Supporting Information

Sulfonate-Containing Thiiranes as Selective Gelatinase Inhibitors

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Content:

General information

All organic reagents were purchased from either Sigma-Aldrich Chemical Company (St Louis, MO, USA) or Acros Organics (Geel, Belgium), unless otherwise stated. All reactions were performed under an atmosphere of nitrogen, unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Varian UnityPlus 300, or a Varian INOVA-500 spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are reported in ppm using tetramethylsilane as an internal standard on the δ scale. Reactions were monitored by TLC on 0.20 mm E. Merck Silica Gel plates ($60F_{254}$) with visualization of product bands by UV fluorescence (λ = 254 nm) and/or aqueous cerium sulfate staining, followed by heating. Flash chromatography was carried out with silica gel 60, 230–400 mesh (0.040–0.063 mm particle size) purchased from EM Science (Gibbstown, NJ, USA). High-resolution mass spectra were obtained at the Department of Chemistry and Biochemistry, University of Notre Dame by FAB ionization, using a JEOL AX505HA mass spectrometer or by ESI ionization, using a BRUKER microTOF II mass spectrometer. MMP-2 was purchased from Calbiochem (EMD Chemicals). MMP-9 (native, human, recombinant, Catalytic Domain) was acquired from Enzo Life Sciences Inc. Human recombinant active MMP-7 and catalytic domains of MMP-3 and MMP-14 were purchased from EMD Biosciences (La Jolla, CA, USA). Catalytic domain of human recombinant MMP-1 was from Biomol International (Plymouth Meeting, PA, USA). Fluorogenic substrates were purchased from Peptides International (Louisville, KY, USA), (MOCAcPLGL(Dpa)AR-NH_2, MOCAcRPKPVE(L-norvalyl)WRK(2,4-

dinitrophenyl)-NH_2), and (MOCAcKPLGL(Dpa)AR-NH_2) from R&D Systems (Minneapolis, MN, USA). Substrate hydrolysis was measured with a Varian Cary Eclipse fluorescence spectrophotometer. The methodology for enzyme inhibition studies were as reported previously.[1]

Syntheses of sulfonyl derivates of 2: General procedure: A solution of the thiirane **4**[2] (100 mg, 0.30 mmol) in dichloromethane (41 mL) was mixed with Et_3N (84 μL, 0.60 mmol) at ice-water temperature, followed by the addition of sulfonyl chloride (0.33 mmol). After 1 hr of stirring at ice-water temperature, the solution was mixed with 10 mL of water and the layers were separated. The organic layer was dried over Na_2SO_4 and the solution was concentrated *in vacuo* to afford the crude product, which was subsequently purified by column chromatography on silica gel. The purity of the final compounds was determined by analytical HPLC on a Perkin-Elmer series 200 instrument with a YMC basic column (5 μm, 4.6 mm i.d. × 15 cm, YMC Inc., Wilmington, NC, USA) connected to a YMC basic guard cartridge (5 μm, 4.0 mm i.d. × 2 cm) using 50% acetonitrile/50% water (or 60% acetonitrile/40% water) over 20 min at 1 mL/min. Purity of all final samples (**2a**-**2d** and **3a**-**3d**) was >95%.

4-(4-(Thiiranylmethylsulfonyl)phenoxy)-phenylethanesulfonate (2b). yield 87%.^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59 (t, J = 7.5 Hz, 3H), 2.18 (dd, J = 5.2, 1.6 Hz, 1H), 2.56 (dd, J = 6.0, 1.2 Hz, 1H), 3.06-3.11 (m, 1H), 3.22 (dd, J = 14.4, 7.8 Hz, 1H), 3.35 (q, J = 7.4 Hz, 2H), 3.52 (dd, J = 14.4, 5.8 Hz, 1H), 7.13 (d, J = 8.8 Hz, 4H), 7.35 (d, J = 9.0 Hz,

2H), 7.91 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 8.5, 24.3, 26.2, 45.4, 62.8, 118.2, 121.7, 124.2, 131.1, 132.8, 145.7, 153.8, 162.4; HRMS (FAB) calcd for $C_{17}H_{19}O_6S_3$ (M + H^+) 415.0344, found 415.0340.

4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl propane-1-sulfonate (2c). yield 85%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16 (t, J = 7.4 Hz, 3H), 2.02-2.10 (m, 2H), 2.18 (dd, J = 5.0, 1.6 Hz, 1H), 2.56 (dd, J = 5.9, 1.4 Hz, 1H), 3.06-3.11 (m, 1H), 3.22 (dd, J = 14.5, 7.6 Hz, 1H), 3.29 (t, J = 2.1 Hz, 1H), 3.29 (t, J = 7.9 Hz, 1H), 3.52 (dd, J = 14.1, 5.5 Hz, 1H), 7.13 (d, J = 8.6 Hz, 4H), 7.34 (d, J = 9.0 Hz, 2H), 7.91 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.9, 17.4, 24.2, 26.2, 52.2, 62.5, 118.1, 121.6, 124.1, 130.9, 132.6, 145.6, 153.6, 162.3; HRMS (FAB) calcd for $C_{18}H_{21}O_6S_3$ (M + H^+) 429.0500, found 429.0501.

4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl propane-2-sulfonate (2d). yield 82%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (d, J = 6.9 Hz, 6H), 2.18 (dd, J = 5.0, 1.6 Hz, 1H), 2.56 (dd, J = 5.9, 1.0 Hz, 1H), 3.06-3.10 (m, 1H), 3.22 (dd, J = 14.1, 7.6 Hz, 1H), 3.52 (dd, J = 13.8, 6.2 Hz, 1H), 3.52-3.57 (m, 1H), 7.12 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.9, 24.3, 26.2, 52.9, 62.8, 118.2, 121.7, 124.2, 131.0, 132.7, 145.6, 153.5, 162.5; HRMS (FAB) calcd for $C_{18}H_{21}O_6S_3$ (M + H^+) 429.0500, found 429.0498.

4-(Allylthio)phenyl acetate (7). To a stirred solution of 4-(Allylthio)phenol (**6**)[3] (3.86 g, 23.22 mmol) in CH_2Cl_2 (40 mL) were added DIPEA (6.13 ml, 34.83 mmol) and acetic anhydride (2.86 ml, 30.19 mmol) at ice-water temperature. The reaction mixture was

stirred at room temperature overnight and then quenched with H_2O. The resulting mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation of solvents, the residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 1/10 to 1/6) to give **7** as a colorless oil (3.71 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.26 (s, 3H), 3.50 (d, J = 6.8 Hz, 2H), 5.04-5.12 (m, 2H), 5.81-5.89 (m, 1H), 6.99-7.02 (m, 2H), 7.33-7.36 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9, 37.5, 117.6, 121.8, 131.1, 133.3, 149.1, 169.1; HRMS (ESI) calcd for $C_{11}H_{12}O_2S$ (M+Na^+) 231.0450, found 231.0448.

4-(Oxiran-2-ylmethylsulfonyl)phenyl acetate (8). To a stirred solution of compound **7** (10.87 g, 52.19 mmol) in CH_2Cl_2 (150 mL) was added *m*-CPBA (58.48 g, 260.95 mmol) at ice-water temperature, and the mixture was subsequently stirred at room temperature for 4 days. An additional portion of *m*-CPBA (29.24 g, 130.48 mmol) was added and stirred for another 4 days. The reaction was filtered and the filtrate was concentrated *in vacuo*. The residue was taken up in ethyl acetate and washed with a saturated $Na_2S_2O_3$ solution, followed by a saturated $NaHCO_3$ solution and water, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography to give the epoxide **8** (9.2 g, 68.8%) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.45 (dd, J = 4.8, 2.4 Hz, 1H), 2.79-2.80 (m, 1H), 3.27-3.33 (m, 3H), 7.33 (d, J = 8.8 Hz, 2H), 7.97 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9, 45.5, 45.6, 59.2, 122.5, 129.8, 136.1, 154.8, 168.3; HRMS (FAB) calcd for $C_{11}H_{12}O_5S$ (M+H^+) 257.0484, found 257.0482.

4-(Thiiran-2-ylmethylsulfonyl)phenyl acetate (**9**). To a stirred solution of epoxide **8** (7 g, 27.3 mmol) in MeOH-CH_2Cl_2 (10:1, 100 mL) was added thiourea (5.2 g, 68.2 mmol) at room temperature, and the mixture was stirred overnight. After concentration under reduced pressure, the residue partitioned between ethyl acetate and water. The EtOAc layer was separated and washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The title compound and a deacetylated portion can be seen by TLC, so the residue was subjected to the next reaction without purification. A portion of the purified title compound was characterized, as follows. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (dd, J = 5.2, 1.8 Hz, 1H), 2.35 (s, 3H), 2.54 (dd, J = 5.8, 1.5 Hz, 1H), 3.03-3.08 (m, 1H), 3.20 (dd, J = 14.3, 7.9 Hz, 1H), 3.55 (dd, J = 14.3, 5.7 Hz, 1H), 7.35 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ21.0, 24.1, 25.8, 62.4, 122.6, 130.1, 135.7, 155.0, 168.3; HRMS (FAB) calcd for $C_{11}H_{12}O_4S_2$ ($M+H^+$) 273.0255, found 273.0253.

4-(Thiiran-2-ylmethylsulfonyl)phenol (10): To a stirred solution of crude acetate thiirane **9** from the previous step (7.4 g) in MeOH-CH_2Cl_2 (10:1, 100 mL) was added imidazole (1.8 g, 27.17 mmol) at room temperature, and the mixture was stirred overnight. After concentration under reduced pressure, the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the desire product **10** (4.91 g, 78.5 %, 2 steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ2.12 (dd, J = 5.1, 1.9 Hz, 1H), 2.51 (dd, J = 6.2, 1.2 Hz, 1H),

3.01-3.06 (m, 1H), 3.22 (dd, J = 14.3, 7.7 Hz, 1H), 3.53 (dd, J = 14.2, 5.8 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1, 26.0, 62.7, 116.4, 129.3, 130.8, 161.4; HRMS (FAB) calcd for $C_9H_{10}O_3S_2$ ($M+H^+$) 231.0150, found 231.0163.

Syntheses of sulfonyl derivates of 10: General procedure: A solution of the thiirane **10** (150 mg, 0.65 mmol) in dichloromethane (10 mL) was mixed with Et_3N (0.1 mL, 0.716 mmol, 1.1 eq) at ice-water temperature, followed by addition of the sulfonyl chloride (1.1 eq, 0.716 mmol). The reaction was stirred for 10 min and was quenched with a 10% solution of NH_4Cl. The reaction mixture was diluted with ethyl acetate, washed with water, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The desired product was purified by silica gel column chromatography.

4-(Thiiran-2-ylmethylsulfonyl)phenyl methanesulfonate (3a): yield 89.6%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.17 (dd, J = 5.2, 1.8 Hz, 1H), 2.56 (dd, J = 5.9, 1.5 Hz, 1H), 3.04-3.09 (m, 1H), 3.25 (s, 3H), 3.24-3.29 (m, 1H), 3.50 (dd, J = 14.4, 6.0 Hz, 1H), 7.51 (d, J = 9.0 Hz, 2H), 8.02 (d, J = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.0, 25.7, 38.3, 62.5, 123.0, 130.8, 137.5, 153.0; HRMS (ESI) calcd for $C_{10}H_{12}O_5S_3$ ($M+Na^+$) 330.9739, found 330.9729.

4-(Thiiran-2-ylmethylsulfonyl)phenyl ethanesulfonate (3b): yield 94.0%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58 (t, J = 7.4 Hz, 3H), 2.17 (dd, J = 5.2, 1.8 Hz, 1H), 2.55 (dd, J = 6.2, 1.8 Hz, 1H), 3.03-3.08 (m, 1H), 3.26 (dd, J = 14.4, 7.4 Hz, 1H), 3.38 q, J = 7.5 Hz, 2H), 3.51 (dd, J = 14.4, 6.0 Hz, 1H), 7.49-7.52 (m, 2H), 7.99-8.02 (m, 2H); ^{13}C NMR (125

MHz, $CDCl_3$) δ 8.22, 24.0, 25.7, 46.0, 62.4, 122.9, 130.7, 137.1, 153.0; HRMS (ESI) calcd for $C_{11}H_{14}O_5S_3$ ($M+Na^+$) 344.9896, found 344.9882.

4-(Thiiran-2-ylmethylsulfonyl)phenyl propane-1-sulfonate (3c): yield 94.7%. ^1H NMR (500 MHz, $CDCl_3$) δ 1.15 (t, J = 7.5 Hz, 3H), 2.05 (dq, J = 15.3, 7.6 Hz, 2H), 2.17 (dd, J = 5.1, 1.9 Hz, 1H), 2.56 (dd, J = 6.2, 1.8 Hz, 1H), 3.04-3.08 (m, 1H), 3.25 (dd, J = 14.4, 7.6 Hz, 1H), 3.30-3.32 (m, 2H), 3.51 (dd, J = 14.4, 6.0 Hz, 1H), 7.50 (d, J = 9.0 Hz, 2H), 8.00 (d, J = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.8, 17.3, 24.1, 25.8, 53.0, 62.5, 122.9, 130.7, 137.2, 153.1; HRMS (ESI) calcd for $C_{12}H_{16}O_5S_3$ ($M+Na^+$) 359.0052, found 359.0056

4-(Thiiran-2-ylmethylsulfonyl)phenyl propane-2-sulfonate (3d): yield 94.4%. ^1H NMR (500 MHz, $CDCl_3$) δ 1.58 (d, J = 6.8 Hz, 6H), 2.16 (dd, J = 5.2, 1.8 Hz, 1H), 2.54 (dd, J = 6.1, 1.5 Hz, 1H), 3.03-3.07 (m, 1H), 3.26 (dd, J = 14.4, 7.6 Hz, 1H), 3.49-3.59 (m, 2H), 7.50 (d, J = 9.0 Hz, 2H), 8.00 (d, J = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.6, 23.9, 25.7, 53.5, 62.2, 122.8, 130.5, 136.8, 153.0; HRMS (ESI) calcd for $C_{12}H_{16}O_5S_3$ ($M+Na^+$) 359.0052, found 359.0056.

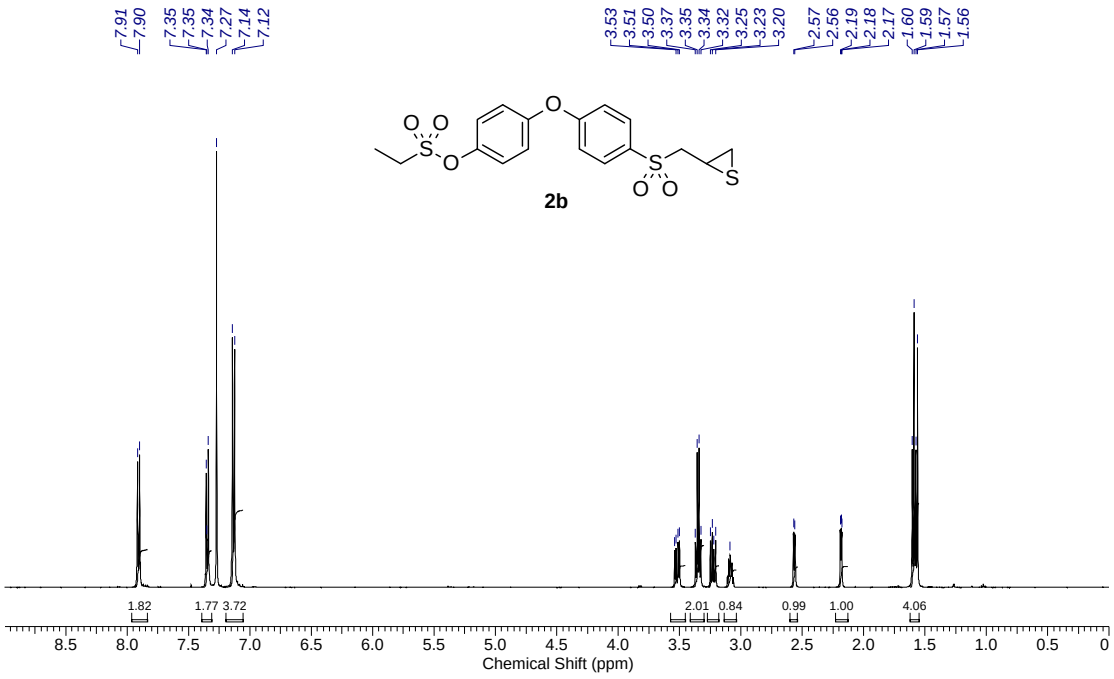
Solubility assay: The water solubility of each compound was determined at room temperature. The molar extinction coefficient, ε, was calculated by plotting absorbance at 245 nm for **1** and **2a-d** and at 225 nm for compounds **3a-d** versus concentration. The absorbance of a saturated solution of each compound in water was measured at the appropriate wavelength. The concentration was calculated using Beer's Law. Assays were performed in triplicate.

Half-life determination: The half-lives were determined in microsomal incubations. Rat liver microsomes (0.5 mg; BD Biosciences, Woburn, MA, USA) in 100 mM potassium phosphate buffer at pH 7.4 and 0.5 mM NADPH in a total volume of 0.5 mL were used. The compounds were dissolved in acetonitrile and added to the microsomal incubations to a final concentration of 100 μM for compounds **1** and **2a-d** and to a final concentration of 200 μM for compounds **3a-d**. The mixture was incubated for 60 min at 37 ^{o}C. At six time points, the reaction was terminated by the addition of one volume of internal standard in acetonitrile. The following were used as internal standards: 40 μM of **4** in acetonitrile for **1**, **2a**, and **2b**, 40 μM of **2a** in acetonitrile for **2c** and **2d**, and 80 μM of **10** in acetonitrile for compounds **3a-d**. The half-life of each compound was determined from the HPLC data using the first order rate law.

The HPLC system was composed of a Perkin-Elmer series 200 quaternary pump (Perkin-Elmer Corp., Norwalk, CT, USA), a Perkin-Elmer UV detector series 200, and a Perkin-Elmer autosampler series 200. Samples were analyzed on a YMC 5 μM basic 4.6 mm i.d. × 15 cm column (YMC Inc., Wilmington, NC, USA) connected to a YMC 5 μM basic 4.0 mm × 2 cm guard cartridge. The mobile phase for **2b** consisted of elution at 1 mL/min with 50% A / 50% B for 15 min. Effluent was monitored by UV detection at 245 nm. The mobile phase for **1**, **2a**, **2c-d**, and **3a-d** consisted of elution at 1 mL/min with 90% A/10% B for 5 min, followed by a 20-min linear gradient to 10% A/90% B, then 10% A/90% B for 5 min, and finally a 1-min linear gradient to 90% A/10% B. Effluent was monitored by UV detection at 245 nm for **1** and **2a-d** and at 225 nm for compounds **3a-d**.

Intrinsic clearance determination: The intrinsic clearance of the compounds was determined using the equation below.[4]

$$CL_{int,app} = \left[\frac{0.693}{in\,vitro\,half\ life}\right] \cdot \left[\frac{incubation\,volume}{mg\ of\ microsomal\ protein}\right] \cdot \left[\frac{45\,mg\ microsomal\ protein}{g\ liver}\right] \cdot \left[\frac{45\,g\ liver}{kg\,body\,weight}\right]$$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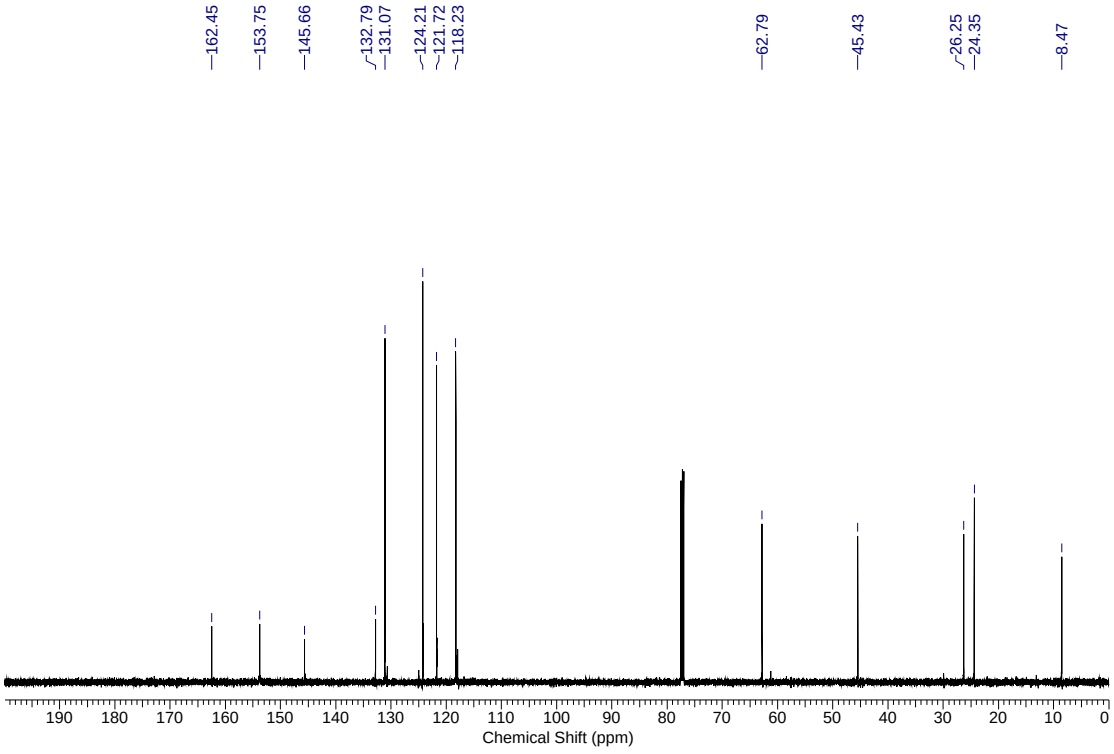
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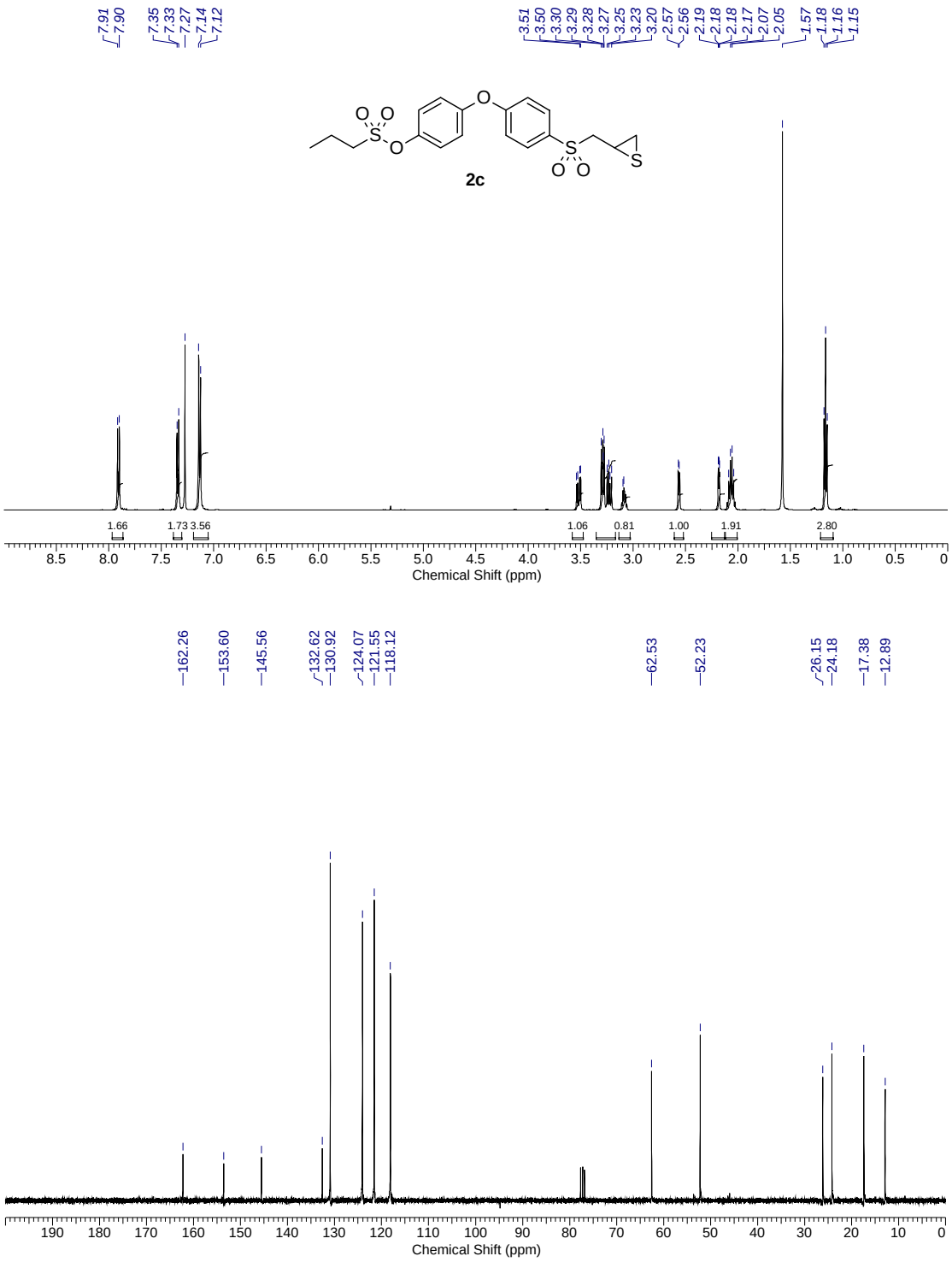
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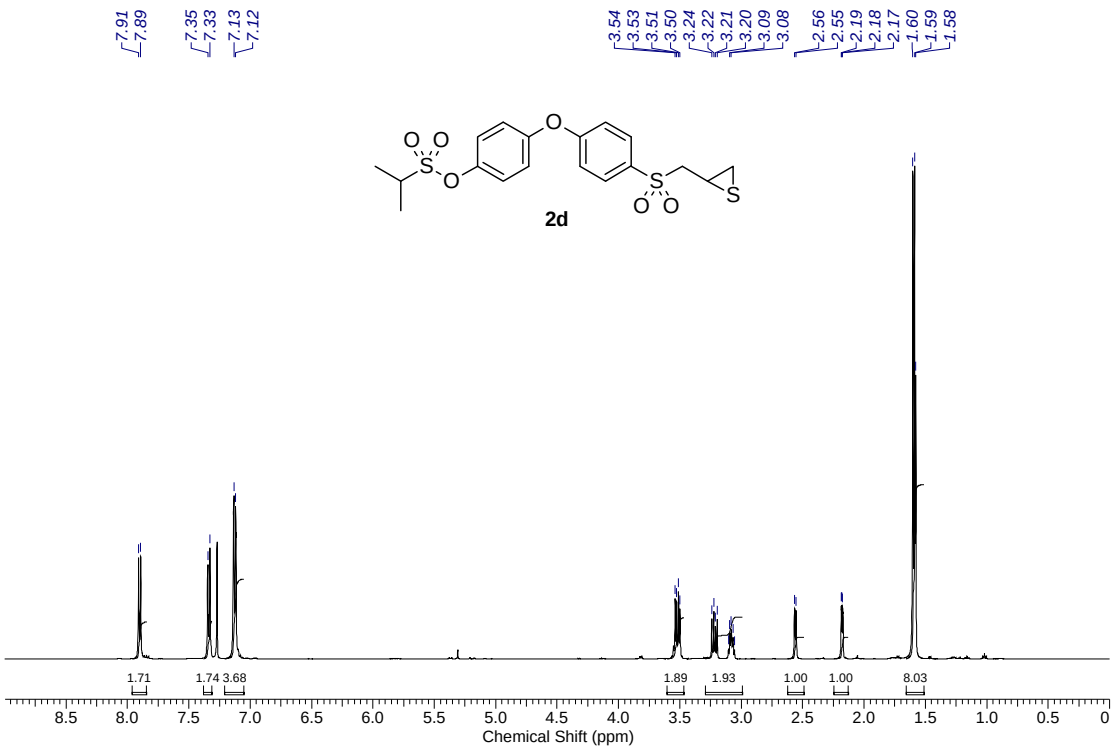
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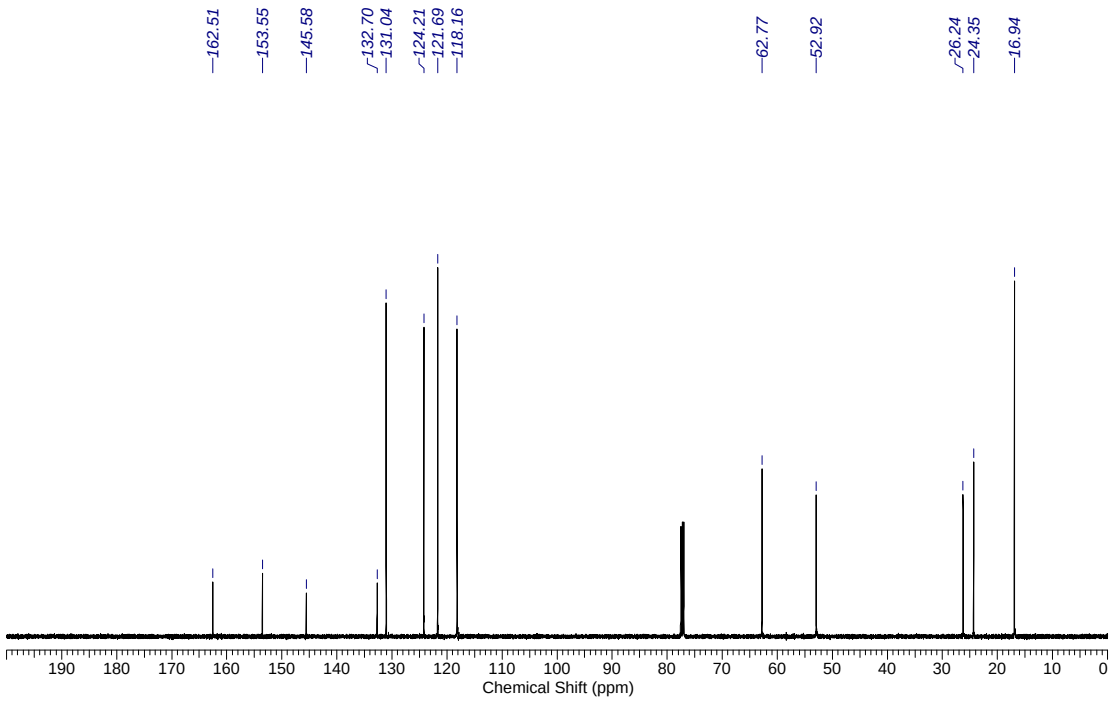
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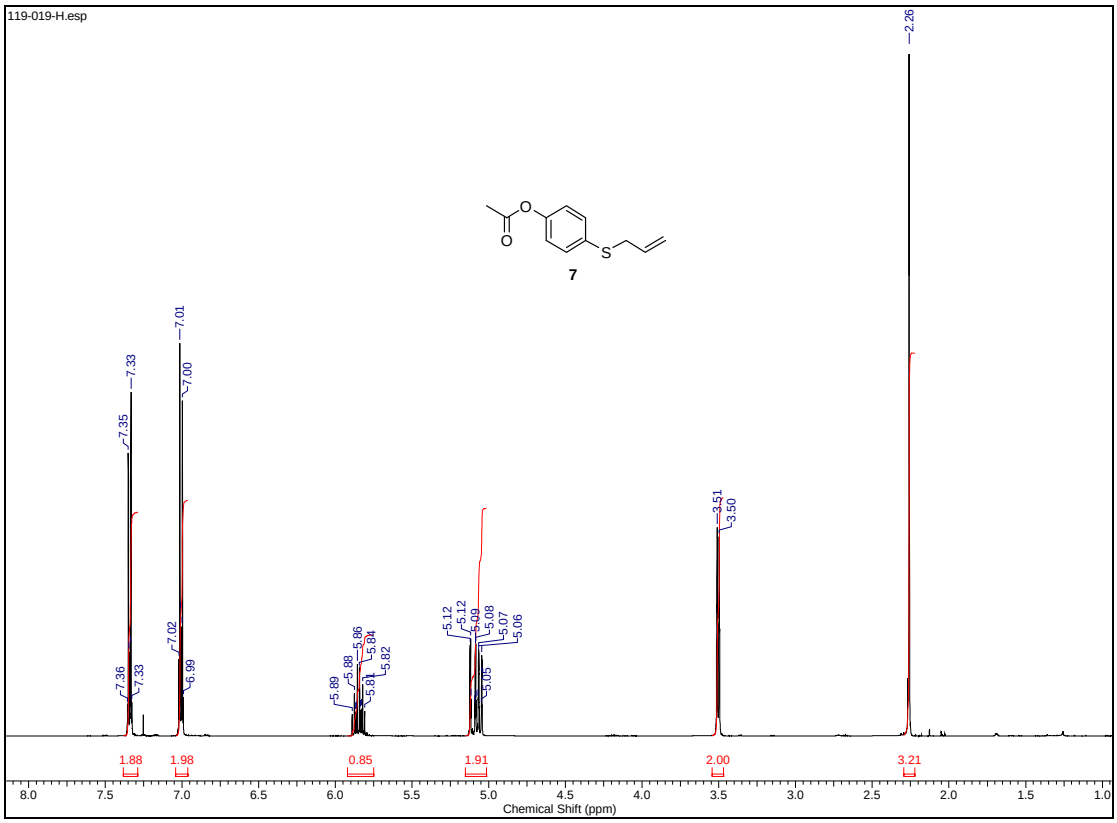
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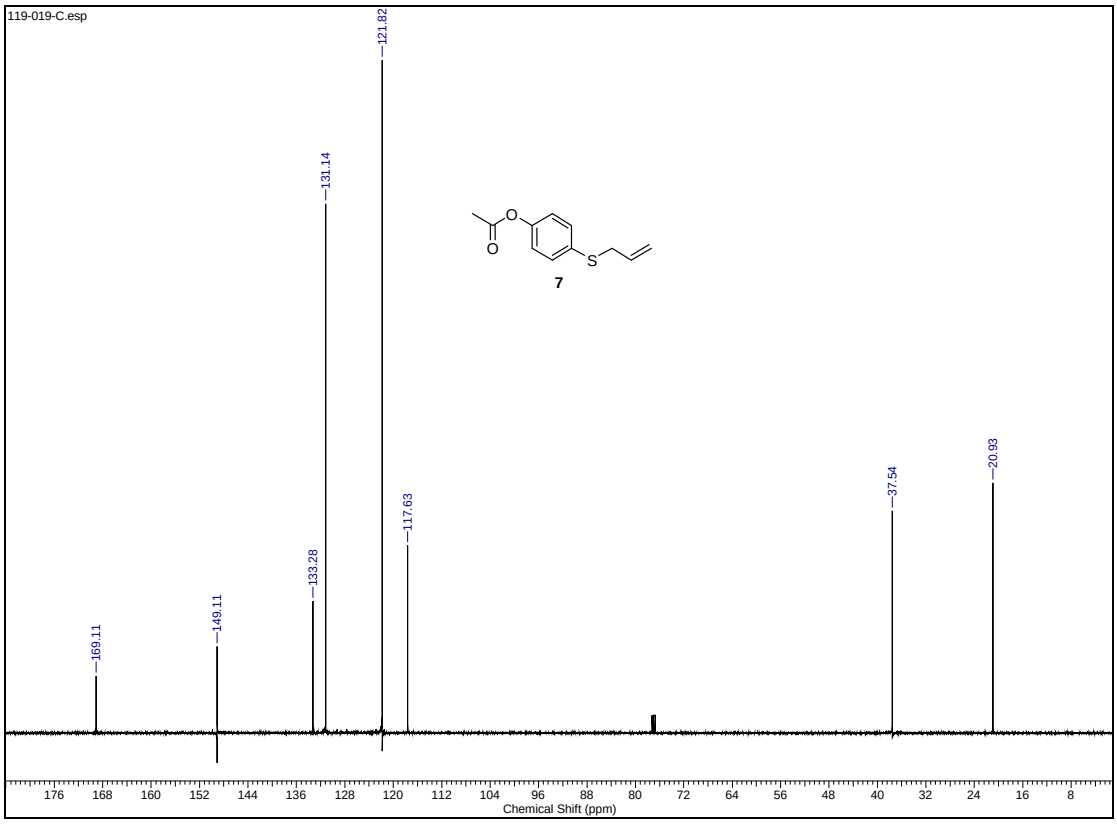
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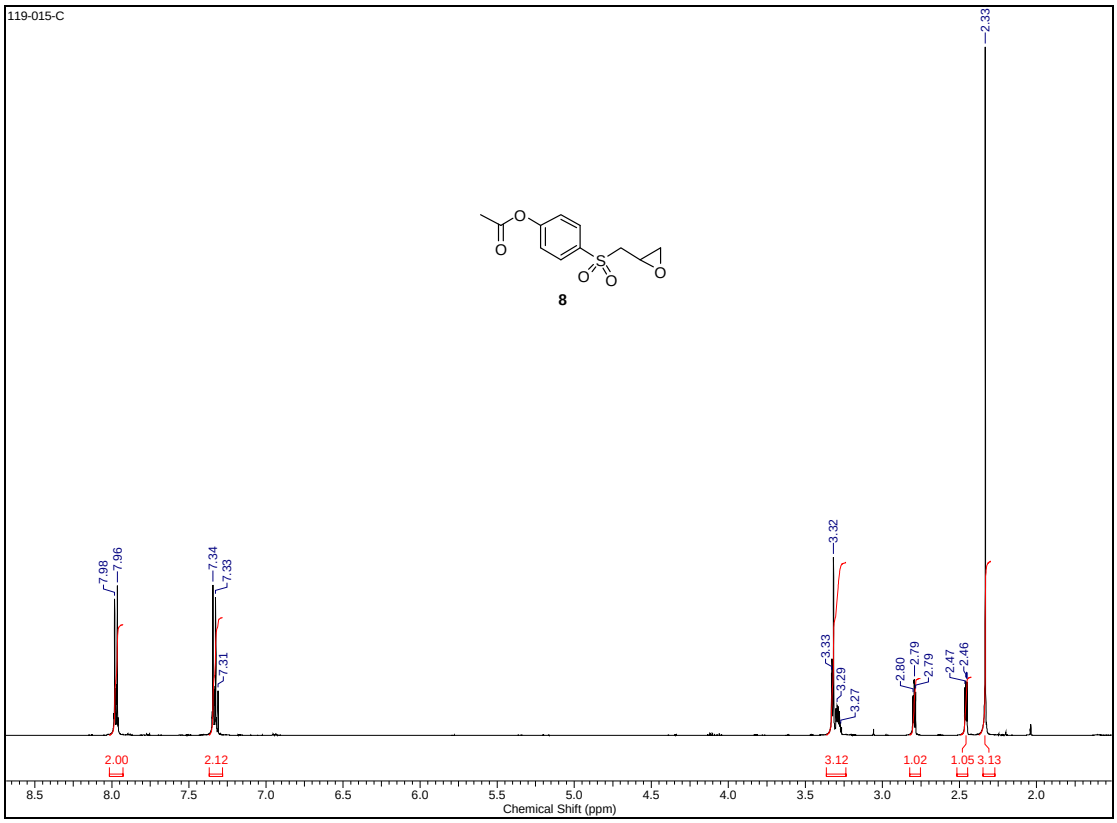
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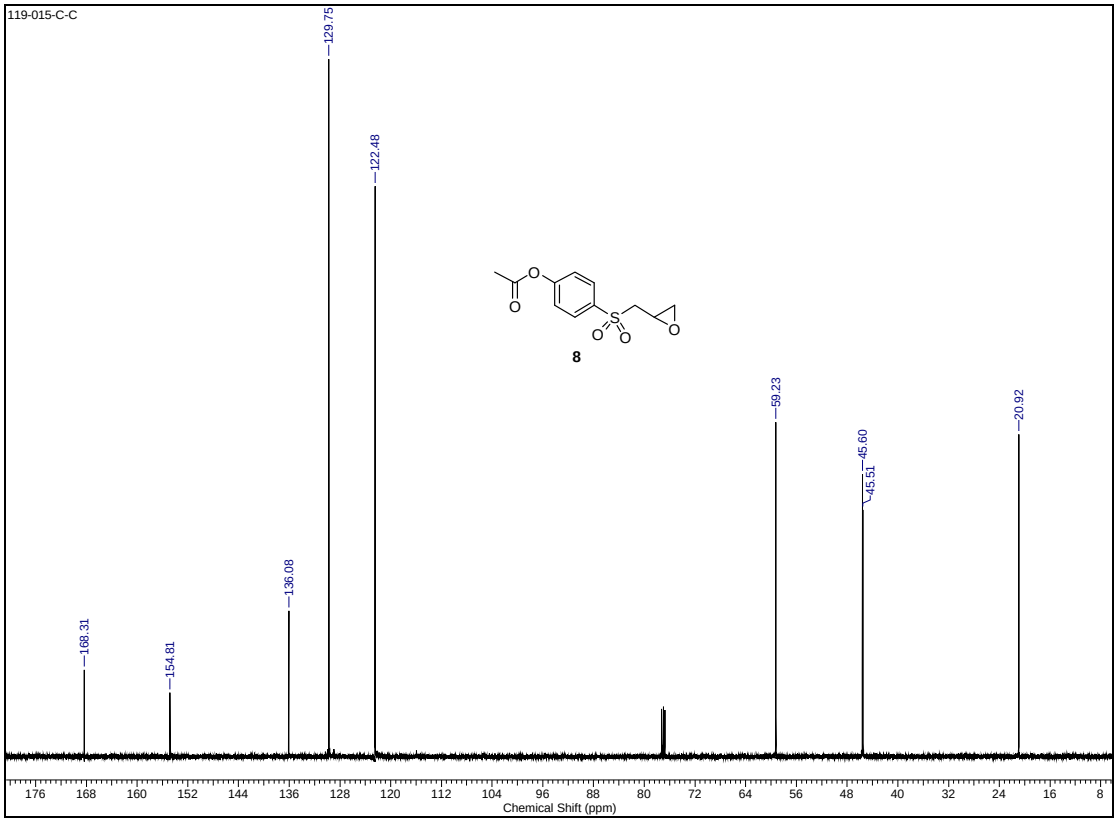
Chemical Shift (ppm)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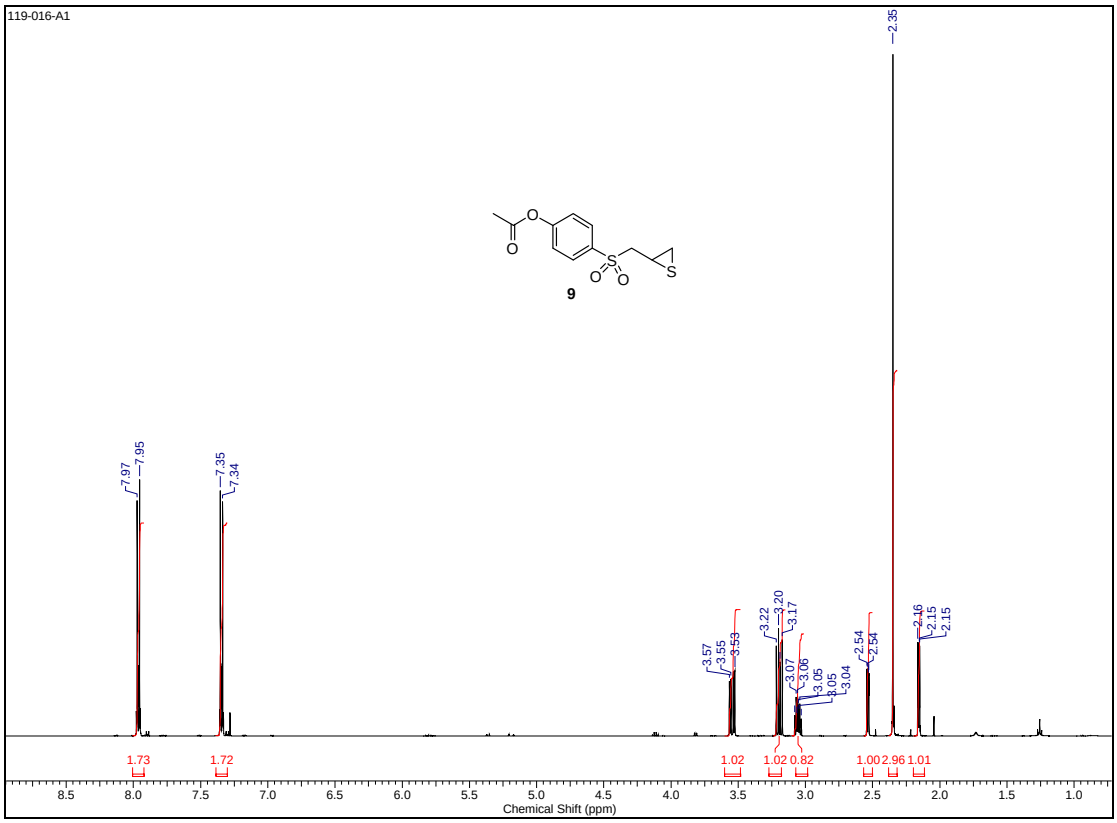
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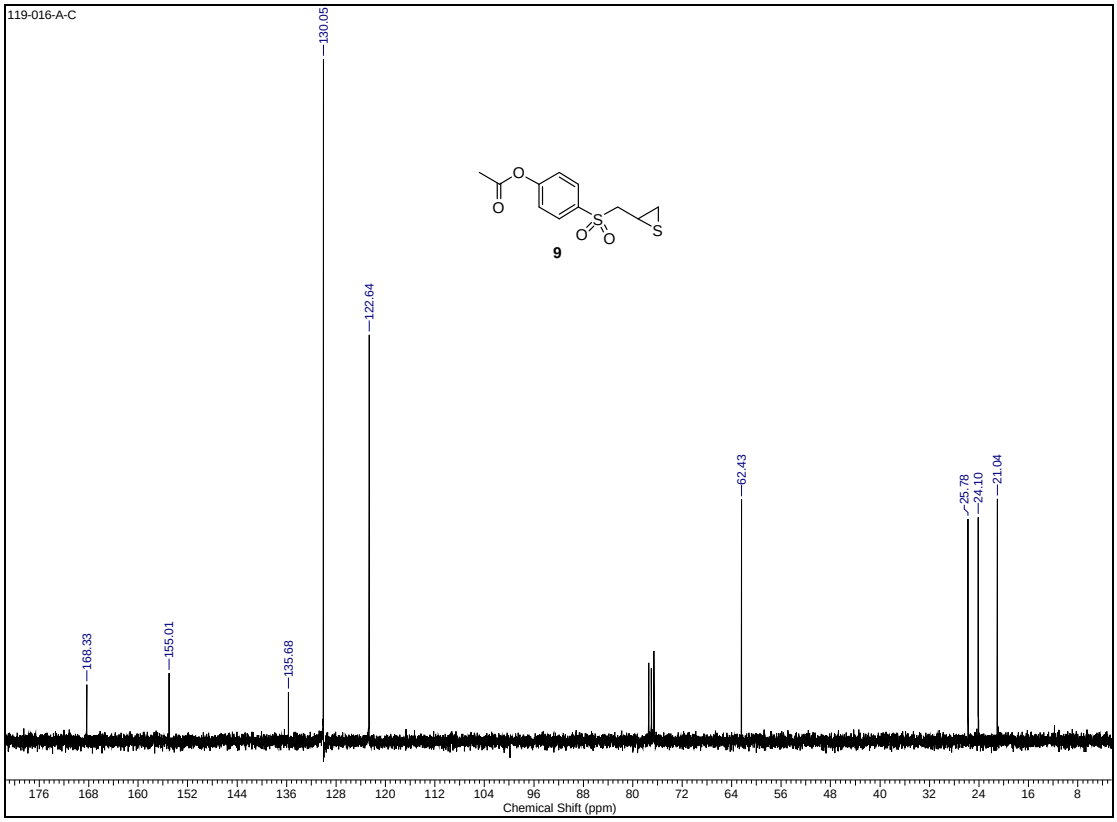
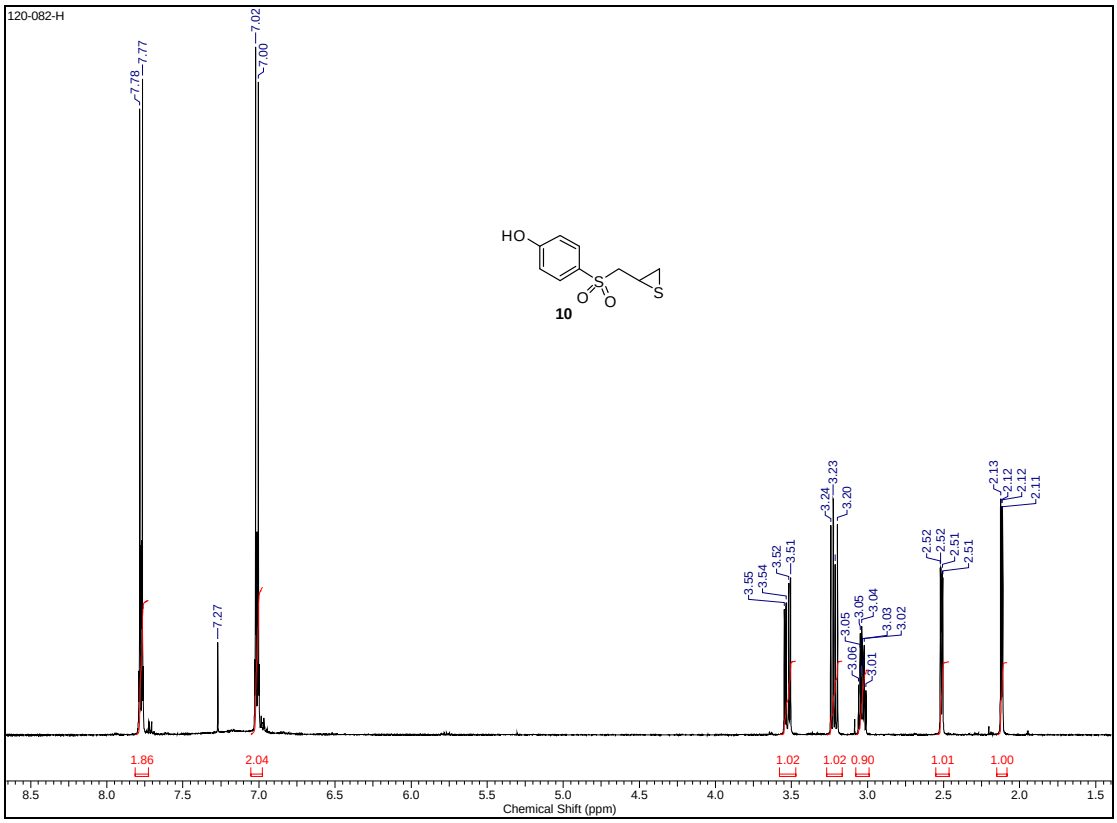
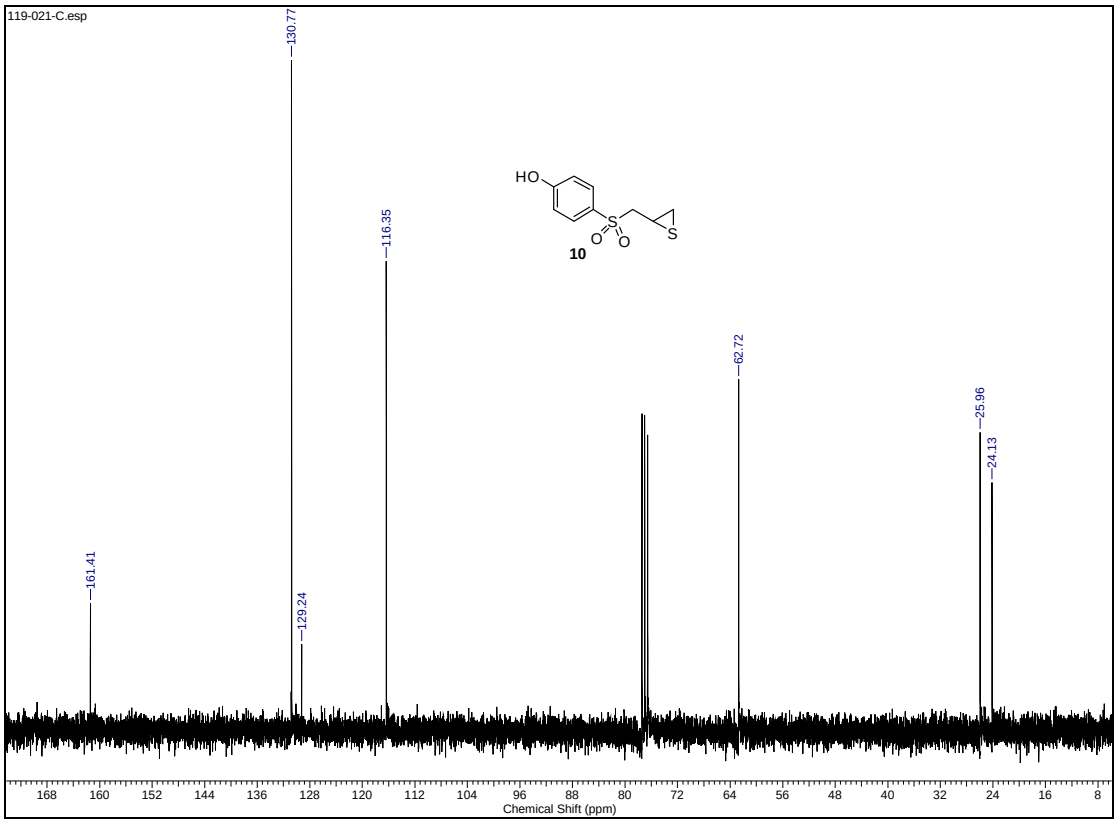
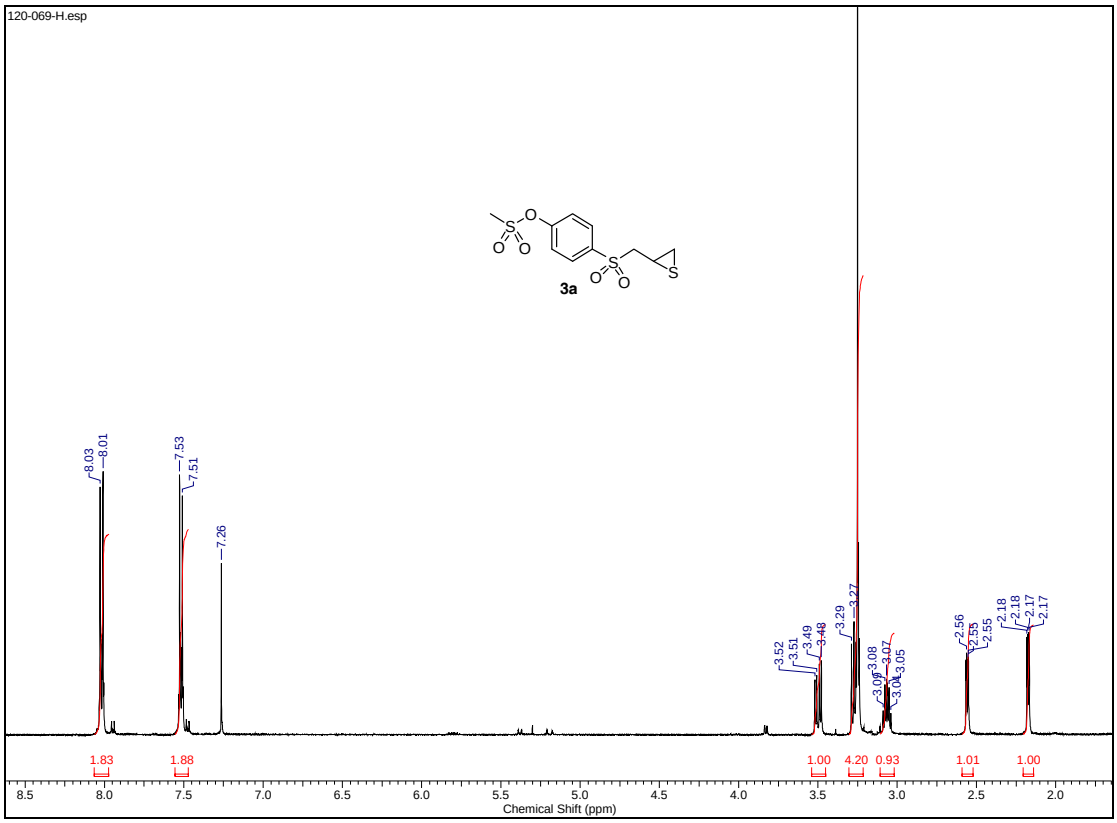
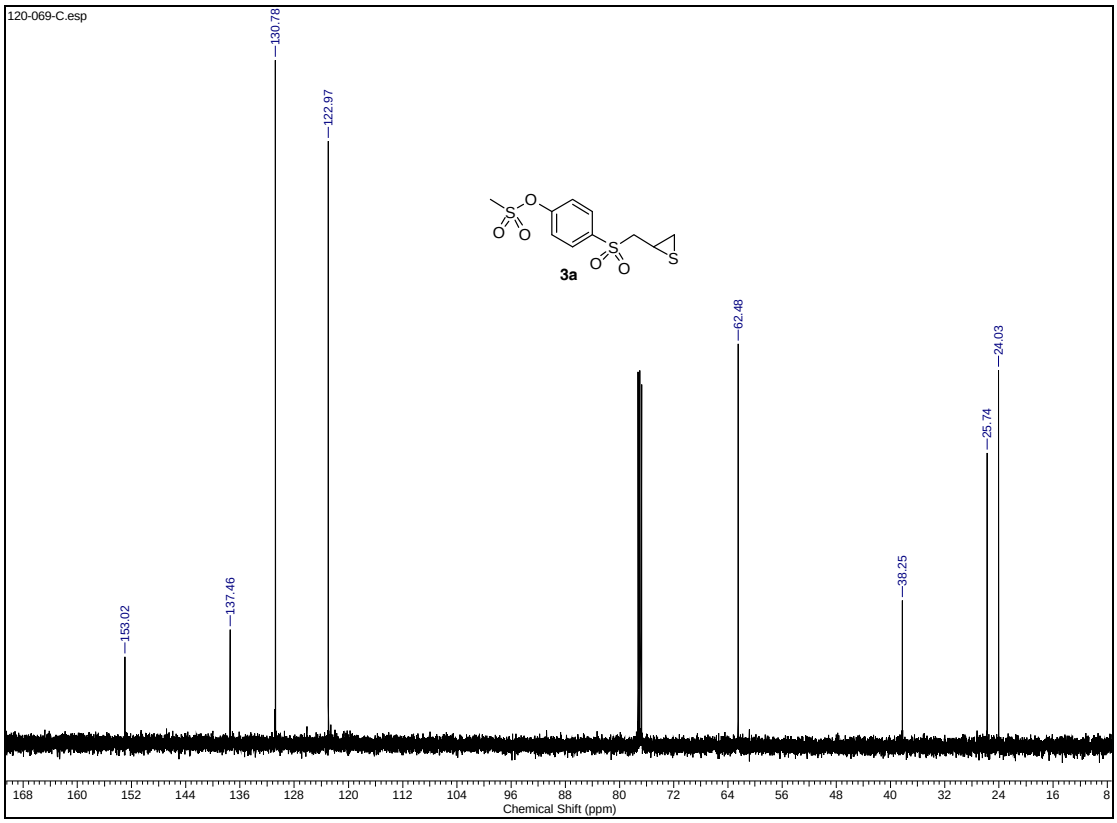
3.04
2.54
2.54
2.35
2.16
2.15
2.15
1.73
1.72
1.02
1.02
0.82
1.00
2.96
1.01
Chemical Shift (ppm)


119-016-A-C
9
130.05
122.64
62.43
25.78
24.10
21.04
168.33
155.01
135.68
Chemical Shift (ppm)


120-082-H
7.78
7.77
7.02
7.00
7.27
3.55
3.54
3.52
3.51
3.24
3.23
3.20
3.06
3.05
3.04
3.03
3.02
3.01
2.52
2.52
2.51
2.51
2.13
2.12
2.12
2.11
HO
O
S
O
S
10
1.86
2.04
1.02
1.02
0.90
1.01
1.00
Chemical Shift (ppm)


119-021-C.esp
130.77
116.35
62.72
25.96
24.13
161.41
129.24
HO
O
S
O
S
10
Chemical Shift (ppm)


120-069-H.esp
3a
8.03
8.01
7.53
7.51
7.26
3.52
3.51
3.49
3.48
3.29
3.27
3.08
3.07
3.05
2.56
2.55
2.18
2.17
2.17
1.83
1.88
1.00
4.20
0.93
1.01
1.00
Chemical Shift (ppm)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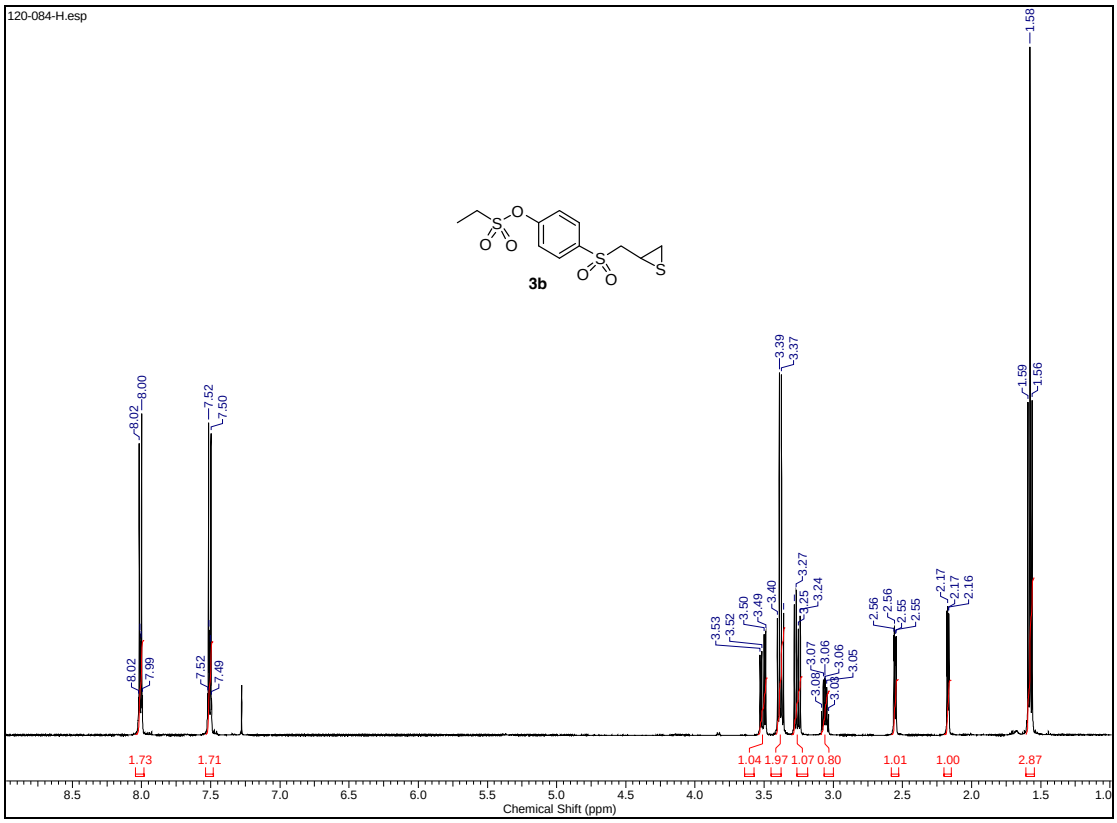
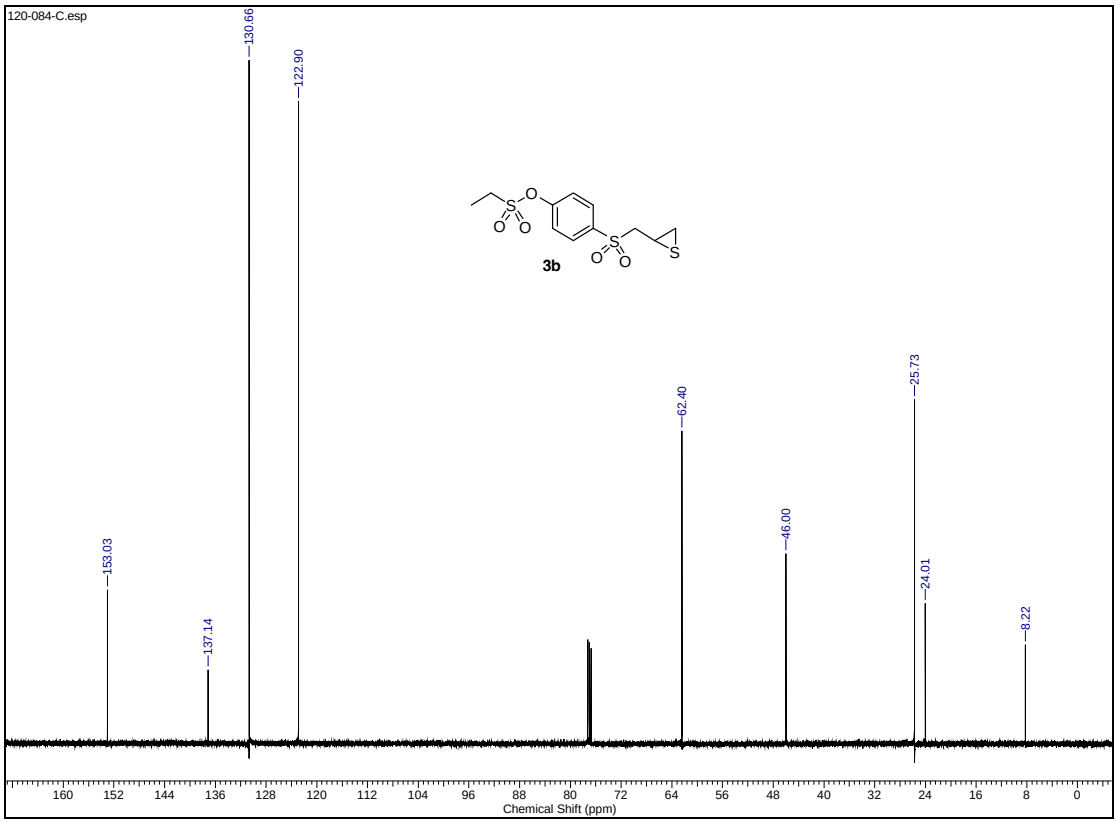
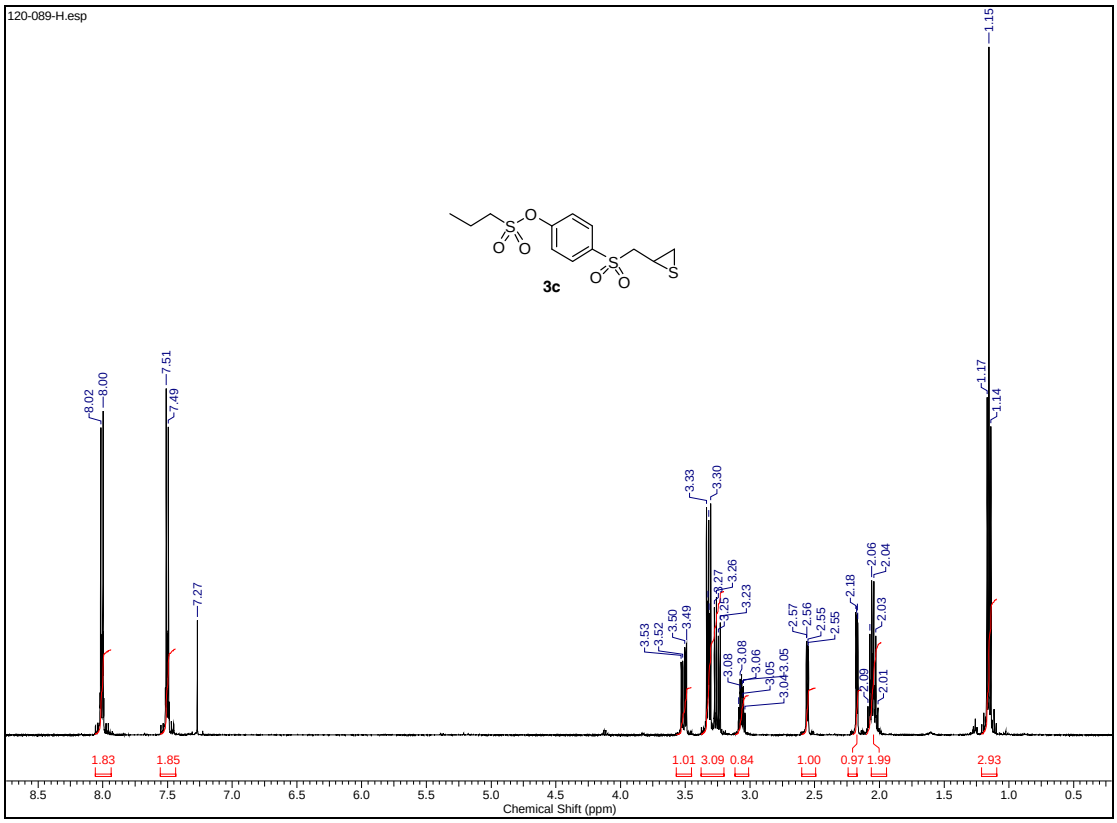
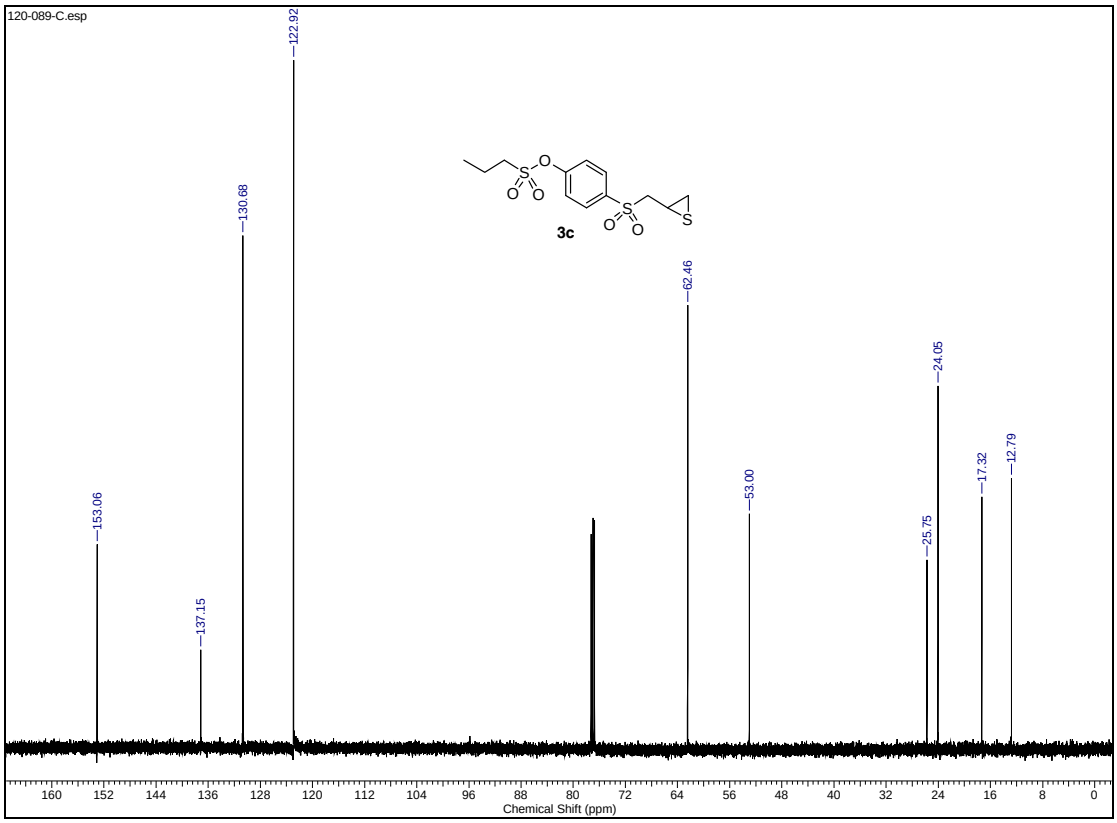
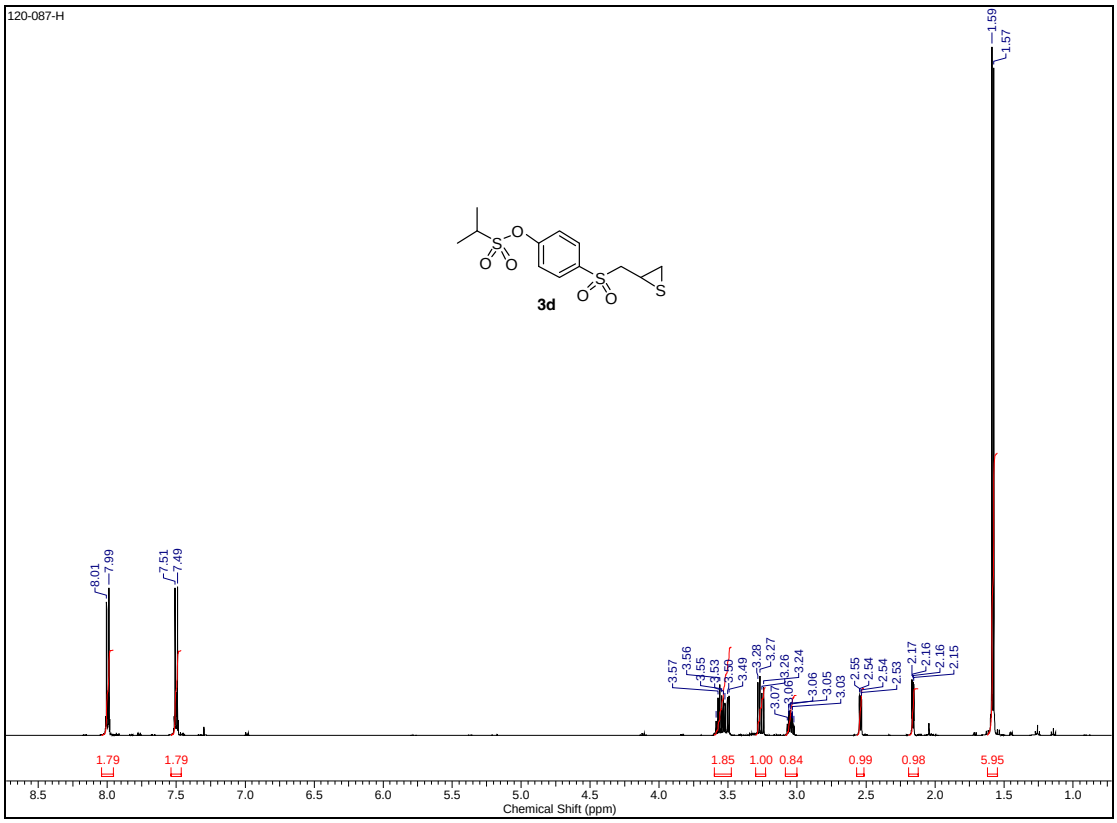
3a
130.78
122.97
62.48
24.03
25.74
38.25
153.02
137.46
Chemical Shift (ppm)

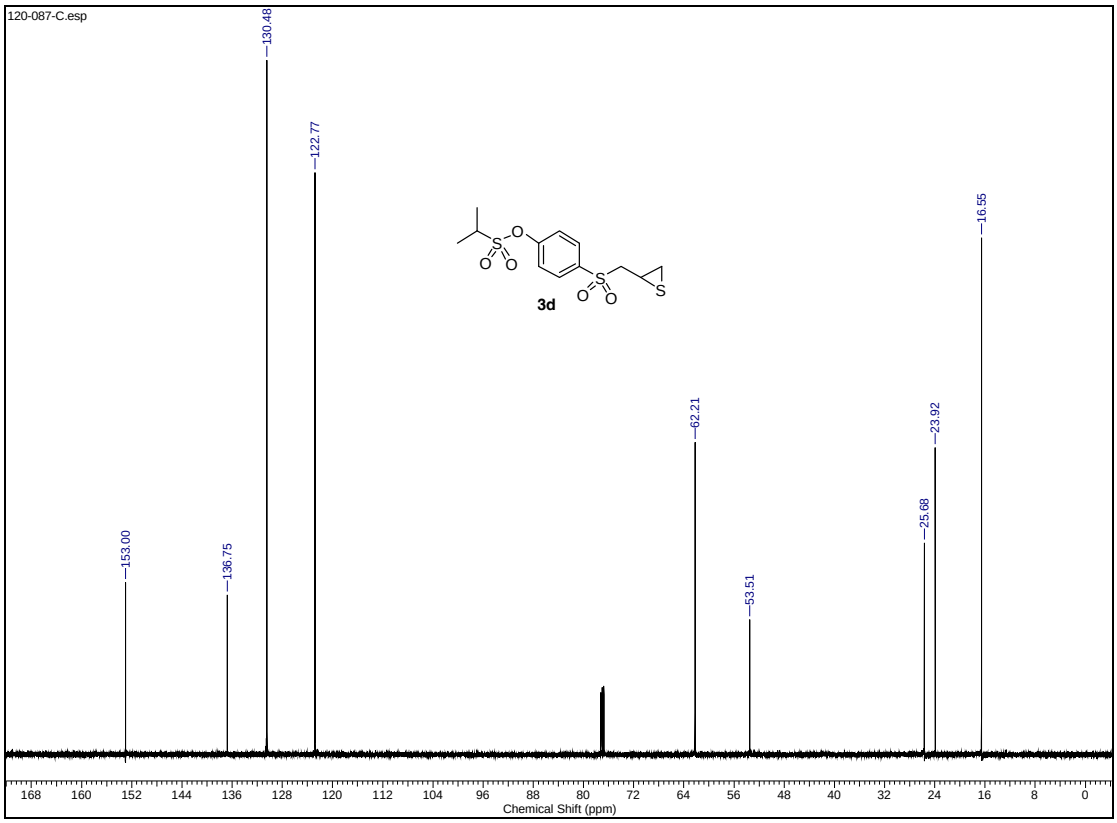

120-084-H.esp
3b
Chemical Shift (ppm)


120-084-C.esp
3b
Chemical Shift (ppm)


120-089-H.esp
3c
Chemical Shift (ppm)


120-089-C.esp
3c
Chemical Shift (ppm)


120-087-H
3d
Chemical Shift (ppm)


120-087-C.esp
3d
Chemical Shift (ppm)

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