in water). The reaction mixture was mixed and incubated at 37 °C. After 5 min, the reaction was quenched by adding 290 μL of ACN followed by the addition of **5** (10 μL, 1 mM solution in ACN) and monitoring the time-dependent fluorescence response of **5** at 519 nm for 60 min at room temperature. For the control reaction (no enzyme), sodium phosphate buffer (60.6 μL) was added in place of ACY-1 solution to the reaction. In the presence of the inhibitor, 200 units of ACY-1 were first incubated with EDTA (4 and 8 mM) for 15 min at 37 °C before adding NAC and following the rest of the procedure as described above.

Section E: NMR spectral data





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6.66
6.64
6.61
6.59
6.58
6.57
6.56
3.82
MeO
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FL-OH
0.93
0.96
0.95
0.97
1.01
1.08
1.02
0.98
0.97
1.99
3.04
12.5
11.5
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124.47
113.32
112.33
111.50
109.80
102.73
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FL-OH
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200
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180
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120
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30
20
10
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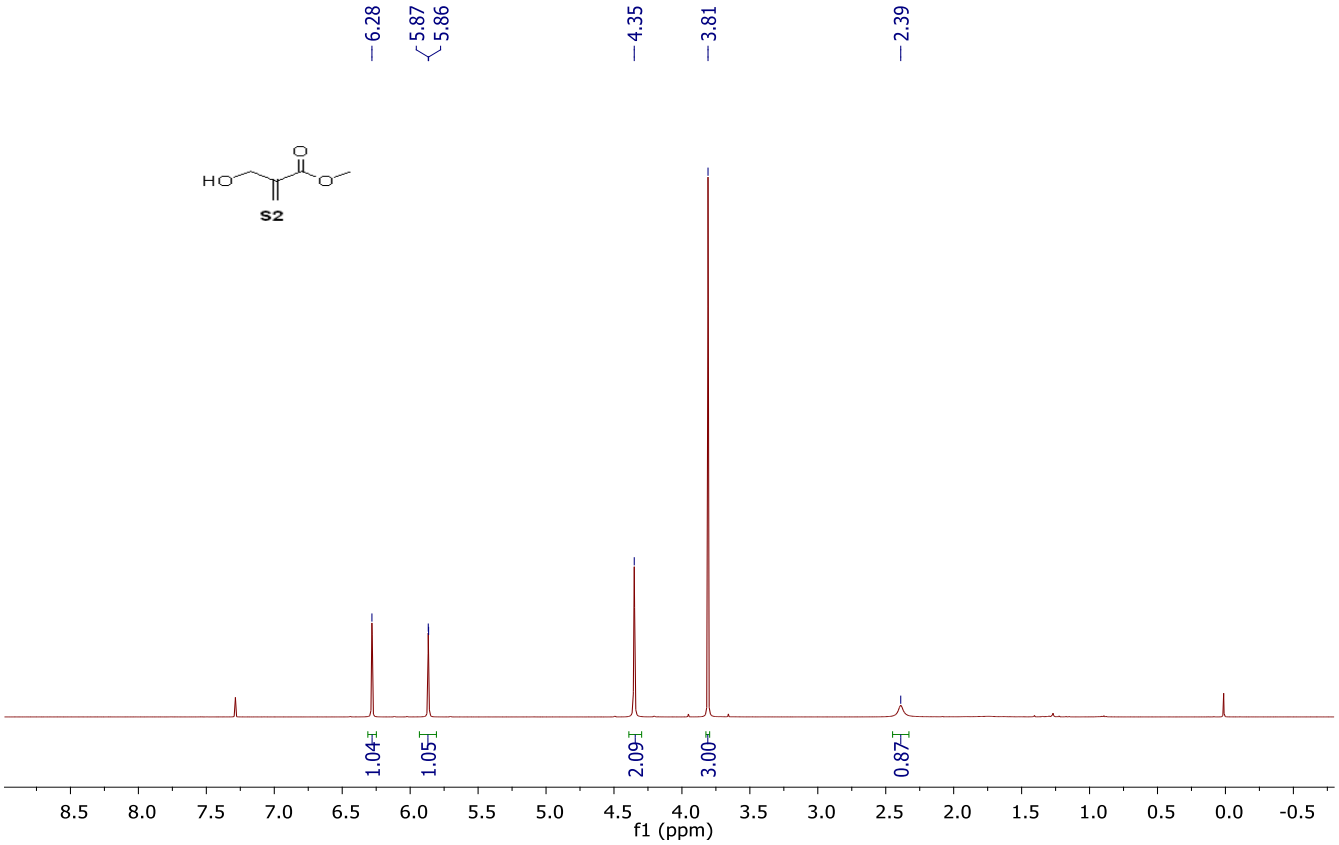
f1 (ppm)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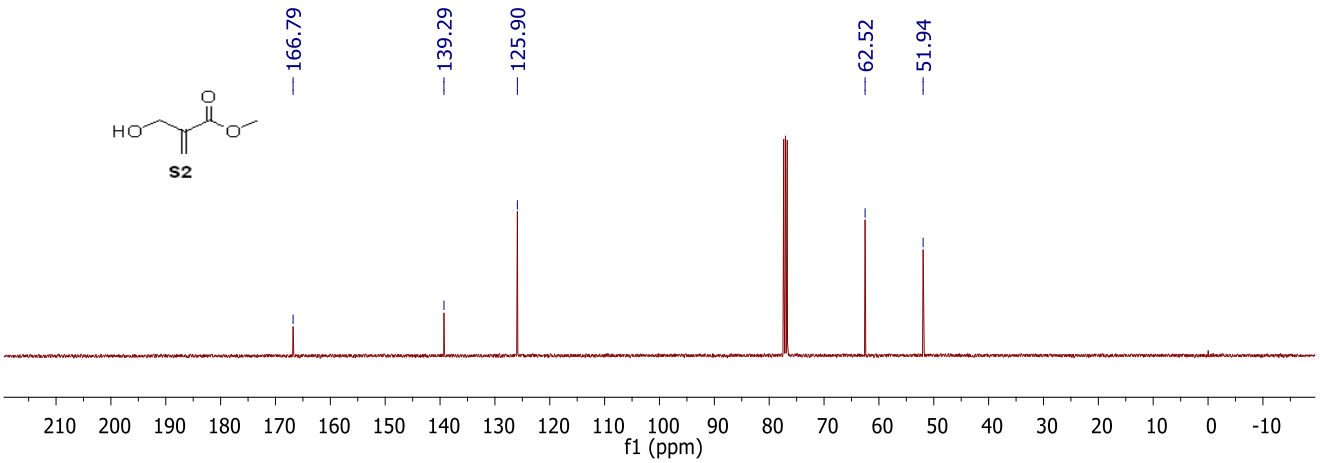
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O
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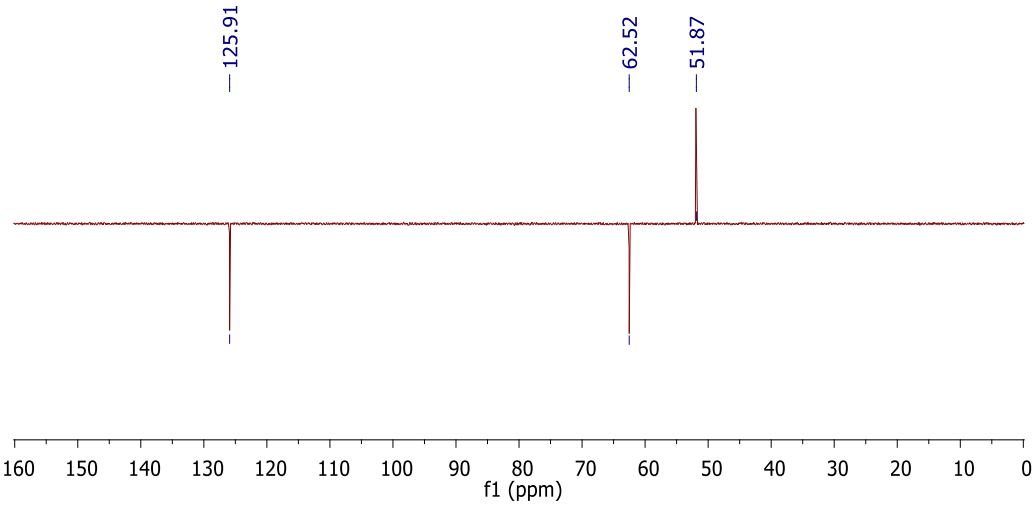
f1 (ppm)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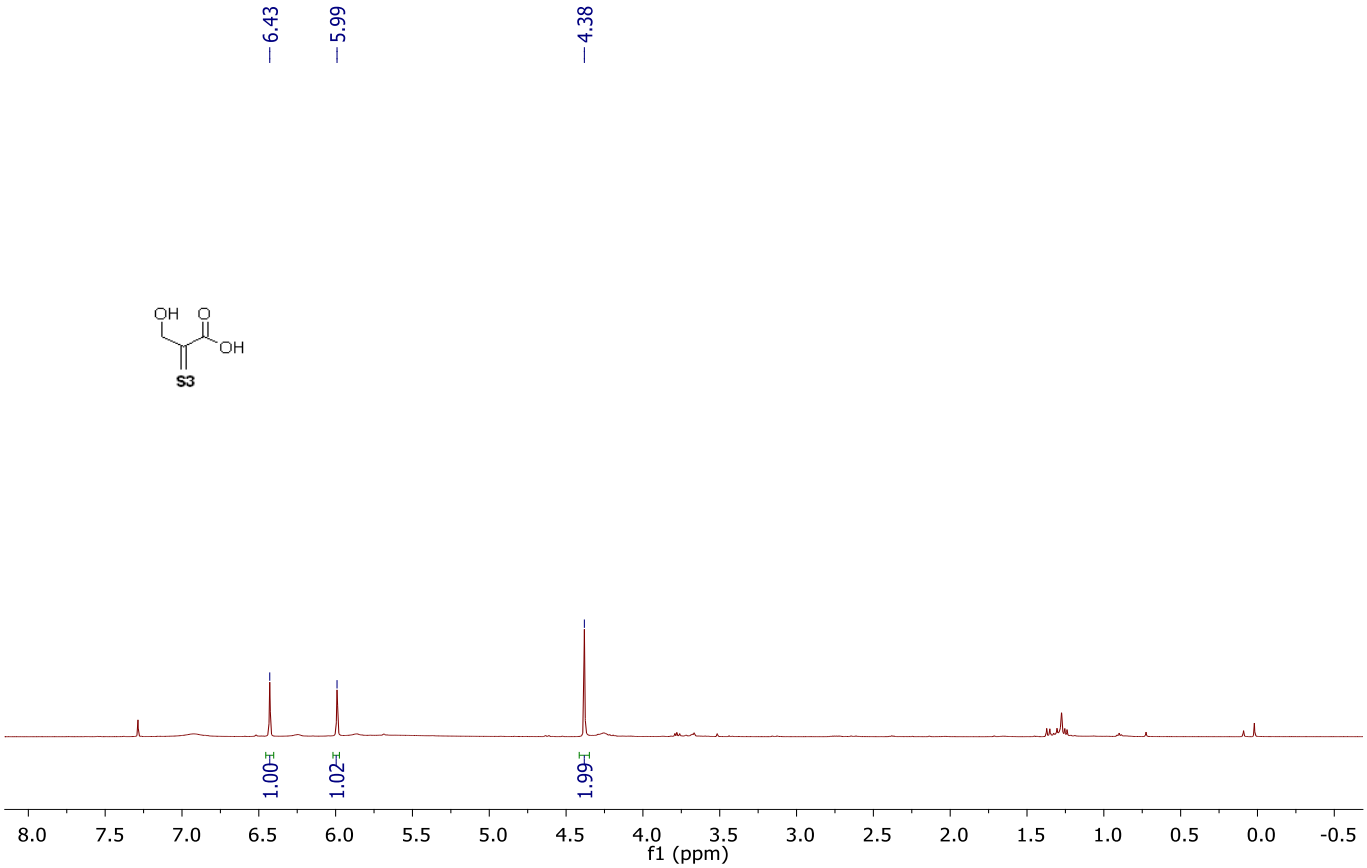
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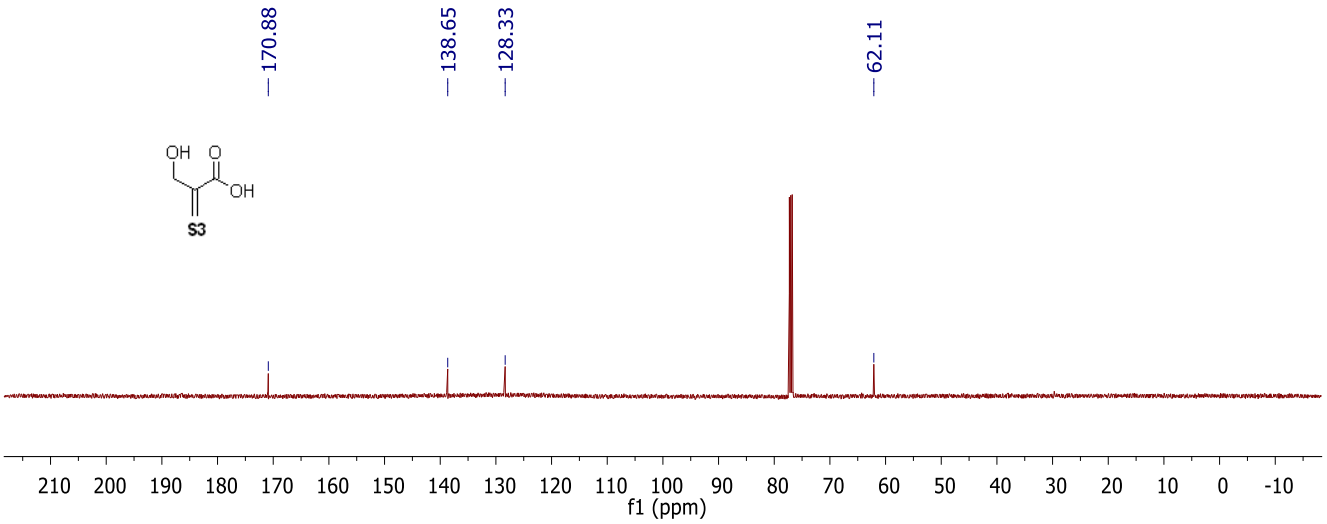
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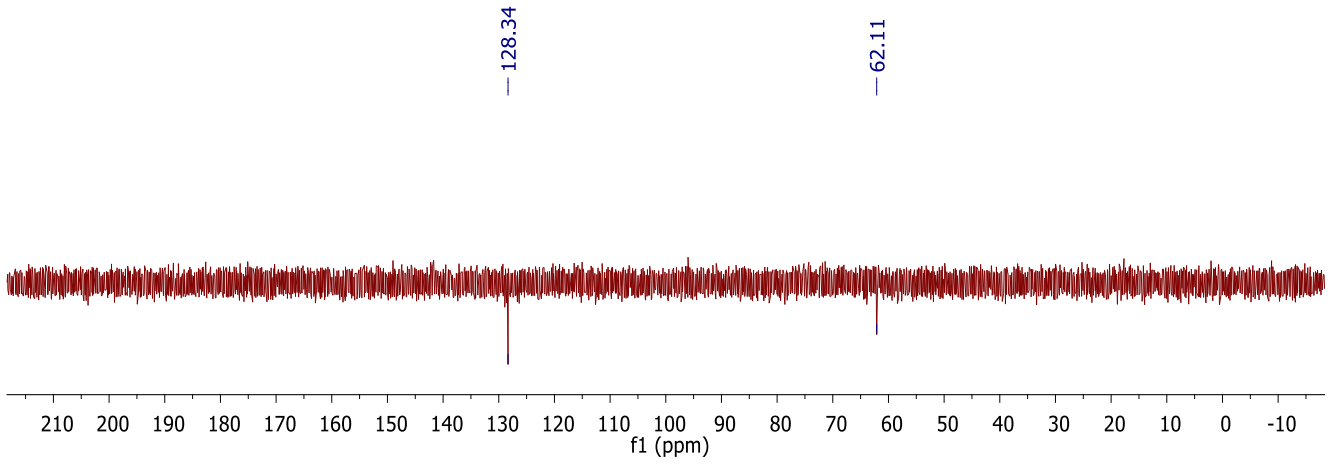
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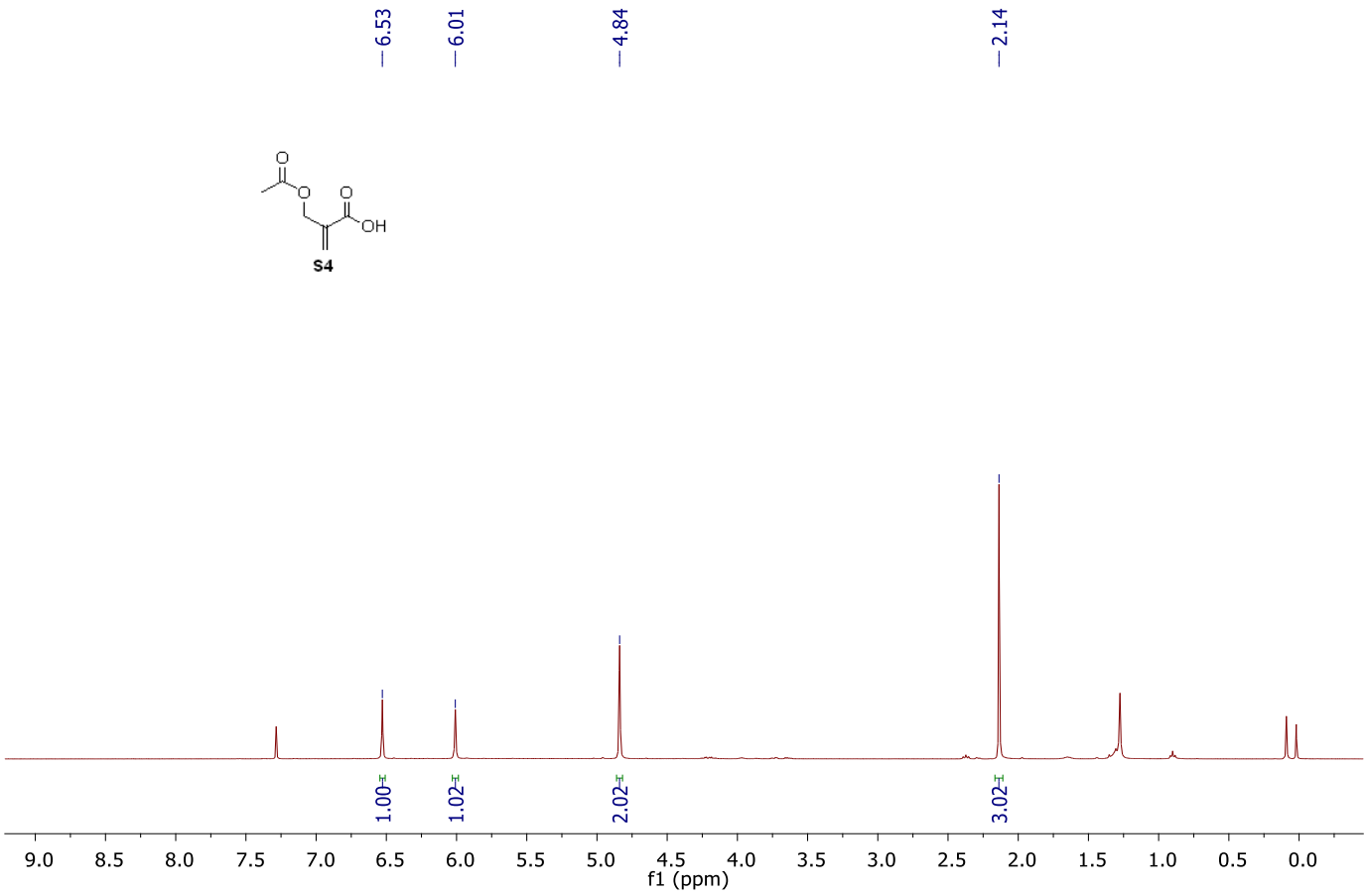
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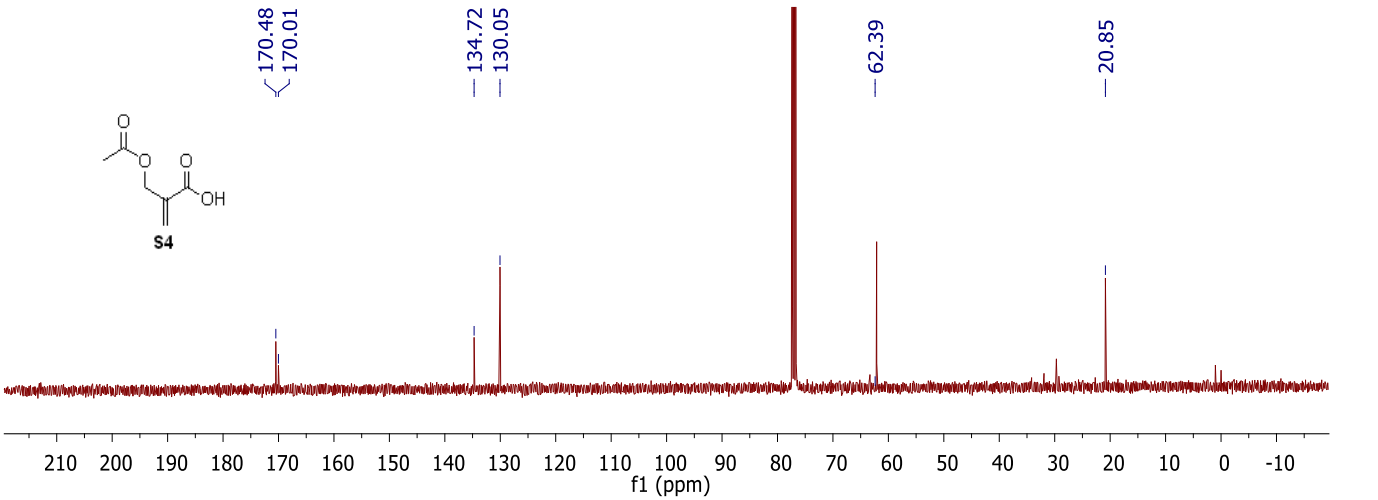
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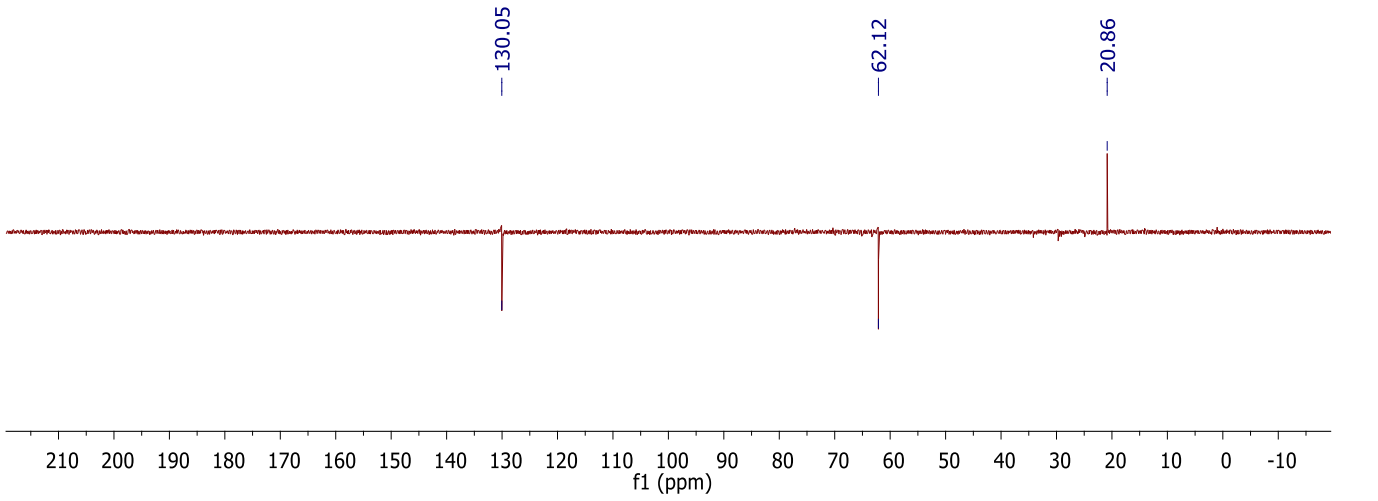
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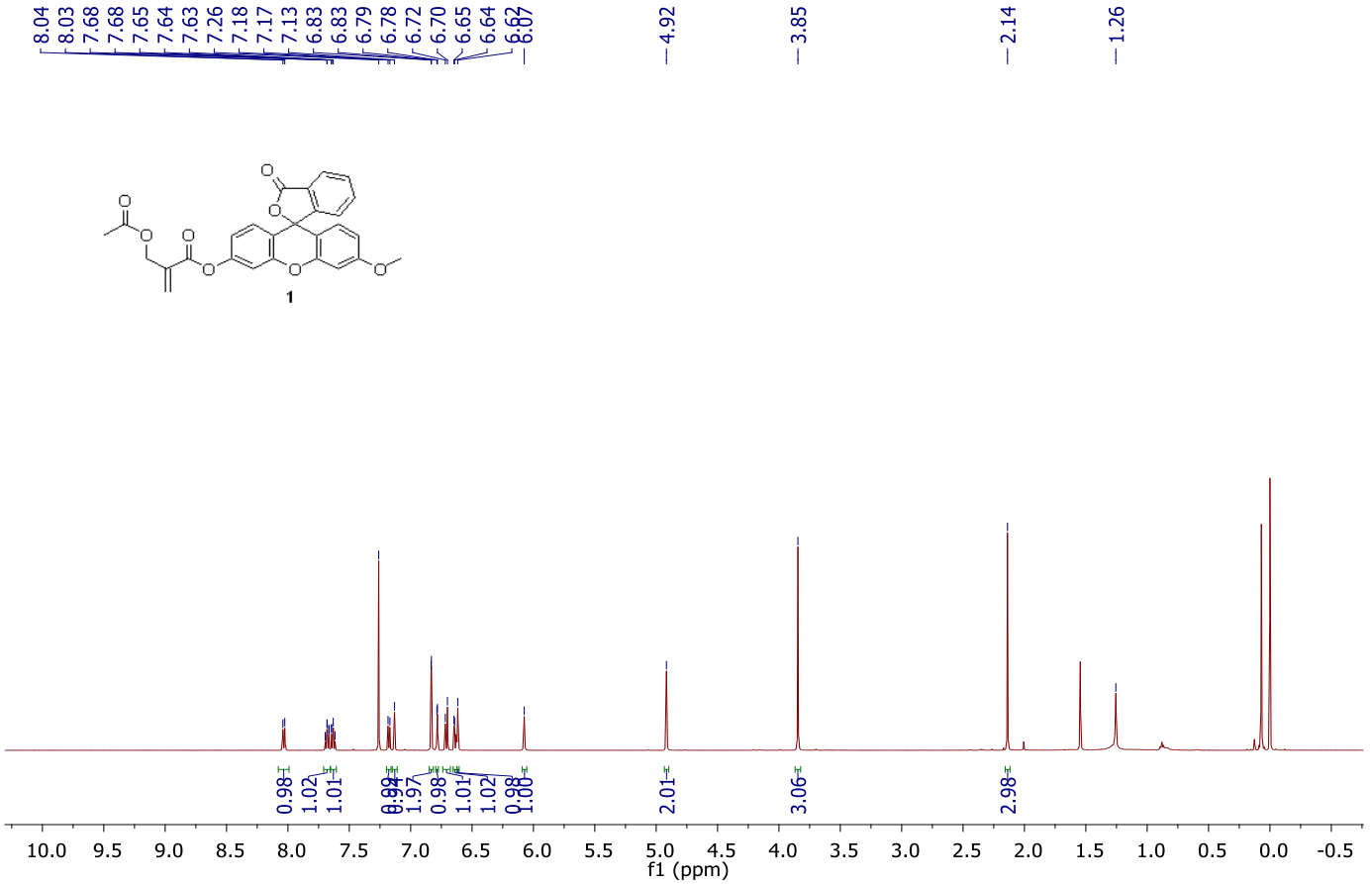
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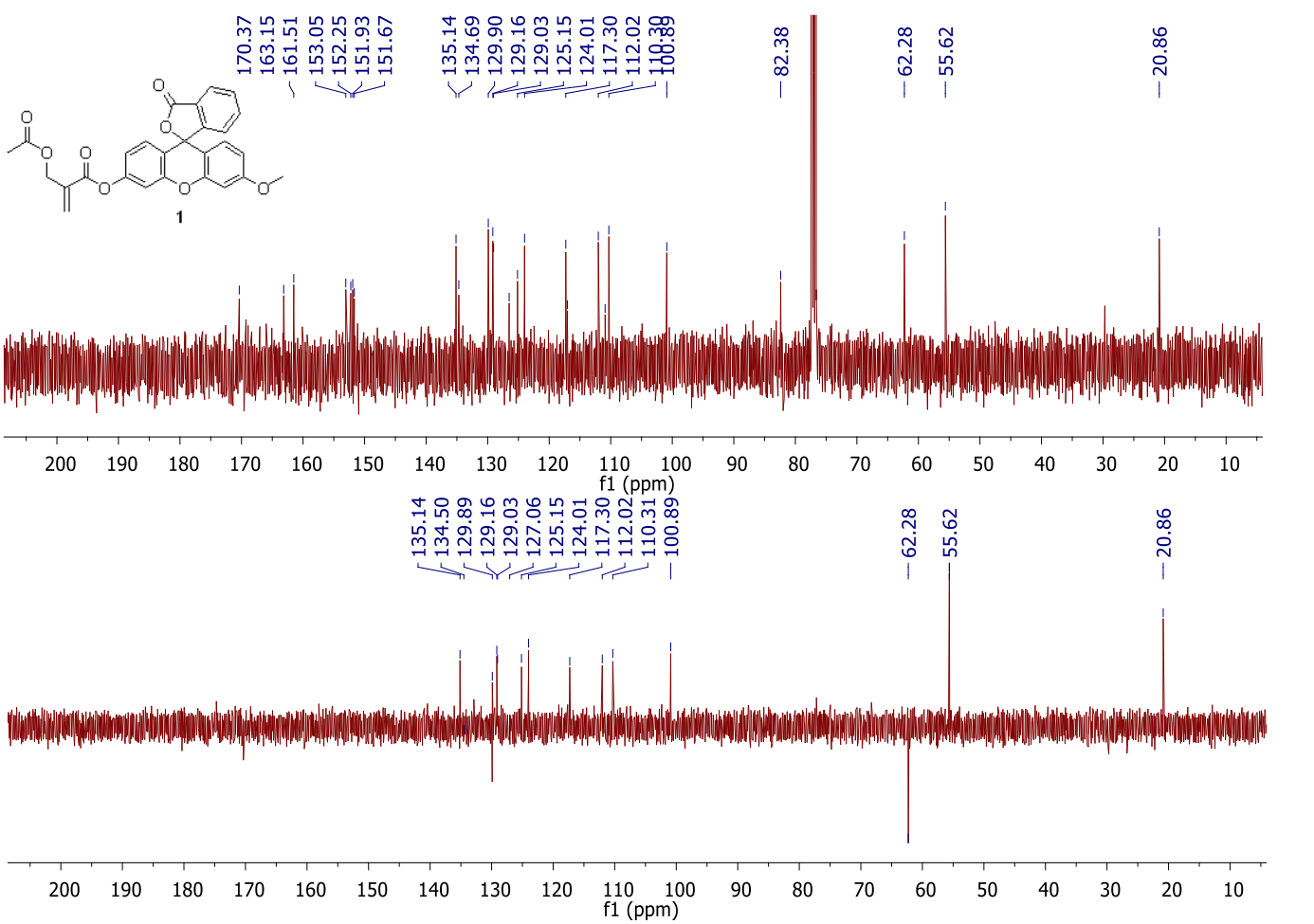
f1 (ppm)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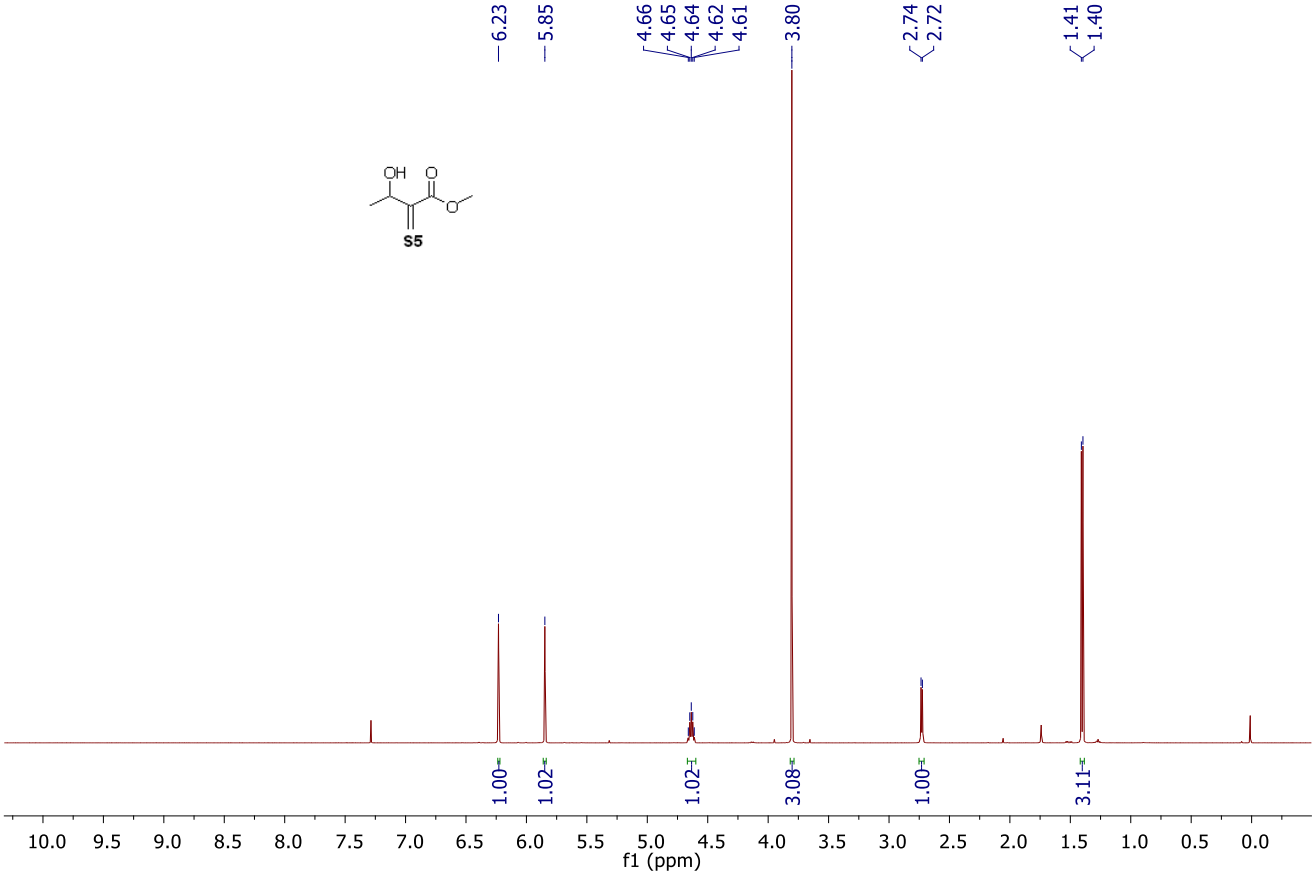
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1.02
1.02
3.08
1.00
3.11
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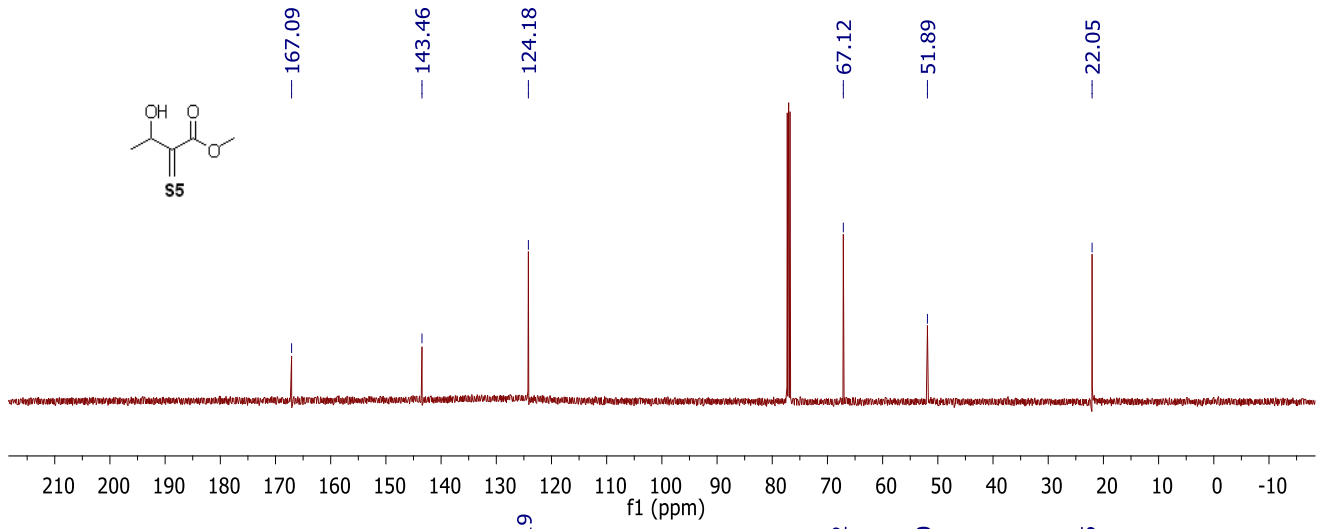
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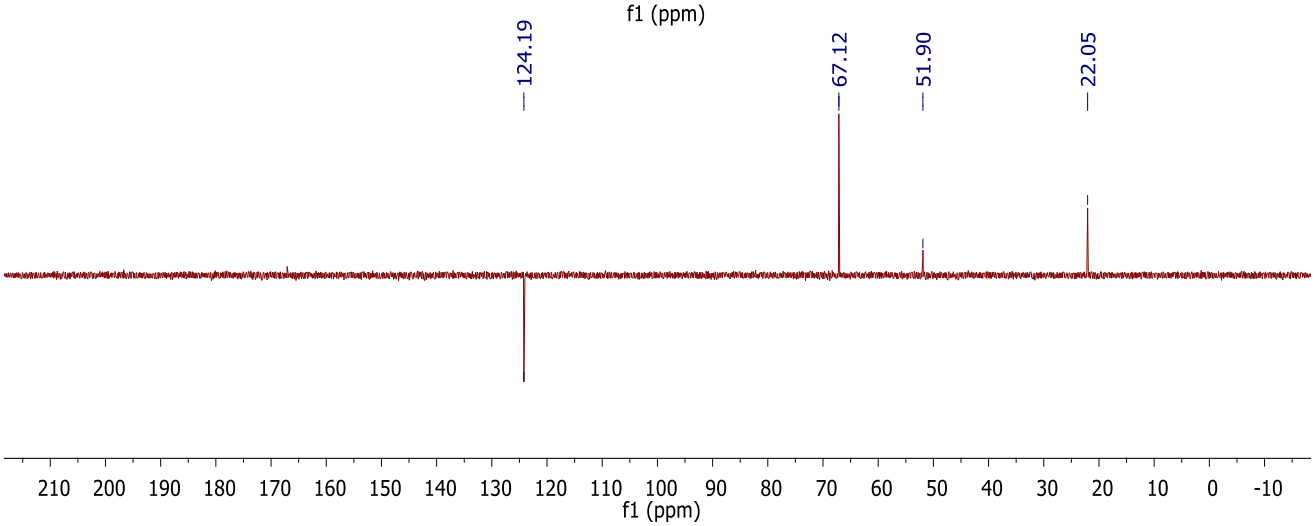
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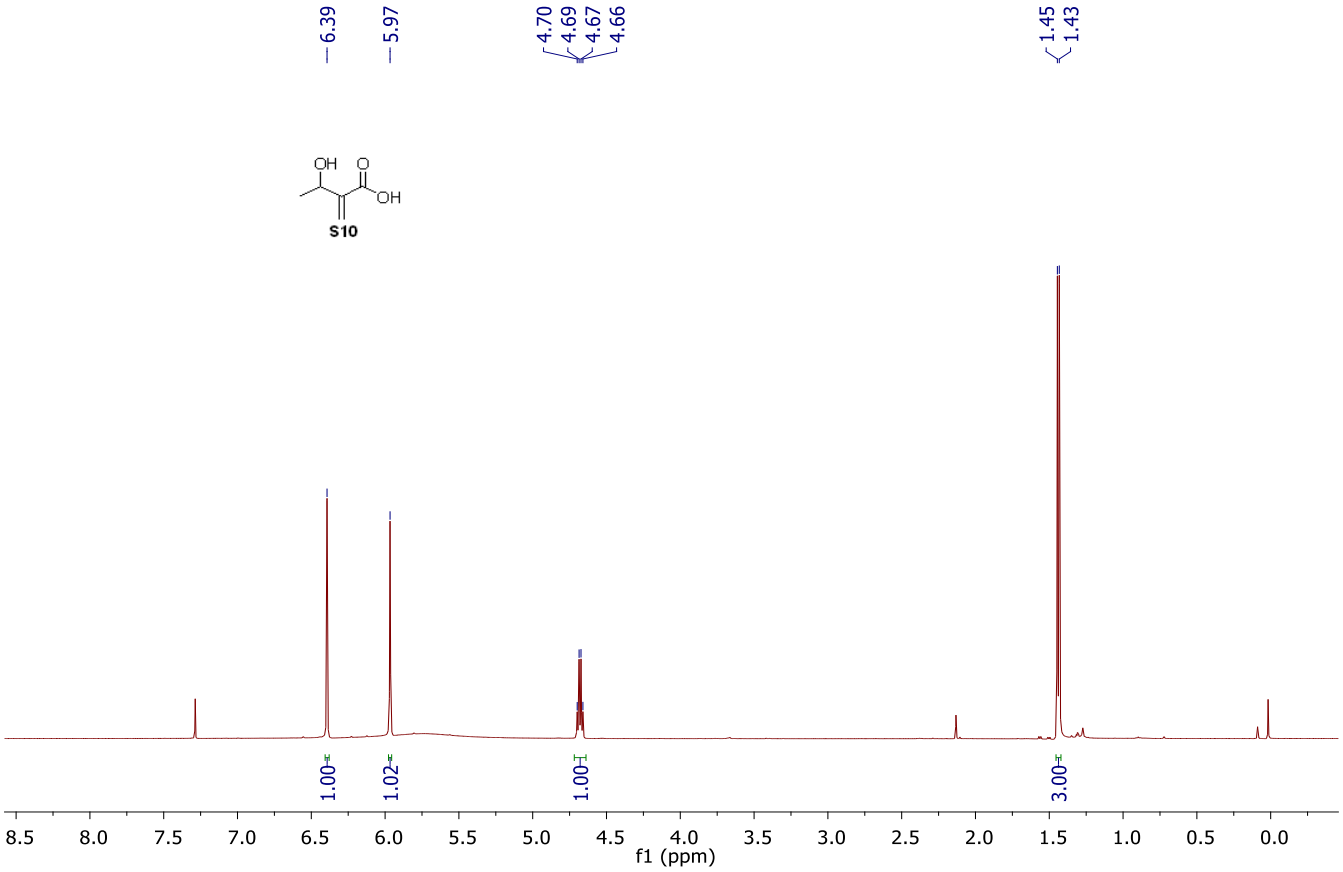
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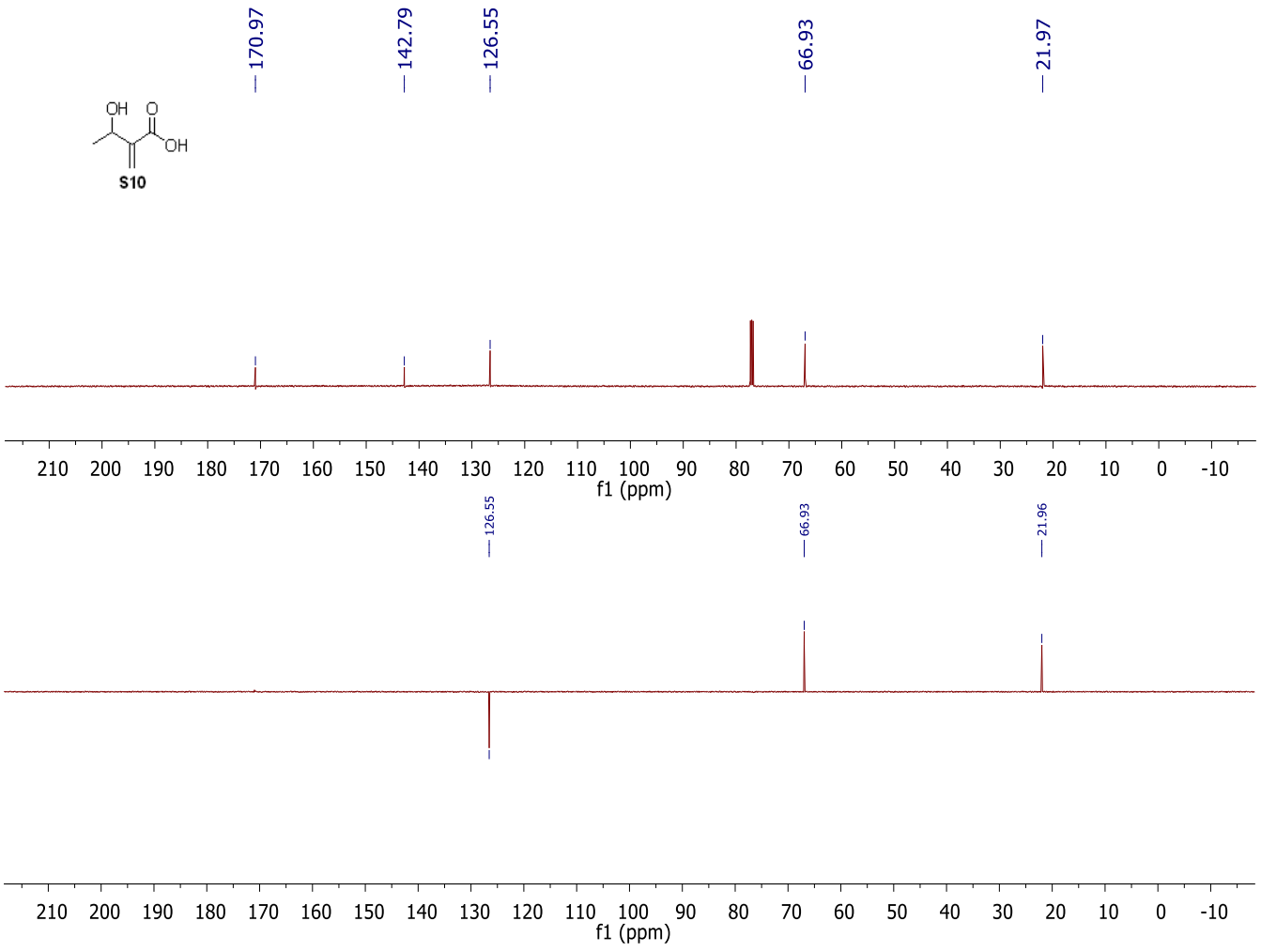
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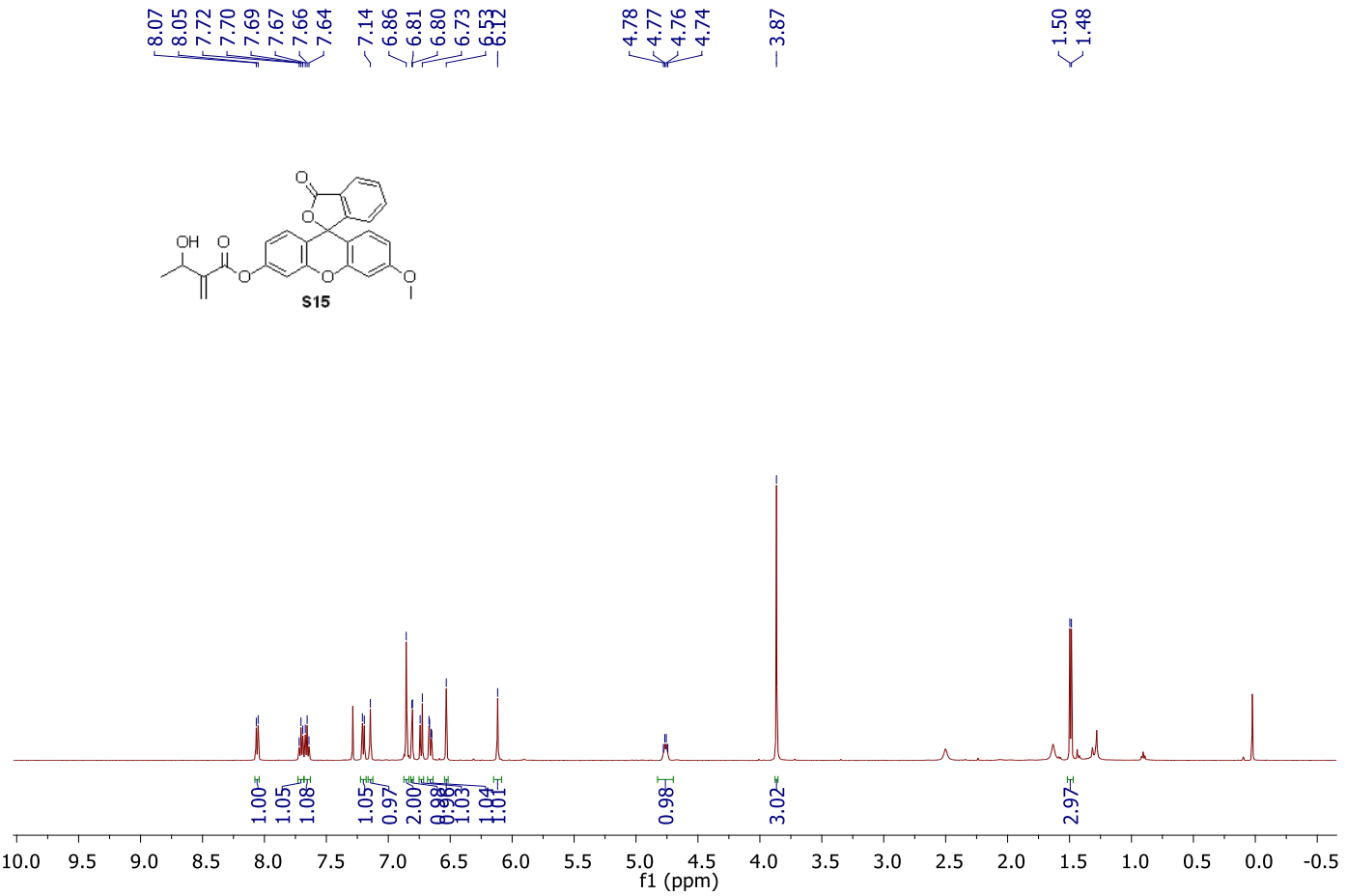
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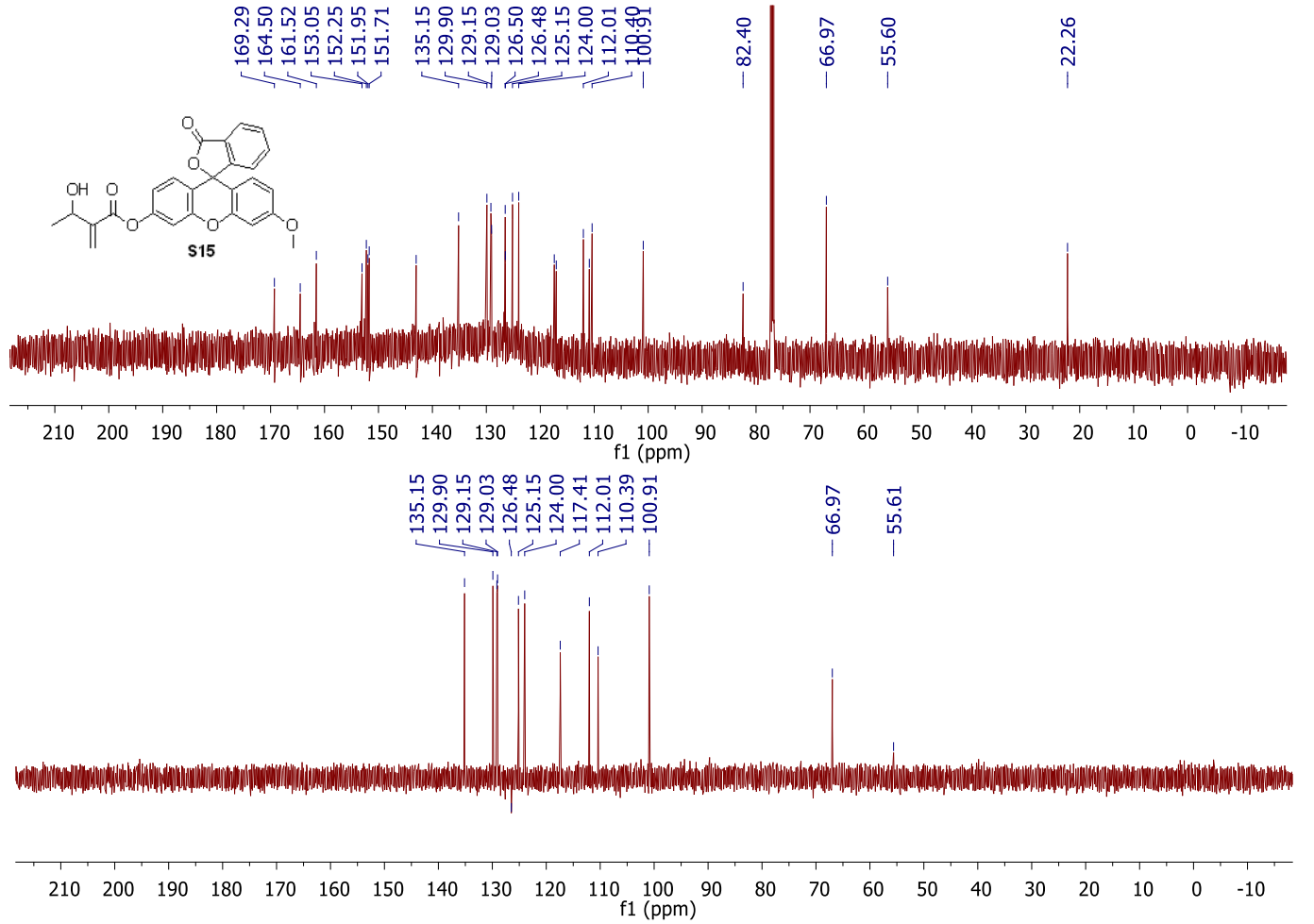
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O
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O
S15
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f1 (ppm)

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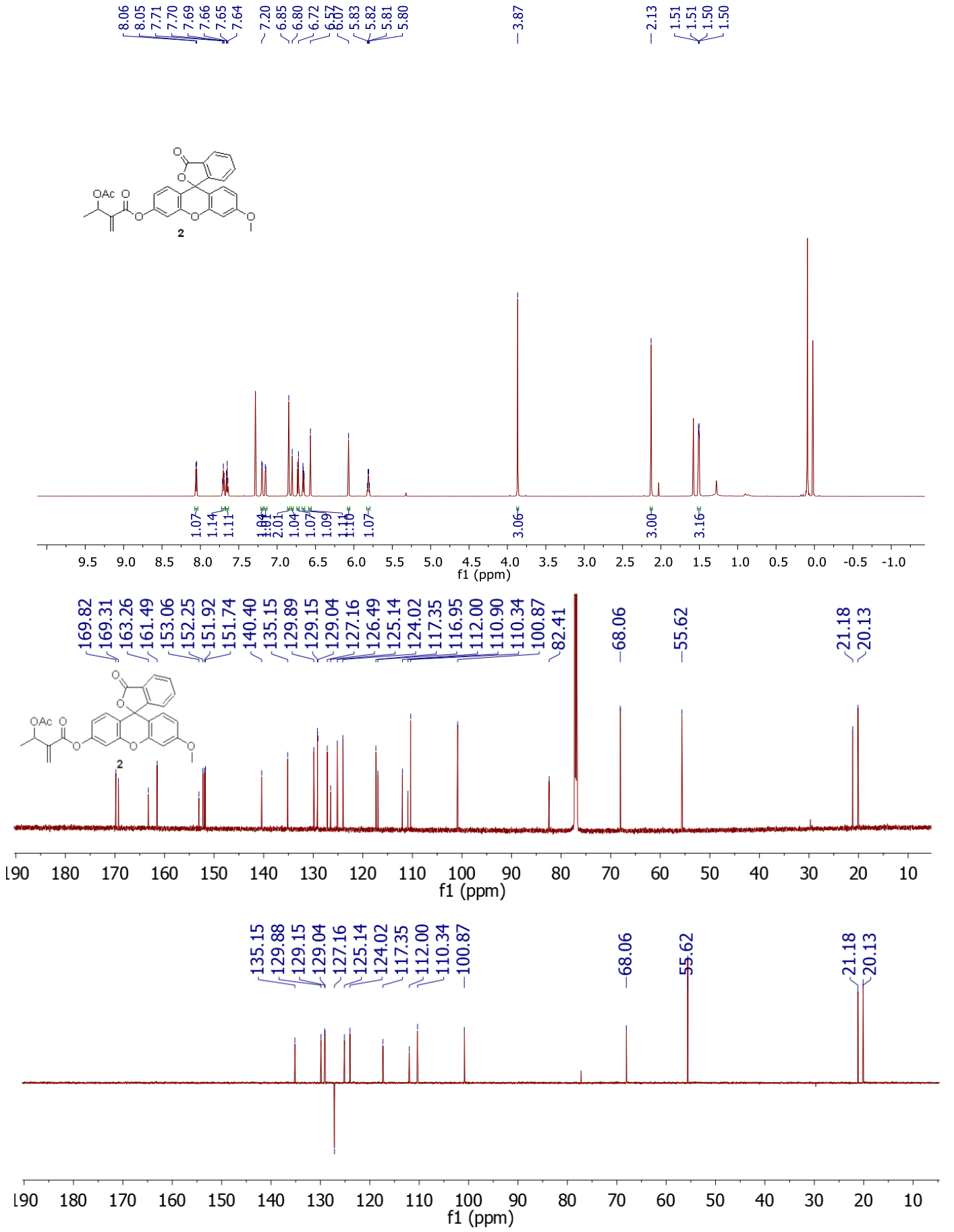
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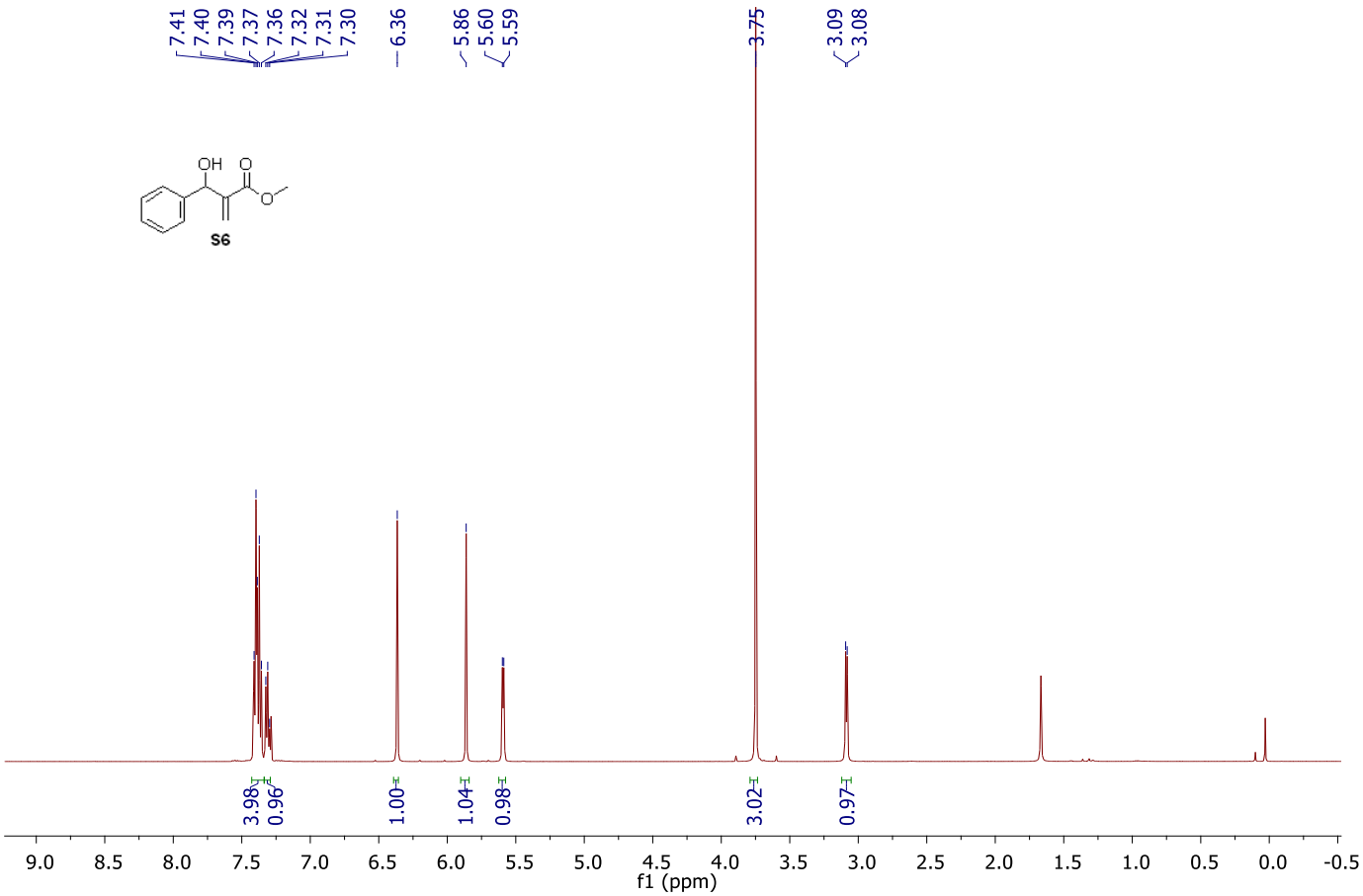
20.13
f1 (ppm)
135.15
129.88
129.15
129.04
127.16
125.14
124.02
117.35
112.00
110.34
100.87
68.06
55.62
21.18
20.13
f1 (ppm)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7.40
7.39
7.37
7.36
7.32
7.31
7.30
6.36
5.86
5.60
5.59
3.75
3.09
3.08
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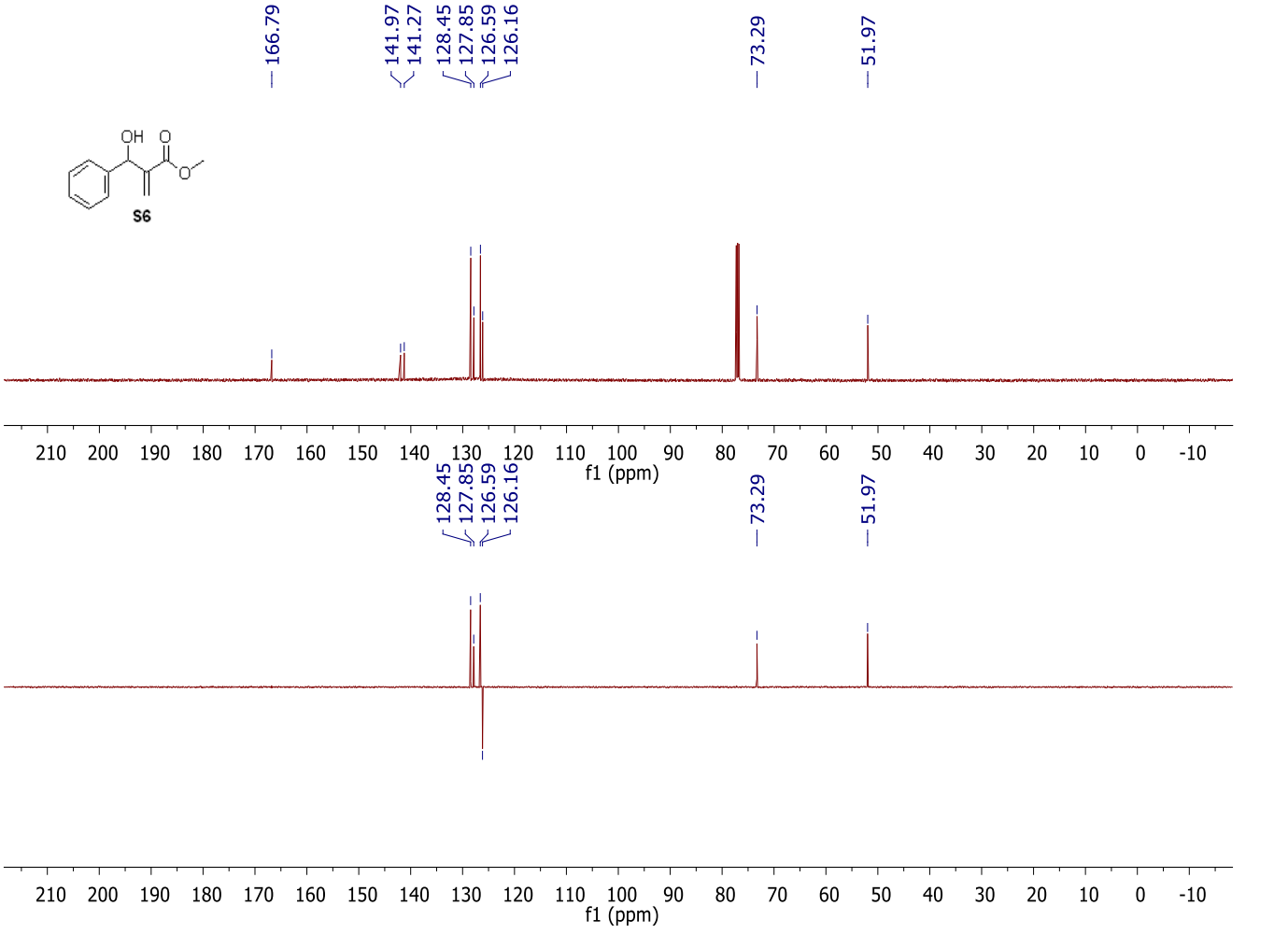
O
O
S6
3.98
0.96
1.00
1.04
0.98
3.02
0.97
f1 (ppm)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141.97
141.27
128.45
127.85
126.59
126.16
73.29
51.97
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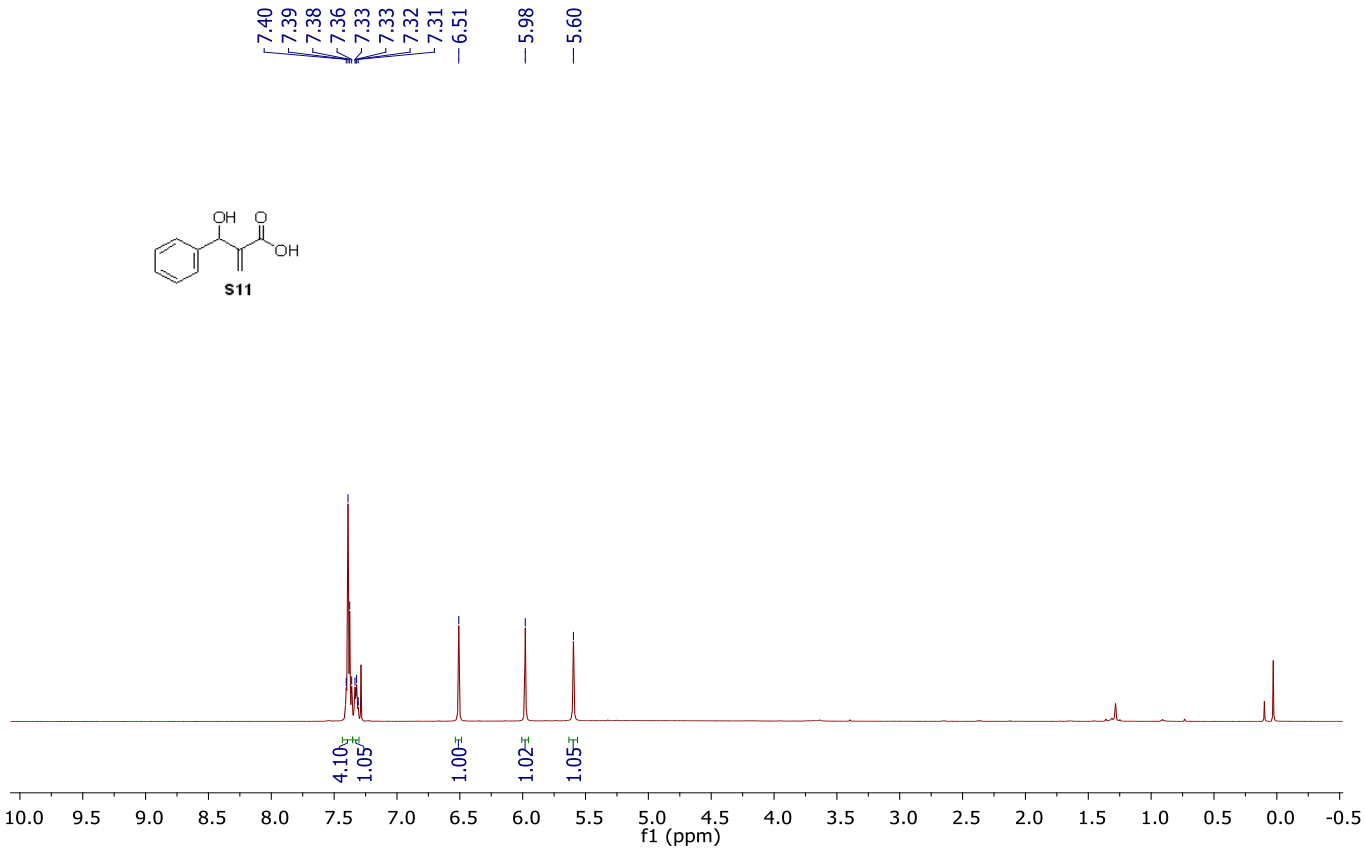
O
O
S6
f1 (ppm)
128.45
127.85
126.59
126.16
73.29
51.97
f1 (ppm)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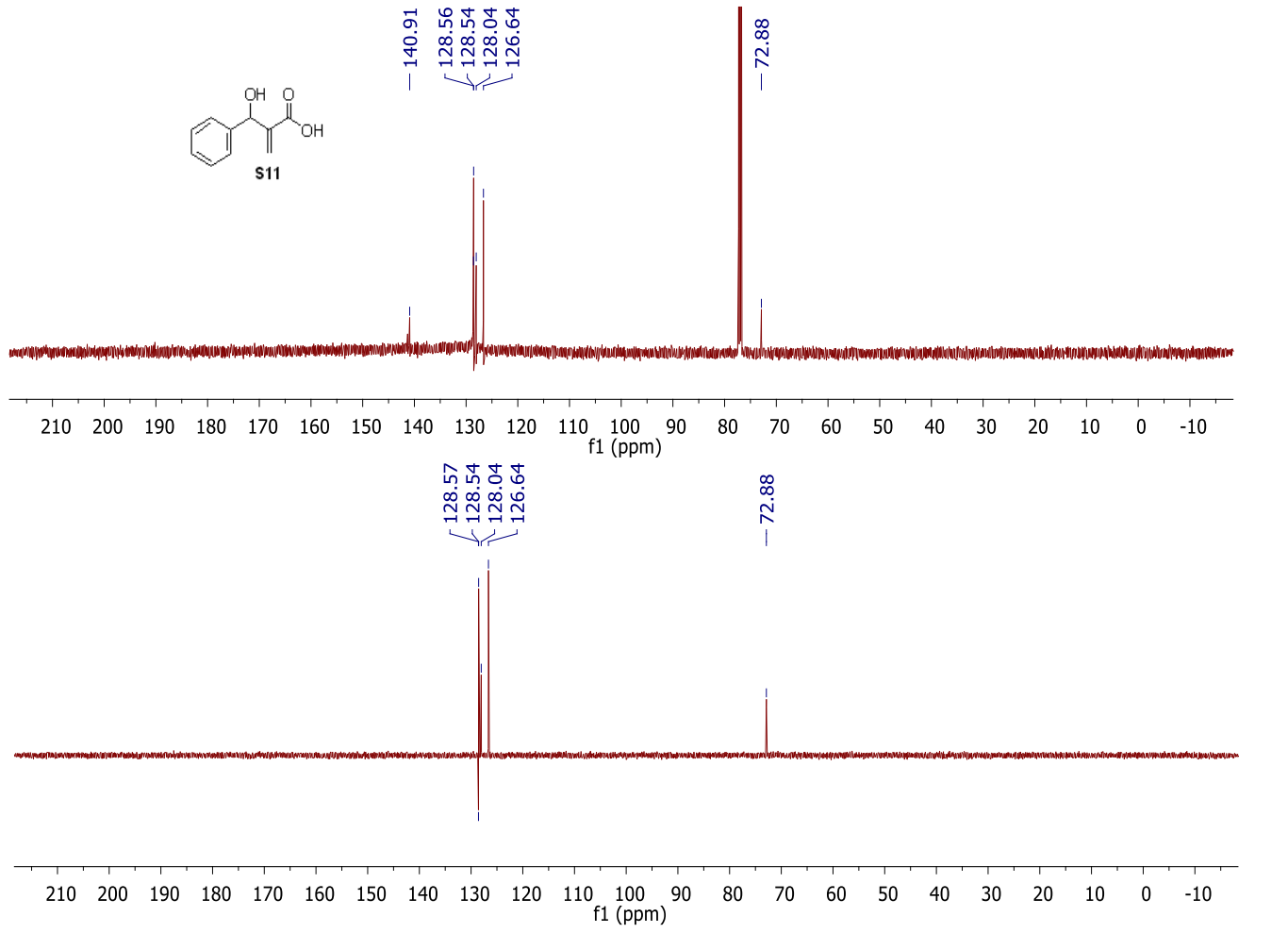
7.39
7.38
7.36
7.33
7.33
7.32
7.31
6.51
5.98
5.60
OH
O
OH
S11
4.10
1.05
1.00
1.02
1.05
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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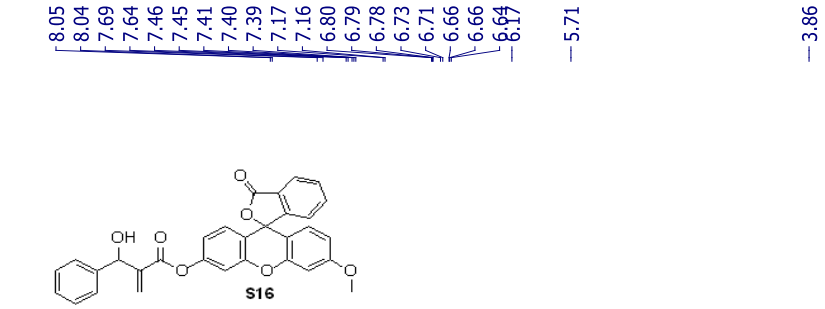
128.56
128.54
128.04
126.64
72.88
OH
O
OH
S11
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

128.57
128.54
128.04
126.64
72.88
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.04
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7.64
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7.45
7.41
7.40
7.39
7.17
7.16
6.80
6.79
6.78
6.73
6.71
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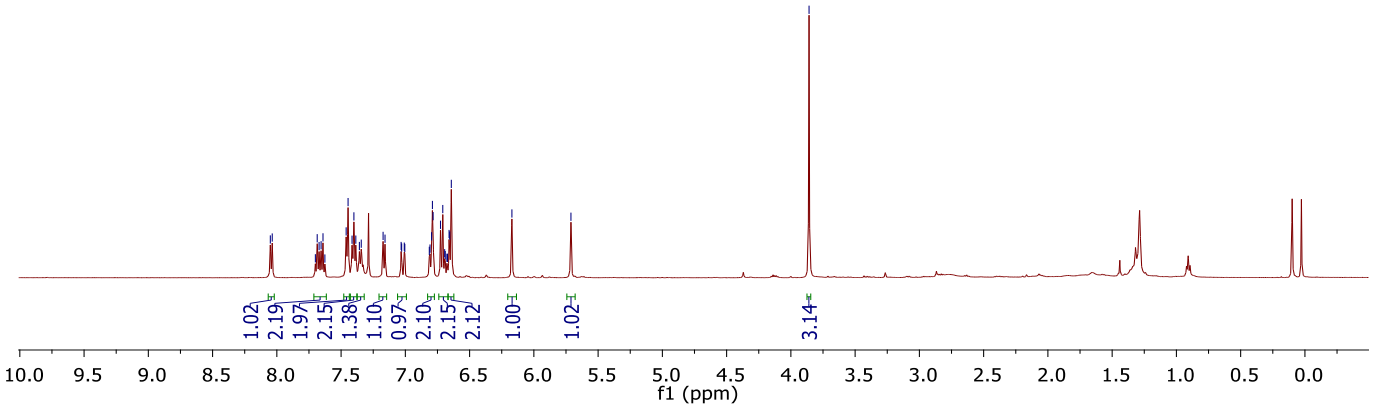
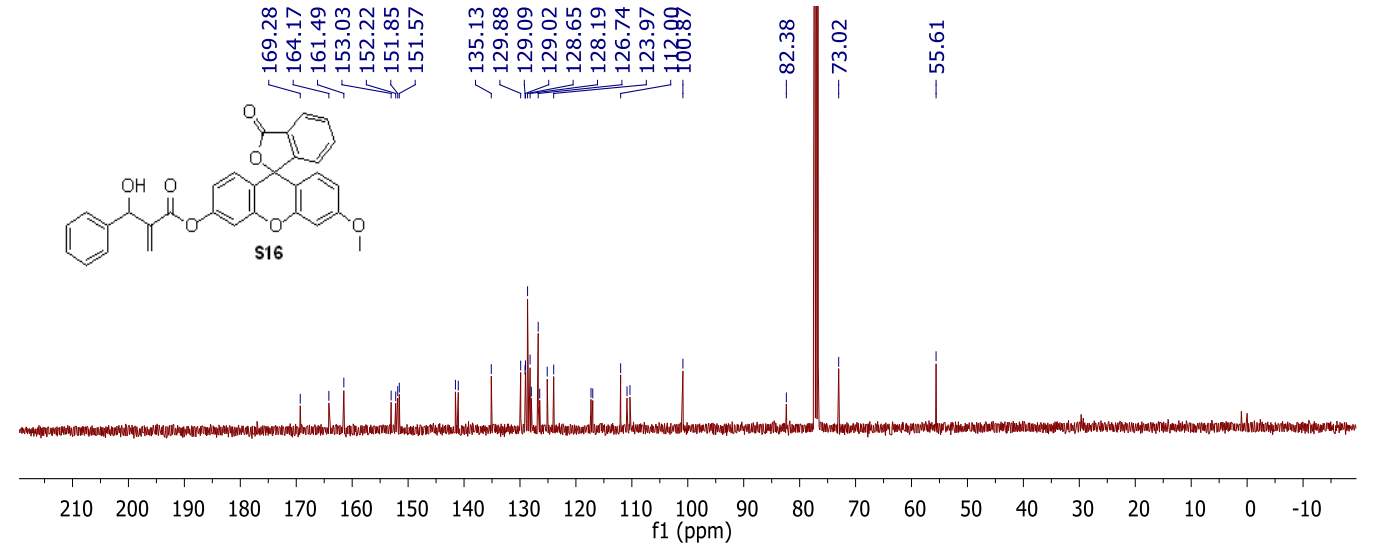
6.66
6.64
6.17
5.71
3.86
OH
O
O
O
O
O
S16


1.02
2.19
1.97
2.15
1.38
1.10
0.97
2.10
2.15
2.12
1.00
1.02
3.14
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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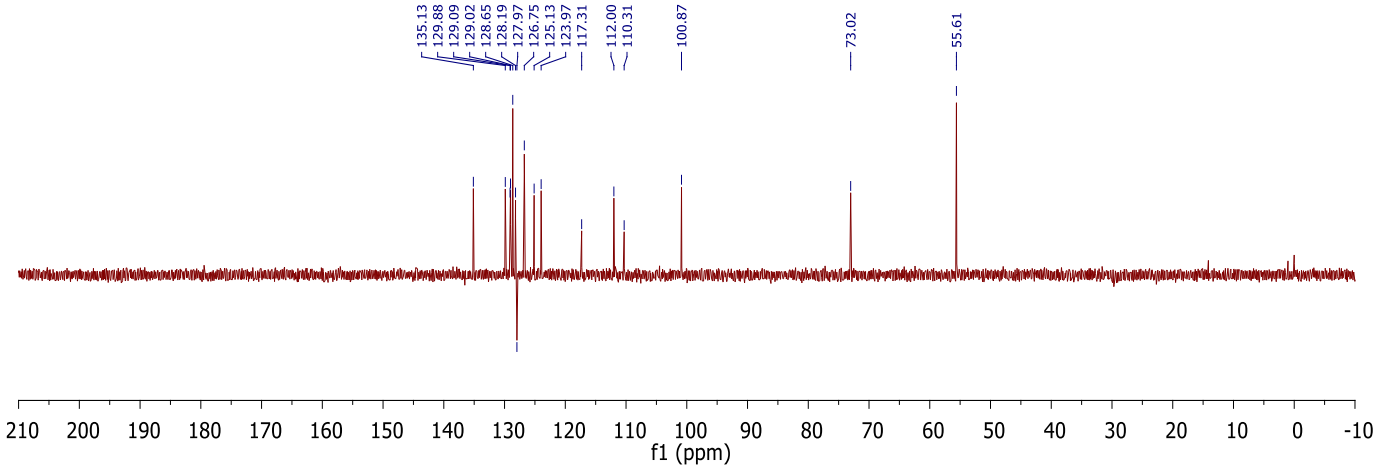
164.17
161.49
153.03
152.22
151.85
151.57
135.13
129.88
129.09
129.02
128.65
128.19
126.74
123.97
82.38
73.02
55.61
OH
O
O
O
O
O
S16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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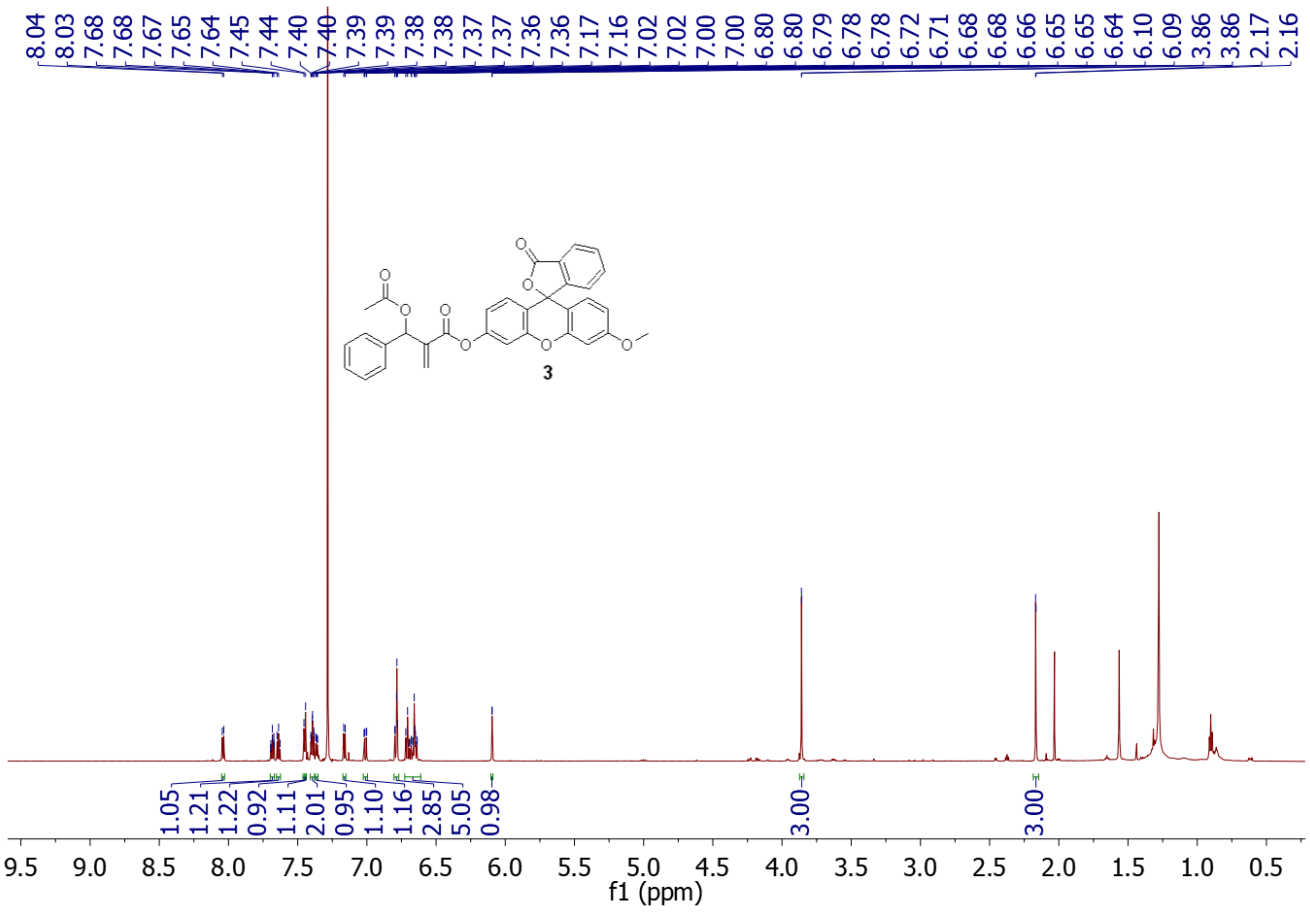
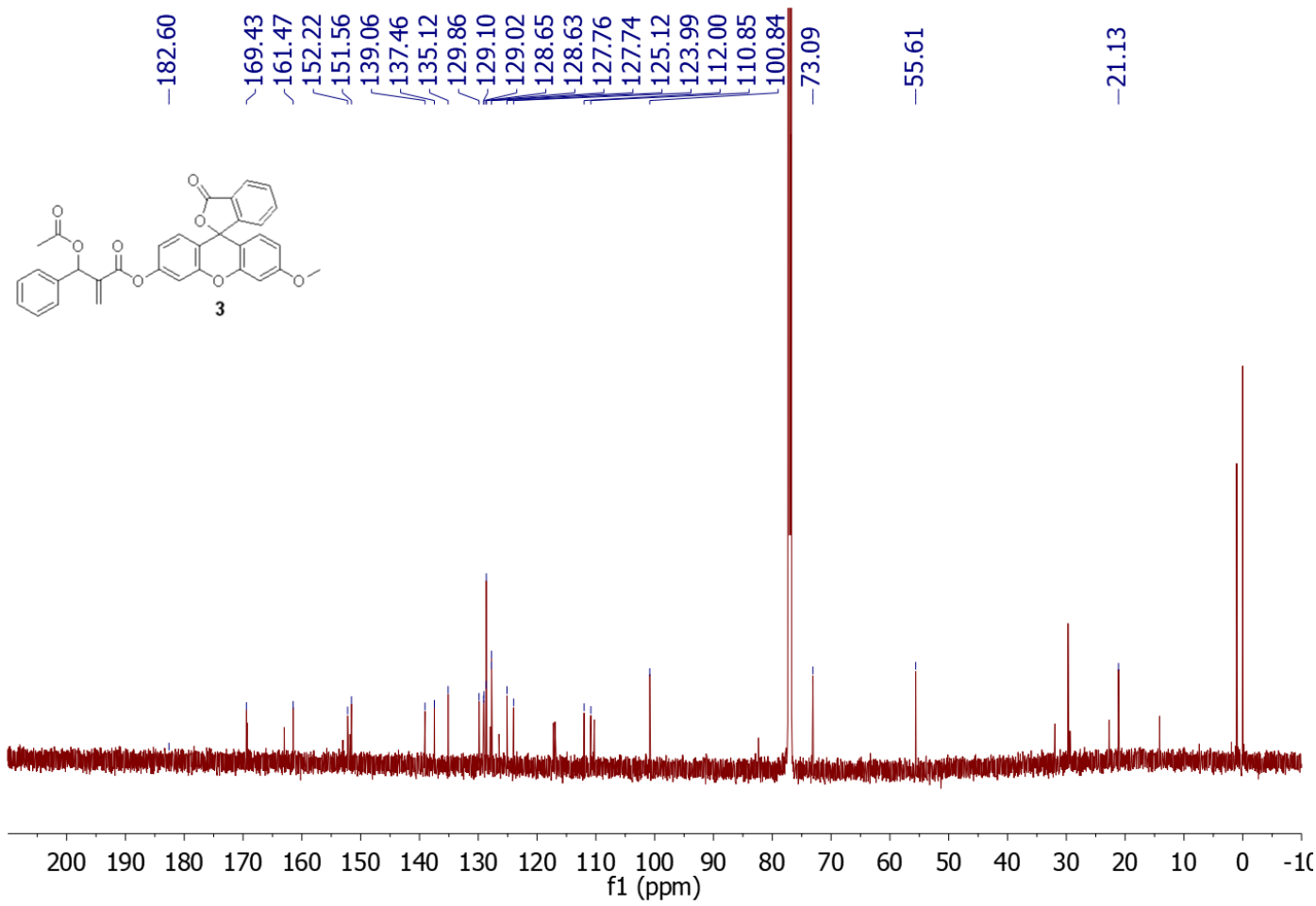
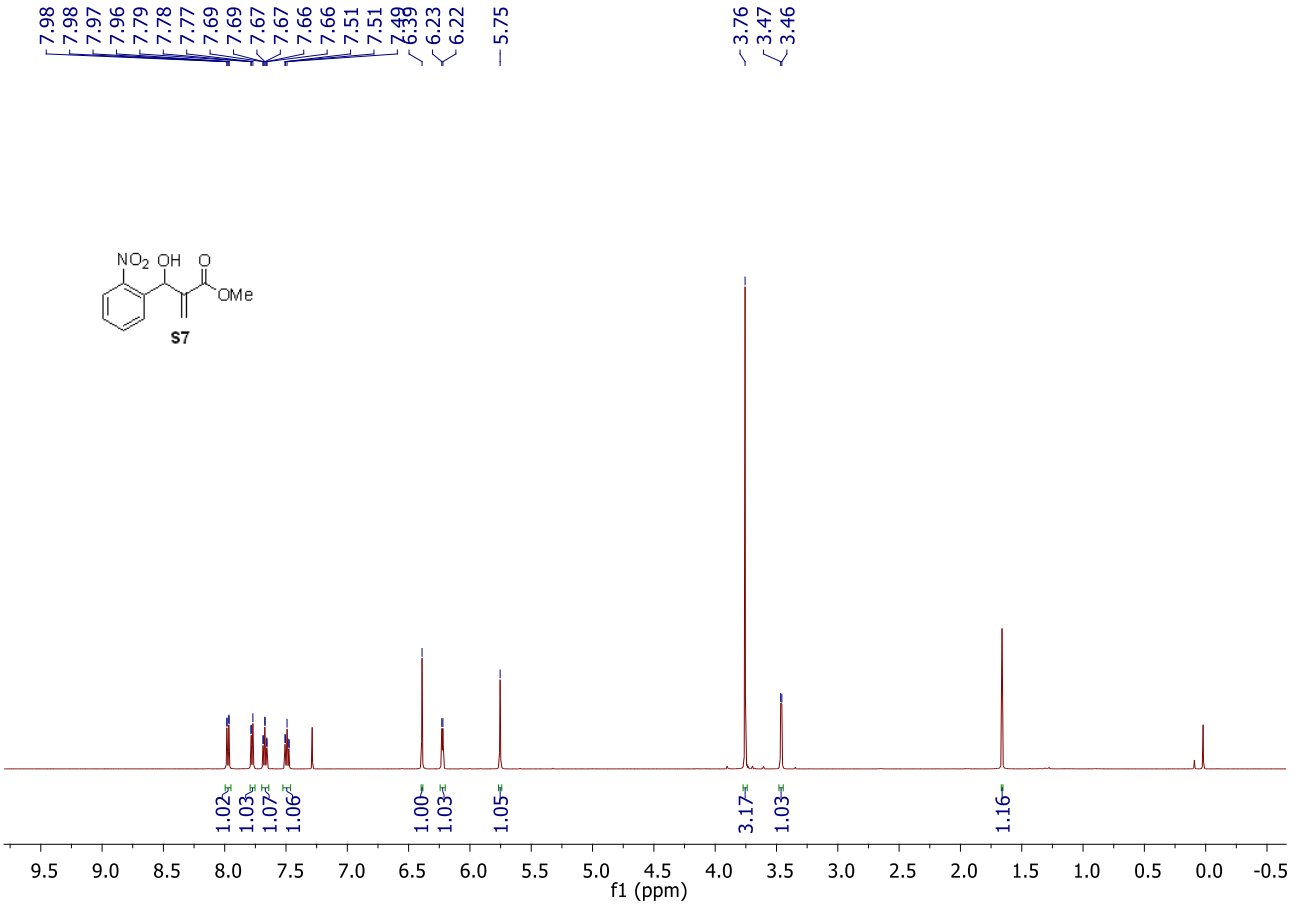
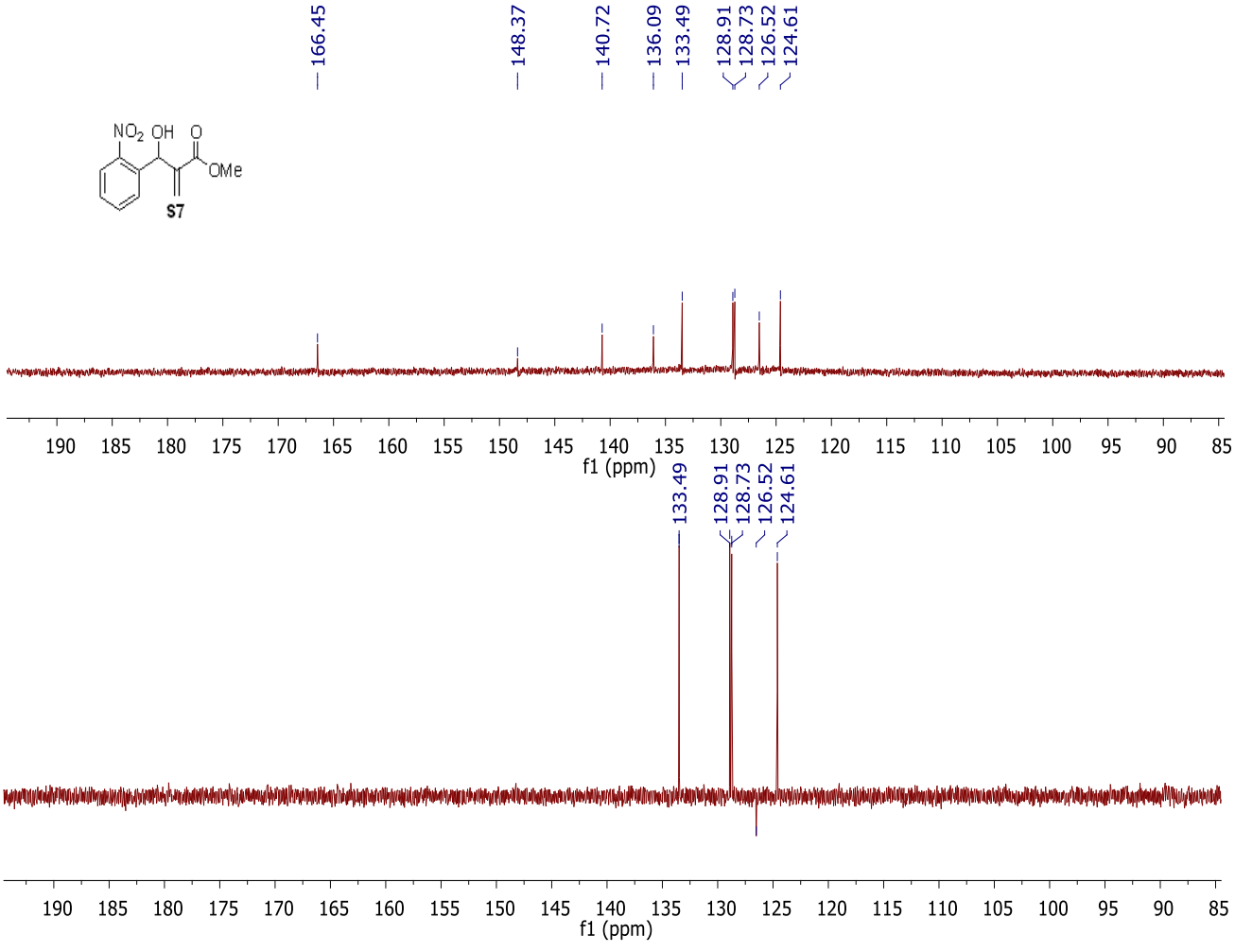
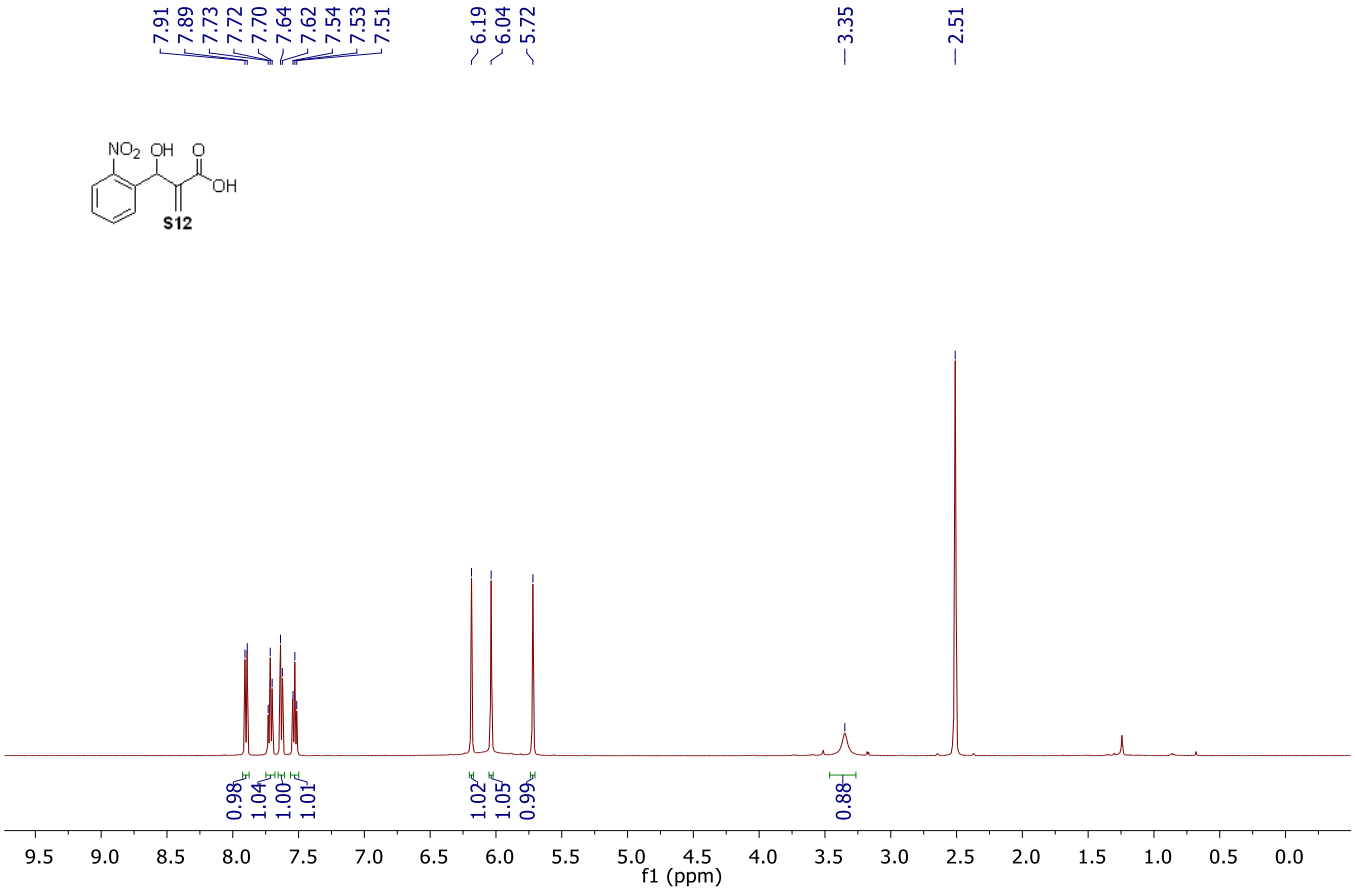
129.88
129.09
129.02
128.65
128.19
127.97
126.75
125.13
123.97
117.31
112.00
110.31
100.87
73.02
55.61
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

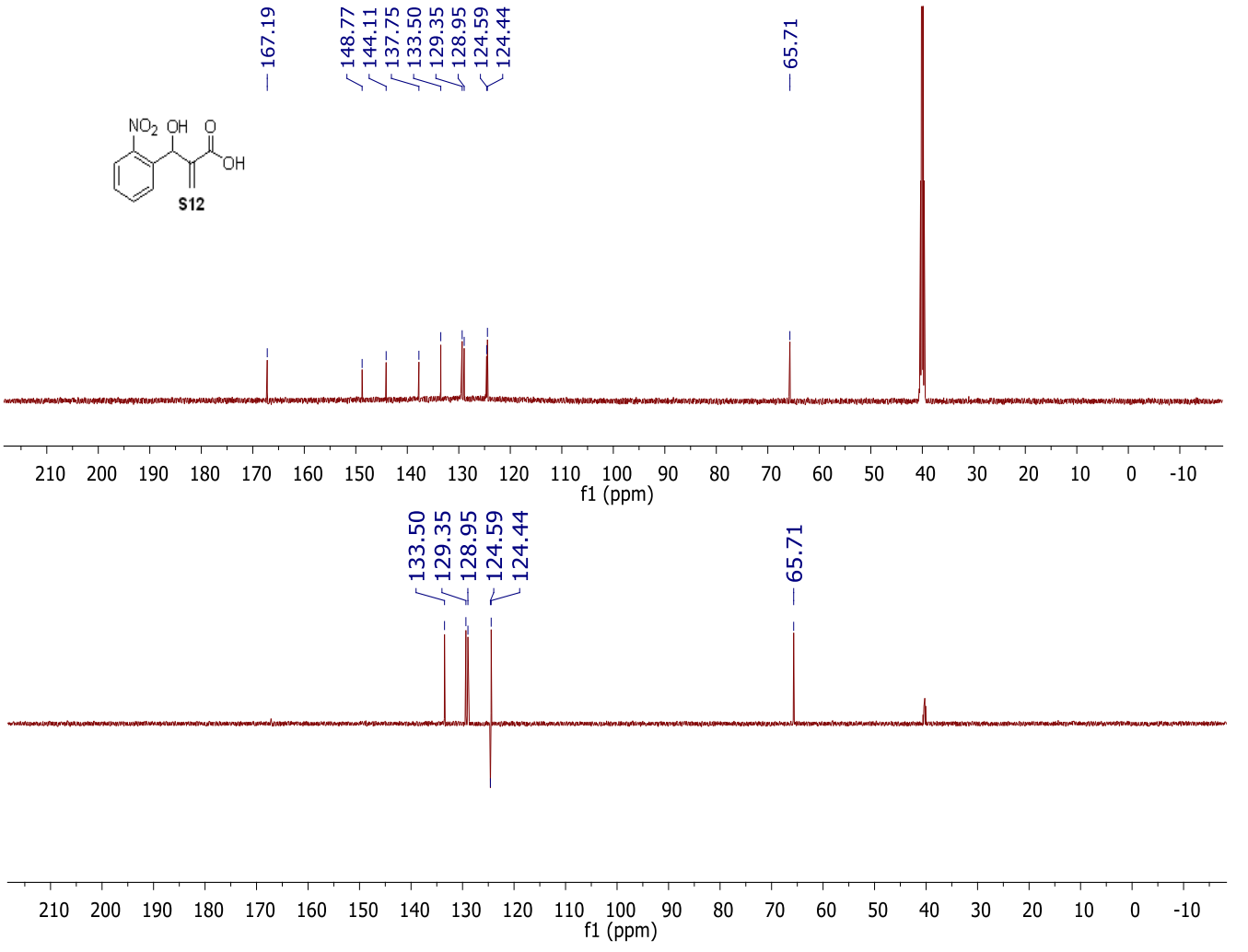

8.04 8.03 7.68 7.68 7.67 7.65 7.64 7.45 7.44 7.40 7.40 7.39 7.39 7.38 7.38 7.37 7.37 7.36 7.36 7.17 7.16 7.02 7.02 7.00 7.00 6.80 6.80 6.79 6.78 6.78 6.72 6.71 6.68 6.68 6.66 6.65 6.65 6.64 6.10 6.09 3.86 3.86 2.17 2.16
3
1.05 1.21 1.22 0.92 1.11 2.01 0.95 1.10 1.16 2.85 5.05 0.98 3.00 3.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


182.60 169.43 161.47 152.22 151.56 139.06 137.46 135.12 129.86 129.10 129.02 128.65 128.63 127.76 127.74 125.12 123.99 112.00 110.85 100.84 73.09 55.61 21.13
3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

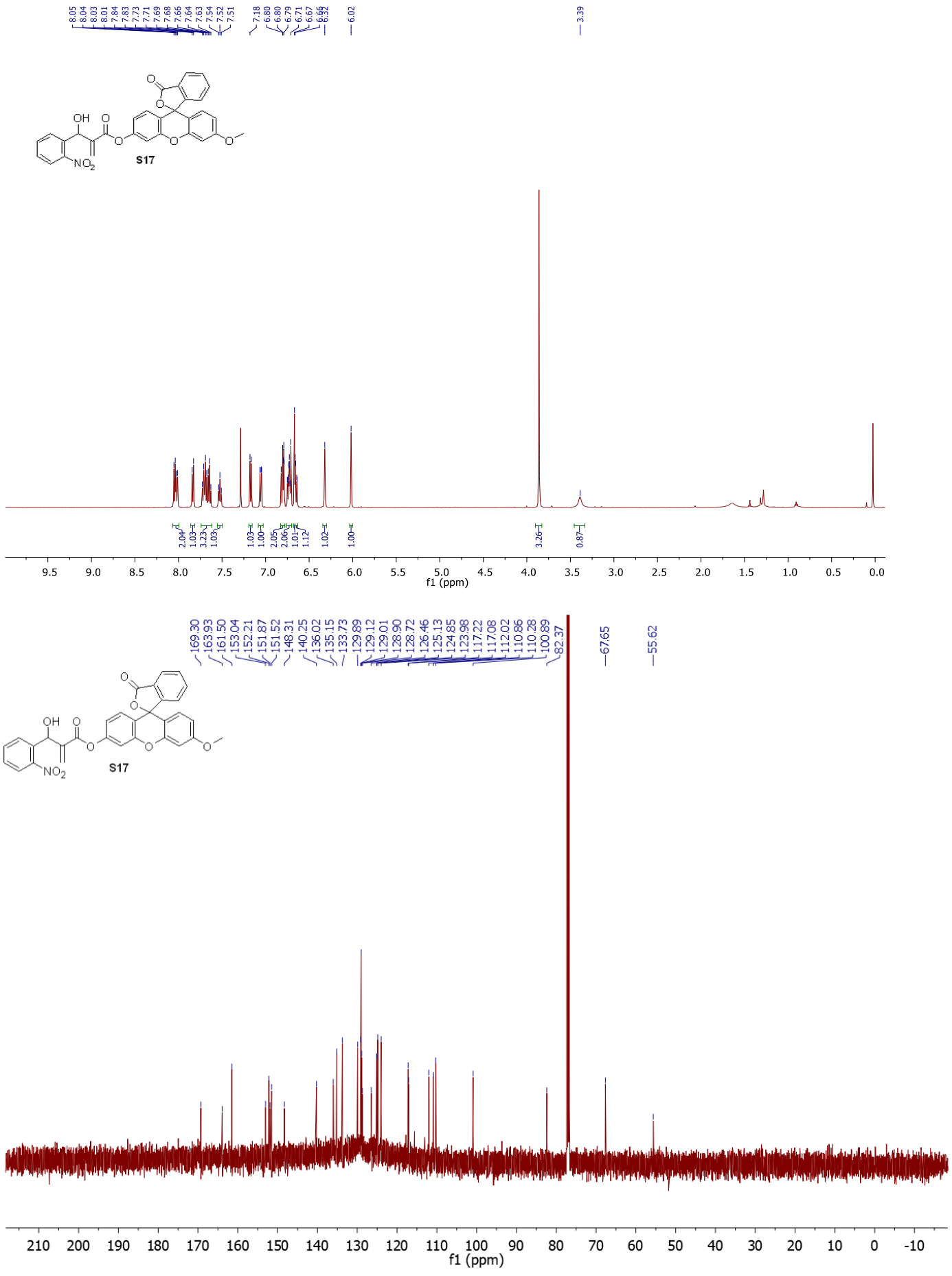

7.98
7.98
7.97
7.96
7.79
7.78
7.77
7.69
7.69
7.67
7.67
7.66
7.66
7.51
7.49
6.39
6.23
6.22
5.75
3.76
3.47
3.46
NO2 OH O
OMe
S7
1.02
1.03
1.07
1.06
1.00
1.03
1.05
3.17
1.03
1.16
f1 (ppm)


166.45
148.37
140.72
136.09
133.49
128.91
128.73
126.52
124.61
NO2 OH O
OMe
S7
f1 (ppm)
133.49
128.91
128.73
126.52
124.61
f1 (ppm)


7.91
7.89
7.73
7.72
7.70
7.64
7.62
7.54
7.53
7.51
6.19
6.04
5.72
3.35
2.51
NO2 OH O
OH
S12
0.98
1.04
1.00
1.01
1.02
1.05
0.99
0.88
f1 (ppm)


167.19
148.77
144.11
137.75
133.50
129.35
128.95
124.59
124.44
65.71
NO2 OH O
OH
S12
f1 (ppm)

133.50
129.35
128.95
124.59
124.44
65.71
f1 (ppm)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8.03
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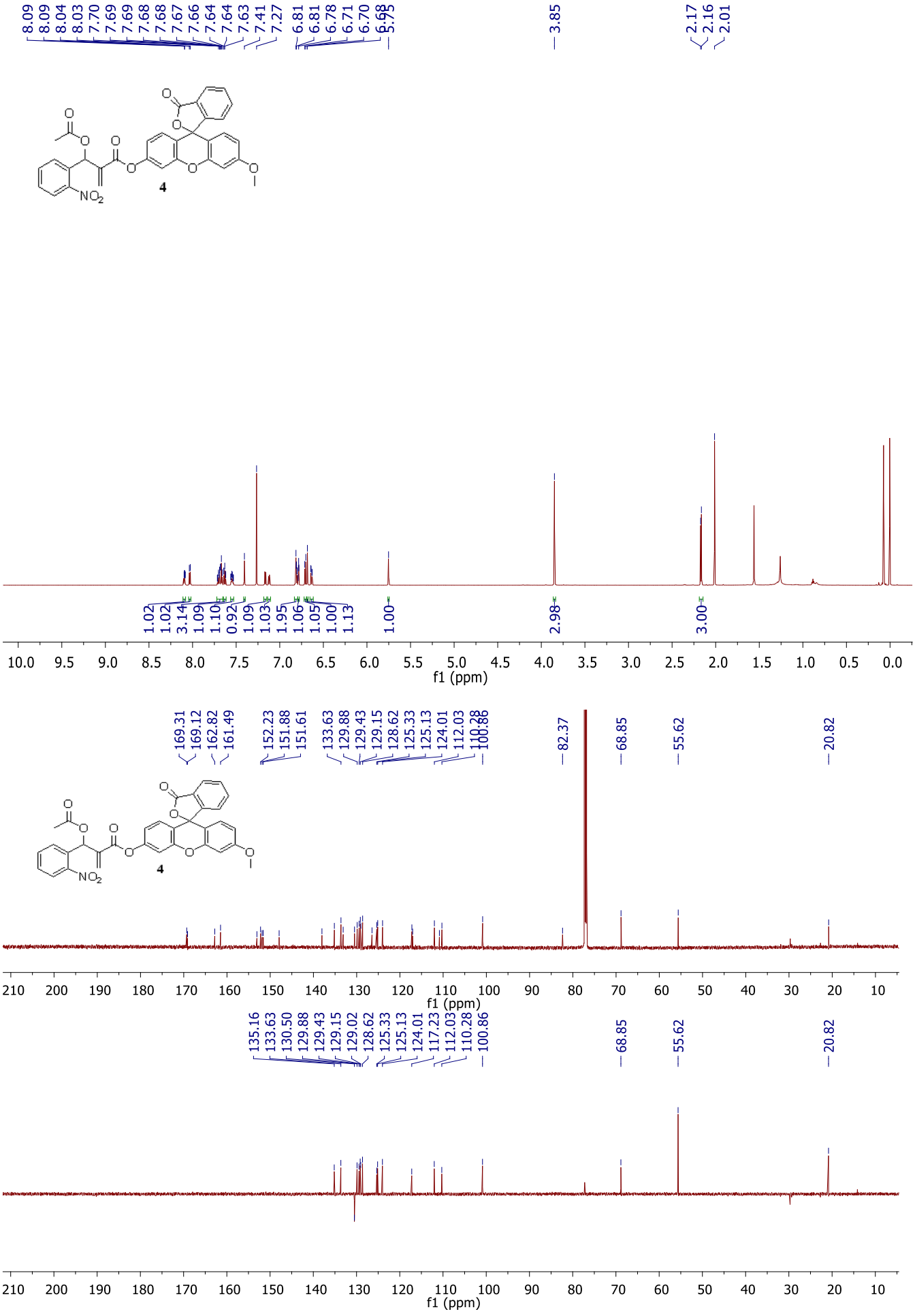
7.83
7.73
7.71
7.69
7.68
7.66
7.64
7.63
7.54
7.52
7.51
7.18
6.80
6.80
6.79
6.71
6.67
6.66
6.32
6.02
3.39
OH
O
O
O
O
O
NO2
S17
2.04
1.03
3.23
1.03
1.03
1.00
2.05
2.06
1.01
1.12
1.02
1.00
3.26
0.87
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
169.30
163.93
161.50
153.04
152.21
151.87
151.52
148.31
140.25
136.02
135.15
133.73
129.89
129.12
129.01
128.90
128.72
126.46
125.13
124.85
123.98
117.22
117.08
112.02
110.86
110.28
100.89
82.37
67.65
55.62
OH
O
O
O
O
O
NO2
S17
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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8.09
8.04
8.03
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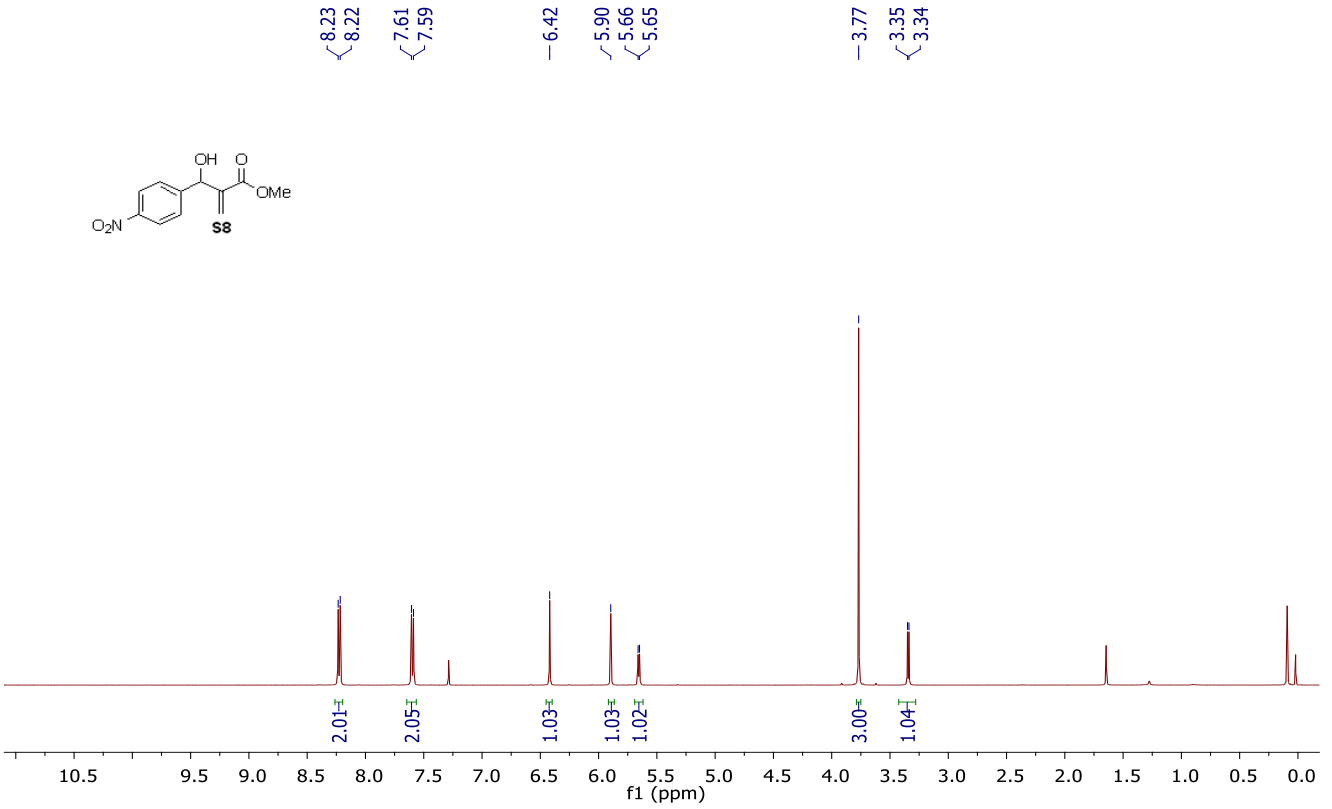
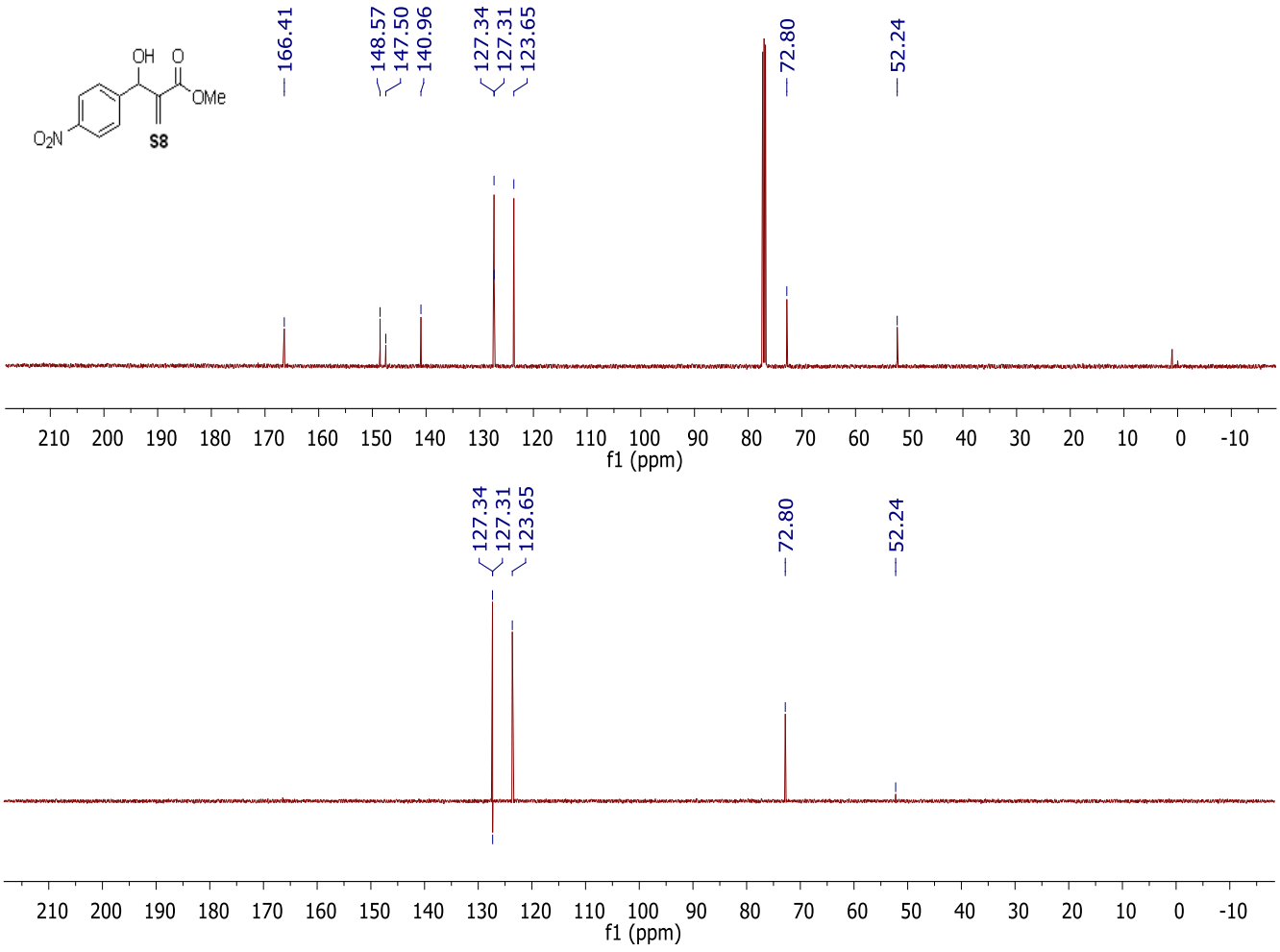
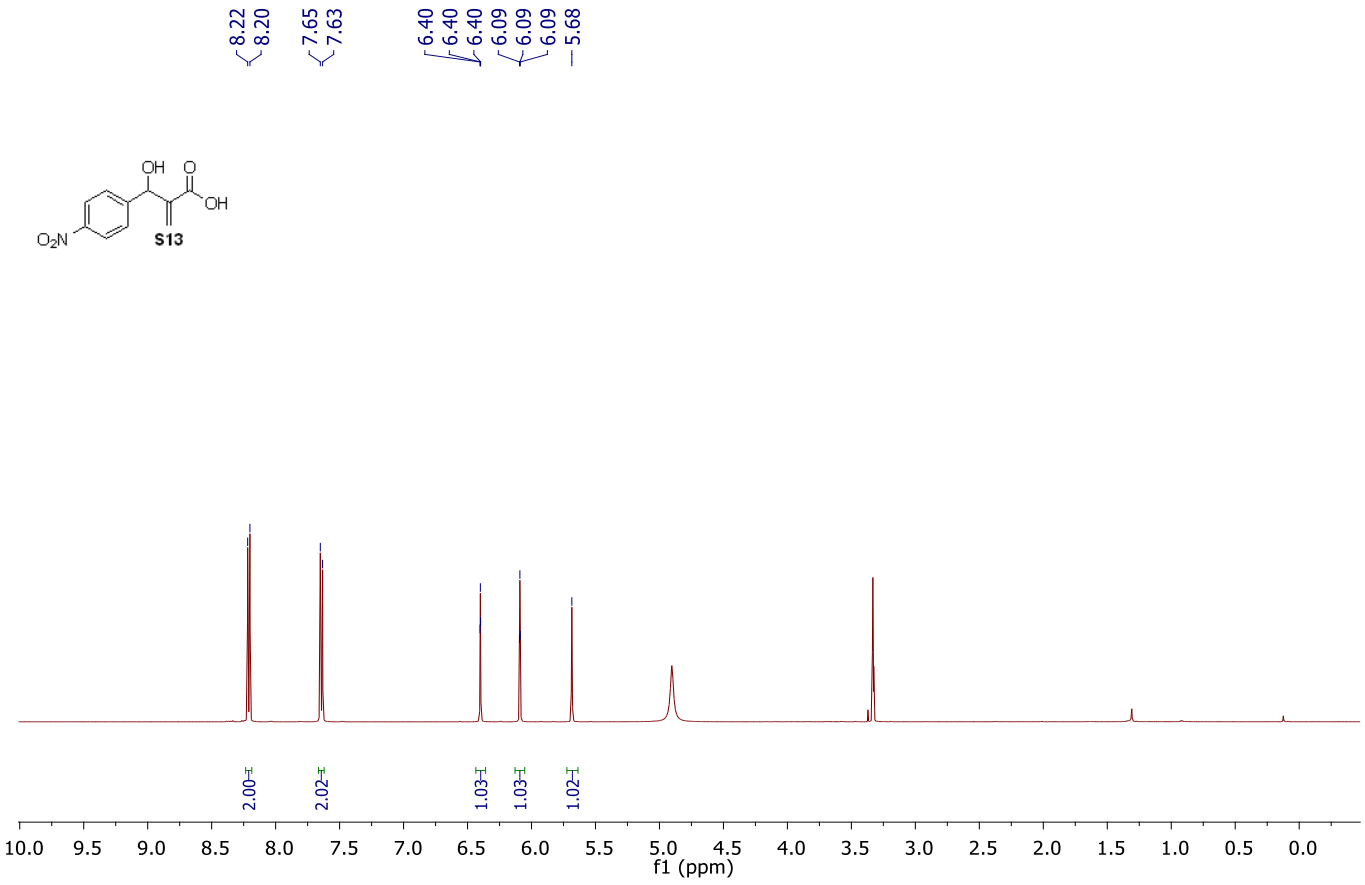
7.69
7.69
7.68
7.68
7.67
7.66
7.64
7.64
7.63
7.41
7.27
6.81
6.81
6.78
6.71
6.70
6.68
5.75
3.85
2.17
2.16
2.01
4
1.02
1.02
3.14
1.09
1.10
0.92
1.09
1.03
1.95
1.06
1.05
1.00
1.13
1.00
2.98
3.00
f1 (ppm)
169.31
169.12
162.82
161.49
152.23
151.88
151.61
133.63
129.88
129.43
129.15
128.62
125.33
125.13
124.01
112.03
110.28
100.86
82.37
68.85
55.62
20.82
4
f1 (ppm)
135.16
133.63
130.50
129.88
129.43
129.15
129.02
128.62
125.33
125.13
124.01
117.23
112.03
110.28
100.86
68.85
55.62
20.82
f1 (ppm)


8.23
8.22
7.61
7.59
6.42
5.90
5.66
5.65
3.77
3.35
3.34
OH
O
OMe
O2N
S8
2.01
2.05
1.03
1.03
1.02
3.00
1.04
f1 (ppm)


166.41
148.57
147.50
140.96
127.34
127.31
123.65
72.80
52.24
OH
O
OMe
O2N
S8
f1 (ppm)

127.34
127.31
123.65
72.80
52.24
f1 (ppm)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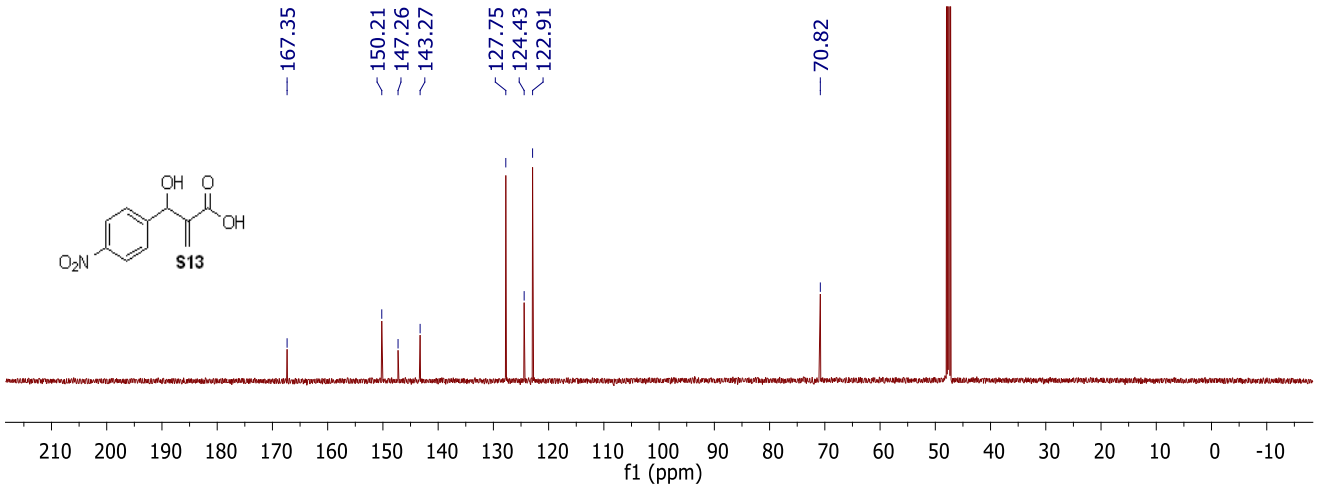
8.20
7.65
7.63
6.40
6.40
6.40
6.09
6.09
6.09
5.68
OH
O
OH
O2N
S13
2.00
2.02
1.03
1.03
1.02
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


167.35
150.21
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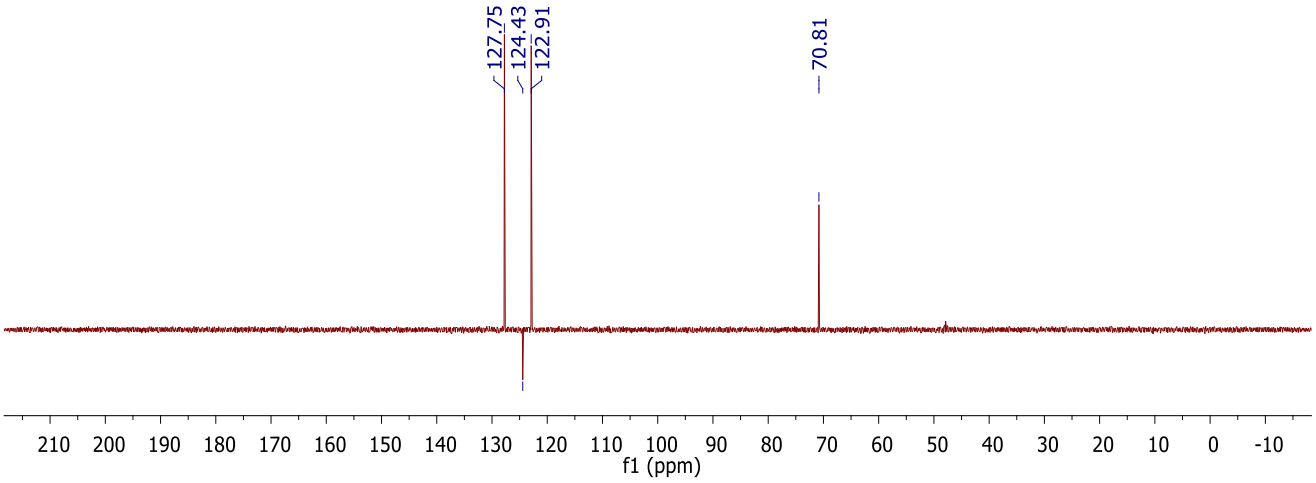
143.27
127.75
124.43
122.91
70.82
OH
O
OH
O2N
S13
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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122.91
70.81
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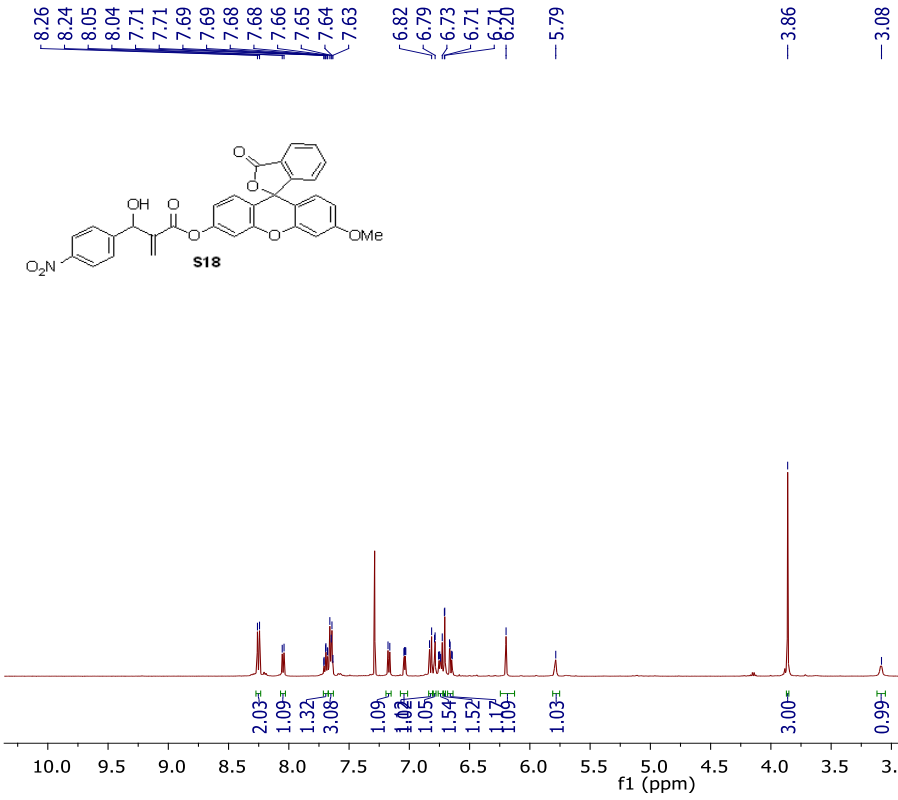
f1 (ppm)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8.04
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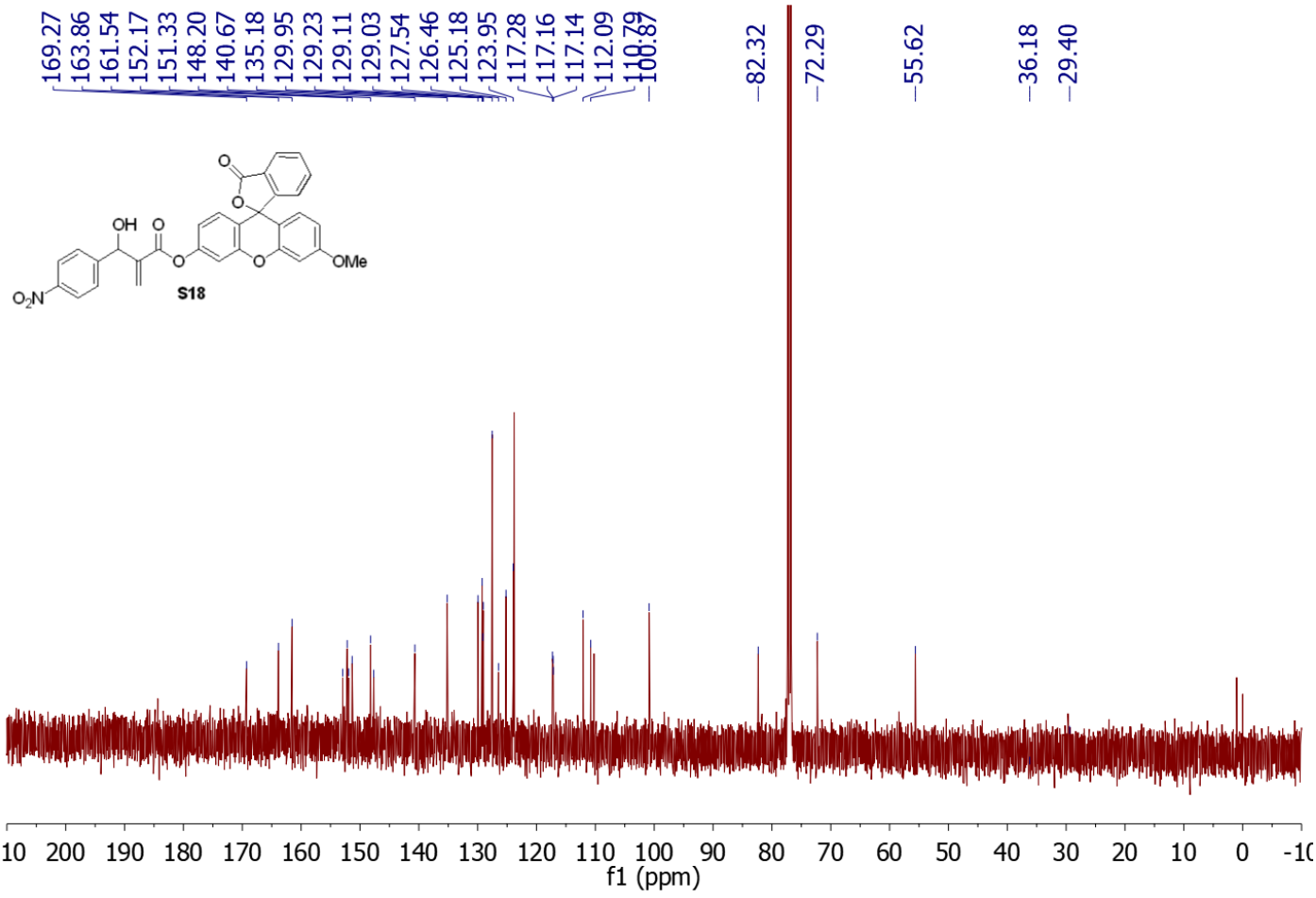
7.71
7.69
7.69
7.68
7.68
7.66
7.65
7.64
7.63
6.82
6.79
6.73
6.71
6.20
5.79
3.86
3.08
OH
O
O
O
O
OMe
O2N
S18
2.03
1.09
1.32
3.08
1.09
1.05
1.54
1.52
1.17
1.09
1.03
3.00
0.99
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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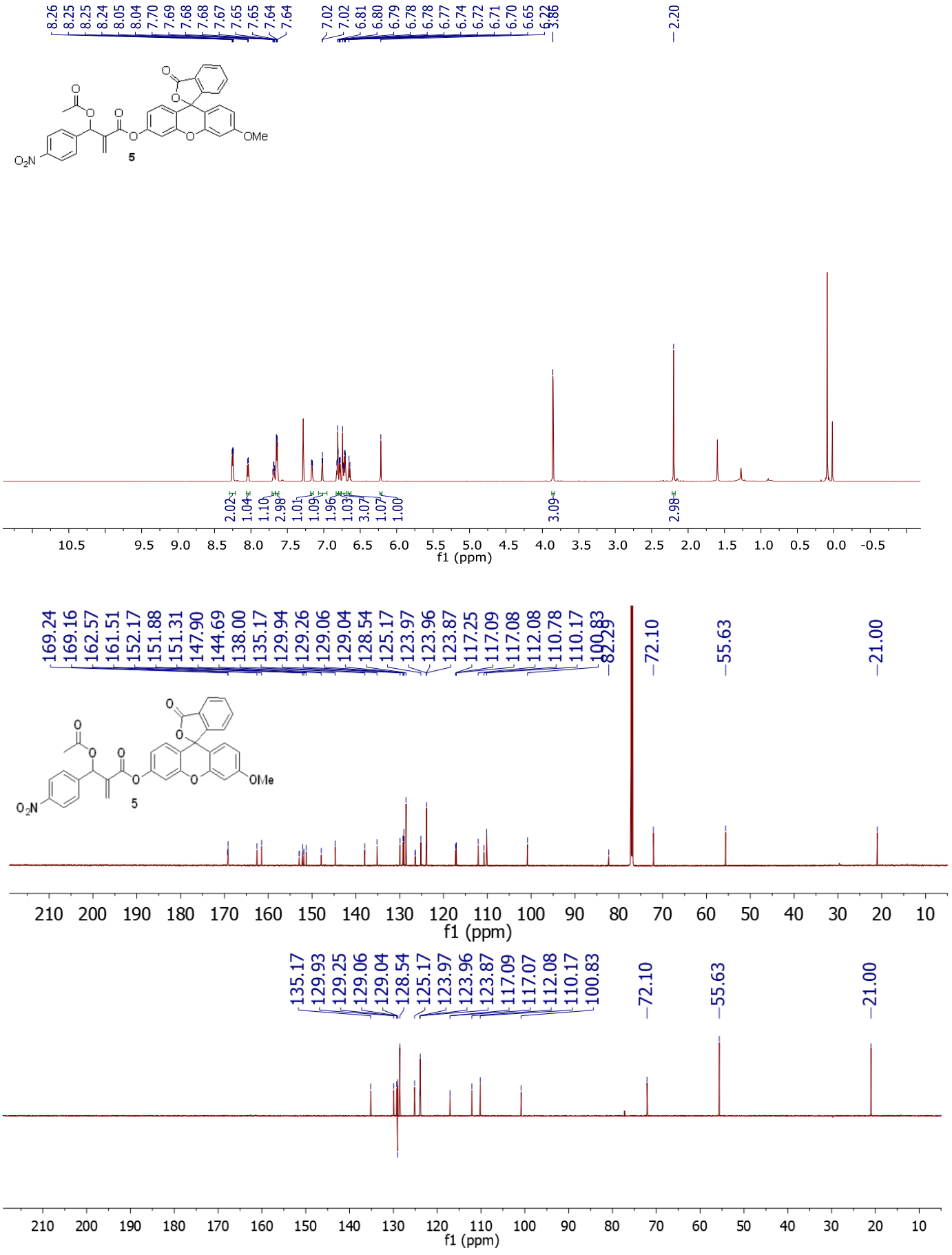
129.23
129.11
129.03
127.54
126.46
125.18
123.95
117.28
117.16
117.14
112.09
82.32
72.29
55.62
36.18
29.40
OH
O
O
O
O
OMe
O2N
S18
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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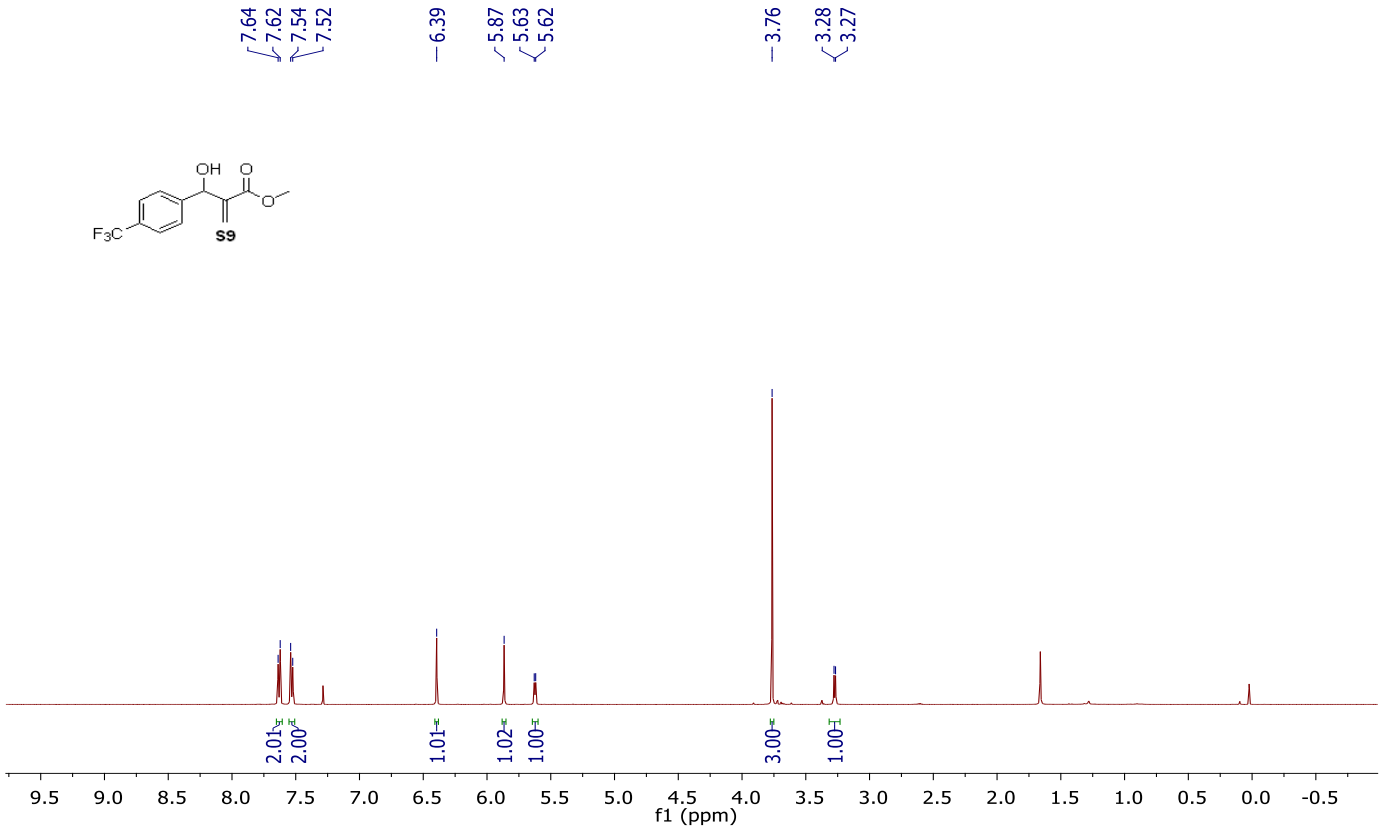
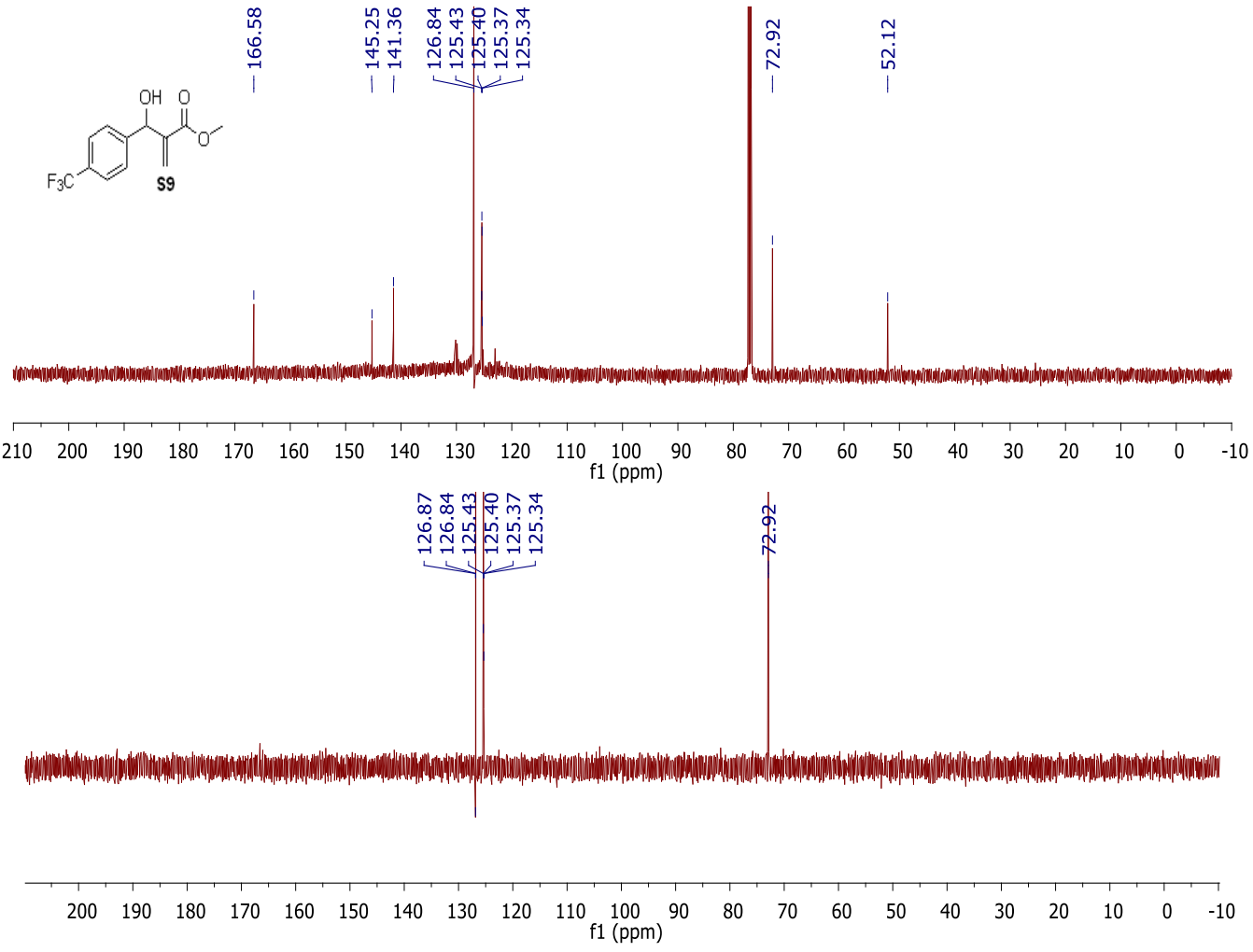
8.25
8.25
8.24
8.05
8.04
7.70
7.69
7.68
7.68
7.67
7.65
7.65
7.64
7.64
7.02
7.02
6.81
6.80
6.79
6.78
6.78
6.77
6.74
6.72
6.71
6.70
6.65
6.22
3.86
2.20
OMe
O_2N
5
2.02
1.04
1.10
2.98
1.01
1.09
1.96
1.03
3.07
1.07
1.00
3.09
2.98
f1 (ppm)
169.24
169.16
162.57
161.51
152.17
151.88
151.31
147.90
144.69
138.00
135.17
129.94
129.26
129.06
129.04
128.54
125.17
123.97
123.96
123.87
117.25
117.09
117.08
112.08
110.78
110.17
100.83
82.29
72.10
55.63
21.00
f1 (ppm)
135.17
129.93
129.25
129.06
129.04
128.54
125.17
123.97
123.96
123.87
117.09
117.07
112.08
110.17
100.83
72.10
55.63
21.00
f1 (ppm)


7.64
7.62
7.54
7.52
6.39
5.87
5.63
5.62
3.76
3.28
3.27
OH
O
O
F3C
S9
2.01
2.00
1.01
1.02
1.00
3.00
1.00
f1 (ppm)


166.58
145.25
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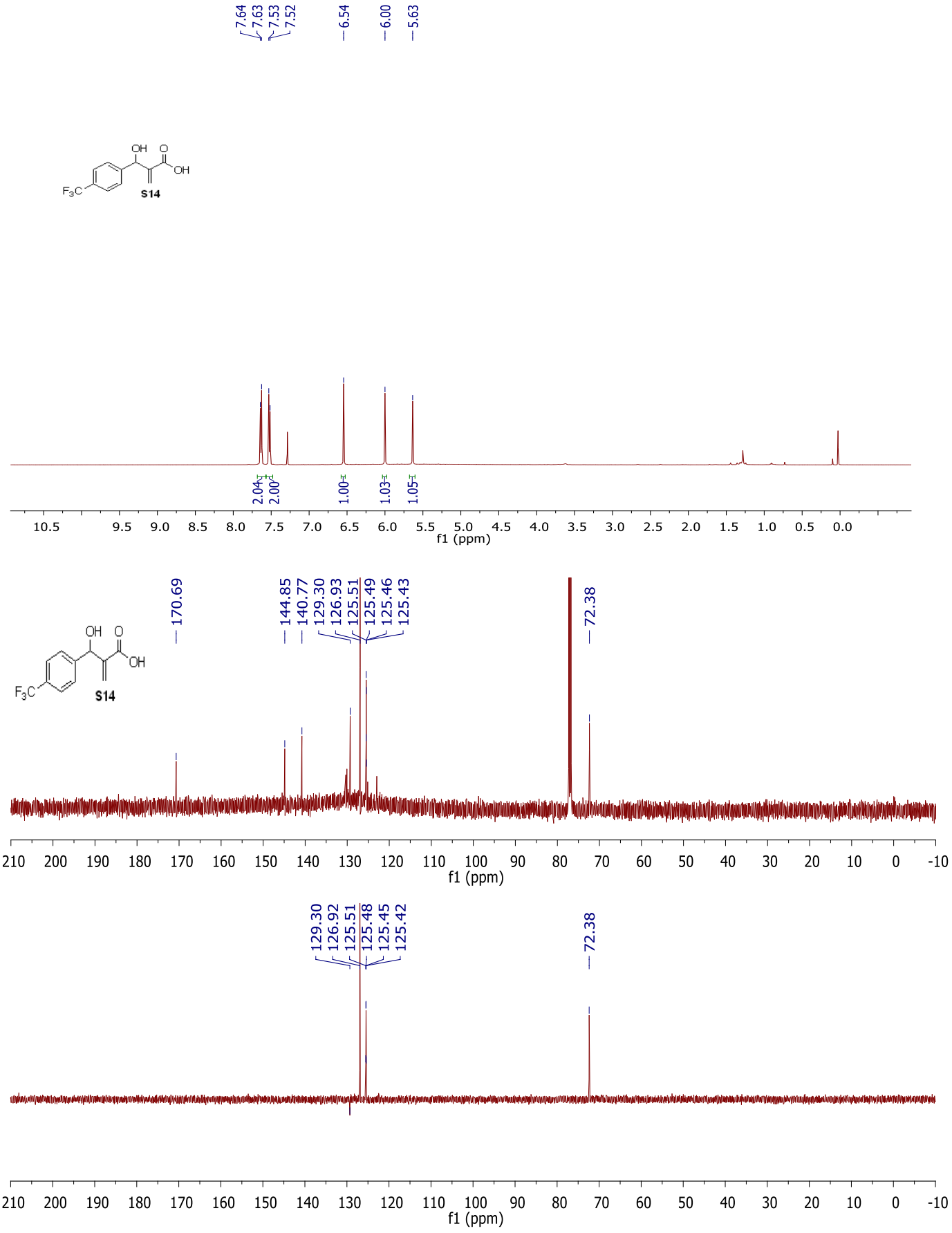
126.84
125.43
125.40
125.37
125.34
72.92
52.12
OH
O
O
F3C
S9
f1 (ppm)

126.87
126.84
125.43
125.40
125.37
125.34
72.92
f1 (ppm)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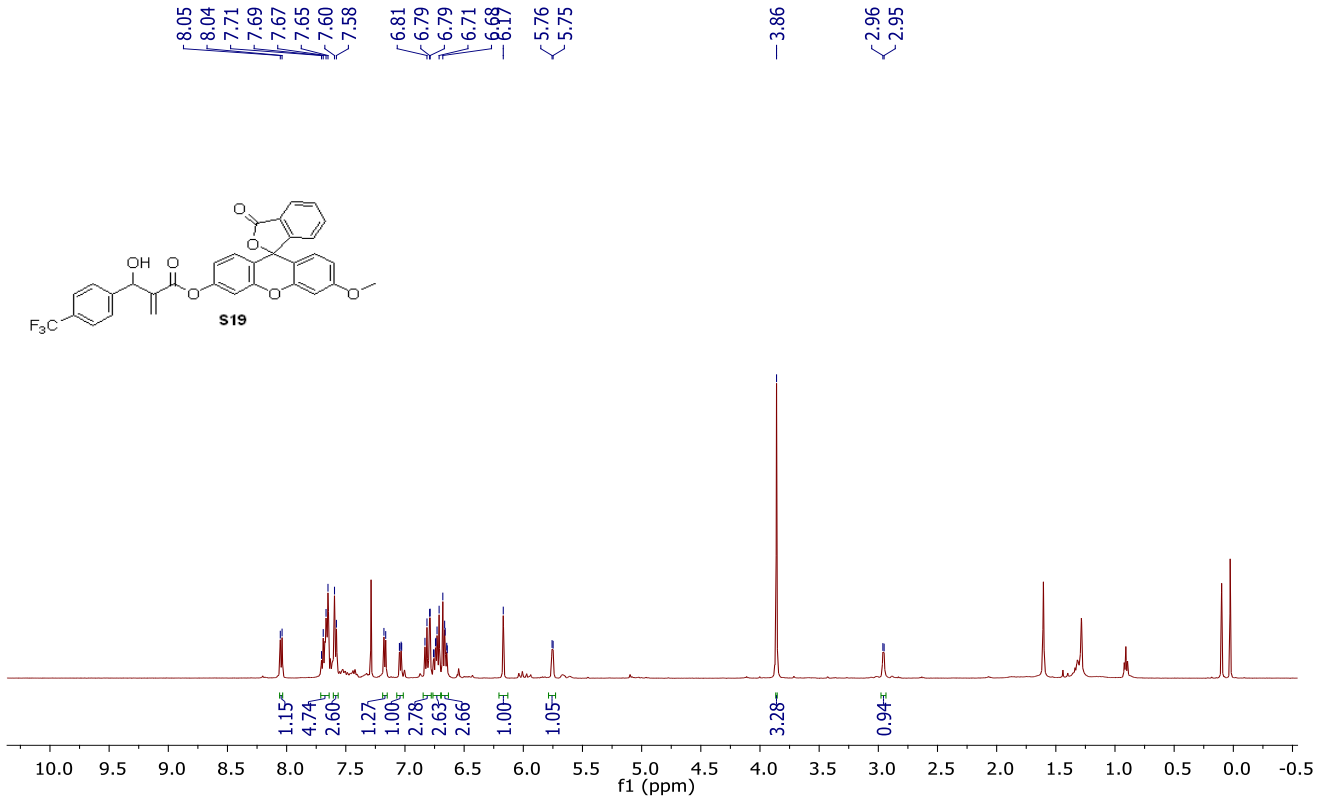
7.63
7.53
7.52
6.54
6.00
5.63
OH
O
OH
F3C
S14
2.04
2.00
1.00
1.03
1.05
f1 (ppm)
170.69
144.85
140.77
129.30
126.93
125.51
125.49
125.46
125.43
72.38
f1 (ppm)
129.30
126.92
125.51
125.48
125.45
125.42
72.38
f1 (ppm)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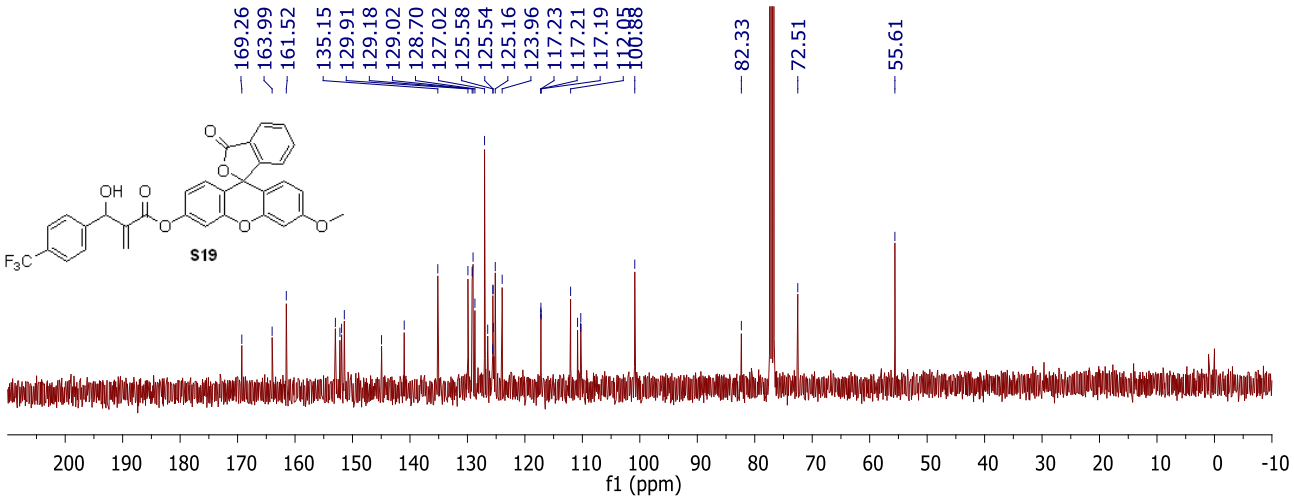
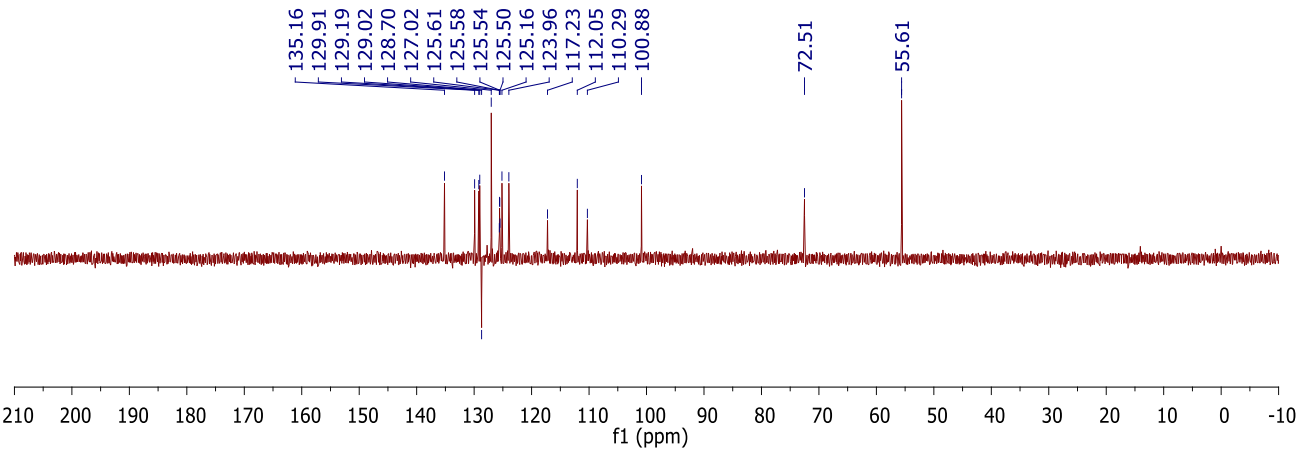
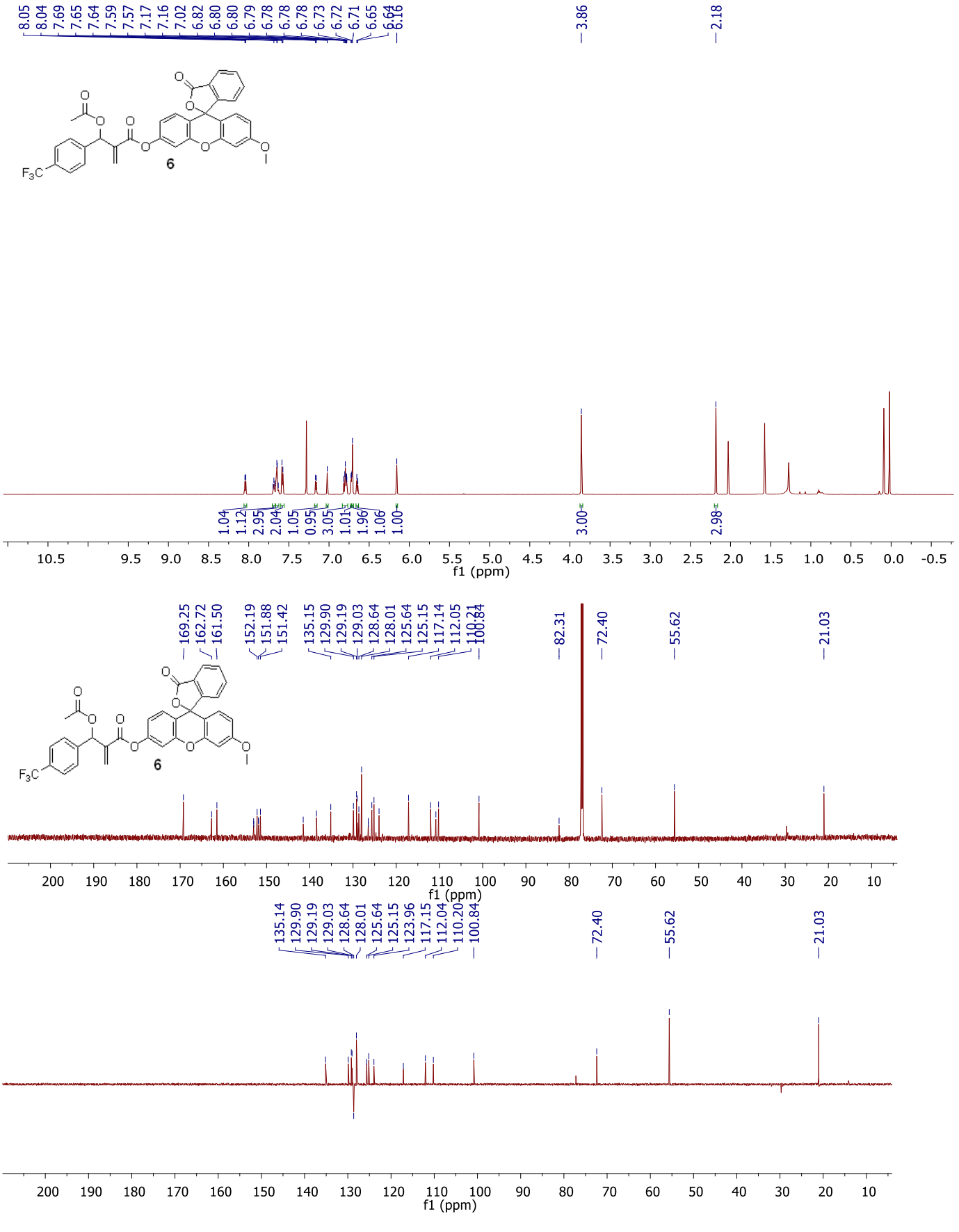
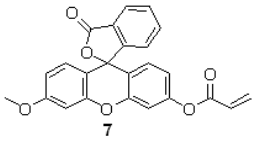
8.04
7.71
7.69
7.67
7.65
7.60
7.58
6.81
6.79
6.79
6.71
6.68
6.67
5.76
5.75
3.86
2.96
2.95
OH
O
O
O
O
O
F3C
S19
1.15
4.74
2.60
1.27
1.00
2.78
2.63
2.66
1.00
1.05
3.28
0.94
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

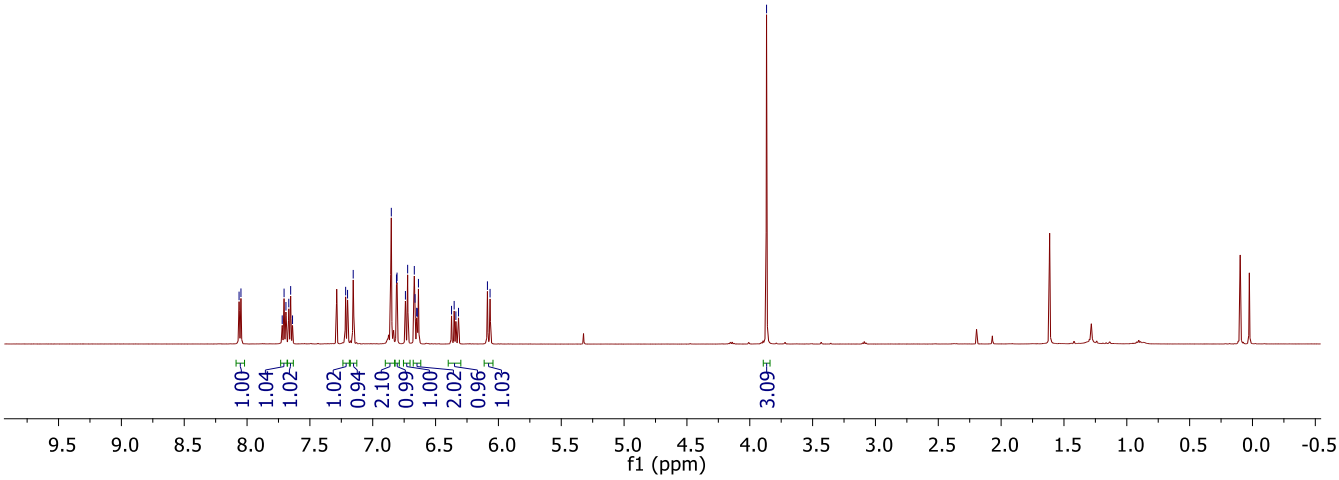

169.26
163.99
161.52
135.15
129.91
129.18
129.02
128.70
127.02
125.58
125.54
125.16
123.96
117.23
117.21
117.19
112.05
110.88
82.33
72.51
55.61
OH
O
O
O
O
O
F3C
S19
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

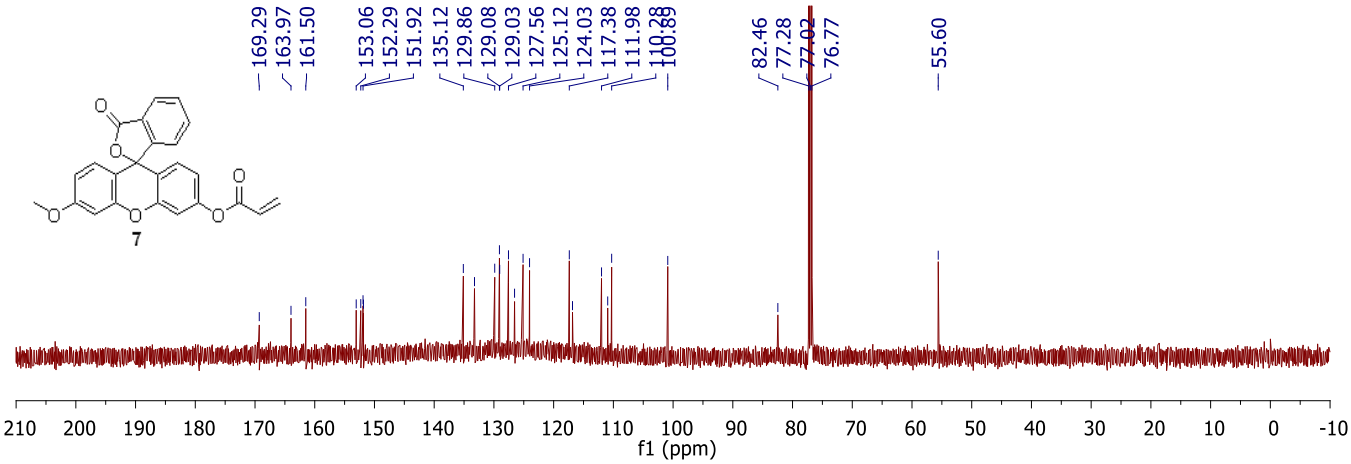

135.16
129.91
129.19
129.02
128.70
127.02
125.61
125.58
125.54
125.50
125.16
123.96
117.23
112.05
110.29
100.88
72.51
55.61
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


8.05
8.04
7.69
7.65
7.64
7.59
7.57
7.17
7.16
7.02
6.82
6.80
6.80
6.79
6.78
6.78
6.78
6.73
6.72
6.71
6.65
6.64
6.16
3.86
2.18
6
1.04
1.12
2.95
2.04
1.05
0.95
3.05
1.01
1.96
1.06
1.00
3.00
2.98
f1 (ppm)
169.25
162.72
161.50
152.19
151.88
151.42
135.15
129.90
129.19
129.03
128.64
128.01
125.64
125.15
117.14
112.05
110.21
100.84
82.31
72.40
55.62
21.03
6
f1 (ppm)
135.14
129.90
129.19
129.03
128.64
128.01
125.64
125.15
123.96
117.15
112.04
110.20
100.84
72.40
55.62
21.03
f1 (ppm)

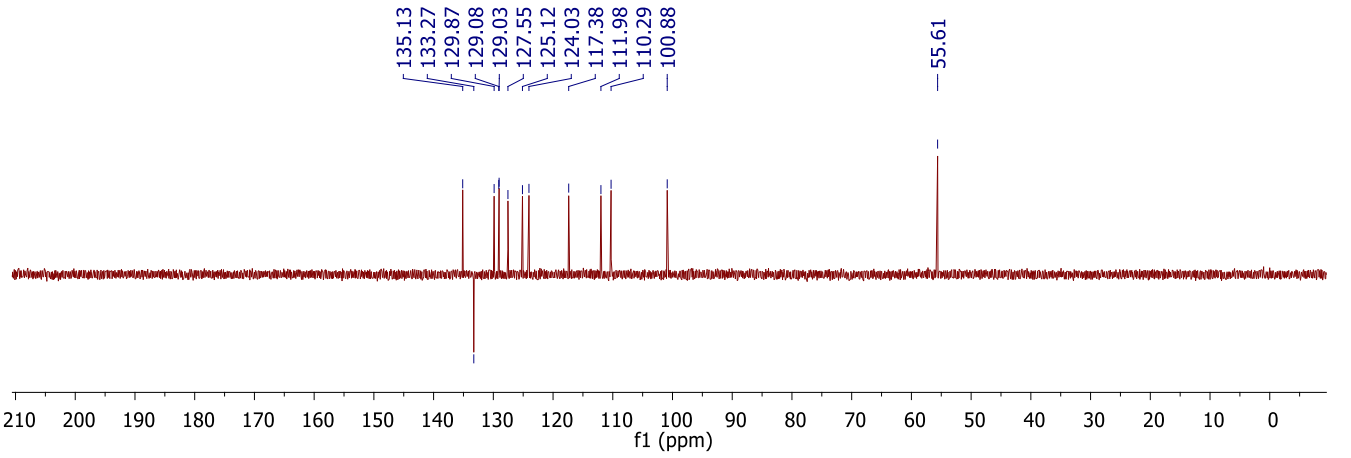

8.06
8.05
7.72
7.71
7.69
7.67
7.65
7.64
7.22
7.20
7.16
6.85
6.81
6.81
6.74
6.72
6.67
6.66
6.65
6.65
6.64
6.37
6.35
6.32
6.09
3.87

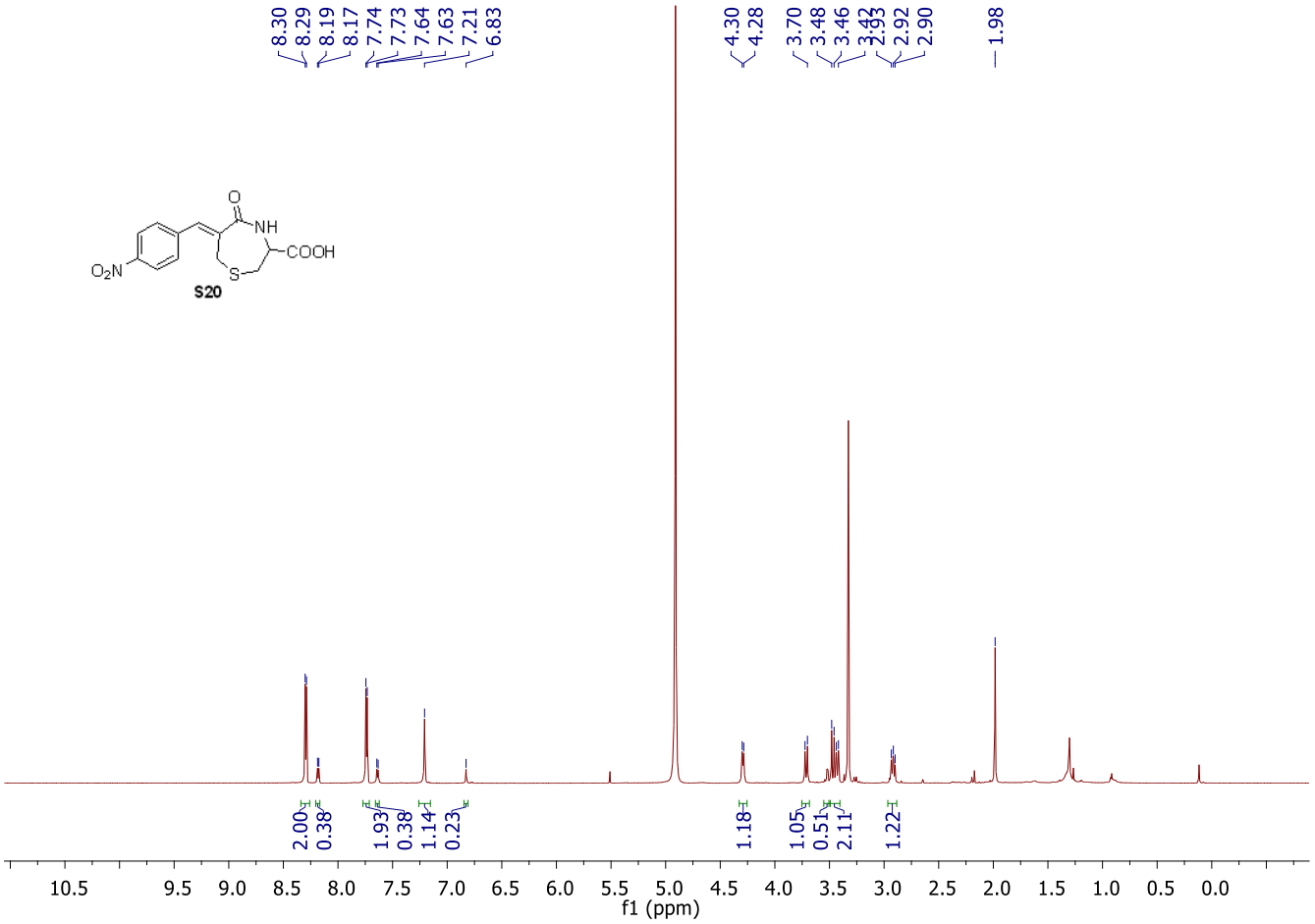

7

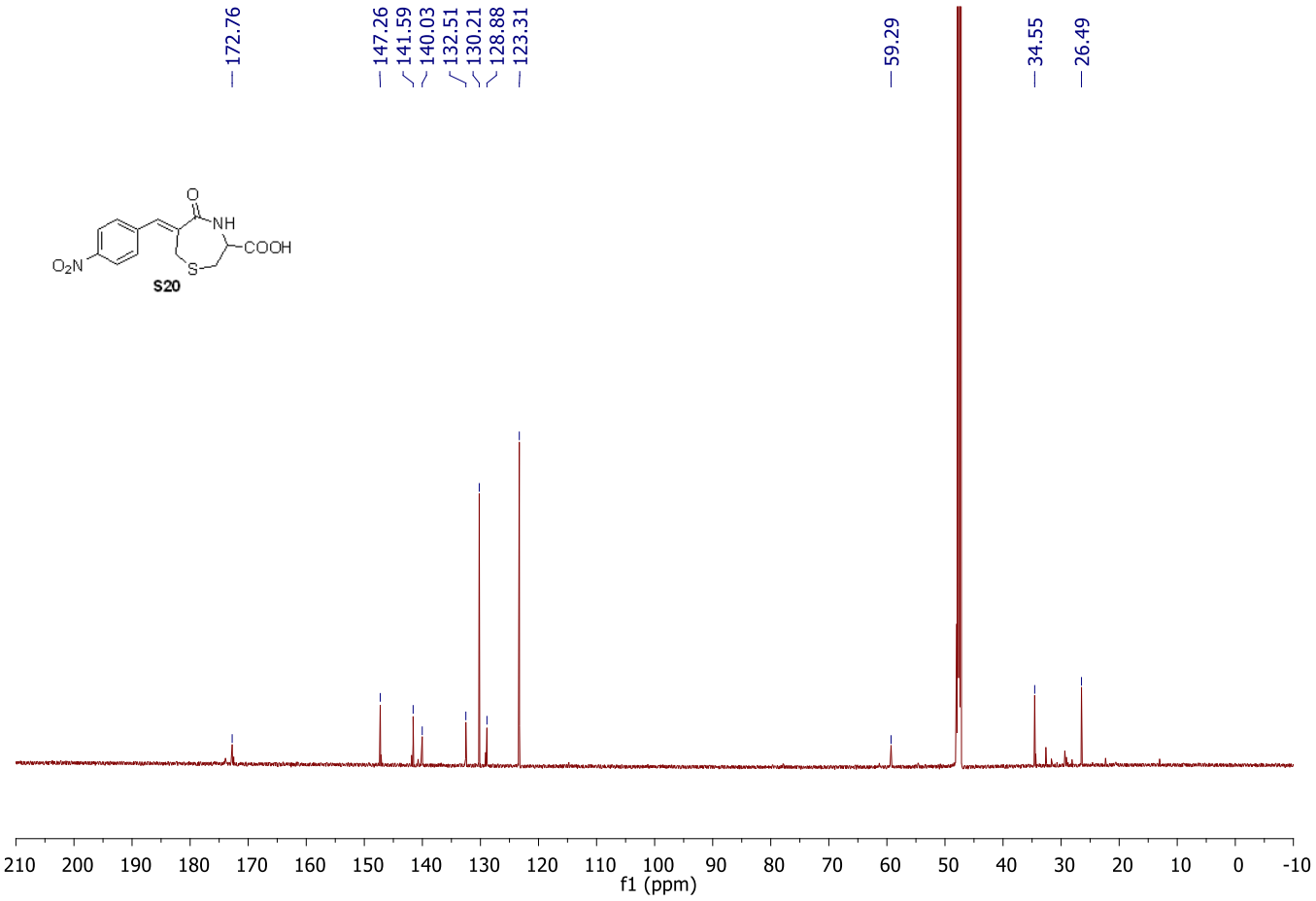

1.00
1.04
1.02
1.02
0.94
2.10
0.99
1.00
2.02
0.96
1.03
3.09
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


169.29
163.97
161.50
153.06
152.29
151.92
135.12
129.86
129.08
129.03
127.56
125.12
124.03
117.38
111.98
110.28
100.88
82.46
77.28
77.02
76.77
55.60
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

7


135.13
133.27
129.87
129.08
129.03
127.55
125.12
124.03
117.38
111.98
110.29
100.88
55.61
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


8.30
8.29
8.19
8.17
7.74
7.73
7.64
7.63
7.21
6.83
4.30
4.28
3.70
3.48
3.46
2.93
2.92
2.90
1.98
O
NH
COOH
O2N
S
S20
2.00
0.38
1.93
0.38
1.14
0.23
1.18
1.05
0.51
2.11
1.22
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


172.76
147.26
141.59
140.03
132.51
130.21
128.88
123.31
59.29
34.55
26.49
O
NH
COOH
O2N
S
S20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Section F: References

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