Divergent synthesis of Thapsigargin analogs

Hang Chu, Georg Dünstl, Jakob Felding, and Phil S. Baran

The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Corresponding Authors:

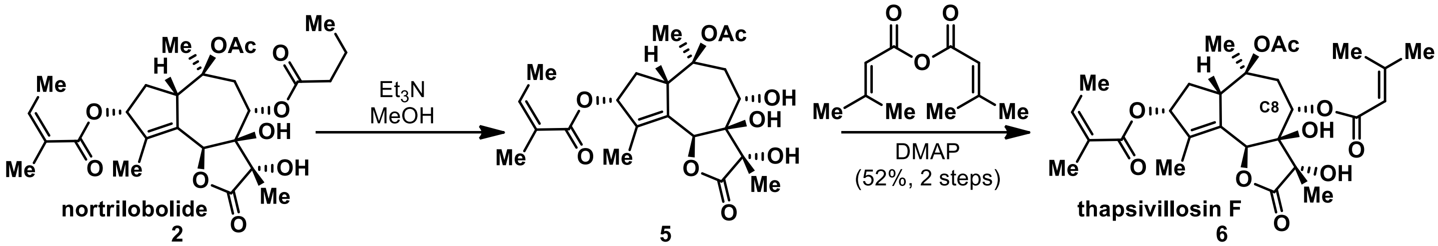
E-mail: pbaran@scripps.edu

Supporting Information

Table of Contents

General Procedures. All reactions were performed using flame-dried round-bottomed flasks or reaction vessels unless otherwise stated. Where appropriate, reactions were carried out under an inert atmosphere of argon with dry solvents, unless otherwise stated. Dry dichloromethane (DCM), tetrahydrofuran (THF), benzene (PhH), acetonitrile (MeCN) and methanol (MeOH) were obtained by passing the previously degassed solvents through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using ultraviolet light as visualizing agent and an acidic mixture of *p*-anisaldehyde as developing agent. NMR spectra were recorded on a Bruker DRX-600 spectrometer and were calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16). The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were recorded on an Agilent Mass spectrometer using ESI-TOF (electrospray ionization-time of flight). The UCSD small molecule X-ray facility collected and analyzed all X-ray diffraction data.

Thapsivillosin F. (6)

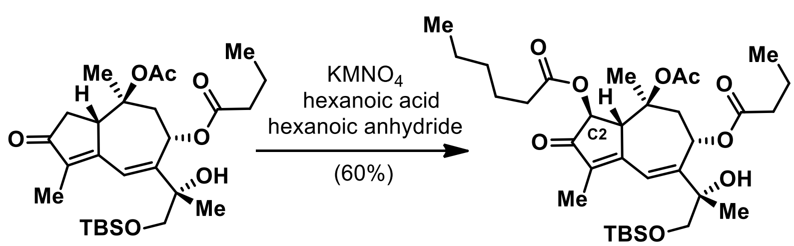


Nortrilobolide (**2**) (3mg, 0.0059 mmol), was dissolved in methanol (0.15 mL) and then triethyl amine (15 μL) was added via syringe. The reaction mixture was heated to 60 °C until TLC indicated full consumption of the starting material (ca. 30 min). Upon completion, the reaction was concentrated under reduced pressure. The crude alcohol was dissolved in dichloromethane (0.15 mL). Senecioic anhydride (ca. 10 mg) and DMAP (5 mg) were added sequentially. The reaction was stirred at room temperature until TLC indicated the full consumption of the starting material (ca. 2h). The reaction mixture was then purified directly via preparative TLC (1:1 EtOAc:Hexanes) to deliver thapsivillosin F (52% yield, 1.6 mg) as an amorphous solid.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.11 (dddd, *J* = 8.8, 7.3, 5.9, 1.7 Hz, 1H), 5.73 (q, *J* = 1.9 Hz, 1H), 5.65 – 5.62 (m, 1H), 5.59 (dt, *J* = 7.9, 3.7 Hz, 2H), 4.23 (s, 1H), 3.00 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.59 (dt, *J* = 14.8, 8.5 Hz, 1H), 2.33 (dt, *J* = 8.6, 4.5 Hz, 3H), 2.19 (d, *J* = 1.3 Hz, 3H), 2.18 (d, *J* = 1.3 Hz, 1H), 2.02 (dd, *J* = 7.3, 1.6 Hz, 2H), 1.96 (s, 3H), 1.94 – 1.89 (m, 9H), 1.68 (dt, *J* = 14.8, 5.4 Hz, 1H), 1.51 (s, 3H), 1.34 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.8, 170.5, 167.7, 165.2, 160.1, 144.3, 138.7, 130.8, 127.9, 115.4, 85.3, 79.7, 79.3, 78.9, 66.6, 51.5, 39.0, 32.4, 27.7, 22.9, 22.0, 20.9, 20.6, 16.6, 16.0, 13.3.

HRMS (ESI) *m/z*: calculated for $C_{27}H_{36}O_{10}$ $[M+H]^+$ 521.2381 found 521.2387.



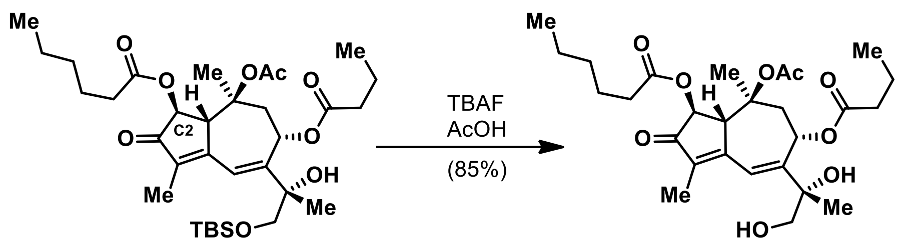
Experimental: To a culture tube equipped with a magnetic stir bar was added $KMnO_4$ (20 mg, 0.124 mmol, 2.1 equiv.) followed by 1.55 mL of benzene. The solution was heated to 85 °C before hexanoic acid (0.285 mL, 2.065 mmol, 35 equiv.) and hexanoic anhydride (114 mg, 0.531 mmol, 9 equiv.) were sequentially added. The solution was stirred for 30 min before compound **8** (30 mg, 0.0590 mmol) was added as a solution in benzene (0.4 mL) via syringe dropwise. The reaction mixture was stirred at 85 °C for 20 hours before it was cooled to room temperature. The black reaction mixture was passed through a column of basic alumina (column washed with EtOAc). The resulting organic layer was washed with saturated $NaHCO_3$ (4x), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via silica gel chromatography (1:5 EtOAc:Hexanes) to yield hexanoate containing some hexanoic acid. This residue was diluted with 1:5 EtOAc:Hexanes and washed further with saturated $NaHCO_3$ (4x). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford hexanoate **9** (22 mg, 60 %) as a yellow oil.

Physical State: yellow oil

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.07 (d, *J* = 0.9 Hz, 1H), 5.91 (dd, *J* = 5.6, 3.6 Hz, 1H), 5.32 (d, *J* = 3.2 Hz, 1H), 3.87 (t, *J* = 2.7 Hz, 1H), 3.66 (d, *J* = 9.7 Hz, 1H), 3.50 (d, *J* = 9.8 Hz, 1H), 2.90 (s, 1H), 2.81 (dd, *J* = 15.1, 3.6 Hz, 1H), 2.66 (dd, *J* = 15.1, 5.7 Hz, 1H), 2.44 – 2.29 (m, 2H), 2.26 (td, *J* = 7.4, 3.3 Hz, 2H), 1.94 (s, 3H), 1.88 (d, *J* = 2.3 Hz, 3H), 1.65 (dt, *J* = 14.9, 7.5 Hz, 3H), 1.35 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.89 (s, 10H), 0.08 (d, *J* = 3.6 Hz, 7H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 201.9, 172.7, 172.3, 170.4, 157.8, 150.3, 138.8, 123.9, 82.4, 76.2, 72.8, 69.6, 67.6, 54.3, 42.2, 36.8, 34.0, 31.3, 25.9, 24.6, 22.5, 22.4, 21.5, 18.3, 14.0, 13.9, 8.9, -5.3, -5.3.

HRMS (ESI) *m/z*: calculated for $C_{33}H_{55}O_9Si$ $[M+H]^+$ 623.3610, found 623.3609



9 **10**

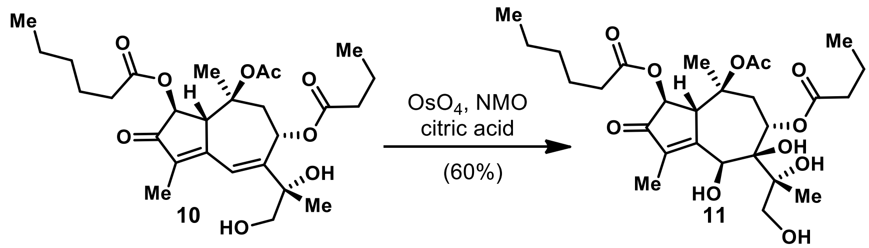
Experimental: Compound **9** (22 mg, 0.0353 mmol) was dissolved in THF (0.35 mL, 0.1 M). The reaction mixture was then cooled to 0 °C before TBAF (1M sln in THF)/AcOH (2:1 v/v, 0.353mL/0.176 mL, 10 equiv.) was added dropwise via syringe. The reaction mixture was stirred at 0 °C until TLC indicated complete consumption of the starting material. The reaction mixture was then diluted with EtOAc and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via column chromatography (1:1 EtOAc:Hexanes) to afford **10** (85%, 15 mg) as an amorphous solid.

Physical State: amorphous solid

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.02 (d, *J* = 1.0 Hz, 1H), 5.99 (dd, *J* = 6.1, 3.6 Hz, 1H), 5.30 (d, *J* = 3.2 Hz, 1H), 3.93 (t, *J* = 2.7 Hz, 1H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.54 (d, *J* = 11.0 Hz, 1H), 2.82 (dd, *J* = 15.1, 3.7 Hz, 1H), 2.65 (dd, *J* = 15.1, 6.1 Hz, 1H), 2.34 (ddd, *J* = 8.0, 7.2, 6.0 Hz, 2H), 2.28 (td, *J* = 7.3, 1.0 Hz, 2H), 1.95 (s, 3H), 1.88 (d, *J* = 2.3 Hz, 3H), 1.71 – 1.64 (m, 1H), 1.36 (d, *J* = 3.6 Hz, 6H), 1.32 (q, *J* = 3.6 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H), 0.91 – 0.85 (m, 3H).

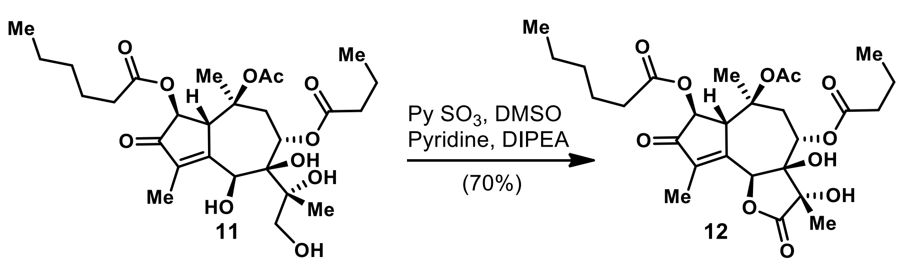
^{13}C NMR (151 MHz, $CDCl_3$) δ 201.9, 172.7, 172.4, 170.5, 157.6, 149.8, 139.1, 124.0, 82.2, 76.5, 72.9, 69.3, 67.6, 54.1, 42.4, 36.7, 34.0, 31.3, 25.0, 24.6, 22.5, 22.4, 21.8, 18.3, 14.0, 13.9, 8.9.

HRMS (ESI) *m/z*: calculated for $C_{27}H_{41}O_9$ $[M+H]^+$ 509.2745, found 509.2742



Experimental: Compound **10** (12 mg, 0.0236 mmol) was dissolved in *t*BuOH/H_2O/Acetone (1:1:1 0.24 mL, 0.1 M). Citric acid (9.1 mg, 0.0471 mmol, 2 equiv.) and NMO (5.5 mg, 0.0471 mmol, 2 equiv.) were added as solids sequentially. OsO4 (0.1 M solution in H_2O, 0.6 mg, 0.1 equiv.) was added via syringe. The solution was stirred at 50 °C for 4.5 hours (note: starting material and product coelute on TLC. Anisaldehyde shows SM to be brown while the product is green). The reaction mixture was then cooled to room temperature and diluted with H_2O. The mixture was extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (1:1 EtOAc:Hexanes) to afford tetraol **11** (8 mg, 60%) as an amorphous solid.

Physical State: amorphous solid
^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.40 (dd, *J* = 5.7, 2.3 Hz, 1H), 5.28 (s, 1H), 5.18 (d, *J* = 6.0 Hz, 1H), 4.65 (s, 1H), 4.48 – 4.28 (m, 2H), 4.10 (d, *J* = 6.7 Hz, 1H), 3.95 (dd, *J* = 11.6, 4.3 Hz, 1H), 3.48 (dd, *J* = 11.6, 6.0 Hz, 1H), 3.19 (dt, *J* = 14.5, 1.8 Hz, 1H), 2.90 (t, *J* = 5.9 Hz, 1H), 2.42 (dd, *J* = 14.7, 5.6 Hz, 1H), 2.31 (td, *J* = 7.4, 1.7 Hz, 2H), 2.20 (td, *J* = 7.4, 3.8 Hz, 2H), 1.99 (s, 3H), 1.96 (d, *J* = 1.9 Hz, 3H), 1.66 (s, 4H), 1.63 – 1.58 (m, 3H), 1.30 (dt, *J* = 6.2, 2.1 Hz, 4H), 1.24 (s, 3H), 1.16 (s, 3H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.91 – 0.80 (m, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 203.3, 172.7, 171.7, 170.7, 167.0, 142.1, 83.4, 77.7, 77.2, 71.9, 71.5, 69.1, 67.6, 52.6, 36.6, 36.4, 34.1, 31.3, 24.7, 23.1, 22.7, 22.4, 22.2, 18.0, 14.1, 13.8, 9.4.
HRMS (ESI) *m/z*: calculated for $C_{27}H_{43}O_{11}$ $[M+H]^+$ 543.2807, found 543.2809



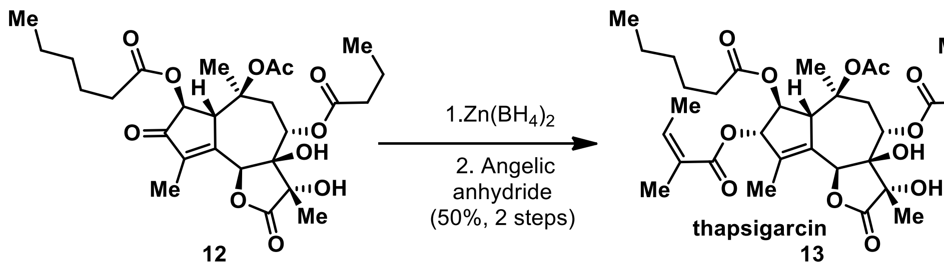
Experimental: To a CH_2Cl_2 solution (0.2 mL) containing tetraol **11** (5 mg, 0.0092 mmol) was added DIPEA (24 mg, 0.184 mmol, 20 equiv.). The reaction mixture was cooled to 0 °C before a premixed solution of $PySO_3$ (30 mg, 0.184 mmol, 20 equiv.) and pyridine (22, 0.276 mmol, 30 equiv.) in DMSO (0.4 mL) was added dropwise over 16 min. The resulting solution was stirred at 0 °C for 30 min. The reaction mixture was diluted with 1:4 EtOAc:Hexanes. Then, it was sequentially washed with saturated $NaHCO_3$, 1M HCl, and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC (1:2 EtOAc:Hexanes) to afford lactone **12** (3.5 mg, 70%) as an amorphous solid.

Physical State: amorphous solid

^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.83 (s, 1H), 5.67 (t, *J* = 3.8 Hz, 1H), 5.16 (d, *J* = 3.3 Hz, 1H), 4.54 (d, *J* = 3.2 Hz, 1H), 3.44 (s, 1H), 3.21 (dd, *J* = 14.8, 3.7 Hz, 1H), 2.61 (s, 1H), 2.39 – 2.31 (m, 2H), 2.31 – 2.25 (m, 3H), 2.00 (dd, *J* = 2.4, 1.6 Hz, 3H), 1.95 (s, 3H), 1.66 – 1.64 (m, 1H), 1.50 (s, 3H), 1.38 (s, 3H), 1.36 – 1.29 (m, 4H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.92 – 0.84 (m, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 201.4, 174.5, 173.0, 172.5, 170.7, 156.1, 142.4, 83.8, 79.2, 78.7, 77.9, 73.3, 66.2, 51.9, 38.9, 36.7, 33.9, 31.3, 24.5, 22.9, 22.6, 22.4, 18.1, 16.4, 14.0, 13.8, 10.4.

HRMS (ESI) *m/z*: calculated for $C_{27}H_{39}O_{11}$ $[M+H]^+$ 539.2487, found 539.2485



Experimental: To lactone **12** (2 mg, 0.0037 mmol) in Et_2O (0.2 mL) was added $Zn(BH_4)_2$ (0.5 M Et_2O solution, 0.2 mL) dropwise at – 20 °C. The resulting solution was stirred at that temperature for 4 hours. The reaction mixture was then diluted with EtOAc (1 mL) and ice water was added dropwise. The aqueous layer was extracted EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC (1:2 EtOAc:Hexanes) to afford intermediate alcohol (1.7 mg, 84%) as an amorphous solid.

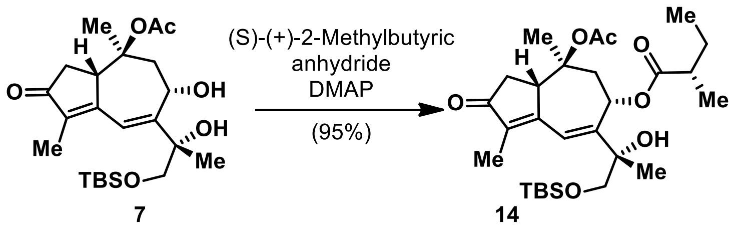
To the alcohol was added neat angelic anhydride (ca. 15 mg) and $NaHCO_3$ (10 mg). The resulting mixture was heated to 80 °C for 16 hours. The resulting mixture was purified directly via preparative TLC (1:2 EtOAc:Hex) to afford thapsigarcin (60%, 1.1 mg) as a white film.

Physical State: white film

^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.11 – 6.00 (m, 1H), 5.71 (s, 1H), 5.66 (s, 1H), 5.61 (t, *J* = 3.8 Hz, 1H), 5.50 (t, *J* = 3.3 Hz, 1H), 4.16 (s, 1H), 2.93 (dd, *J* = 14.9, 3.6 Hz, 1H), 2.41 (dd, *J* = 14.8, 4.1 Hz, 1H), 2.38 – 2.24 (m, 4H), 2.22 (s, 1H), 2.18 (s, 1H), 2.00 (dq, *J* = 7.3, 1.6 Hz, 3H), 1.93 – 1.83 (m, 9H), 1.63 (dt, *J* = 12.2, 6.1 Hz, 3H), 1.51 (s, 3H), 1.42 (s, 3H), 1.38 – 1.27 (m, 3H), 1.25 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.89 (q, *J* = 4.0, 3.1 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.9, 172.6, 172.4, 170.5, 167.2, 142.6, 138.9, 129.8, 127.6, 84.2, 84.1, 78.9, 78.8, 77.9, 76.9, 66.2, 58.1, 38.5, 36.6, 34.4, 31.4, 29.9, 24.7, 22.8, 22.7, 22.5, 20.7, 18.2, 16.50, 15.97, 14.06, 13.8, 13.2.

HRMS (ESI) *m/z*: calculated for $C_{32}H_{46}O_{12}Na$ [M+Na] 645.2898, found 645.2881.



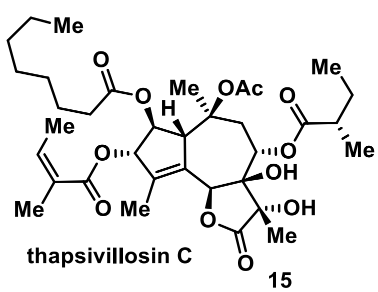
Experimental: Compound **7** (100 mg, 0.228 mmol) was dissolved in CH_2Cl_2 (2.28 mL) before (S)-(+)-2-methylbutyric anhydride (55 mg, 1.3 equiv., 0.297 mmol) and DMAP (22.3 mg, 0.8 equiv.) were added sequentially. The resulting solution was stirred at room temperature for 12 hours. The reaction mixture was then diluted with EtOAc and washed with saturated NH_4Cl. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (1:4 EtOAc:Hexanes → 1:3 EtOAc:Hexanes) to yield compound **14** (113 mg, 95%) as a yellow oil. This compound was carried forward to thapsivillosin C with procedures analogous to those implemented for thapsigarcin.

Physical State: yellow oil.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.03 (s, 1H), 5.87 (dd, J = 5.7, 3.1 Hz, 1H), 4.00 – 3.75 (m, 1H), 3.68 (d, J = 9.7 Hz, 1H), 3.47 (d, J = 9.8 Hz, 1H), 2.91 (s, 1H), 2.76 (dd, J = 15.1, 3.1 Hz, 1H), 2.57 (dd, J = 15.1, 5.7 Hz, 1H), 2.52 – 2.30 (m, 2H), 2.28 (q, J = 7.0 Hz, 1H), 1.96 (s, 3H), 1.80 (d, J = 2.1 Hz, 3H), 1.73 – 1.58 (m, 1H), 1.40 (dp, J = 14.4, 7.3 Hz, 1H), 1.27 (s, 3H), 1.23 (s, 3H), 1.12 (d, J = 7.0 Hz, 3H), 0.86 (s, 11H), 0.06 (d, J = 1.2 Hz, 6H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 208.0, 175.4, 170.4, 161.1, 150.1, 140.5, 124.9, 83.7, 76.0, 69.4, 67.7, 49.2, 42.6, 41.5, 37.0, 26.5, 25.9, 24.5, 22.4, 20.1, 18.3, 16.5, 11.8, 8.8, -5.3, -5.4.

HRMS (ESI) *m/z*: calculated for $C_{28}H_{47}O_7Si$ $[M+H]^+$ 523.3085, found 523.3084



15

Physical State: white film.

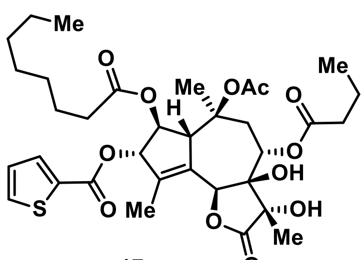
^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.11 (qd, *J* = 7.2 Hz, 1H), 5.71 (s, 1H), 5.64 (s, 1H), 5.61 (t, *J* = 3.8 Hz, 1H), 5.53 – 5.49 (m, 1H), 4.20 (s, 1H), 2.97 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.42 – 2.26 (m, 4H), 2.23 (s, 1H), 2.00 (dd, *J* = 7.3, 1.6 Hz, 3H), 1.92 (q, *J* = 1.5 Hz, 3H), 1.90 (s, 3H), 1.89 – 1.86 (m, 3H), 1.70 (dt, *J* = 14.2, 7.2 Hz, 1H), 1.61 (m, 2H), 1.52 (s, 3H), 1.43 (s, 3H), 1.35 – 1.23 (m, 5H), 1.15 (dd, *J* = 7.1, 2.7 Hz, 3H), 0.94 – 0.84 (m, 6H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 175.3, 174.8, 172.6, 170.6, 167.2, 142.6, 138.9, 129.8, 127.6, 84.2, 84.1, 79.0, 78.7, 77.9, 76.9, 66.3, 58.0, 41.5, 38.6, 34.4, 31.8, 29.2, 29.1, 26.3, 25.0, 23.0, 22.8, 22.7, 20.74, 16.55, 16.46, 16.0, 14.2, 13.2, 11.8.

HRMS (ESI) *m/z*: calculated for $C_{35}H_{52}O_{12}Na$ $[M+Na]^+$ 687.3351, found 687.3353

Preparation of C3 analogs:

Compound **17**, **18**, **19**, **20** were prepared according to the following general procedure. Compound **16** was dissolved in CH_2Cl_2 (0.2 mL/3 mg of **16**). DCC (4 eq.), carboxylic acid (4 eq.) and DMAP (2 eq.) were added sequentially to the reaction mixture. Upon completion of the reaction determined by TLC analysis, the reaction mixture was purified directly via preparative TLC (1:2 EtOAc:Hexanes) to afford the corresponding C3 analog.



17

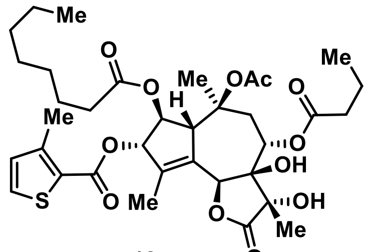
Yield: (from 2 mg of **16**, 85% yield, 2 mg)

Physical State: amorphous solid.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.94 – 7.80 (m, 1H), 7.58 (dt, *J* = 4.9, 1.1 Hz, 1H), 7.15 – 7.09 (m, 1H), 5.78 (s, 1H), 5.69 (s, 1H), 5.65 – 5.60 (m, 1H), 5.57 (t, *J* = 3.2 Hz, 1H), 4.23 (s, 1H), 2.97 (dd, *J* = 15.1, 3.7 Hz, 1H), 2.47 (s, 1H), 2.40 (dd, *J* = 14.8, 4.1 Hz, 1H), 2.36 – 2.24 (m, 5H), 1.96 – 1.92 (m, 5H), 1.90 (d, *J* = 1.1 Hz, 3H), 1.67 (dd, *J* = 18.1, 10.7 Hz, 3H), 1.51 (s, 3H), 1.47 (s, 3H), 1.41 – 1.23 (m, 8H), 1.14 (dq, *J* = 22.7, 11.2, 10.6 Hz, 2H), 0.99 – 0.93 (m, 3H), 0.87 (t, *J* = 6.8 Hz, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.9, 172.6, 172.4, 170.6, 161.5, 142.2, 134.1, 133.3, 132.9, 130.6, 128.0, 85.3, 84.2, 78.9, 78.8, 77.6, 77.5, 76.8, 66.2, 58.3, 38.6, 36.7, 34.4, 33.9, 31.8, 29.2, 29.1, 25.70, 25.02, 24.98, 23.0, 22.8, 22.7, 18.2, 16.5, 14.2, 13.9, 13.2.

HRMS (ESI) *m/z*: calculated for $C_{34}H_{46}O_{12}SNa$ $[M+Na]^+$ 701.2602, found 701.2622



18

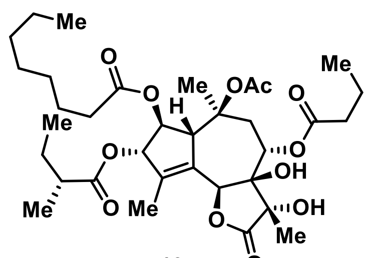
Yield: (from 2 mg of **16**, 85% yield, 2 mg)

Physical State: amorphous solid.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.40 (d, *J* = 5.0 Hz, 1H), 6.93 (d, *J* = 5.0 Hz, 1H), 5.76 (s, 1H), 5.68 (s, 1H), 5.63 (t, *J* = 3.8 Hz, 1H), 5.56 (d, *J* = 3.2 Hz, 1H), 4.25 (s, 1H), 2.99 (dd, *J* = 14.9, 3.6 Hz, 1H), 2.86 (s, 1H), 2.56 (s, 3H), 2.44 – 2.11 (m, 6H), 1.91 (d, *J* = 12.2 Hz, 9H), 1.75 – 1.54 (m, 11H), 1.51 (s, 3H), 1.46 (s, 3H), 1.40 – 1.31 (m, 1H), 1.19 – 1.05 (m, 3H), 0.96 (t, *J* = 7.4 Hz, 3H), 0.87 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 175.1, 172.6, 172.5, 170.7, 161.9, 147.1, 142.2, 132.0, 130.6, 126.2, 84.8, 84.4, 78.9, 78.8, 77.7, 76.9, 66.3, 58.2, 49.5, 38.5, 36.7, 34.4, 34.0, 31.8, 29.2, 29.1, 25.7, 25.04, 25.00, 22.98, 22.8, 22.7, 18.2, 16.5, 16.2, 14.2, 13.9, 13.2.

HRMS (ESI) *m/z*: calculated for $C_{35}H_{48}O_{12}SNa$ $[M+Na]^+$ 715.2775, found 715.2759



19

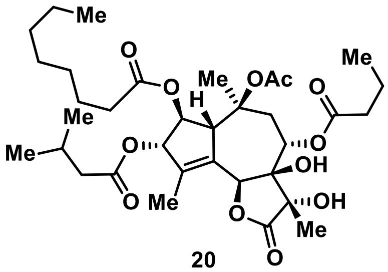
Yield: (from 3 mg of **16**, 82% yield, 2.8 mg)

Physical State: amorphous solid.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.65 (q, *J* = 1.9 Hz, 1H), 5.64 – 5.62 (m, 1H), 5.61 (t, *J* = 3.9 Hz, 1H), 5.46 (dd, *J* = 4.0, 3.0 Hz, 1H), 4.15 (s, 1H), 2.93 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.54 (s, 1H), 2.46 – 2.35 (m, 2H), 2.28 (dddt, *J* = 15.5, 14.3, 8.4, 6.8 Hz, 5H), 1.89 (s, 3H), 1.84 (dt, *J* = 2.6, 1.3 Hz, 3H), 1.76 – 1.69 (m, 1H), 1.50 (s, 3H), 1.43 (s, 3H), 1.33 – 1.23 (m, 11H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.95 (td, *J* = 7.4, 2.7 Hz, 6H), 0.87 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 176.0, 174.9, 172.6, 172.5, 170.6, 142.3, 129.7, 84.3, 84.1, 78.9, 78.7, 77.8, 66.2, 57.9, 41.2, 38.5, 36.7, 34.4, 31.8, 29.2, 29.1, 26.8, 25.0, 22.9, 22.8, 22.7, 18.2, 16.8, 16.49, 14.22, 13.85, 13.0, 11.7.

HRMS (ESI) *m/z*: calculated for $C_{34}H_{52}O_{12}Na$ $[M+Na]^+$ 675.3364, found 675.3351



20

Yield: (from 2 mg of **16**, 80% yield, 1.8 mg)

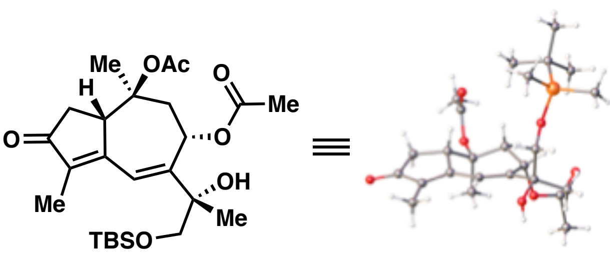
Physical State: amorphous solid.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.67 (s, 1H), 5.65 (s, 1H), 5.63 (t, *J* = 3.8 Hz, 1H), 5.48 (dd, *J* = 3.9, 2.9 Hz, 1H), 4.17 (s, 1H), 2.94 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.52 (s, 1H), 2.44 (dd, *J* = 14.8, 4.1 Hz, 1H), 2.38 – 2.23 (m, 6H), 2.16 (dq, *J* = 13.6, 6.7 Hz, 1H), 1.95 (dd, *J* = 10.7, 6.8 Hz, 3H), 1.92 (s, 3H), 1.88 (dt, *J* = 2.6, 1.3 Hz, 3H), 1.72 (dt, *J* = 13.5, 3.9 Hz, 2H), 1.66 (d, *J* = 7.4 Hz, 1H), 1.53 (s, 3H), 1.45 (s, 3H), 1.42 – 1.32 (m, 10H), 1.21 – 1.09 (m, 3H), 1.01 (dd, *J* = 6.6, 3.6 Hz, 6H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H).

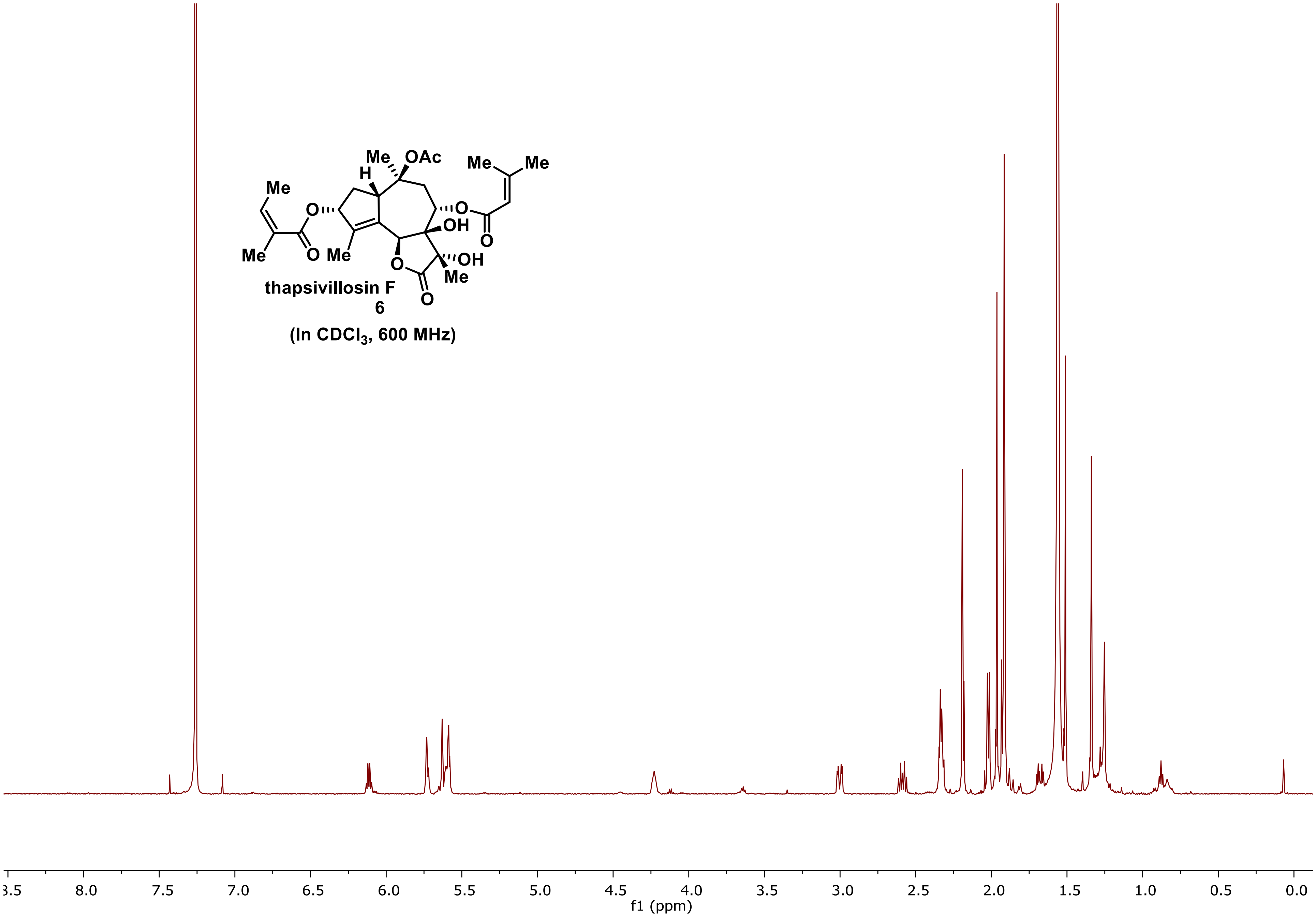
^{13}C NMR (151 MHz, $CDCl_3$) δ 174.9, 172.6, 172.5, 172.4, 170.5, 142.3, 129.8, 84.3, 84.2, 78.9, 78.8, 77.9, 76.9, 66.2, 58.0, 49.4, 43.5, 38.5, 36.7, 34.4, 34.1, 31.8, 29.2, 29.1, 25.9, 25.8, 25.1, 25.0, 22.85, 22.75, 22.66, 22.6, 22.5, 18.2, 16.5, 14.2, 13.9, 13.1.

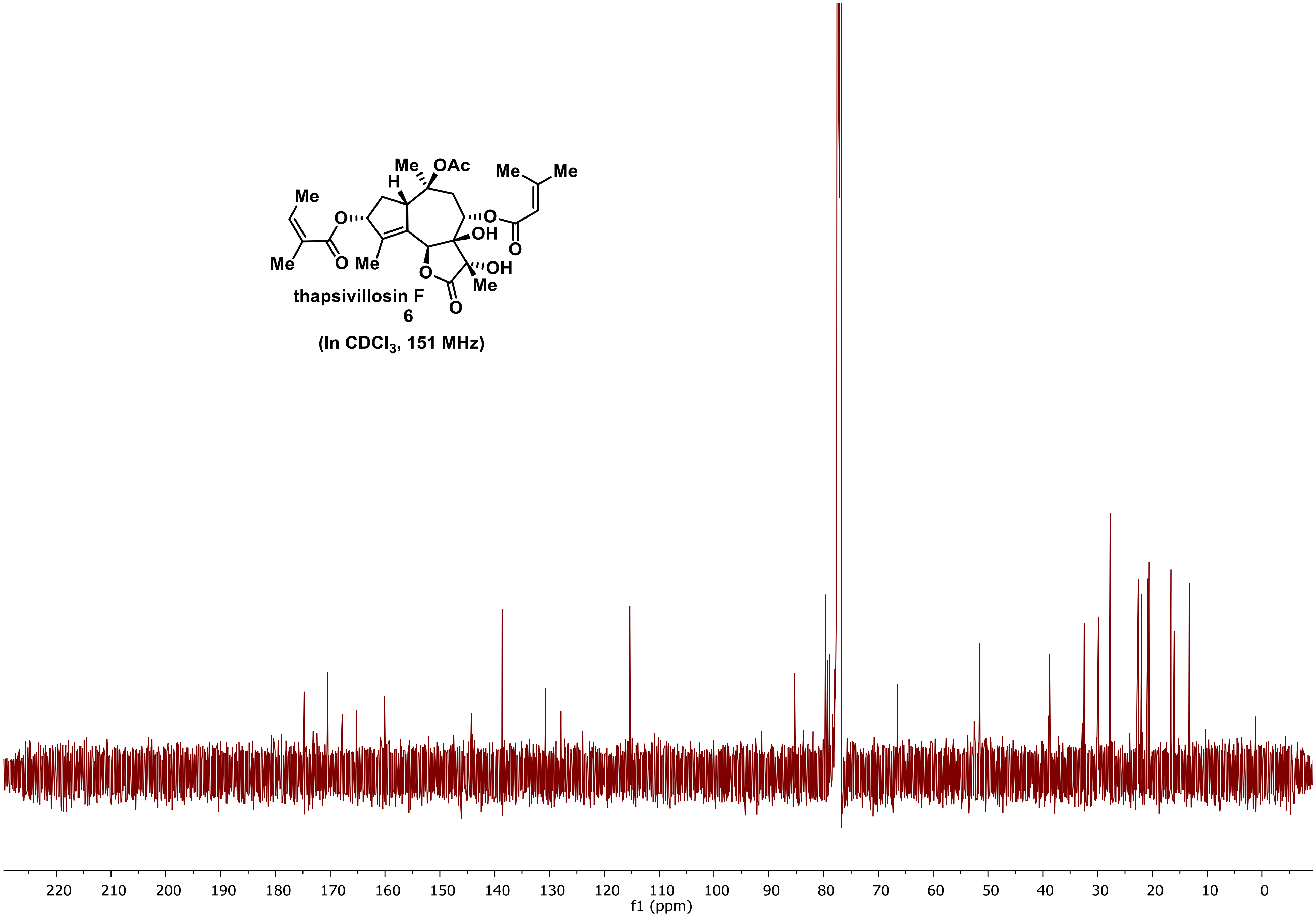
HRMS (ESI) *m/z*: calculated for $C_{34}H_{52}O_{12}Na$ $[M+Na]^+$ 675.3351, found 675.3354

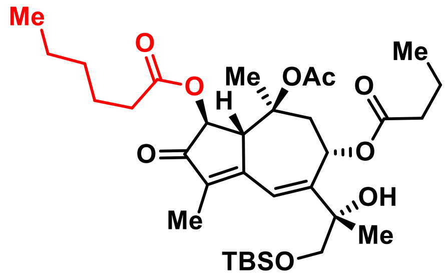
Figure S-1. X-ray structure of **S1**



S1

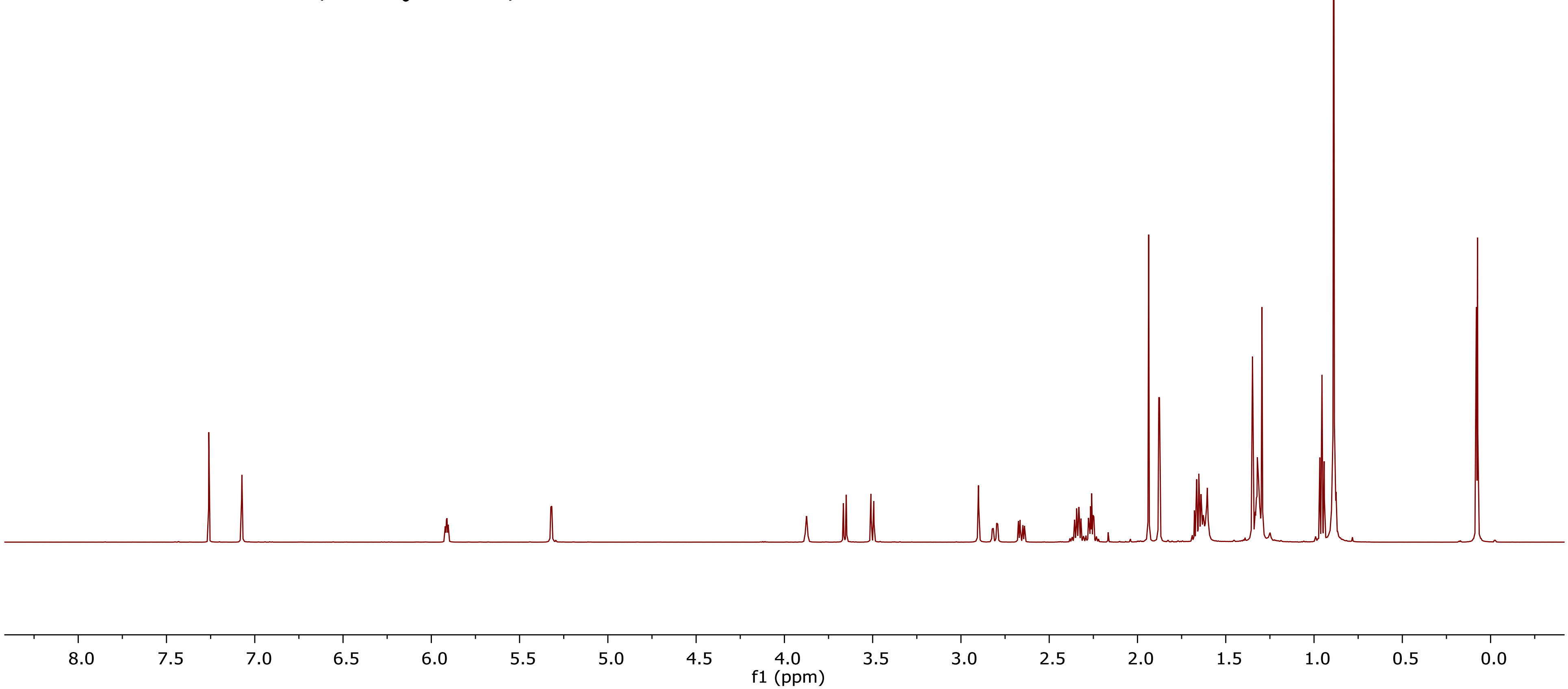

thapsivillosin F
6
(In CDCl$_3$, 600 MHz)
Me
OAc
H
Me
Me
Me
O
OH
O
O
Me
Me
O
OH
Me
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

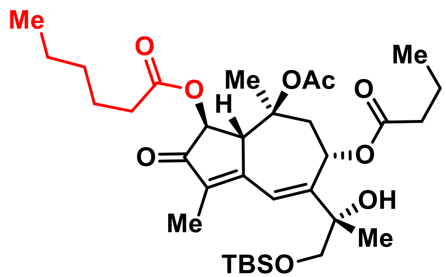

thapsivillosin F
6
(In CDCl$_3$, 151 MHz)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



9

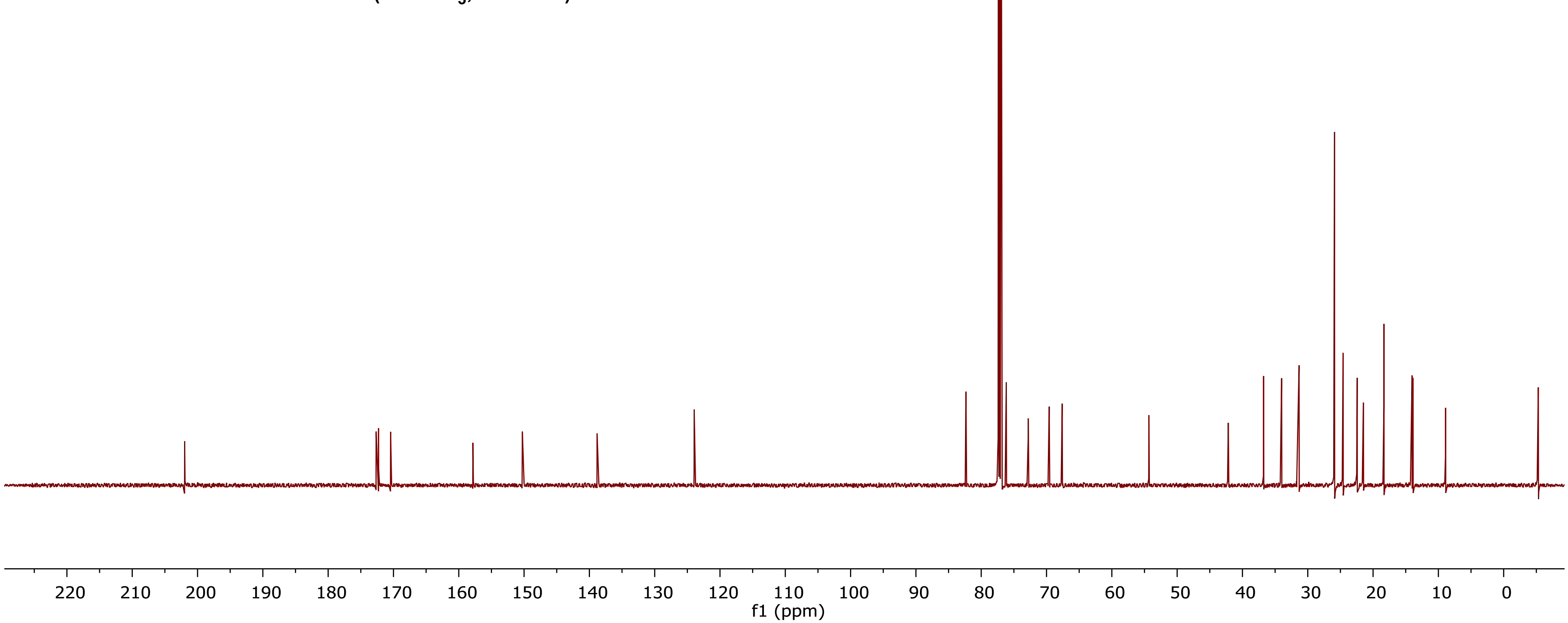
(In $CDCl_3$, 600 MHz)

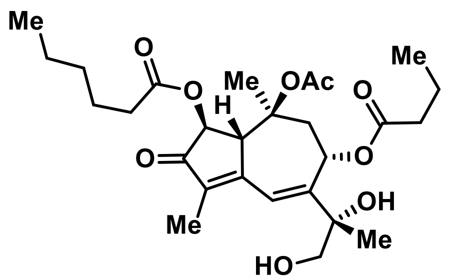




9

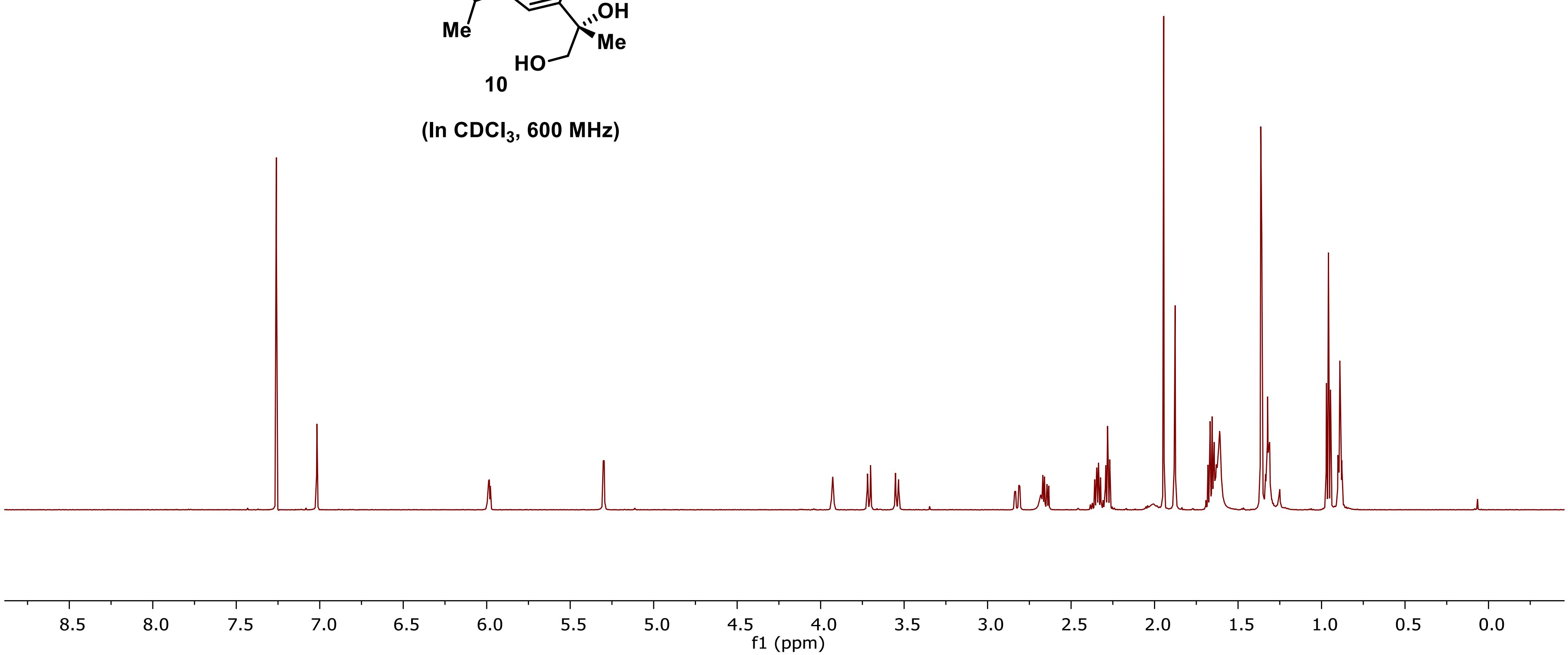
(In $CDCl_3$, 151 MHz)

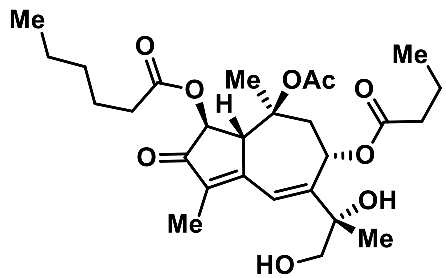




10

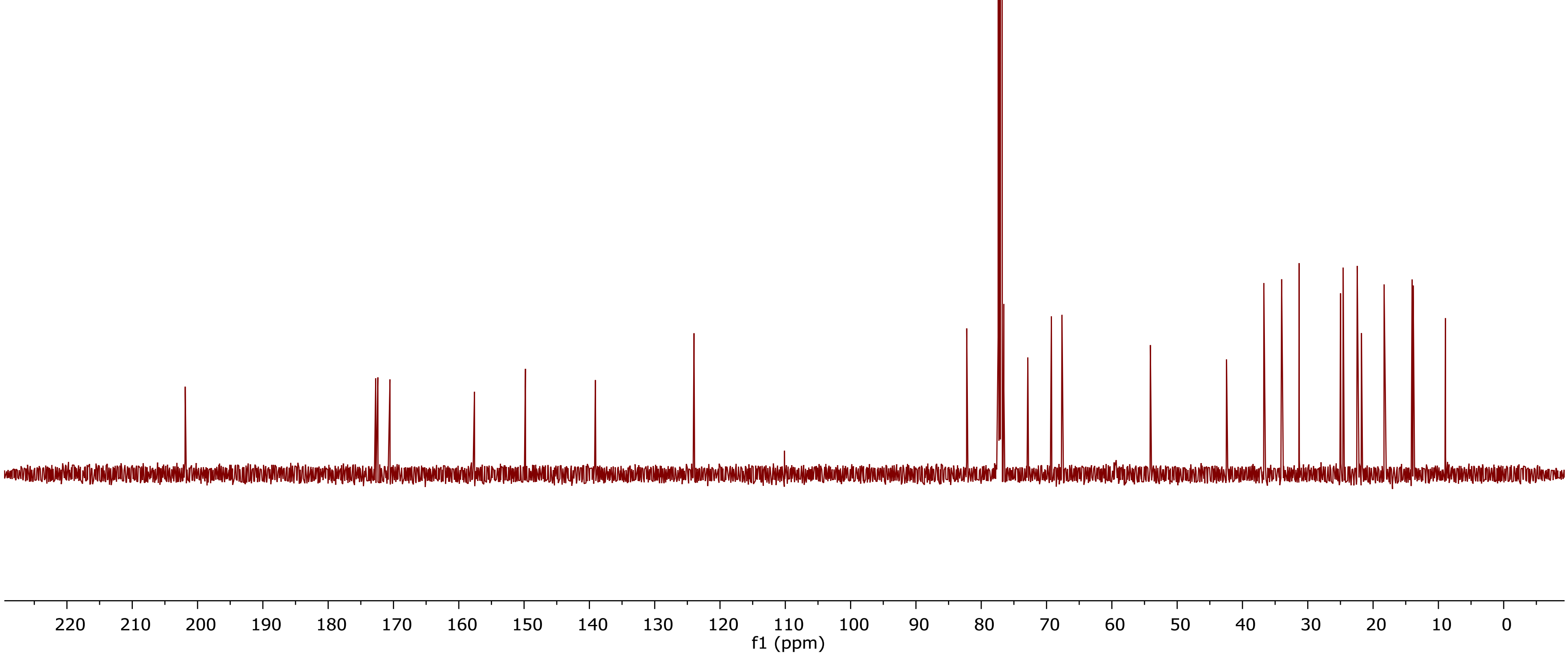
(In $CDCl_3$, 600 MHz)

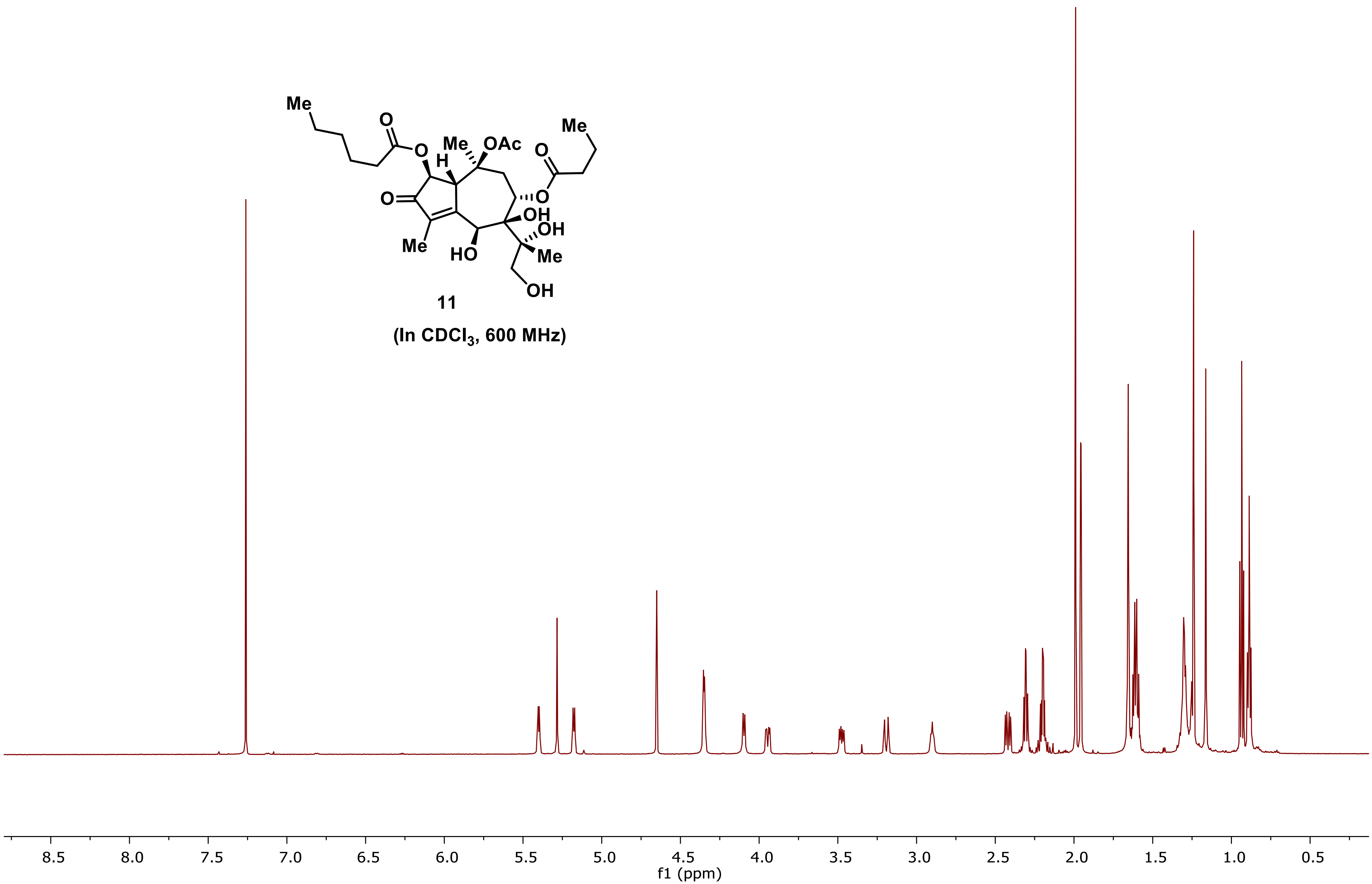




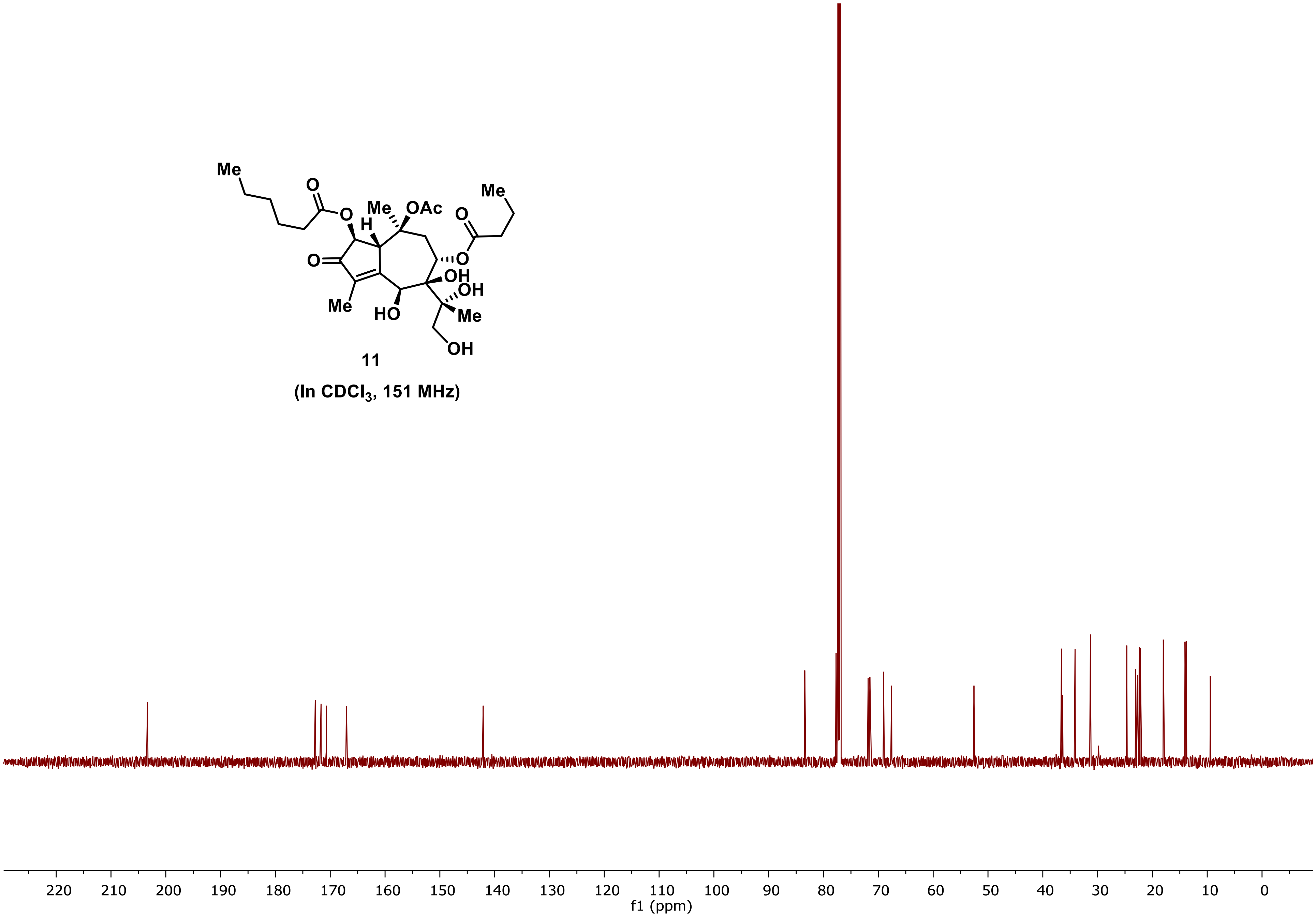
10

(In $CDCl_3$, 151 MHz)



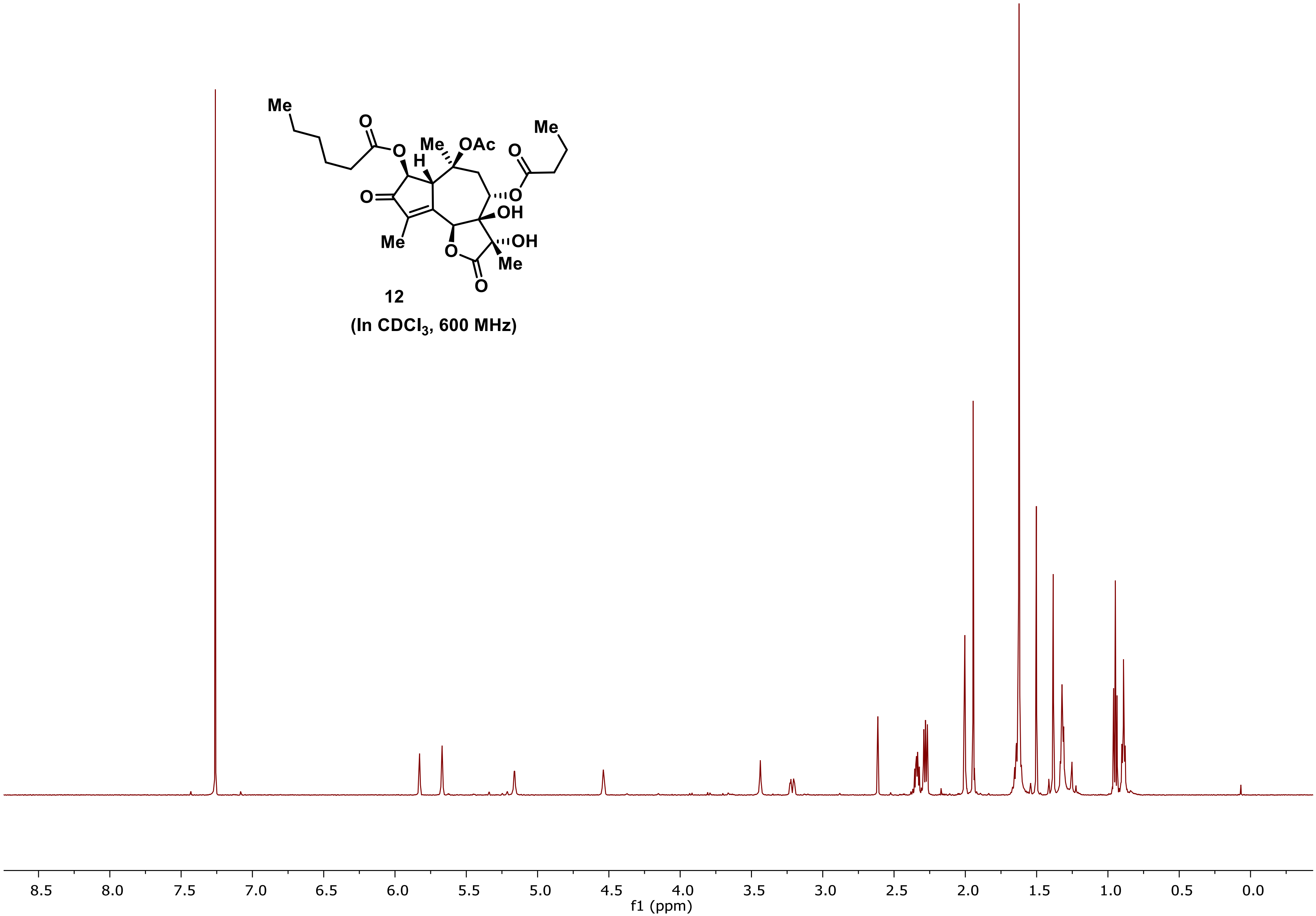


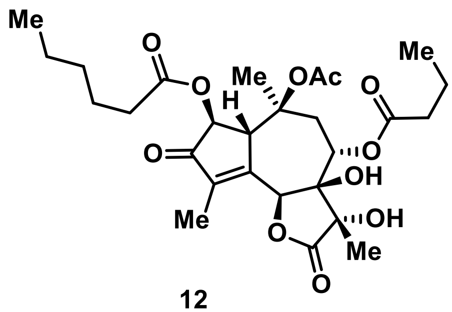
Me
O
O
Me
OAc
H
Me
O
O
O
OH
OH
Me
Me
HO
OH
11
(In CDCl3, 600 MHz)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)



11

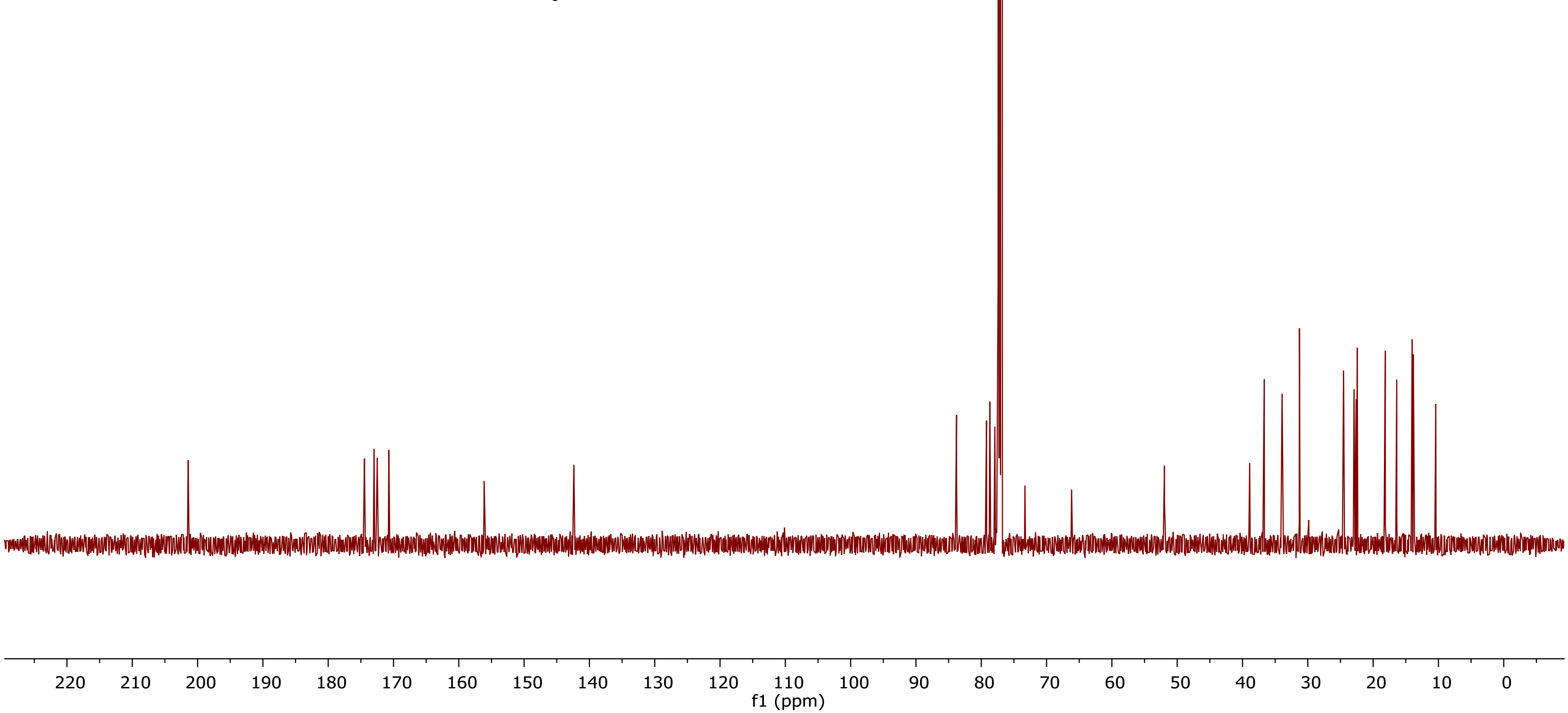
(In $CDCl_3$, 151 MHz)

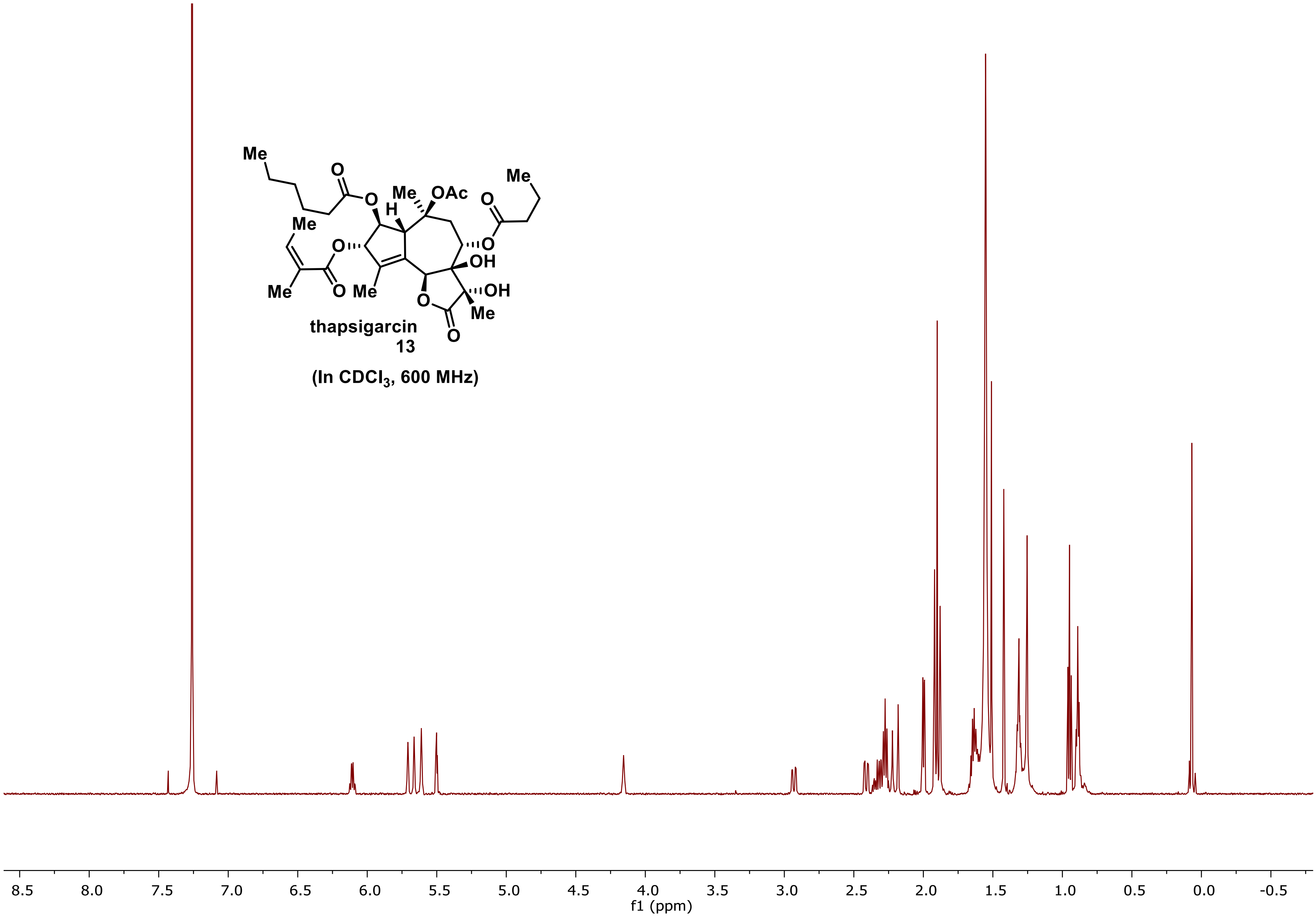

Me
O
Me
OAc
Me
O
O
H
O
O
OH
Me
OH
O
Me
O
12
(In CDCl$_3$, 600 MHz)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

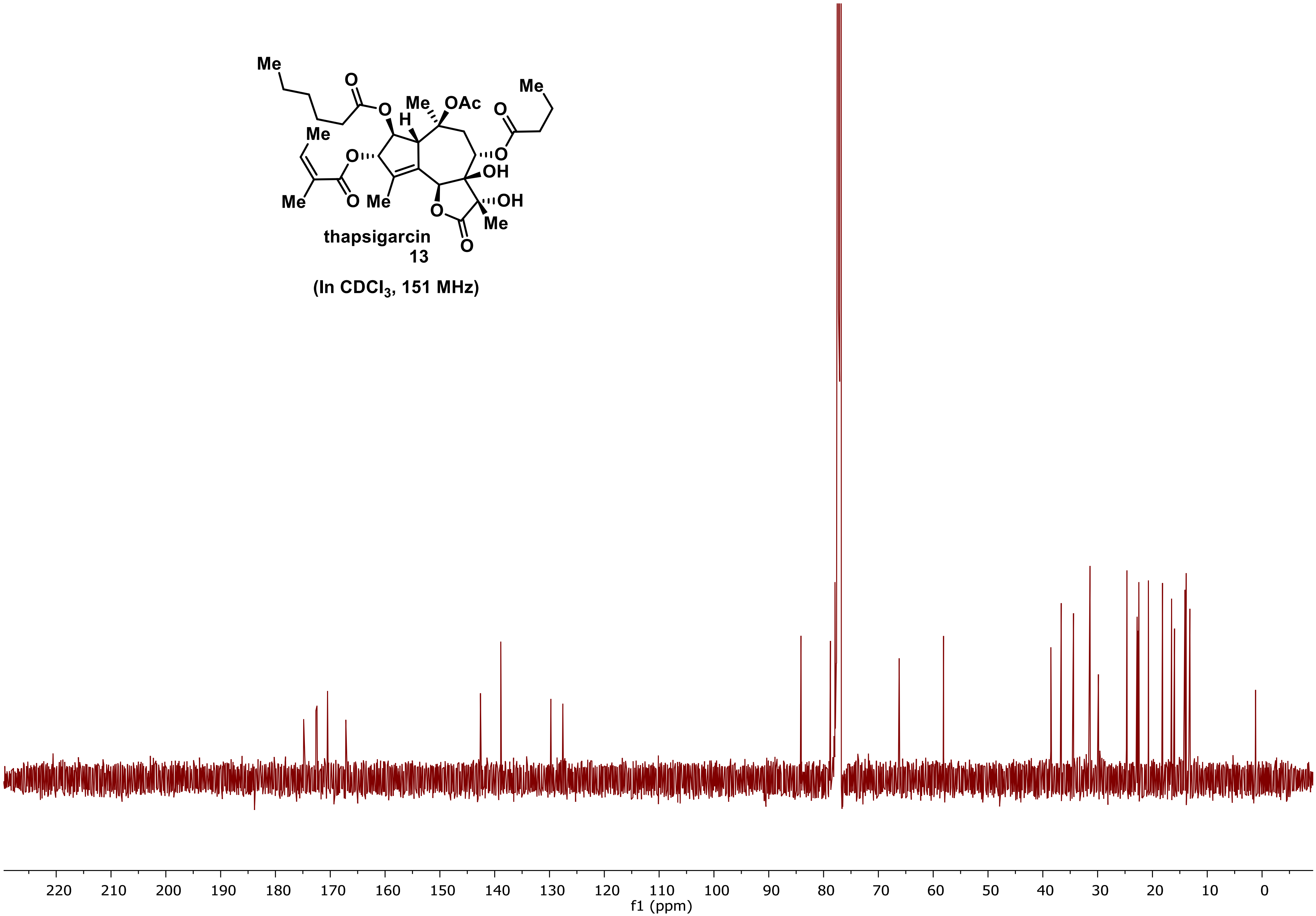


12

(In $CDCl_3$, 151 MHz)

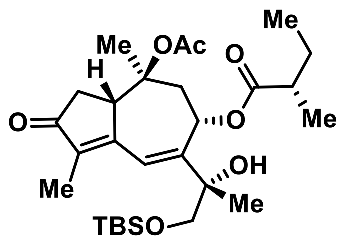



thapsigarcin
13
(In CDCl$_3$, 600 MHz)
Me
O
O
Me
OAc
H
Me
O
O
OH
OH
Me
O
O
Me
O
O
Me
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



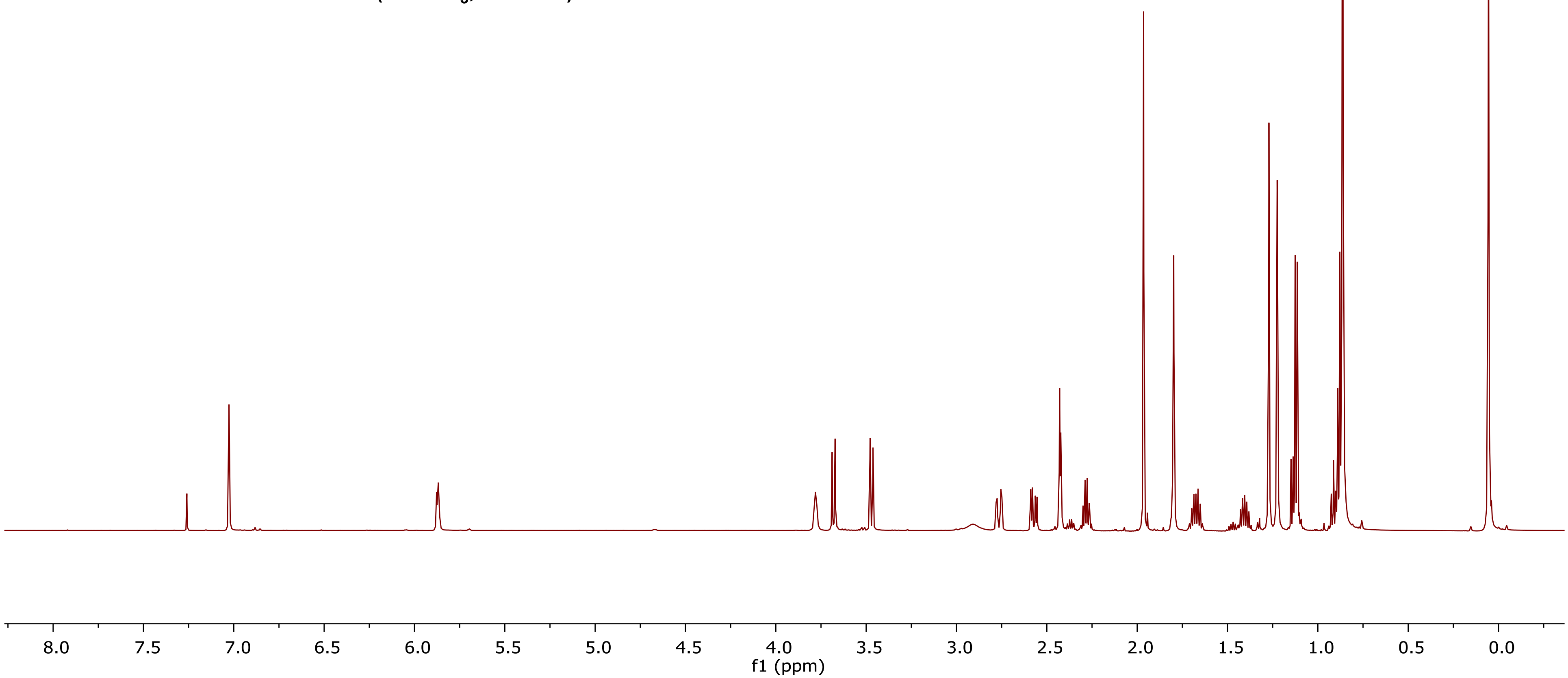
thapsigarcin
13

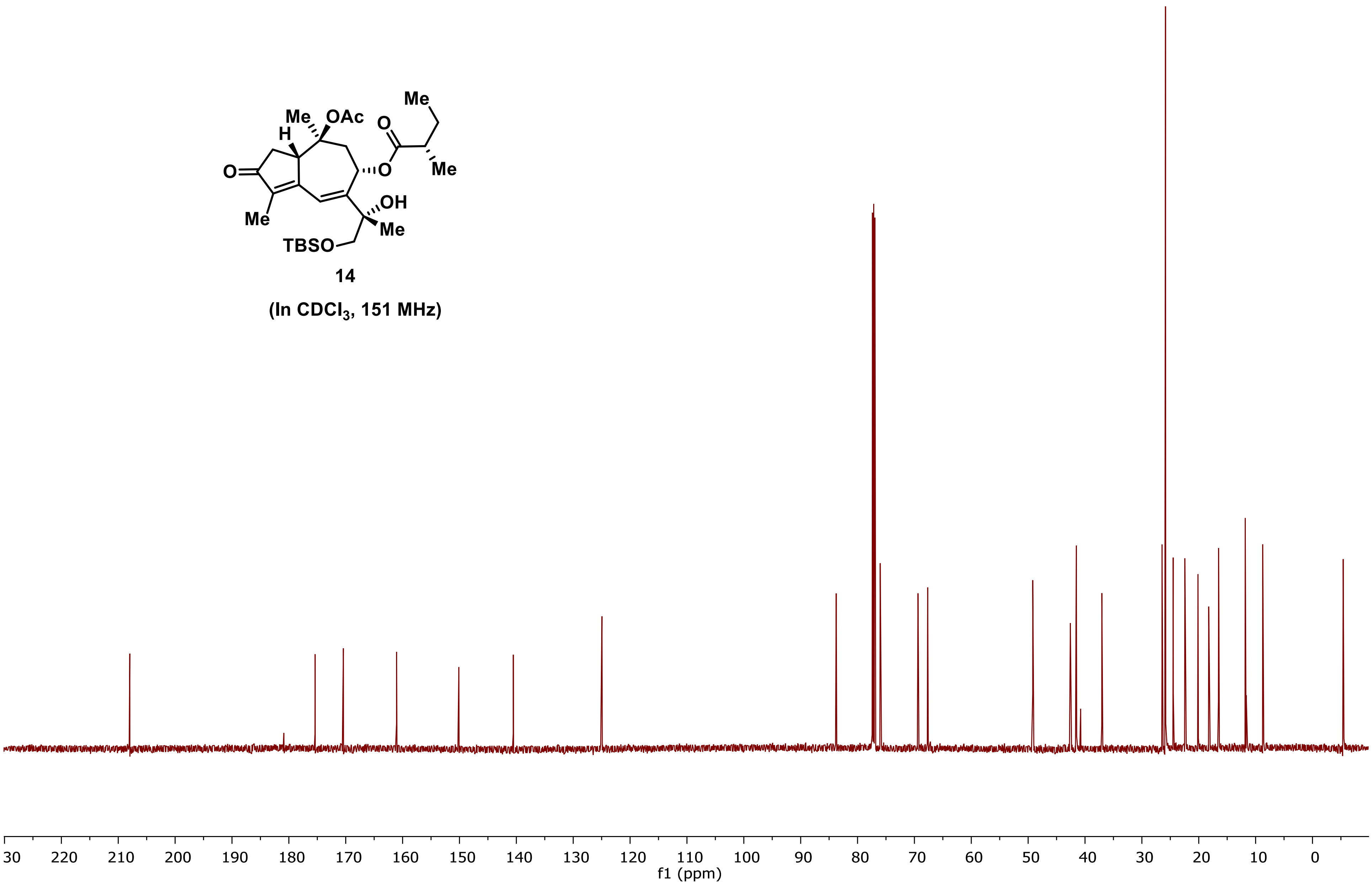
(In $CDCl_3$, 151 MHz)



14

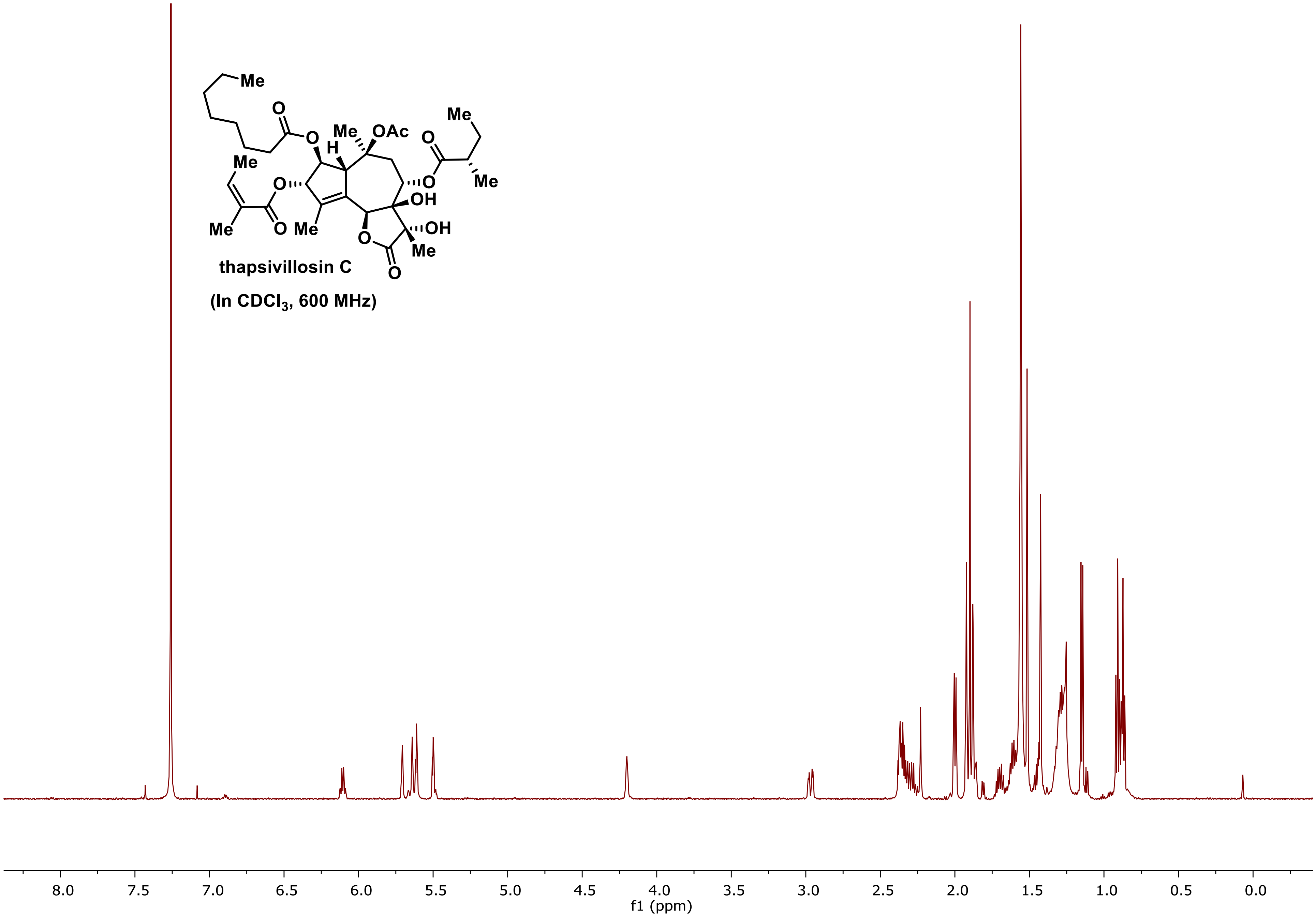
(In $CDCl_3$, 600 MHz)

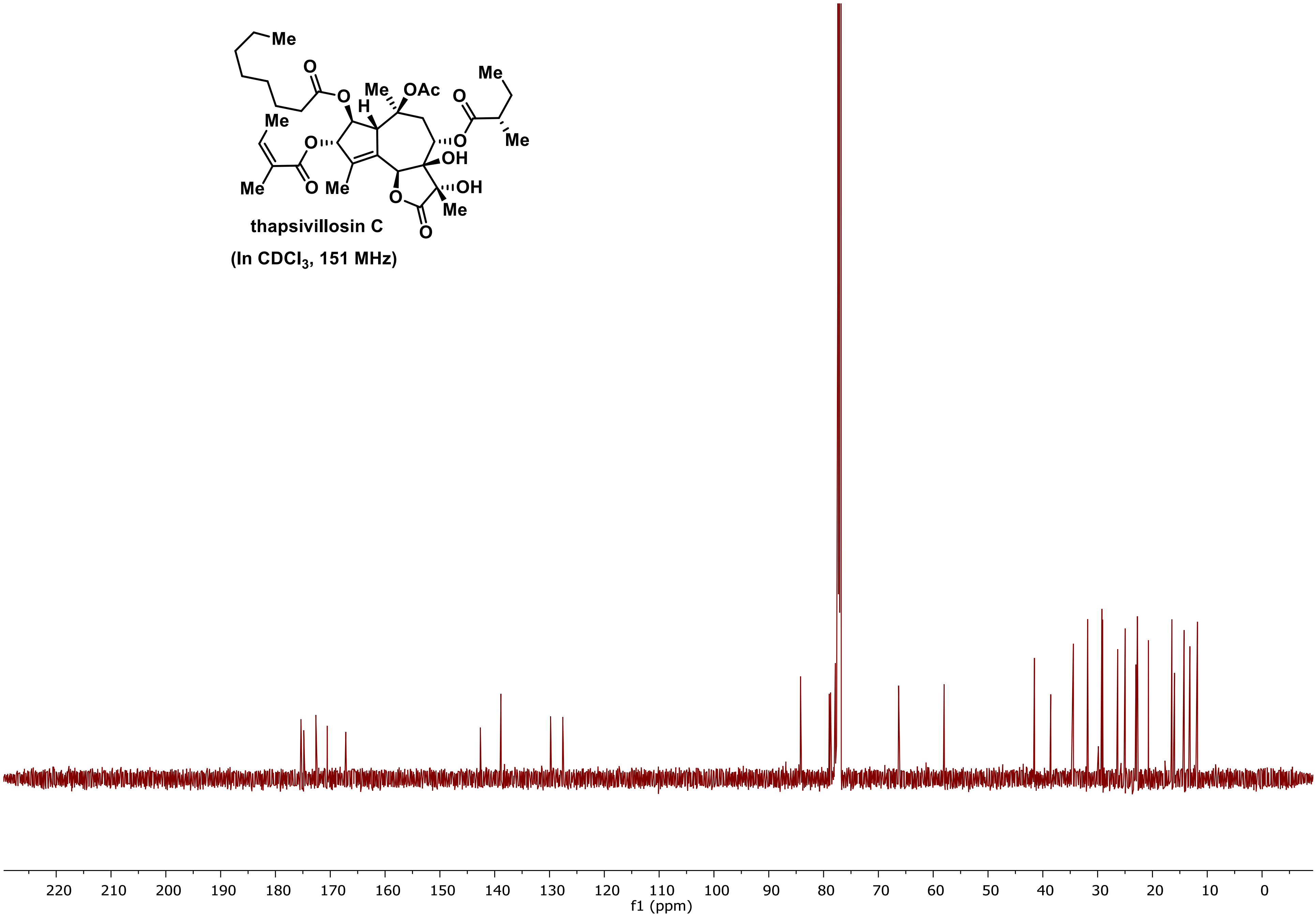


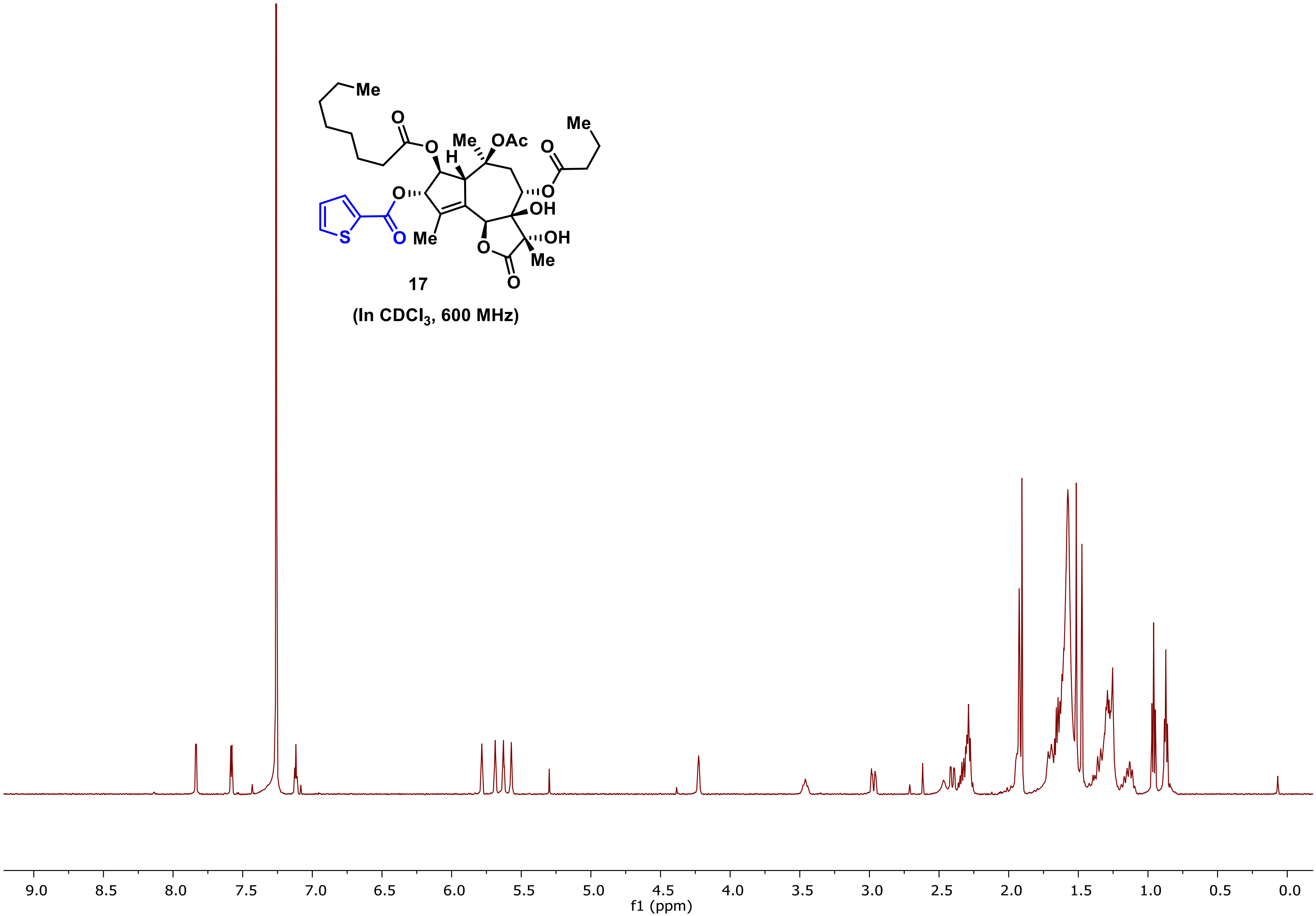


14

(In $CDCl_3$, 151 MHz)

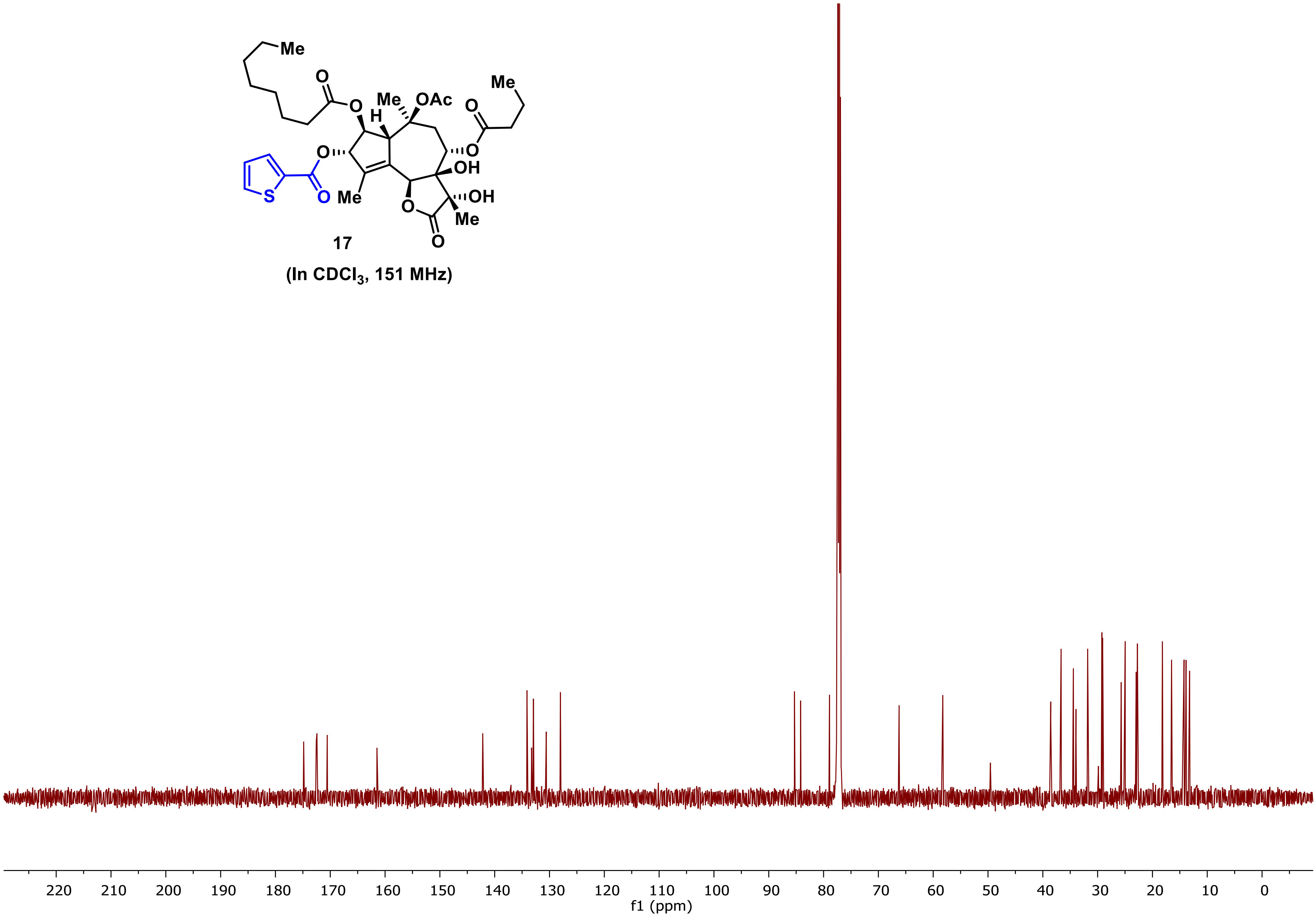

thapsivillosin C
(In CDCl3, 600 MHz)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

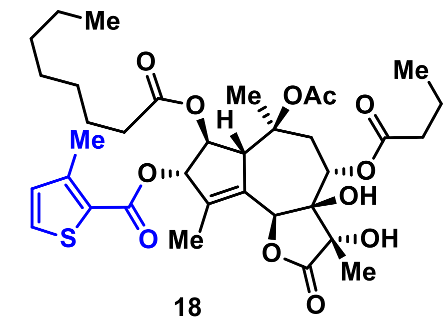

thapsivillosin C
(In $CDCl_3$, 151 MHz)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



17

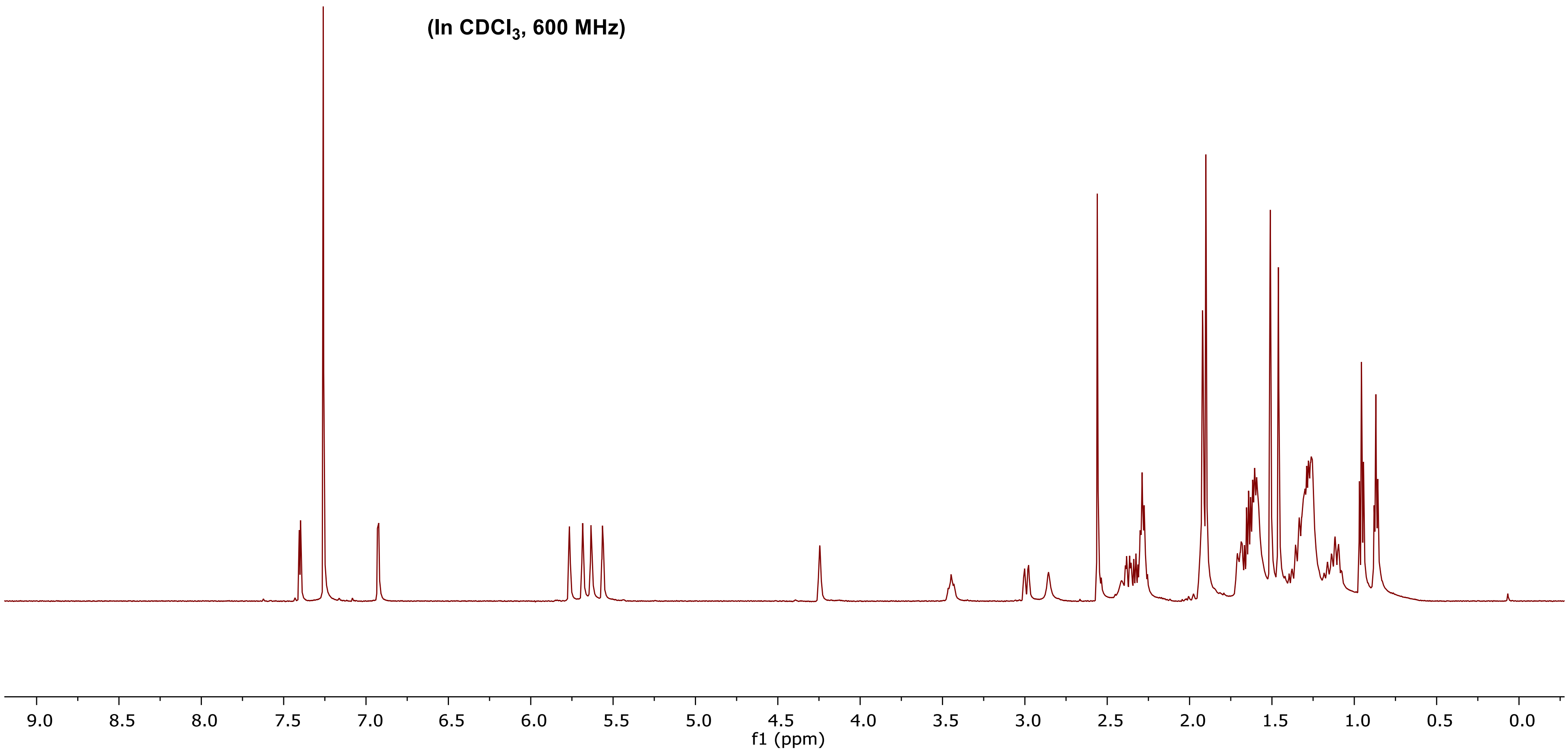
(In $CDCl_3$, 600 MHz)

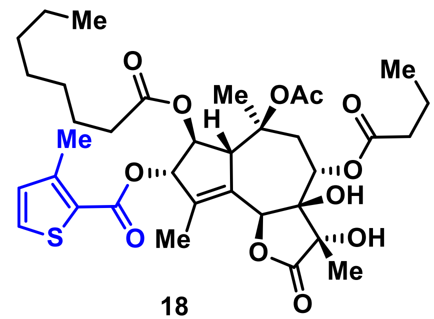

Me
O
Me
OAc
Me
H
O
O
O
O
S
O
OH
Me
O
OH
Me
O
17
(In $CDCl_3$, 151 MHz)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



18

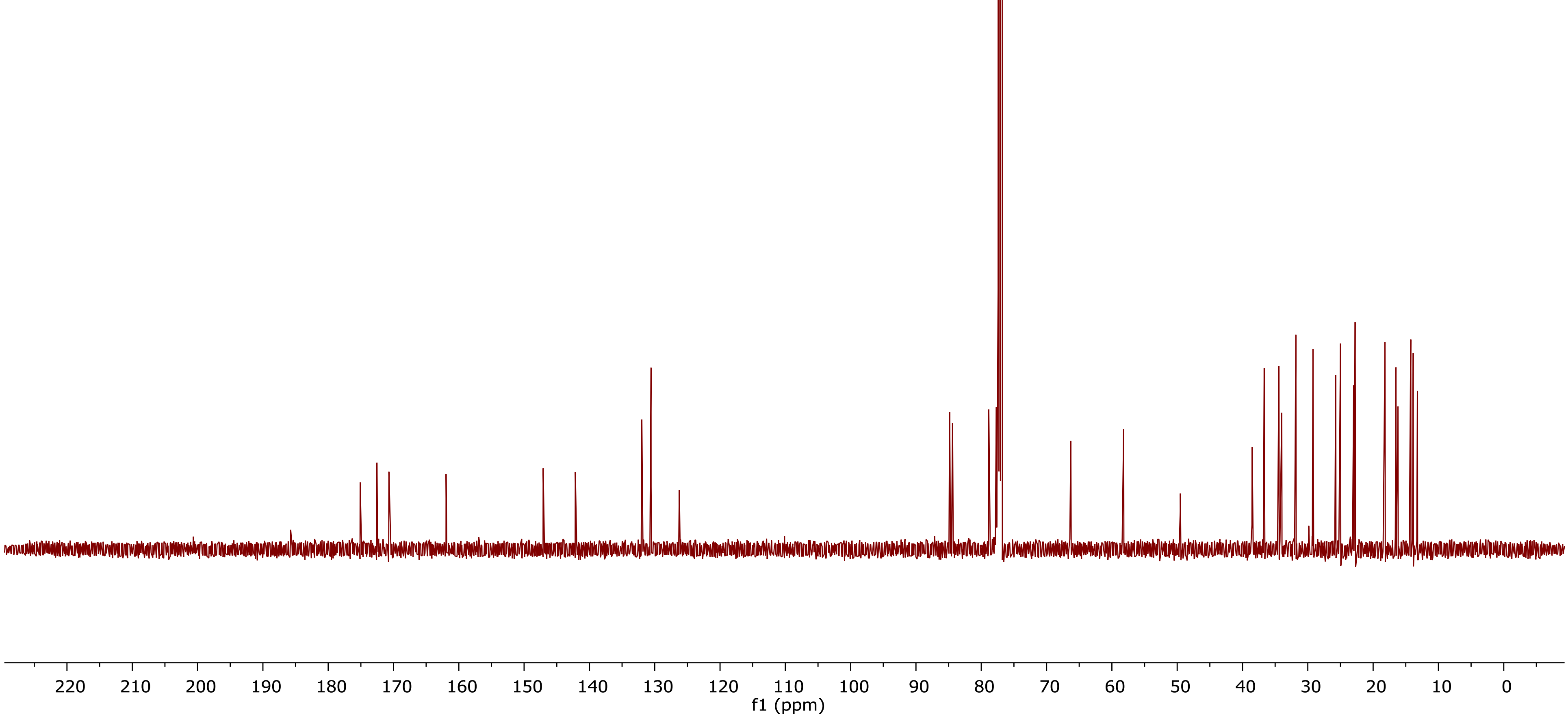
(In $CDCl_3$, 600 MHz)

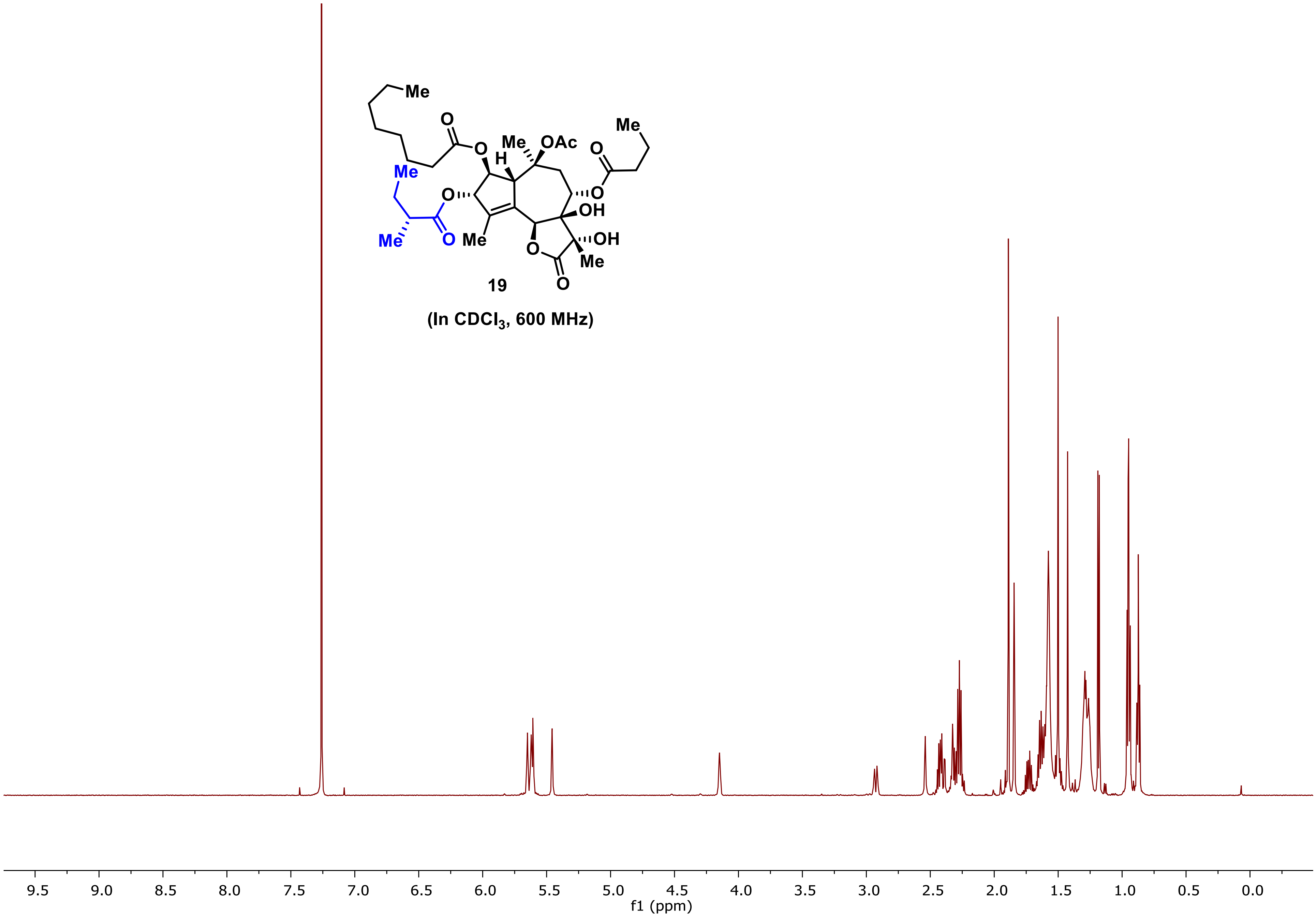


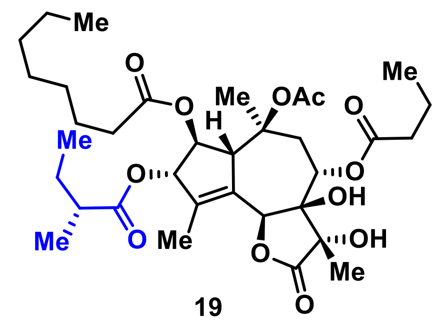


18

(In $CDCl_3$, 151 MHz)

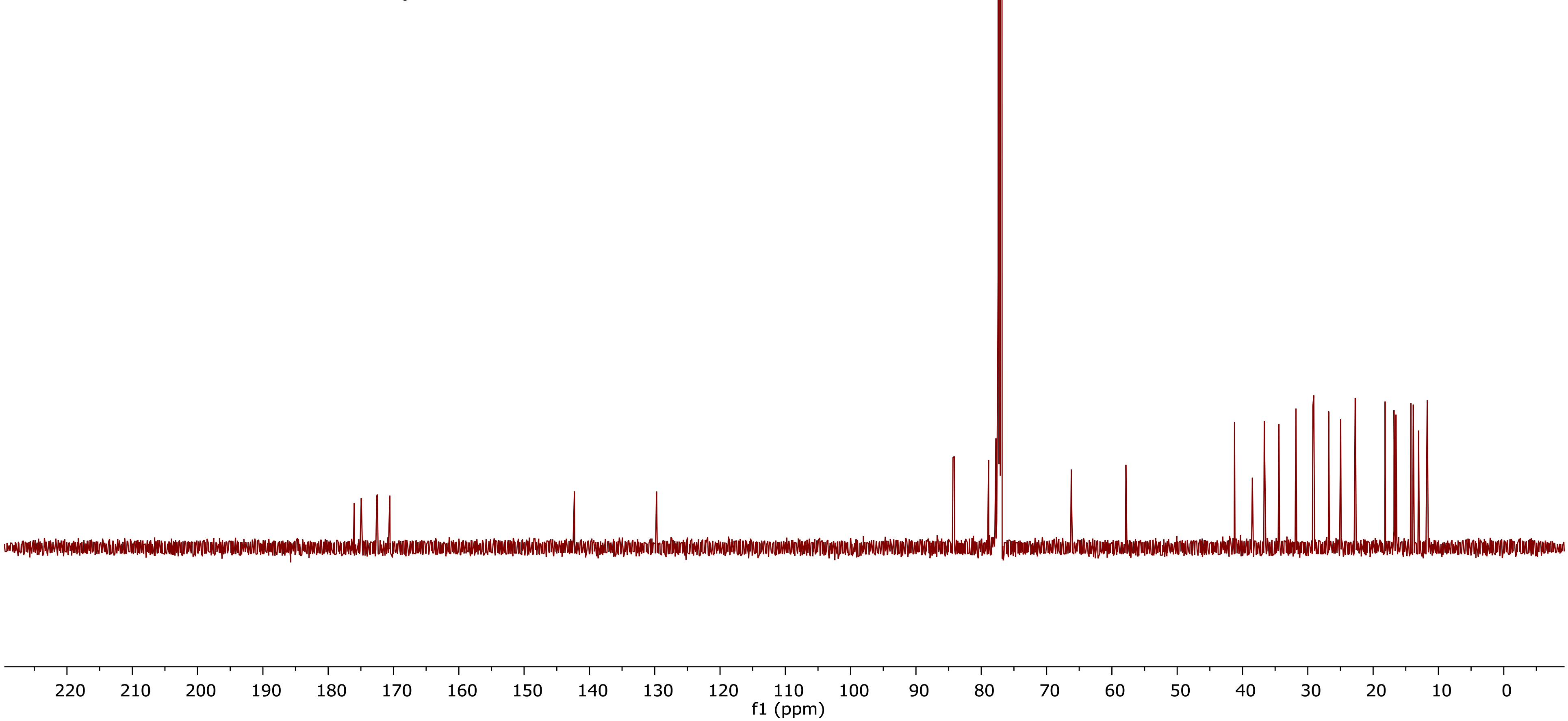


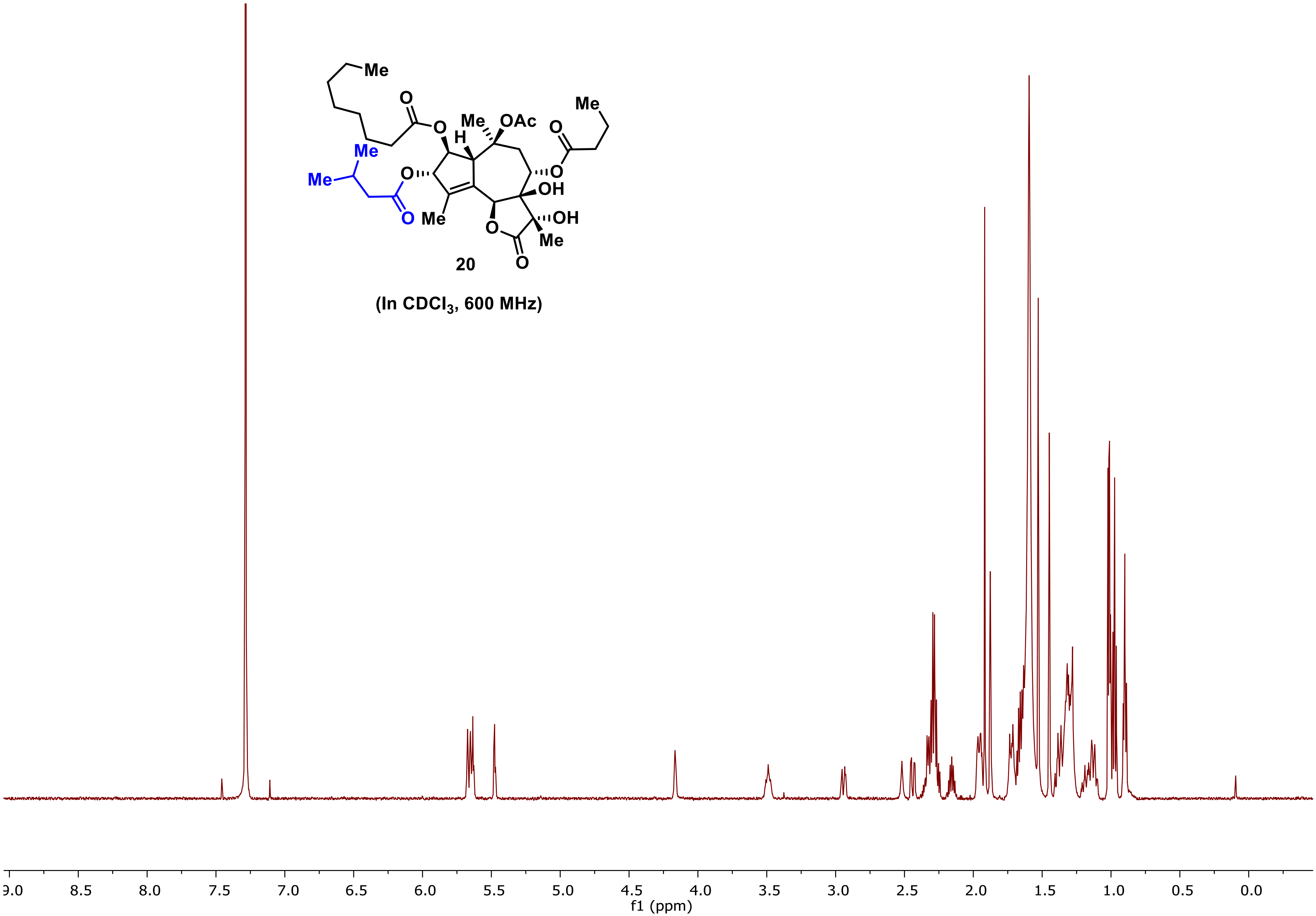

19
(In CDCl$_3$, 600 MHz)
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)



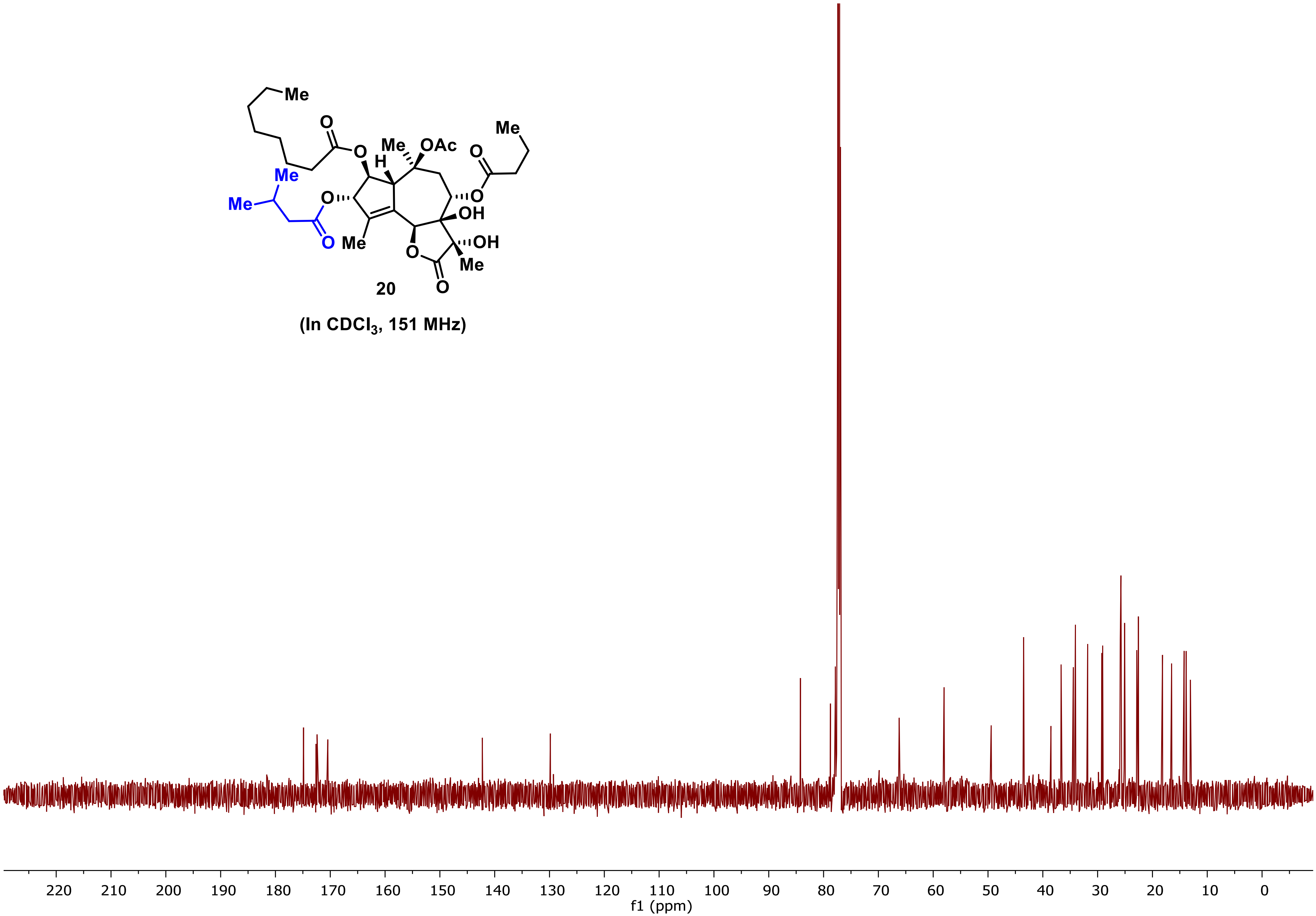
19

(In $CDCl_3$, 151 MHz)





Me
O
O
Me
OAc
O
Me
H
Me
Me
O
O
OH
OH
O
Me
O
Me
O
O
20
(In CDCl3, 600 MHz)
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)



20

(In $CDCl_3$, 151 MHz)

SERCA inhibition experiments

SERCA inhibition assay

Inhibition of SERCA results in a slow and steady increase of cytosolic Ca^{2+} concentration [Ca^{2+}] due to leakage of calcium ions from the endoplasmatic reticulum. This disturbance of intracellular calcium homeostasis can be quantified using cell-permeable, calcium-sensitive fluorogenic dyes like Fura-2 or Calcium 6 **(1)**. In the present work, increase in fluorescence of FLUO-4 NW (ThermoFisher Scientific) in HeLa cells after addition of thapsigargin and its exemplified analogues, respectively, was quantified on the FLIPR-Tetra platform (Molecular Devices) according to the manufacturer's instructions **(2)**. The potency of thapsigargin and the exemplified analogues in eliciting this response is used as a proxy for their potency as SERCA inhibitors.

Briefly, HeLa cells were seeded at a density of 25,000 cells/well (cell culture medium MEM with 10% FBS, 100 ul per well) in 96-well plates and incubated overnight at 37°C in humidified air with 5% CO_2. In preparation for the assay, cells were incubated with FLUO-4 NW as per the manufacturer's instructions (30 min at 37°C in humidified air with 5% CO_2 followed by 30 min at room temperature).

Test compounds were prepared in 100% DMSO and diluted with assay buffer (HBSS, 20 mM HEPES) to 6X final concentration. For each compound, 10-point titrations covering an in-assay range of 10 uM – 38 pM were generated. At t=0, 20 ul of 6X test compound stocks were added to HeLa cells loaded with FLUO-4 NW in 100 ul assay buffer and increase in fluorescence was recorded on the FLIPR-Tetra platform over 300 seconds for each well (excitation LED 470-495 nm, emission filter 515-575 nm). For each compound, signal maxima for 10-point titrations were fitted to a log agonist vs response curve model (GraphPad Prism) after normalizing effect of the test compounds to the signal maxima observed for the positive control 10 mM thapsigargin (=100% inhibition) and the background buffer control (HBSS + 20 mM HEPES, 0.2% DMSO) over the total measurement time. Relative EC50 defined as the concentration of test compound producing a maximum fluorescence increase midway between the fitted top and bottom was calculated for each compound and is reported as IC50 for inhibition of SERCA. Results represent average +/- standard deviation of three independent determinations run in duplicate on individual plates. All experiments were performed at HD Biosciences La Jolla, San Diego, CA.

(1) Li S, Hao B, Lu Y, Yu P, Lee H-C, et al. (2012). PLoS ONE 7(2): e31905.
(2) https://mdc.custhelp.com/euf/assets/content/product_insert_R8194_5024582-FLIPR%20Calcium%206%20Evaluation%20Kit%20Product%20Insert.C.pdf

Results from individual SERCA assay runs:
Run #1

	Thapsigargin		Cpd 6		Cpd 13		Cpd 15		Cpd 17		Cpd 18		Cpd 19		Cpd 20	
Conc [M]	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**
1.00E-05	89.0	92.9	96.9	101.7	92.5	96.4	100.4	110.0	97.6	98.0	93.9	93.2	87.4	89.0	89.7	90.6
2.50E-06	105.2	104.2	101.1	97.7	102.9	105.3	102.1	108.9	101.8	104.6	101.0	99.7	100.3	94.8	90.5	86.0
6.25E-07	101.4	109.0	86.0	76.0	104.1	104.5	94.2	100.6	99.3	102.6	100.3	101.9	102.6	103.2	60.2	57.2
1.56E-07	103.0	102.6	40.2	31.7	78.7	70.6	36.2	33.8	83.7	78.7	99.4	96.0	80.8	74.8	13.2	16.1
3.91E-08	69.2	57.2	10.7	8.6	30.7	23.6	10.3	8.0	25.7	23.2	45.2	43.2	24.4	33.1	2.8	3.4
9.77E-09	9.7	10.1	4.6	4.6	7.9	6.8	3.8	2.3	6.2	6.1	10.0	8.2	5.7	7.1	1.2	1.0
2.44E-09	0.8	1.4	2.5	4.1	4.2	4.6	2.8	1.2	1.4	1.3	1.6	1.2	1.4	3.6	-2.6	0.6
6.10E-10	-0.2	0.1	3.3	3.1	4.1	3.6	2.2	1.2	0.4	0.2	0.8	0.0	-0.4	1.0	-0.2	-0.3
1.53E-10	-0.4	-0.1	2.5	2.6	3.2	3.5	2.5	1.0	0.2	0.2	0.0	0.4	-0.5	1.5	-0.1	-0.3
3.81E-11	0.4	-0.1	1.0	1.7	1.9	2.0	1.0	0.4	-0.1	0.3	0.1	0.1	-0.7	-0.1	-0.1	0.1
	IC50: 30.6		**IC50: 245.7 nM**		**IC50: 81.3 nM**		**IC50: 220.3 nM**		**IC50: 73.4 nM**		**IC50: 42.7 nM**		**IC50:65.2 nM**		**IC50: 435.7 nM**	

Run #2

	Thapsigargin		Cpd 6		Cpd 13		Cpd 15		Cpd 17		Cpd 18		Cpd 19		Cpd 20	
Conc [M]	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**	**%Inh_1**	**%Inh_2**
1.00E-05	97.0	98.6	92.0	93.1	86.6	95.0	99.8	89.4	98.0	92.9	95.4	92.0	95.8	100.5	91.7	94.7
2.50E-06	92.9	100.8	93.2	87.1	91.1	92.0	95.0	~~75.6~~ *	94.4	92.2	93.1	86.7	88.1	98.1	91.7	91.3
6.25E-07	90.4	100.6	86.9	65.5	93.0	90.8	91.5	88.4	94.3	89.0	92.3	87.1	91.7	99.4	84.1	70.7
1.56E-07	87.0	89.8	55.6	17.6	86.2	64.9	40.9	21.2	84.4	82.3	93.4	85.8	87.8	80.0	24.4	10.1
3.91E-08	75.1	49.1	2.8	-1.7	26.8	9.7	4.3	11.1	16.6	12.3	58.5	60.1	45.8	14.1	0.3	0.9
9.77E-09	9.8	10.5	-1.1	-3.0	-0.6	-0.1	1.2	2.7	0.1	0.3	5.4	5.5	0.0	1.0	-0.9	0.4
2.44E-09	1.0	0.1	-0.3	-2.9	-2.9	-4.2	0.0	0.0	-1.4	-0.3	0.3	0.1	1.3	-1.1	-2.1	-0.5
6.10E-10	-0.2	-1.8	-2.0	-3.0	-1.8	-2.5	-1.0	-0.7	-0.9	-1.6	-2.4	-1.1	0.3	-0.7	-1.4	-0.4
1.53E-10	-0.8	0.1	-3.3	-2.8	-2.1	-0.8	-1.1	1.4	-3.5	-2.7	-1.2	0.5	-0.1	-2.8	1.1	-2.0
3.81E-11	-0.2	-2.3	-1.8	-1.3	-2.2	0.7	0.0	-2.2	-0.6	0.6	~~30.9~~ *	3.1	0.3	-0.2	0.8	21.4
	IC50: 28.5 nM		**IC50: 203.9 nM**		**IC50: 73.0 nM**		**IC50: 220 nM**		**IC50: 71.5 nM**		**IC50: 30.2 nM**		**IC50: 57.7 nM**		**IC50: 310.5 nM**	

**) Censored value*

Run #3

	Thapsigargin		Cpd 6		Cpd 13		Cpd 15		Cpd 17		Cpd 18		Cpd 19		Cpd 20	
Conc [M]	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2	%Inh _1	%Inh _2
1.00E-05	101.9	107.0	99.6	100.9	100.0	97.3	102.2	93.6	95.1	101.5	100.7	97.0	99.6	108.4	96.6	90.7
2.50E-06	101.9	104.7	102.5	96.4	100.2	105.2	100.9	91.1	79.8	79.6	93.2	91.7	96.9	106.4	72.4	72.9
6.25E-07	99.5	93.7	94.1	80.5	104.4	101.1	96.7	90.1	68.8	73.6	85.6	83.7	102.9	104.2	40.2	47.8
1.56E-07	90.0	67.6	30.1	12.1	94.4	72.5	6.2	7.7	53.6	52.4	57.0	74.3	81.4	71.9	-10.6	-14.4
3.91E-08	55.6	37.6	0.8	-0.4	5.8	7.2	27.6	2.9	-4.5	-7.5	-6.6	2.2	8.8	8.1	-11.8	-15.0
9.77E-09	-1.3	-2.7	-4.7	-3.7	-2.2	-0.2	-1.3	0.0	-16.9	-11.9	-6.6	-0.8	4.6	2.2	-12.4	-13.2
2.44E-09	1.1	-4.1	-1.1	-3.4	-5.9	-1.6	-1.6	-0.9	-16.9	-14.7	-7.7	-2.7	-0.2	-1.6	-8.6	-16.0
6.10E-10	0.6	-5.0	0.2	-1.3	-3.6	-4.6	-3.2	-1.1	-14.9	-10.3	-4.2	-1.3	1.9	1.0	-10.0	-14.6
1.53E-10	-0.7	-1.7	-1.3	-2.6	-1.8	-1.8	-4.2	0.7	-11.6	-10.6	-2.6	0.2	-0.6	-4.3	-9.8	-12.8
3.81E-11	2.9	-1.6	-0.4	-2.0	-2.5	-0.5	2.4	-0.6	-5.9	-4.1	-2.2	-0.6	2.6	1.2	-4.0	-8.0
	IC50: 50.7 nM		IC50: 268.3 nM		IC50: 89.9 nM		IC50: 298.3 nM		IC50: 116.7 nM		IC50: 118.9 nM		IC50: 103.7 nM		IC50: 576.0 nM	