SUPPORTING INFORMATION

for

Synthesis and biological evaluation of semi-synthetic albocycline analogs

Samer S. Daher,[a] Kevin P. Franklin,[a] Tyler Scherzi,[b] Paul M. Dunman,[b] and

Rodrigo B. Andrade[a,*]

[a] Department of Chemistry, Temple University, Philadelphia, PA 19122

[b] Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Table of Contents

General Methods: All reactions containing water or air sensitive reagents were performed in oven-dried glassware under nitrogen or argon. Tetrahydrofuran, toluene and dichloromethane were passed through two columns of neutral alumina. Triethylamine was distilled from CaH_2 prior to use. 4 Å molecular sieves were activated by flame drying under vacuum prior to use. All other reagents were purchased from commercial sources and used without further purification. All solvents for work-up procedures were used as received. Flash column chromatography was performed using ICN Silitech 32-63 D 60Å silica gel and activated neutral Brockmann I Alumina with the indicated solvents. Thin layer chromatography was performed on Merck $60F_{254}$ silica gel plates and Sorbtech W/UV254 alumina N TLC plates. Detection was performed using UV light, $KMnO_4$ stain, PMA stain and subsequent heating. ^1H and ^{13}C NMR spectra were recorded at the indicated field strength in $CDCl_3$ at rt. Chemical shifts are indicated in parts per million (ppm) downfield from tetramethylsilane (TMS, δ= 0.00) and referenced to the $CDCl_3$. Splitting patterns are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Optical rotations were measured on a Perkin-Elmer 341 Polarimeter at room temperature, using the sodium D line.

Modified Protocol Based on the Upjohn Patent for Isolating Albocycline via Culturing *S. maizeus*

All glassware and media prepared should be sterilized through autoclave. First, we transferred 100 μL *S. maizeus* cell stock solution into 6 mL Bennett's growth medium (ATCC Medium # 174). Second, the mixture was shaken at 28 °C (200 rpm) for ~4 days. At this stage, the cell culture would turn brown in color and many small beads would form inside the medium. Third, we added 6 mL of this cell culture into 2 L flask containing 500 mL of Bennett's medium as the production medium. Fourth, the production flask was incubated at 28 °C (200 rpm) for 4−5 days. Finally, an equal volume of ethyl acetate (500 mL) was added to the flask, and the mixture was vigorously shaken. Upon settling, the organic layer containing albocycline was transferred to a round-bottomed flask and concentrated *in vacuo*. Albocycline was subsequently purified by flash silica gel column chromatography.[1] The detection of albocycline was routinely performed by TLC (EtOAc/hexane = 30/70 with $KMNO_4$ stain), which had a retention factor (R_f) of ~ 0.35. Upon completion of this process, around 50−70 mg of albocycline (less polar spot) and 12-15 mg of cineromycin B (more polar spot) can be obtained from 2L of *S. maizeus* cell culture. A schematic diagram illustrating the major steps of isolation is shown below (Figure S-1).

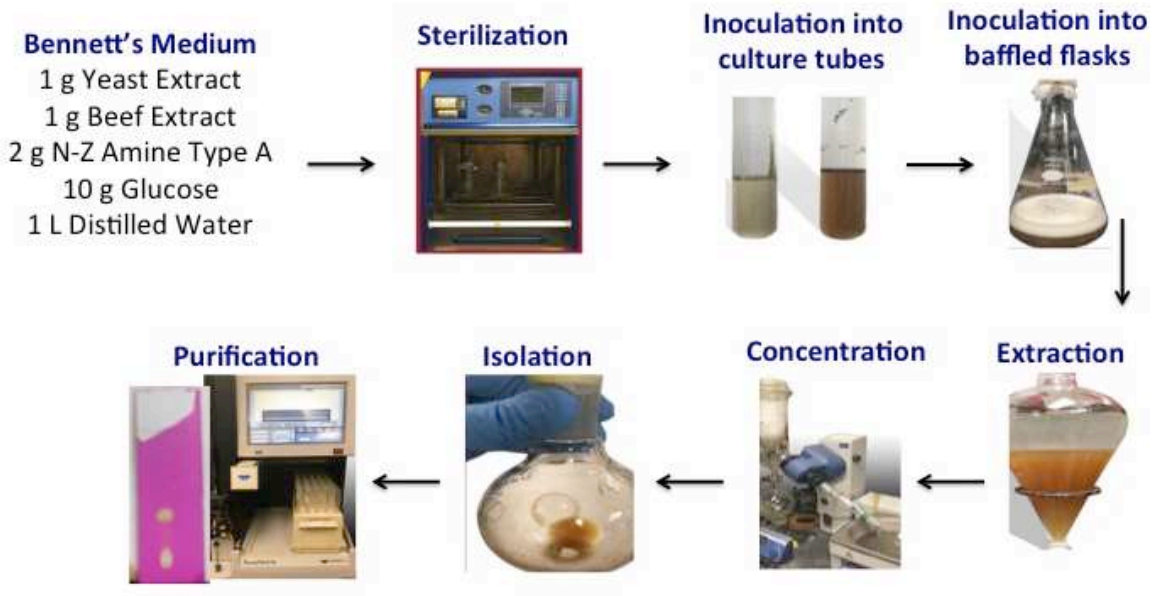


Figure S-1. Schematic illustration of albocycline culturing from *S. maizeus*

Minimum Inhibitory Concentration (MIC) Methods For *S. aureus*

1- Dissolve compound in enough volume of DMSO/other solvent to reach a final stock concentration of 12.8 mg/mL.

2- Serial dilute stock concentration (volumes can be increased proportionately for larger stock volumes).

a. 5 µL of 12.8 mg/mL + 5 µL DMSO = 6.4 mg/mL
b. 5 µL of 6.4 mg/mL + 5 µL DMSO = 3.2 mg/mL
c. 5 µL of 3.2 mg/mL + 5 µL DMSO = 1.6 mg/mL
d. 5 µL of 1.6 mg/mL + 5 µL DMSO = 0.8 mg/mL
e. 5 µL of 0.8 mg/mL + 5 µL DMSO = 0.4 mg/mL
f. 5 µL of 0.4 mg/mL + 5 µL DMSO = 0.2 mg/mL
g. 5 µL of 0.2 mg/mL + 5 µL DMSO = 0.1 mg/mL
h. 5 µL of 0.1 mg/mL + 5 µL DMSO = 0.05 mg/mL
i. 5 µL of 0.05 mg/mL + 5 µL DMSO = 0.025 mg/mL

3- Add 88 µL of Mueller Hinton (MH) broth to wells of a 96 well plate.

4- 2 µL of each compound or DMSO control to corresponding wells containing 88 µL of MH.

a. 2 µL of DMSO Control (-) = 0 µg/mL
b. 2 µL of DMSO Control (+) = 0 µg/mL
c. 2 µL of 0.025 mg/mL = 0.5 µg /mL
d. 2 µL of 0.05 mg/mL = 1 µg /mL
e. 2 µL of 0.1 mg/mL = 2 µg /mL
f. 2 µL of 0.2 mg/mL = 4 µg /mL

g. 2 µL of 0.4 mg/mL =8 µg /mL

h. 2 µL of 0.8 mg/mL = 16 µg/mL

i. 2 µL of 1.6 mg/mL = 32 µg /mL

j. 2 µL of 3.2 mg/mL = 64 µg /mL

k. 2 µL of 6.4 mg/mL = 128 µg/mL

l. 2 µL of 12.8 mg/mL = 256 µg/mL

Inoculum Procedure

1- Transfer 3-5 colonies from a plate to 5 mL of MH (or serial dilute 1:100 from an overnight culture).

2- Incubate at 37°C with shaking at 200 rpm until culture reaches an OD_{600} of 0.18 (~ $1x10^8$ cells/mL).

3- Add 0.3 mL from (2) to 9.7 mL of MH (~$3x10^6$ cells/mL).

4- Add 10 µL from (3) to corresponding wells containing 90 µL of MH + Compound ($3x10^5$ cells/mL).

a. **DO NOT** add bacteria to Control wells (-).

b. Perform individual tests in **technical duplicate**.

c. **Confirm bacterial concentration in starter culture**

   i. Remove 100 µL of culture from (2)

   ii. Add to 900 µL of PBS or 0.8% NaCl; Serial dilute 10^{-3} and 10^{-4} and spread plate.

iii. Incubate plate for 16-24 hrs at 37°C; Enumerate colonies and back-calculate starting bacteria concentration using the following formula: $CFU/mL = \frac{(\#\ of\ colonies) * (dilution\ factor)}{volume\ of\ culture\ plate}$

5- Incubate 96-well plate for 16-24 hrs at 37°C; Determine MIC by denoting lowest antimicrobial concentration exhibiting visual growth inhibition.

MIC Protocol and Results For Gram-negative bacteria

1- A series of two fold dilutions were done in 96-well microtiter plates starting with 10 mg/mL concentration of ALB (**1**), **15A** (major diastereomer), **15B** (minor diastereomer) in DMSO. DMSO was used a solvent control. Kanamycin (25 mg/mL) was used as a positive control.

2- Wells were inoculated with a 1:100 dilution of overnight cultures of *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa* PA10.

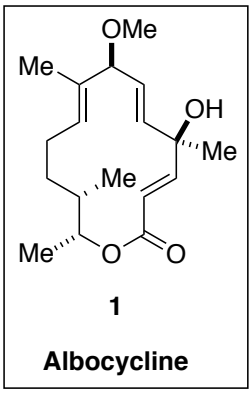
3- After incubating at 37°C for 24 hours, inhibition was scored by examining plates for the lowest well that showed inhibition of growth.

4- Results:

a. No inhibition of ALB (1), 15A, 15B at 2.5 mg/mL (5mg/mL still showed DMSO inhibition).
b. Kanamycin displayed the following MICs: 3.1 mg/mL against *Acinetobacter baumannii,* 24 µg/mL against *Escherichia coli*, and 780 µg/mL against *Pseudomonas aeruginosa.*

Experimental Procedures and Characterization of New Compounds

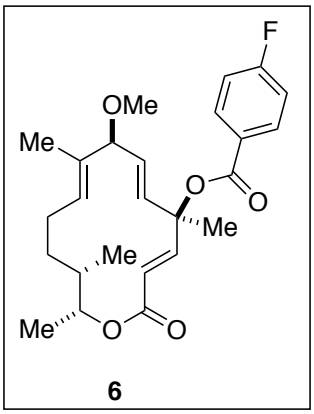
The spectral data of cineromycin B (**5**) was matching the literature references: *Russ. Chem. Bull.* 2007, **56**, 815.



1

Albocycline

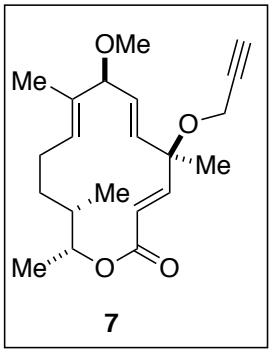
(–)-Albocycline (**1**). $[\alpha]^{23}_{D}$ –110 (*c* 0.62, $CHCl_3$); IR ($CHCl_3$) 3443, 2975, 2930, 1715, 1512, 1452, 1382, 1250 cm^{-1}; ^1H NMR (500 MHz, Chloroform-*d*) δ 6.88 (d, *J* = 15.5 Hz, 1H), 5.87 (d, *J* = 15.5 Hz, 1H), 5.78 (d, *J* = 16.2 Hz, 1H), 5.64 (d, *J* = 16.1 Hz, 1H), 5.29 (s, 1H), 4.54 (s, 1H), 4.07 (s, 1H), 3.30 (s, 3H), 2.17 (s, 1H), 1.86 (s, 2H), 1.64 (s, 3H), 1.54 (s, 3H), 1.43 (s, 1H), 1.18 (s, 5H), 0.87 (s, 3H);^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 155.0, 136.9, 136.3, 131.1, 129.5, 115.7, 85.1, 75.9, 73.6, 57.4, 39.4, 34.5, 27.4, 25.0, 18.2, 16.0, 14.3; HRMS (ESI) calc'd for $C_{18}H_{28}O_4$ + Na = 331.1885, found 331.1892.



6

Ester 6. To a solution of albocycline (**1**) (40 mg, 0.13 mmol) in 3 mL of dry pyridine was added (23 mg, 0.14 mmol) of *p*-fluorobenzoyl chloride. The mixture was heated to 80 °C under argon atmosphere for 80 hours, the allowed to cool to rt, and pyridine was removed under reduced pressure. The crude mixture was washed successively with brine (4 mL), and extracted with EtOAc (3 × 3 mL). The combined organic layers were washed were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified *via* flash chromatography eluting with Ethyl acetate/hexane 0-40% to give the desired product in 43%. $[\alpha]^{23}_{D}$ –26.2 (*c* 0.58, $CHCl_3$); IR ($CHCl_3$) 2979, 2929, 2450, 2160, 2027, 1977, 1702, 1600, 1521, 1344, 1215, 1060, 749 cm^{-1};1H NMR (500 MHz, Chloroform-d) δ 8.14 (s, 2H), 7.15 (s,

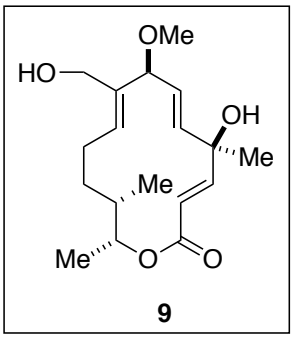
2H), 6.90 (s, 1H), 5.89 (s, 1H), 5.78 (d, *J* = 16.9 Hz, 1H), 5.65 (d, *J* = 22.3 Hz, 1H), 5.29 (s, 1H), 4.57 (s, 1H), 4.07 (s, 1H), 3.30 (s, 3H), 2.17 (s, 1H), 1.87 (s, 1H), 1.64 (s, 4H), 1.45 (s, 2H), 1.24 (d, *J* = 14.6 Hz, 7H), 0.89 (s, 4H); 13C NMR (126 MHz, CDCl3) δ 169.6, 167.4, 166.3, 165.4, 154.7, 136.7, 136.0, 133.0, 132.9, 130.9, 129.3, 115.9, 115.7, 115.5, 84.9, 75.7, 73.3, 57.1, 39.1, 34.3, 27.2, 24.8, 17.9, 15.8, 14.1; HRMS (ESI) calc'd for $C_{25}H_{31}FO_5$ + Na = 453.2156 found 453.2140



7

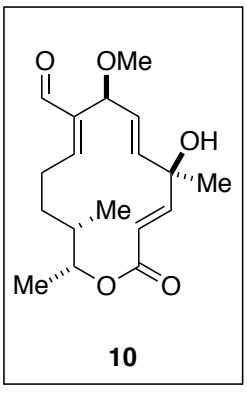
Ether 7. A flame-dried vial equipped with a stirrer bar was evacuated and purged with argon three times. Then, a solution of albocycline (19 mg, 0.06 mmol) in DMF (2 mL) was added followed by propargyl bromide (11 mg, 0.09 mmol), and tetrabutylammonium iodide (3 mg, 0.008 mmol). The reaction mixture was cooled to 0 °C, at which NaH (60% in mineral oil, 5 mg, 0.12 mmol) was slowly added. The reaction mixture was stirred at 0 ^{o}C for 20 mins, warmed to rt over 10 mins, and then stirred at 80 °C for 12 hours. The mixture was cooled to rt and quenched with H_2O (4 mL). The organic layer was separated, and the aqueous layer was extracted with (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography eluting with 4% EtOAc/hexane to afford a bright yellow liquid (11.5 mg, 55%). $[\alpha]^{23}_{D}$ –39.8 (*c* 0.44, $CHCl_3$); IR ($CHCl_3$) 3292, 2979, 1712, 1455, 1278, 1093, 990, 668 cm^{-1}; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.71 (d, *J* = 15.6 Hz, 1H), 5.89 (d, *J* = 15.6 Hz, 1H), 5.66 (d, *J* = 20.9 Hz, 2H), 5.27 (s, 1H), 4.60 (s, 1H), 4.18 (s, 2H), 4.03 (s, 1H), 3.30 (s, 3H), 2.43 (s, 1H), 2.17 (s, 1H), 1.85 (s, 1H), 1.65 (s, 3H), 1.57 (s, 2H), 1.39 (s, 1H), 1.22 (s, 5H), 0.87 (s, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 153. 1, 136.7, 134.0, 133.0, 129.9, 117.1,

85.1, 81.2, 79.6, 76.0, 74.5, 57.6, 52.8, 40.1, 34.7, 25.5, 22.6, 18.6, 16.5, 14.9; HRMS (ESI) calc'd for $C_{21}H_{30}O_4$ + H = 347.2144 found 347.2155.



9

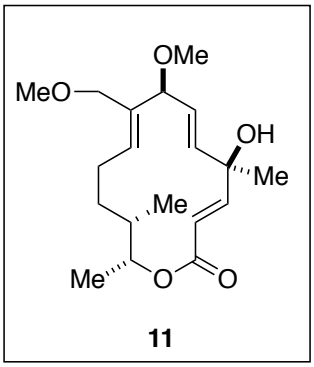
Allylic Alcohol 9. To a solution of albocycline (20 mg, 0.06 mmol) in CH_2Cl_2 (2 mL), was added selenium dioxide (6 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 48 hours. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography eluting with 80% EtOAc/hexane to afford **9** as a yellowish liquid (7 mg, 35%). $[\alpha]^{23}_D$ −18.7 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3395, 2972, 2928, 2160, 2030, 1710, 1452, 1379, 1250, 732 cm^{-1}; ^1H NMR (500 MHz, Chloroform-*d*) δ 6.95 (d, *J* = 15.7 Hz, 1H), 5.92 (s, 1H), 5.75 (d, *J* = 15.8 Hz, 1H), 5.53 (d, *J* = 22.6 Hz, 1H), 5.33 (s, 1H), 4.60 (s, 2H), 4.06 (d, *J* = 46.6 Hz, 2H), 3.35 (s, 3H), 2.56 (s, 1H), 2.17 (s, 1H), 2.04 (s, 1H), 1.84 (s, 1H), 1.51 (s, 4H), 1.29 (s, 5H), 0.92 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 153.7, 137.2, 136.3, 129.6, 128.7, 117.3, 80.8, 77.0, 73.0, 66.1, 57.0, 41.3, 34.2, 27.2, 26.2, 19.0, 16.3 ; HRMS (ESI) calc'd for $C_{18}H_{28}O_5$+ H = 325.1937 found 325.1949.



10

Aldehyde 10. To a solution of 16-hydroxyalbocycline (**9**, 10 mg, 0.03 mmol) in CH_2Cl_2 (2 mL), was added DMP (19 mg, 0.05 mmol), and $NaHCO_3$ (10 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 2 hours. After confirming the completion of reaction via TLC, the reaction was quenched with H_2O/saturated aqueous bicarbonate/thiosulfate (2 mL/1 mL/1 mL). The resulting reaction mixture was stirred for additional 2 hours, and then the organic layer was separated. The aqueous layer

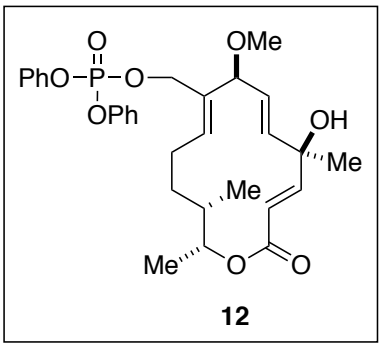
was extracted with CH_2Cl_2 (3 × 4 mL). The combined organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 10-12% EtOAc/hexane to afford **10** a colorless liquid (5.5 mg, 57%). $[\alpha]^{23}_D$ −74.5 (*c* 0.40, $CHCl_3$); IR ($CDCl_3$) 3464, 2978, 2159, 2030, 1682, 971, 557 cm^{-1}; ^1H NMR (500 MHz, Chloroform-*d*) δ 9.37 (s, 1H), 6.69 (d, *J* = 15.6 Hz, 1H), 6.60 (dd, *J* = 8.0, 6.2 Hz, 1H), 5.95 (d, *J* = 15.7 Hz, 1H), 5.85 (dd, *J* = 16.0, 1.5 Hz, 1H), 5.57 (dd, *J* = 16.0, 5.7 Hz, 1H), 5.04 – 4.74 (m, 2H), 3.25 (s, 3H), 2.78 (dtd, *J* = 16.5, 8.4, 6.8 Hz, 1H), 2.38 (ddt, *J* = 16.1, 9.1, 6.5 Hz, 1H), 1.87 – 1.67 (m, 2H), 1.62 – 1.51 (m, 3H), 1.35 – 1.22 (m, 5H), 0.97 (d, *J* = 7.0 Hz, 3H).;^{13}C NMR (101 MHz, $CDCl_3$) δ 193.6, 165.9, 160.0, 154.8, 141.6, 135.9, 130.9, 116.8, 76.1, 74.0, 73.4, 56.8, 37.9, 31.3, 26.1, 25.6, 19.1, 17.3; HRMS (ESI) calc'd for $C_{18}H_{26}O_5$ + Na = 345.1780 found 345.1765.



11

Ether 11. Into a flame-dried vial with freshly activated 4Å molecular sieves (150 mg), was added a solution of 16-hydroxyalbocycline (**9**, 9 mg, 0.03 mmol) in CH_2Cl_2 (2 mL). The mixture was cooled to 0 °C at which proton sponge (9 mg, 0.04 mmol) followed by Me_3OBF_4 (9 mg, 0.06 mmol) were added successively. The reaction mixture was stirred at 0 °C for 2 hours, diluted with CH_2Cl_2 (5 mL), and filtered through a short pad of Celite. The filter cake was washed with CH_2Cl_2 (3 mL). The filtrate was washed with cold 1N HCl (12 mL), and layers were quickly separated. The organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 17 % EtOAc/hexane to afford **11** a colorless liquid in 55%. $[\alpha]^{23}_D$ −26.9 (*c* 0.26, $CHCl_3$); IR ($CDCl_3$)

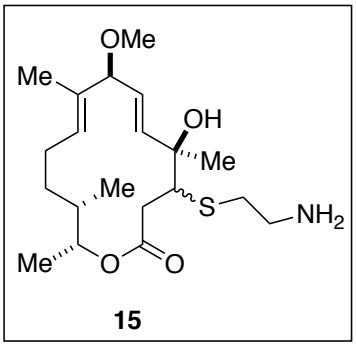
3427, 2980, 2930, 2822, 2359, 2323, 1714, 1250, 1106 cm^{-1}; ^{1}H NMR (500 MHz, Chloroform-d) δ 6.91 (d, J = 15.7 Hz, 1H), 5.92 (d, *J* = 15.8 Hz, 1H), 5.75 (dd, *J* = 15.8, 1.2 Hz, 1H), 5.66 – 5.54 (m, 1H), 5.53 – 5.41 (m, 1H), 4.65 (dd, *J* = 8.8, 6.3 Hz, 1H), 4.44 (d, *J* = 6.6 Hz, 1H), 3.91 (d, *J* = 12.3 Hz, 1H), 3.79 (d, *J* = 12.3 Hz, 1H), 3.30 (d, *J* = 6.4 Hz, 6H), 2.17 (ddt, *J* = 18.4, 12.3, 5.3 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.76 (s, 1H), 1.49 (s, 3H), 1.30 – 1.25 (m, 6H), 1.16 – 1.07 (m, 1H), 0.91 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CDCl3) δ 166.8, 154.3, 136.1, 135.0, 131.2, 130.8, 117.8, 80.1, 77.1, 74.3, 73.5, 58.2, 57.3, 40.8, 34.1, 26.8, 26.4, 19.3, 16.7; HRMS (ESI) calc'd for $C_{19}H_{30}O_5$ + Na = 361.1991 found 361.2007.



12

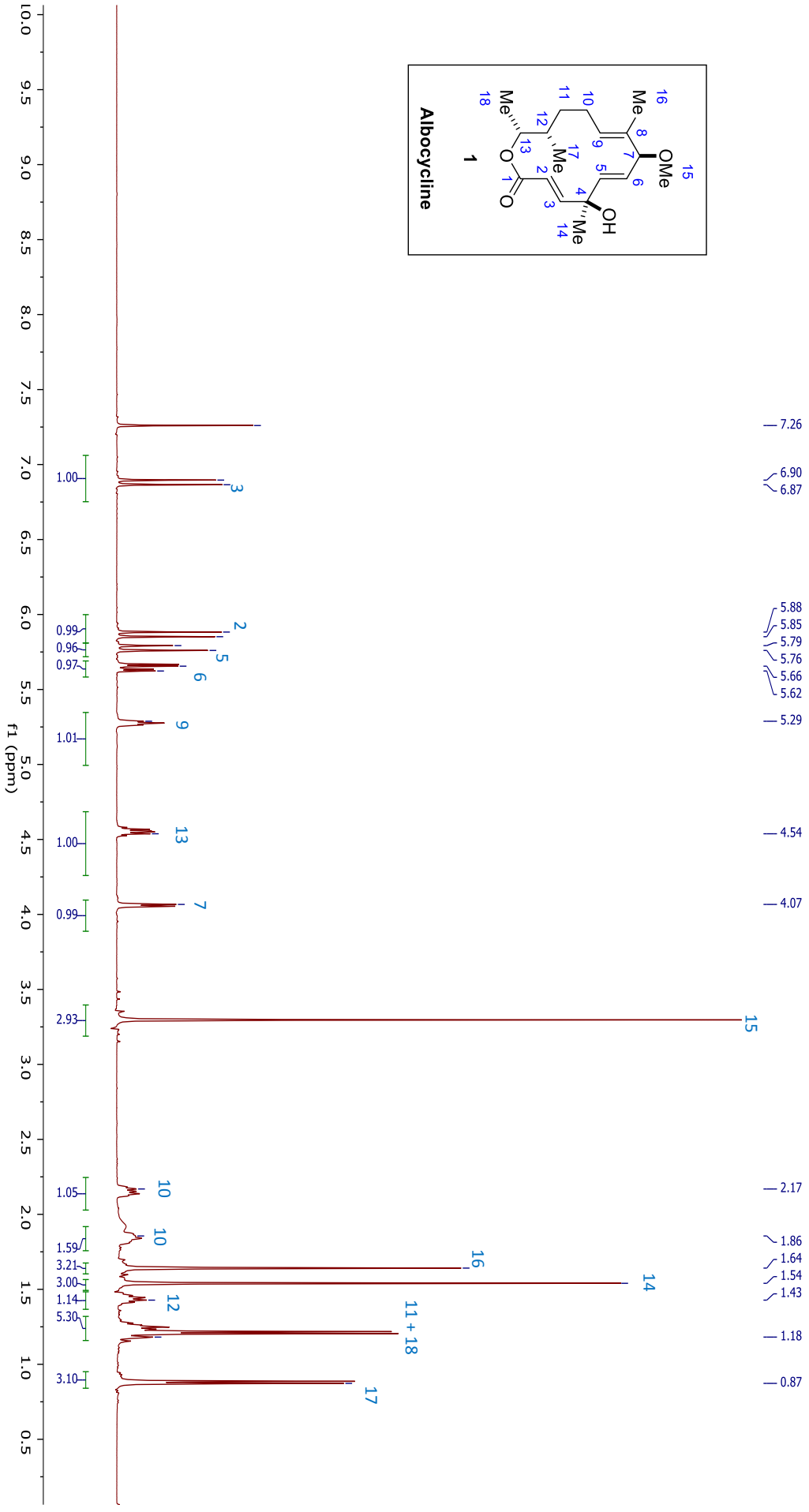
Phosphonate 12. Into a flame-dried vial was added a solution of 16-hydroxyalbocycline (**9**, 7 mg, 0.02 mmol) in THF (2 mL). The mixture was cooled to 0 °C under argon atmosphere, at which (8.0 mg, 0.03 mmol) of diphenylphosphorylazide (DPPA) followed by DBU (5 mg, 0.03 mmol) were added. The mixture was stirred at 0 °C for 30 mins, in which the reaction mixture turned yellow, and upon checking TLC, it was confirmed the consumption of starting material. The reaction was then quenched with H_2O (2 mL), and extracted with EtOAc (3 × 3 mL). The combined organic layers were washed were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified *via* flash chromatography eluting with 20 % EtOAc /hexane to afford **12** as an orange liquid (4 mg, 45%). $[\alpha]^{23}_{D}$ −5.2 (*c* 1.26, $CHCl_3$); IR ($CDCl_3$) 2928, 2160, 2029, 1700, 1652, 1214, 1128, 750 cm^{-1}; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37 – 7.31 (m, 4H), 7.23 – 7.15 (m, 6H), 6.92 (dd, *J* = 15.9, 0.7 Hz, 1H), 5.89 (dd, *J* = 15.8, 0.8 Hz, 1H), 5.74 (dd, *J* = 15.9, 1.3 Hz, 1H), 5.58 – 5.44 (m, 2H), 4.81 – 4.73 (m, 1H), 4.70 – 4.56 (m, 2H), 4.50 (d, *J* =

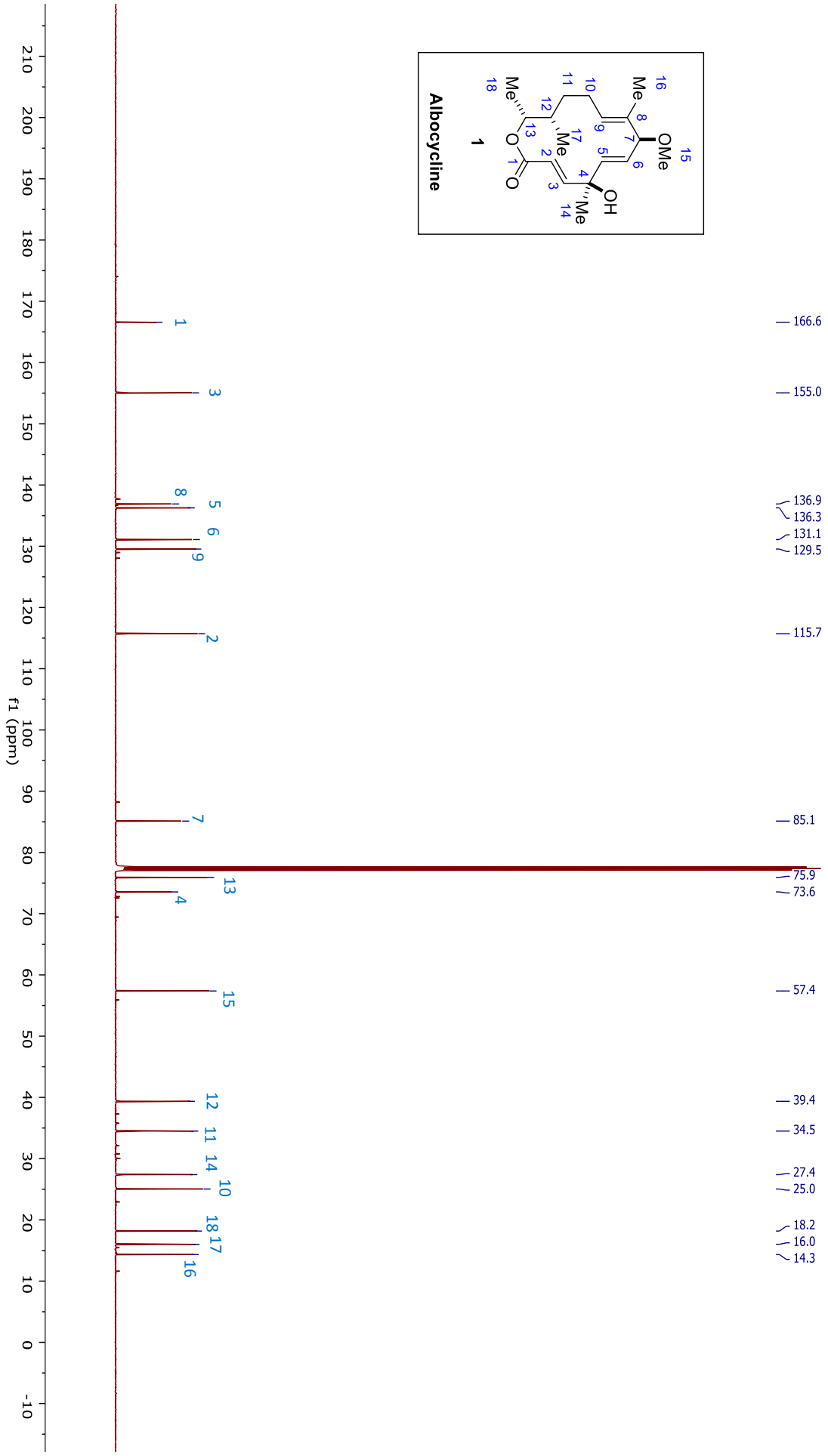
6.2 Hz, 1H), 3.28 (d, *J* = 0.7 Hz, 3H), 2.14 – 1.98 (m, 1H), 1.94 – 1.84 (m, 2H), 1.49 (d, *J* = 8.6 Hz, 1H), 1.46 (s, 3H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.26 – 1.14 (m, 3H), 0.95 (dtd, *J* = 12.8, 8.5, 4.3 Hz, 1H), 0.89 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, CDCl3) δ 166.5, 154.2, 150.7, 150.6, 135.9, 133.3, 133. 4 133.2, 129.9, 129.9, 129.5, 125.4, 125.4, 120.2, 120.2, 117.8, 79.0, 76.9, 73.0, 70.2, 70.1, 57.2, 41.1, 33.8, 27.1, 26.0, 19.1, 16.4; HRMS (ESI) calc'd for $C_{30}H_{37}O_8P$ + Na = 579.2226 found 579.2245.

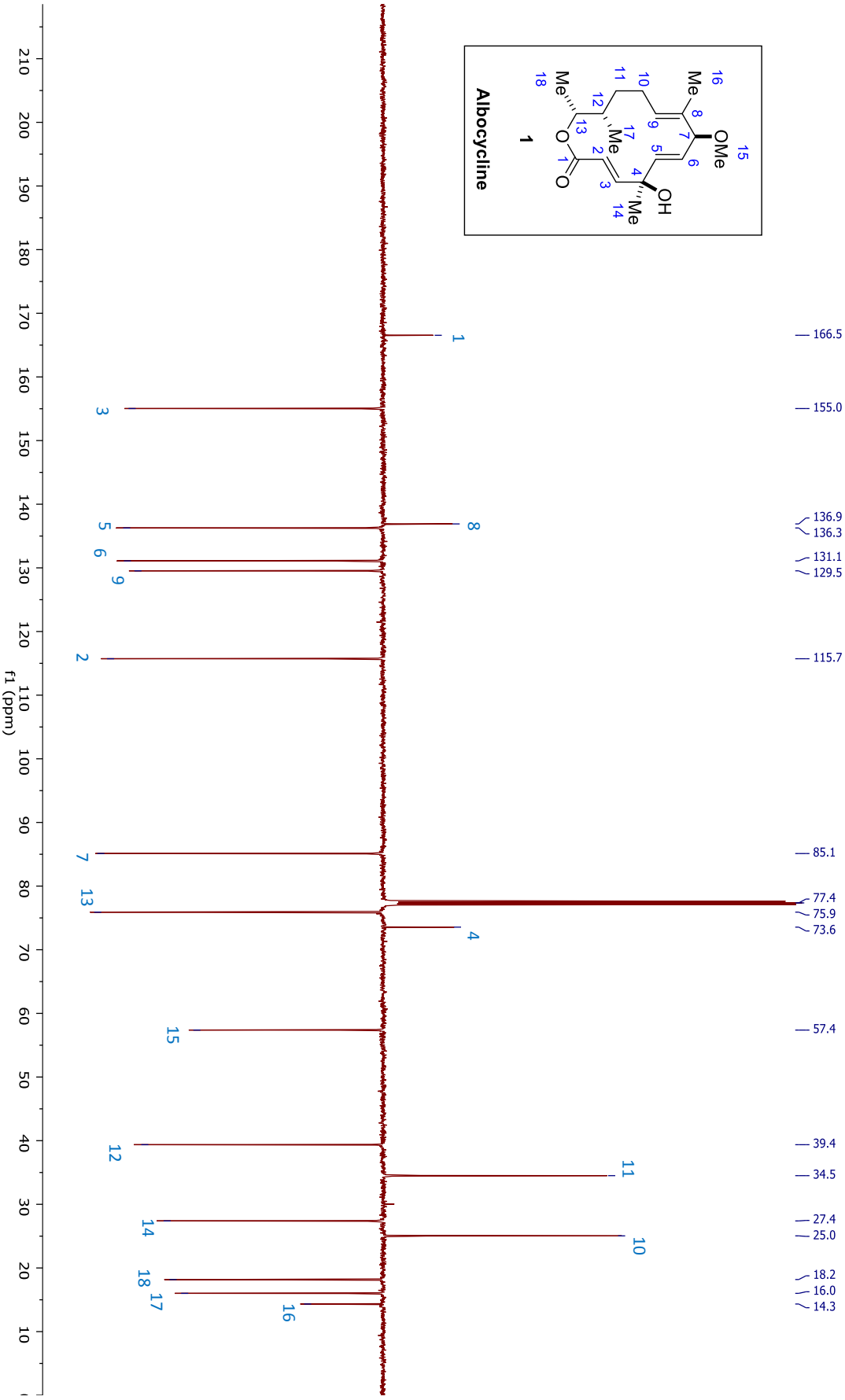


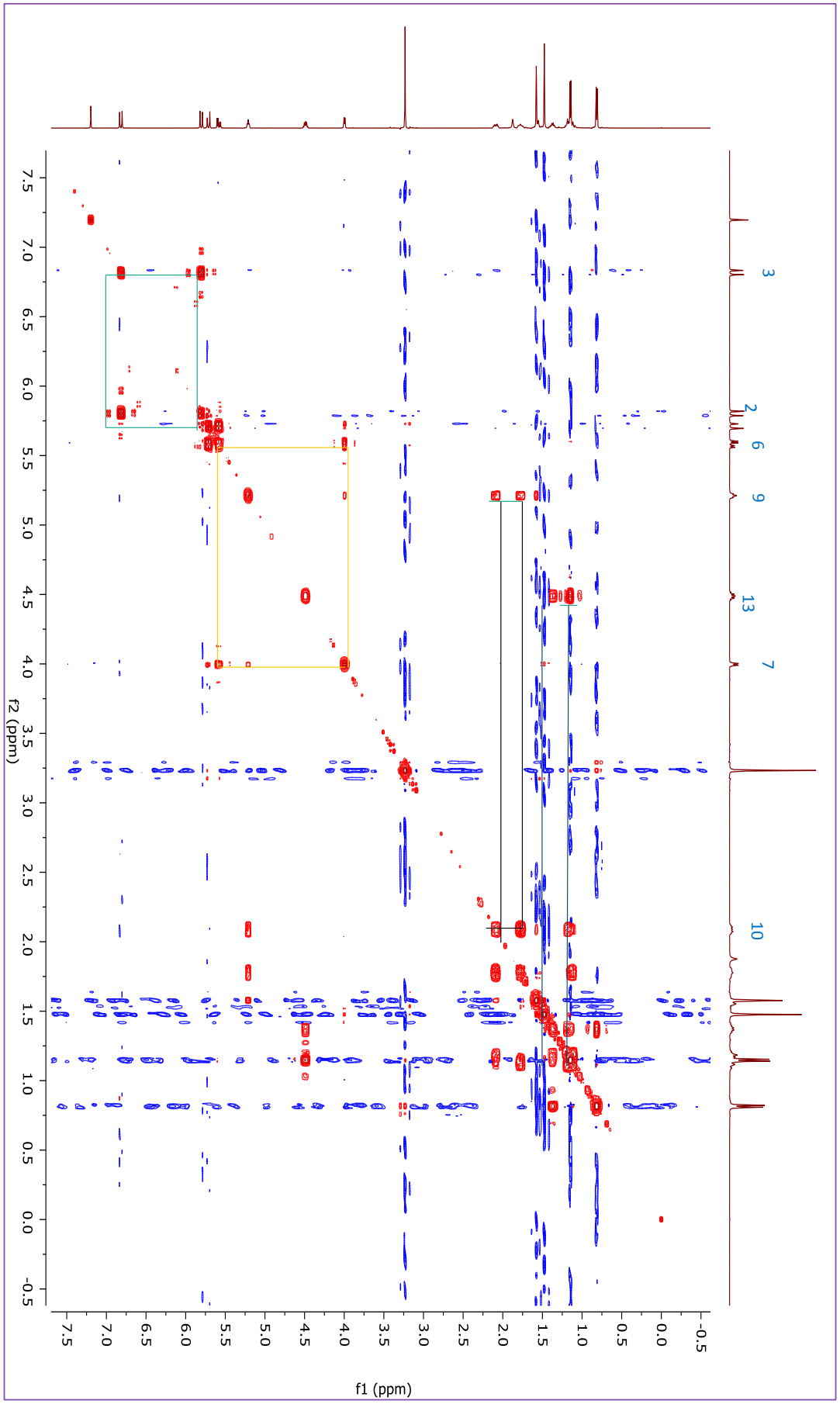
15

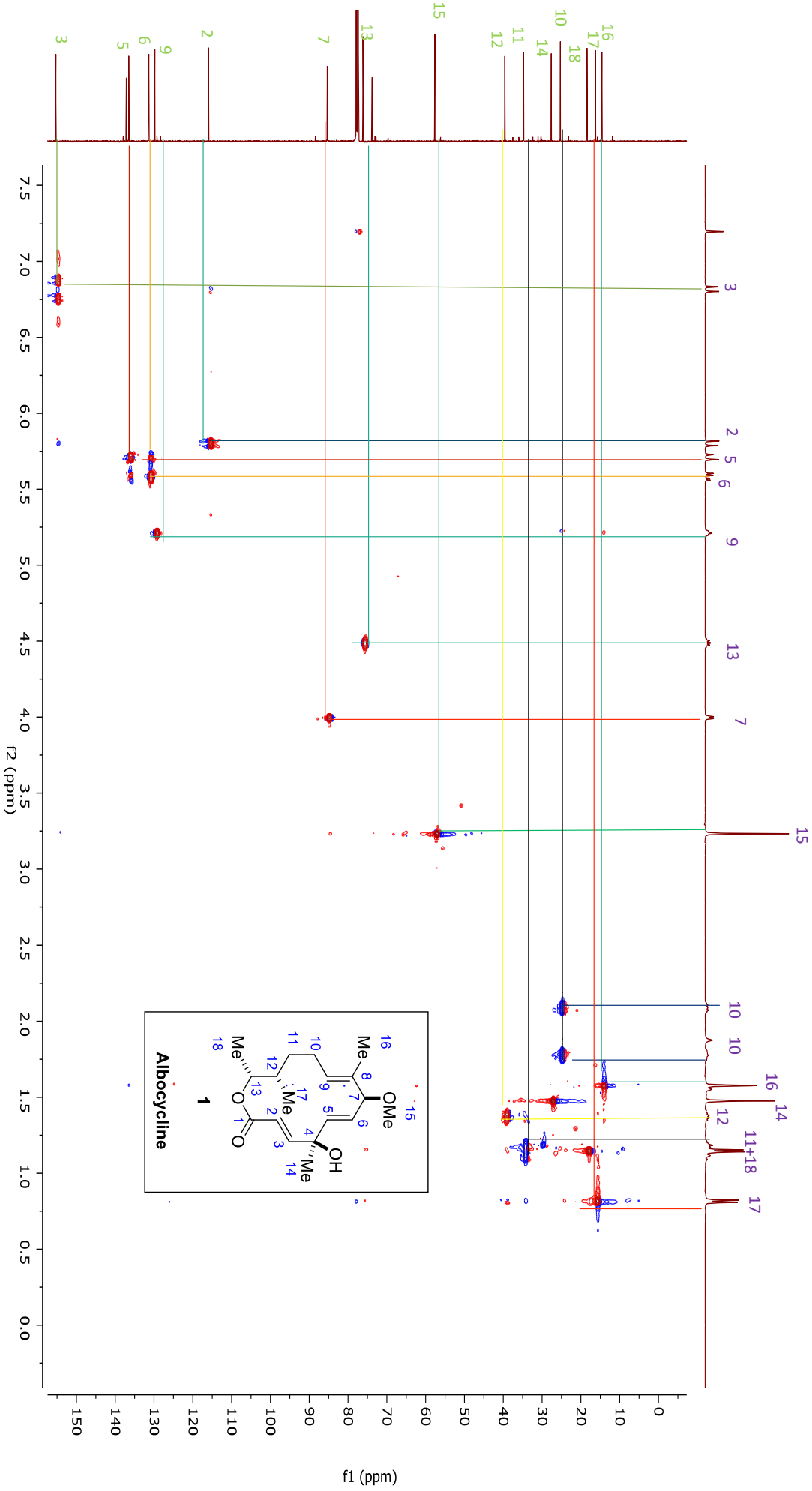
Amine 15. To a solution of albocycline **1** (17 mg, 0.06 mmol) in CH_2Cl_2 (1.5 mL), was added 2-aminothiol (4 mg, 0.06 mmol), and the reaction mixture was stirred at room temperature for 6 hours. The solvent was removed under reduced pressure, and the product was purified by reverse-phase chromatography (H_2O: CH_3CN) and then lyophilized to afford 2:1 mixture of diastereomers in 40% as a white foam, which were separated and the major diastereomer was fully characterized. $[\alpha]^{23}_D$ −19.6 (*c* 0.26, $CHCl_3$); IR ($CDCl_3$) 2928, 24450, 2160, 2029, 1977, 1716, 1378, 1093, 904, 726 cm^{-1}; ^{1}H NMR (500 MHz, Chloroform-d) δ 5.86 (dd, *J* = 15.7, 5.4 Hz, 1H), 5.48 – 5.29 (m, 2H), 4.58 (p, *J* = 6.3 Hz, 1H), 4.16 – 3.97 (m, 1H), 3.21 (s, 3H), 3.13 (s, 2H), 3.00 – 2.83 (m, 3H), 2.79 (dd, *J* = 15.1, 4.2 Hz, 1H), 2.59 (dd, *J* = 15.1, 5.6 Hz, 1H), 2.28 (td, *J* = 15.3, 14.9, 5.6 Hz, 1H), 1.95 (dd, *J* = 14.5, 6.4 Hz, 1H), 1.65 (p, *J* = 6.6 Hz, 1H), 1.57 (s, 3H), 1.45 (s, 3H), 1.33 (d, *J* = 6.4 Hz, 2H), 1.15 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CDCl3) δ 171.5, 135.9, 135.4, 130.0, 128.6, 87.7, 75.5, 74.1, 55.9, 53.5, 39.6, 37.4, 35.8, 32.8, 30.5, 28.2, 23.5, 15.9, 15.5, 11.3; HRMS (ESI) calc'd for $C_{20}H_{35}NO_4S$ + H = 386.2287 found 386.2279.

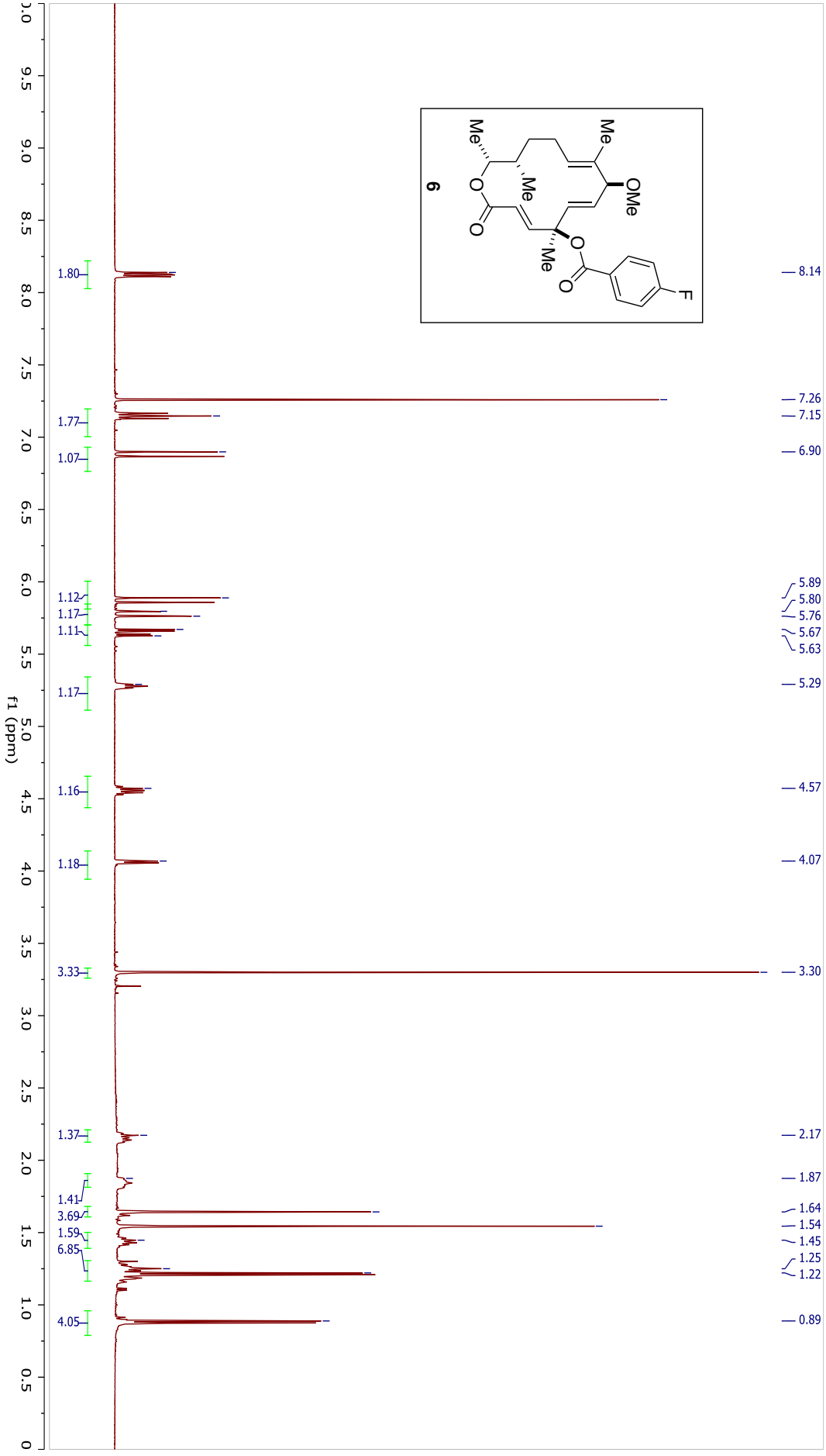

Albocycline
1
OMe
OH
Me
Me
Me
O
O
f1 (ppm)
7.26
6.90
6.87
5.88
5.85
5.79
5.76
5.66
5.62
5.29
4.54
4.07
2.17
1.86
1.64
1.54
1.43
1.18
0.87
1.00
0.99
0.96
0.97
1.01
1.00
0.99
2.93
1.05
1.59
3.21
3.00
1.14
5.30
3.10
11 + 18

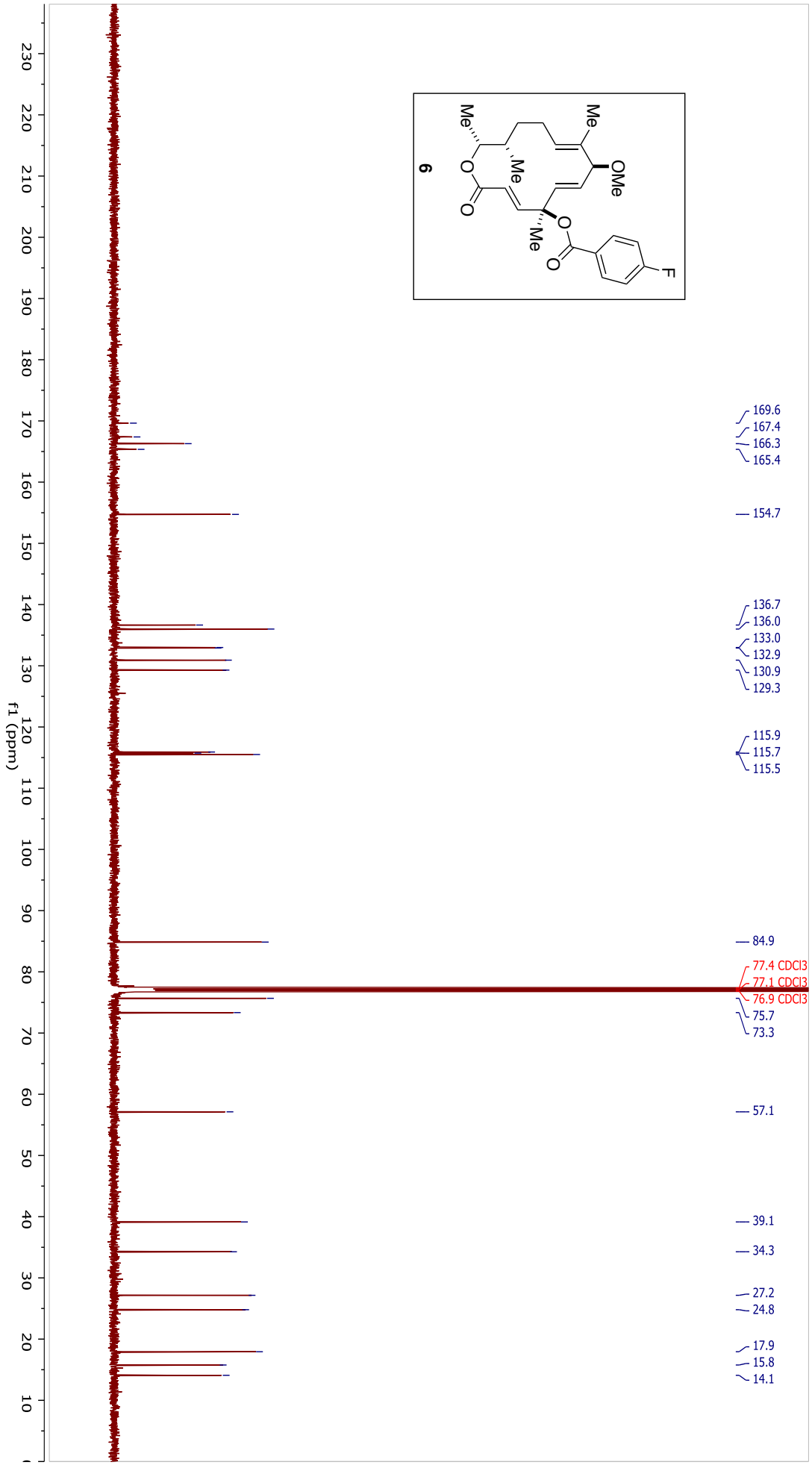

Albocycline
1
f1 (ppm)
166.6
155.0
136.9
136.3
131.1
129.5
115.7
85.1
75.9
73.6
57.4
39.4
34.5
27.4
25.0
18.2
16.0
14.3

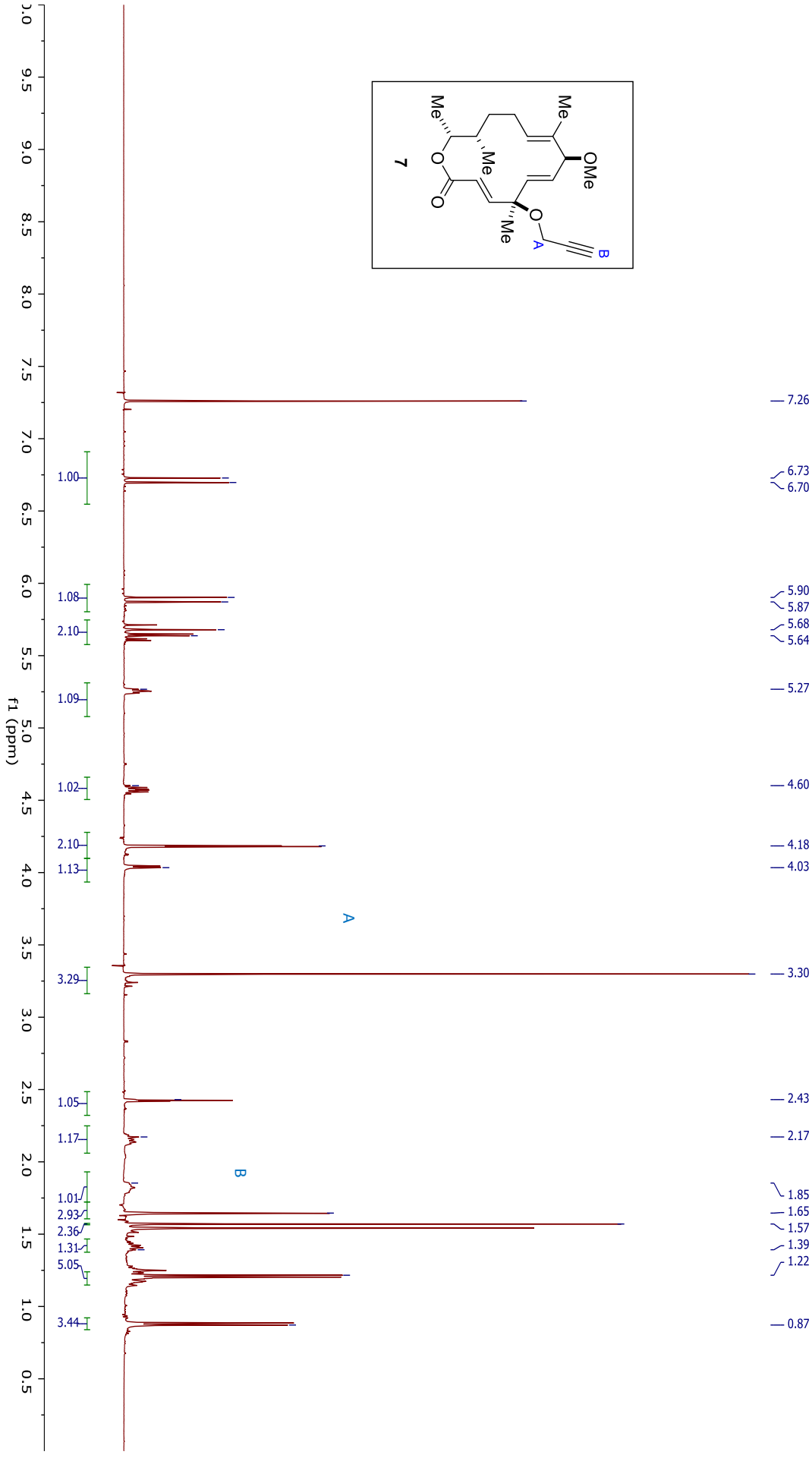

Albocycline
1
f1 (ppm)
166.5
155.0
136.9
136.3
131.1
129.5
115.7
85.1
77.4
75.9
73.6
57.4
39.4
34.5
27.4
25.0
18.2
16.0
14.3

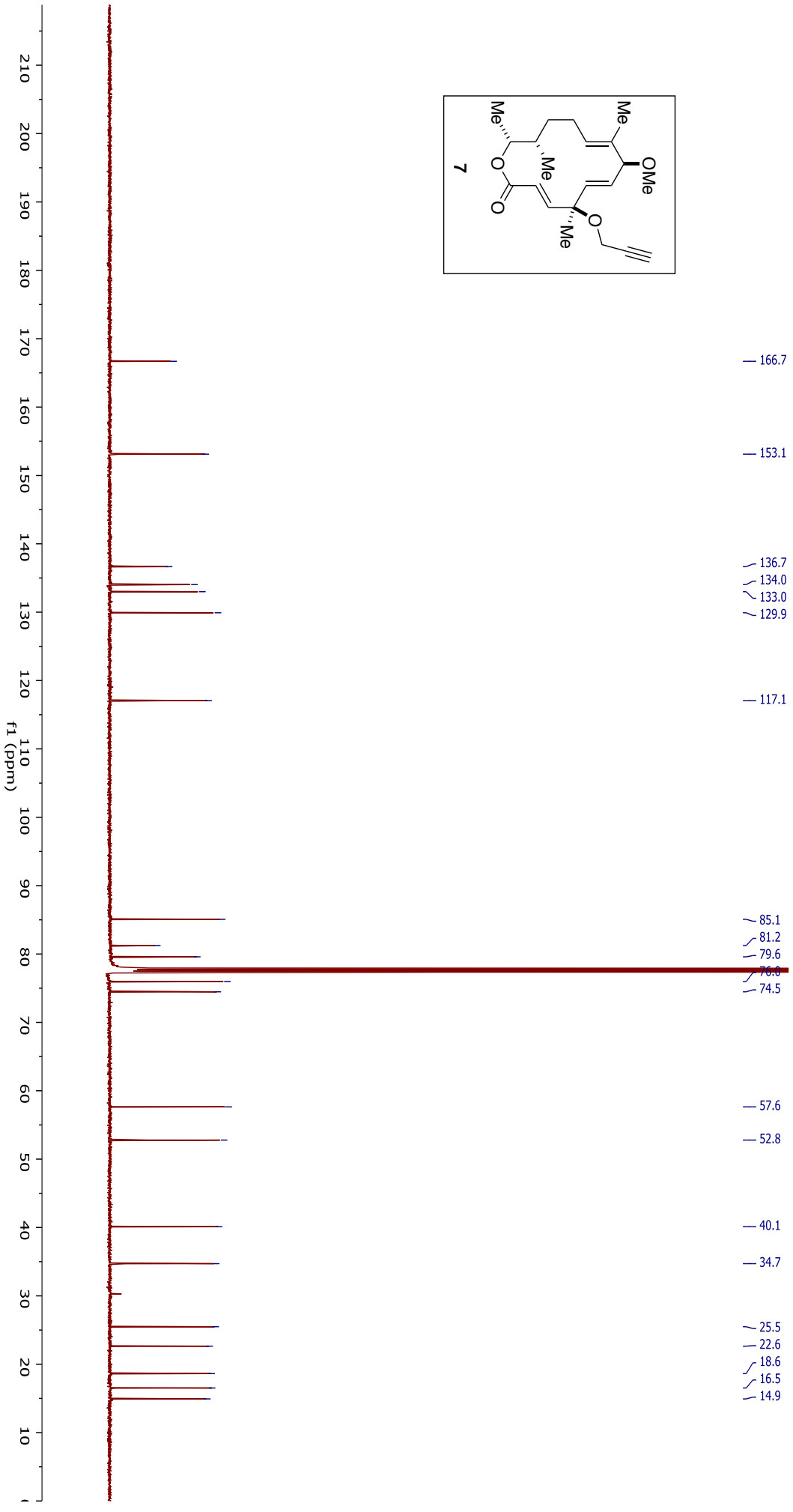

Albocycline
1
f1 (ppm)
f2 (ppm)

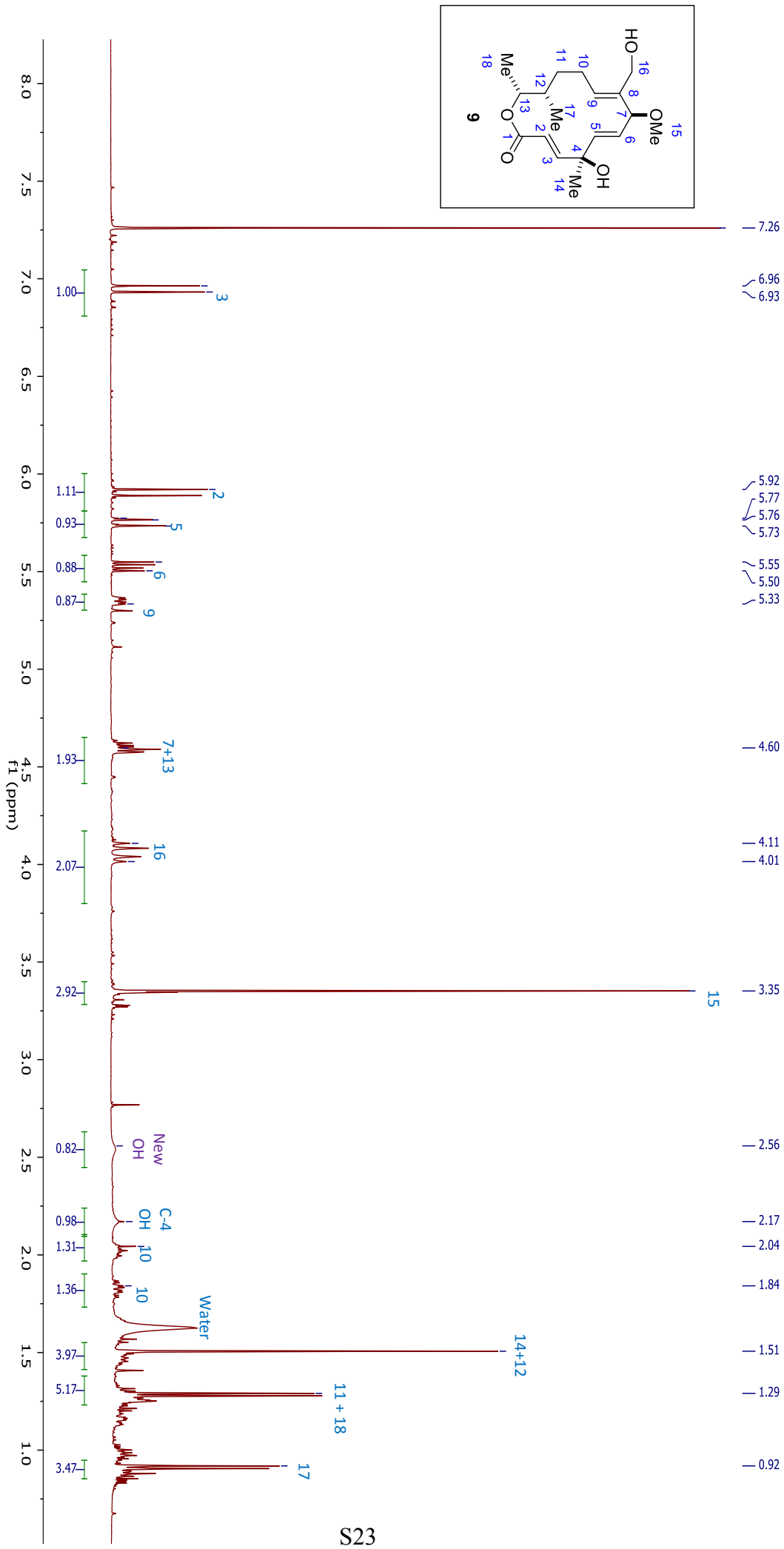

f1 (ppm)
f2 (ppm)
Albocycline
1

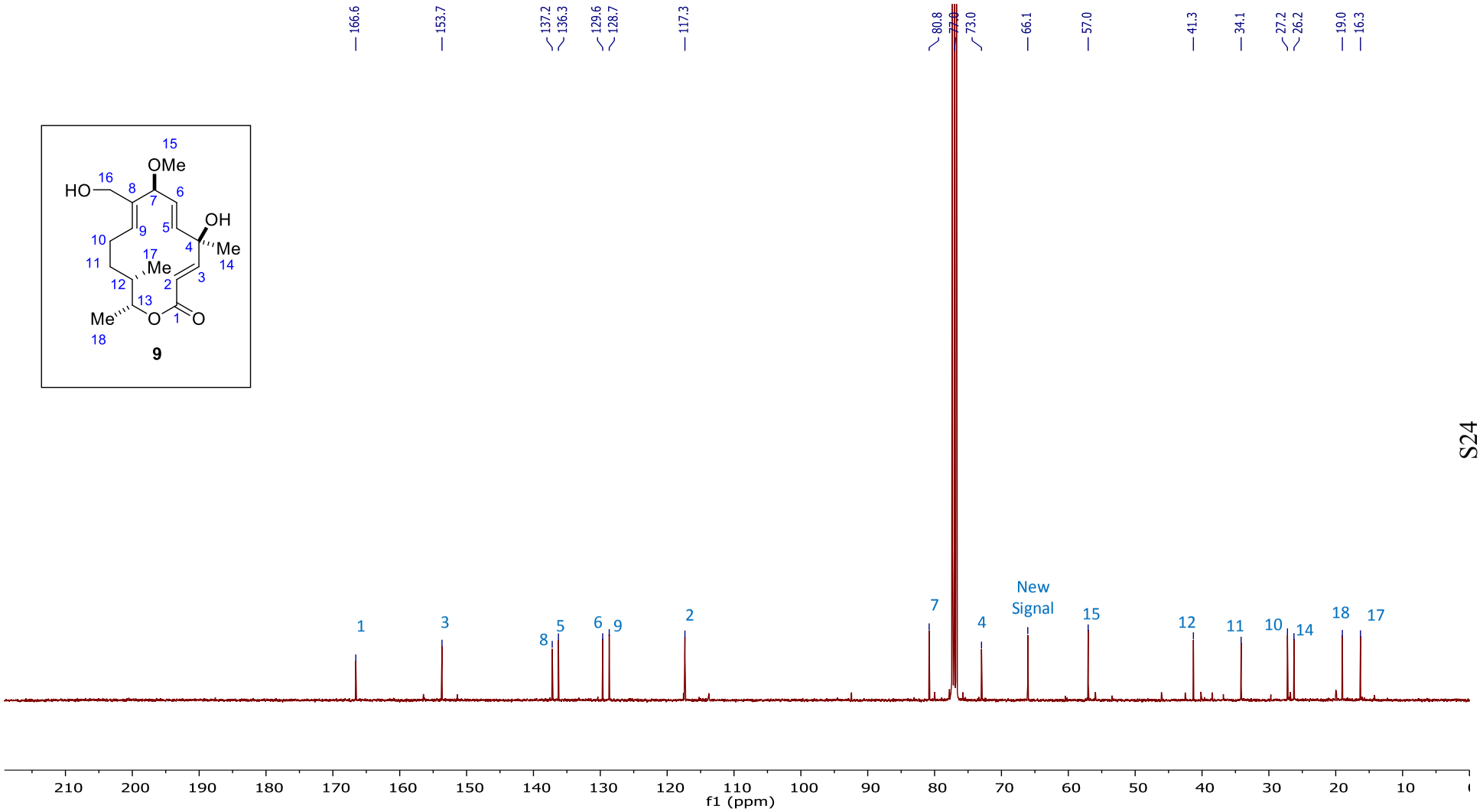

6
Me
Me
Me
OMe
Me
O
O
O
O
O
F
f1 (ppm)
1.80
1.77
1.07
1.12
1.17
1.11
1.17
1.16
1.18
3.33
1.37
1.41
3.69
1.59
6.85
4.05
8.14
7.26
7.15
6.90
5.89
5.80
5.76
5.67
5.63
5.29
4.57
4.07
3.30
2.17
1.87
1.64
1.54
1.45
1.25
1.22
0.89

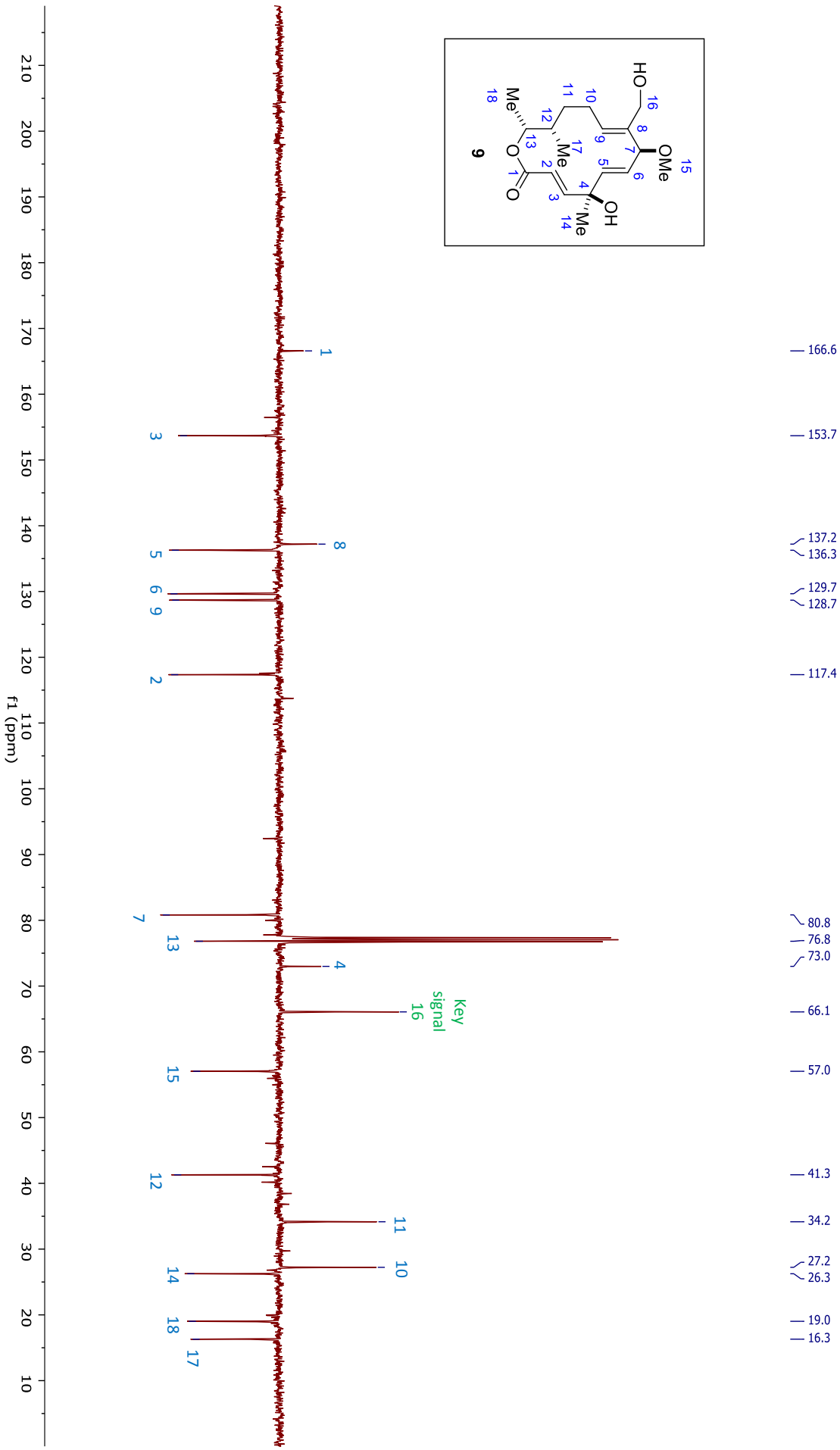

6
Me
Me
Me
OMe
O
O
O
O
F
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
169.6
167.4
166.3
165.4
154.7
136.7
136.0
133.0
132.9
130.9
129.3
115.9
115.7
115.5
84.9
77.4 CDCl3
77.1 CDCl3
76.9 CDCl3
75.7
73.3
57.1
39.1
34.3
27.2
24.8
17.9
15.8
14.1

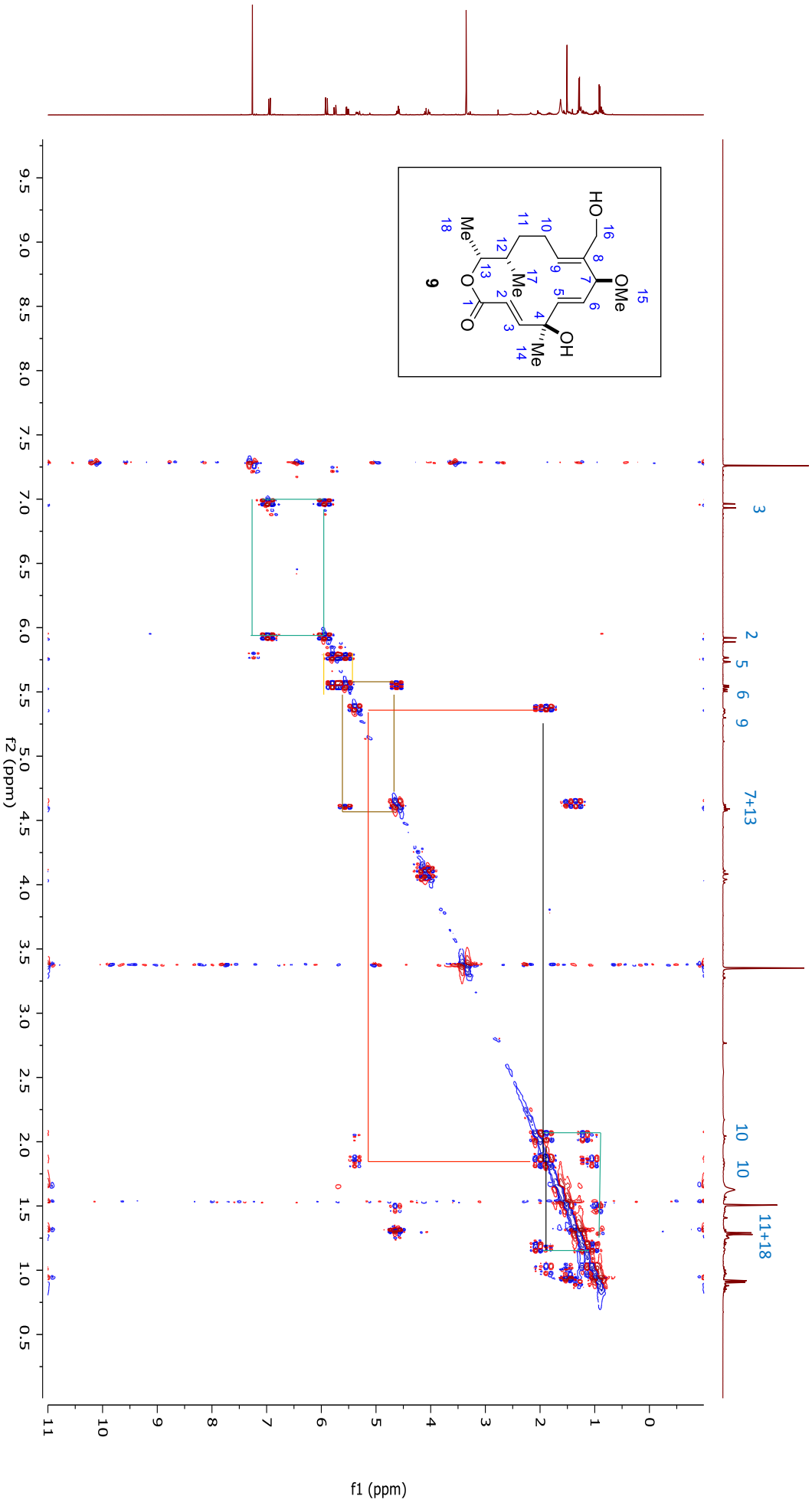

7
Me
OMe
Me
Me
Me
O
O
O
A
B
f1 (ppm)
1.00
1.08
2.10
1.09
1.02
2.10
1.13
3.29
1.05
1.17
1.01
2.93
2.36
1.31
5.05
3.44
7.26
6.73
6.70
5.90
5.87
5.68
5.64
5.27
4.60
4.18
4.03
3.30
2.43
2.17
1.85
1.65
1.57
1.39
1.22
0.87

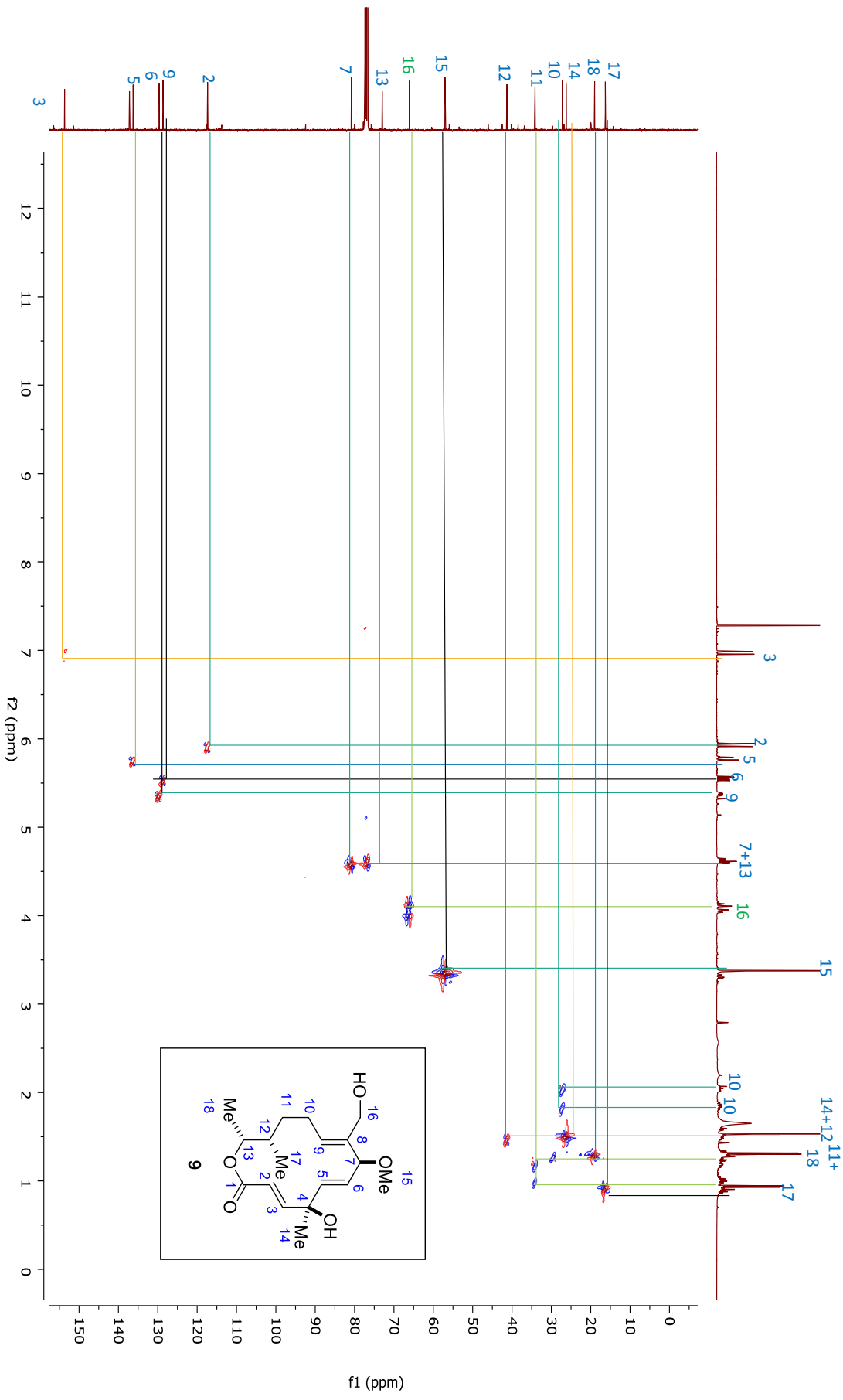

7
Me
Me
OMe
Me
O
O
O
Me
166.7
153.1
136.7
134.0
133.0
129.9
117.1
85.1
81.2
79.6
76.8
74.5
57.6
52.8
40.1
34.7
25.5
22.6
18.6
16.5
14.9
f1 (ppm)

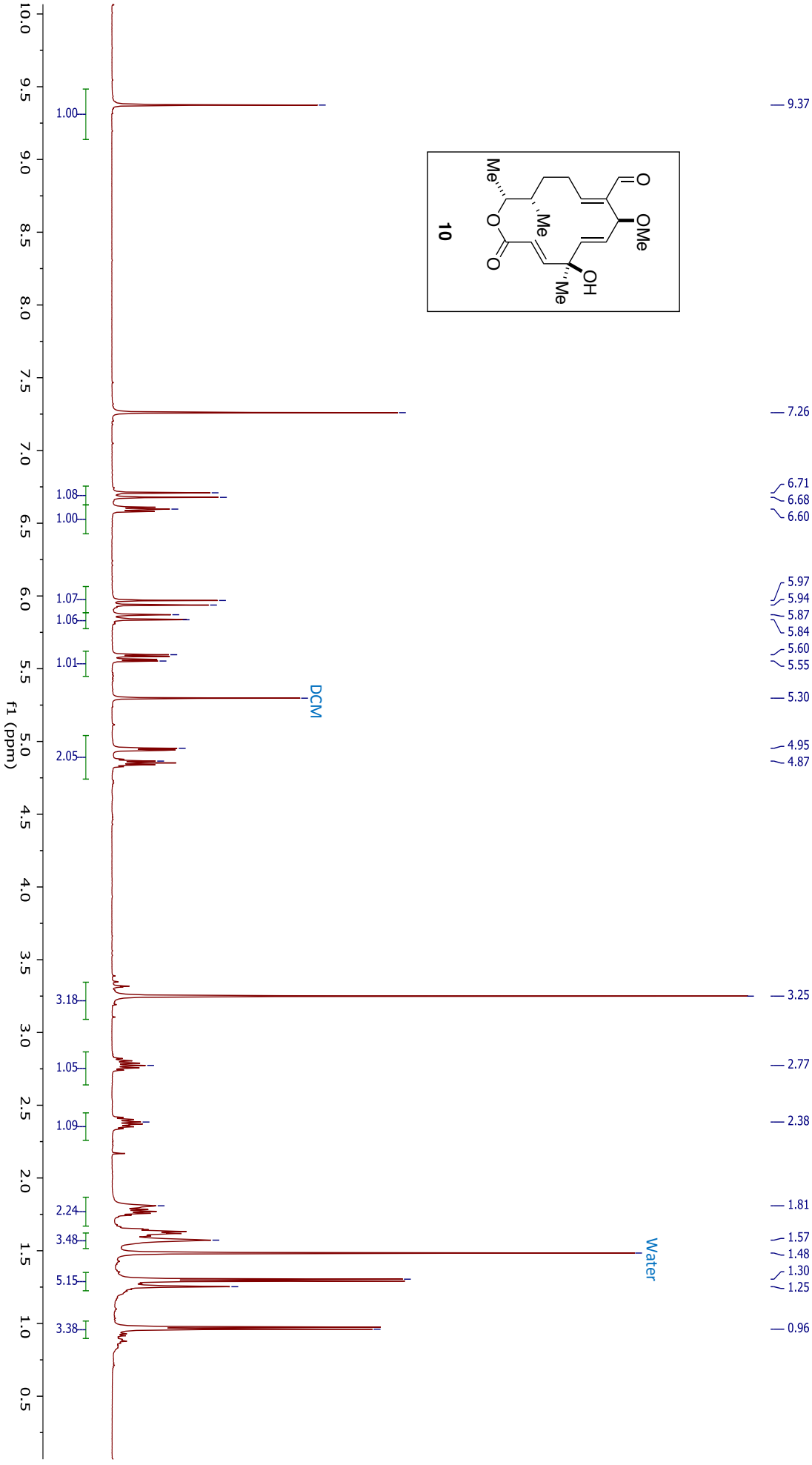

9
HO
OMe
OH
Me
Me
Me
O
O
1.00
1.11
0.93
0.88
0.87
1.93
2.07
2.92
0.82
0.98
1.31
1.36
3.97
5.17
3.47
3
2
5
6
9
7+13
16
15
New OH
C-4 OH
10
10
Water
14+12
11 + 18
17
7.26
6.96
6.93
5.92
5.77
5.76
5.73
5.55
5.50
5.33
4.60
4.11
4.01
3.35
2.56
2.17
2.04
1.84
1.51
1.29
0.92
f1 (ppm)

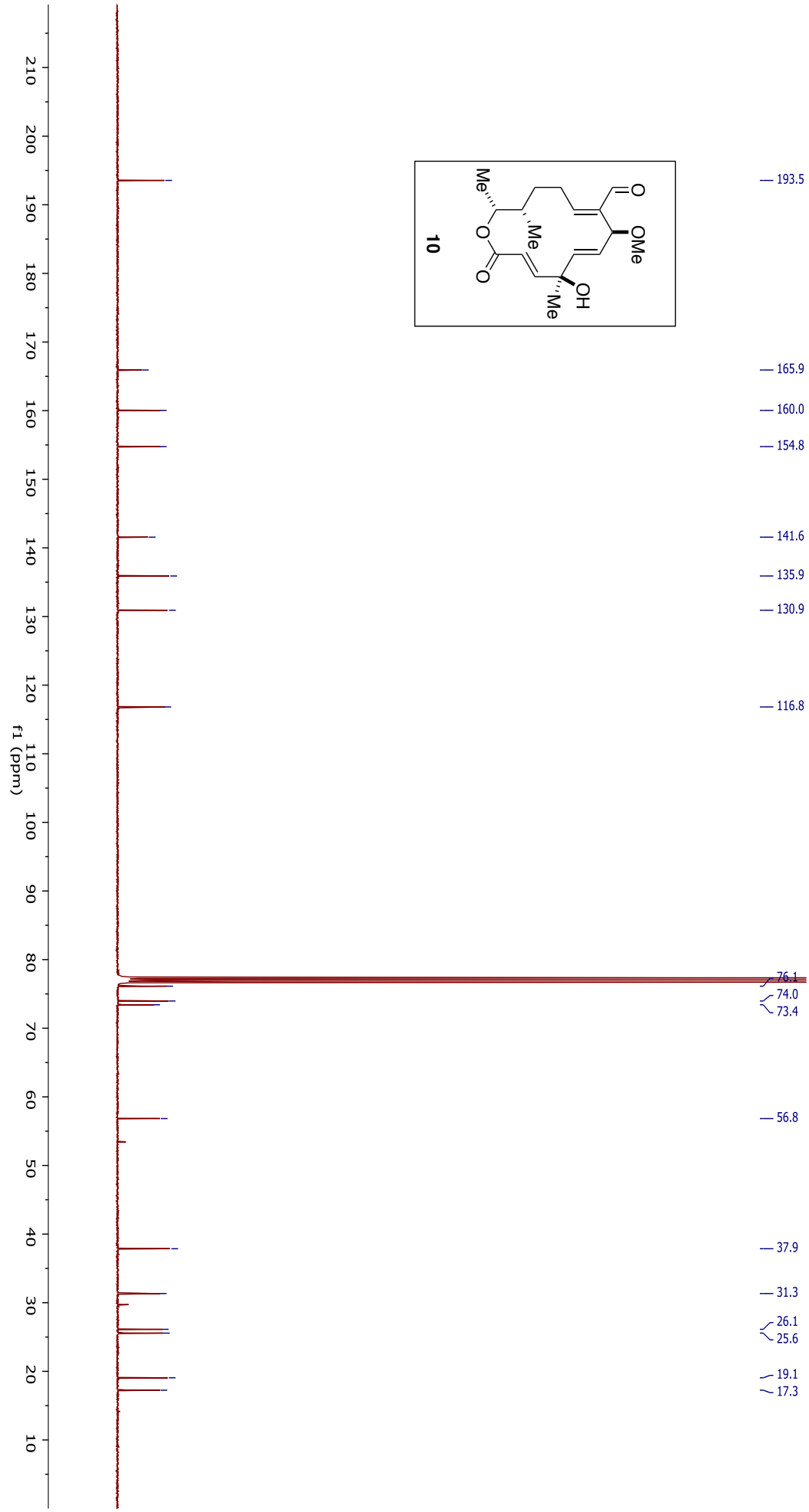

166.6
153.7
137.2
136.3
129.6
128.7
117.3
80.8
77.0
73.0
66.1
57.0
41.3
34.1
27.2
26.2
19.0
16.3
OMe
HO
OH
Me
Me
Me
O
O
9
1
3
8
5
6
9
2
7
4
New Signal
15
12
11
10
14
18
17
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

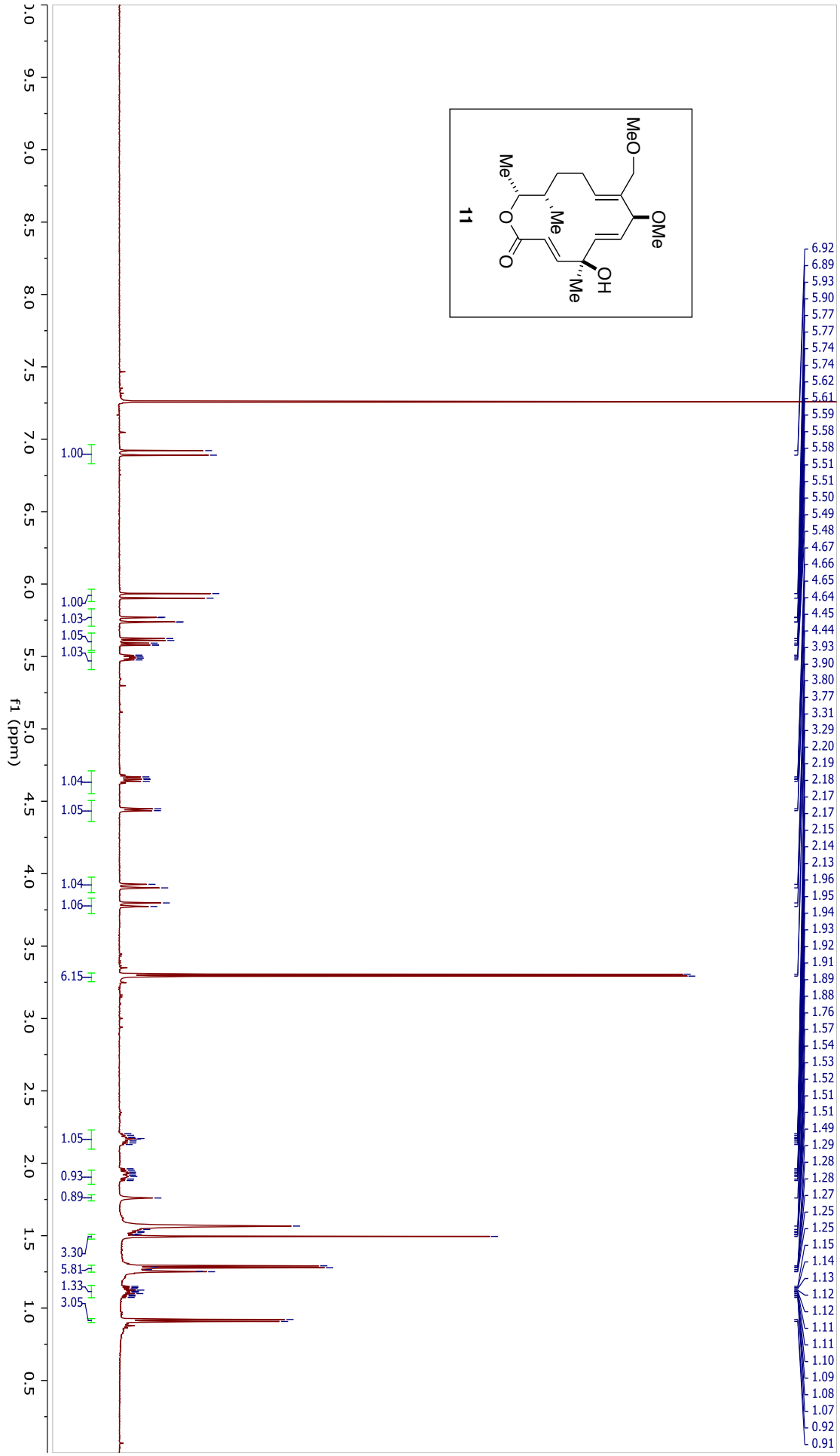

HO
16
OMe
15
8
7
6
10
11
9
5
12
17
Me
2
4
13
O
1
3
O
Me
14
OH
18
9
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
3
8
5
6
9
2
7
13
4
16
15
12
11
10
14
18
17
Key signal
166.6
153.7
137.2
136.3
129.7
128.7
117.4
80.8
76.8
73.0
66.1
57.0
41.3
34.2
27.2
26.3
19.0
16.3

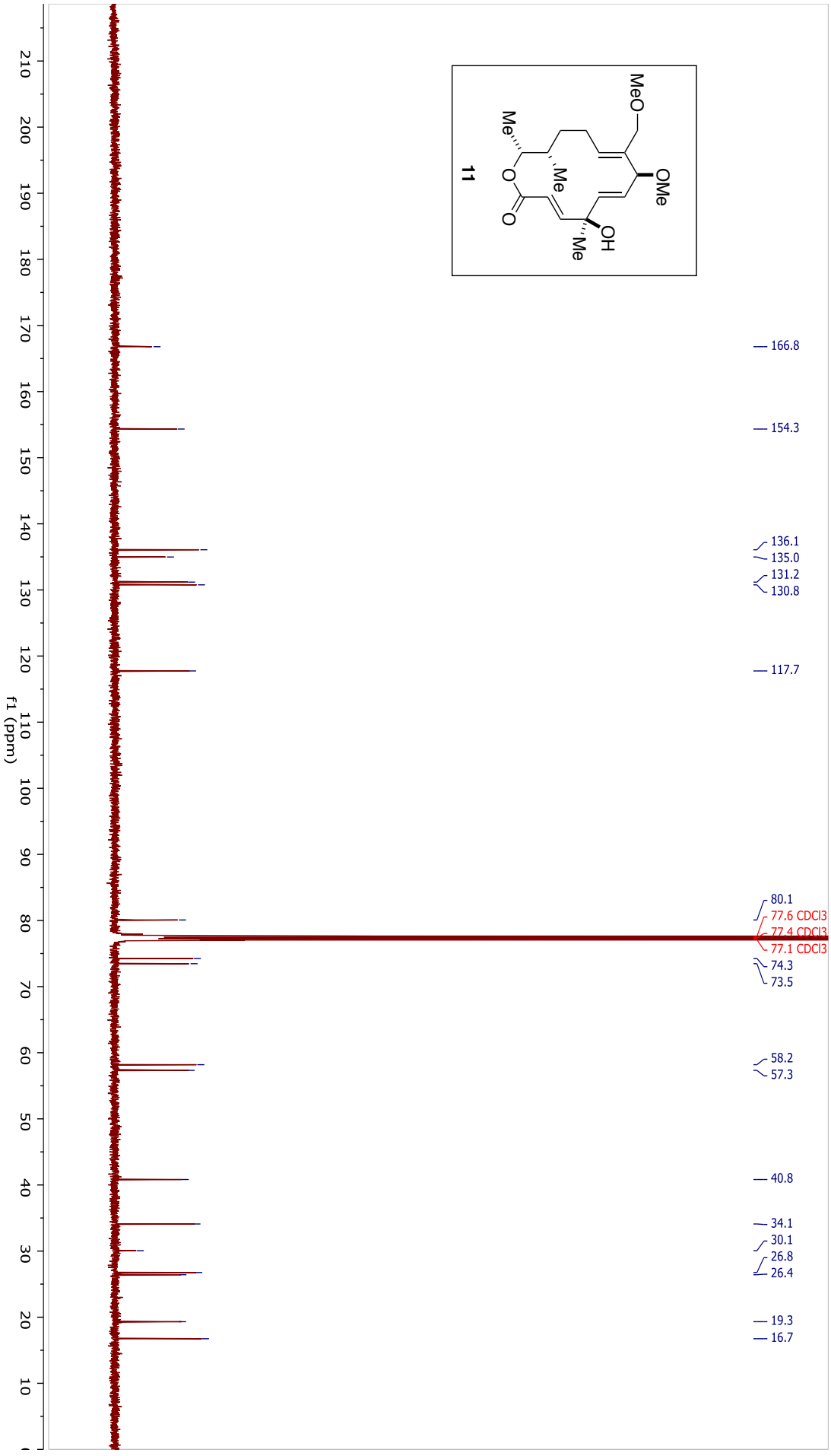

f2 (ppm)
f1 (ppm)
HO
OMe
OH
Me
Me
Me
O
O
9
3
2
5
6
9
7+13
10
10
11+18

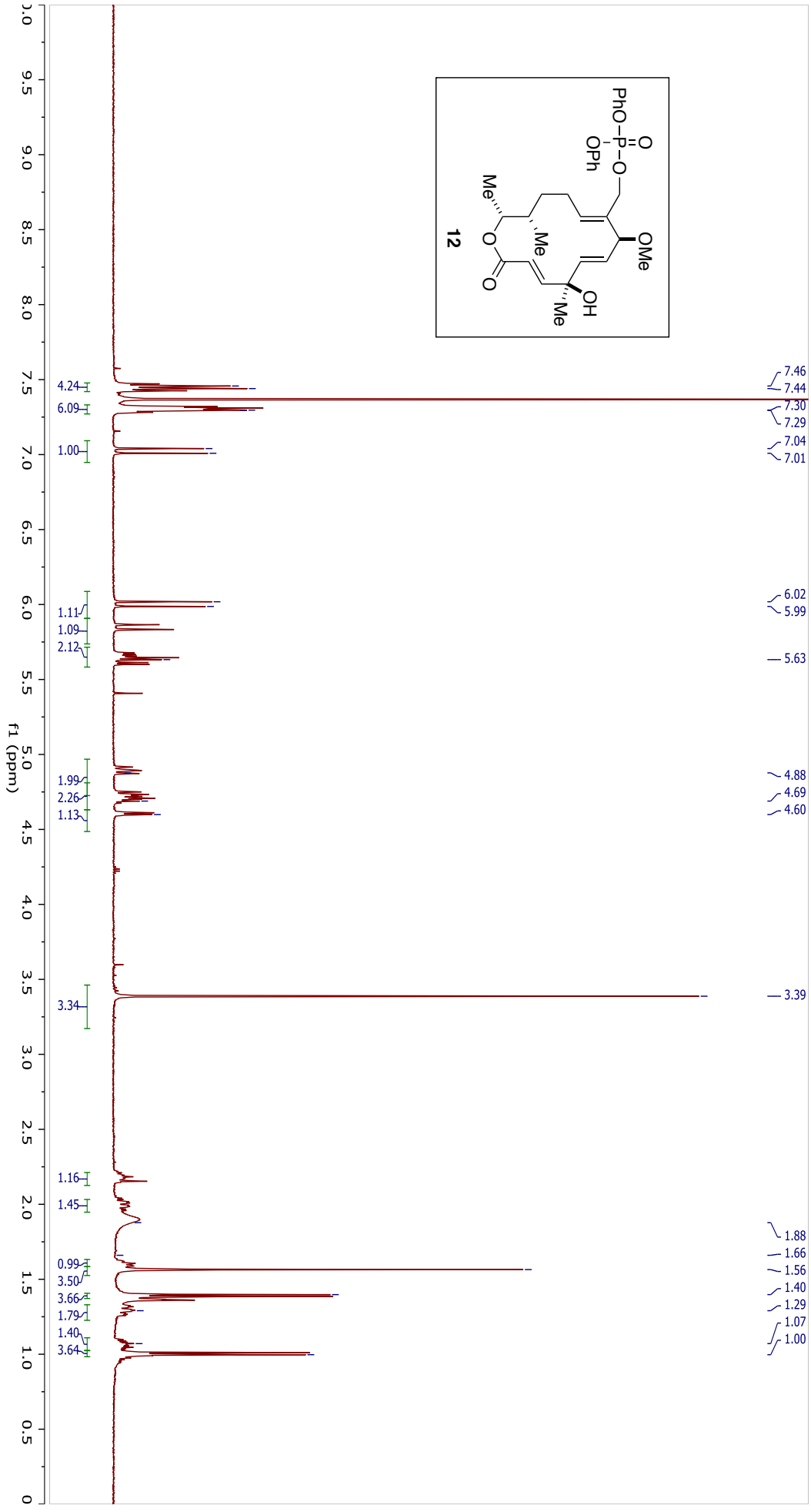

f1 (ppm)
f2 (ppm)
9

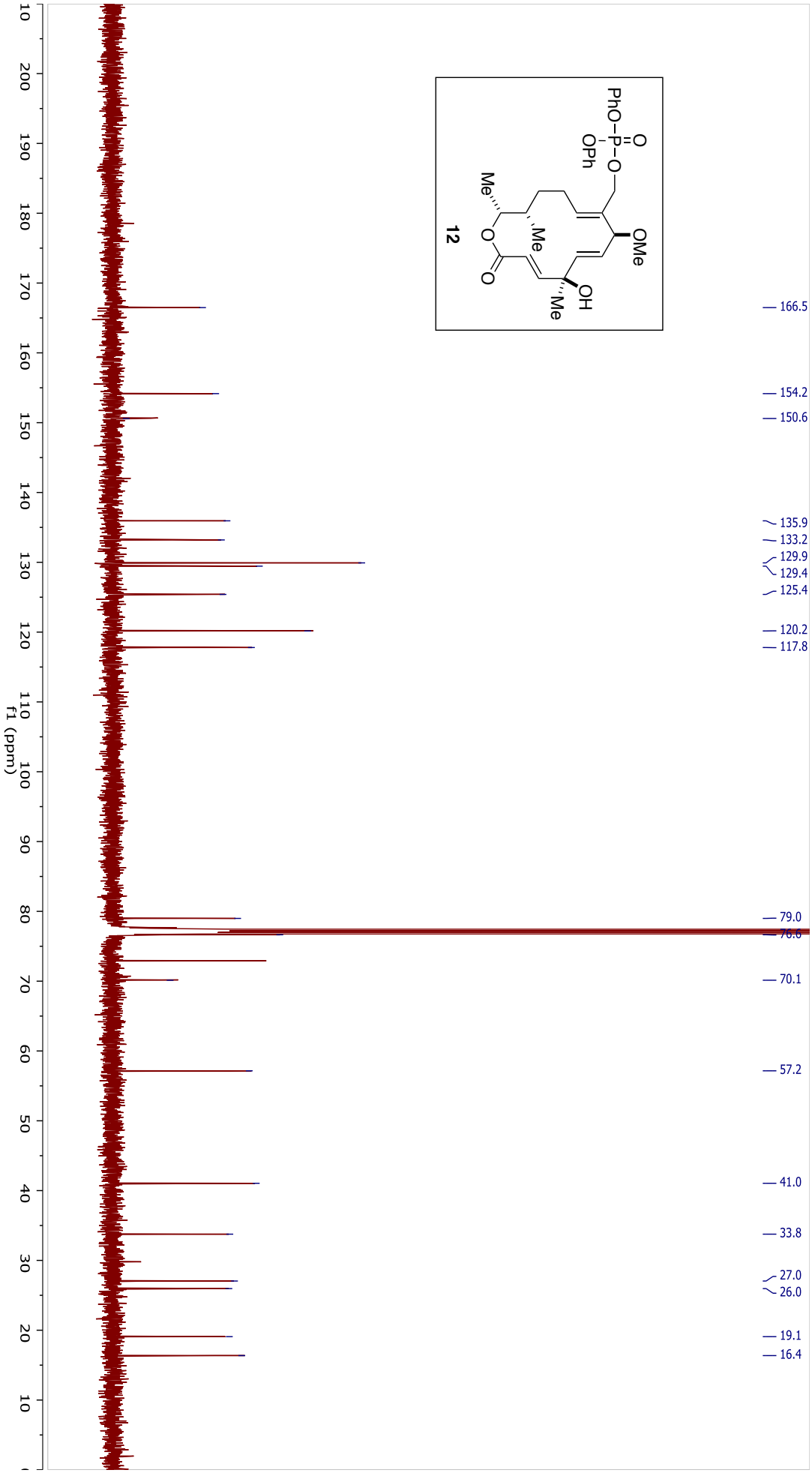

10
Me
Me
Me
OH
OMe
O
O
O
f1 (ppm)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.00
1.08
1.00
1.07
1.06
1.01
2.05
3.18
1.05
1.09
2.24
3.48
5.15
3.38
DCM
Water
9.37
7.26
6.71
6.68
6.60
5.97
5.94
5.87
5.84
5.60
5.55
5.30
4.95
4.87
3.25
2.77
2.38
1.81
1.57
1.48
1.30
1.25
0.96

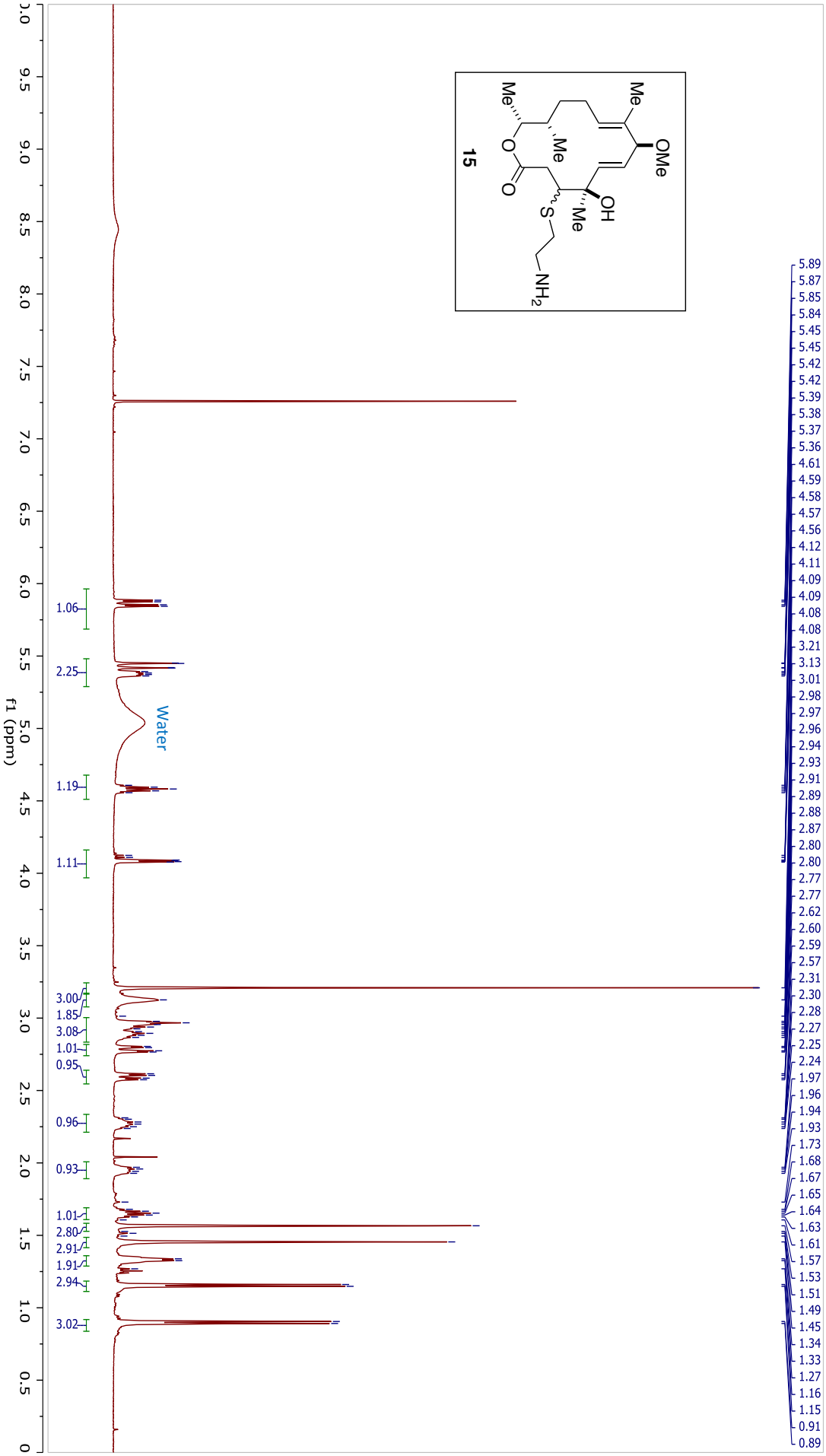

10
Me
O
Me
O
O
OMe
OH
Me
193.5
165.9
160.0
154.8
141.6
135.9
130.9
116.8
76.1
74.0
73.4
56.8
37.9
31.3
26.1
25.6
19.1
17.3
f1 (ppm)

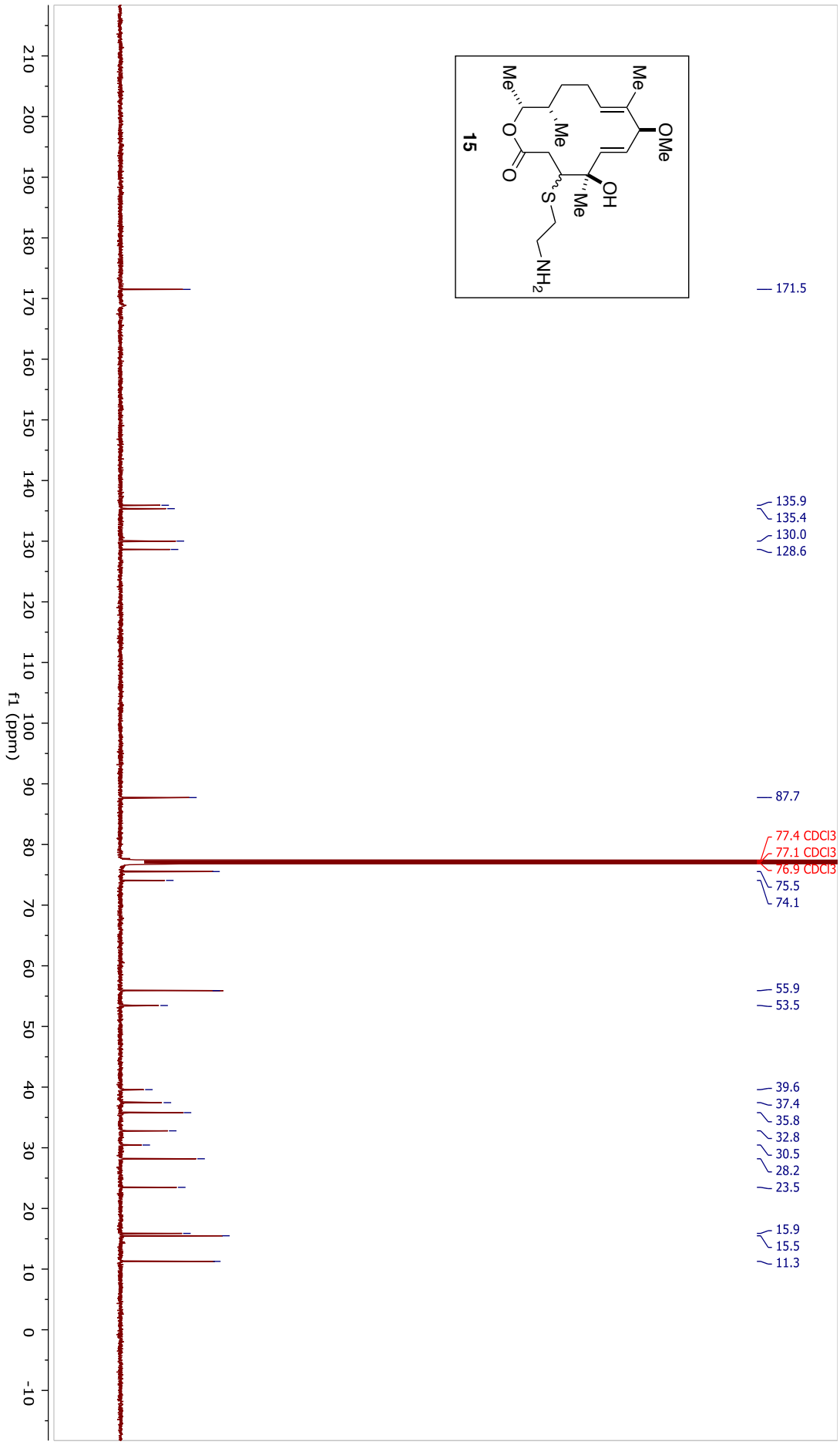

11
MeO
OMe
Me
Me
O
O
OH
Me
f1 (ppm)
1.00
1.00
1.03
1.05
1.03
1.04
1.05
1.04
1.06
6.15
1.05
0.93
0.89
3.30
5.81
1.33
3.05
6.92
6.89
5.93
5.90
5.77
5.77
5.74
5.74
5.62
5.61
5.59
5.58
5.58
5.51
5.51
5.51
5.50
5.49
5.48
4.67
4.66
4.65
4.64
4.45
4.44
3.93
3.90
3.80
3.77
3.31
3.29
2.20
2.19
2.18
2.17
2.17
2.15
2.14
2.13
1.96
1.95
1.94
1.93
1.92
1.91
1.89
1.88
1.76
1.57
1.54
1.53
1.52
1.51
1.51
1.49
1.29
1.28
1.28
1.27
1.25
1.25
1.15
1.14
1.13
1.12
1.12
1.11
1.11
1.10
1.09
1.08
1.07
0.92
0.91


MeO
OMe
Me
Me
O
O
OH
Me
11
166.8
154.3
136.1
135.0
131.2
130.8
117.7
80.1
77.6 CDCl3
77.4 CDCl3
77.1 CDCl3
74.3
73.5
58.2
57.3
40.8
34.1
30.1
26.8
26.4
19.3
16.7
f1 (ppm)


12
PhO–P–O
OPh
O
OMe
OH
Me
Me
Me
O
O
f1 (ppm)
4.24
6.09
1.00
1.11
1.09
2.12
1.99
2.26
1.13
3.34
1.16
1.45
0.99
3.50
3.66
1.79
1.40
3.64
7.46
7.44
7.30
7.29
7.04
7.01
6.02
5.99
5.63
4.88
4.69
4.60
3.39
1.88
1.66
1.56
1.40
1.29
1.07
1.00


12
Me
O
O
Me
OH
Me
OMe
PhO–P–O
OPh
O
166.5
154.2
150.6
135.9
133.2
129.9
129.4
125.4
120.2
117.8
79.0
70.1
57.2
41.0
33.8
27.0
26.0
19.1
16.4
f1 (ppm)


15
Me
OMe
Me
O
Me
O
OH
Me
S
NH2
Water
1.06
2.25
1.19
1.11
3.00
1.85
3.08
1.01
0.95
0.96
0.93
1.01
2.80
2.91
1.91
2.94
3.02
f1 (ppm)


15
Me
OMe
Me
O
Me
O
S
OH
Me
NH2
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
171.5
135.9
135.4
130.0
128.6
87.7
77.4 CDCl3
77.1 CDCl3
76.9 CDCl3
75.5
74.1
55.9
53.5
39.6
37.4
35.8
32.8
30.5
28.2
23.5
15.9
15.5
11.3