Supporting Information

Development of a Highly Potent, Selective, and Cell-Active Inhibitor of Cysteine Cathepsin L – A Hybrid Design Approach

Dibyendu Dana,[$] Shatarupa De,[$,≠] Pratikkumar Rathod,[$,‡,≠] Anibal R. Davalos,[$] Daniel A. Novoa,[$] Suneeta Paroly,[Φ] Viviana M. Torres,[$] Nisar Afzal,[$] Ravi S. Lankalapalli,[$,†] Susan A. Rotenberg[$], Emmanuel J. Chang,[‡] Gopal Subramaniam[$], Sanjai Kumar[$,*]

[$]Department of Chemistry and Biochemistry, Queens College and the Graduate Center of the City University of New York, Queens, New York 11367-1597, USA; [‡]Department of Chemistry, York College of the City University of New York, Jamaica, New York, 11451, USA, [Φ]Bard High School Early College Queens, 30-20 Thomson Avenue, Long Island City, New York, 11101, USA; [≠]Equal contributions; [†]Current address: Agroprocessing and Natural Products Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram 695019, Kerala, India
*Corresponding Author: Email: Sanjai.Kumar@qc.cuny.edu (Sanjai Kumar); Phone: +1 718 997 4120; fax: +1 718 997 5531

Table of Contents

A. Synthesis and Characterization

A.1 General:

^{1}H NMR spectra were recorded at either 400 or 500 MHz using $CDCl_3$ or DMSO as the solvent. ^{13}C NMR spectra were recorded at either 125 or 100 MHz using $CDCl_3$ or DMSO as the solvent. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C), DMSO (2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (td), and multiplet (m). The mass spectra were acquired using a 6520 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (Agilent Technologies, Santa Clara, CA, USA). 4-ethynylbenzyl alcohol was purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) was purchased from peptide international (Louisville, Kentucky, USA). All anhydrous solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials were purchased from Fisher Scientific Inc. (Pittsburgh, PA, USA).

A.2. Synthesis of library of aryl vinylsulfonate ester compounds (1-27):

Synthesis of all aryl vinylsulfonates were carried out using a literature reported protocol [1].

4-Bromophenyl ethenesulfonate (1): Pale yellow semi solid. R_f = 0.46. Hexane: EtOAc (8:2). Yield: 61%.^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 6.19 (d, *J* = 10.0Hz, 1H), 6.36 (d, *J* = 15.1Hz, 1H), 6.67 (dd, *J* = 15.1Hz, 10.0Hz, 1H), 7.12 (d, *J* = 10.2Hz, 2H), 7.50 (d, *J* = 10.2Hz, 2H). ^{13}C NMR (100 MHz $CDCl_3$) δ ppm: 120.9, 123.7 131.7, 132.3, 133.0, 148.3. HRMS [ESI-MS +ve] calculated for $(C_8H_7BrO_3S+H)^+$: 262.9378, observed: 262.9375.

Quinoline-8-yl ethenesulfonate (2): Colorless solid R_f = 0.21.Hexane: EtOAc (8:2). Yield: 65%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.05 (d, *J*= 10.2Hz, 1H), 6.35 (d, *J*= 15.1Hz, 1H), 7.11 (dd, *J*= 15.1Hz and 10.2Hz, 1H), 7.50 (dd, *J*= 5.2Hz and 5.3Hz, 1H), 7.56 (m, 1H), 7.72 (d, *J*= 5.3Hz, 1H), 7.81 (d, *J*= 5.3Hz, 1H), 8.25 (d, J= 5.3Hz, 1H), 8.98 (s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 122.1, 123.3, 126.2, 127.2, 129.7, 130.2, 133.4, 136.1, 141.3,145.3, 150.9. HRMS [ESI-MS +ve] calculated for $(C_{11}H_9NO_3S+H)^+$: 236.0376, observed: 236.0375.

5-Nitroquinoline-8-yl ethenesulfonate (3): Colorless solid. R_f = 0.24. Hexane: EtOAc (8:2). Yield: 68%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.15 (d, *J*= 8.2Hz, 1H), 6.45 (d, *J*= 16.1Hz, 1H), 7.11, (dd, *J*= 16.1Hz and 8.2Hz, 1H), 7.74 (dd, *J*= 4.1Hz and 8.2Hz, 1H), 7.83 (d, *J*= 8.5Hz, 1H), 8.44 (d, *J*= 8.5Hz, 1H), 9.07 (d, *J*= 8.3Hz, 1H), 9.11 (d, *J*= 4.1Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 121.5, 122.9, 124.8, 124.9, 131.3, 132.4, 133.2, 141.2, 143.7, 149.7, 151.9. HRMS [ESI-MS +ve] calculated for $(C_{11}H_8N_2O_5S+H)^+$: 281.0227, observed: 281.0227 .

[1, 1'-Biphenyl]-4-yl ethenesulfonate (4): white powder. R_f = 0.28. Hexane: EtOAc (9:1). Yield: 76%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.19 (d, *J*= 10.1Hz, 1H), 6.41 (d, *J*= 16.6Hz, 1H), 6.70 (dd, *J*= 10.1Hz and 16.6Hz, 1H), 7.30 (m, 2H), 7.33-7.47 (m, 3H), 7.57 (m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 122.6, 127.1, 127.8, 128.5, 128.9, 131.8, 132.1, 139.8, 140.6, 148.7. HRMS [ESI-MS +ve] calculated for $(C_{14}H_{12}O_3S+Na)^+$: 283.0399, observed: 283.0399.

4'-Nitro-[1, 1'-biphenyl]-4-yl ethenesulfonate (5): yellow powder. R_f = 0.25. Hexane: EtOAc (8:2). Yield: 72%. ^{1}H NMR (400MHz, DMSO-D_6) δ ppm: 6.35 (d, *J*= 16.8Hz, 1H), 6.43 (d, *J*= 10.1 Hz, 1H), 7.30 (dd, *J*= 10.1Hz and 16.8Hz, 1H), 7.46 (d, *J*= 8.7Hz, 2H), 7.90 (d, *J*= 8.5Hz, 2H), 7.98 (d, *J*= 8.7Hz, 2H), 8.32 (d, *J*= 8.7Hz, 2H). ^{13}C NMR (100MHz, DMSO-D_6) δ ppm: 123.1, 124.1, 128.0, 129.1, 132.2, 133.6, 136.9, 145.1, 146.9, 149.5. HRMS [ESI-MS +ve] calculated for ($C_{14}H_{11}NO_5S$+H): 328.0250, observed: 328.0247.

Methyl 2-((vinylsulfonyl) oxy) benzoate (6): Viscous liquid R_f = 0.51. Hexane: EtOAc (8:2). Yield: 67%. ^{1}H NMR (500MHz, $CDCl_3$) δ ppm: 3.93 (s, 3H), 6.16 (d, *J*= 10.1Hz, 1H), 6.37 (d, *J*= 14.9Hz, 1H), 6.82 (dd, *J*= 14.91Hz and 10.1Hz, 1H), 7.39 (m, 2H), 7.57 (t, *J*= 10.4Hz, 1H), 7.97 (d, *J*= 10.4Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 52.5, 124.1, 124.8, 127.2, 131.3, 132.1, 132.7, 133.7, 147.8, 164.9. HRMS [ESI-MS +ve] calculated for $(C_{10}H_{10}O_5S+Na)^+$: 265.0141, observed: 265.0141.

Methyl 4-((vinylsulfonyl) oxy) benzoate (7): white powder. R_f = 0.59. Hexane: EtOAc (8:2). Yield: 62%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 3.92 (s, 3H), 6.23 (d, *J*= 10.2Hz, 1H), 6.39 (d, *J*= 14.9Hz, 1H), 6.73 (dd, *J*= 14.9Hz and 10.2Hz, 1H), 7.31 (d, *J*= 10.1 Hz, 2H), 8.08 (d, *J*= 10.1Hz, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 52.4, 122.2, 129.2, 131.5, 131.9, 132.3, 152.6, 165.9. HRMS [ESI-MS +ve] calculated for $(C_{10}H_{10}O_5S+H)^+$: 243.0322, observed: 243.0322.

4-Cyanophenyl ethenesulfonate (8): Yellow powder. R_f = 0.20. Hexane: EtOAc (9:1). Yield: 73%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.25 (d, *J*= 9.9Hz, 1H), 6.43 (d, *J*= 16.6Hz, 1H), 6.70 (dd, *J*= 16.6Hz and 9.9Hz, 1H), 7.37 (d, *J*= 8.7Hz, 2H), 7.72 (d, *J*= 8.6Hz, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 111.4, 117.6, 123.3, 131.8, 132.7, 134.1, 152.2. HRMS [ESI-MS +ve] calculated for ($C_9H_7NO_3S$+H): 210.0219, observed: 210.0221.

4-Nitrophenyl ethenesulfonate (9): white powder. R_f = 0.49. Hexane: EtOAc (8:2). Yield: 69%. ^{1}H NMR (500MHz, $CDCl_3$) δ ppm: 6.30 (d, *J*= 9.7Hz, 1H), 6.46 (d, *J*= 14.8Hz, 1H), 6.74 (dd, *J*= 14.8Hz and 9.7Hz, 1H), 7.44 (d, *J*= 9.8Hz, 2H), 8.30 (d, *J*= 9.8Hz, 2H). ^{13}C NMR (125MHz, $CDCl_3$) δ ppm: 123.1, 125.6, 131.5, 133.2, 146.3, 153.5. HRMS [ESI-MS +ve] calculated for $(C_8H_7NO_5S+H)^+$: 230.0118, observed: 230.0118.

5-Fluoro-2-nitrophenyl ethenesulfonate (10): yellow viscous liquid. R_f = 0.29. Hexane: EtOAc (9:1). Yield: 56%.^{1}H NMR (400Hz, $CDCl_3$) δ ppm: 6.27 (d, *J*= 9.6 Hz, 1H), 6.52 (d, *J*= 15.8 Hz, 1H), 6.76 (dd, *J*= 15.8 Hz and 9.6 Hz, 1Hz), 7.23 (m,1H), 7.31 (m, 1H), 8.17 (m, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 113.5 (d, J_{C-F}= 26.1 Hz), 115.0 (d, J_{C-F}= 23.2 Hz), 128.1 (d, J_{C-F}= 10.1

Hz), 132.1, 133.2, 139.0, 143.8, 165.3 (d, J_{C-F}= 258.2 Hz).HRMS [ESI-MS +ve] calculated for $(C_8H_6FNO_5S+Na)^+$: 269.9843, observed: 269.9841.

4-Chlorophenyl ethenesulfonate (11): pale yellow viscous liquid. R_f = 0.27 Hexane: EtOAc (9:1). Yield: 68%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.21 (d, *J*= 9.9Hz, 1H), 6.39 (d, *J*= 16.7Hz, 1H), 6.67 (dd, *J*= 16.7Hz and 9.9Hz, 1H), 7.19 (d, *J*= 9.0, 2H), 7.36 (d, *J*= 9.0, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 123.7, 129.9, 131.7, 132.3, 133.1, 147.7. HRMS [ESI-MS +ve] calculated for $(C_8H_7ClO_3S+H)^+$: 218.9877, observed: 218.9877.

2-Chlorophenyl ethenesulfonate (12): pale yellow viscous liquid. R_f = 0.25. Hexane: EtOAc (9.5:0.5). Yield: 68%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.18 (d, *J*= 10.4Hz, 1H), 6.37 (d, *J*= 16.6Hz, 1H), 6.78 (dd, *J*= 16.6Hz and 10.4Hz, 1H), 7.25 (t, *J*= 7.7Hz, 1H), 7.30 (t, *J*= 7.7Hz, 1H), 7.42 (d, *J*= 8.1Hz, 1H), 7.44 (d, *J*= 8.1Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 124.5, 127.1, 128.1, 128.2, 130.9, 131.8, 132.4, 145.3. HRMS [ESI-MS +ve] calculated for $(C_8H_7ClO_3S+Na)^+$: 240.9697, observed: 240.9699.

3-Chlorophenyl ethenesulfonate (13): colorless viscous liquid. R_f = 0.25 Hexane: EtOAc (9.5:0.5). Yield: 70%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.21 (d, *J*= 9.9Hz, 1H), 6.40 (d, *J*= 16.7Hz, 1H), 6.67 (dd, *J*= 16.7Hz and 9.9Hz, 1H), 7.16 (m, 1H), 7.26 (m, 1H), 7.31 (m, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 120.6, 122.8, 127.7, 130.6, 131.8, 132.3, 135.1, 149.6. HRMS [ESI-MS +ve] calculated for $(C_8H_7ClO_3S+H)^+$: 218.9877, observed: 218.9877.

4-Chloro-2-fluorophenyl ethenesulfonate (14): colorless viscous liquid. R_f = 0.48 Hexane: EtOAc (8:2). Yield: 63%.^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.22 (d, *J*= 10.0Hz, 1H), 6.39 (d, *J*= 16.6Hz, 1H), 6.72 (d, *J*= 16.6Hz and 10.0Hz, 1H), 7.21 (m, 2H), 7.34 (m, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 117.9 (d, J_{C-F}= 21.1 Hz, 1C), 125.2 (d, J_{C-F}= 3.6 Hz, 1C), 125.8, 131.7, 132.4, 133.5 (d, J_{C-F}= 8.8 Hz, 1C), 135.4 (d, J_{C-F}= 12.6 Hz, 1C), 154.1 (d, J_{C-F}= 250.1 Hz, 1C).HRMS [ESI-MS +ve] calculated for $(C_8H_6ClFO_3S+Na)^+$: 258.9602, observed: 258.9604.

4-Chloro-3-fluorophenyl ethenesulfonate (15): colorless viscous liquid. R_f = Hexane: EtOAc (9:1). Yield: 64%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.25 (d, J=9.9 Hz, 1H), 6.41 (d, J=16.6 Hz, 1H), 6.68 (dd, J= 16.6 Hz and 9.9 Hz, 1H), 7.01 (m, 1H), 7.12 (m, 1H), 7.43 (m, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 111.6 (d, J_{C-F}= 24.2 Hz) 119.0 (d, J_{C-F}= 4.1 Hz) 120.3 (d, J_{C-F}= 17.7 Hz) 131.1, 131.6, 132.8, 148.2 (d, J_{C-F}= 9.3 Hz,), 158.0 (d, J_{C-F}= 251.8 Hz) HRMS [ESI-MS +ve] calculated for $(C_8H_6ClFO_3S+H)^+$: 236.9783, observed: 236.9783.

4-Chloro-3-(trifluoromethyl) phenyl ethenesulfonate (16): colorless viscous liquid. R_f = 0.32. Hexane: EtOAc (9:1). Yield: 57%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.27 (d, *J*= 9.8Hz, 1H), 6.42 (d, *J*= 16.6Hz, 1H), 6.71 (d, *J*= 16.6Hz and 9.8Hz, 1H), 7.40 (m, 1H), 7.55 (m, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 121.7 (q, J_{C-F}= 121.9 Hz), 122.2 (q, J_{C-F}= 270.1 Hz), 126.9, 131.1, 132.5, 133.0, 133.1, 147.3 HRMS [ESI-MS +ve] calculated for $(C_9H_6ClF_3O_3S+Na)^+$: 308.9570, observed: 308.9572.

4-Chloro-2, 3, 5, 6-tetrafluorophenyl ethenesulfonate (17): colorless liquid. R_f = 0.24 Hexane: EtOAc (9.8:0.2). Yield: 66%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.35 (d, *J*= 9.9Hz, 1H), 6.56 (d, *J*= 16.5Hz, 1H), 6.81 (dd, *J*= 16.5Hz and 9.9Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 131.8, 133.1, 140.3, 143.1, 143.2, 145.6 HRMS [ESI-MS +ve] calculated for $(C_8H_3ClF4O_3S+Na)^+$: 312.9320, observed: 312.9323.

4-Chloro-3-methylphenyl ethenesulfonate (18): colorless viscous liquid. R_f = 0.32. Hexane: EtOAc (9:1). Yield: 80%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 2.35 (s, 3H), 6.19 (d, *J*= 10.3Hz, 1H), 6.35 (d, *J*= 16.7Hz, 1H), 6.68 (dd, *J*= 10.3Hz and 16.7 Hz, 1H), 7.00 (dd, *J*= 2.9Hz and 8.8 Hz, 1H), 7.10 (d, *J*= 2.8Hz, 1H), 7.30 (d, *J*= 8.6Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 20.2, 120.9, 124.6, 130.1, 131.8, 132.3, 133.1, 138.1, 147.6. HRMS [ESI-MS +ve] calculated for $(C_9H_9ClO_3S+Na)^+$: 254.9853, observed: 254.9855.

4-Chloro-3, 5-dimethylphenyl ethenesulfonate (19): white powder. R_f = 0.43. Hexane: EtOAc (8:2). Yield: 59%.^{1}H NMR (500MHz, $CDCl_3$) δ ppm: 2.27 (s, 6H), 6.10 (d, *J*= 5.5Hz, 1H), 6.28 (d, *J*= 20.0Hz, 1H), 6.59 (dd, *J*= 20.0Hz and 5.5Hz, 1H), 6.87 (s, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 20.8, 121.8, 131.9, 132.1, 133.3, 138.6, 146.9 HRMS [ESI-MS +ve] calculated for $(C_{10}H_{11}ClO_3S+Na)^+$: 269.0010, observed: 269.0010.

4-Chloro-2-nitrophenyl ethenesulfonate (20): pale yellow viscous liquid. R_f = 0.24 Hexane: EtOAc (9:1). Yield: 72%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.29 (d, *J*= 9.9Hz, 1H), 6.49 (d, *J*= 16.7Hz, 1H), 6.81 (dd, *J*= 16.7Hz and 9.9Hz, 1H), 7.52 (d, *J*= 8.9, 1H), 7.65 (dd, *J*= 8.9Hz and 2.5Hz, 1H), 8.01 (d, *J*= 2.5Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 126.1, 126.7, 131.9, 133.1, 133.4, 134.5, 139.8, 142.5. HRMS [ESI-MS +ve] calculated for $(C_8H_6ClNO_5S+Na)^+$: 285.9547, observed: 285.9547.

3, 4-Dichlorophenyl ethenesulfonate (21): colorless viscous liquid. R_f = 0.33. Hexane: EtOAc (9:1). Yield: 67%.^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.25 (d, *J*= 9.9Hz, 1H), 6.41 (d, *J*= 16.6Hz, 1H), 6.68 (dd, *J*= 16.6Hz and 9.9Hz, 1H), 7.12 (dd, *J*= 8.8Hz and 2.7Hz, 1H), 7.39 (d, *J*= 2.7Hz, 1H), 7.47 (d, *J*= 8.8Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 121.9, 124.5, 131.2, 131.5, 131.7, 132.8, 133.4, 147.6. HRMS [ESI-MS +ve] calculated for $(C_8H_6Cl_2O_3S+H)^+$: 252.9487, observed: 252.9489.

2, 4, 5-Trichlorophenyl ethenesulfonate (22): white powder. R_f = 0.48 Hexane: EtOAc (8:2). Yield: 59%.^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.26 (d, *J*= 8.5Hz, 1H), 6.45 (d, *J*= 16.1Hz, 1H), 6.77 (dd, *J*= 16.1Hz and 8.5Hz, 1H), 7.56 (s, 1H), 7.57 (s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 125.9, 126.3,131.4, 131.9, 132.1,132.7, 143.8. HRMS [ESI-MS +ve] calculated for $(C_8H_5Cl_3O_3S+H)^+$: 286.9098, observed: 286.9101.

2, 4, 6-Trichlorophenyl ethenesulfonate (23): white powder. R_f = 0.44 Hexane: EtOAc (9:1). Yield: 63%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.26 (d, *J*= 10.3Hz, 1H), 6.56 (d, *J*= 16.5Hz, 1H), 6.94 (d, *J*= 16.5 Hz and 10.3Hz, 1H), 7.4 (s, *2*H), ^{13}C NMR (100MHz, $CDCl_3$) δ ppm:

126.7, 128.3, 128.8, 130.6, 131.4, 139.5. HRMS [ESI-MS +ve] calculated for $(C_8H_5Cl_3O_3S+Na)^+$: 308.8917, observed: 308.8919.

Methyl 5-chloro-2-((vinylsulfonyl) oxy) benzoate (24): pale yellow viscous liquid. R_f = 0.23. Hexane: EtOAc (9.5:0.5). Yield: 60%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 3.95 (s, 3H), 6.18 (d, *J*= 10.0Hz, 1H), 6.37 (d, *J*= 16.7Hz, 1H), 6.81 (dd, *J*= 16.7Hz and 10.0Hz, 1H), 7.34 (d, *J*= 8.7Hz, 1H), 7.50 (d, *J*= 8.7Hz, 1H), 7.93 (s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 52.7, 125.5, 126.1, 131.8, 132.4, 132.9, 133.5, 133.88, 146.2, 163.6. HRMS [ESI-MS +ve] calculated for $(C_{10}H_9ClO_5S+Na)^+$: 298.9751, observed: 298.9751.

4-Fluorophenyl ethenesulfonate (25): colorless viscous liquid. R_f = 0.25. Hexane: EtOAc (9:1). Yield: 69%.^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.10 (d, *J*= 10.0Hz, 1H), 6.37 (d, *J*= 16.7Hz, 1H), 6.66 (dd, *J*= 10.0Hz and 16.7Hz, 1H), 7.10 (m, 2H), 7.22 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 116.6 (d, J_{C-F}= 29.0Hz,), 124.0 (d, J_{C-F}= 9.2 Hz,), 131.8, 132.1, 145.1 (d, J_{C-F}= 3.5Hz,), 161.7 (d, J_{C-F}= 247.3Hz, 1C). HRMS [ESI-MS +ve] calculated for $(C_8H_7FO_3S+H)^+$: 203.0173, observed: 203.0174.

4-Iodophenyl ethenesulfonate (26): colorless viscous liquid. R_f = 0.29. Hexane: EtOAc (9:1). Yield: 64%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 6.19 (d, *J*= 10.0Hz, 1H), 6.37 (d, *J*= 16.8Hz, 1H), 6.65 (dd, *J*= 16.8Hz and 10.0Hz, 1H), 6.99 (d, *J*= 8.7Hz, 2H), 7.70 (d, *J*= 8.7Hz, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 91.9, 124.4, 131.7, 132.3, 139.1, 149.2. HRMS [ESI-MS +ve] calculated for $(C_8H_7IO_3S+Na)^+$: 332.9053, observed: 332.9056.

2-Ethylphenyl ethenesulfonate (27): pale yellow viscous liquid. R_f = 0.37. Hexane: EtOAc (9:1). Yield: 64%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm: 1.22 (t, *J*= 7.6Hz, 3H), 2.71 (q, *J*= 7.6Hz, 2H), 6.14 (d, *J*= 9.9Hz, 1H), 6.38 (d, *J*= 16.6Hz, 1H), 6.72 (dd, *J*= 16.6Hz and 9.9Hz, 1H), 7.23 (m, 4H).^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 14.2, 23.1, 121.9, 127.1, 127.4, 130.1, 131.2, 132.7, 137.0, 147.6. HRMS [ESI-MS +ve] calculated for $(C_{10}H_{12}O_3S+Na)^+$: 235.0399, observed: 235.0401.

A.3 The synthetic scheme utilized in the development of cathepsin L inhibitor KD-1

Inhibitor **KD-1** was synthesized using the following scheme (Figure S1).

(i) **Synthesis of ethyl (diethoxyphosphoryl)methanesulfonate (I)** This compound was synthesized by following the literature reported protocol[2] and its characterization was found to be consistent with the previously reported NMR spectra[3].

(ii) **Synthesis of *tert*-butyl (4-methyl-1-oxopentan-2-yl)carbamate (II)** This compound was synthesized by following the literature reported protocol and its characterization was found to be consistent with the previously reported NMR spectra [4].

(iii) **Synthesis of ethyl (E)-3-((*tert*-butoxycarbonyl)amino)-5-methylhex-1-ene-1-**

sulfonate (III) This compound was prepared using a literature reported protocol with appropriate modification[5] . A solution of ethyl (diethoxyphosphoryl)methanesulfonate (483 mg, 1.86 mmol, 1 equiv) in anhydrous tetrahydrofuran (4 mL) was added dropwise to a stirring suspension of sodium hydride (58 mg, 2.41 mmol) in anhydrous tetrahydrofuran (3 mL) at 0°C under inert condition. After 30 min, a solution of N-BOC-L-leucinal in anhydrous tetrahydrofuran (5 mL) was added to the reaction mixture. Stirring was continued for an hour. The reaction was quenched by an addition of 5 mL of

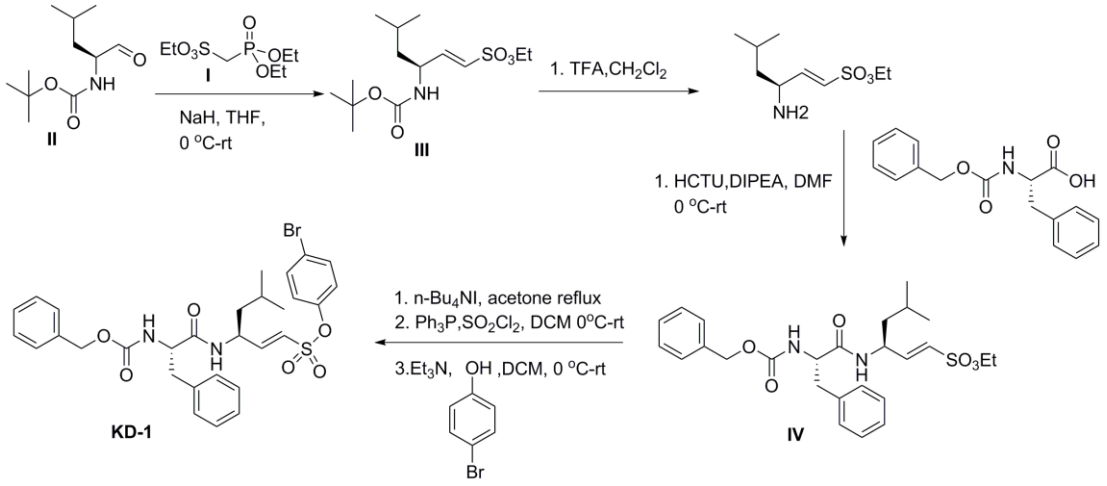


Figure S1. Scheme utilized for the synthesis of inhibitor **KD-1**

saturated ammonium chloride solution. The solution was concentrated under vacuum, diluted with water (10 mL), and extracted with ethylacetate (3×25 mL). The combined organic extracts were washed brine and dried over sodium sulfate, filtered, and concentrated under vacuum. Purification by flash column chromatography (silica gel; 90:10 hexane/ethylacetate) provided 419 mg (yield: 70%) of product. R_f = 0.6 (80:20 hexane/ethylacetate). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 0.94 (m, 6H), 1.37 (t, *J*= 6.9 Hz, 3H), 1.44 (s,11H), 1.70 (m,1H), 4.16 (q, *J*= 6.9 Hz, 2H), 4.37 (m,1H), 4.73 (m,1H), 6.31 (d, 15.2 Hz, 1H), 6.79 (dd, *J*= 15.2 and 5.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 15.1, 22.0, 23.1, 24.8, 28.3, 43.4, 49.7, 67.3, 80.1, 124.4, 149.5, 155.3. HRMS [ESI-MS +ve] calculated for $(C_{14}H_{27}NO_5S + Na)^+$: 344.1508, observed 344.1528.

(iv) **Synthesis of Ethyl (E)-3-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-5-methylhex-1-ene-1-sulfonate (IV)** To a stirred solution of the BOC-protected amine **III** (180 mg, 0.56 mmol) was added 25% solution of trifluoroacetic acid in dichloromethane (5 ml) and stirred for 1 hr. The solution was removed in vacuo. The residue was dissolved in DMF(2 ml). To this was added a cocktail of N-Cbz-L-phenylalanine (168 mg, 0.56 mmol), HCTU (232mg, 0.56 mmol) and *N*,*N*-diisopropylethylamine (375µl, 2.15 mmol) as a solution in anhydrous DMF (5 ml) at 0°C. Once the addition was done, ice bath was removed and the reaction was stirred overnight. The reaction was then diluted with 50 ml of ethyl acetate and washed with saturated

ammonium chloride solution (25 ml), saturated sodium bicarbonate solution, and with brine (25 ml). The organic layer was dried over sodium sulfate and concentrated in vacuo. Purification by flash column chromatography (silica gel; 80:20 hexane/ethylacetate) provided 169 mg (yield: 60%) of product. R_f =0.21(80:20 hexane/ethylacetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.86 (m, 6H), 1.31 (m, 2H), 1.37 (t, J= 6.9 Hz, 3H), 1.51 (m,1H), 3.05 (dd, J= 14.4 and 7.7 Hz, 1H), 3.11 (dd, J= 14.4 and 6.4 Hz, 1H), 4.13 (m, 2H), 4.38 (m, 1H), 4.62 (m, 1H), 5.08 (m, 2H), 5.35 (bs, 1H), 5.94 (d, 15.4 Hz, 2H), 6.61 (dd, J= 15.4 and 5.2 Hz, 1H), 7.17 (m, 2H), 7.32 (m,8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.9, 21.9, 22.7, 24.6, 38.2, 42.7, 48.0, 56.5, 67.0, 67.3, 124.5, 127.6, 128.2, 128.4, 128.6, 129.2, 129.2, 135.8, 135.9, 147.7, 156.1, 170.6. HRMS [ESI-MS +ve] calculated for $(C_{26}H_{34}N2O_6S + H)^+$: 503.2217, observed 503.2218.

(v) **Synthesis of 4-bromophenyl (E)-3-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-5-methylhex-1-ene-1-sulfonate (KD-1)** This compound was prepared using a literature reported protocol with appropriate modification [6]. To a stirred solution of **IV** (150 mg, 0.3 mmol) in acetone was added tetrabutyl ammonium iodide (45 mg, 0.3 mmol) and refluxed for overnight. The mixture was concentrated in vacuo and was dissolved in anhydrous dichloromethane (2 ml). This was added to a stirred solution of triphenyl phosphine (157 mg, 0.6 mmol) and sulfuryl chloride (53 µl, 0.664 mmol) in anhydrous dichloromethane (4 ml) at 0°C. Once the addition was done, ice bath was removed and the reaction was stirred overnight. The mixture was then concentrated in vacuo and passed through silica (oven dried) using hexane and ethyl acetate (90:10) to recover sulfonyl chloride as white solid. Subsequently, the sulfonyl chloride (100mg, 0.23 mmol) was dissolved in anhydrous dichloromethane (3 ml). 4-bromophenol (60 mg, 0.345 mmol) was added to the solution followed by addition of triethylamine (100 µl, 0.69 mmol) at 0°C. The mixture was stirred for one hr and diluted with ethyl acetate (30 ml) followed by washing with saturated ammonium chloride solution (15 ml), saturated sodium bicarbonate solution (15ml ×3), and with brine (15 ml). The organic layer was dried over sodium sulfate and concentrated in vacuo. Purification by flash column chromatography (silica gel; 90:10 hexane/ethylacetate) provided 40 mg (overall yield: 22%) of the product as white solid. R_f =0.37 (80:20 hexane/ethylacetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.82 (m, 6H), 1.22 (m, 2H), 1.41 (m, 1H), 3.0 (dd, J= 13.7 and 7.9 Hz, 1H), 3.11 (dd, J= 13.7 and 6.4 Hz, 1H), 4.38 (m,1H), 4.52 (m, 1H), 5.07(m, 2H), 5.4 (bs,1H), 5.93(bs, 1H), 6.03(d, J=15.1 Hz, 1H), 6.46(dd, J= 15.1 and 5.2 Hz, 1H), 7.08 (m, 2H), 7.13 (m, 2H), 7.33 (m, 8H), 7.51 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.8, 22.5, 24.5, 38.3, 42.2, 48.2, 56.6, 67.4 120.8, 123.7, 124.5, 127.6, 128.2, 128.4, 128.6, 129.1, 129.1, 132.9, 135.8, 135.8, 148.3, 150.1, 156.1, 170.6. HRMS [ESI-MS +ve] calculated for $(C_{30}H_{33}BrN_2O_6S +H)^+$: 629.1322, observed 629.1315.

B. Screening and Enzymology

B.1 General:

Following enzymes and their substrates were purchased from Enzo Life Sciences (Farmingdale, NY, USA): cathepsin L (BML-SE201), cathepsin K (BML-SE553), cathepsin B (BML-SE198), cathepsin D (BML-SE199), cathepsin S (BML-SE453), cathepsin H (BML-SE200), cathepsin G (BML-SE283), human PTP1B (BML-SE332), Z-Arg-Arg-pNA (BML-P138), Z-Gly-Pro-Arg-AMC (BML-P142), Z-Phe-Arg-AMC (BML-P139), H-Arg-AMC.2HCl (BML-P135), Suc-Ala-Ala-Pro-Phe-pNA (BML-P141), Mca-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH_2 (BML-P145), Z-Val-Val-Arg-AMC (BML-P199). N-alpha-Benzoyl-DL-arginine-p-nitroanilide (BAPNA) (B4875), trypsin (T1426), para-nitrophenylphosphate were purchased from Sigma–Aldrich (St. Louis, MO, USA). All the biological grade buffers were used and were purchased from Sigma–Aldrich (St. Louis, MO, USA). All enzyme kinetics experiments (unless otherwise stated) were carried out at 30°C in appropriate buffer condition with 5% DMSO concentration. For measuring the initial rates of enzyme catalyzed reaction, a temperature-controlled steady-state arc lamp fluorometer equipped with Felix 32 software (Photon Technology Instrument, Birmingham, NJ, USA) and a UV–Vis spectro-photometer (Modelk25; Perkin Elmer Inc., Waltham, MA, USA) was used.

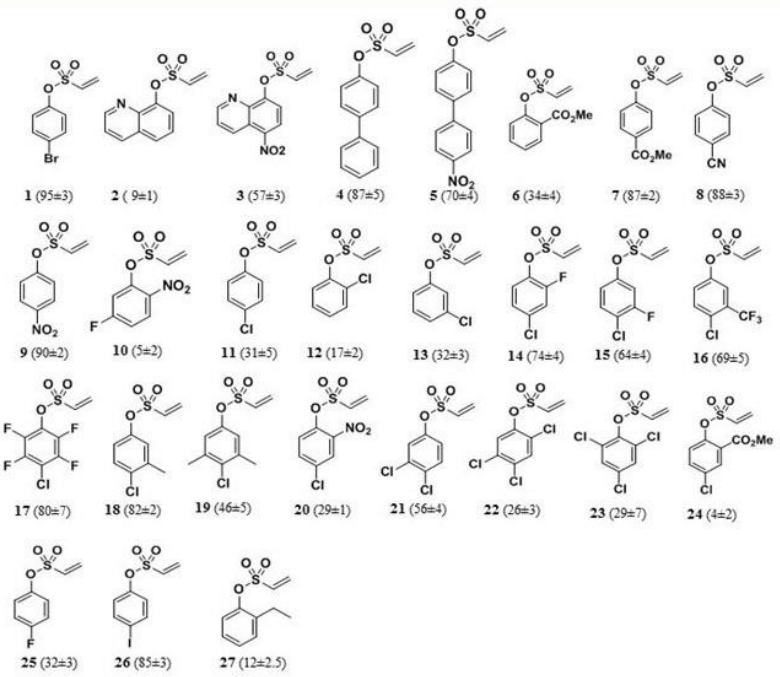


Figure S2. Chemical structures of the synthesized aryl vinylsulfonate ester compound library (1-27), designed to target the prime site residues of cathepsin L. Time-dependent inhibitory screen was performed under pseudo-first order condition at 10 μM inhibitor concentration. In parenthesis, % inhibition is calculated from two independent experiments and compound 1 is identified as the lead inhibitor.

B.2 Screening of aryl vinylsulfonate library compounds:

The inactivation reaction of human liver cathepsin L (net 50 nM) by 10 μM of inhibitor was performed under pseudo first order condition ([I]>>[E]) in a 0.5 ml eppendorf tube maintained at 30°C in a temperature controlled bath. A previously reported cathepsin L assay

procedure was employed [7, 8]. Cathepsin L enzyme was first activated for 10 min in sodium acetate buffer (400 mM, pH 5.5) containing 8 mM DTT and 4 mM Na_2EDTA. The inactivation reaction was initiated by the addition of inhibitor where DMSO concentration was maintained at 5%. After suitable time intervals, an aliquot of 8µl of incubation mixture was withdrawn and added to an assay mixture (net assay volume 200µl) that contained 5 µM of Z-FR-AMC (K_m= 2.2 µM) substrate at 30°C. A progress curve was recorded (excitation: 365 nm; emission: 440 nm) and the enzyme activity was determined by measuring the initial rates of substrate turnover (Figure S2).

B.3 Determination of IC_{50} of KD-1 against cathepsin L:

Briefly the IC_{50} experiment was performed as follows: Active cathespsin L (160 pM, as determined by active site titration using E-64 [9]) was added to acetate assay buffer that contained KD-1 (concentration ranging from 0 pM to 15 nM) and Z-FR-AMC (5 µM) at 30°C. Net DMSO concentration was maintained at 8%. Cathepsin L activity was monitored fluorometrically (excitation: 365 nm; emission: 440 nm) for 100 seconds at 30°C. The experimental data thus obtained were plotted using SigmaPlot software and fitted using Enzyme Kinetics module to obtain the IC_{50} value (Figure S3).

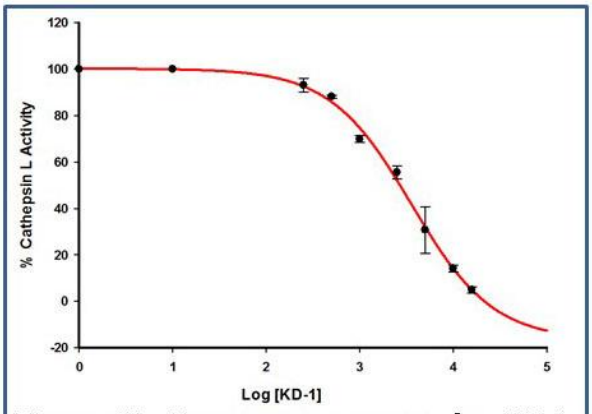


Figure S3. Dose–response curves for **KD-1**-mediated inhibition of human liver cathepsin L. The points are experimental and the line joining them is the fit to obtain the IC_{50} values as 3.6 ± 0.1 nM.

B.4 Determination of kinetic inactivation parameters of KD-1 against cathepsin L:

The inactivation reaction of human liver cathepsin L (net 160 pM) by appropriate concentrations of KD-1 was performed under pseudo first order condition ([I]>>[E]) in a fluorescence quartz cuvette maintained at 30°C. Briefly, the inactivation reaction was performed by subsequent addition of active cathespsin L (160 pM) and 5 µM of Z-FR-AMC in acetate buffer (pH 5.5) containing KD-1 (concentration ranging from 1 nM to 20 nM). Net DMSO concentration was maintained at 8%. The reaction was monitored fluorometrically (excitation: 365 nm; emission: 440 nm) for 3 minutes at 30°C. Subsequently, the progress curves were analyzed using non-linear regression according the following equation [10]:

$$P = v_z [1- \exp (-k_{obs} * t)] / k_{obs};$$

where [P] is the product, v_z is the initial velocity (at time zero), and k_{obs} is the pseudo-first order rate constant.

Pseudo-first order rate constants k_{obs} were plotted against appropriate inhibitor concentrations [I], and 2nd order inactivation rate constant k_{inact} was calculated from the slope of the plot according to following equation [10] (Figure S4);

$$k_{obs} = k_{inact}[I] / [1+ ([S]/K_M)]$$

where k_{obs} is the pseudo-first order rate constant, [I] is the inhibitor concentration, [S] is the substrate concentration, with a given K_M . The data were analyzed using KaleidaGraph (version 3.52).

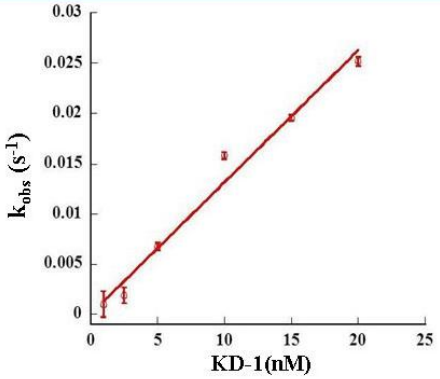


Figure S4. Determination of second order inactivation rate constant (k_{inact}) for **KD-1**-mediated inhibition of human liver cathepsin L. The k_{obs} obtained from the progress curve fit were plotted against appropriate inhibitor concentrations [**KD-1**]. The slope of the line yielded the enzyme inactivation rate constant k_{inact}.

B.5 Selectivity profile of KD-1 against other enzymes

Determination of kinetic inactivation parameters of KD-1 against cathepsin S

A previously reported cathepsin S assay procedure was followed with suitable modification [11]. Thus, recombinant human cathepsin S enzyme was activated in the 100 mM potassium phosphate buffer (pH 6.5) containing 2.5 mM DTT, 2.5 mM Na_2EDTA for 10 min. The inactivation reaction of cathepsin S (net 1 nM) by appropriate concentrations of KD-1 was performed under pseudo first order condition ([I]>>[E]) in a fluorescence quartz cuvette maintained at 30°C. Briefly, the inactivation reaction was initiated by subsequent addition of active cathepsin L and 50 μM of Z-VVR-AMC (K_M = 17.7 μM) in phosphate buffer (pH 6.5) containing KD-1 (concentration ranging from 20 nM to 200 nM). Net DMSO concentration was maintained at 8%.. The reaction was monitored fluorometrically (excitation: 365 nm; emission: 440 nm) for 3 minutes at 30°C. The progress curves were analyzed using the same equations used for cathepsin L assay.

Cathepsin K inhibition assay

For assaying cathepsin K activity, a previously published protocol was followed with appropriate modification [12]. Thus, recombinant human cathepsin K enzyme was activated in the 50 mM sodium acetate buffer (pH 5.5) containing 2.5 mM DTT, 2.5 mM Na_2EDTA for 10 min. The inactivation reaction of cathepsin K (net 50 nM) with inhibitor KD-1(250 nM) was initiated under pseudo first order condition ([I]>>[E]). After suitable time intervals, an aliquot of 10 μl of incubation mixture was withdrawn and the enzyme activity was monitored fluorometrically in assay buffer (200 μl; 7.5% DMSO) containing 200 μM of Z-GPR-AMC (K_M =68 μM; Ex/Em: 365/440 nm). The data thus obtained was plotted and fitted to the following equation:

$$A_t = A_f – (A_f – A_0) \exp(-k_{obs}*t)$$

where A_t = enzyme activity at time t of cathepsin K inactivation, A_f = final cathepsin K activity at infinite time of inactivation, A_0 = initial enzyme activity at zero time of inactivation, and k_{obs} = pseudo-first order rate constant of inactivation. The data were analyzed using KaleidaGraph (version 3.52).

Cathepsin H inhibition assay

For assaying cathepsin H activity, a previously published protocol was followed with appropriate modification [7]. Thus, human liver cathepsin H enzyme was activated in the 200 mM KH_2PO_4 / 200 mM Na_2HPO_4 buffer (pH 6.8) containing 5 mM DTT, 2 mM Na_2EDTA for 10 min. The inactivation reaction of cathepsin H (net 330 nM) with inhibitor KD-1 (100 μM) was initiated under pseudo first order condition ([I]>>[E]). After suitable time intervals, an aliquot of 10 μl of incubation mixture was withdrawn and the enzyme activity was monitored fluorometrically in assay buffer (200 μl; 8% DMSO) containing 300 μM of H-R-AMC.2HCl (K_M = 150 μM; Ex/Em: 365/440 nm).

*Assays involving cathepsin B, cathepsin D, cathepsin G, trypsin and PTP1B were carried out according to the literature reported protocol [13].

C. Biological and Cell-Based Studies

Inhibition of Cathepsin L's Collagenase Activity by KD-1

This experiment was carried out by following slight modification of a literature reported protocol [14]. Human skin type I collagen (Calbiochem) was dissolved in 100 mM acetic acid for 24 h at room temperature to give a final concentration of 1.0 mg/ml. In 500 μl eppendorf tubes, different concentrations of KD-1 (0 nM, 300 nM, 500 nM and 1000 nM) were incubated with 200 nM of active cathepsin L (Assay Condition: 100 mM sodium acetate pH 5.5, 1 mM EDTA, 3 mM DTT, 2% DMSO, 30°C) for 30 minutes. Reaction was initiated by addition of 6 μg of collagen (Total reaction volume 26 μl). Reactions were quenched after 6 days by addition of 10 μl of Laemmli's SDS- Sample Buffer (4X, reducing) and heated to 95°C for 10 minutes. 20 μl of reaction mixtures were (3.3 μg of type I collagen) loaded to 4-15% gradient Bio-Rad Mini-Protean® TGX™ Gels. Gels were stained in Coomassie Brilliant Blue, G250 and imaging was performed using scanner.

Cell culture: All the cell culture reagents were purchased from Invitrogen (Carlsbad, CA, USA). MDA-MB-231cells (American Type Culture Collection, Manassas, VA) were cultured on 10-cm plates (BD biosciences) at 37°C and 5% CO_2 in Iscove's Modified Dulbecco's medium with L-glutamine (IMDM), 10% fetal bovine serum, and antibiotics (1% penicillin/streptomycin and 0.5μg/ml fungizone) as described earlier [15]. Cells were passaged at

1:3 ratio every 3–4 days and subcultured by 0.25% trypsin-EDTA treatment. Cells were re-plated one day prior to experiment so that the cell density was 80-90% on the day of the experiment.

Confocal microscopy

For live-cell imaging, cells were trypsinized at ~ 80% confluency, seeded to Poly-D-lysine coated 35mm-glass bottom dishes (MatTek; Ashland, MA) and grown overnight in complete medium at 37°C, as described previously (Dana et al. 2013). Cells were treated with (0.05% Dimethyl-sulfoxide, DMSO) or Cathepsin-L inhibitor (750nM) inhibitors overnight, as mentioned in the text. Prior to imaging, cells were treated with the substrate (Z-FR-AMC) for 2-3 mins.

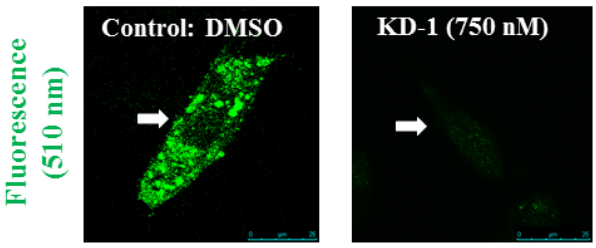


Figure S5. Another representative image (in addition to the one shown in Figure 2B) indicating the effect of **KD-1** on intracellular cathepsin L activity in metastatic MDA-MB-231 breast cancer cell line.

Images of live cells were acquired using an inverted Leica TCS-SP5 confocal microscope (Leica; IL) by Plan apochromat 63x1.4 oil objective lens. An argon ion laser (25%) was used to generate excitation at 488nm, and pinholes were typically set to 1–1.5 Airy units.

Wound-healing cell migration assay: For wound healing experiments, MDA-MB-231 cells were grown to confluence in a 6-well plate. Prior creating wound with a sterile pipette tip, the cells were treated with the vehicle control (0.05% Dimethyl-sulfoxide, DMSO) or Cathepsin-L inhibitor (750nM) and maintained at 37°C. Floating cells were removed by washing once with PBS. Images were captured by 4x objective at different time intervals.

Images were captured right after the scratch ('wound'), t=0h, to record the initial area of the wounds, followed by image acquisition at t=22hrs to measure the recovery of the wounded area by cell migration under different conditions. The area of the 'wound' was quantified by ImageJ software1.4 by polygon selection mode [16 17]. The migration of cells towards the denuded area ('wound') was expressed as a percentage of wound closure as:

$$\% \text{ of wound closure} = [(A_{t=0\,h} - A_{t=\Delta\,h})/A_{t=0\,h}] \times 100\%,$$

where $A_{t=0\,h}$ is the area of the wound measured immediately after the scratch, and $A_{t=\Delta\,h}$ is the area of the wound after 22 hrs.

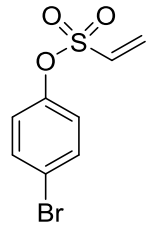
Statistical analysis

Each experiment was performed a minimum of three times, and differences between groups were determined using the unpaired Student's *t* test. The data were evaluated as the mean $\pm$ 1 s.d.

C. Appendix

^{1}H and ^{13}C NMR spectra:

4-Bromophenyl ethenesulfonate (1):



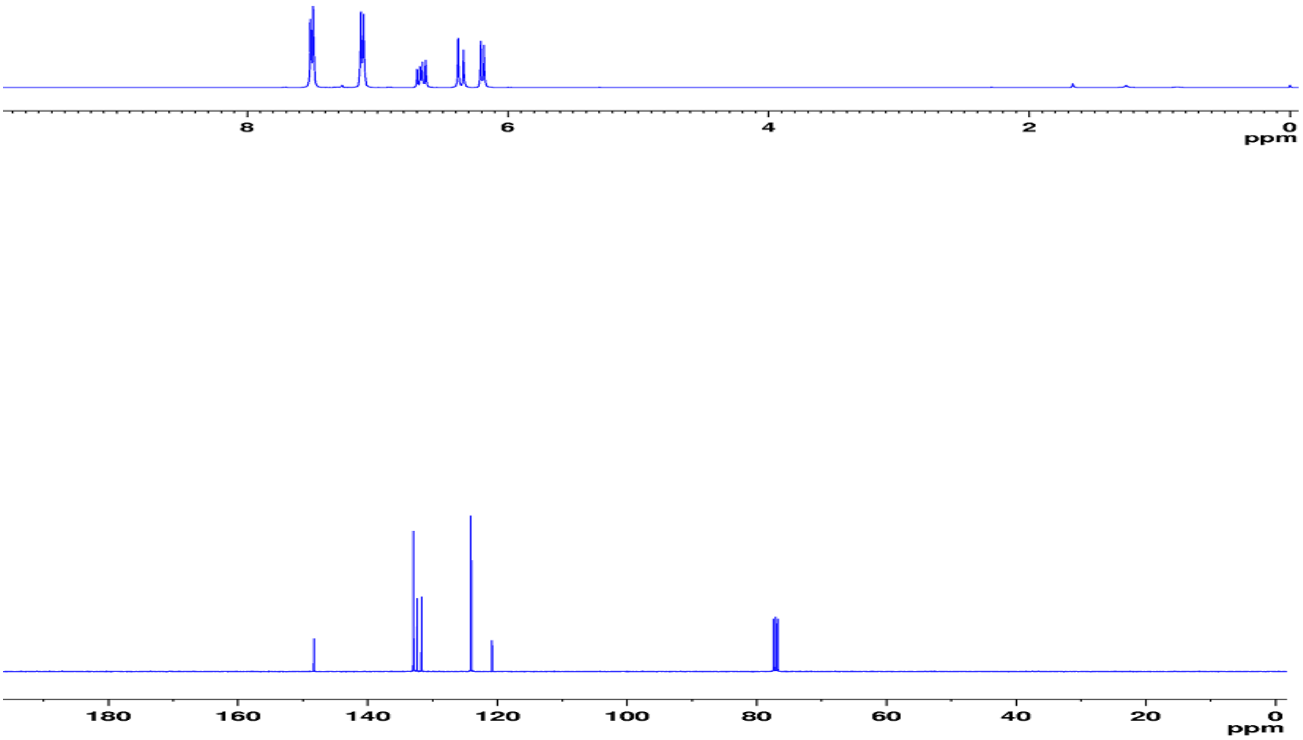


Figure: ^{1}H and ^{13}C NMR spectrum of 1

Quinolin-8-yl ethenesulfonate (2):

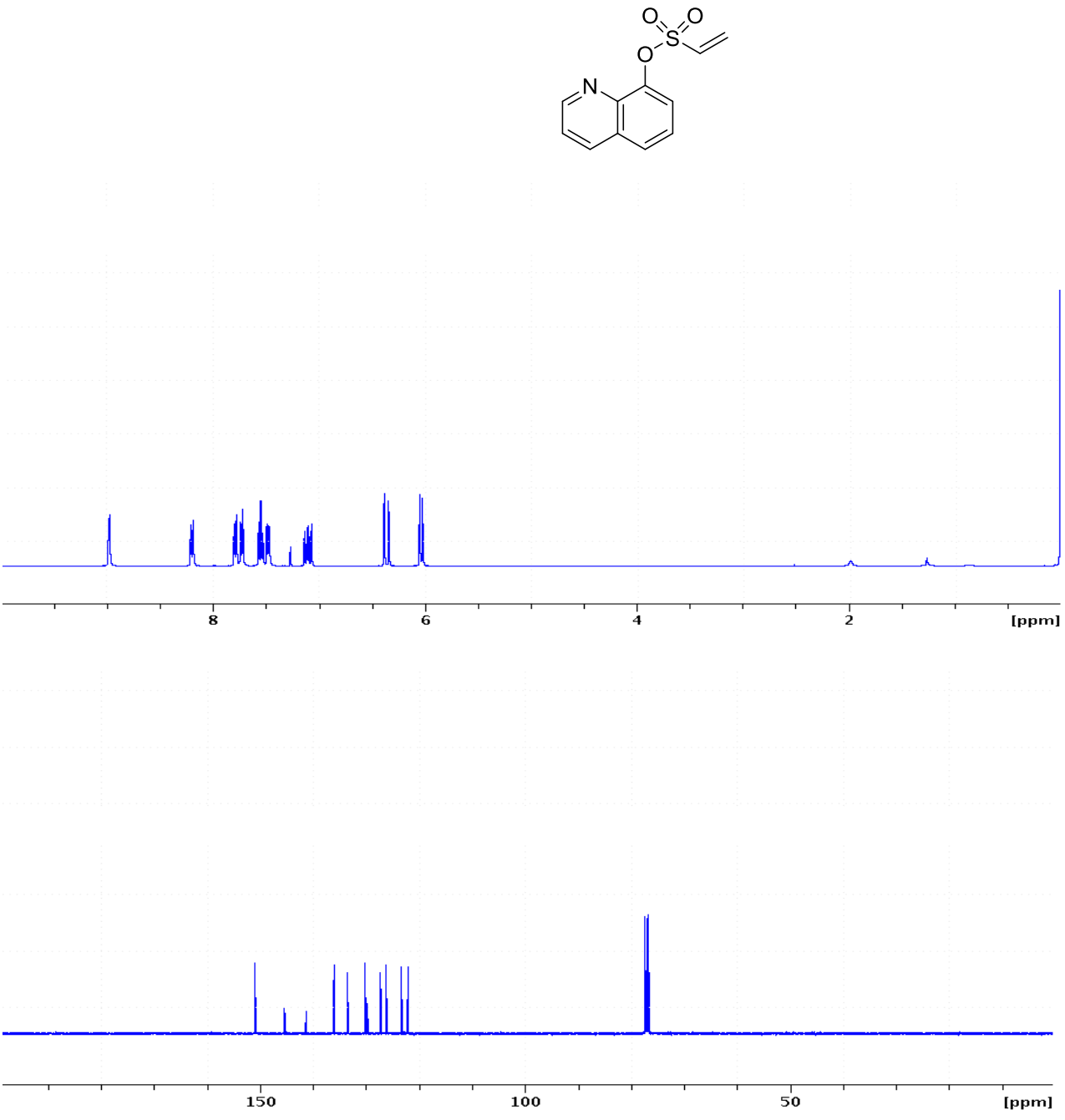


Figure. ^{1}H and ^{13}C NMR spectrum of 2.

5-Nitroquinolin-8-yl ethenesulfonate (3):

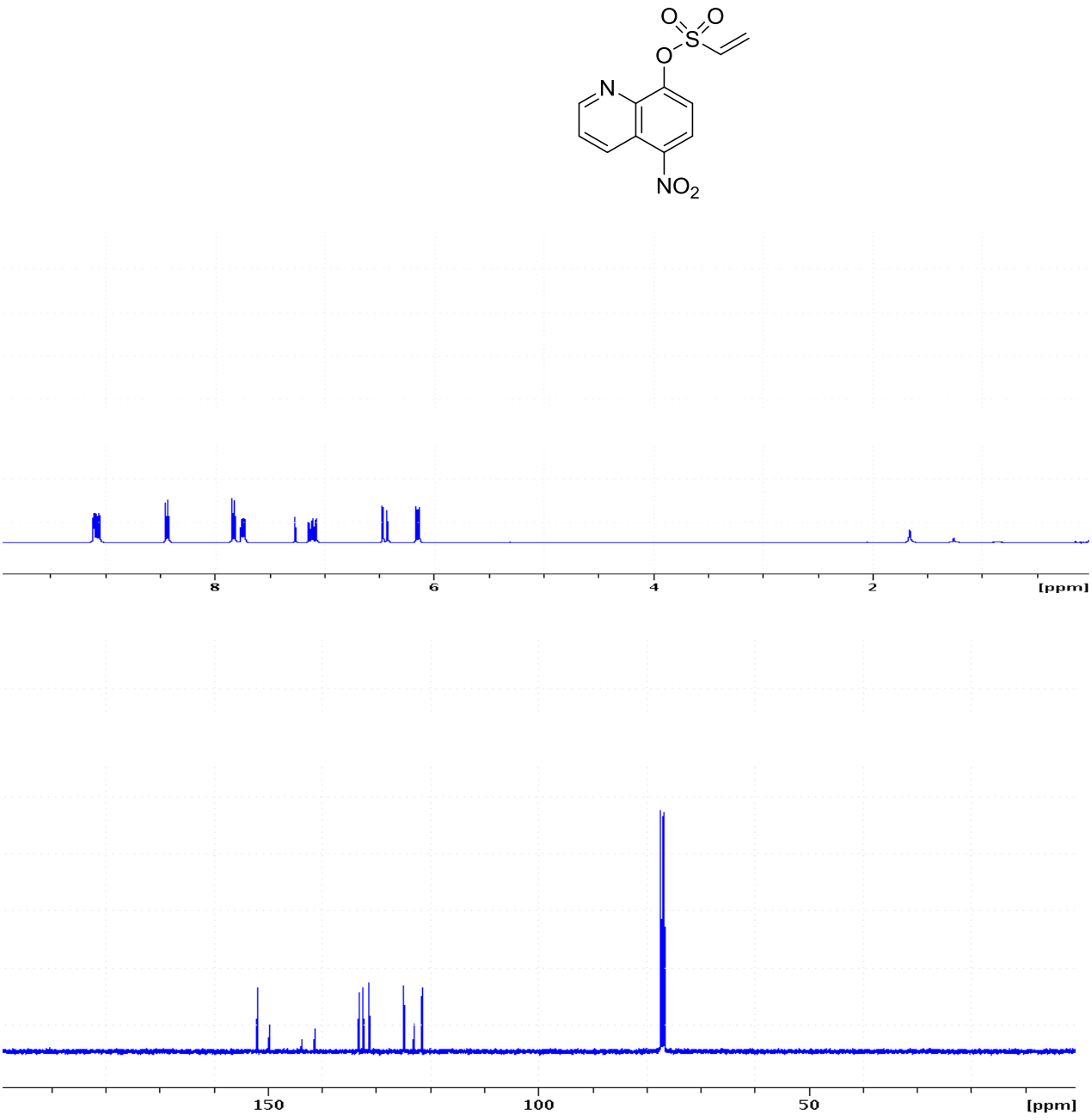


Figure. ^{1}H and ^{13}C NMR spectrum of 3.

[1,1’-Biphenyl]-4-yl ethenesulfonate (4):

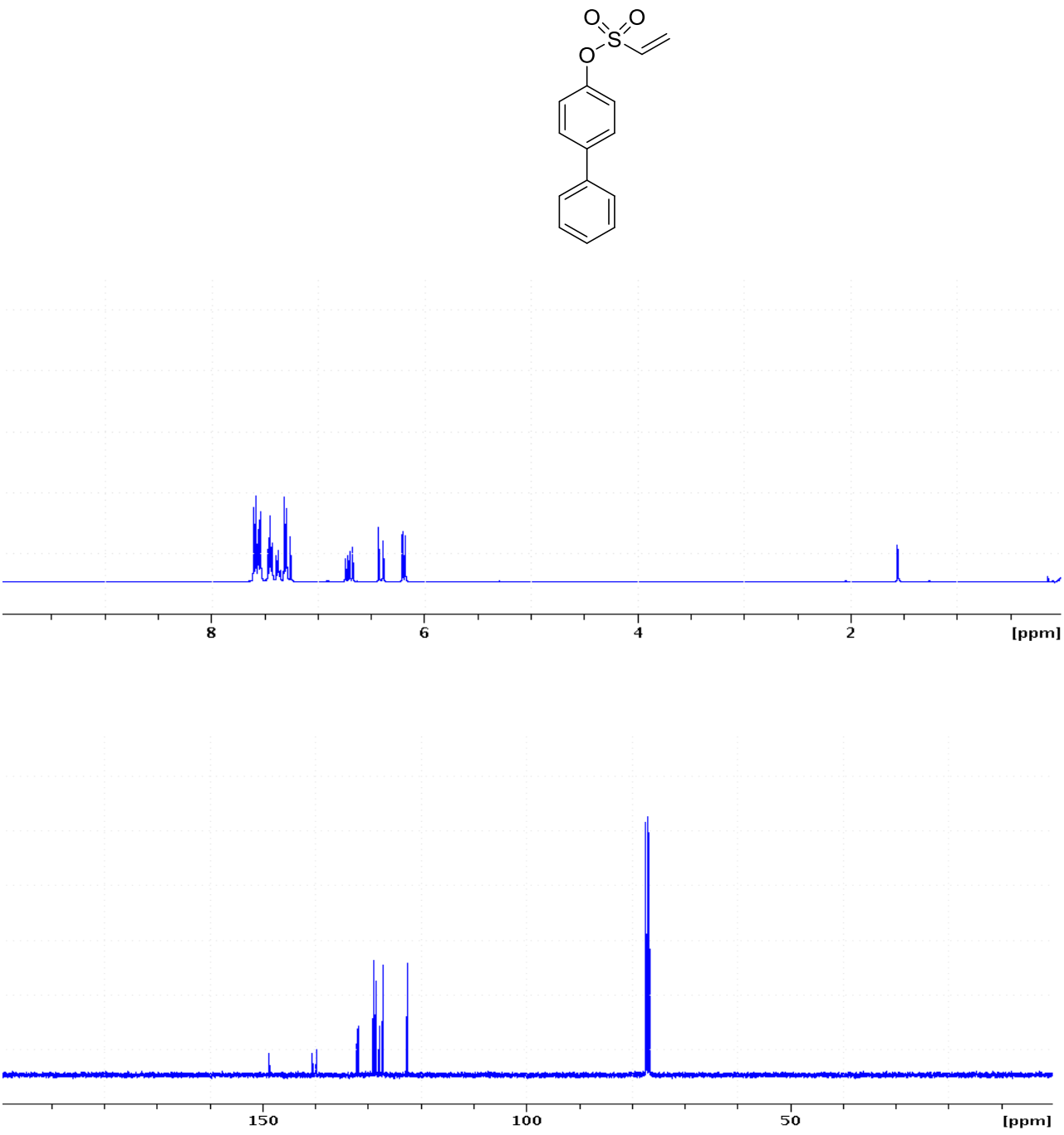


Figure. ^{1}H and ^{13}C NMR spectrum of 4.

4’-Nitro-[1, 1’-biphenyl]-4-yl ethenesulfonate (5):

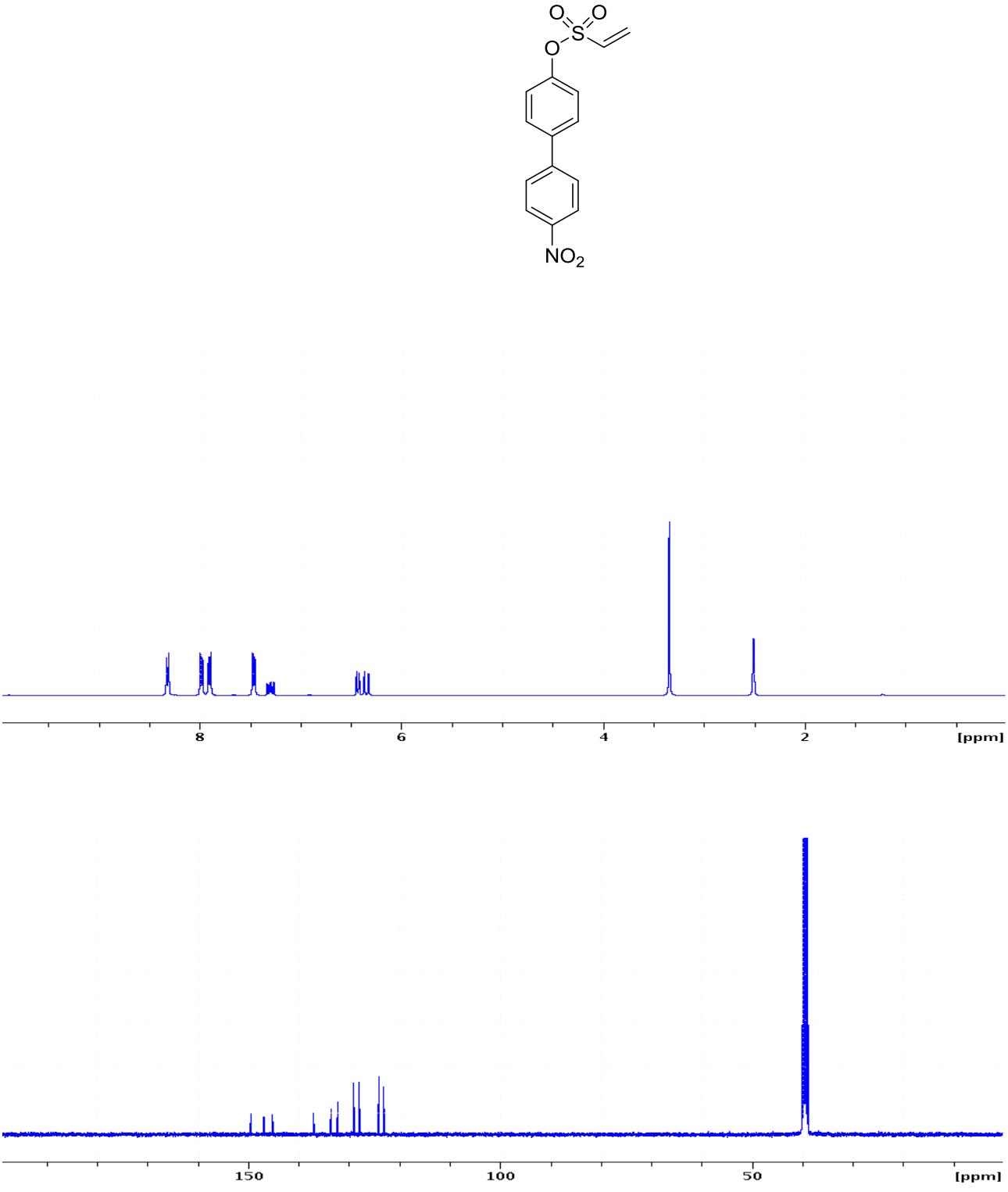


Figure. ^{1}H and ^{13}C NMR spectrum of 5.

Methyl 2-((vinylsulfonyl) oxy) benzoate (6):

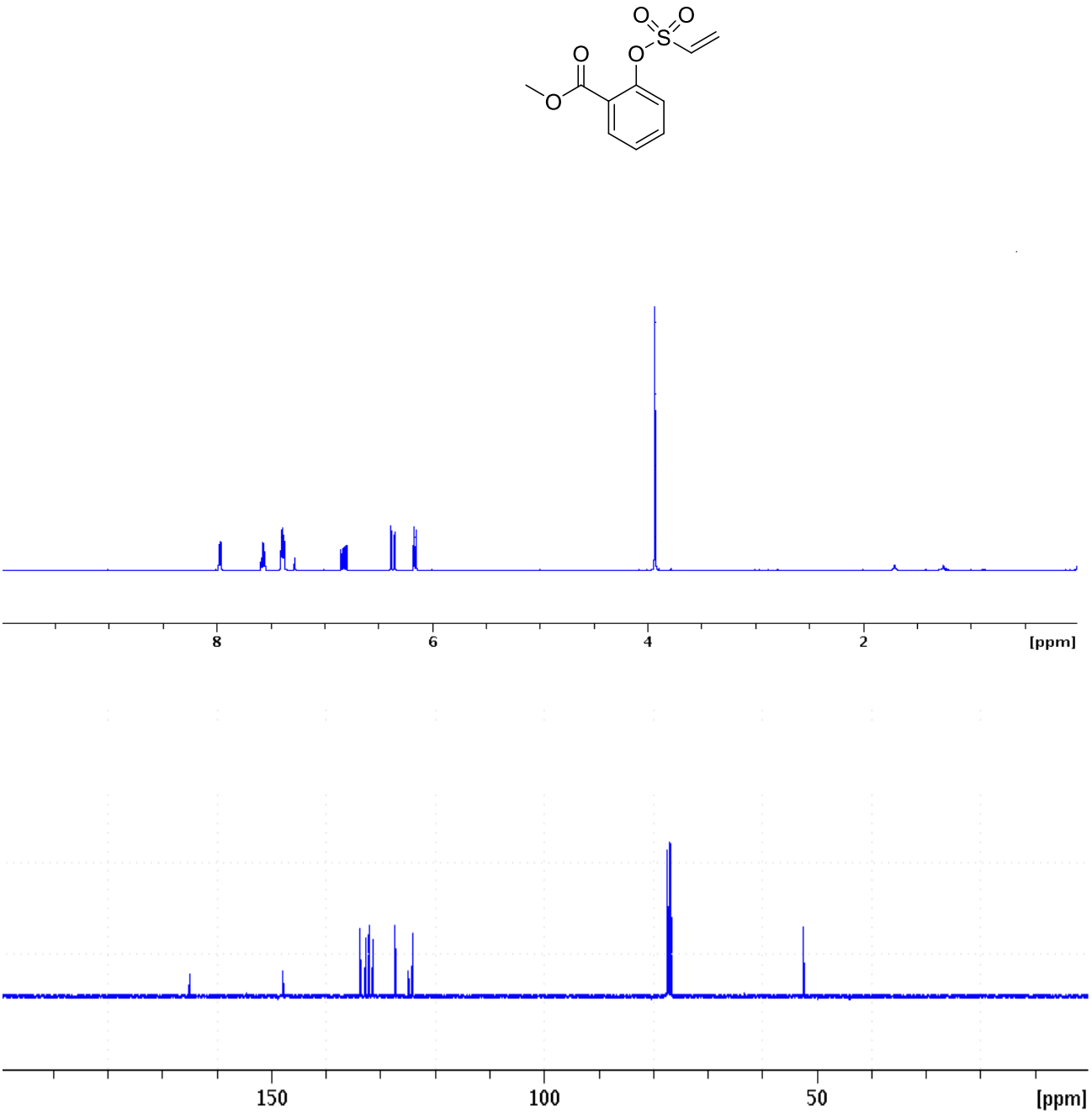


Figure. ^{1}H and ^{13}C NMR spectrum of 6.

Methyl 4-((vinylsulfonyl) oxy) benzoate (7):

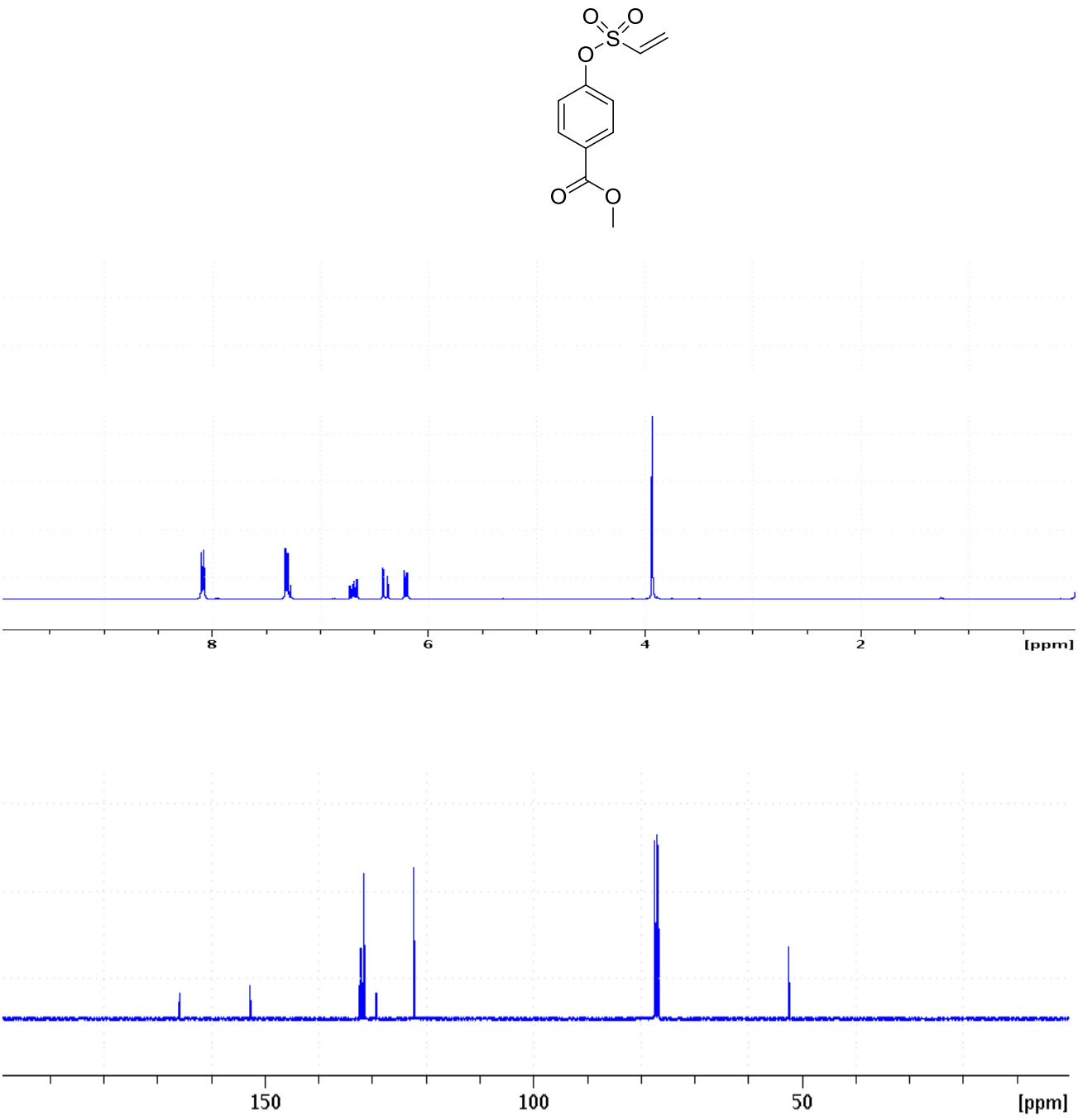


Figure. ^{1}H and ^{13}C NMR spectrum of 7.

4-Cyanophenyl ethenesulfonate (8):

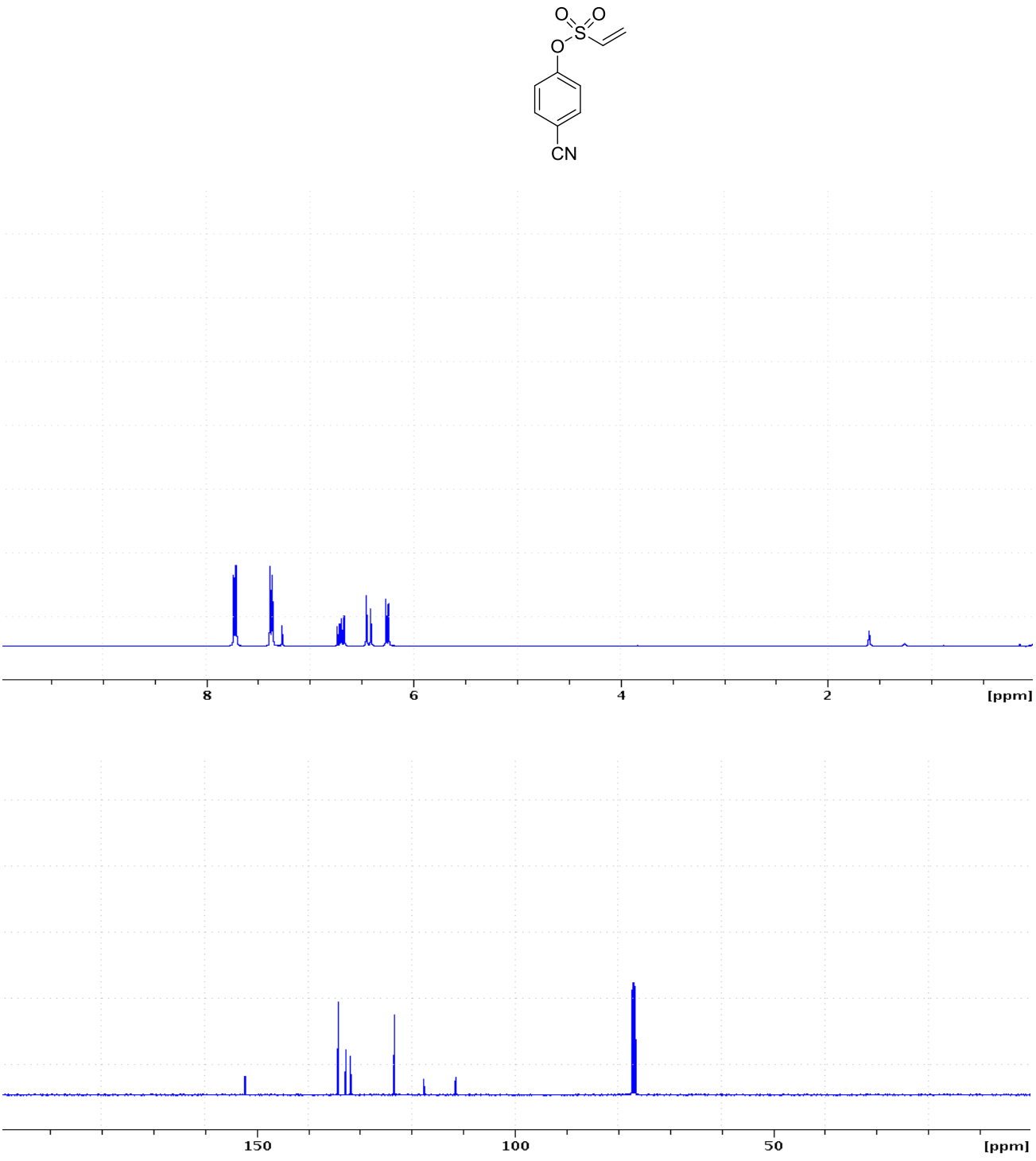


Figure. ^{1}H and ^{13}C NMR spectrum of 8.

4-Nitrophenyl ethenesulfonate (9):

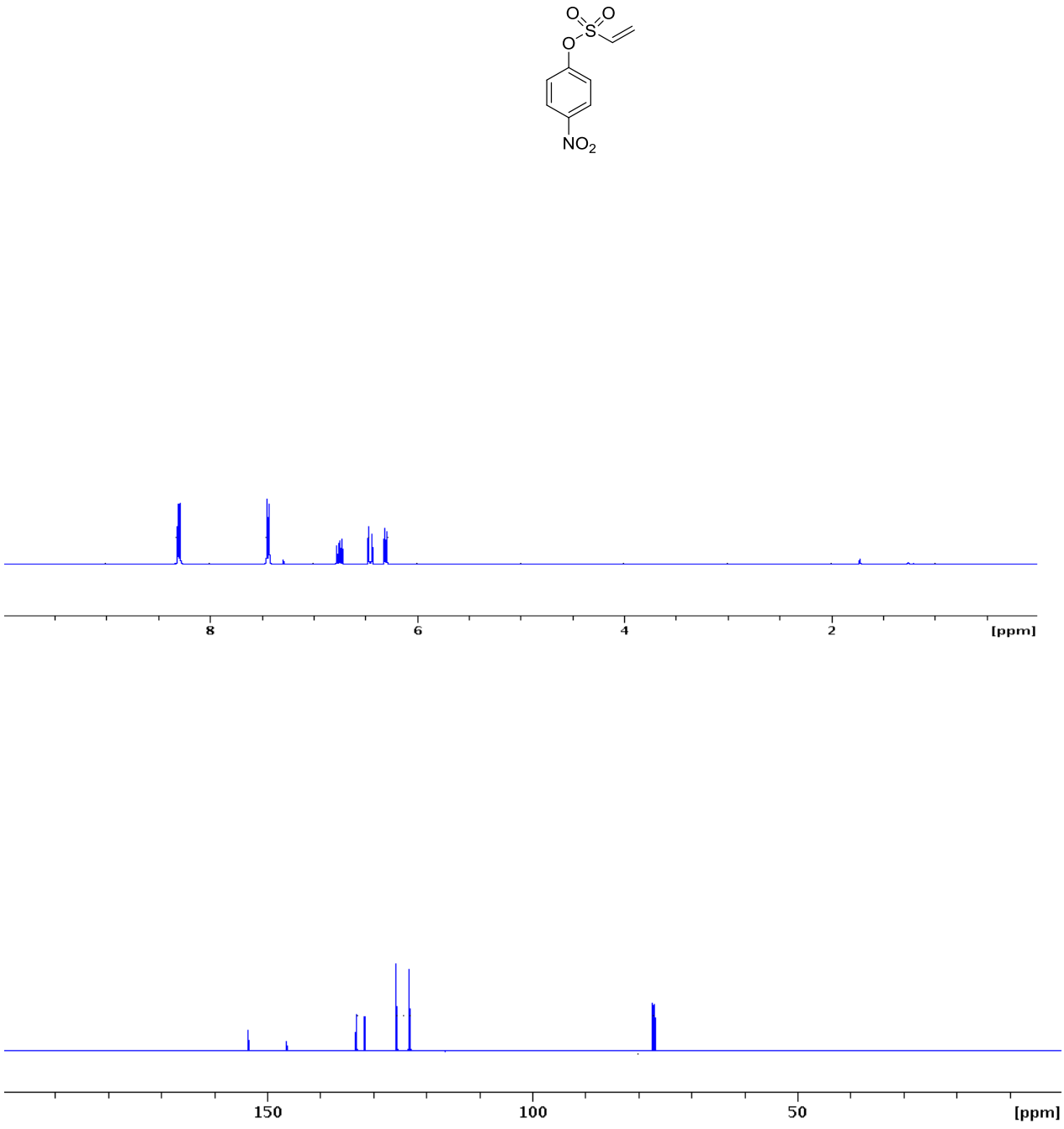
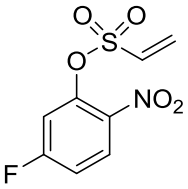


Figure. ^{1}H and ^{13}C NMR spectrum of 9.

5-Fluoro-2-nitrophenyl ethenesulfonate (10):



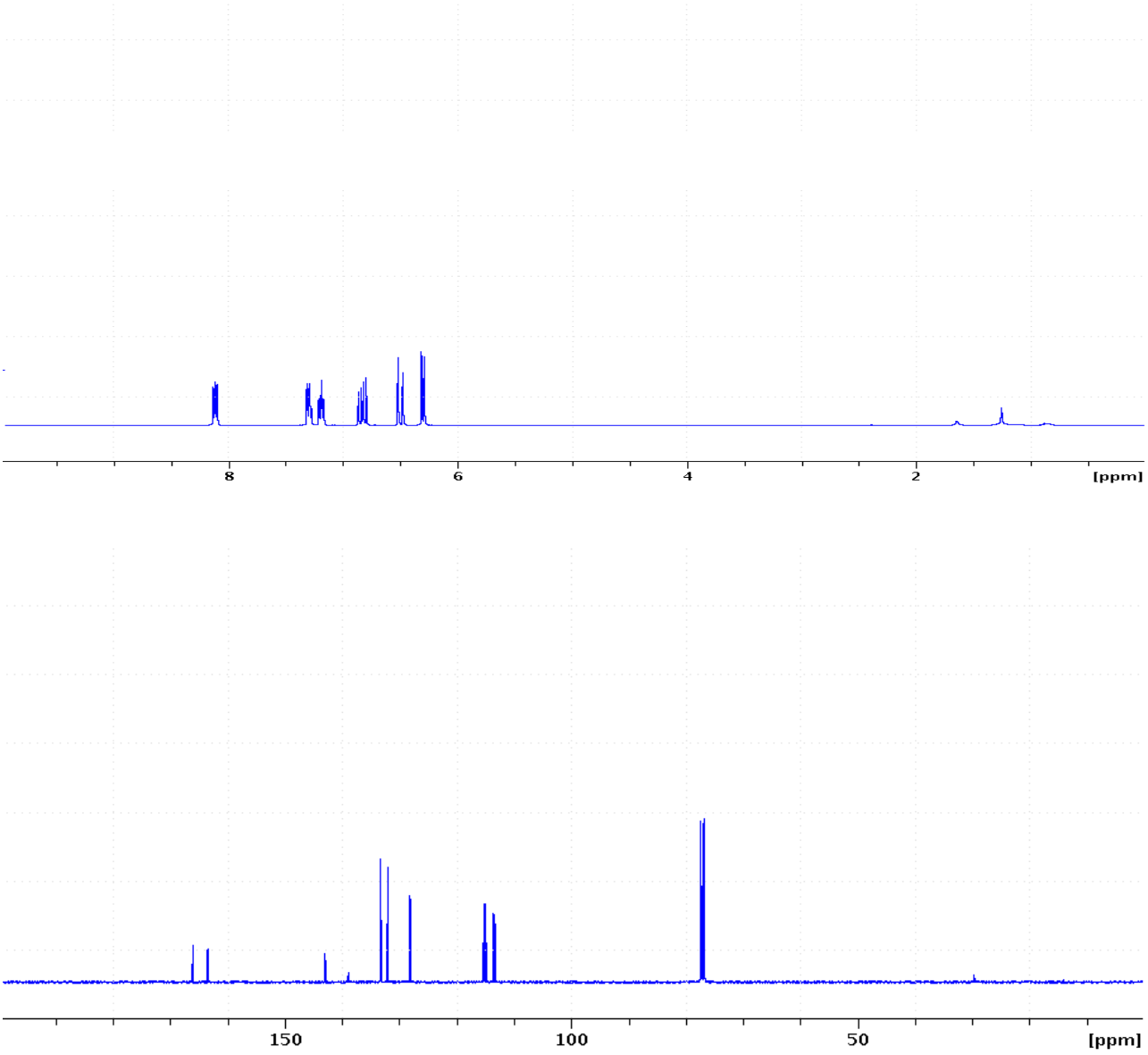
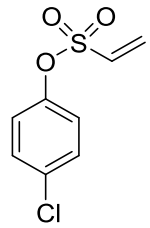
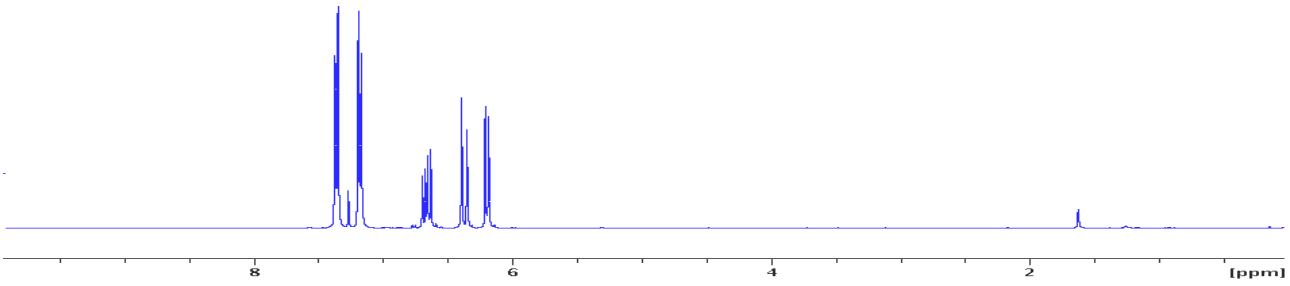


Figure. ^{1}H and ^{13}C NMR spectrum of 10

4-Chlorophenyl ethenesulfonate (11):





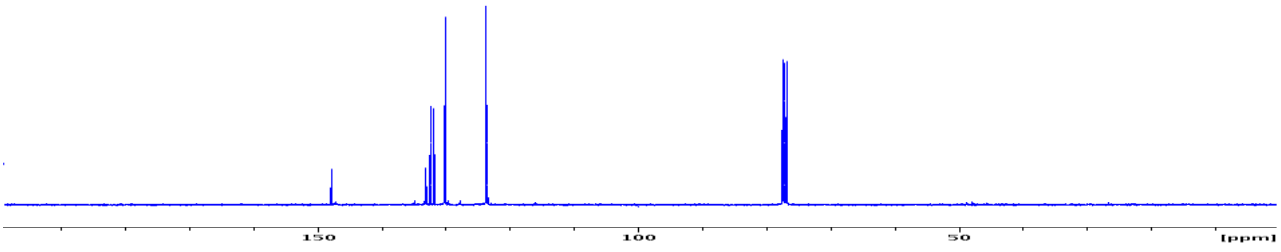
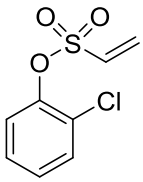


Figure. ^{1}H and ^{13}C NMR spectrum of 11

2-Chlorophenyl ethenesulfonate (12):



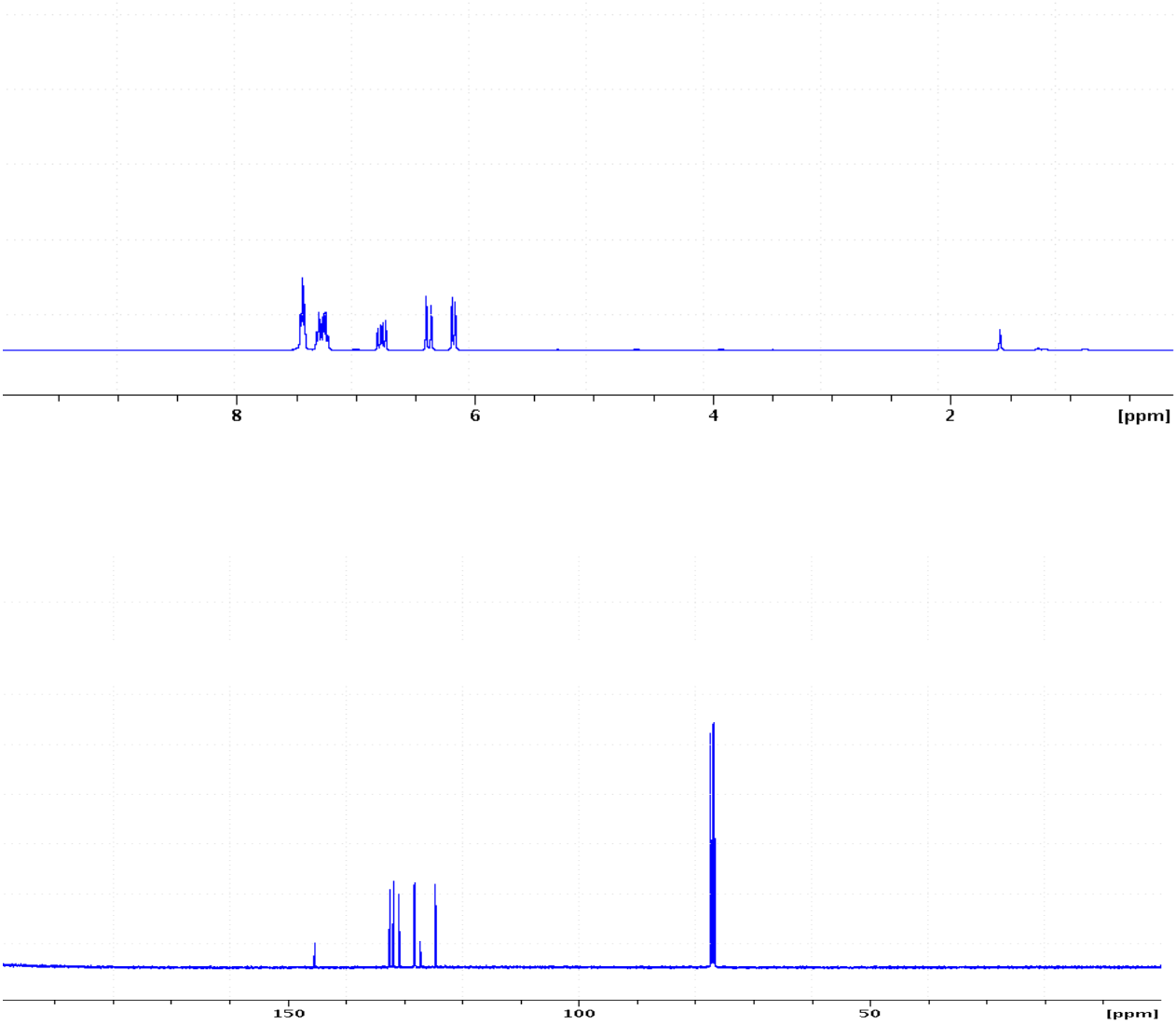


Figure. ^{1}H and ^{13}C NMR spectrum of 12.

3-Chlorophenyl ethenesulfonate (13):

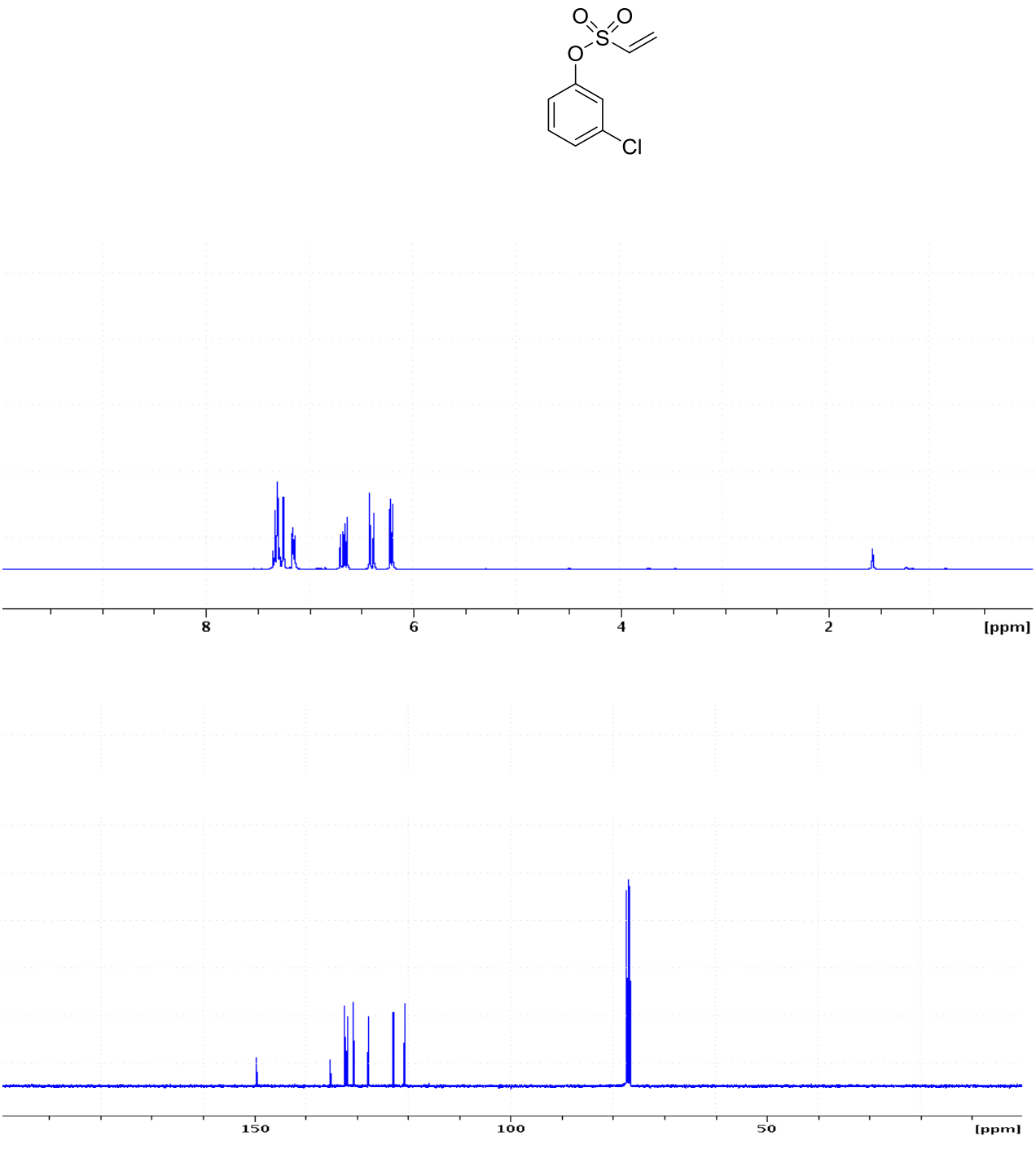


Figure. ^{1}H and ^{13}C NMR spectrum of 13

4-Chloro-2-fluorophenyl ethenesulfonate (14):

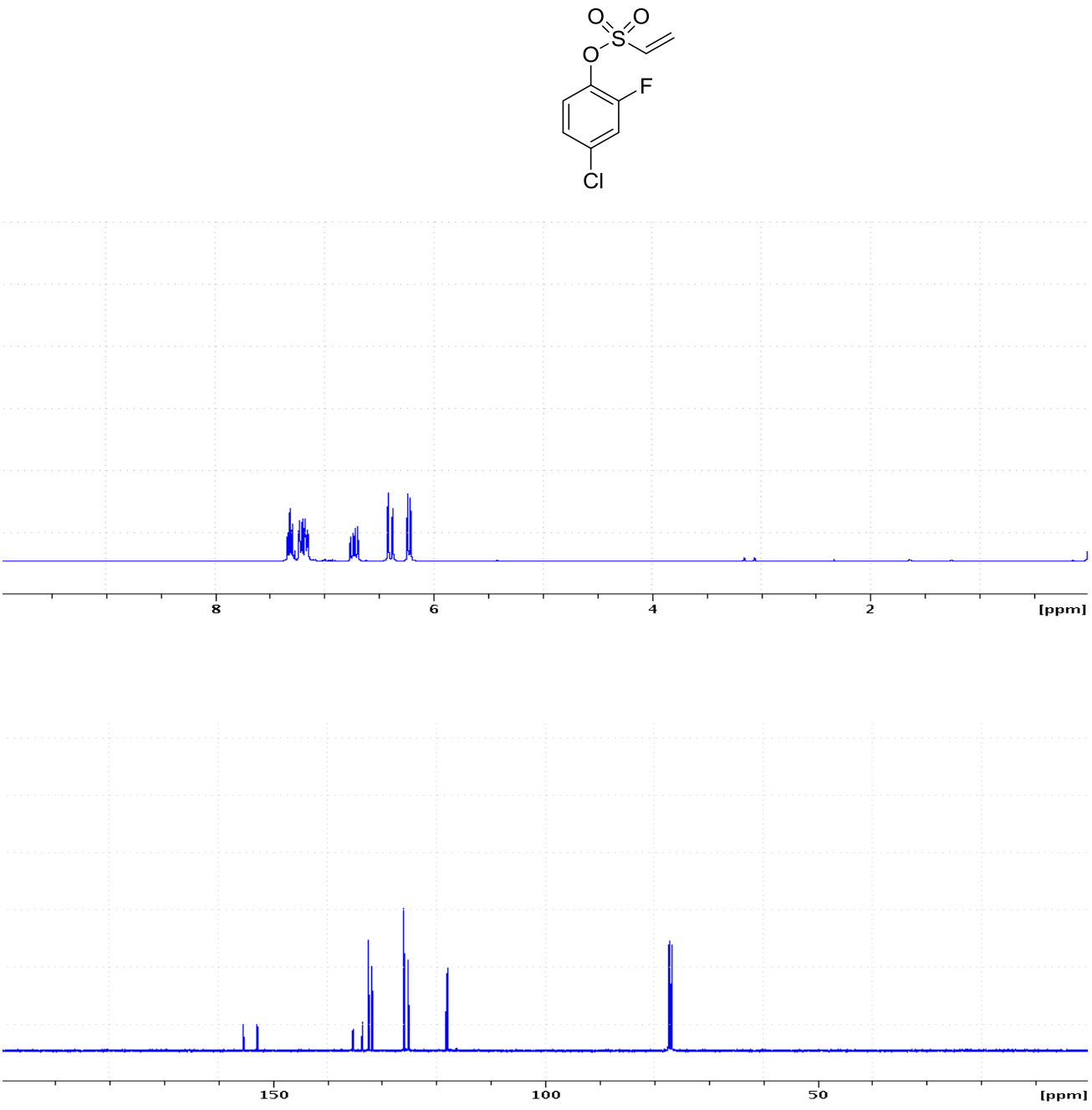


Figure. ^{1}H and ^{13}C NMR spectrum of 14

4-Chloro-3-fluorophenyl ethenesulfoante (15):

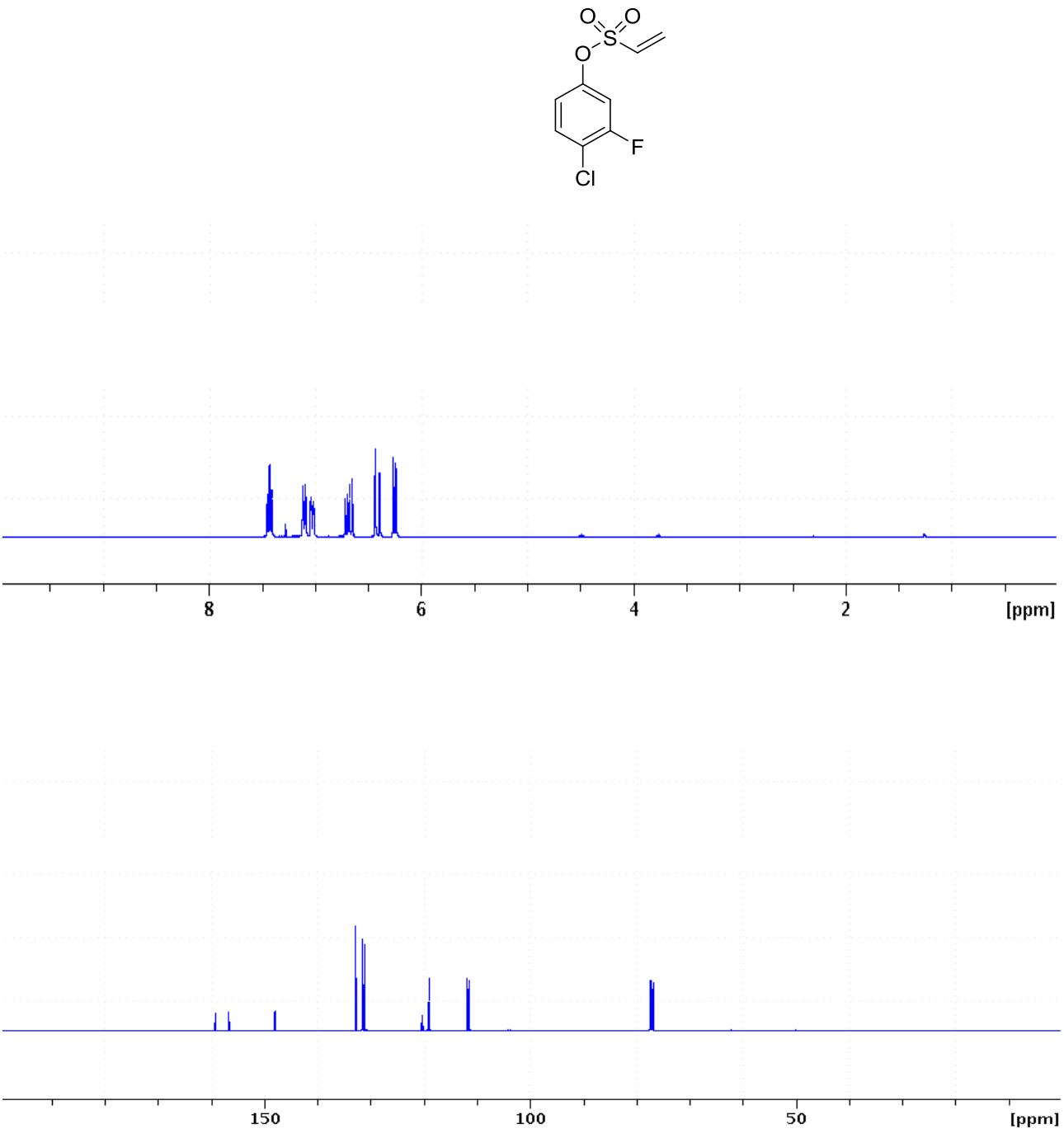


Figure. ^{1}H and ^{13}C NMR spectrum of 15.

4-Chloro-3-(trifluoromethyl) phenyl ethenesulfonate (16):

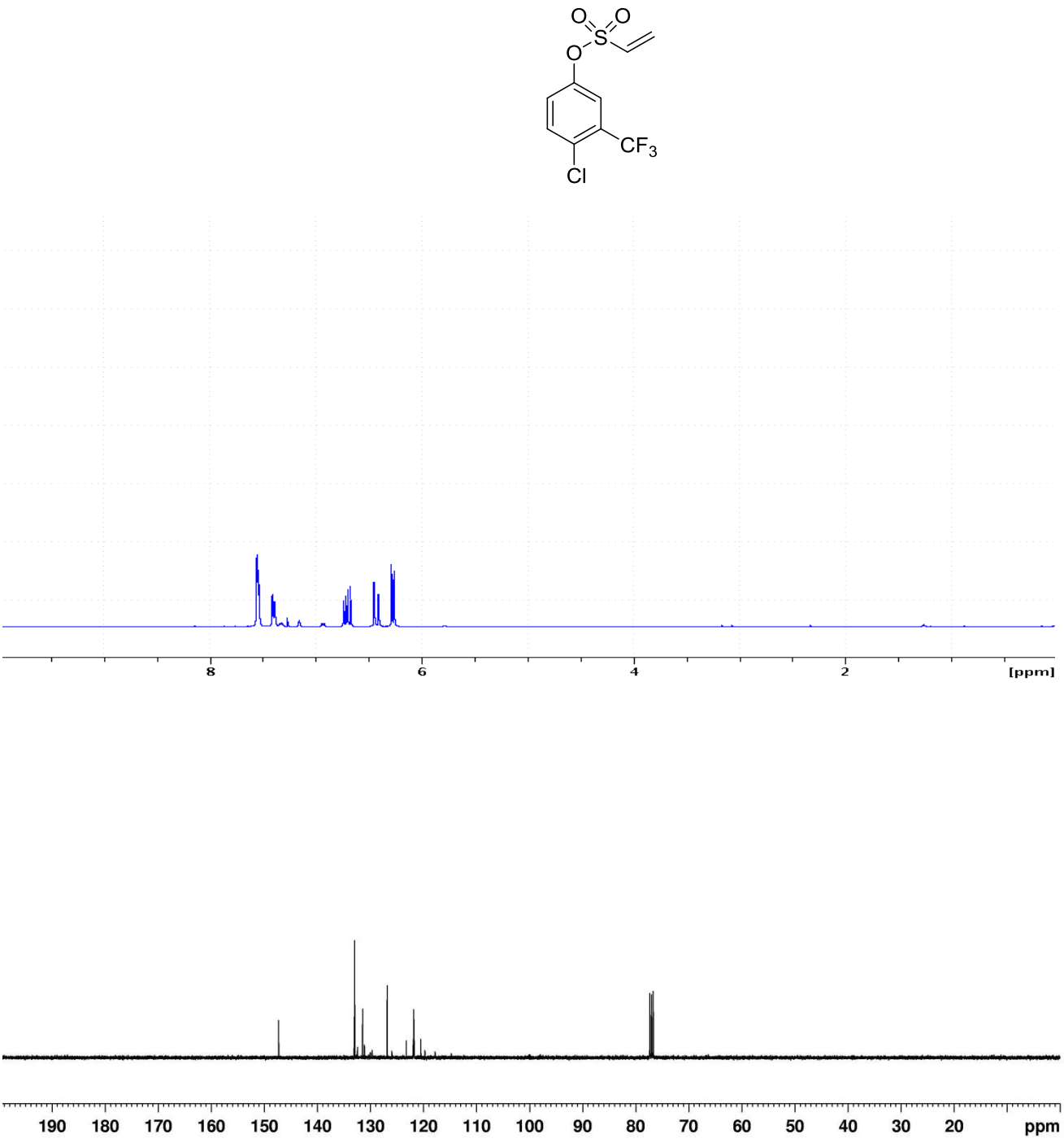
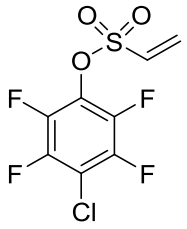


Figure. ^{1}H and ^{13}C NMR spectrum for FN-16

4-Chloro-2, 3, 5, 6-tetrafluorophenyl ethenesulfonate (17):



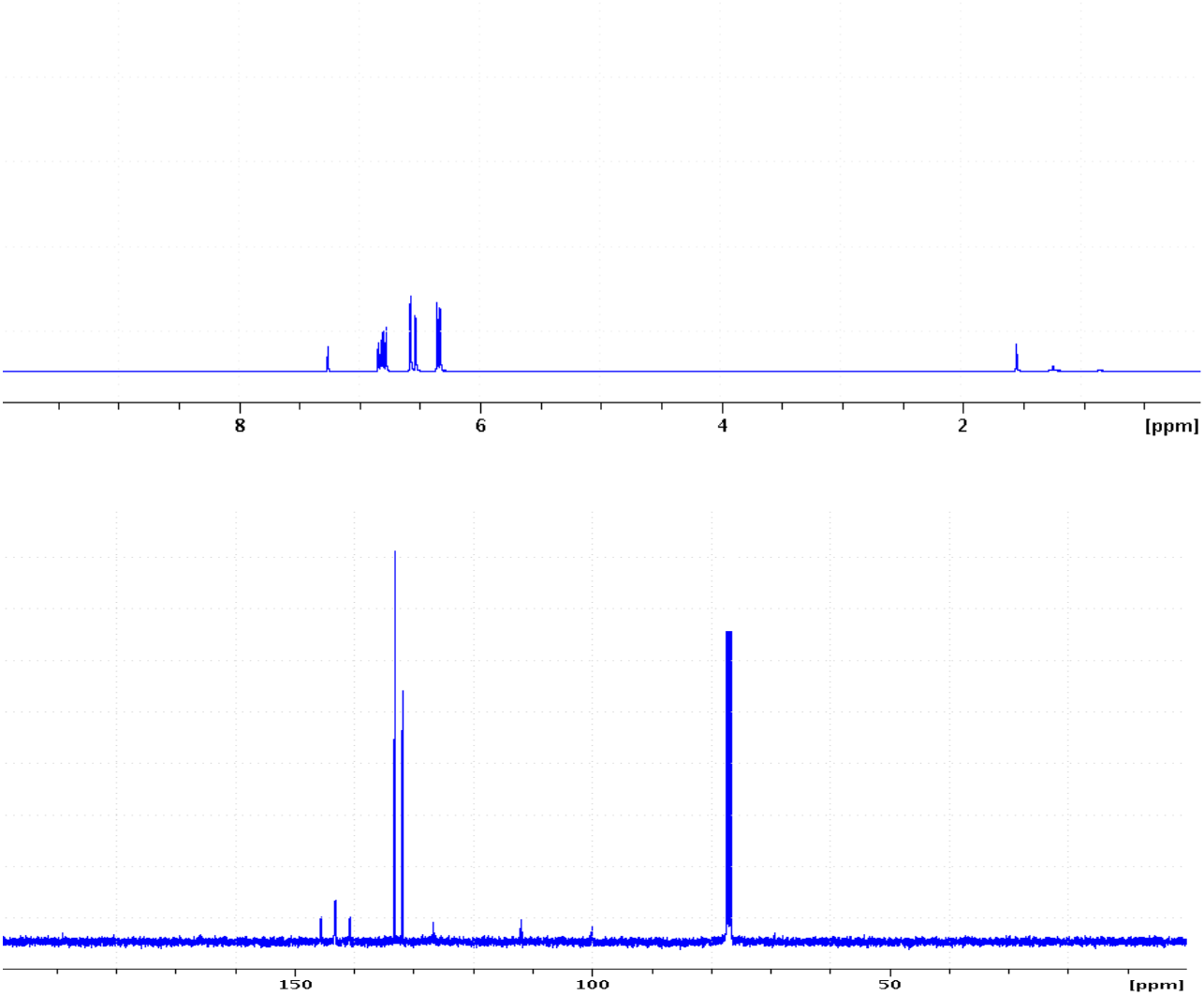


Figure. ^{1}H and ^{13}C NMR spectrum of 17

4-Chloro-3-methylphenyl ethenesulfonate (18):

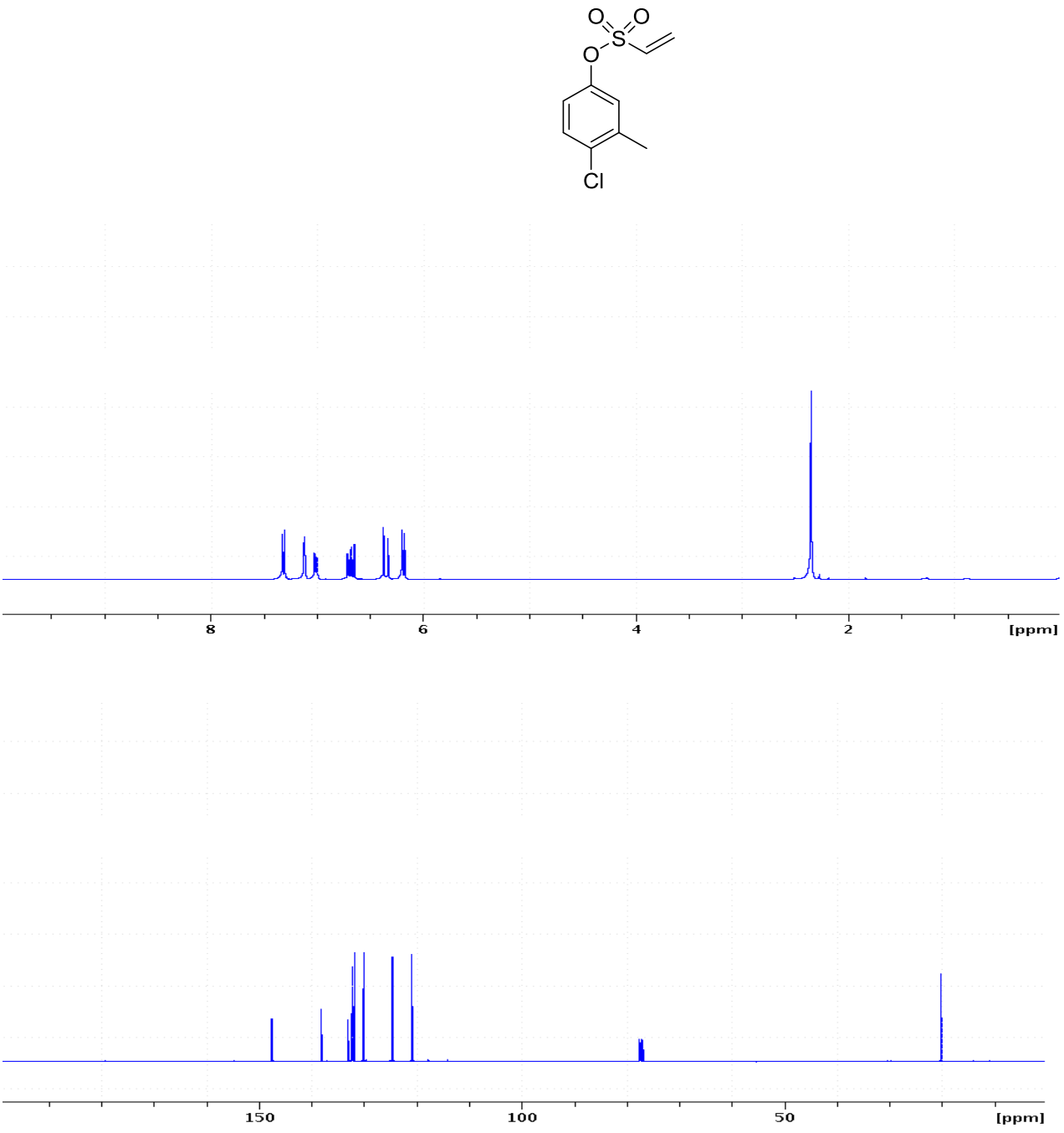


Figure. ^{1}H and ^{13}C NMR spectrum of 18

4-Chloro-3, 5-dimethylphenyl ethenesulfonate (19):

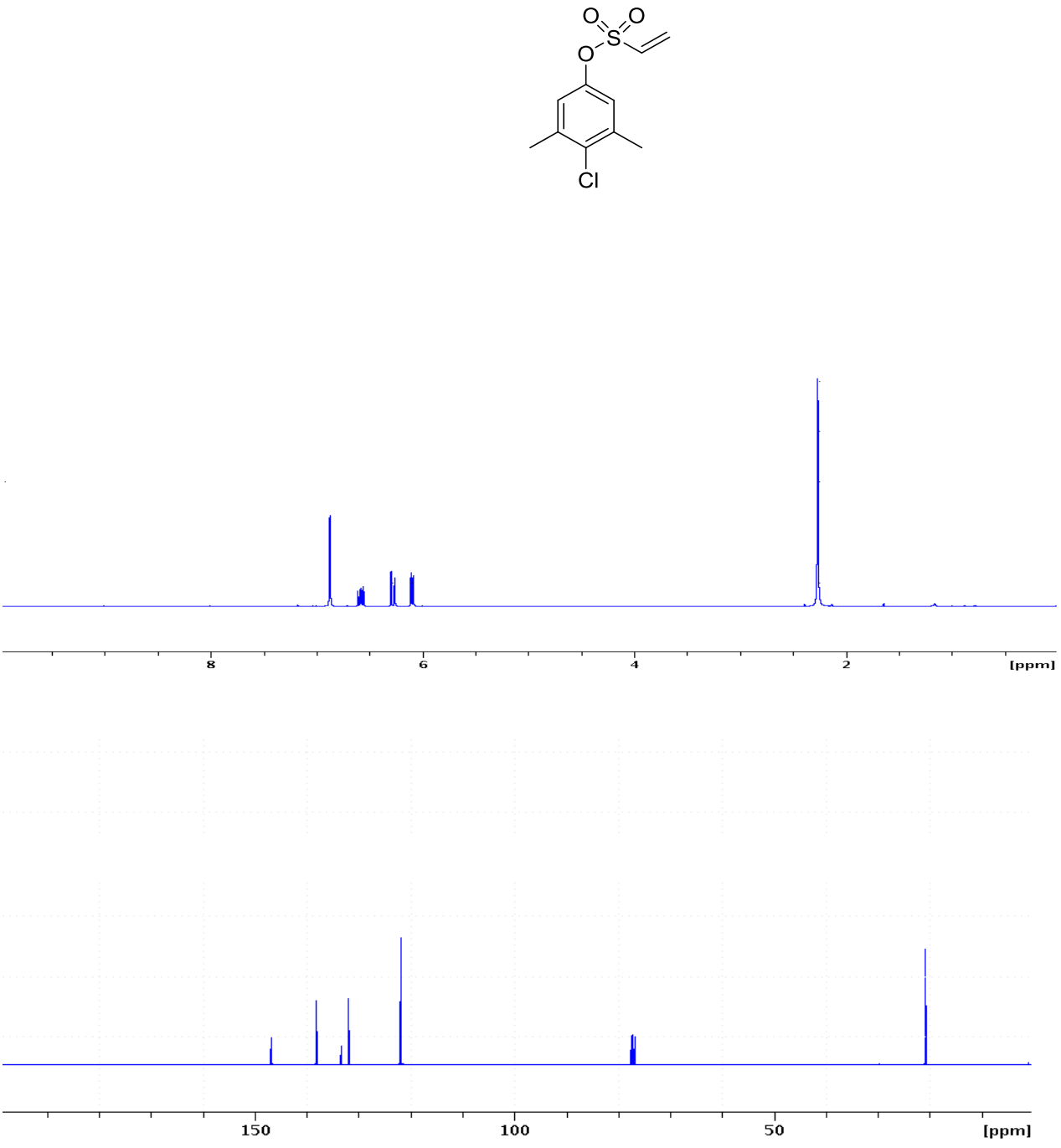


Figure. ^{1}H and ^{13}C NMR spectrum of 19

4-Chloro-2-nitrophenyl sulfonate (20):

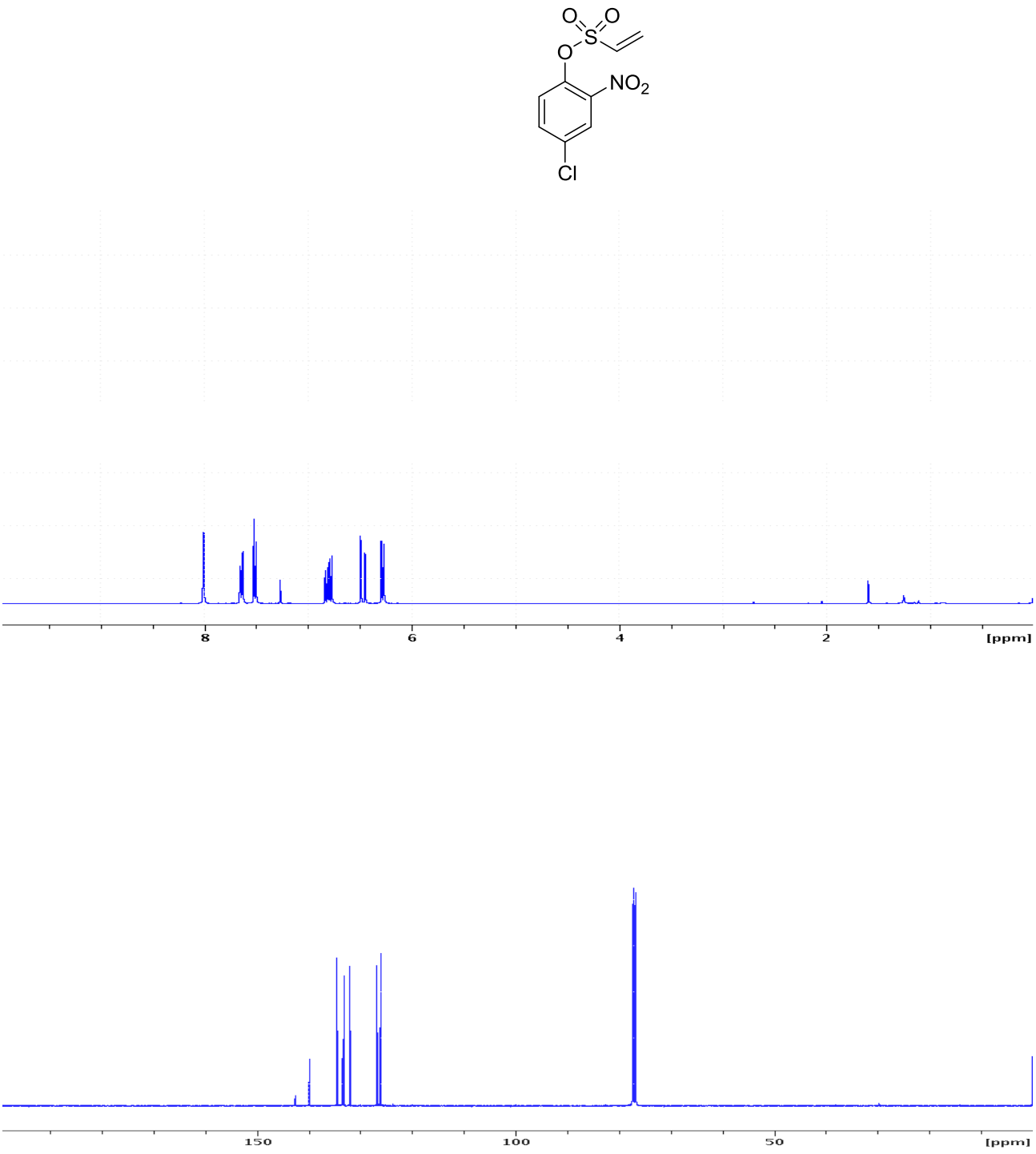


Figure. ^{1}H and ^{13}C NMR spectrum of 20.

3, 4-Dichlorophenyl ethenesulfonate (21):

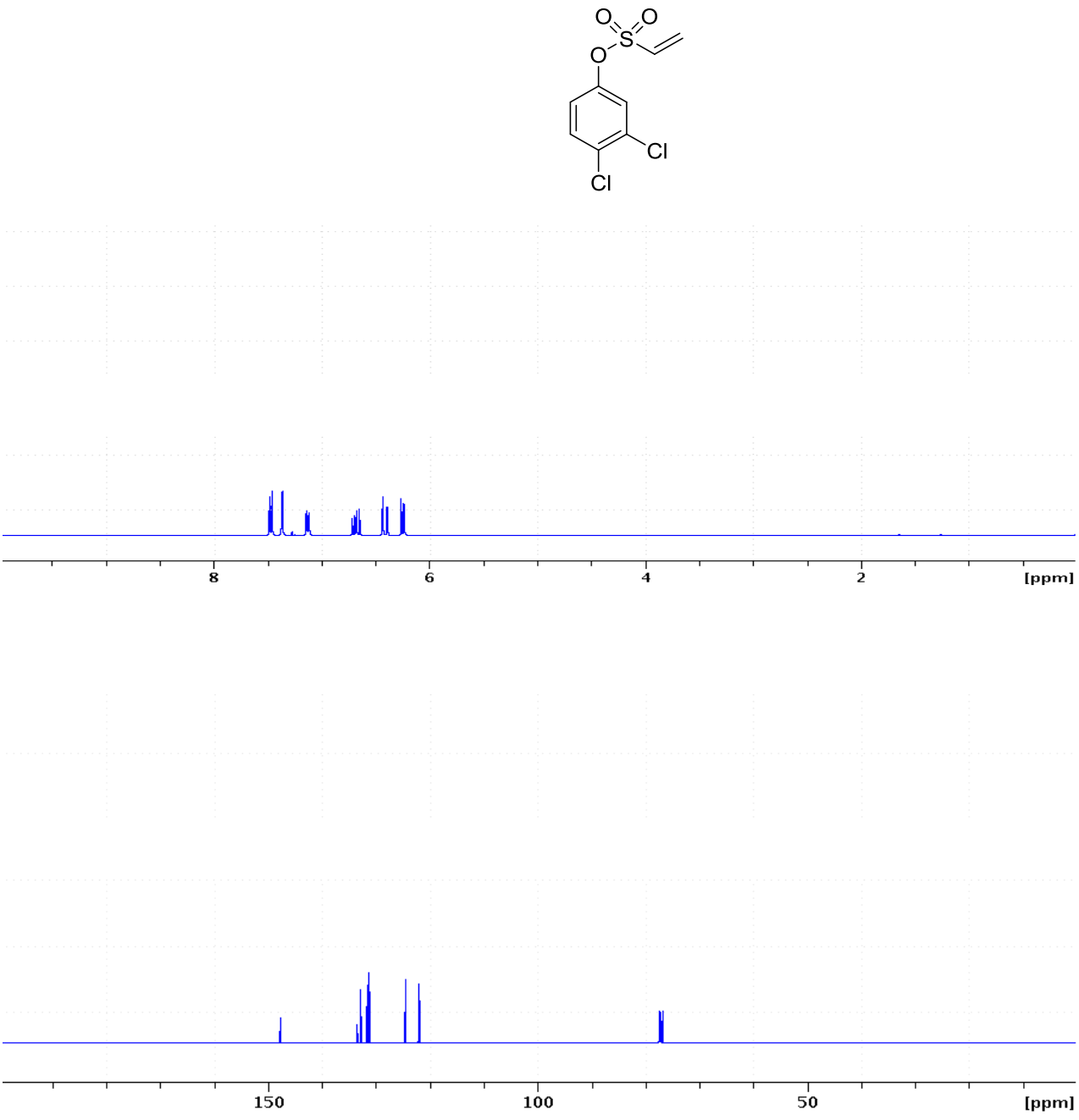
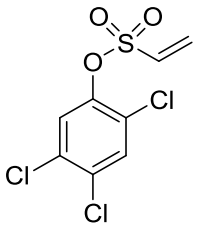
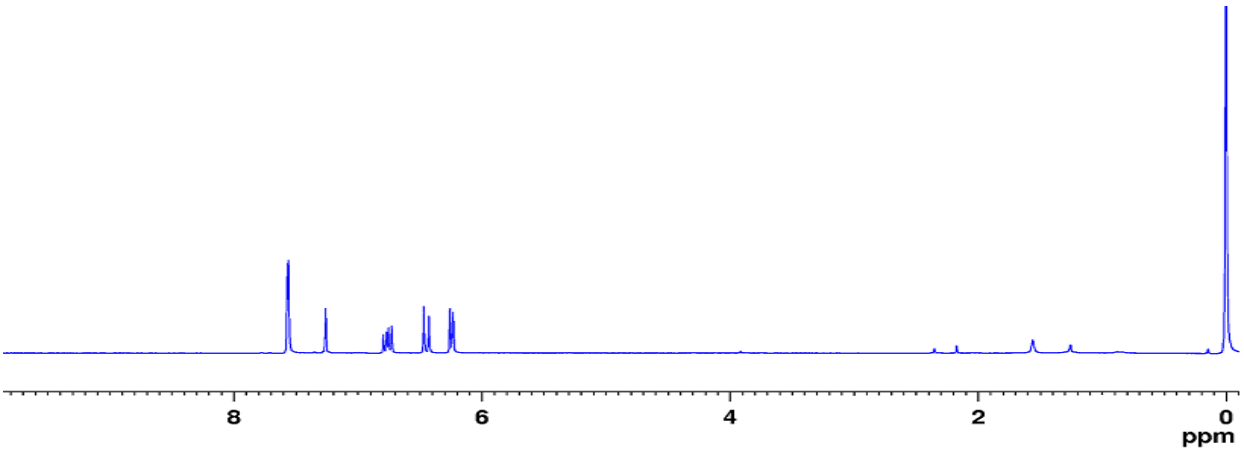


Figure. ^{1}H and ^{13}C NMR spectrum of 21.

2, 4, 5- Trichlorophenyl ethenesulfonate (22):





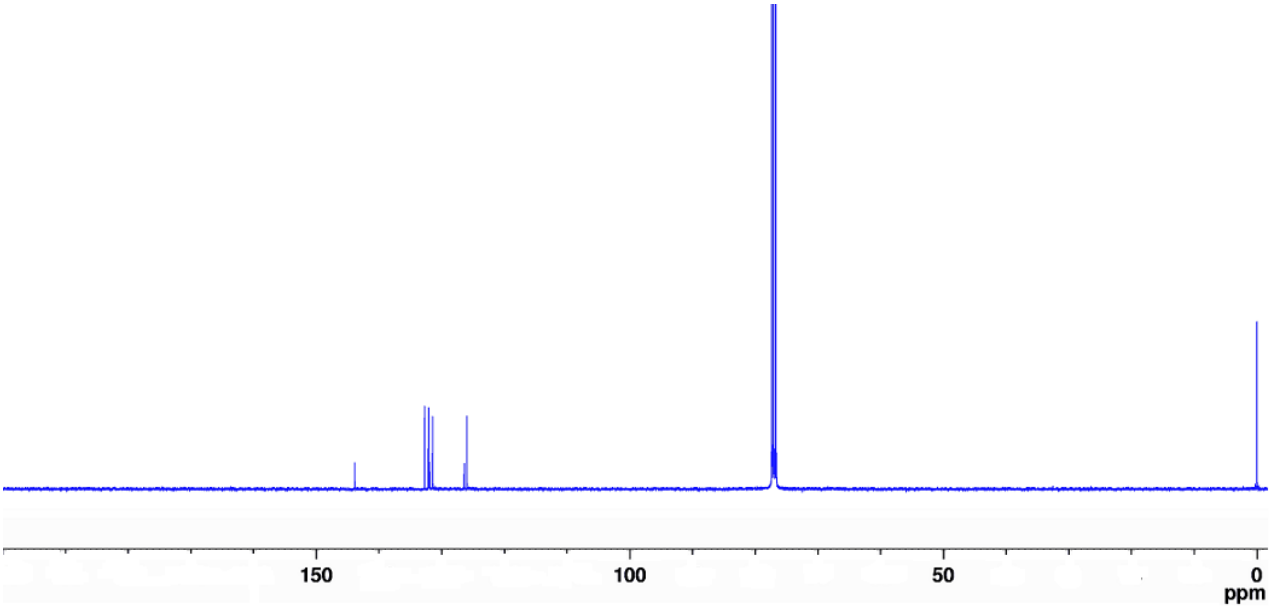
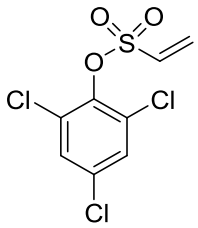
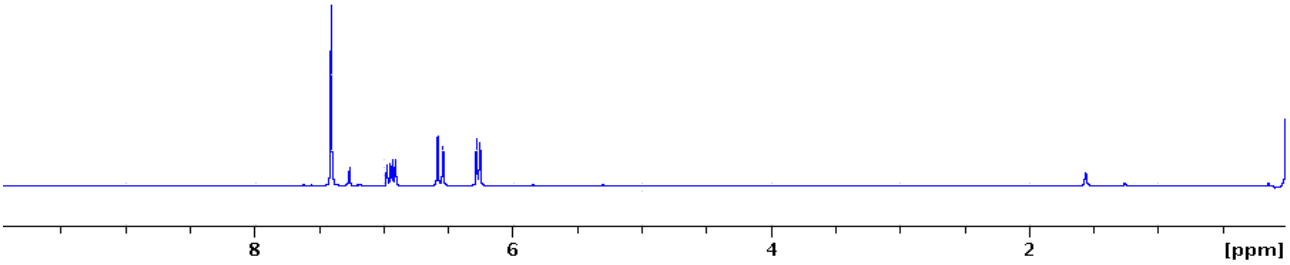


Figure. ^{1}H and ^{13}C NMR spectrum of 22.

2, 4, 6-Trichlorophenyl ethenesulfonate (23):





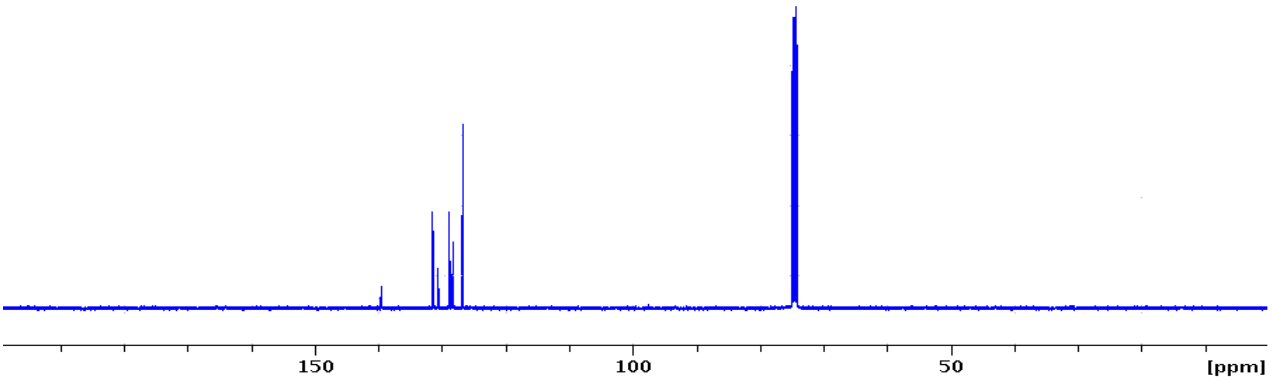


Figure. ^{1}H and ^{13}C NMR spectrum of 23.

Methyl 5-chloro-2-((vinylsulfonyl) oxy) benzoate (24):

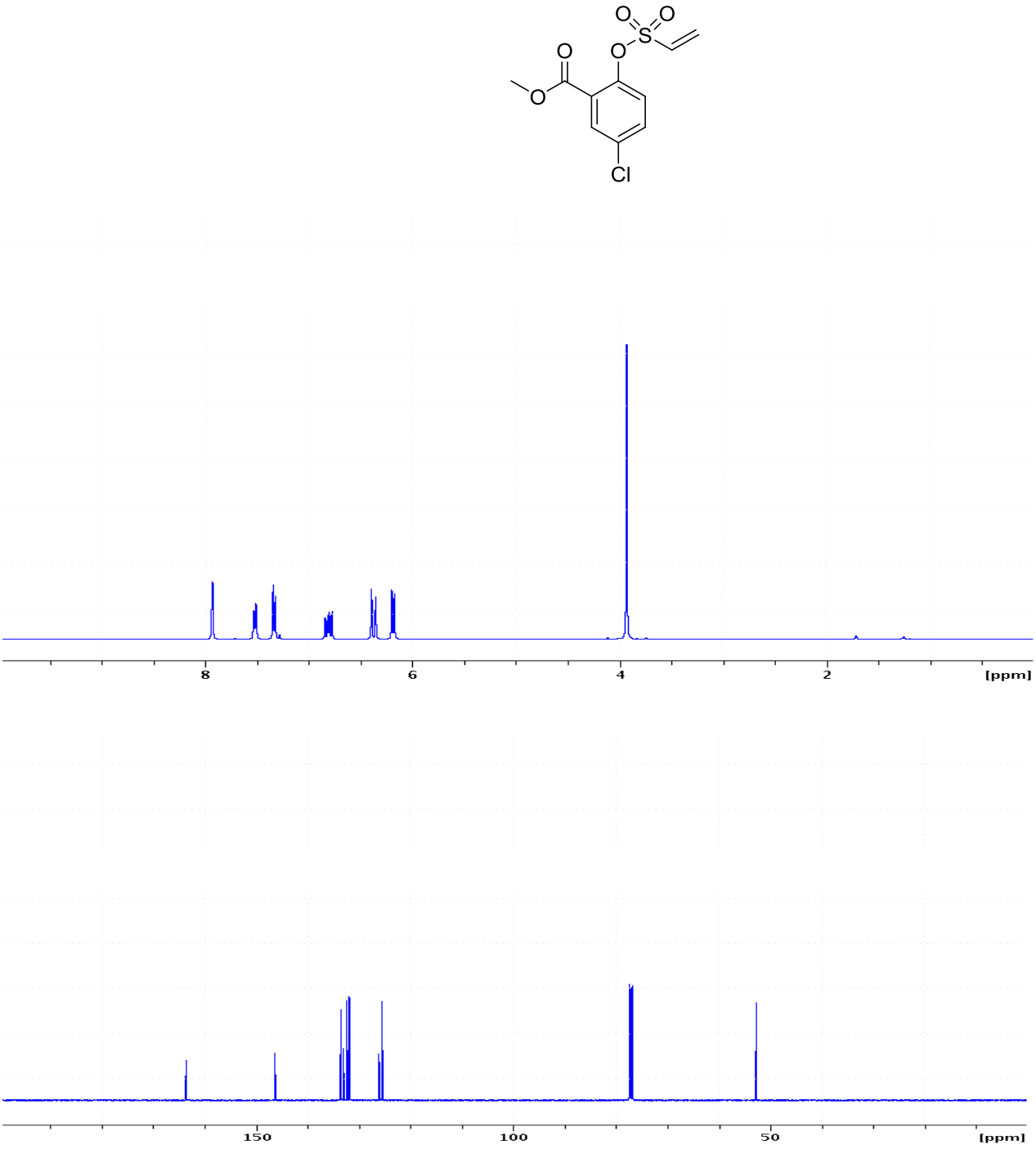
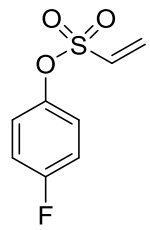
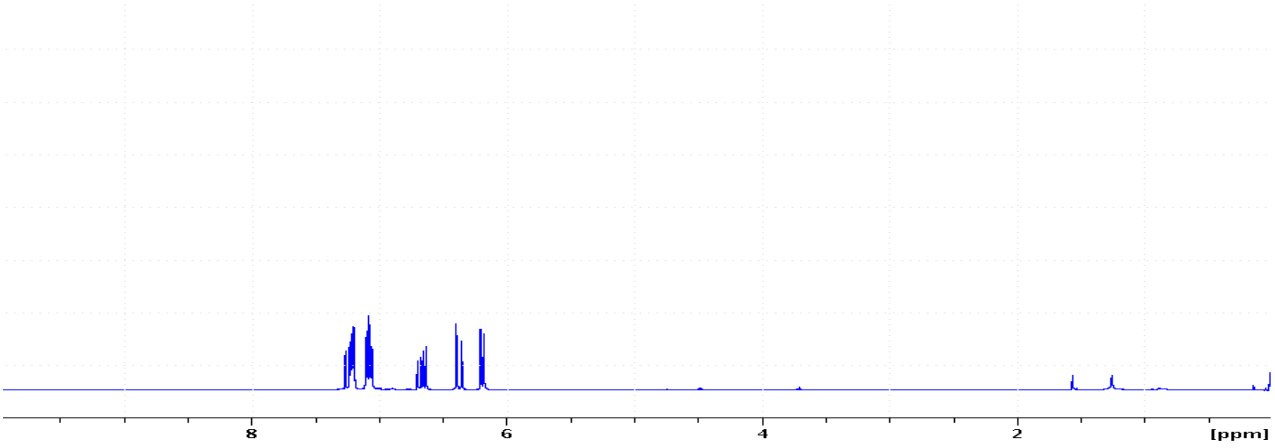


Figure. ^{1}H and ^{13}C NMR spectrum of 24

4-Fluorophenyl ethenesulfonate (25):





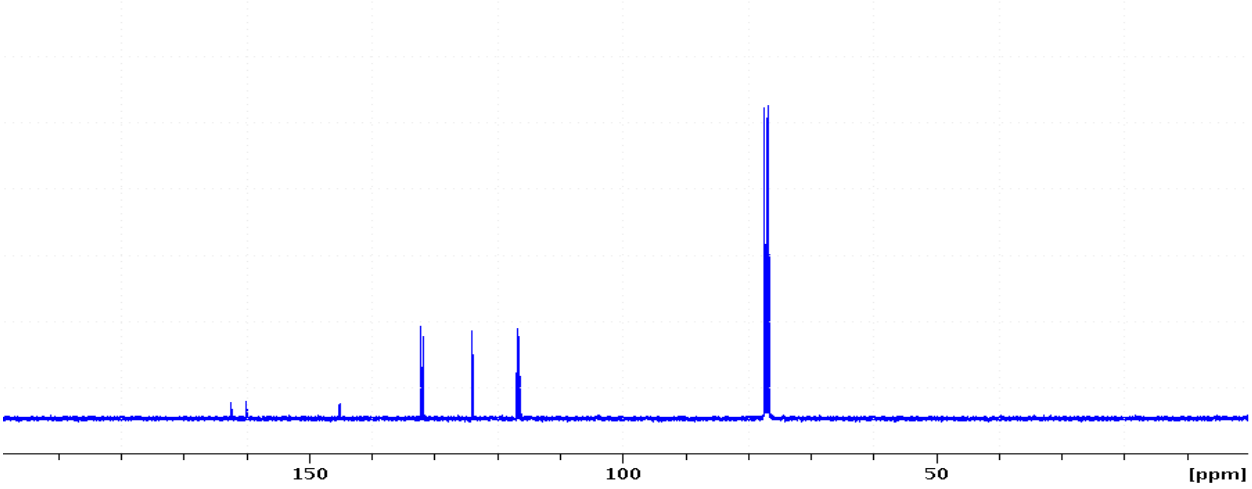
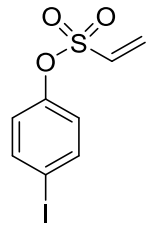
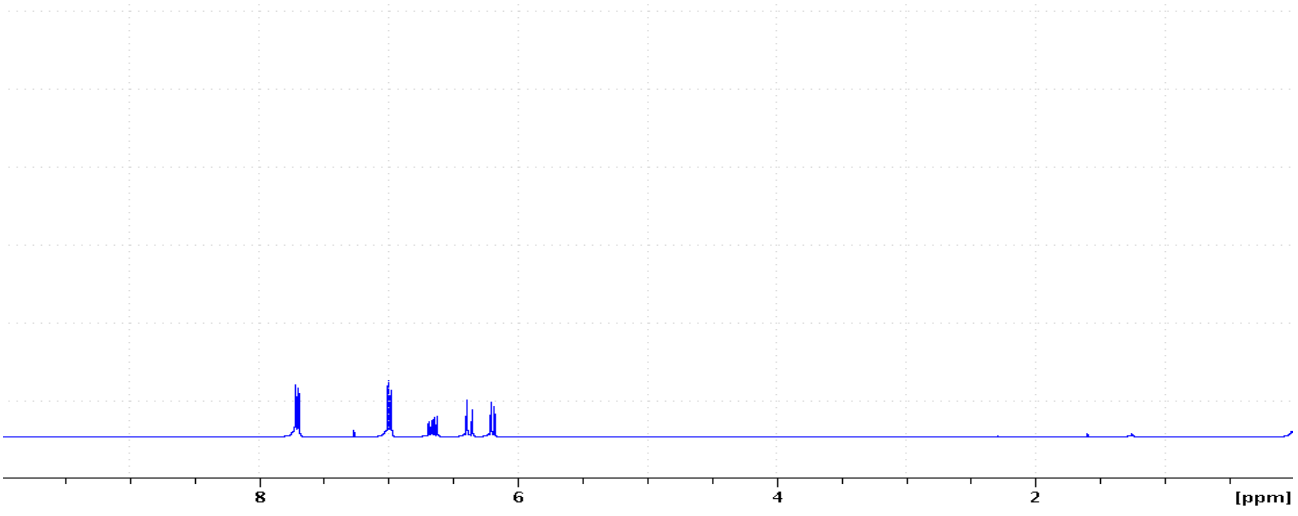


Figure. ^{1}H and ^{13}C NMR spectrum of 25.

4-Iodophenyl ethenesulfonate (26):





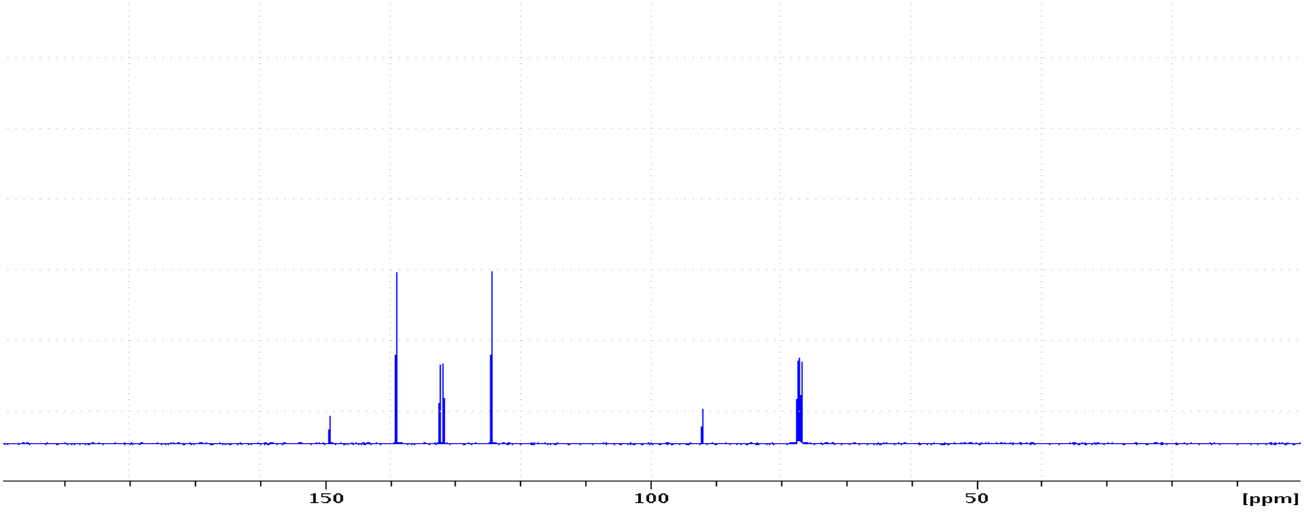
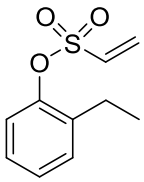
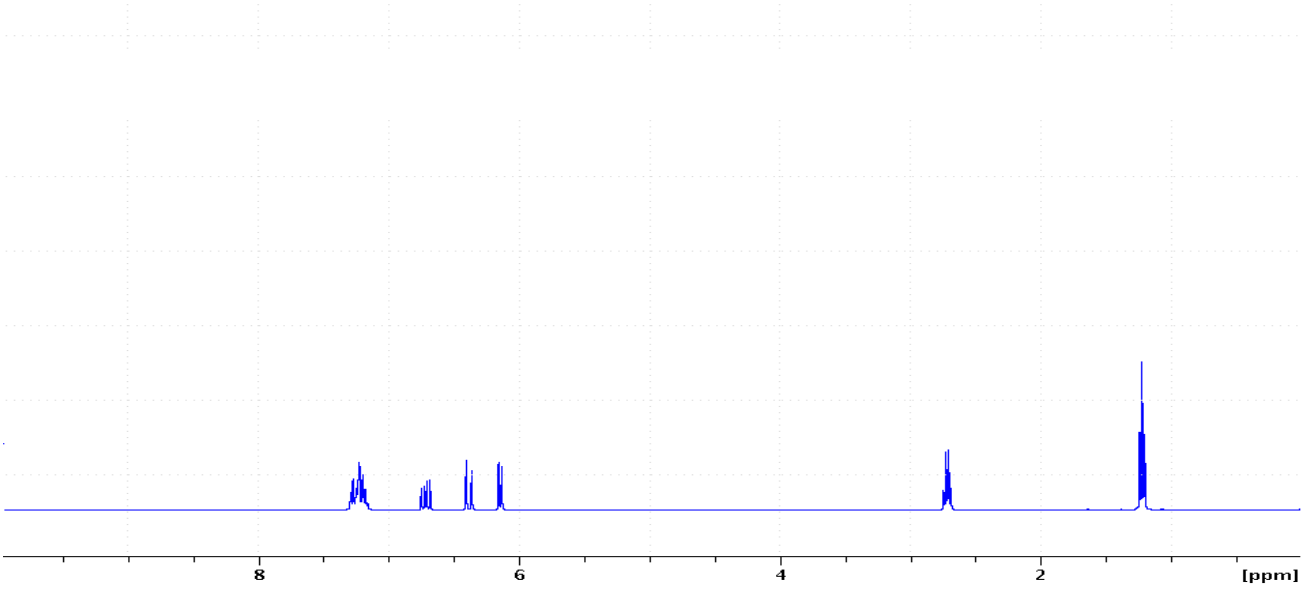


Figure. ^{1}H and ^{13}C NMR spectrum of 26.

2-Ethylphenyl ethenesulfonate (27):





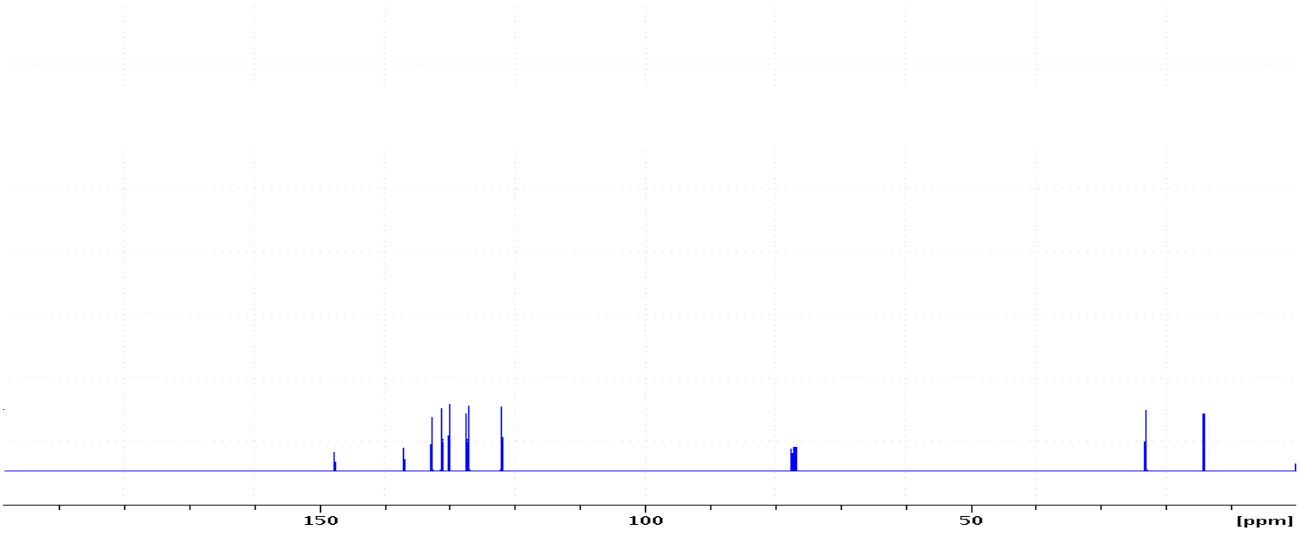
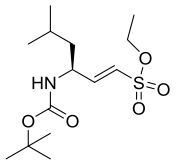
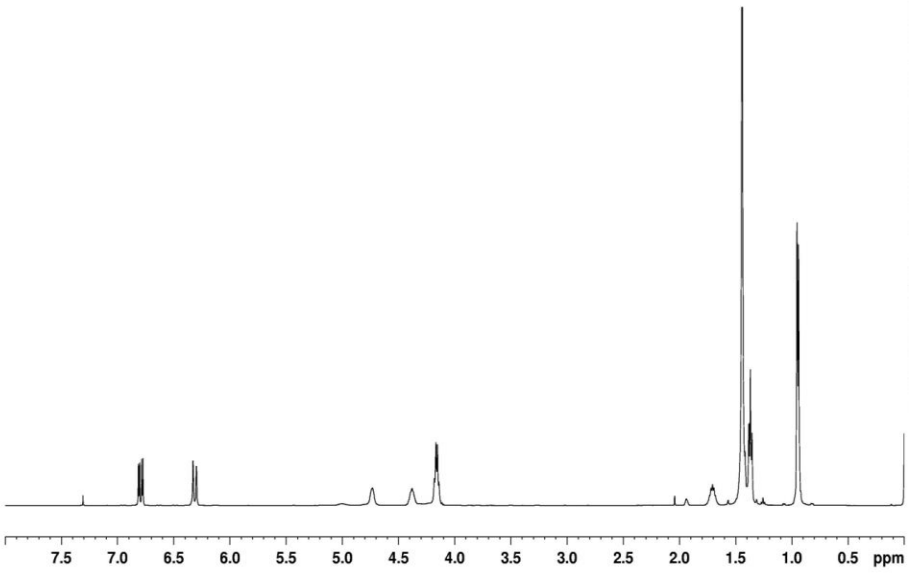


Figure. ^{1}H and ^{13}C NMR spectrum of 27

Ethyl (E)-3-((*tert*-butoxycarbonyl)amino)-5-methylhex-1-ene-1-sulfonate (III)





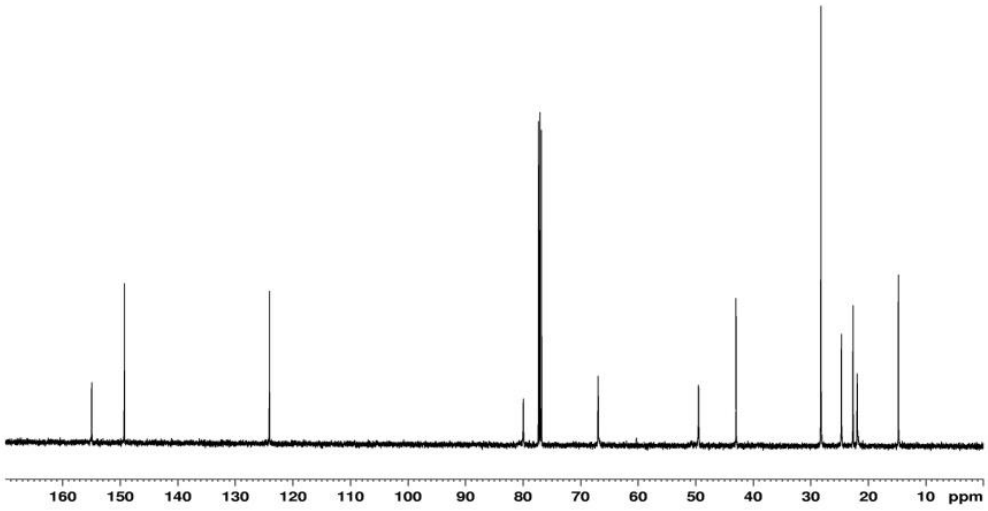
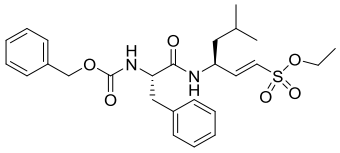
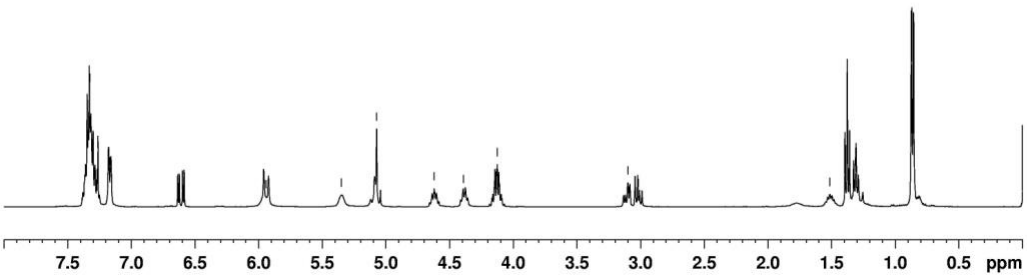


Figure. ^{1}H and ^{13}C NMR spectrum of III.

Ethyl(E)-3-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-5-methylhex-1-ene-1-sulfonate (IV)





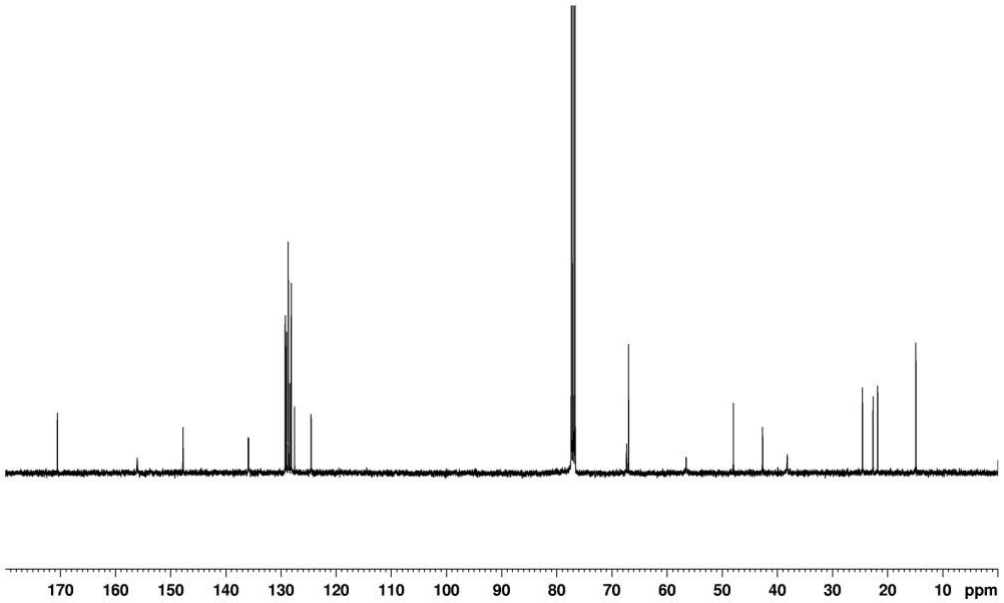
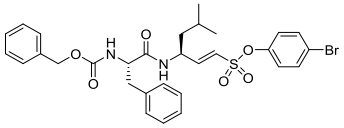
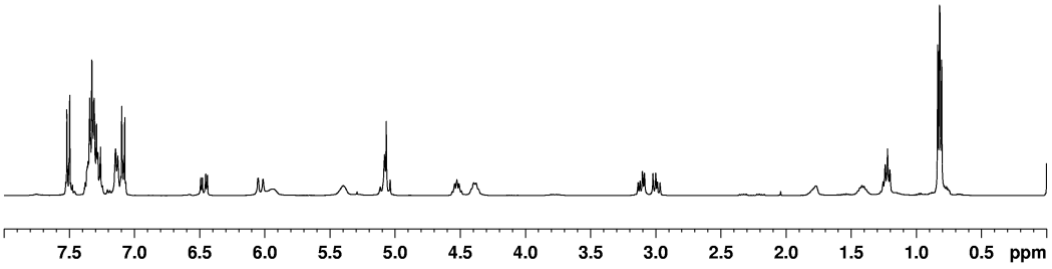


Figure. ^{1}H and ^{13}C NMR spectrum of IV.

4-bromophenyl(E)-3-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-5-methylhex-1-ene-1-sulfonate (KD-1)





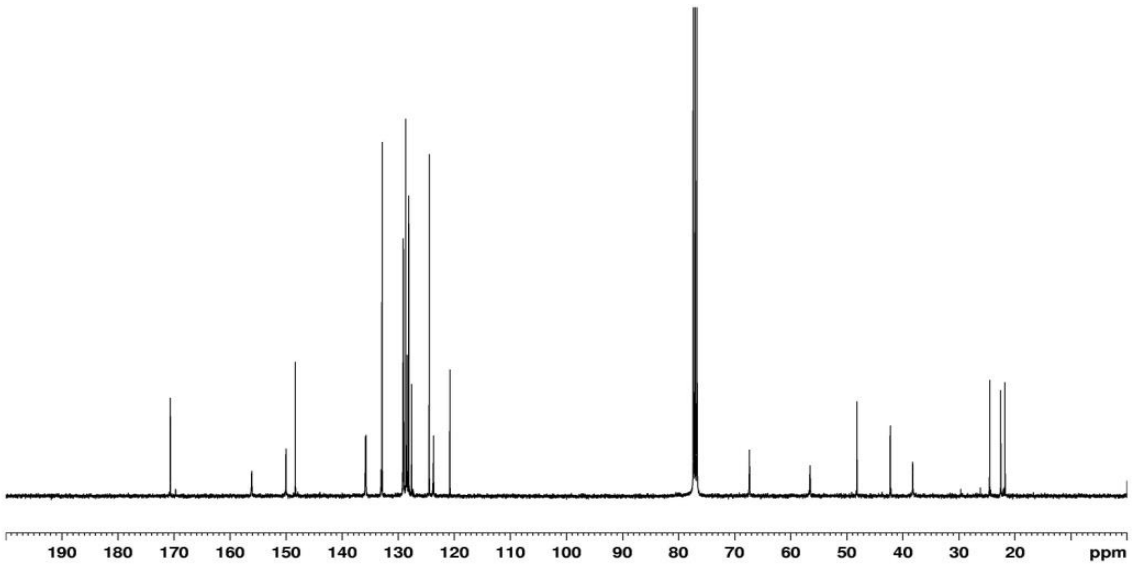


Figure. ^{1}H and ^{13}C NMR spectrum of KD-1.

E. References

1. R. J. Fitzmaurice, J. M. Ahern and S. Caddick, *Org. Biomol. Chem.*, 2009, 7, 235-237.
2. J. C. Carretero, M. Demillequand and L. Ghosez, *Tetrahedron*, 1987, 43, 5125-5134.
3. J. Liu, F. Jiang, Y. Jin, Y. Zhang, J. Liu, W. Liu and L. Fu, *Eur. J. Med. Chem.*, 2012, 57, 10-20.
4. D. Blomberg, K. Brickmann and J. Kihlberg, *Tetrahedron*, 2006, 62, 10937-10944.
5. D. Dana, A. R. Davalos, G. Subramaniam, N. Afzal, W. H. Hersh and S. Kumar, *Tetrahedron Lett.*, 2013, 54, 2717-2721.
6. W. R. Roush, S. L. Gwaltney, II, J. Cheng, K. A. Scheidt, J. H. McKerrow and E. Hansell, *J. Am. Chem. Soc.*, 1998, 120, 10994-10995.
7. A. J. Barrett and H. Kirschke, *Methods in enzymology*, 1981, 80 Pt C, 535-561.
8. Y. Choe, F. Leonetti, D. C. Greenbaum, F. Lecaille, M. Bogyo, D. Bromme, J. A. Ellman and C. S. Craik, *J Biol Chem*, 2006, 281, 12824-12832.
9. A. J. Barrett, A. A. Kembhavi, M. A. Brown, H. Kirschke, C. G. Knight, M. Tamai and K. Hanada, *The Biochemical journal*, 1982, 201, 189-198.
10. A. Torkar, B. Lenarcic, T. Lah, V. Dive and L. Devel, *Bioorganic & medicinal chemistry letters*, 2013, 23, 2968-2973.
11. D. Bromme, P. R. Bonneau, P. Lachance, B. Wiederanders, H. Kirschke, C. Peters, D. Y. Thomas, A. C. Storer and T. Vernet, *The Journal of biological chemistry*, 1993, 268, 4832-4838.
12. F. Buhling, A. Gerber, C. Hackel, S. Kruger, T. Kohnlein, D. Bromme, D. Reinhold, S. Ansorge and T. Welte, *Am J Respir Cell Mol Biol*, 1999, 20, 612-619.
13. D. Dana, A. R. Davalos, S. De, P. Rathod, R. K. Gamage, J. Huestis, N. Afzal, Y. Zavlanov, S. S. Paroly, S. A. Rotenberg, G. Subramaniam, K. J. Mark, E. J. Chang and S. Kumar, *Bioorganic & medicinal chemistry*, 2013, 21, 2975-2987.
14. G. E. Chavarria, M. R. Horsman, W. M. Arispe, G. D. Kumar, S. E. Chen, T. E. Strecker, E. N. Parker, D. J. Chaplin, K. G. Pinney and M. L. Trawick, *European journal of medicinal chemistry*, 2012, 58, 568-572.
15. T. P. Abeyweera, X. Chen and S. A. Rotenberg, *The Journal of biological chemistry*, 2009, 284, 17648-17656.
16. W. Li, J. Fan, M. Chen, S. Guan, D. Sawcer, G. M. Bokoch and D. T. Woodley, *Molecular biology of the cell*, 2004, 15, 294-309.
17. P. Y. Yue, E. P. Leung, N. K. Mak and R. N. Wong, *Journal of biomolecular screening*, 2010, 15, 427-433.