Desmethyl Macrolides: Synthesis and Evaluation of 4-Desmethyl Telithromycin

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SUPPORTING INFORMATION

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General Methods. All reactions containing moisture or air sensitive reagents were performed in oven-dried glassware under nitrogen or argon. *N,N*-Dimethylformamide (DMF), tetrahydrofuran (THF), toluene and dichloromethane were passed through two columns of neutral alumina prior to use. Anhydrous dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich and subjected to three cycles of freeze-pump-thaw before use. Pyridine, 2,6-lutidine, acetone, *i*-Pr_2NEt, and Et_3N were all distilled from CaH_2 prior to use. Molecular sieves (4Å) were activated by flame-drying under vacuum prior to use. AgOTf was purchased from Sigma-Aldrich and azeotroped with dry toluene prior to use. Compounds **7**,[1] **9**,[2] **19**,[3, 4] and **22**[5] were prepared according to known literature procedures. 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 with ICN Silitech 32-63 D 60Å silica gel using the indicated solvents. All HF reactions are performed in Nalgene containers. Thin layer chromatography was performed on Merck 60 F_{254} silica gel plates. Detection was performed using UV light, $KMnO_4$ stain, or PMA stain followed by 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, $\delta = 0.00$) and referenced to the $CDCl_3$. Splitting patterns are abbreviated as follows: s (singlet), d (doublet), bs (broad singlet), bd (broad doublet), t (triplet), q (quartet) and m (multiplet). Dissociation constants (K_d) were determined by fluorescence polarization using a Tecan F200 plate reader and following procedure of Yan for determining K_d of macrolide antibiotics by competitive binding with a BODIPY-labeled Erythromycin A.[6] Data was fit using Graphpad Prism using Wang's cubic derived equation for the direct determination of K_d for a competitive binding assay.[7-9]

Computational Methods.

Conformationally Sampled Pharmacophore

Calculations were performed with the program CHARMM, version C36a2.[10] Force field parameters were obtained using a combination of the CHARMM carbohydrate[11-15] and CGenFF[16-18] force fields and the TIP3P water model.[19] All structures were initially minimized by 500 steps of steepest descent (SD) in the gas phase using infinite nonbond lists. The minimized structures were then immersed in a cubic waterbox with a side length of approximately 48 Å. Any waters with an oxygen within 2.5 Å of solute non-hydrogen atoms were deleted. The box length was chosen on the basis that it extends 14 Å beyond the maximum distance between the solute non-hydrogen atoms. The entire system was then subjected to 2000 steps of SD minimization, with a harmonic restraint of 50 kcal/mol/Å on the solute non-hydrogen atoms, followed by 1000 steps of conjugate gradient (CG) minimization with a harmonic restraint of 0.5 kcal/mol/Å on the solute non-hydrogen atoms. In preparation for the production simulation, the systems were equilibrated for 100 ps in the NVT ensemble (T = 298.0 K) followed by 400 ps in the NPT ensemble (T = 298.0 K, P = 1 atm with a piston mass of 1000.0 amu and gamma value of 25.0 per picosecond)[20-24] allowing equilibration of the water molecules around the solutes. All dynamics were performed using SHAKE for the covalent bonds involving hydrogens[25] and a 2 fs integration timestep with a force switching function applied from 10 to 12 Å for the Lennard-Jones interactions and a non-bonded cutoff list at 14 Å. The electrostatics were treated using Particle Mesh Ewald with a kappa equal to 0.29, a sixth order spline and ~ 1 Å grid spacing, with

a real space cutoff of 12 Å and a switching function applied to the forces from 10 to 12 Å.[26] Non-bond lists were updated heuristically during dynamics. Conformational sampling was achieved using Hamiltonian Replica Exchange Molecular Dynamics (HREX MD) [27-29] in the NPT ensemble. In this method, a set of simulations is run in parallel, in which to each simulation (or replica) a biasing potential is applied to dihedral angles along the compound's backbone. Comprehensive sampling is achieved by gradually increasing the biasing potential in the replicas, allowing the compound to surmount dihedral transition energy barriers and escape local minima. For these simulations the dihedral angles were arbitrarily selected to be representative of the whole backbone and correspond to the following: C3-C4-C5-C6, C2-C1-O-C13, O-C13-C12-C11, and C8-C9-C10-C11. The biasing potential was applied to the force constants of the dihedral angles using 5 replicas so that the first replica was unperturbed and the final replica contained dihedral angle force constants of opposite sign from their original values. Force constants for the intermediate replicas were obtained by linear interpolation. Exchanges were attempted every 1 ps, with coordinates saved every 2 ps for a total simulation time of 10 ns/replica. Only coordinates stored in the unperturbed replica were used for analysis. Conformationally Sampled Pharmacophore (CSP) analysis included the probability distributions for distances between relevant atoms in the compound. Distributions reported correspond to bin sizes of 0.2 Å for Figures 3A-C and 0.7 Å for Figure 3D.

Ligand-bound Molecular Dynamics

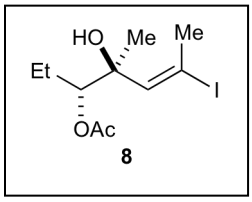
Calculations were performed with the program CHARMM, version C35b6[10] and the CHARMM additive force field including the protein,[30-33] nucleic acid,[34-37] carbohydrate,[11-15, 30, 38] and CGenFF[16-18] parameters and the TIP3P water model.[19] Coordinates were obtained from the protein crystal database (PDB ID 3OAT),[39] with hydrogens added using the HBUILd facility in CHARMM. All molecular dynamics (MD) simulations were performed using a stochastic boundary-based approach that has been presented previously.[40] Briefly, the system was truncated to the region of interest around telithromycin by deleting residues outside of 40 Å of telithromycin's center of mass. Residues were considered within 40 Å if one atom was within the distance criterion. This truncation scheme reduced the number of atoms, making the MD simulations less computationally expensive. Then, three regions within the sphere were defined. Bases and residues containing one or more atoms within 28 Å comprised the dynamic region, those not in the dynamic region containing one or more atoms within 34 Å comprised the buffer region, and the remainder comprised the outer reservoir region. Atoms within the reservoir region were fixed for all calculations, while varying harmonic restraints were used on atoms within the buffer and dynamic regions as described below. Water was maintained within the sphere using a spherical, quartic restraining potential as implemented in the MMFP module of CHARMM[41] using a 1 kcal/mol/Å force constant and offset parameter (P1) of 2.5 that was applied to the water oxygen atoms.

Prior to dynamics, the entire system was first subjected to 250 steps of steepest descent (SD)[42] minimization with a harmonic restraint of 5 kcal/mol/Å on non-hydrogen atoms within the dynamic region and a mass-weighted harmonic restraint of 10 kcal/mol/Å on non-hydrogen atoms within the buffer region, followed by 250 steps of Adopted-Basis Newton Rhapson

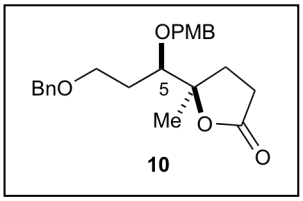
(ABNR)[42] using the same restraints. Equilibration consisted of 400 ps (20 cycles) of Grand Canonical Monte Carlo/Molecular Dynamics (GCMC/MD) using the aforementioned restraints. GCMC/MD is implemented within the MC module in CHARMM and has been described previously.[43]

Following equilibration, the C4-desmethyl telithromycin and mutant/modified A2058 ribosomes were generated. Inactive water molecules from the GCMC/MD equilibration were deleted and patches were applied to telithromycin and A2058 in order to generate C4-desmethyl telithromycin with WT, A2058G, N6-monomethyl (MAD), and N6, N6'-dimethyl A2058 (DMAD). Atoms modified during the patch were subjected to minimization for 200 steps SD and 200 steps CG, and the entire system was allowed to relax for 50 steps SD and 50 steps CG. Parameters for the N6-mono and N6,N6'-dimethyl A2058 have been developed in our lab previously.[40] Two monomethyl systems were studied due to the high energy barrier for the C6-N6 torsion, in which the methyl group in MAD1 is oriented toward telithromycin's desosamine sugar and away from it in MAD2.

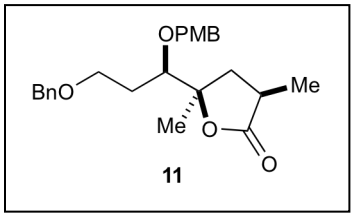
All systems were then subjected to 5 ns of Langevin dynamics[44, 45] at 298 K with a friction coefficient of 5/ps and a 2 fs integration timestep using the "leapfrog" Verlet integrator.[46] All dynamics were performed using SHAKE for the covalent bonds involving hydrogens.[25] Nonbond lists were updated heuristically during dynamics with a cutoff of 16 Å, the forces truncated at 12 Å and a switching function applied to the forces from 10 to 12 Å for both electrostatic and van der Waals energy terms. Interaction energies reported were calculated using the last 4 ns of the simulation, with the same non-bonded cutoffs as used during dynamics. Snapshots were written every 10 ps. The neutral group surrounding the C4 methyl [C3(=O)-C4(H_2)-C5] was used so as not to calculate the interaction between species with non-integer charge.



Vinyl iodide 8: Acetic anhydride (74.5 mg, 0,73 mmol) was added to a solution of **7** (165 mg, 0.61 mmol), Et_3N (73.6 mg, 0.73 mmol) and DMAP (7.3 mg, 0.06 mmol) in CH_2Cl_2 (6 mL) at 0 °C. The solution was stirred overnight while warming to rt. The solution was then diluted with sat. aq. NH_4Cl (10 mL) and the aqueous fraction extracted with EtOAc (3 x 20 mL). The organic fraction was washed with brine (10 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 166 mg (87%) of **8** as a white solid. $[\alpha]^{23}_D$ +74.6 (c 1.5, $CHCl_3$); IR (neat) 3499, 2971, 2932, 1713, 1373, 1247, 1189, 1049, 964, 844; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 – 6.02 (m, 1H), 4.78 (dd, *J* = 10.3, 3.0 Hz, 1H), 2.70 (d, *J* = 1.5 Hz, 3H), 2.12 (s, 3H), 1.76 – 1.52 (m, 2H), 1.28 (s, 3H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.16, 141.83, 99.94, 80.48, 77.88, 30.26, 26.29, 22.45, 20.95, 10.56; HRMS (ESI) calc'd for $C_{10}H_{17}IO_3$ + Na = 335.0120, found 335.0117.

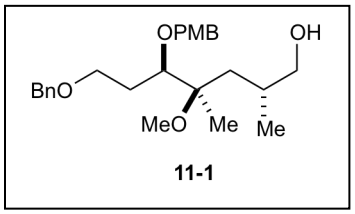


Lactone 10: A solution of (*R*)-5-((*R*)-3-(benzyloxy)-1-hydroxypropyl)-5-methyldihydrofuran-2(3H)-one (**9**) (1.1 g, 4.16 mmol) in DMF (5 mL) was cannulated into a suspension of NaH (60%, 200 mg, 5 mmol) in DMF (15 mL) at 0 °C. The resulting mixture was stirred for 20 min at 0 °C before adding PMBCl (783 mg, 5 mmol). The solution was allowed to gradually warm to rt and after 4 hours cooled back to 0 °C and slowly quenched with water until the bubbling of H_2 ceased. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3 x 40 mL). The combined organic fractions were then washed with H_2O (2 x 20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to afford 1.03 g (65%) of **10**. $[\alpha]^{23}_D$ +34.0 (c 1.5, $CHCl_3$); IR (neat) 3509, 2934, 2861, 1765, 1611, 1512, 1453, 1244, 1075, 1028, 941, 820, 737, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 7.20 (d, *J* = 8.7 , 2H), 6.84 (d, *J* = 8.7, 2H), 4.70 (d, *J* = 11.0 Hz, 1H), 4.50 – 4.40 (m, 3H), 3.62 (dt, *J* = 9.7, 4.8 Hz, 1H), 3.59 – 3.52 (m, 2H), 2.60 – 2.53 (m, 2H), 2.12 – 2.02 (m, 1H), 1.90 (ddd, *J* = 12.9, 8.9, 5.9 Hz, 1H), 1.86 – 1.77 (m, 1H), 1.65 (ddt, *J* = 14.2, 9.6, 4.3 Hz, 1H), 1.38 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.83, 159.30, 138.28, 130.50, 129.65 (2C), 128.41 (2C), 127.77 (2C), 127.69, 113.75 (2C), 89.44, 80.68, 73.88, 73.07, 66.45, 55.25, 31.29, 30.98, 28.79, 21.36; HRMS (ESI) calc'd for $C_{23}H_{28}O_5$ + H = 385.2015, found 385.2006.



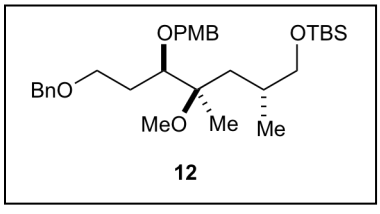
Lactone 11: *n*-BuLi (1.0 mL, 2.41 M) was added drop wise to a stirring solution of diisoproplylamine (253 mg, 2.5 mmol) in THF (5 mL) at -78 °C and stirred for 10 min. The solution was warmed to 0 °C and stirred for an additional 20 min before cooling back to -78 °C. Lactone **10** (503 mg, 1.31 mmol) in THF (3 mL) was cannulated into the solution and stirred at -78 °C for 2 h. MeI (1.99 g, 14 mmol) in THF (3 mL) was cannulated into the solution and stirred for 1 h. Saturated NH_4Cl (25 mL) was added to the solution and was extracted with Et_2O (3 x 50 mL). The combined organic fractions were washed with brine (25 mL) and dried over Na_2SO_4. The dried solvent was removed under reduced pressure and dried under high vacuum before proceeding to the next step. *n*-BuLi (1.0 mL, 2.41 M) was added drop wise to a stirring solution of diisoproplylamine

(253 mg, 2.5 mmol) in THF (5 mL) at -78 °C and stirred for 10 min. The solution was warmed to 0 °C and stirred for an additional 20 min before cooling back to -78 °C. The product (vide supra) (510 mg, 1.31 mmol) in THF (3 mL) was cannulated into the solution and stirred for 30 min, then warmed to -45 °C for 1.5 h. The solution was cooled back to -78 °C and triphenylacetic acid (755 mg, 2.62 mmol) in THF (5 mL) was cannulated into the solution and stirred for 2 h while slowly warming to rt. Saturated NH_4Cl (25 mL) was added and the mixture was extracted with Et_2O (3 x 50 mL). The combined organic fractions were washed with brine (25 mL) and dried over Na_2SO_4. The dried solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 276 mg (54%) of **11**. $[\alpha]^{23}_D$ +40.6 (c 1.5, $CHCl_3$); IR (neat) 2970, 2933, 2867, 1764, 1612, 1513, 1246, 1089, 1033, 821, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.80 (d, *J* = 10.9 Hz, 1H), 4.50 – 4.38 (m, 3H), 3.78 (s, 3H), 3.62 (dd, *J* = 10.3, 2.0 Hz, 1H), 3.58 – 3.50 (m, 2H), 2.85 – 2.71 (m, 1H), 2.12 (dd, *J* = 12.5, 8.8 Hz, 1H), 1.79 – 1.50 (m, 3H), 1.37 (s, 3H), 1.27 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.00, 159.16, 138.24, 130.27, 129.76 (2C), 128.41 (2C), 127.79 (2C), 127.70, 113.71 (2C), 87.27, 81.05, 73.94, 73.10, 66.40, 55.24, 39.73, 34.23, 31.04, 19.61, 15.15; HRMS (ESI) calc'd for $C_{24}H_{30}O_5$ + Na = 421.1991, found 421.1982.



11-1

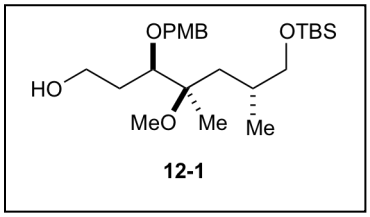
Alcohol 11-1: Lactone **11** (188 mg, 0.47 mmol) in THF (2 mL) was cannulated into a suspension of $LiAlH_4$ (23 mg, 0.61 mmol) in THF (3 mL) at -45 °C. The solution was stirred for 2 h, then allowed to slowly warm to rt over 1 hour. The mixture was diluted with Et_2O (10 mL) and cooled back to 0 °C. Sat'd aq. Na_2SO_4 (10 mL) was added slowly until all H_2 formation ceased. The mixture was extracted with Et_2O (3 x 20 mL). The combined organic fractions were washed with brine, filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-60% EtOAc in hexanes to afford 174 mg (92%) of **11-1** as a colorless oil. $[\alpha]^{23}_D$ +6.6 (c 1.5, $CHCl_3$); IR (neat) 3331, 2954, 2932, 2869, 1612, 1513, 1496, 1245, 1092, 1035, 821, 738, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 5H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 4.44 (m, 4H), 3.71 (s, 3H), 3.58 – 3.50 (m, 2H), 3.47 (dd, *J* = 10.9, 3.4 Hz, 1H), 3.31 (dd, *J* = 8.1, 3.6 Hz, 1H), 3.19 (dd, *J* = 10.9, 8.4 Hz, 1H), 1.97 – 1.83 (m, 2H), 1.66 (ddt, *J* = 14.5, 8.2, 4.9 Hz, 1H), 1.46 (dd, *J* = 14.5, 9.1 Hz, 1H), 1.36 (dd, *J* = 14.5, 3.1 Hz, 1H), 1.11 (s, 3H), 0.79 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.22, 137.92, 130.40, 129.41(2C), 128.40 (2C), 127.80 (2C), 127.72, 113.79 (2C), 83.69, 74.82, 74.07, 73.05, 69.12, 67.20, 55.22, 44.13, 31.47, 30.94, 21.72, 19.81; HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + H = 404.2484, found 404.2481.



12

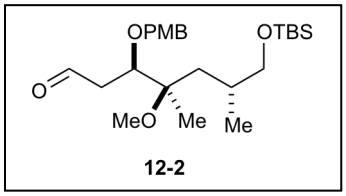
Benzyl ether 12: TBSCl (74 mg, 0.49 mmol) and imidazole (39 mg, 0.57 mmol) were added sequentially to a solution of **11-1** (165 mg, 0.41 mmol) in CH_2Cl_2 (4 mL) at 0 °C and stirred for 2 h while warming to rt. H_2O (4 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 8 mL). The organic fractions were washed with brine (4 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and dried under high vacuum. The crude product was dissolved in CH_2Cl_2 (4 mL). 2,6-DTBMP (740 mg, 3.6 mmol) followed by MeOTf (361 mg, 2.2

mmol) were added and the solution stirred at rt for 48 h. Sat'd aq. $NaHCO_3$ (4 mL) was added and the mixture stirred for 15 min. MeOH (4 mL) was added, and the mixture stirred for 30 min. The mixture was then extracted with Et_2O (3 x 10 mL). The combined organic fractions were washed with brine (5 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure, and the product purified by flash column chromatography eluting with hexanes to recover the 2,6-DTBMP and then 0-40% EtOAc in hexanes to give 157 mg (72%) of **12** as a colorless oil. $[\alpha]^{23}_D$ +15.8 (c 1.5, $CHCl_3$); IR (neat) 2963, 2929, 2855, 1514, 1463, 1248, 1098, 836, 775, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 5H), 7.24 – 7.18 (m, 2H), 6.87 – 6.81 (m, 2H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.55 – 4.40 (m, *J* = 10.9, 8.4 Hz, 3H), 3.79 (s, 3H), 3.65 (dd, *J* = 10.1, 2.3 Hz, 1H), 3.62 – 3.55 (m, 2H), 3.52 – 3.43 (m, 1H), 3.31 (dd, *J* = 9.7, 7.0 Hz, 1H), 3.23 (s, 3H), 1.98 (dddd, *J* = 15.7, 8.9, 6.9, 2.3 Hz, 1H), 1.85 (td, *J* = 13.0, 6.7 Hz, 1H), 1.68 – 1.52 (m, 2H), 1.36 – 1.25 (m, 1H), 1.20 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.94 – 0.88 (m, 9H), 0.04 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.93, 138.66, 131.50, 129.31 (2C), 128.29 (2C), 127.62 (2C), 127.42, 113.60 (2C), 80.48, 80.31, 74.42, 72.78, 69.02, 67.52, 55.23, 49.41, 36.89, 30.99, 30.02, 25.95 (3C), 19.55, 18.62, 18.34, -5.37, -5.39; HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + K = 569.3065, found 569.3047.



12-1

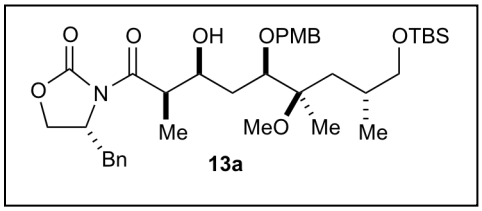
Alcohol 12-1: Raney-Ni in H_2O was washed with EtOH and decanted. Eight spatulas full of Raney-Ni were then added to a solution of **12** (830 mg, 0.1.56 mmol) in EtOH (30 mL). The suspension was then placed under an atmosphere of H_2 and stirred approximately 6 h (Reaction times vary. TLC analysis is necessary to prevent reduction of the PMB ether). The suspension was then filtered over Celite washing with EtOAc. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-20% EtOAc in hexanes to afford 520 mg (75%) of **12-1** as a colorless oil. $[\alpha]^{23}_D$ +9.8 (c 1.5, $CHCl_3$); IR (neat) 3435, 2954, 2929, 2856, 1613, 1514, 1464, 1249, 1085, 836, 775 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.64 (dd, *J* = 75.8, 10.9 Hz, 2H), 3.80 (s, 3H), 3.77 – 3.70 (m, 1H), 3.70 – 3.63 (m, 2H), 3.40 – 3.30 (m, 2H), 3.24 (s, 3H), 2.55 (dd, *J* = 7.4, 4.0 Hz, 1H), 1.90 – 1.82 (m, 1H), 1.81 – 1.75 (m, 1H), 1.69 – 1.62 (m, 1H), 1.58 (dd, *J* = 14.9, 3.8 Hz, 1H), 1.34 (dd, *J* = 15.0, 7.8 Hz, 1H), 1.20 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.89 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.13, 131.01, 129.61 (2C), 113.77 (2C), 99.60, 82.60, 80.79, 74.12, 69.01, 60.93, 55.25, 49.19, 36.62, 32.91, 31.09, 25.93 (3C), 19.45, 18.29, -5.40 (2C); HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + K = 479.2595, found 479.2597.



12-2

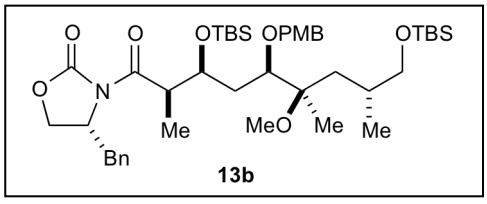
Aldehyde 12-2: DMSO (2.8 g, 35.8 mmol) was added drop wise to a solution of oxalyl chloride (2.2 g, 17.2 mmol) in CH_2Cl_2 (125 mL) at -78 °C. The solution was stirred for 20 min, then alcohol **12-1** (6.3 g, 14.3 mmol) in CH_2Cl_2 (20 mL) was cannulated into the solution and stirred for 45 min at -78 °C. Et_3N (3.6 g, 35.8 mmol) was then added and the solution allowed to warm to rt over 1 h. H_2O (70 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 145 mL). The combined organic fractions were washed with brine (70 mL) and removed under reduced pressure. The product was redissolved in Et_2O (100 mL) and passed through a plug of silica washing with Et_2O (3 x 150

mL). The solvent was removed under reduced pressure and azeotropically dried with toluene (3 x 50 mL). The product was dried under high vacuum for 3 h before taking directly to the next step.



13a

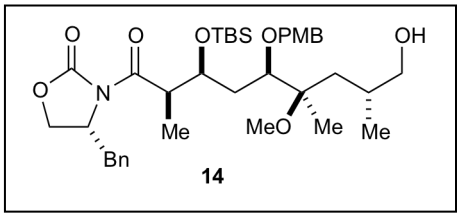
Aldol 13a: Et_3N (2.2 g, 21.5 mmol) was added dropwise to a solution of (*R*)-4-benzyl-3-propionyl-2-oxazolidinone (4.0 g, 17.2 mmol) and Bu_2BOTf (18.6 mL, 1 M) in CH_2Cl_2 (60 mL). The solution changed from red to yellow and was subsequently cooled to -78 °C. Aldehyde **12-2** (6.3 g, 14.3 mmol) in CH_2Cl_2 (10 mL) was cannulated into the solution and stirred at -78 °C for 20 min and then at 0 °C for 1 h. Phosphate buffer (pH 7, 0.2 M aq. Na_2HPO_4:0.1M aq. citric acid, 82:18, 50 mL) and MeOH (150 mL). The solution becomes cloudy and a solution of MeOH:30% H_2O_2 (2:1, 150 mL) was added and stirred at 0 °C for 1 h. The solution was then concentrated under reduced pressure and the remaining aqueous fraction was extracted with EtOAc (3 x 200 mL). The combined organic fractions were washed with sat. $NaHCO_3$ (100 mL), brine (100 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 7.1 g (74%) of **13a** as a colorless oil. $[\alpha]^{23}_D$ +33.6 (c 1.5, $CHCl_3$); IR (neat) 3380, 2953, 2928, 2855, 1753, 1514, 1250, 1094, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 5H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.88 – 6.80 (m, 2H), 4.74 (d, *J* = 11.0 Hz, 1H), 4.65 (qd, *J* = 6.5, 3.1 Hz, 1H), 4.57 (d, *J* = 11.0 Hz, 1H), 4.22 – 4.11 (m, 2H), 4.11 – 4.03 (m, 1H), 3.79 (s, 3H), 3.74 (dd, *J* = 7.0, 3.8 Hz, 1H), 3.66 (dd, *J* = 8.8, 3.5 Hz, 1H), 3.42 (dd, *J* = 9.7, 6.2 Hz, 1H), 3.33 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.2 Hz, 1H), 3.23 (s, 3H), 2.76 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.87 – 1.61 (m, 4H), 1.58 (dd, *J* = 14.9, 4.0 Hz, 1H), 1.35 (dd, *J* = 14.9, 7.7 Hz, 1H), 1.25 (d, *J* = 7.0 Hz, 3H), 1.22 (s, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.90 (s, *J* = 2.8 Hz, 9H), 0.04 (d, *J* = 1.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.98, 159.01, 153.01, 135.29, 130.82, 129.58 (2C), 129.42 (2C), 128.91 (2C), 127.31, 113.66 (2C), 82.61, 80.78, 73.49, 70.50, 69.05, 66.00, 55.31, 55.22, 49.28, 42.56, 37.74, 36.61, 34.42, 31.11, 29.67, 25.95 (3C), 19.67, 18.39, 10.88, -5.37, -5.41; HRMS (ESI) calc'd for $C_{37}H_{57}NO_8Si$ + H = 672.3932, found 672.3903.



13b

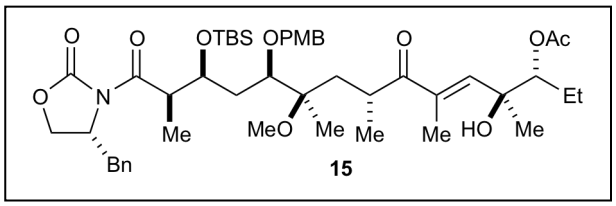
Oxazolidinone 13b: 2,6-Lutidine (150 mg, 1.4 mmol) followed by TBSOTf (291 mg, 1.1 mmol) were added to a solution of **13a** (486 mg, 0.7 mmol) in CH_2Cl_2 (5 mL) at 0 °C and stirred for 30 min. Sat. $NaHCO_3$ (5 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with brine (5 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-10% EtOAc in hexanes to afford 560 mg (95%) of **13b** as a colorless oil. $[\alpha]^{23}_D$ -22.9 (c 1.5, $CHCl_3$); IR (neat) 2953, 2928, 2856, 1780, 1707, 1514, 1463, 1386, 1248, 1094, 837, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.17 (m, 7H), 6.82 (d, *J* = 8.7 Hz, 1H), 4.66 (dd, *J* = 51.7, 11.4 Hz, 2H), 4.54 – 4.44 (m, 1H), 4.21 – 4.14 (m, 1H), 4.08 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.92 – 3.82 (m, 2H), 3.78 (s, 3H), 3.59 (d, *J* = 8.5 Hz, 1H), 3.47 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.33 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.0 Hz, 1H), 3.22 (s, 3H), 2.74 (dd, *J* = 13.3, 9.7 Hz, 1H), 2.08 – 1.95 (m, 1H), 1.81 (dd, *J* = 10.9, 6.6 Hz, 1H), 1.67 – 1.54 (m, 2H), 1.35 – 1.17 (m, 7H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 4.5 Hz, 18H), 0.05 – -0.08 (m, 12H); ^{13}C NMR (101

MHz, $CDCl_3$) δ 175.02, 158.54, 152.98, 135.48, 132.20, 129.45 (2C), 128.89 (2C), 128.57 (2C), 127.26, 113.36 (2C), 80.46, 79.93, 73.24, 70.52, 69.05, 65.84, 55.78, 55.23, 49.44, 42.41, 37.50, 37.28, 36.74, 31.00, 25.96 (3C), 25.85 (3C), 19.78, 18.61, 18.34, 17.96, 10.83, -4.12, -5.27, -5.40 (2C); HRMS (ESI) calc'd for $C_{43}H_{71}NO_8Si_2$ + Na = 808.4616, found 808.4646.



14

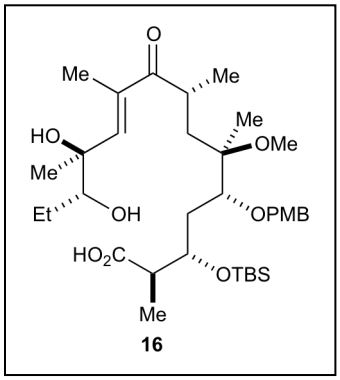
Alcohol 14: CSA (33 mg, 0.14 mmol) was added to a solution of TBS Protected Aldol (560 mg, 0.7 mmol) in MeOH (15 mL) at 0 °C and stirred for 2 h. The MeOH was then removed under reduced pressure and the crude product redissolved in EtOAc (25 mL). The organic fraction was then washed with sat. $NaHCO_3$ (10 mL), brine (10 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-40% EtOAc in hexanes to afford 425 mg (88%) **14** as a colorless oil. $[\alpha]^{23}_D$ -34.4 (c 1.5, $CHCl_3$); IR (neat) 3407, 3029, 2928, 2855, 1774, 1704, 1513, 1380, 1350, 1246, 1207, 1102, 1035, 835, 774, 733, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.22 (m, 7H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.68 (d, *J* = 3.0 Hz, 2H), 4.62 – 4.53 (m, 1H), 4.24 – 4.11 (m, 3H), 4.04 – 3.94 (m, 1H), 3.91 – 3.84 (m, 1H), 3.83 (s, 3H), 3.71 (d, *J* = 8.4 Hz, 1H), 3.63 (s, *J* = 9.1 Hz, 2H), 3.34 (s, *J* = 5.3 Hz, 3H), 3.33 – 3.29 (m, 1H), 2.81 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.17 (dd, *J* = 13.9, 9.4 Hz, 3H), 1.93 (s, 1H), 1.74 – 1.61 (m, 2H), 1.59 – 1.48 (m, 1H), 1.39 (s, 3H), 1.30 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.95 (s, *J* = 13.7 Hz, 9H), 0.05 (d, *J* = 38.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.69, 158.80, 153.16, 135.35, 131.44, 129.45 (2C), 128.91 (2C), 128.63 (2C), 127.32, 113.53 (2C), 79.93, 79.56, 73.73, 70.48, 68.64, 65.97, 55.79, 55.25, 49.95, 42.51, 39.18, 37.49, 37.02, 31.11, 25.83 (3C), 19.72, 19.29, 17.97, 10.47, -4.09, -5.28; HRMS (ESI) calc'd for $C_{37}H_{57}NO_8Si$ + H = 672.3932, found 672.3916.



15

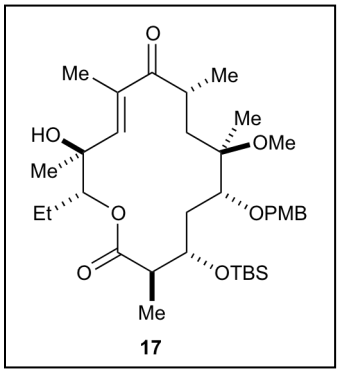
Ketone 15: Dess-Martin periodinane (5.0 g, 11.9 mmol) was added to a solution of **14** (4.0 g, 6.0 mmol) and pyridine (2.4 g, 29.8 mmol) in CH_2Cl_2 (60 mL). The solution was stirred at rt for 2 h. Sat'd aq. $NaHCO_3$ (50 mL), sat'd aq. Na_2SO_3 (50 mL) and H_2O (50 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 200 mL). The combined organic fractions were washed with brine (100 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure. The crude aldehyde (4.0 g, 6.0 mmol) was combined with vinyl iodide **8** (3.7 g, 12 mmol) and azeotropically dried with toluene (3 x 10 mL), dried under high vacuum, dissolved in DMSO (20 mL) and cannulated into a suspension of $CrCl_2$ (2.9 g, 24 mmol) and $NiCl_2$ (29 mg, 0.2 mmol) in DMSO (4 mL). The suspension was stirred at rt for 48 h and then diluted with H_2O (50 mL). The mixture was extracted with EtOAc (5 x 100 mL) and the combined organic fractions were washed with brine (50 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure and dried under high vacuum. The crude NHK product was dissolved in CH_2Cl_2 (60 mL) and pyridine (4.7 g, 59.5 mmol) followed by Dess-Martin periodinane (10 g, 23.8 mmol) were added and the solution stirred at rt for 3 h. Sat'd aq. $NaHCO_3$ (50 mL), sat'd aq. Na_2SO_3 (50 mL) and H_2O (50 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 200 mL). The combined organic fractions were washed with brine (50 mL) and filtered over Na_2SO_4. The solvent was

removed under reduced pressure and the product purified by flash column chromatography eluting with 0–20% EtOAc in hexanes to give 5.4 g (45% over 3 steps) of **15** as a white foam. $[\alpha]^{23}_{D}$ -33.4 (c 1.5, $CHCl_3$); IR (neat) 2955, 2935, 1790, 1733, 1710, 1514, 1463, 1375, 1247, 1098, 1043, 840, 776 cm^{-1}; 1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 7H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.50 (s, 1H), 5.00 (t, *J* = 6.6 Hz, 1H), 4.66 – 4.43 (m, 3H), 4.16 – 4.05 (m, 2H), 4.00 – 3.90 (m, 1H), 3.77 (s, 3H), 3.76 – 3.73 (m, 1H), 3.47 (d, *J* = 7.7 Hz, 1H), 3.44 – 3.33 (m, 1H), 3.26 (dd, *J* = 13.3, 3.0 Hz, 1H), 3.12 (s, 3H), 2.75 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.24 – 2.16 (m, 1H), 2.13 (s, *J* = 2.0 Hz, 3H), 2.02 (s, *J* = 1.1 Hz, 3H), 2.00 – 1.94 (m, 1H), 1.72 – 1.64 (m, 2H), 1.64 – 1.53 (m, 2H), 1.46 (dd, *J* = 14.4, 3.0 Hz, 1H), 1.36 (s, 3H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.20 (s, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.93 – 0.83 (m, 12H), -0.03 (d, *J* = 46.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.05, 175.25, 171.33, 159.09, 153.65, 140.78, 138.89, 135.78, 132.11, 129.84 (2C), 129.28 (2C), 128.96 (2C), 127.67, 113.86 (2C), 80.77, 80.49, 79.83, 77.59, 75.90, 73.82, 70.89, 66.35, 56.19, 55.63, 50.25, 42.95, 39.50, 37.86, 34.70, 26.23 (3C), 26.06, 23.01, 21.31, 20.80, 19.67, 18.35, 13.44, 10.97 (2C), -3.66, -4.95; HRMS (ESI) calc'd for $C_{47}H_{71}NO_{11}Si$ + Na = 876.4694, found 876.4694.



16

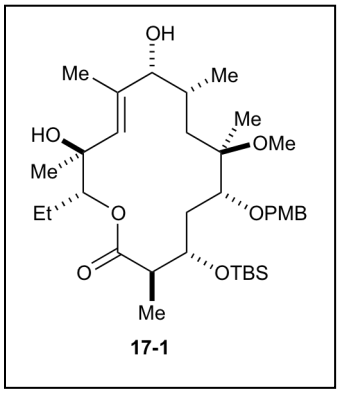
Seco acid 16: 30% H_2O_2 (219 mg, 6.5 mmol) followed by aq. LiOH (1.5 M, 2.42 mmol) were added to a solution of **15** (690 mg, 0.81 mmol) in THF/H_2O (4:1, 8 mL) at 0 °C. The solution was allowed to warm to rt stirring for 48 h. Sat'd aq. Na_2SO_4 (4 mL) and sat'd aq. NH_4Cl (4 mL) were added to the solution and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic fractions were washed with brine (10 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–5% MeOH in CH_2Cl_2 to afford 458 mg (87%) of **16** as a white foam. $[\alpha]^{23}_{D}$ +6.7 (c 1.5, $CHCl_3$); IR (neat) 3428, 2957, 2932, 2856, 1709, 1664, 1515, 1462, 1374, 1250, 1095, 1038, 837, 805, 776 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.51 (d, *J* = 0.9 Hz, 1H), 4.54 (d, *J* = 1.1 Hz, 2H), 4.39 – 4.23 (m, 1H), 3.79 (s, 3H), 3.45 – 3.36 (m, 2H), 3.31 (dd, *J* = 9.5, 2.1 Hz, 1H), 3.13 (s, 3H), 2.64 (dd, *J* = 6.9, 3.5 Hz, 1H), 2.22 (dd, *J* = 14.3, 8.7 Hz, 1H), 2.01 (d, *J* = 0.9 Hz, 3H), 1.93 – 1.81 (m, 1H), 1.59 (ddd, *J* = 16.2, 9.5, 3.9 Hz, 2H), 1.46 – 1.39 (m, 2H), 1.38 (s, 3H), 1.20 (d, *J* = 6.1 Hz, 1H), 1.18 (s, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 0.86 (s, 9H), 0.03 (d, *J* = 14.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.88, 178.96, 159.11, 140.92, 138.04, 130.60, 129.31(2C), 113.73 (2C), 81.57, 80.01, 79.29, 76.24, 74.27, 70.78, 55.24, 50.14, 43.85, 39.23, 36.36, 34.56, 25.77 (3C), 24.95, 24.66, 20.39, 19.22, 17.92, 13.00, 11.09, 9.94, -4.14, -5.05; HRMS (ESI) calc'd for $C_{35}H_{60}NO_9Si$ + Na = 675.3904, found 675.3907.



17

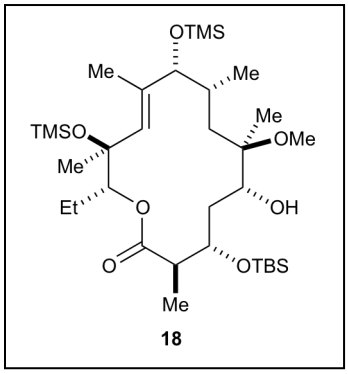
Macrolactone 17: *i*Pr_2NEt (194 mg, 1.5 mmol) and 2,4,6-trichlorobenzoyl chloride (188 mg, 0.8 mmol) were added to an azeotropically dried with toluene (3 x 5 mL) solution of **16** (100 mg, 0.15 mmol) in benzene (15 mL) at rt. After 1 h, an additional amount of DIPEA (194 mg, 1.5 mmol) and 2,4,6-trichlorobenzoyl chloride (376 mg, 1.5 mmol) were added and the solution stirred for 12 h. DMAP (745 mg, 6.1 mmol) was added followed by Benzene (15

mL) and stirred for 1 h. Sat'd aq. NH_4Cl (30 mL) was added and the mixture was extracted with EtOAc (5 x 100 mL). The combined organic fractions were washed with brine (100 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–20% to give 65 mg (65%) of **17** as a white foam. $[\alpha]^{23}_D$ +30.2 (c 1.5, $CHCl_3$); IR (neat) 2929, 2855, 1728, 1667, 1514, 1371, 1249, 1165, 1056, 835, 804, 776, 737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.2 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.53 (d, *J* = 1.0 Hz, 1H), 4.96 (dd, *J* = 10.7, 2.0 Hz, 1H), 4.66 (d, *J* = 5.5 Hz, 2H), 4.11 – 4.03 (m, 1H), 3.79 (s, *J* = 3.7 Hz, 3H), 3.70 (dd, *J* = 10.1, 4.0 Hz, 1H), 3.39 (dd, *J* = 11.9, 7.0 Hz, 1H), 3.19 (s, 3H), 2.68 (dd, *J* = 9.2, 7.3 Hz, 1H), 2.01 (d, *J* = 1.0 Hz, 3H), 1.97 – 1.41 (m, 6H), 1.28 (s, 3H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.13 (s, 3H), 1.11 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.88 (s, 9H), 0.02 (d, *J* = 21.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.40, 176.36, 158.78, 142.30, 138.82, 131.59, 129.05 (2C), 113.47 (2C), 81.13, 80.22, 79.27, 77.21, 73.94, 71.05, 55.25, 49.36, 47.79, 38.61, 37.22, 35.55, 25.96 (3C), 21.65, 21.32, 20.43, 18.69, 18.08, 17.14, 12.83, 10.65, -3.87, -4.94; HRMS (ESI) calc'd for $C_{35}H_{58}NO_9Si$ + Na = 657.3799, found 657.3816.



17-1

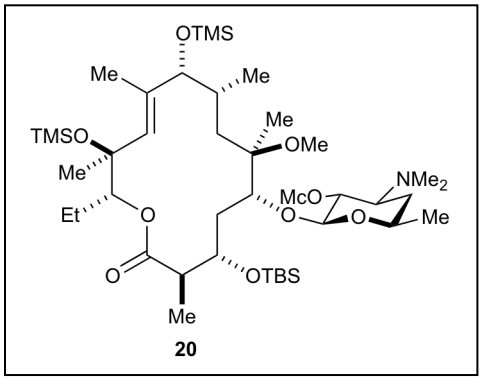
Alcohol 17-1: $CeCl_3$•7 H_2O (105 mg, 0.28 mmol) was added to a solution of **17** (76 mg, 0.12 mmol) in MeOH (2.4 mL) at rt and stirred for 30 min. The solution was cooled to -15 °C and $NaBH_4$ (9.8 mg, 0.26 mmol) was added. The solution was stirred at -15 °C for 15 min and allowed to warm to rt stirring for 30 min. The solution was then diluted with EtOAc (50 mL) and washed with 1M aq. HCl (10 mL), sat'd aq. $NaHCO_3$ (3 x 10 mL) and brine (10 mL). The solution was filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–40% EtOAc in hexanes to afford 72 mg (96%) of **17-1** as a 4.6:1 mixture of separable diastereomers. (The major isomer was taken forward separately for ease of characterization). $[\alpha]^{23}_D$ +14.3 (c 1.5, $CHCl_3$); IR (neat) 2956, 2932, 2856, 1729, 1514, 1463, 1370, 1249, 1171, 1061, 836, 775 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.81 (s, 1H), 4.88 (dd, *J* = 10.4, 2.9 Hz, 1H), 4.69 (d, *J* = 11.0 Hz, 1H), 4.43 (d, *J* = 11.0 Hz, 1H), 3.94 (s, 1H), 3.87 – 3.81 (m, 1H), 3.80 (s, *J* = 7.7 Hz, 3H), 3.59 (dd, *J* = 6.6, 4.4 Hz, 1H), 3.20 (s, 3H), 2.71 (dd, *J* = 8.7, 7.2 Hz, 1H), 2.12 – 2.05 (m, 1H), 1.95 – 1.86 (m, 1H), 1.83 (s, *J* = 0.7 Hz, 3H), 1.83 – 1.78 (m, 1H), 1.77 – 1.69 (m, 1H), 1.68 – 1.57 (m, 2H), 1.40 (s, 3H), 1.27 (s, 3H), 1.18 (d, *J* = 7.1 Hz, 3H), 1.15 (d, *J* = 7.1 Hz, 3H), 1.05 – 0.95 (m, 1H), 0.96 – 0.87 (m, 12H), 0.09 (d, *J* = 4.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.69, 158.74, 142.12, 131.61, 128.69 (2C), 125.62, 113.52 (2C), 83.10, 81.10, 80.73, 79.23, 74.25, 72.46, 71.52, 55.25, 50.90, 48.18, 39.63, 33.67, 30.03, 26.14 (3C), 24.65, 23.42, 21.02, 19.58, 18.26, 16.25, 15.58, 10.84, -3.34, -4.29; HRMS (ESI) calc'd for $C_{35}H_{60}NO_8Si$ + Na = 659.3955, found 659.3949.



18

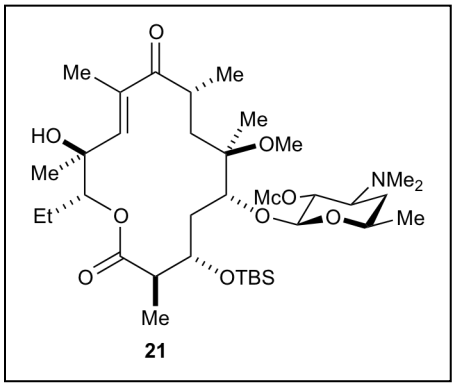
Macrocyclic acceptor 18: TMSOTf (33 mg, 0.15 mmol) was added to a solution of **17-1** (32 mg, 0.05 mmol) and 2,6-lutidine (21 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) at -78 °C. The solution was stirred for 30 min and sat'd aq. $NaHCO_3$ (1 mL). The mixture was extracted with CH_2Cl_2 (3 x 5 mL) and the combined organic fractions were washed with brine (1 mL), filtered over Na_2SO_4 and the solvent

removed under reduced pressure. The product was dissolved in CH_2Cl_2:H_2O (8:1, 1.1 mL) and cooled to 0 °C. DDQ (23 mg, 0.1 mmol) was added and the solution stirred for 30 min. Sat'd aq. $NaHCO_3$ (1 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–10% EtOAc in hexanes to afford 25 mg (76%) of **18** as a white foam. $[\alpha]^{23}_D$ +53.2 (c 1.5, $CHCl_3$); IR (neat) 2957, 2856, 1731, 1250, 1096, 1065, 1047, 864, 838, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.41 (s, 1H), 4.82 (dd, *J* = 8.3, 3.6 Hz, 1H), 4.01 – 3.91 (m, 1H), 3.86 (d, *J* = 9.9 Hz, 1H), 3.78 (s, 1H), 3.14 (s, 3H), 2.56 (dd, *J* = 9.0, 7.2 Hz, 1H), 2.21 (s, 1H), 1.92 – 1.78 (m, 2H), 1.75 (s, 3H), 1.73 – 1.61 (m, 2H), 1.55 (ddd, *J* = 14.9, 10.2, 2.7 Hz, 1H), 1.47 – 1.40 (m, 1H), 1.39 (s, 3H), 1.35 – 1.26 (m, 1H), 1.24 (d, *J* = 7.3 Hz, 3H), 1.09 – 1.05 (m, 6H), 0.93 (t, *J* = 7.5 Hz, 3H), 0.89 (s, 9H), 0.14 (s, 3H), 0.10 (s, 12H), 0.08 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.37, 139.06, 128.80, 82.14, 81.43, 78.42, 76.54, 72.80, 72.00, 49.86, 49.41, 39.84, 31.97, 31.64, 25.91 (3C), 24.28, 23.56, 20.05, 19.52, 17.99, 17.00, 15.33, 11.57, 2.29 (3C), 0.39 (3C), -4.18, -4.71; HRMS (ESI) calc'd for $C_{33}H_{68}O_7Si_3$ + Na = 683.4171, found 683.4172.



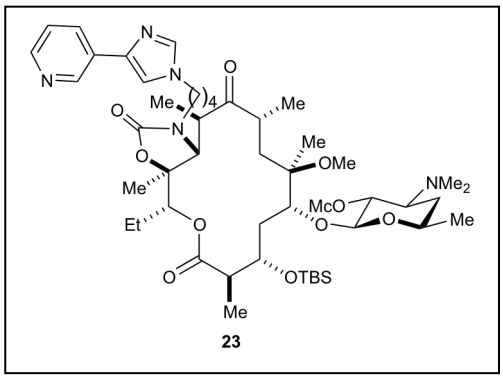
20

Macrocyclic glycoside 20: An azeotropically dried with toluene (3 x 3 mL) solution of **18** (45 mg, 0.07 mmol), desosamine thiopyrimidine donor (134 mg, 0.41 mmol) and 2,6-DTBMP (84 mg, 0.41 mmol) in CH_2Cl_2 (2 mL) was cannulated into a suspension of activated 4 Å molecular sieves and AgOTf (349 mg, 1.36 mmol) in CH_2Cl_2/toluene (4 mL, 1:1) at 0 °C. The mixture was allowed to warm to rt stirring for 12 h. Et_3N (3 mL) was added and stirred for 30 min before filtering over Celite and washing with EtOAc (25 mL). The organic fraction was then washed with sat'd aq. $NaHCO_3$ (3 x 5 mL) and brine (5 mL). The solution was filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–5% MeOH in CH_2Cl_2 to give 40 mg (70%) of **20** as a colorless oil. $[\alpha]^{23}_D$ -12.9 (c 1.5, $CHCl_3$); IR (neat) 2956, 2857, 1756, 1733, 1261, 1163, 1095, 1073, 1052, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.48 (s, 1H), 5.00 (dd, *J* = 9.1, 2.6 Hz, 1H), 4.53 (dd, *J* = 10.5, 7.6 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 4.11 (s, 1H), 3.80 (d, *J* = 6.4 Hz, 1H), 3.74 (s, 4H), 3.52 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.16 (s, 3H), 2.74 (s, 1H), 2.54 – 2.40 (m, 1H), 2.30 (s, 6H), 1.98 (dd, *J* = 15.1, 7.7 Hz, 1H), 1.92 – 1.76 (m, 2H), 1.74 (s, 3H), 1.44 (d, *J* = 14.0 Hz, 4H), 1.37 (s, 3H), 1.28 – 1.20 (m, 8H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.99 – 0.85 (m, 15H), 0.13 (s, 6H), 0.09 (d, *J* = 3.7 Hz, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.87, 155.13, 141.17, 130.60, 98.52, 81.65, 80.39, 78.65, 77.18, 75.87, 75.31, 71.86, 68.66, 62.64, 54.71, 50.57, 48.63, 40.71, 39.36, 34.16, 32.61, 31.24, 29.69, 26.39 (3C), 23.56, 22.75, 20.96, 20.14, 19.65, 18.43, 16.31, 15.45, 11.18, 2.37 (3C), 0.47 (3C), -2.95, -3.83; HRMS (ESI) calc'd for $C_{43}H_{85}NO_{11}Si_3$ + H = 876.5509, found 876.5514.



21

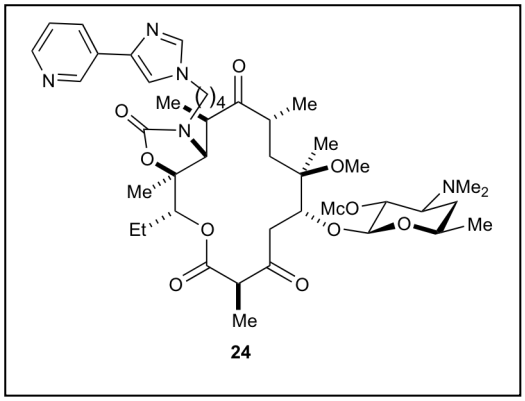
Enone 21: Pyridine (138 mg, 1.75 mmol) followed by 70% HF•pyridine (57 mg, 2.8 mmol) were added to a solution of **20** (30 mg, 0.03 mmol) in THF (1.2 mL) at 0 °C. The solution was allowed to warm to 15 °C stirring for 3 h. The solution was then cooled back to 0 °C and sat'd aq. $NaHCO_3$ was added drop wise until HF quenched. The mixture was then extracted with EtOAc (3 x 5 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was then dissolved in CH_2Cl_2 (0.3 mL) and Dess-Martin periodinane (28 mg, 0.07 mmol) was added and the solution stirred at rt for 3 h. Sat'd aq. $NaHCO_3$ (1 mL), sat'd aq. Na_2SO_3 (1 mL) and H_2O (1 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 10 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–5% MeOH in CH_2Cl_2 to give 16 mg (67%) of **21** as a white foam. $[\alpha]^{23}_D$ +1.6 (c 1.5, $CHCl_3$); IR (neat) 1756, 1732, 1670, 1457, 1441, 1372, 1293, 1265, 1161, 1055, 995, 836, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.46 (d, *J* = 1.0 Hz, 1H), 4.99 (dd, *J* = 10.4, 2.1 Hz, 1H), 4.54 – 4.44 (m, 2H), 4.06 (s, 1H), 3.71 (s, 3H), 3.70 – 3.66 (m, 1H), 3.61 (s, 1H), 3.45 (d, *J* = 5.3 Hz, 1H), 3.22 (s, 3H), 2.82 – 2.69 (m, 1H), 2.46 – 2.31 (m, 1H), 2.28 (s, 6H), 2.00 (d, *J* = 0.8 Hz, 3H), 1.98 – 1.88 (m, 2H), 1.85 – 1.77 (m, 1H), 1.76 – 1.70 (m, 2H), 1.62 – 1.49 (m, 2H), 1.44 (s, 3H), 1.32 (dd, *J* = 23.9, 12.5 Hz, 1H), 1.27 – 1.20 (m, 7H), 1.10 (s, 3H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.96 – 0.88 (m, 12H), 0.13 (d, *J* = 4.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.94, 176.04, 155.20, 141.83, 140.37, 98.54, 79.55, 78.11, 77.21, 76.31, 75.13, 73.58, 71.26, 68.60, 63.05, 54.55, 50.70, 48.05, 40.66 (2C), 38.07, 35.62, 30.60, 26.14 (3C), 23.43, 21.68, 21.09, 20.71, 20.21, 18.23, 16.91, 13.36, 10.77, -3.41, -4.24; HRMS (ESI) calc'd for $C_{37}H_{68}NO_7Si$ + H = 730.4562, found 730.4563.



23

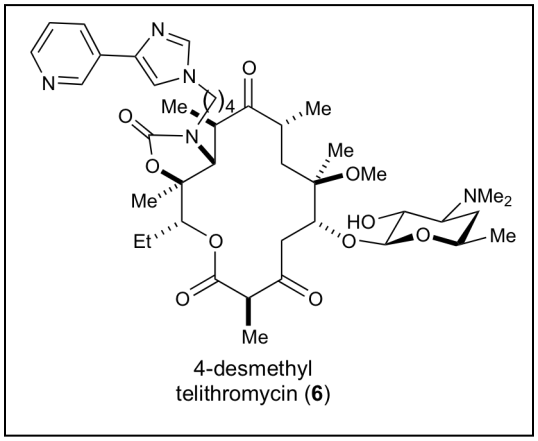
Oxazolidinone 23: 60% NaH in oil (7.2 mg, 0.18 mmol) was added to a solution of **21** (31 mg, 0.04 mmol) and CDI (73 mg, 0.45 mmol) in DMF/THF (0.35 mL, 10:1) at -20 °C. The solution was stirred for 45 min while warming to 0 °C. Sat'd aq. $NaHCO_3$ (2 mL) was added dropwise and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic fractions were washed with NH_4OH (2 x 5 mL) and brine (5 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was dissolved in MeCN/H_2O (1 mL, 9:1). Amine **22** (42 mg, 0.21 mmol) was added and the solution stirred at rt for 72 h. The solvent was then removed under reduced pressure and the product purified by flash column chromatography eluting with 0–5% MeOH in CH_2Cl_2 to give 25 mg (61%) of **23** as yellow foam. $[\alpha]^{23}_D$ -8.8 (c 1.5, $CHCl_3$); IR (neat) 3117, 2929, 2853, 1751, 1457, 1264, 1166, 1061, 836, 776, 665 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.95 (d, *J* = 2.2 Hz, 1H), 8.44 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.08 – 8.02 (m, 1H), 7.56 (d, *J* = 1.1 Hz, 1H), 7.34 (d, *J* = 1.1 Hz, 1H), 7.31 – 7.26 (m, 1H), 4.94 (dd, *J* = 11.0, 1.9

Hz, 1H), 4.50 (dd, *J* = 10.7, 7.5 Hz, 1H), 4.35 (d, *J* = 7.5 Hz, 1H), 4.04 (t, *J* = 7.4 Hz, 2H), 3.76 (s, 3H), 3.74 – 3.58 (m, 4H), 3.52 (dd, *J* = 10.0, 5.4 Hz, 1H), 3.13 – 3.08 (m, 1H), 2.94 (s, 3H), 2.71 (td, *J* = 12.1, 4.3 Hz, 1H), 2.61 – 2.54 (m, 1H), 2.50 (dd, *J* = 9.4, 7.2 Hz, 1H), 2.27 (d, *J* = 9.2 Hz, 6H), 1.94 – 1.82 (m, 4H), 1.78 – 1.65 (m, 3H), 1.63 – 1.49 (m, 3H), 1.44 (d, *J* = 6.5 Hz, 3H), 1.30 (s, 3H), 1.22 (dd, *J* = 12.6, 6.5 Hz, 6H), 1.16 (t, *J* = 7.8 Hz, 3H), 1.12 (d, *J* = 7.1 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.94 (s, 9H), 0.80 (t, *J* = 7.3 Hz, 3H), 0.11 (d, *J* = 14.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.02, 176.58, 157.50, 155.23, 147.49, 146.21, 138.94, 137.81, 132.08, 130.27, 123.61, 115.59, 98.28, 82.76, 78.15, 77.64, 76.35, 75.13, 71.42, 68.84, 62.74, 59.96, 54.67, 50.76, 48.58, 46.86, 45.00, 42.49, 40.54 (3C), 39.96, 39.18, 38.31, 30.32, 28.64, 26.32 (3C), 24.21, 21.91, 20.94, 19.76, 18.84, 18.33, 16.20, 14.18, 13.78, 10.39, -2.93, -3.52; HRMS (ESI) calc'd for $C_{50}H_{81}N_5O_{12}Si$ + H = 972.5279, found 972.5738.



24

Macroketolactone 24: Tris(dimethylamino)sulfonium difluorotrimethylsilicate (36 mg, 0.13 mmol) in DMF (130 μL) was cannulated into a solution of **23** (25 mg, 0.03 mmol) in DMF/H_2O (65:1, 325 μL) at rt and stirred for 14 h. The solution was then diluted with EtOAc (10 mL) and washed with pH 7 phosphate buffer (2 x 2 mL), brine (2 mL), and filtered over Na_2SO_4 and the solvent removed under reduced pressure. The crude alcohol was azeotropically dried with toluene (3 x 2 mL) and used directly in the next step. Me_2S (12.4 mg, 0.2 mmol) was added to a solution of NCS (16 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) at 0 °C and stirred for 5 min before cooling to -20 °C. The crude alcohol in CH_2Cl_2 (1 mL) was cannulated into the solution and stirred for 1.5 h. Et_3N (29 mg, 0.29 mmol) was added and the solution allowed to warm to rt. Sat'd aq. $NaHCO_3$ (3 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with H_2O (3 mL) and brine (3 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0–5% MeOH in CH_2Cl_2 to give 12 mg (53%) of **24**. $[\alpha]^{23}_D$ +3.0 (c 0.97, $CHCl_3$); IR (neat) 2925, 1744, 1714, 1456, 1375, 1264, 1174, 1106, 1052, 1000, 734, 631 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.44 (d, *J* = 3.9 Hz, 1H), 8.12 – 8.03 (m, 1H), 7.54 (s, 1H), 7.33 (s, 1H), 7.31 – 7.27 (m, 1H), 4.95 (dd, *J* = 10.5, 2.3 Hz, 1H), 4.49 (dd, *J* = 10.5, 7.7 Hz, 1H), 4.37 (d, *J* = 8.1 Hz, 1H), 4.24 (d, *J* = 7.6 Hz, 1H), 4.00 (t, *J* = 7.4 Hz, 2H), 3.81 (s, 3H), 3.78 – 3.61 (m, 4H), 3.59 – 3.51 (m, 2H), 3.16 – 3.06 (m, 2H), 2.76 – 2.68 (m, 1H), 2.64 (s, 3H), 2.61 – 2.52 (m, 1H), 2.28 (s, 6H), 2.00 – 1.92 (m, 1H), 1.91 – 1.82 (m, 2H), 1.76 (dd, *J* = 12.3, 3.4 Hz, 1H), 1.67 – 1.52 (m, 6H), 1.51 (s, 3H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.31 (s, 3H), 1.26 (d, *J* = 6.2 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 215.67, 199.33, 169.71, 157.22, 155.14, 147.58, 146.48, 139.20, 137.78, 132.02, 130.33, 123.46, 115.47, 100.58, 82.28, 77.76, 77.50, 75.00, 69.48, 63.21, 60.34, 54.79, 53.32, 50.07, 46.84, 45.10, 43.41, 42.65, 40.58 (2C), 39.04, 38.69, 31.90, 30.45, 28.61, 24.39, 22.45, 20.95, 19.28, 18.53, 14.61, 14.09, 13.97, 10.54; HRMS (ESI) calc'd for $C_{44}H_{65}N_5O_{12}$ + H = 856.4708, found 856.4712.



(−)-4-desmethyl telithromycin (6): Macroketolactone **24** (12.1 mg, 0.014 mmol) dissolved in MeOH (2.8 mL) was stirred at rt for 10 h. The solvent was removed under reduced pressure and the product was purified by flash column chromatography eluting with 0–10% MeOH in CH_2Cl_2 to give 7.5 mg (67%) of **6**. $[\alpha]^{23}_D$ -3.5 (c 0.23, $CHCl_3$); IR (neat) 3649, 2934, 2361, 1748, 1717, 1540, 1521, 1472, 1375, 1286, 1234, 1175, 1108, 1075, 668 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 1.7 Hz, 1H), 8.45 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.10 – 8.03 (m, 1H), 7.54 (d, *J* = 1.2 Hz, 1H), 7.34 (d, *J* = 1.2 Hz, 1H), 7.29 (dd, *J* = 8.2, 5.1 Hz, 1H), 4.96 (dd, *J* = 10.5, 2.3 Hz, 1H), 4.43 (d, *J* = 8.1 Hz, 1H), 4.17 (d, *J* = 7.4 Hz, 1H), 4.01 (t, *J* = 7.3 Hz, 2H), 3.82 – 3.50 (m, 5H), 3.28 (dd, *J* = 18.3, 9.2 Hz, 1H), 3.19 – 3.10 (m, 2H), 2.67 (s, 3H), 2.59 (dd, *J* = 12.5, 5.6 Hz, 1H), 2.54 – 2.39 (m, 2H), 2.27 (s, 6H), 2.02 – 1.79 (m, 5H), 1.76 – 1.59 (m, 5H), 1.50 (s, 3H), 1.33 (d, *J* = 6.3 Hz, 6H), 1.26 (d, *J* = 8.4 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.02 (d, *J* = 6.9 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 215.93, 199.68, 169.78, 157.30, 147.58, 146.41, 139.08, 137.77, 131.99, 130.26, 123.49, 115.49, 103.38, 82.34, 78.27, 77.62, 69.84, 69.27, 65.87, 60.14, 53.25, 50.16, 46.84, 45.24, 44.37, 42.54, 40.22 (2C), 38.99, 38.83, 29.68, 28.63, 28.10, 24.31, 22.32, 21.22, 19.30, 18.54, 14.49, 14.13, 14.00, 10.56; HRMS (ESI) calc'd for $C_{42}H_{63}N_5O_{10}$ + H = 798.4653, found 798.4654.

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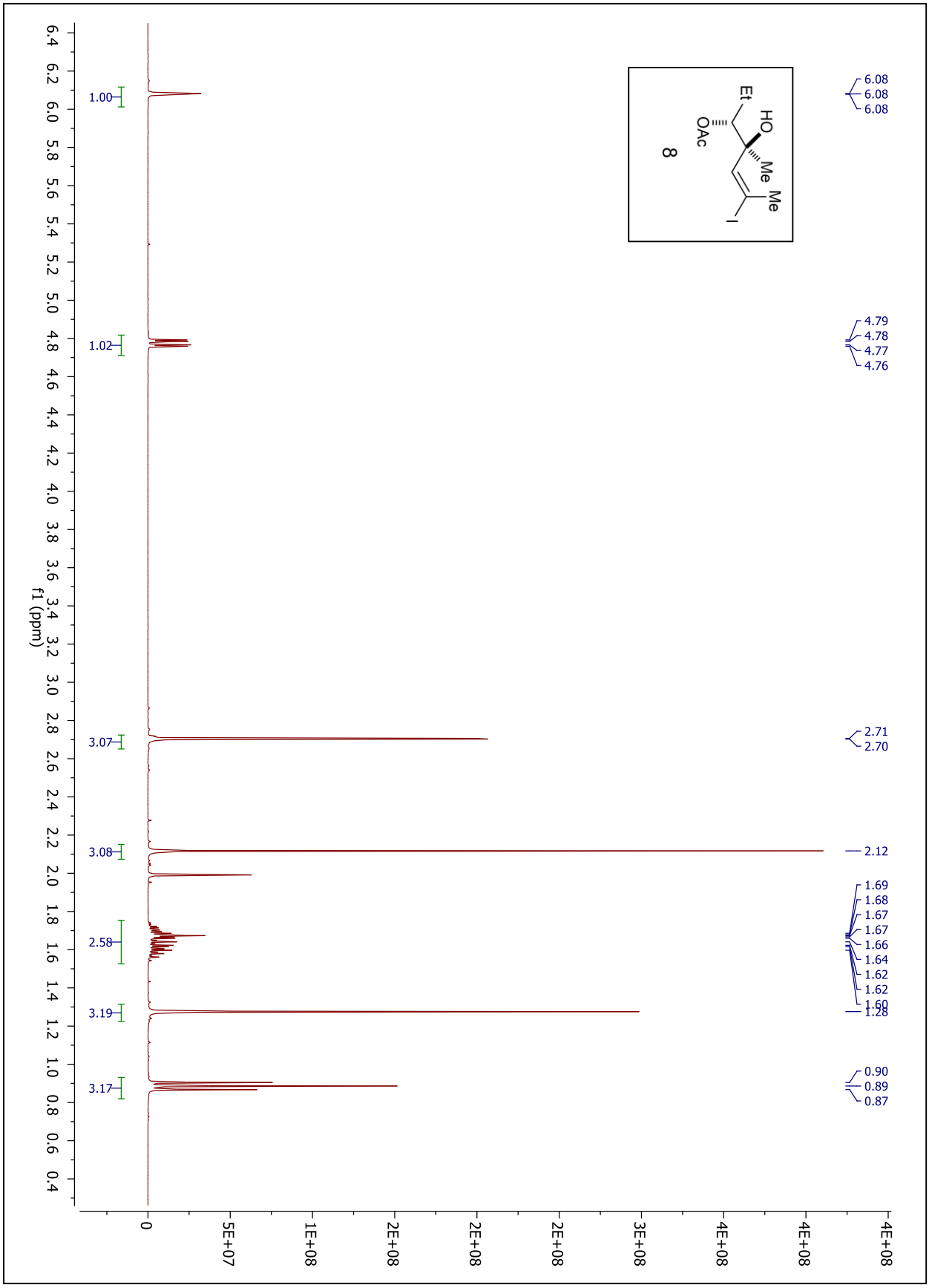
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2.12
1.69
1.68
1.67
1.67
1.66
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1.62
1.60
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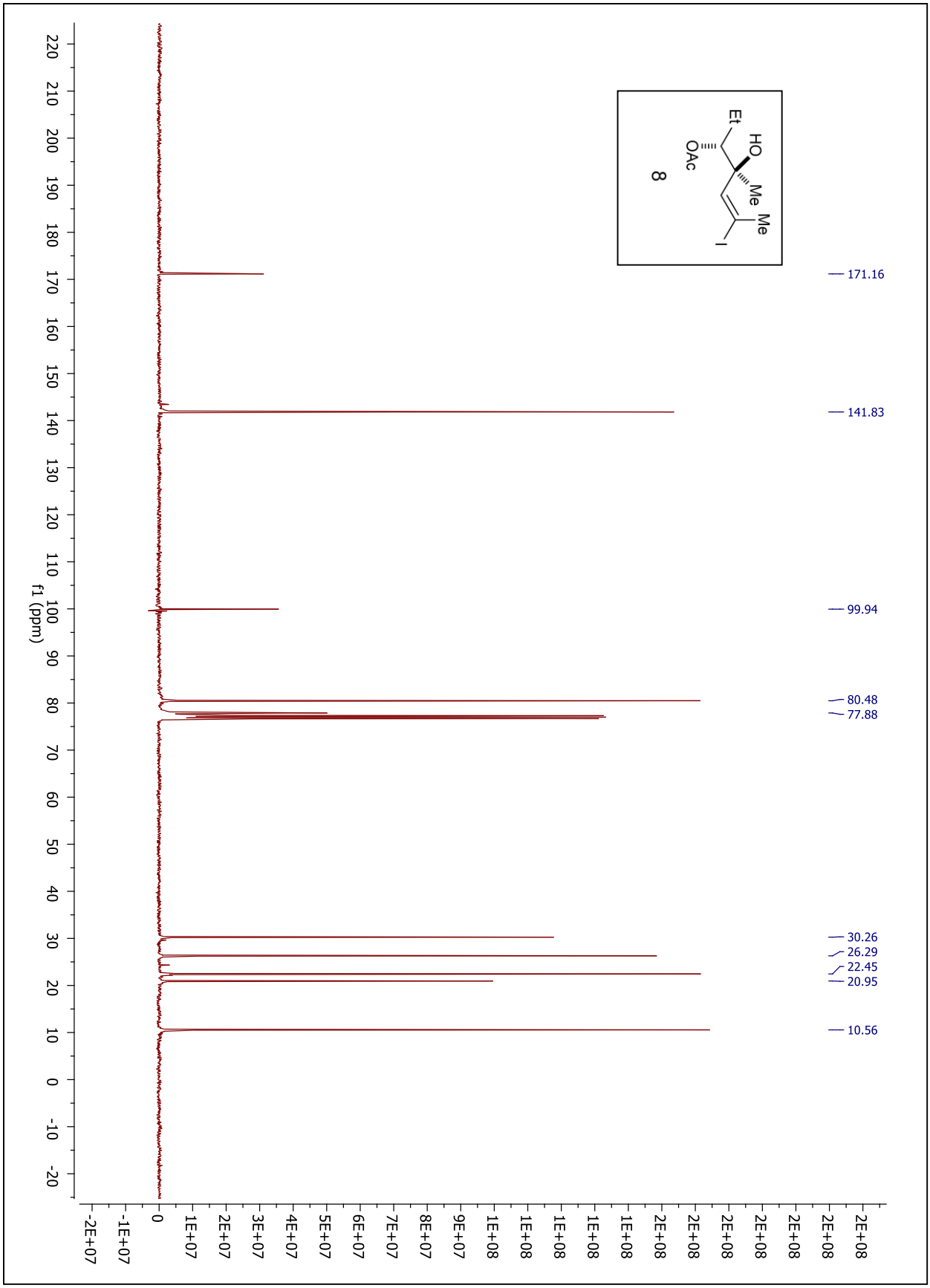
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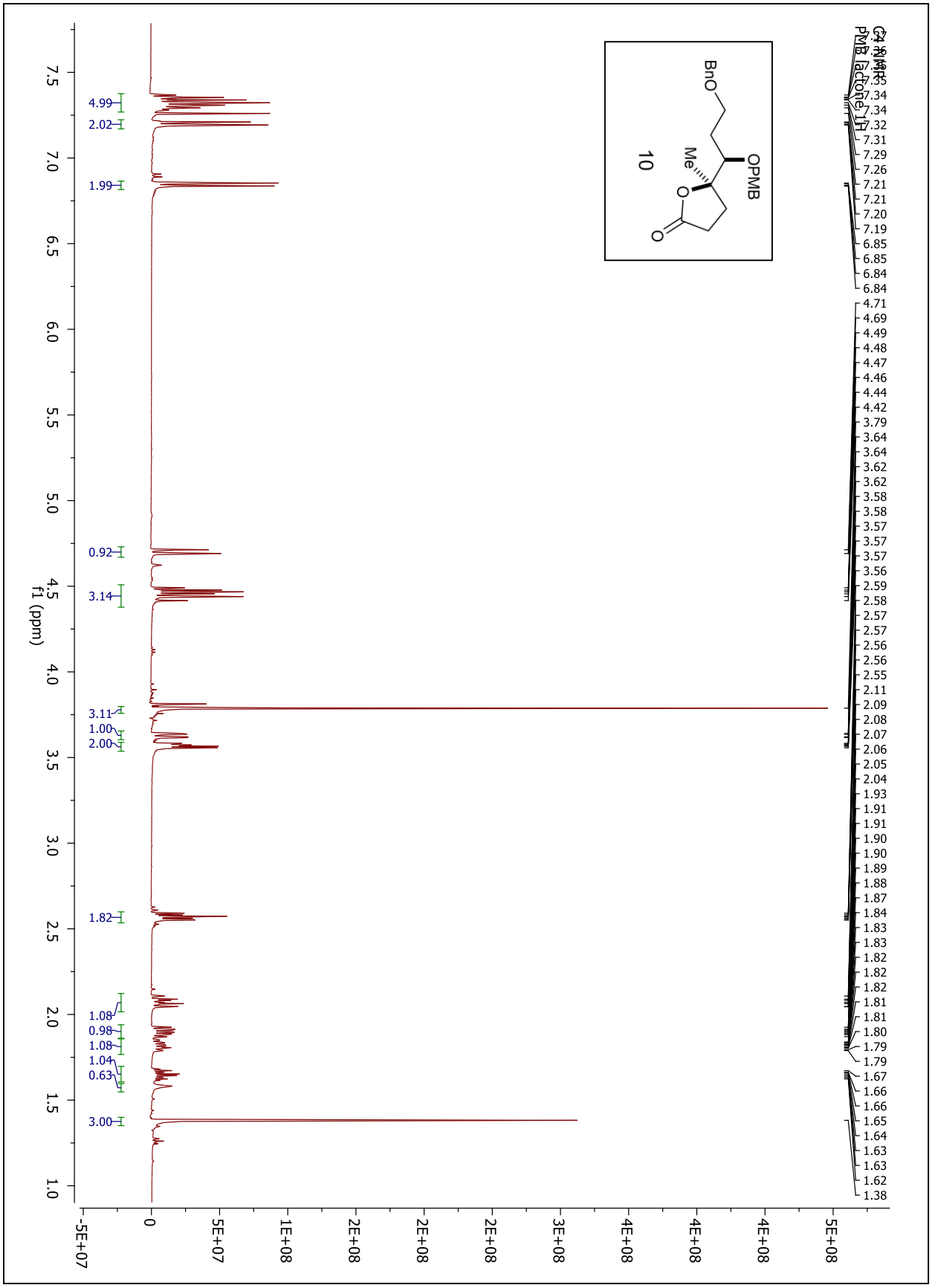
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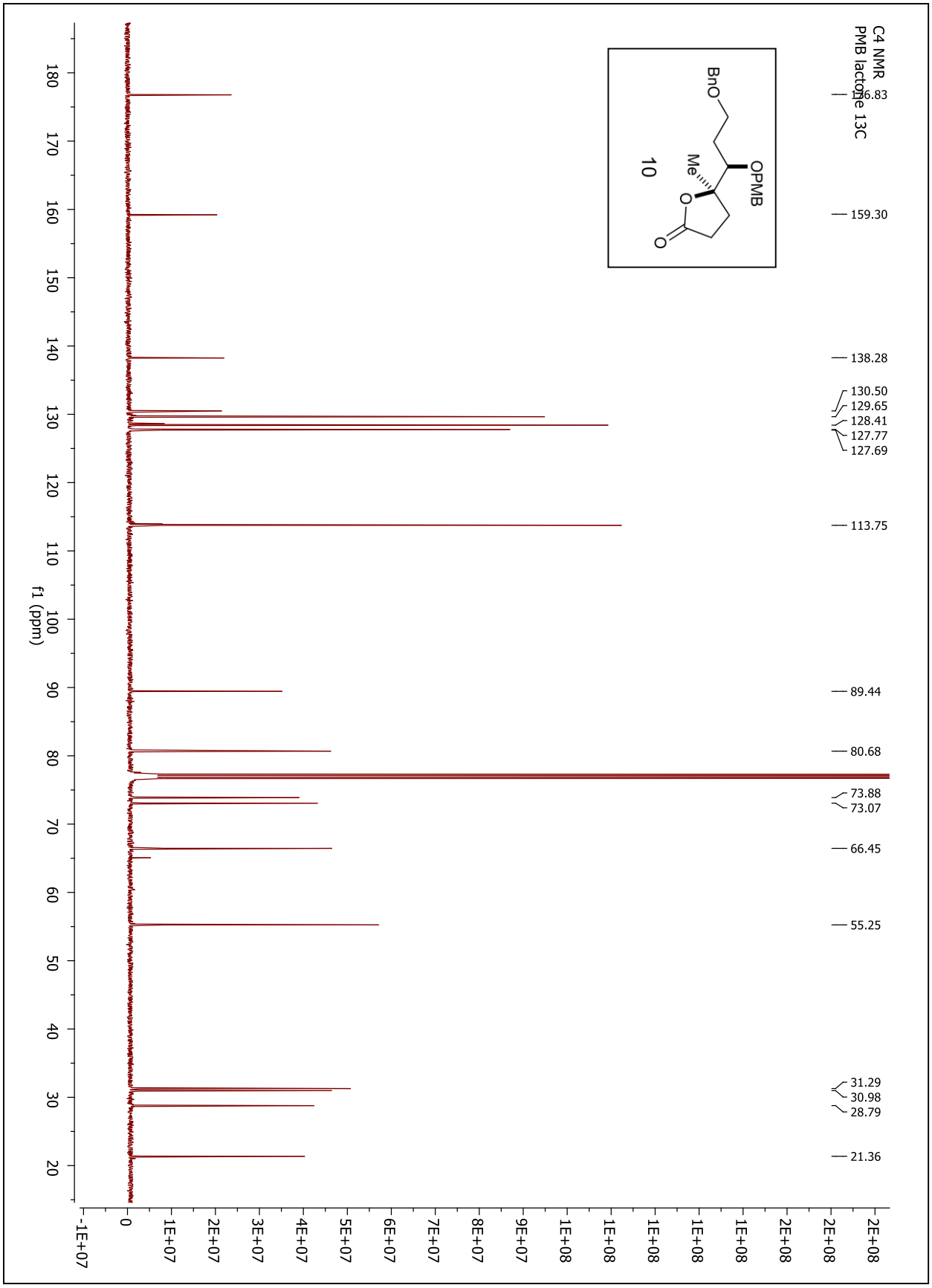
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PMB lactone 13C
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128.41
127.77
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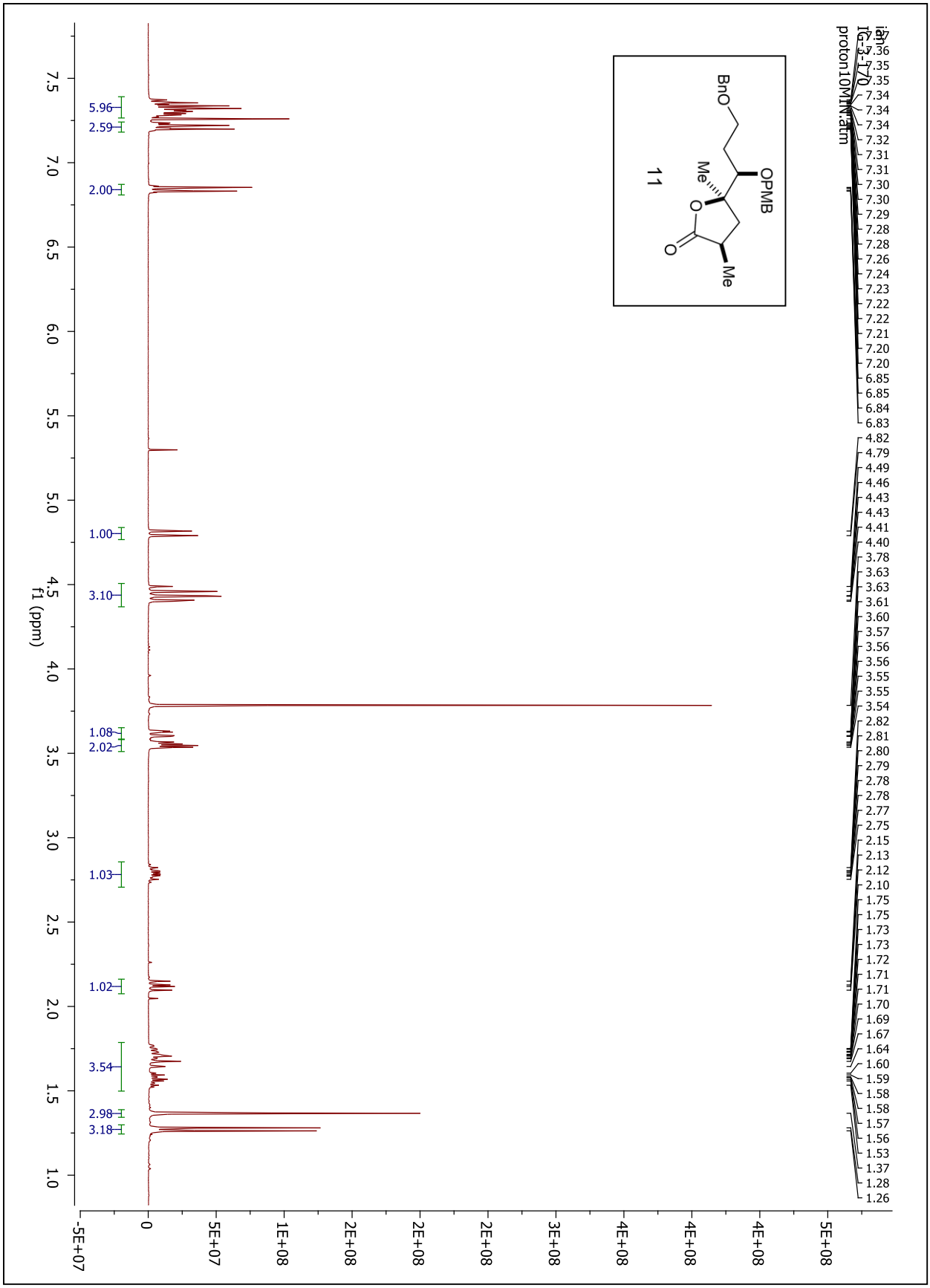
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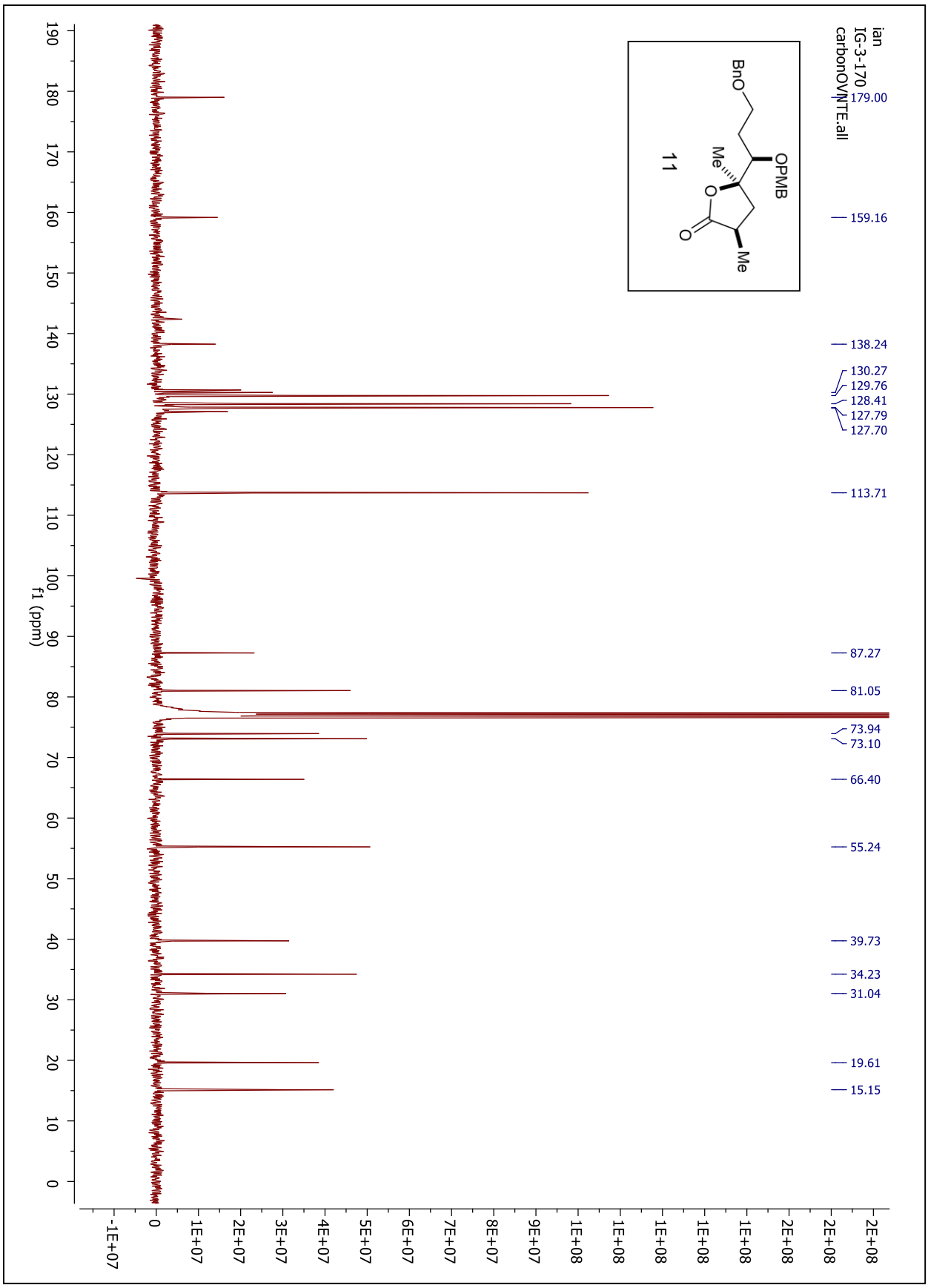
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73.10
66.40
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O
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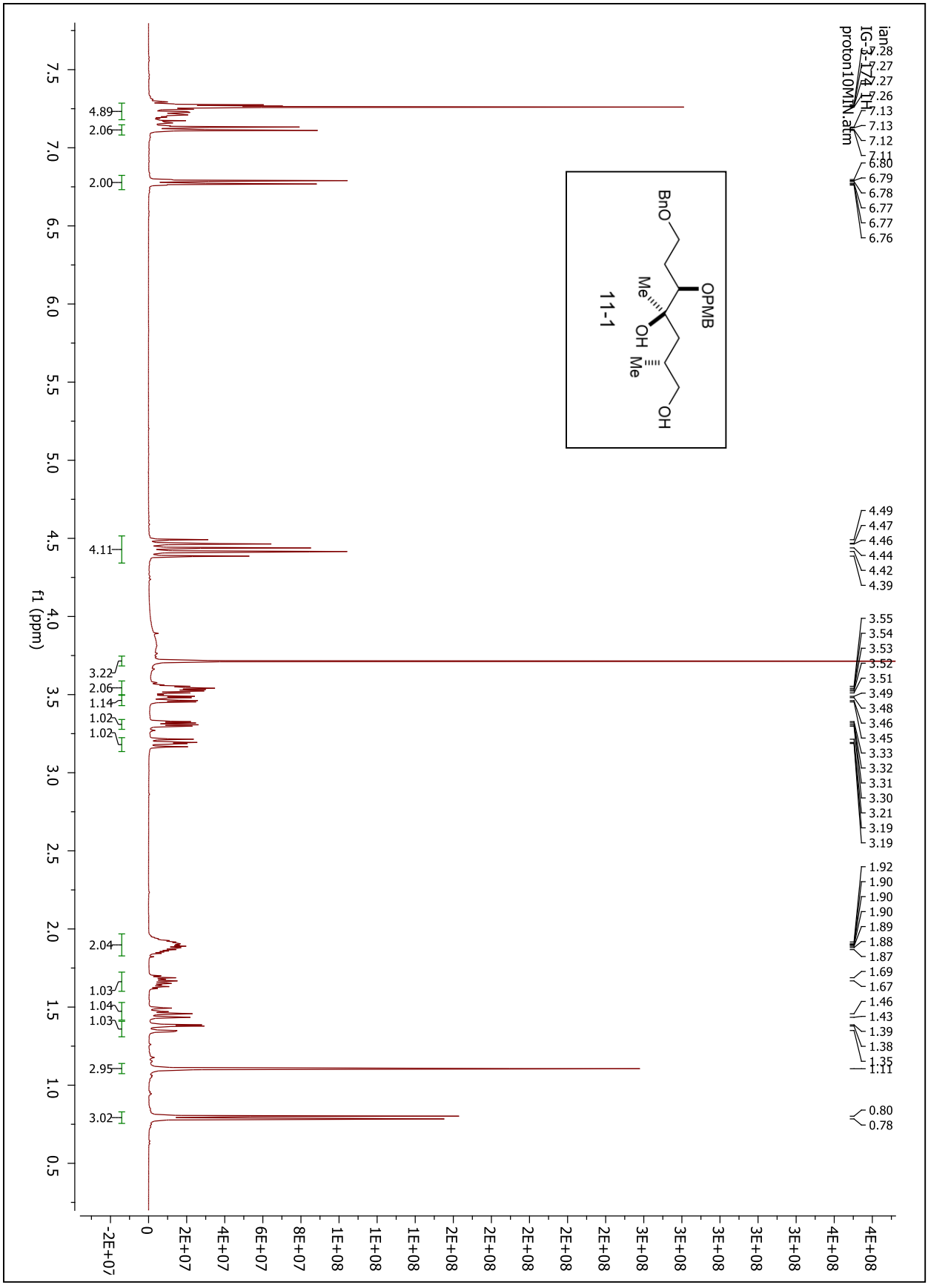
f1 (ppm)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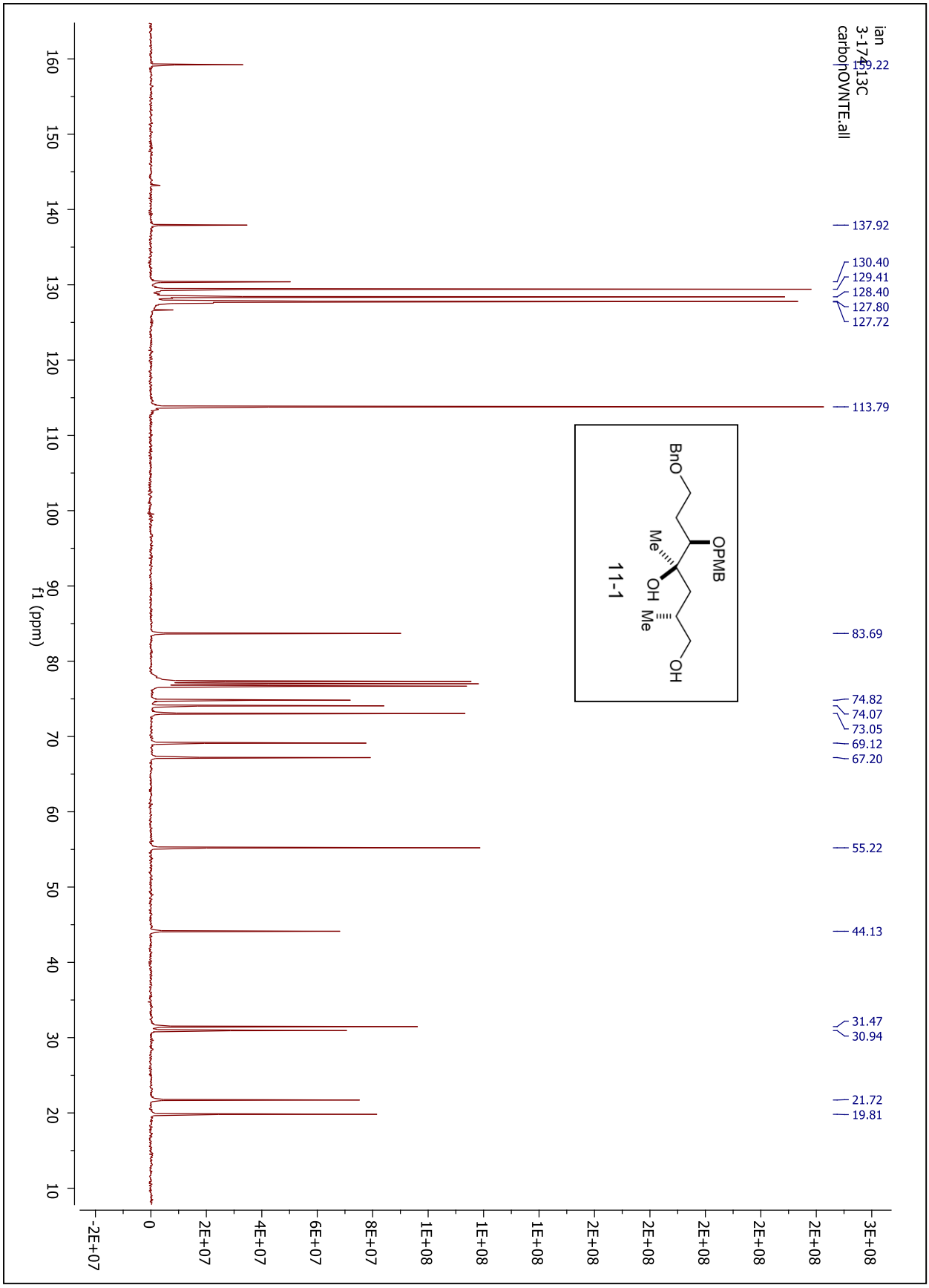
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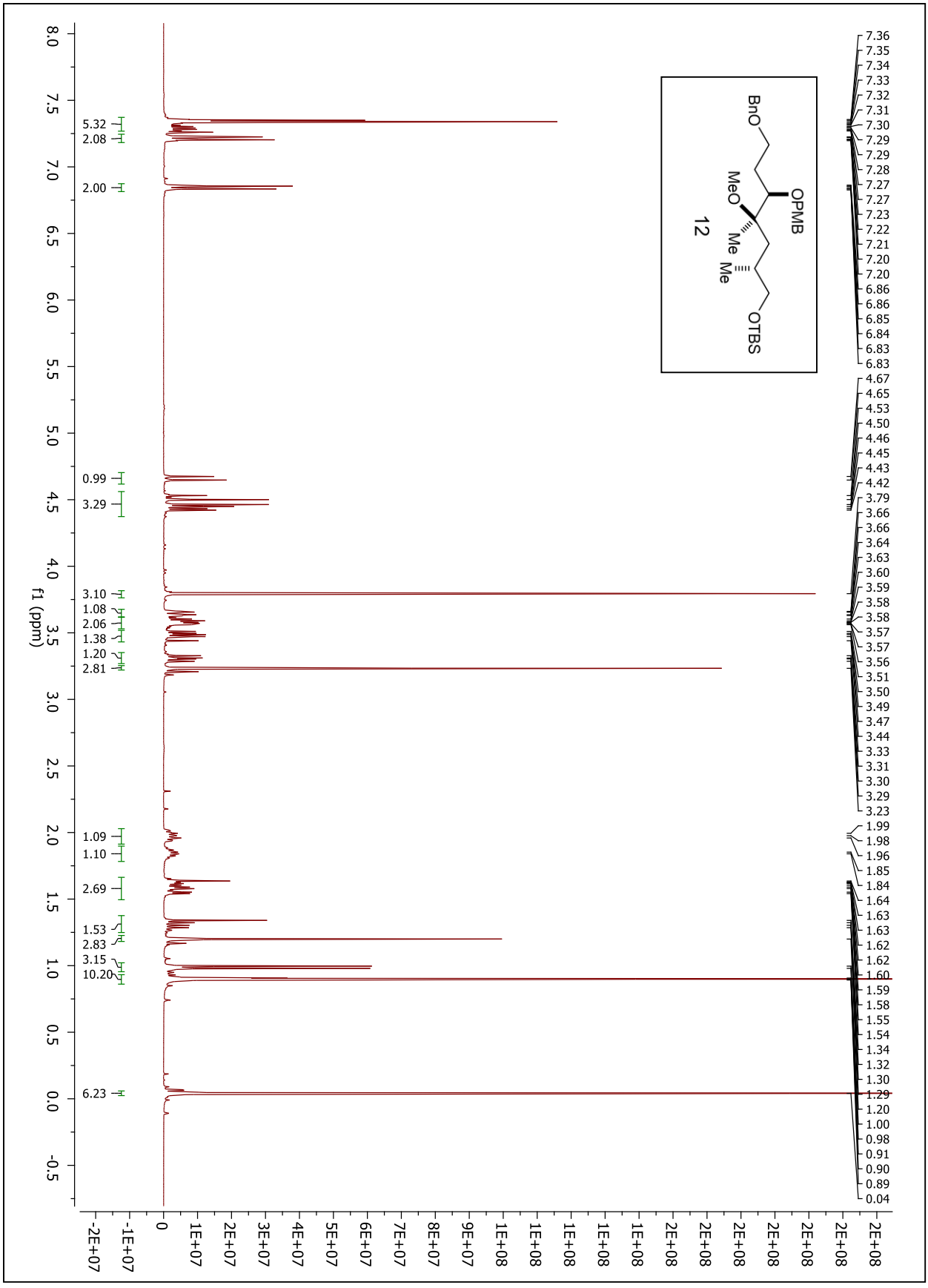
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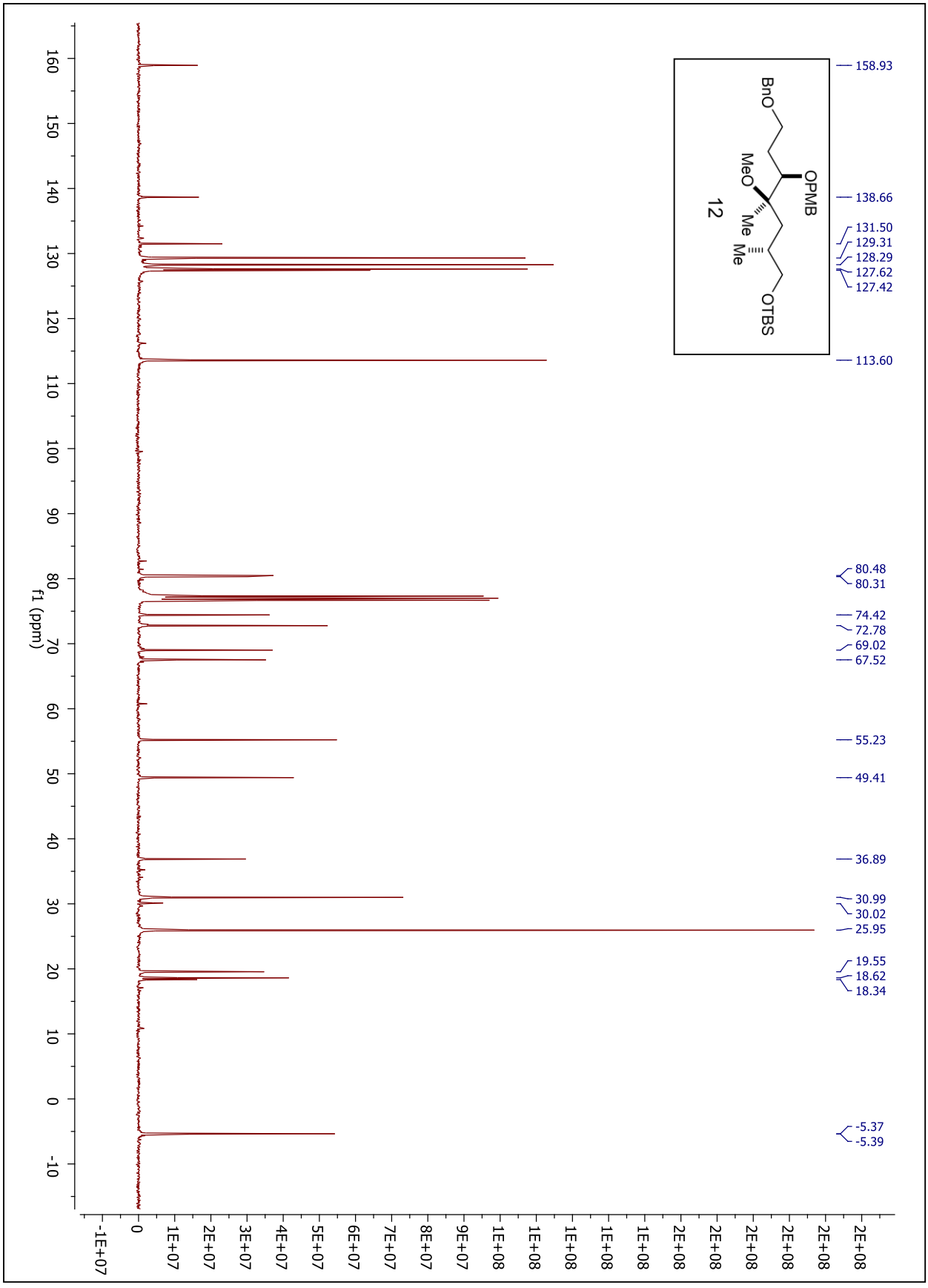
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69.02
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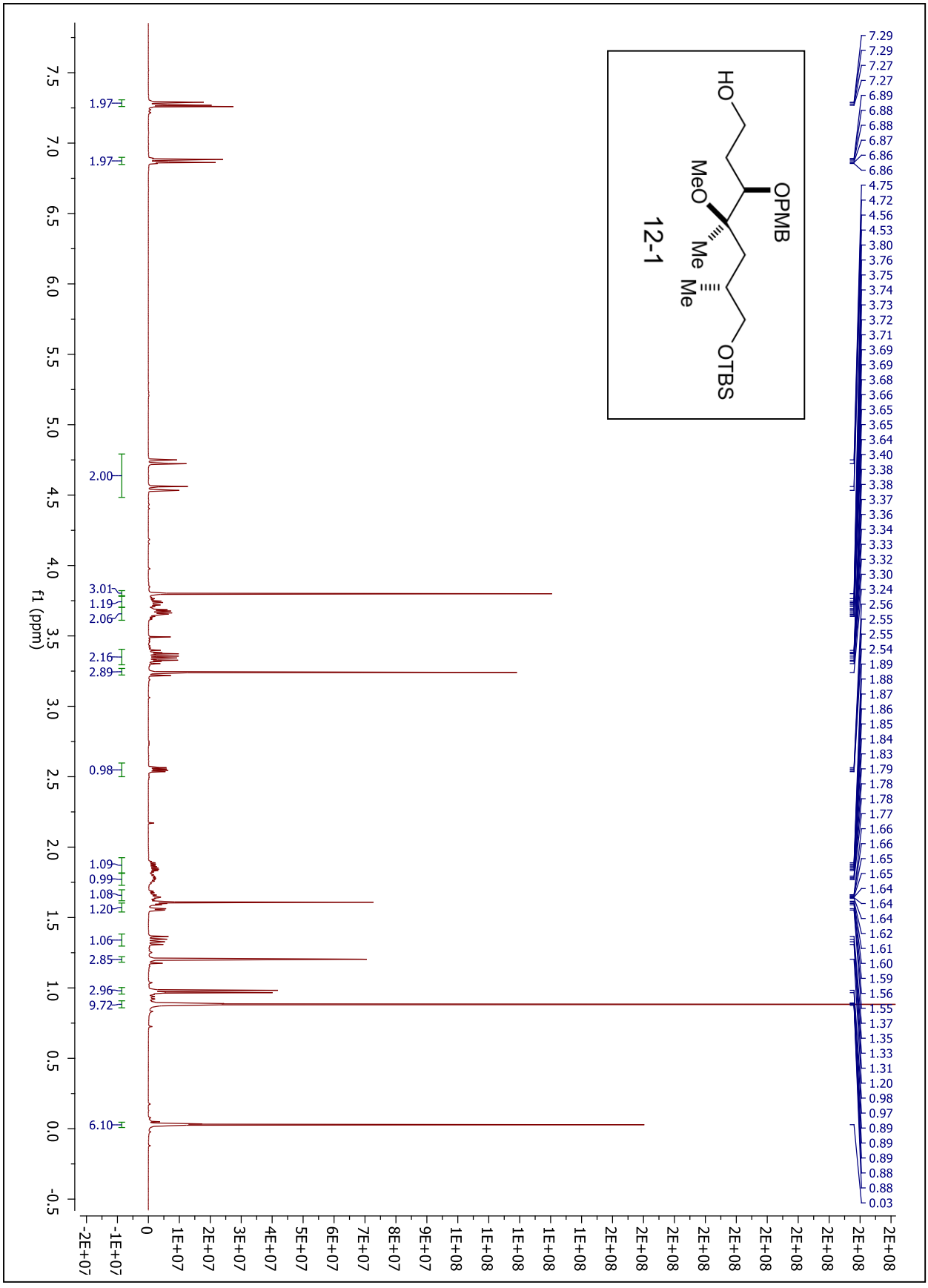
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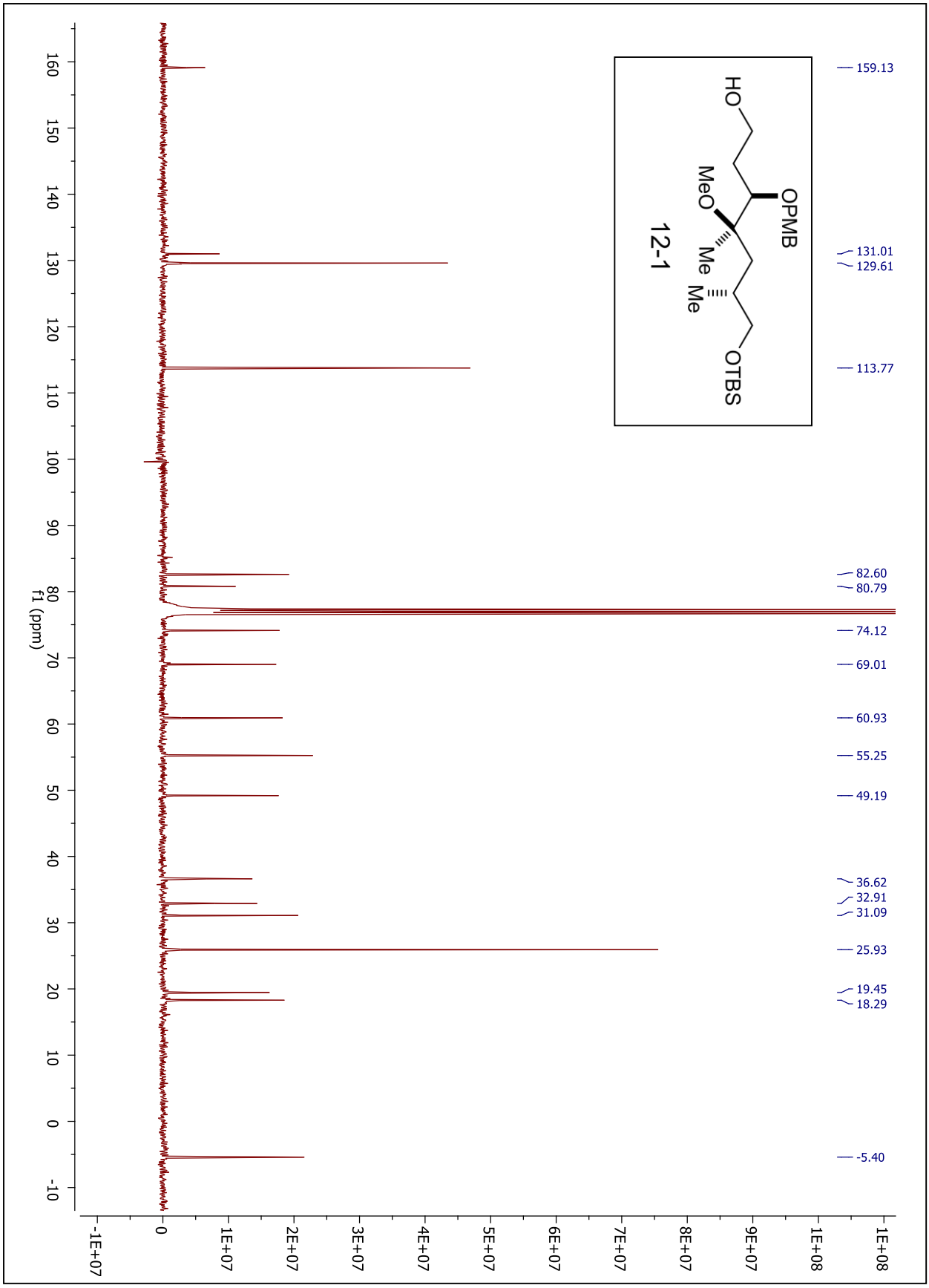
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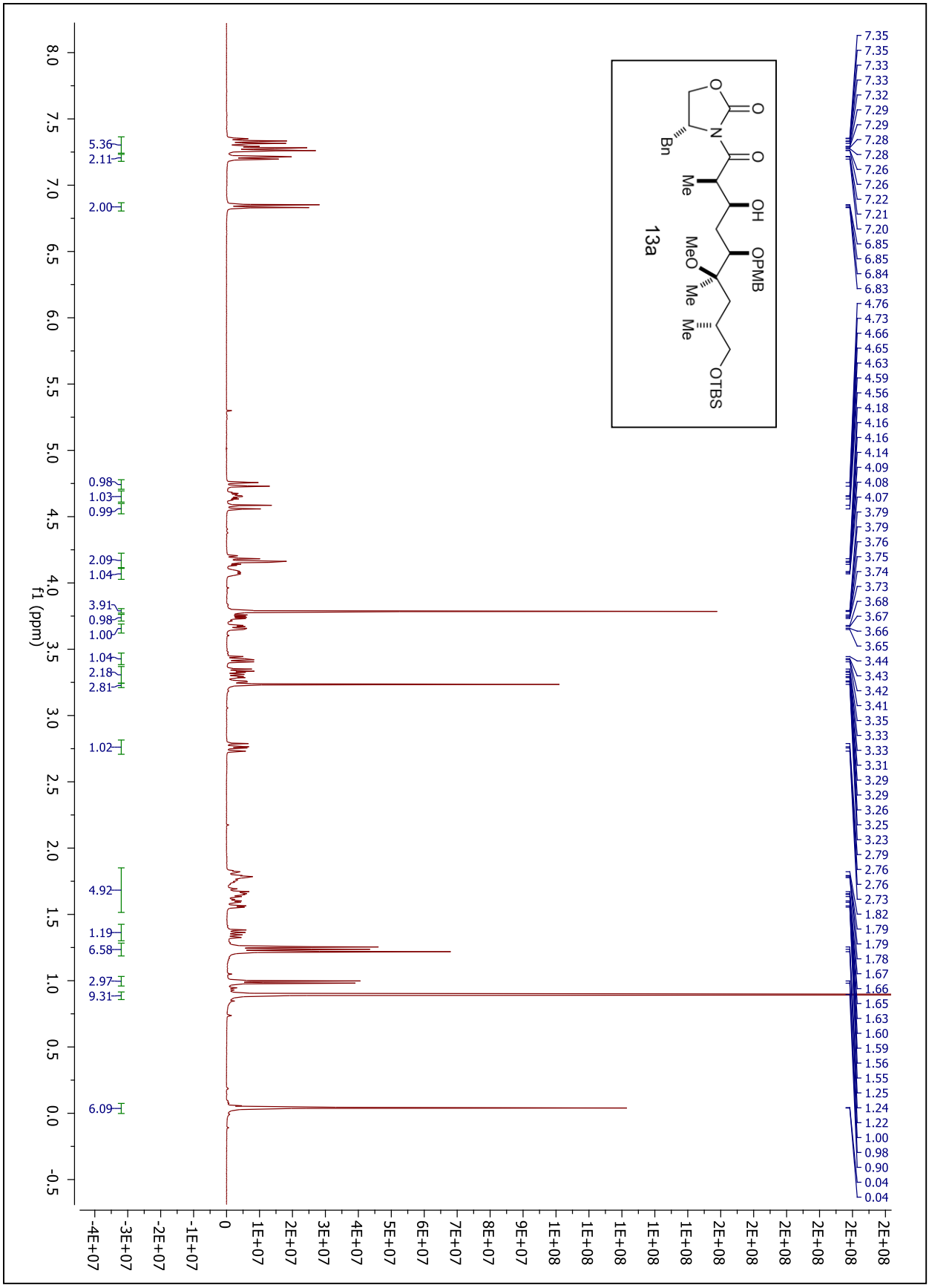
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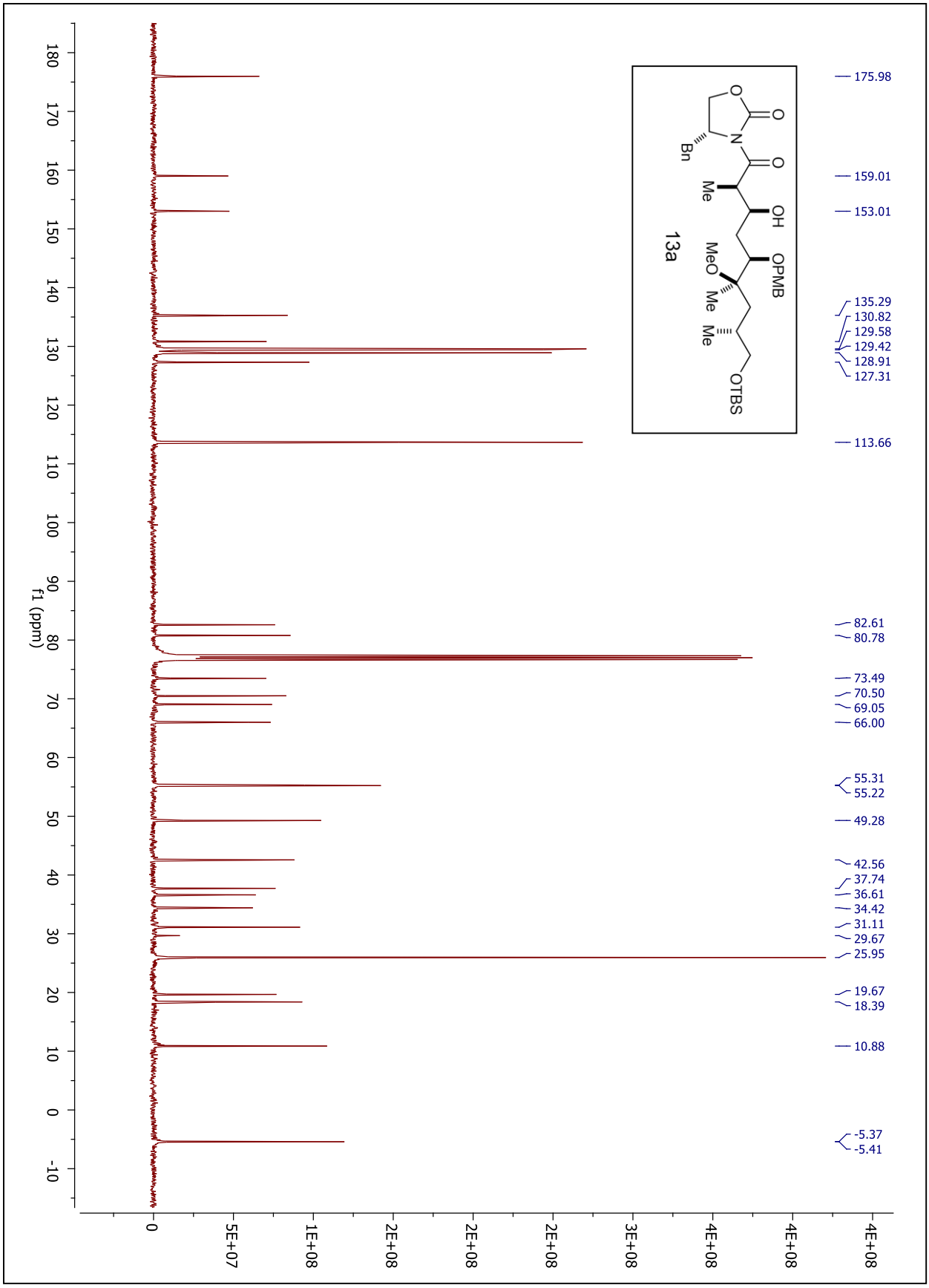
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66.00
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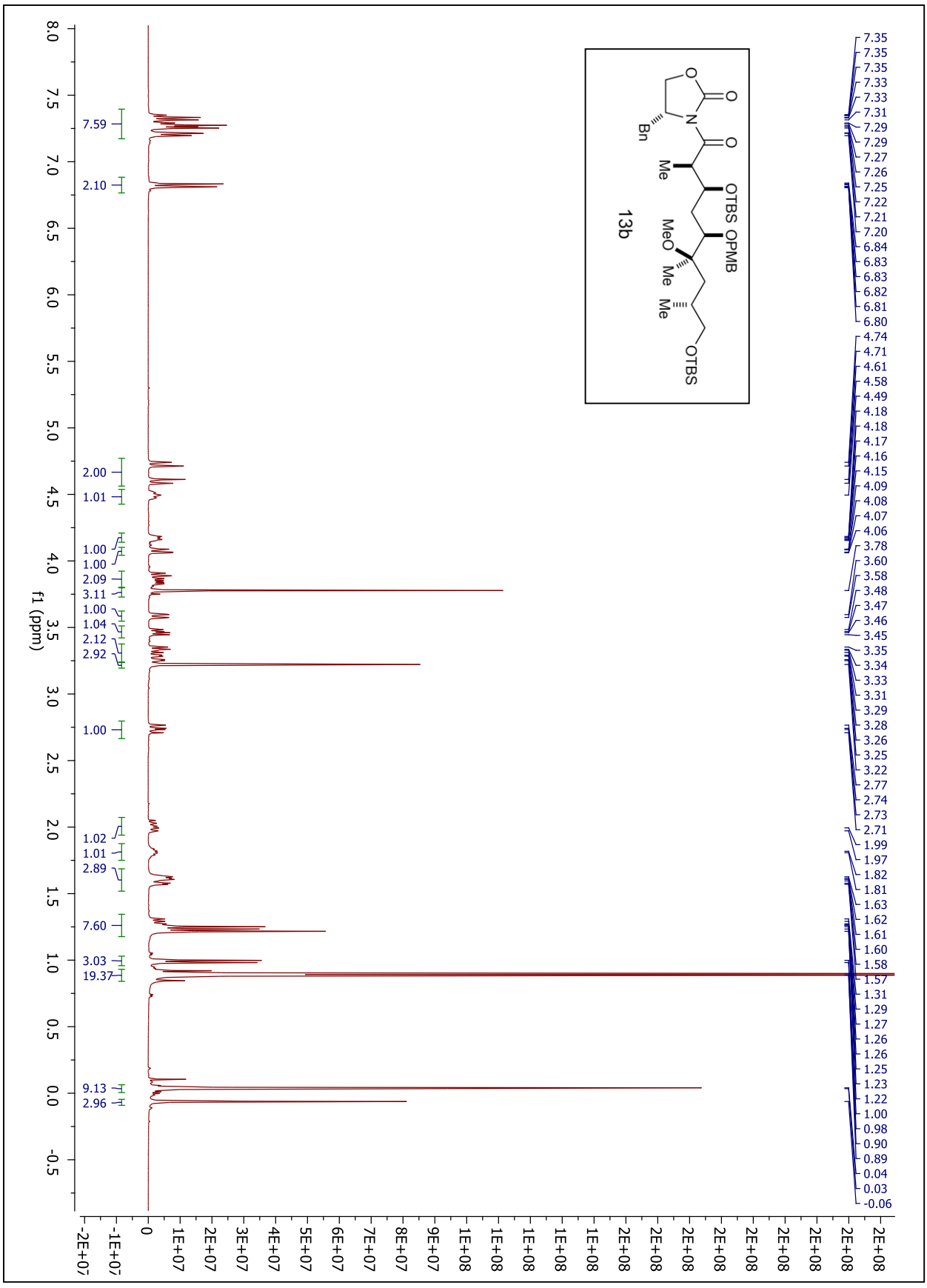
f1 (ppm)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Bn
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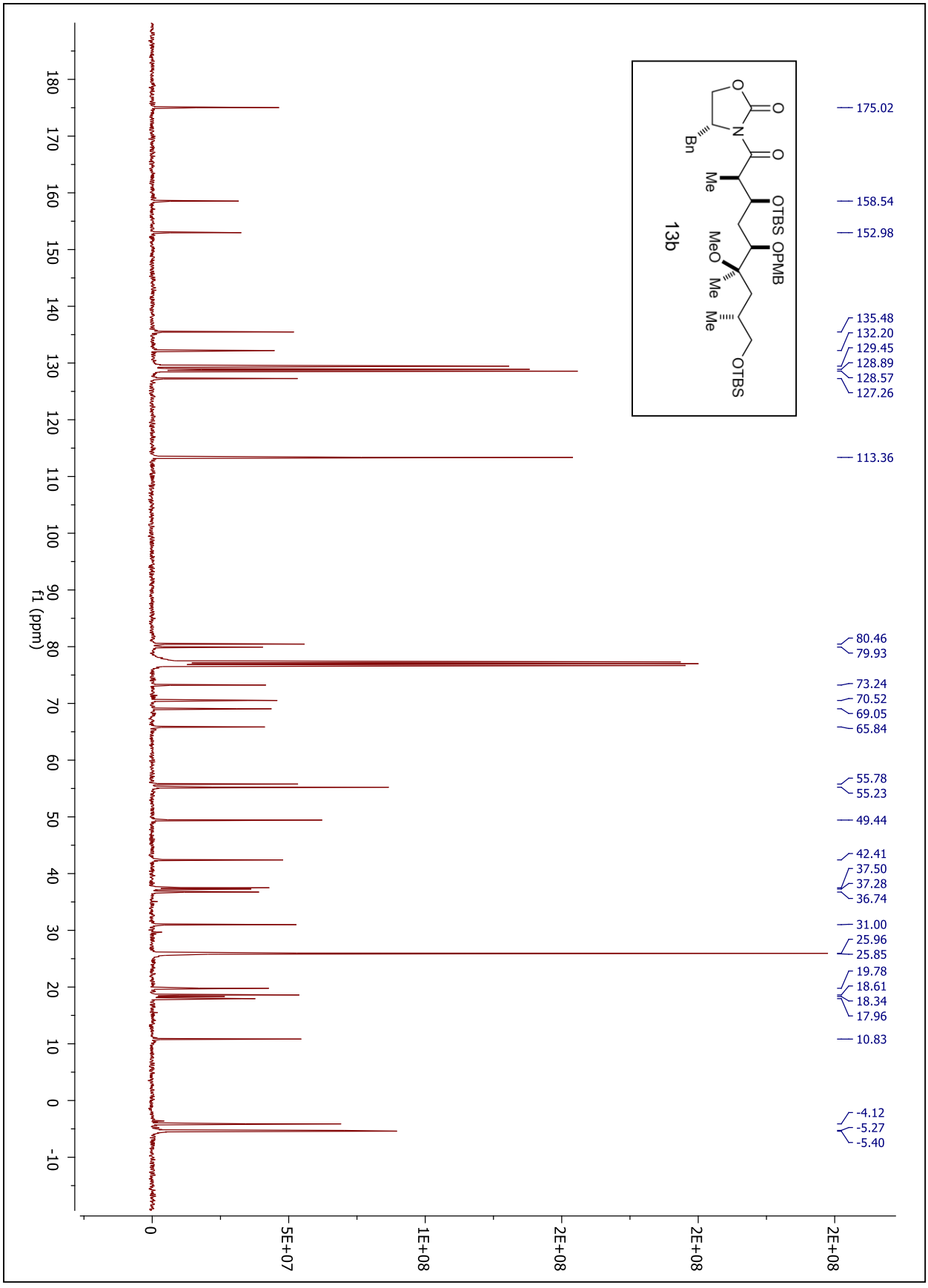
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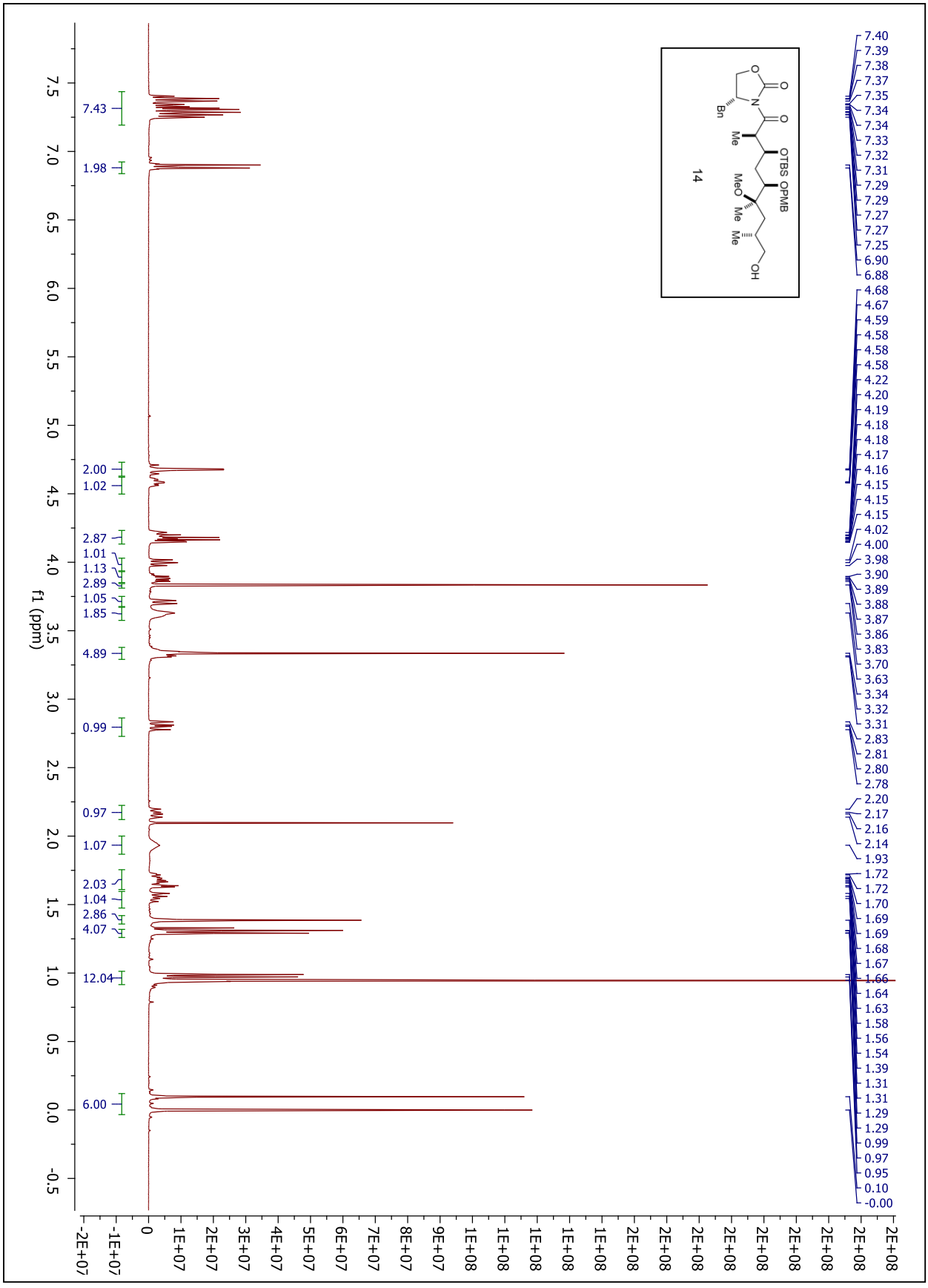
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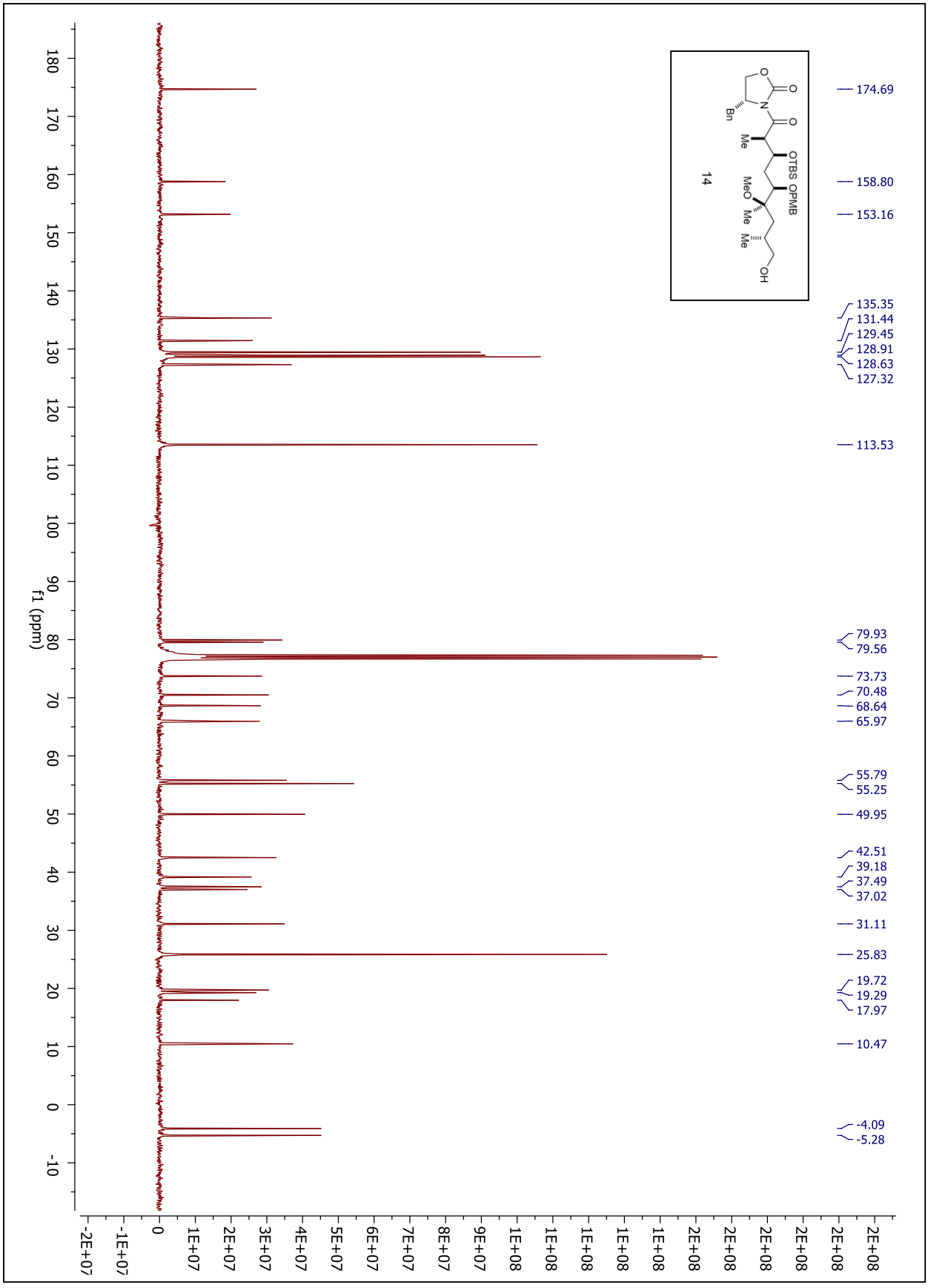
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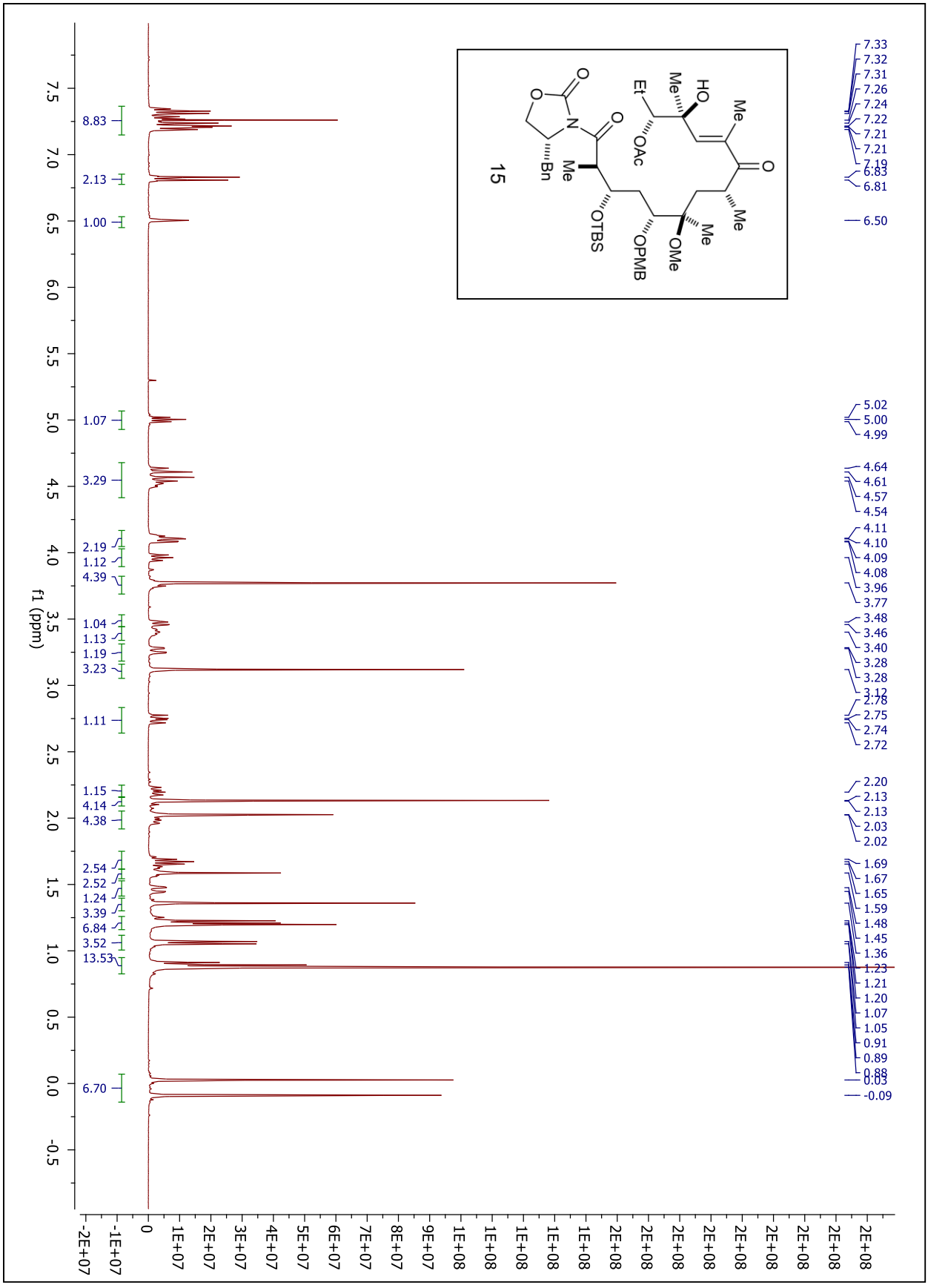
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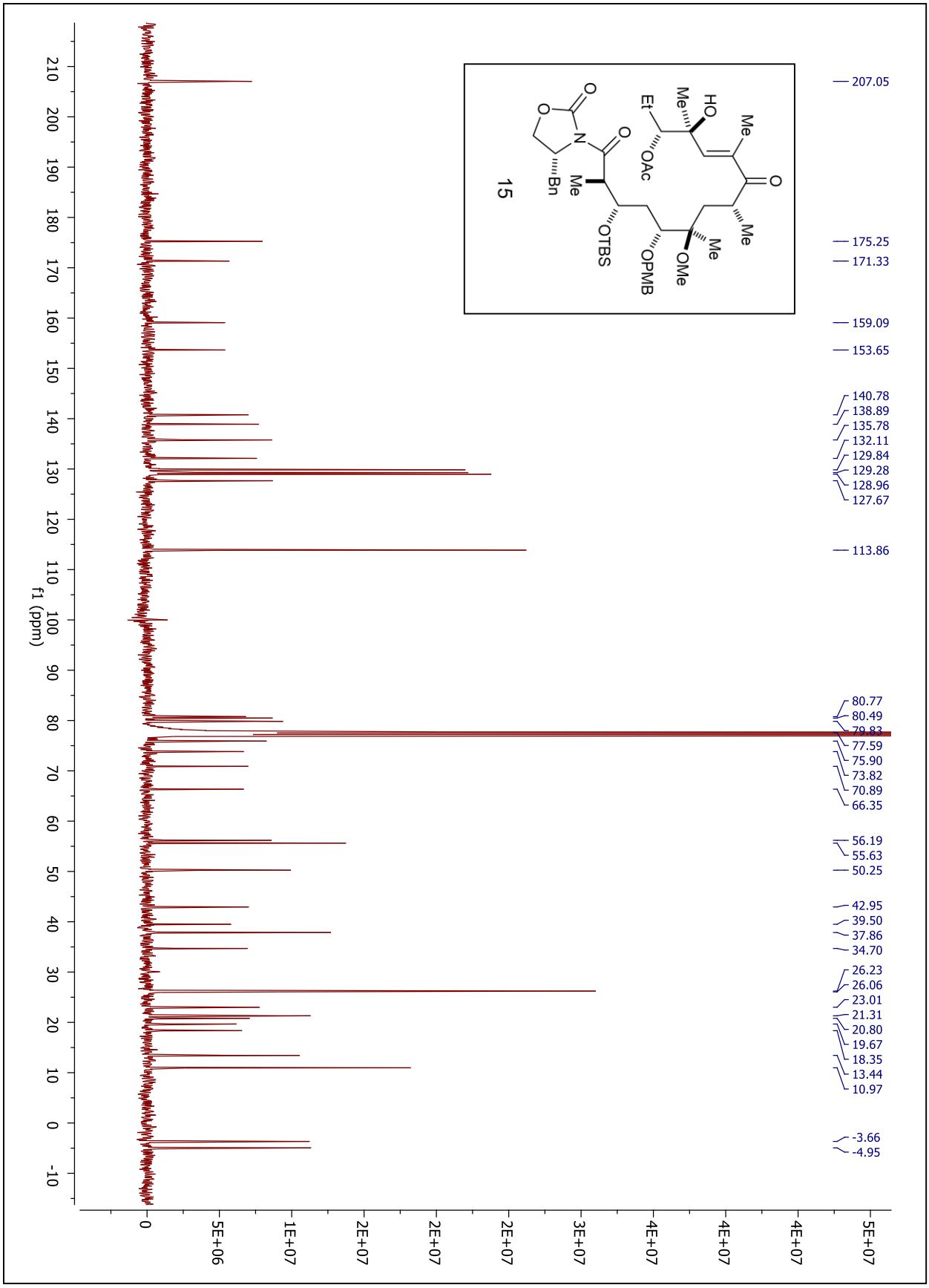
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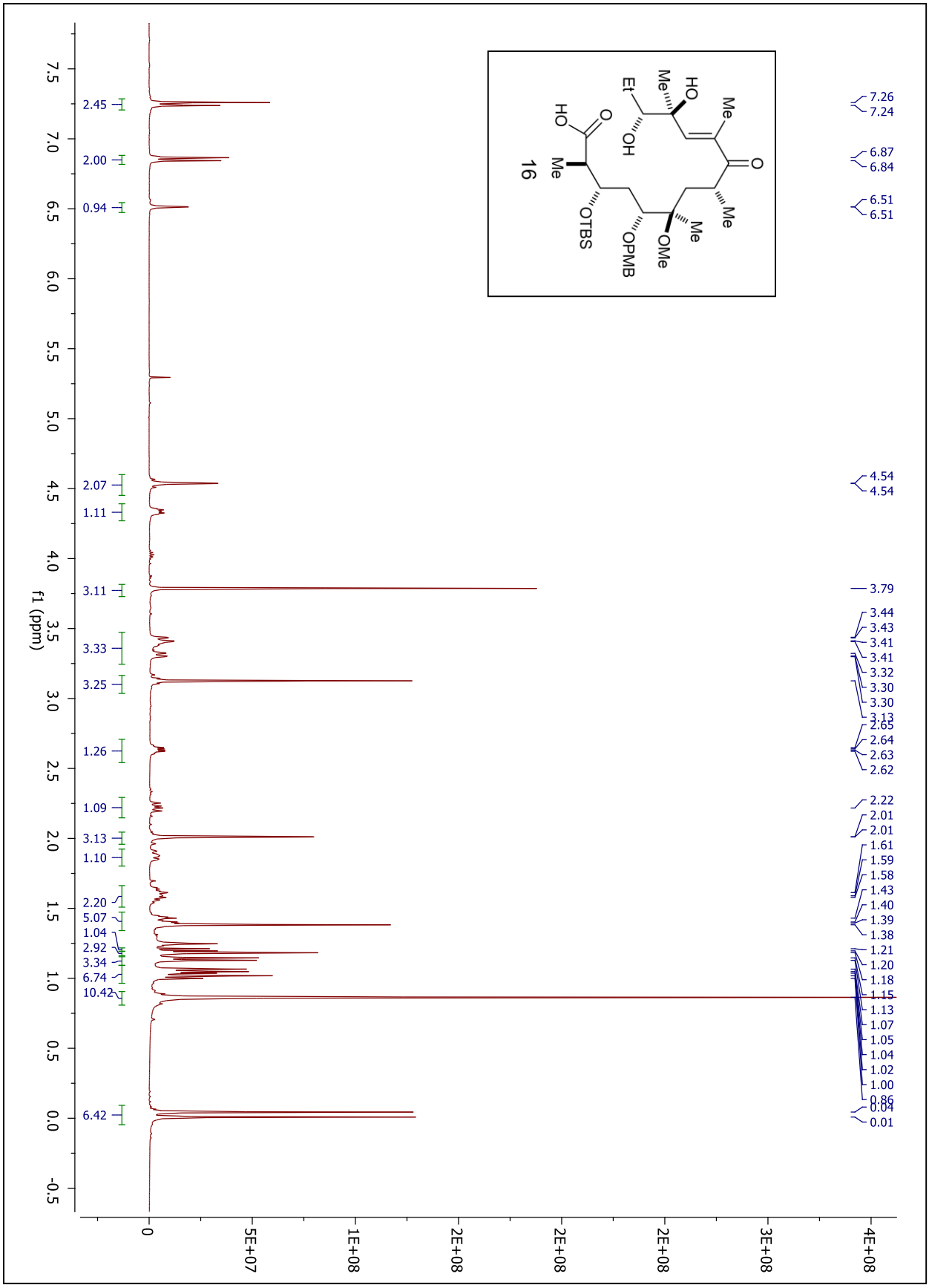
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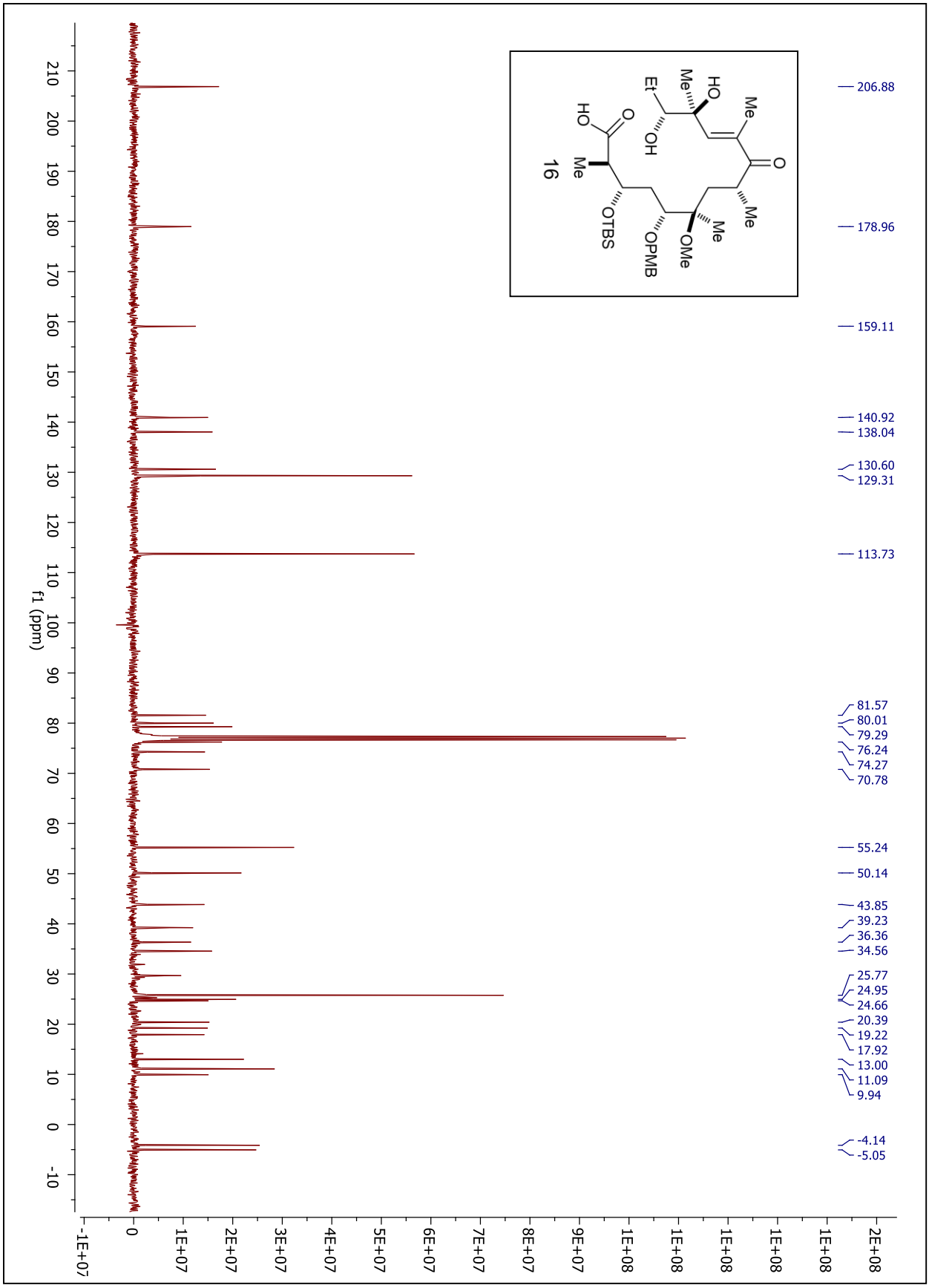
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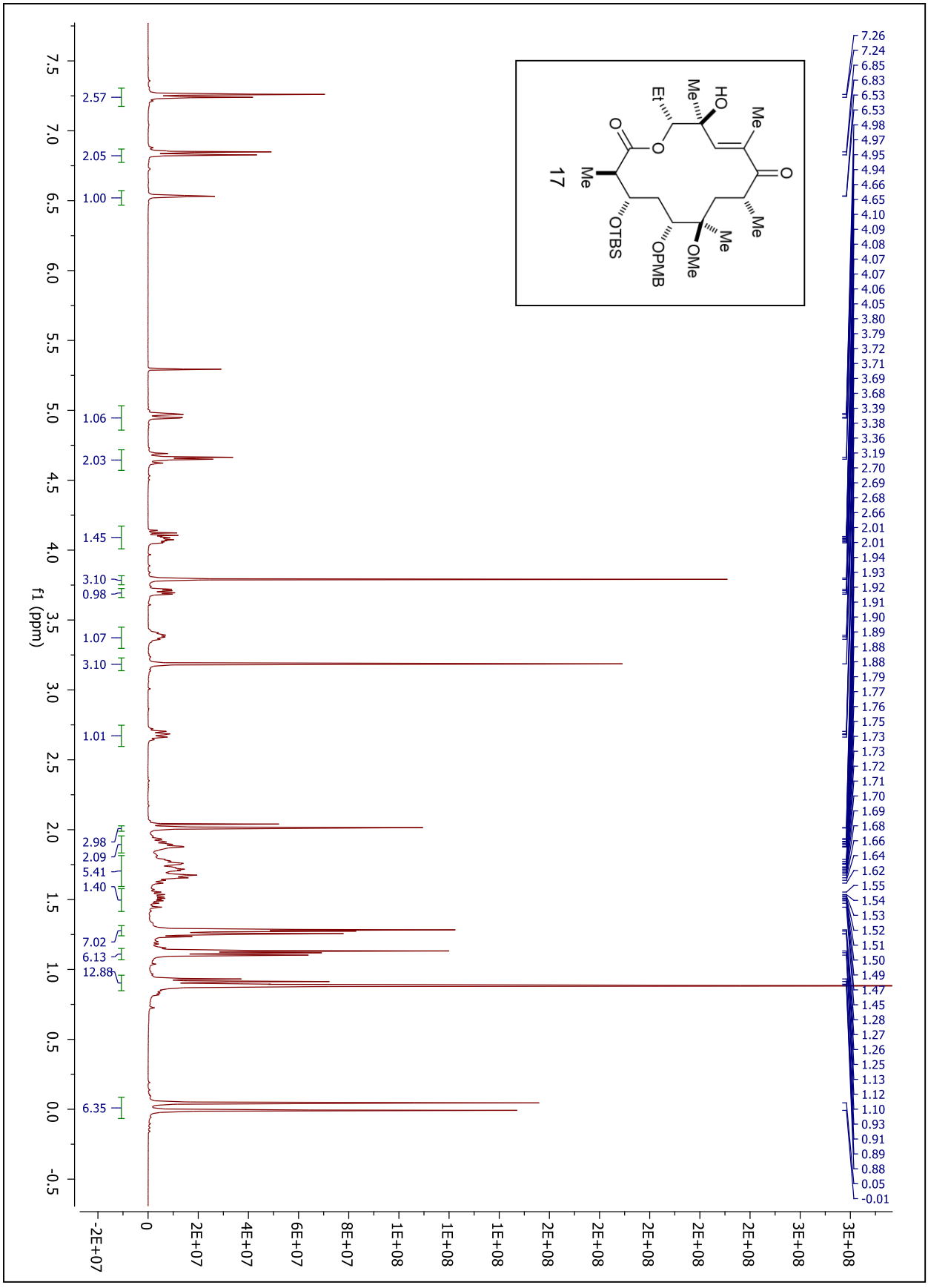
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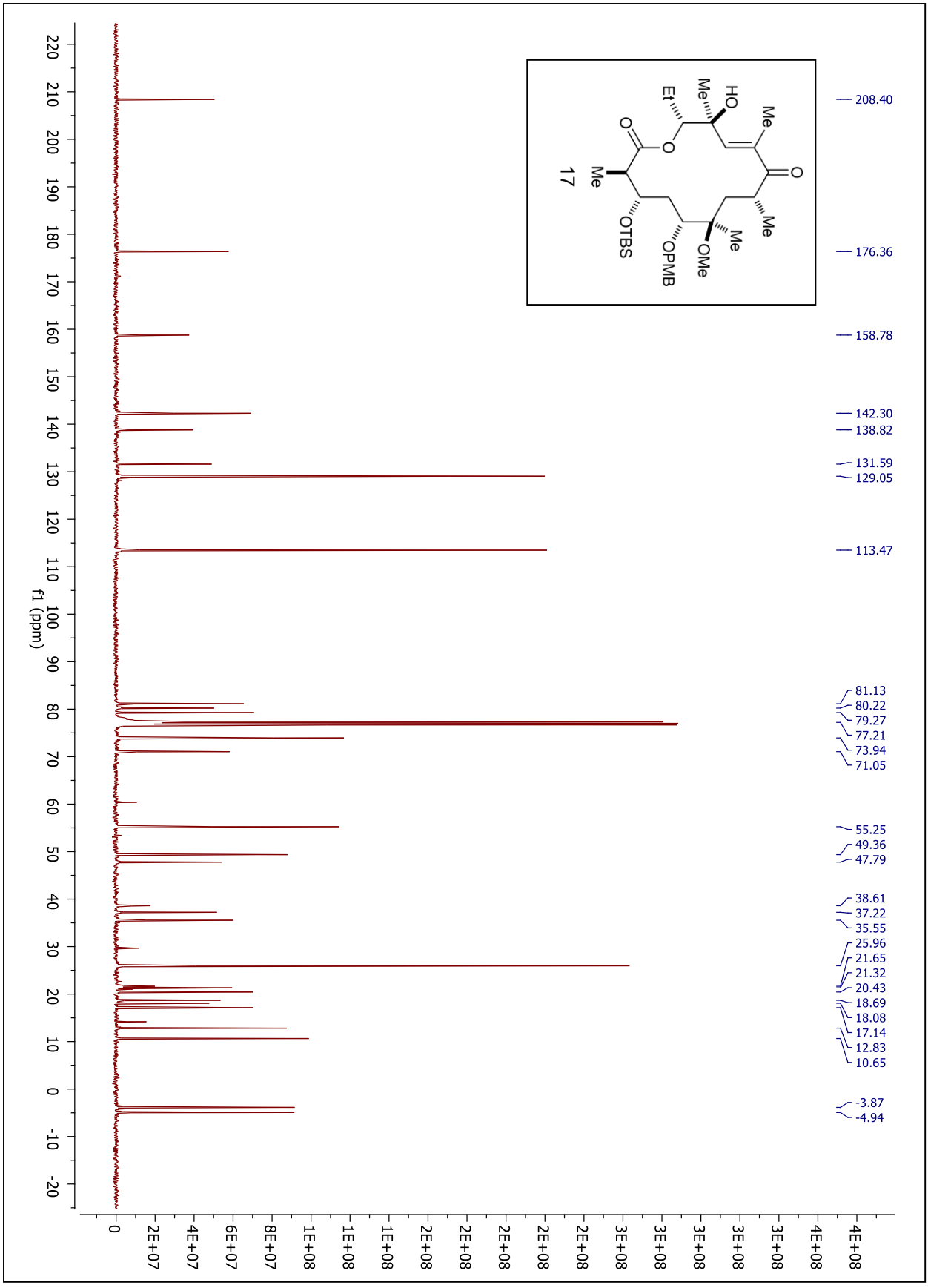
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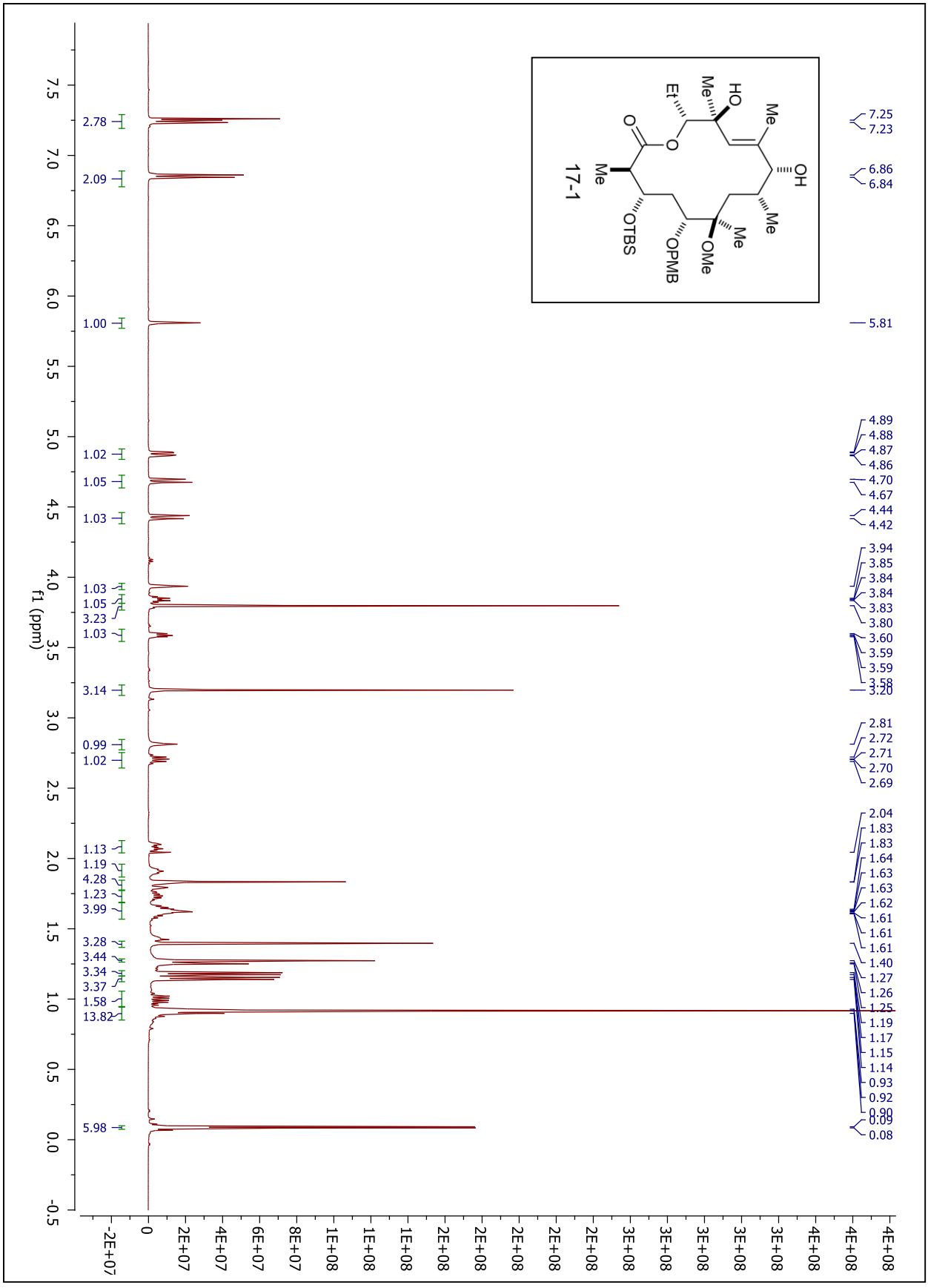
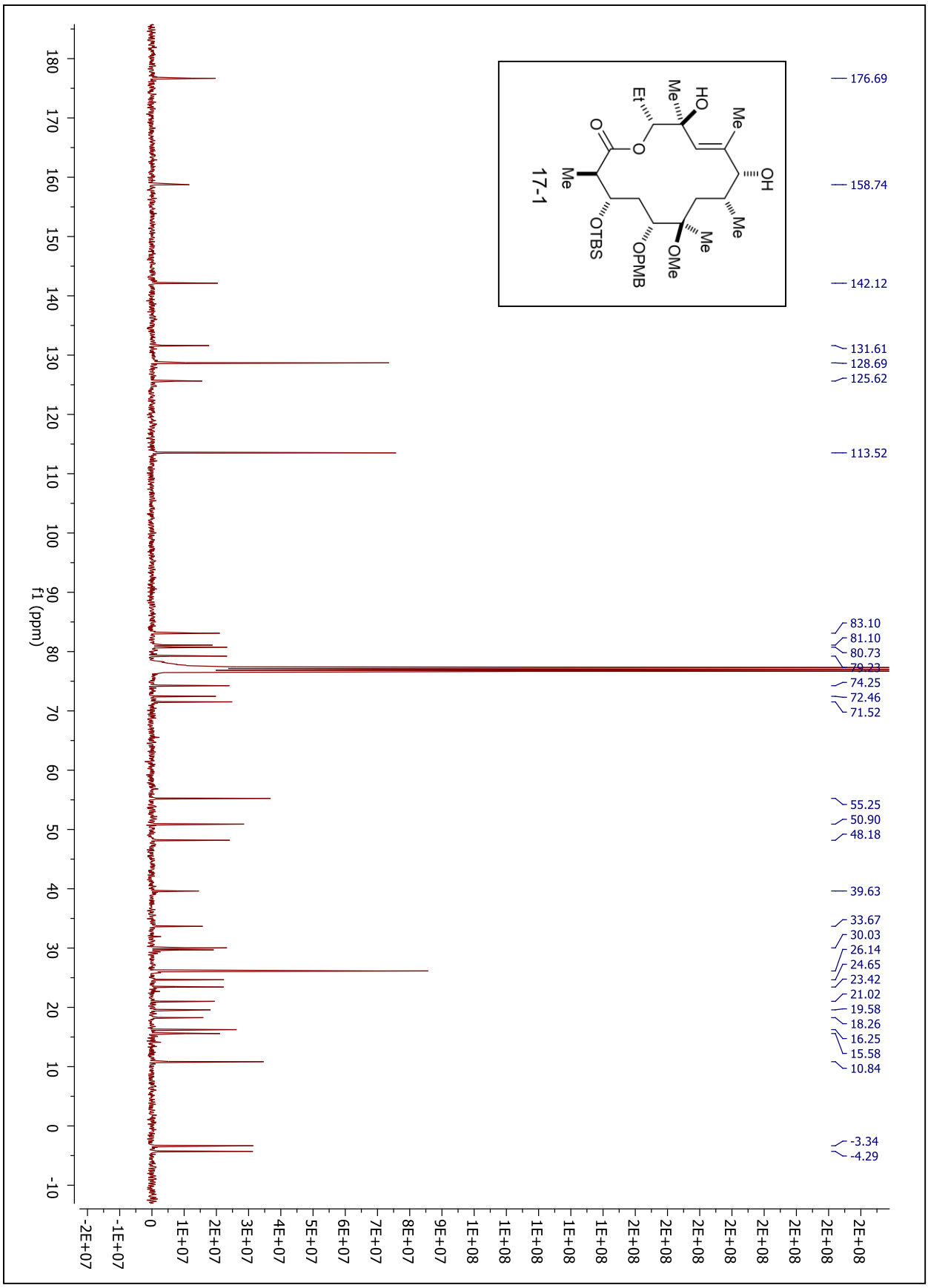
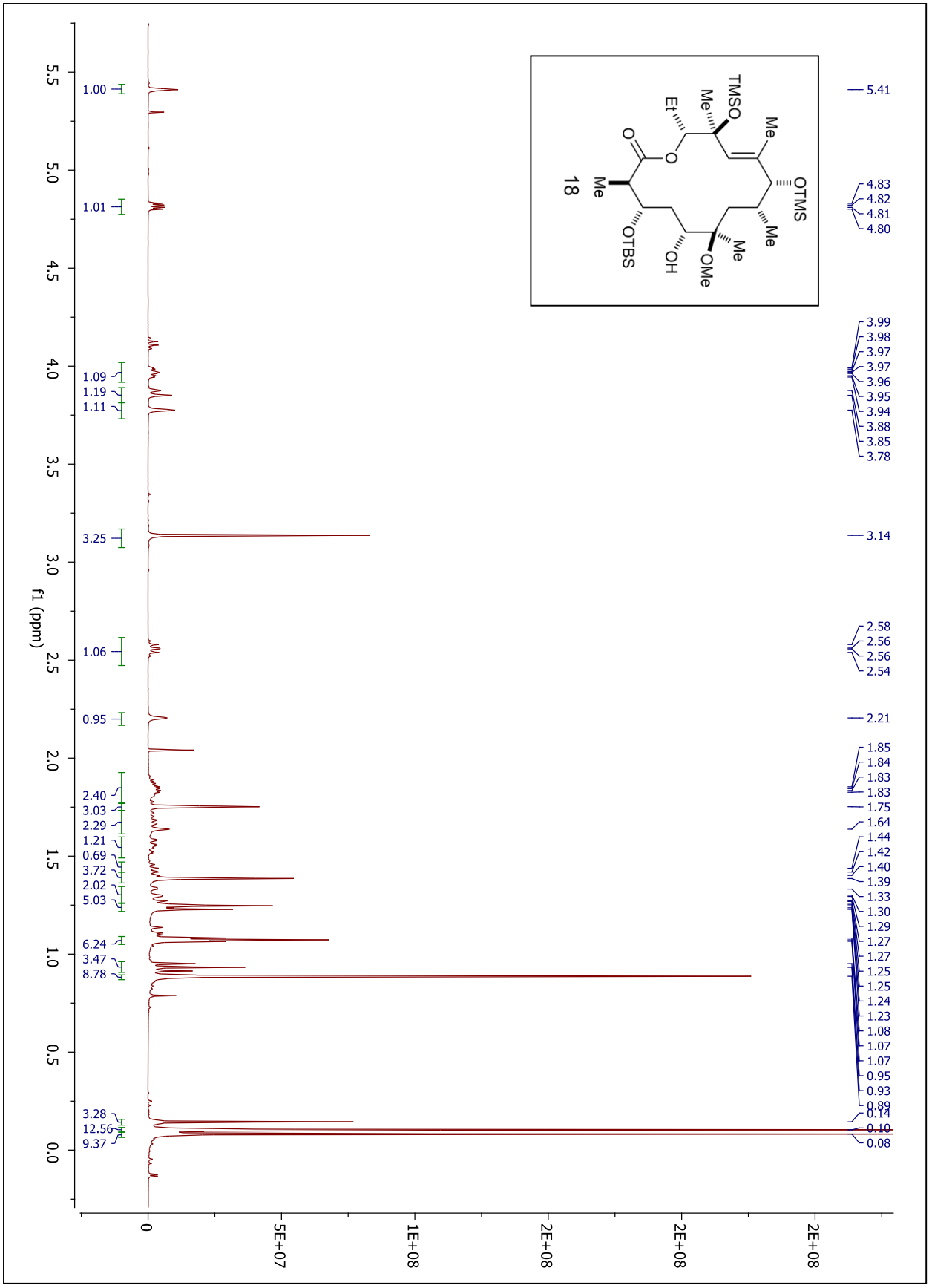
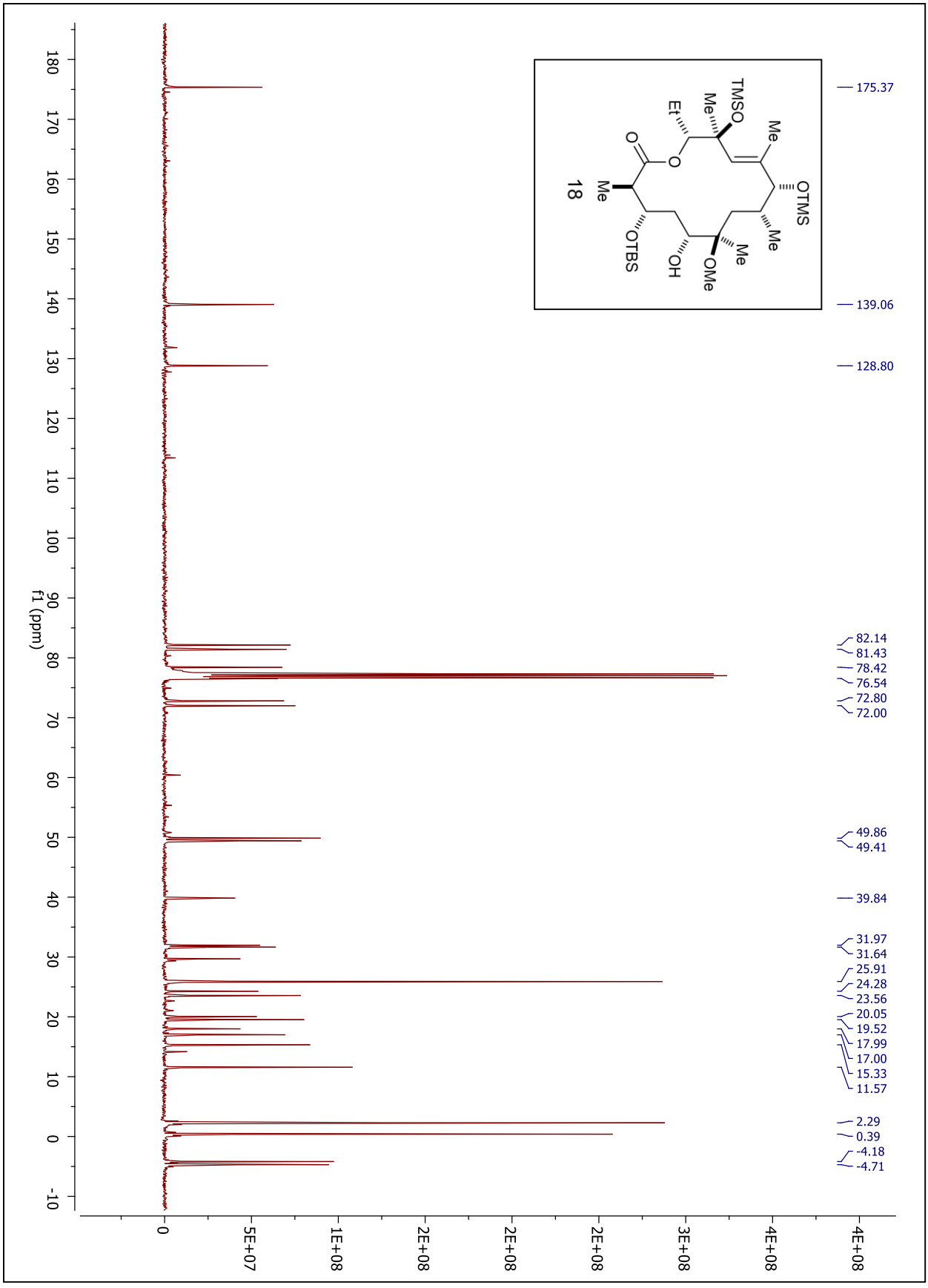
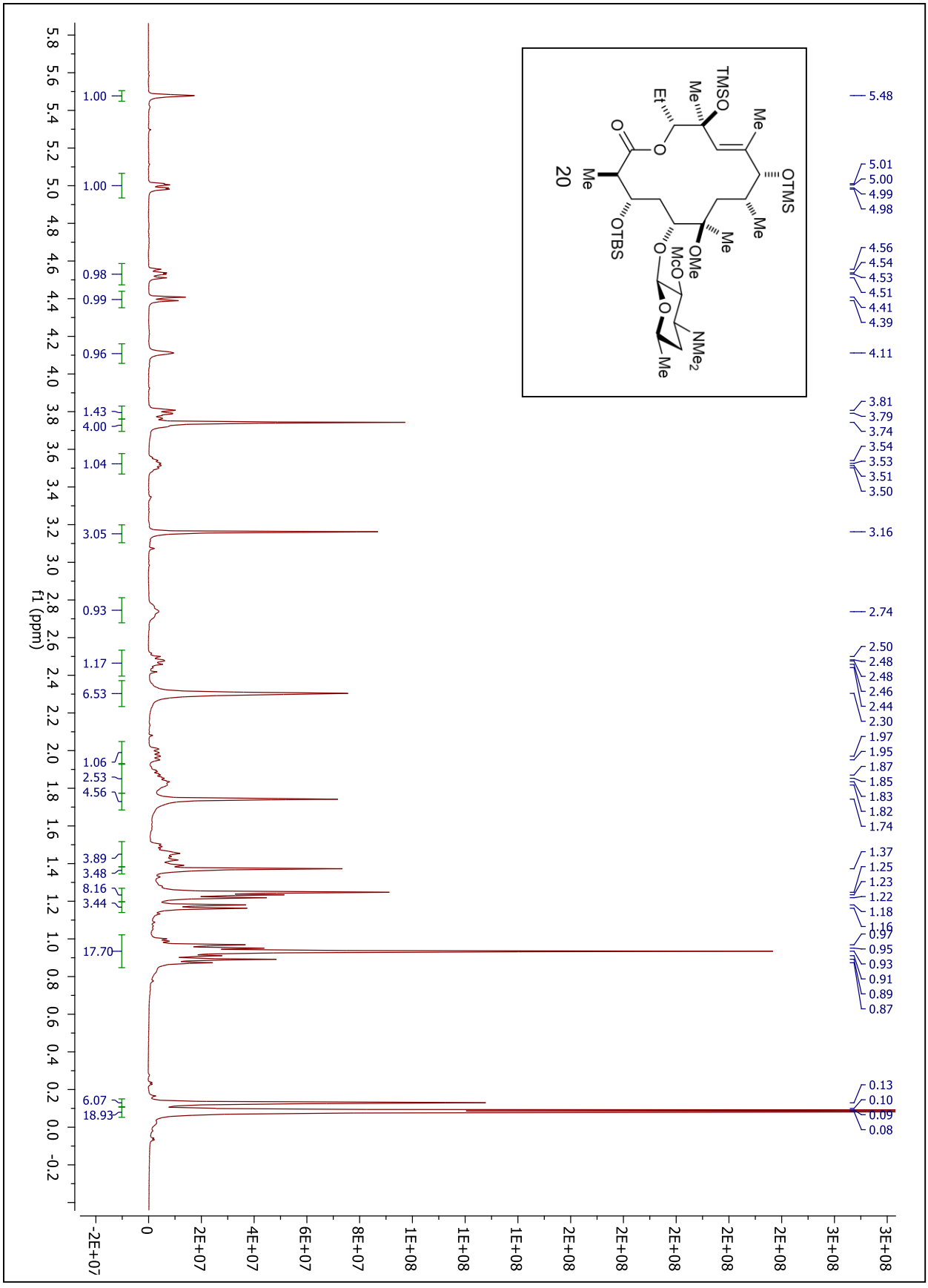
142.30
138.82
131.59
129.05
113.47
81.13
80.22
79.27
77.21
73.94
71.05
55.25
49.36
47.79
38.61
37.22
35.55
25.96
21.65
21.32
20.43
18.69
18.08
17.14
12.83
10.65
-3.87
-4.94
f1 (ppm)

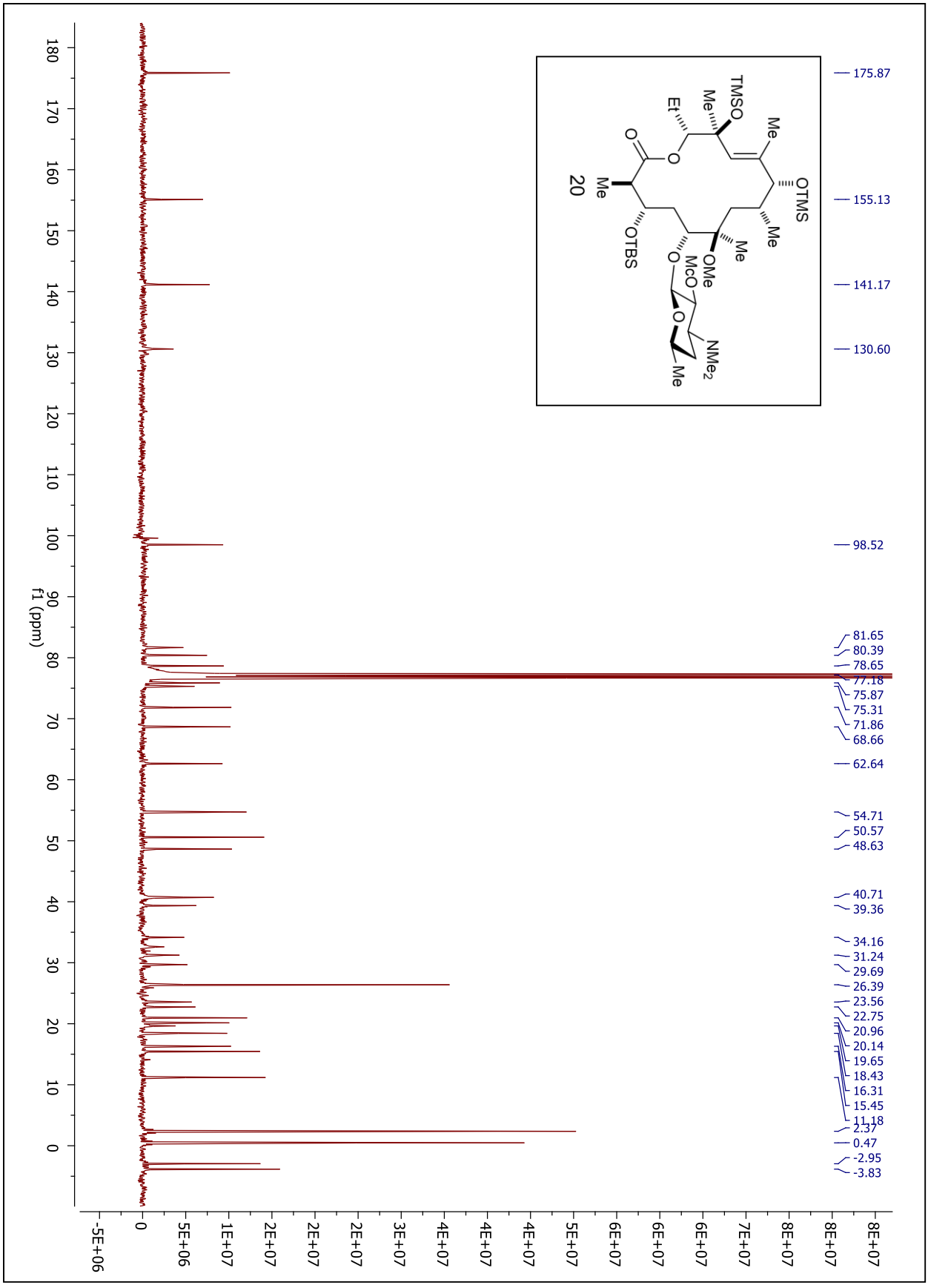

17-1
Et
Me
HO
Me
OH
O
O
Me
Me
OTBS
OPMB
OMe
Me
f1 (ppm)

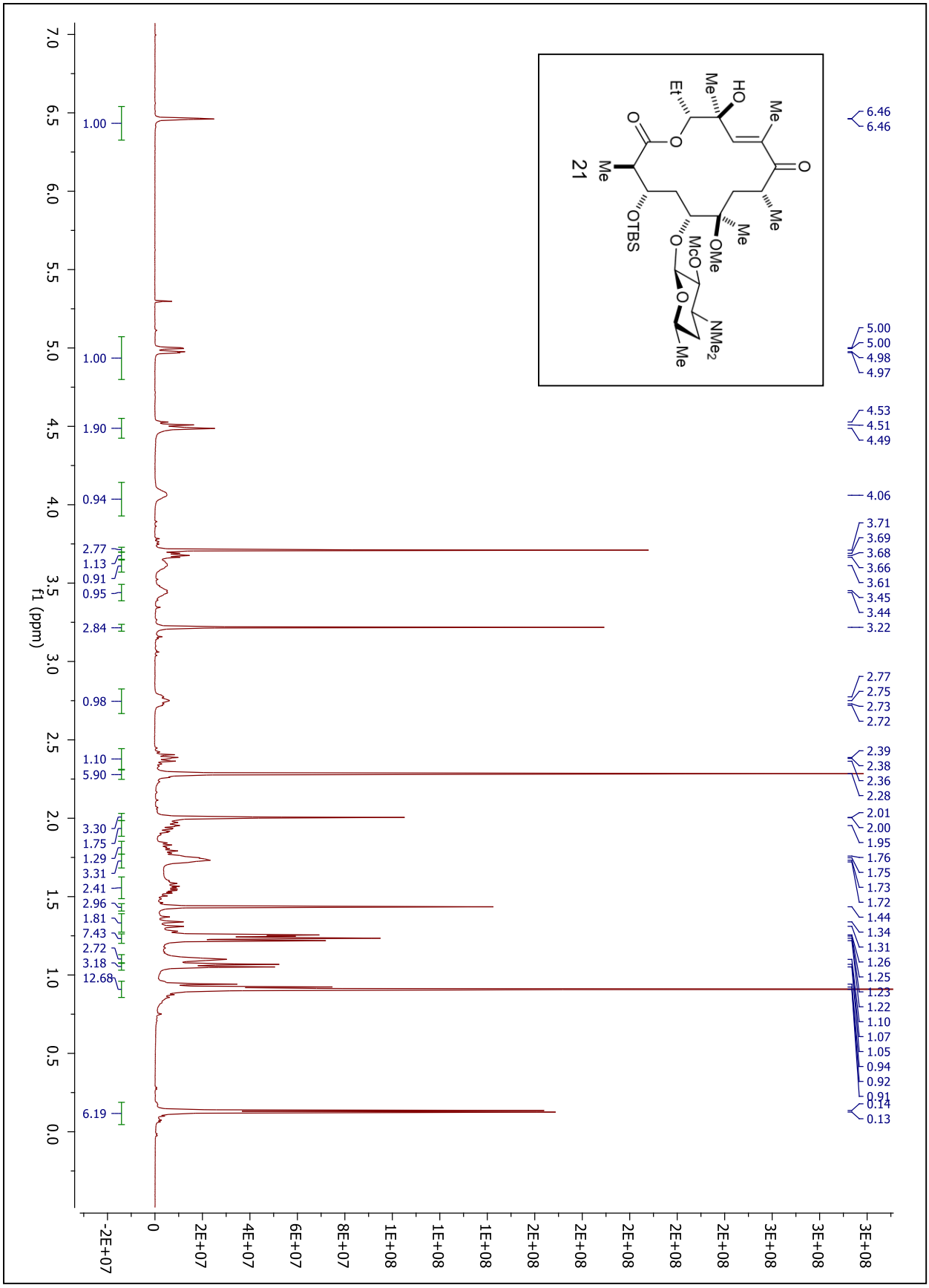

17-1
Me
OH
Me
HO
Me
Et
O
O
Me
OTBS
OPMB
OMe
Me
176.69
158.74
142.12
131.61
128.69
125.62
113.52
83.10
81.10
80.73
79.23
74.25
72.46
71.52
55.25
50.90
48.18
39.63
33.67
30.03
26.14
24.65
23.42
21.02
19.58
18.26
16.25
15.58
10.84
-3.34
-4.29
f1 (ppm)

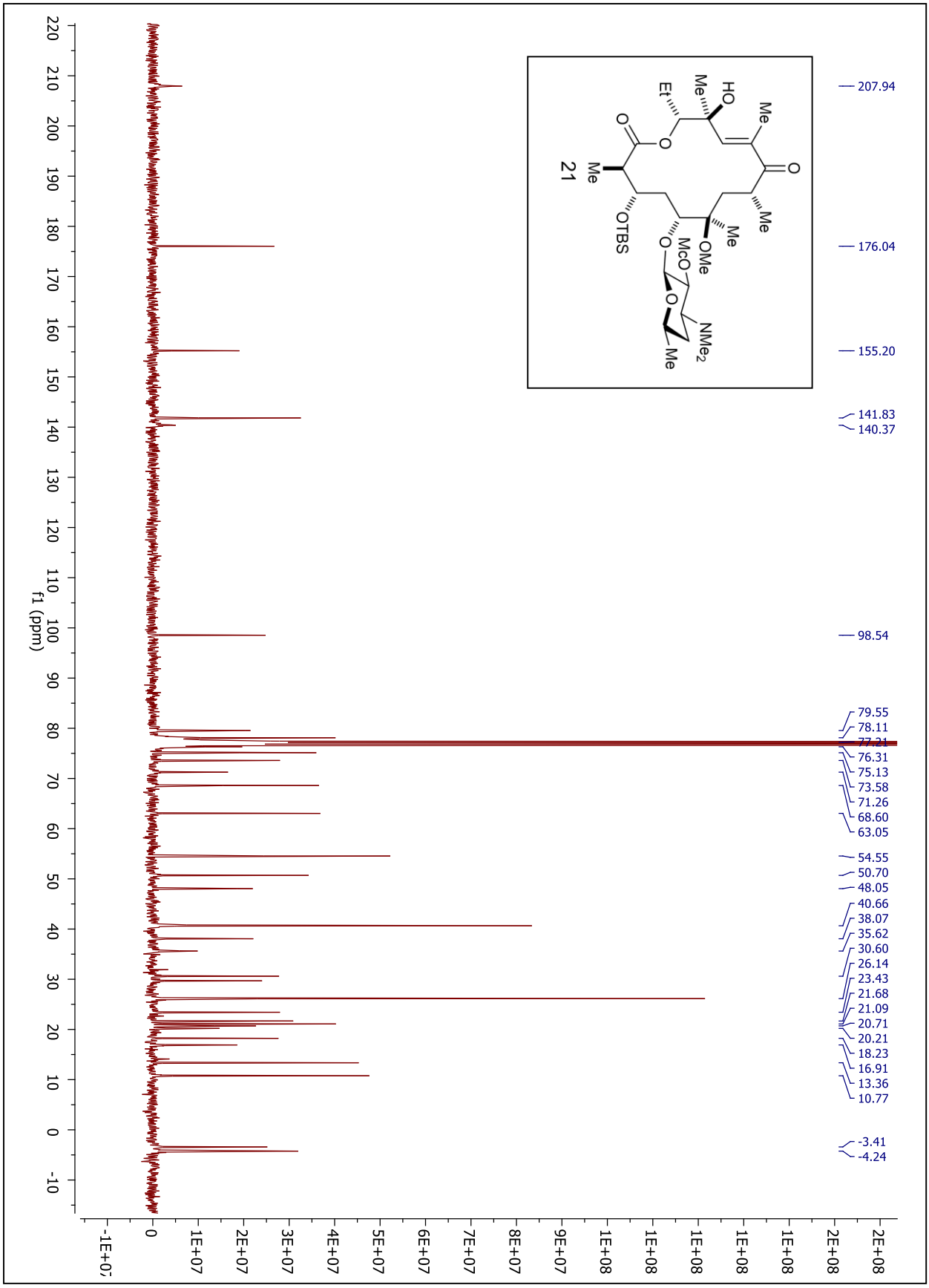

18
TMSO
Me
Et
Me
O
O
Me
OTMS
Me
Me
OTBS
OH
OMe
Me
f1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.00
1.01
1.09
1.19
1.11
3.25
1.06
0.95
2.40
3.03
2.29
1.21
0.69
3.72
2.02
5.03
6.24
3.47
8.78
3.28
12.56
9.37
5.41
4.83
4.82
4.81
4.80
3.99
3.98
3.97
3.97
3.96
3.95
3.94
3.88
3.85
3.78
3.14
2.58
2.56
2.56
2.54
2.21
1.85
1.84
1.83
1.83
1.75
1.64
1.44
1.42
1.40
1.39
1.33
1.30
1.29
1.27
1.27
1.25
1.25
1.24
1.23
1.08
1.07
1.07
0.95
0.93
0.89
0.14
0.10
0.08
0
5E+07
1E+08
2E+08
2E+08
2E+08

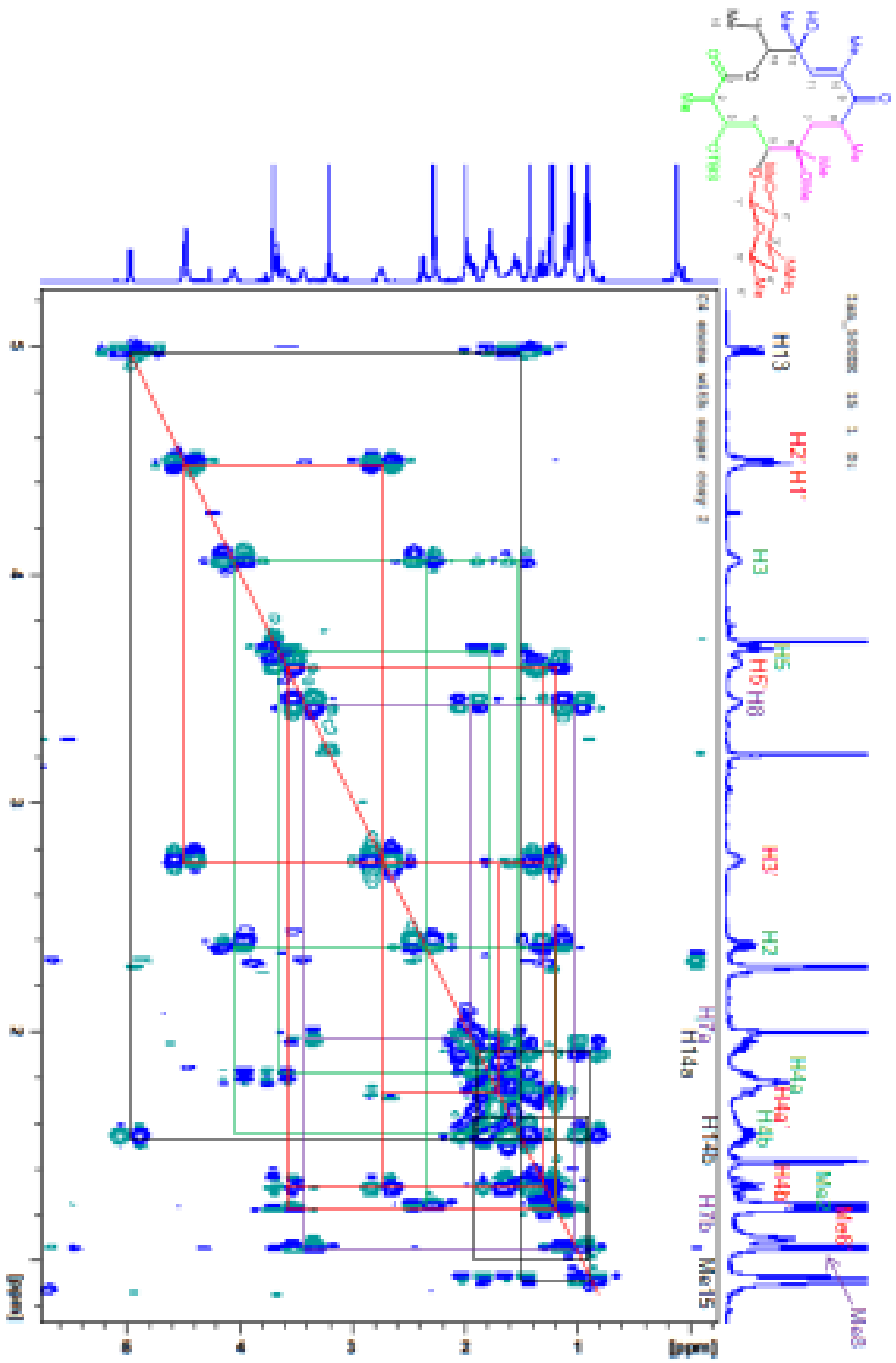

TMSO
Me
Et
O
O
Me
OTMS
Me
Me
OMe
OH
OTBS
Me
18
175.37
139.06
128.80
82.14
81.43
78.42
76.54
72.80
72.00
49.86
49.41
39.84
31.97
31.64
25.91
24.28
23.56
20.05
19.52
17.99
17.00
15.33
11.57
2.29
0.39
-4.18
-4.71
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
0
5E+07
1E+08
2E+08
2E+08
2E+08
3E+08
4E+08
4E+08

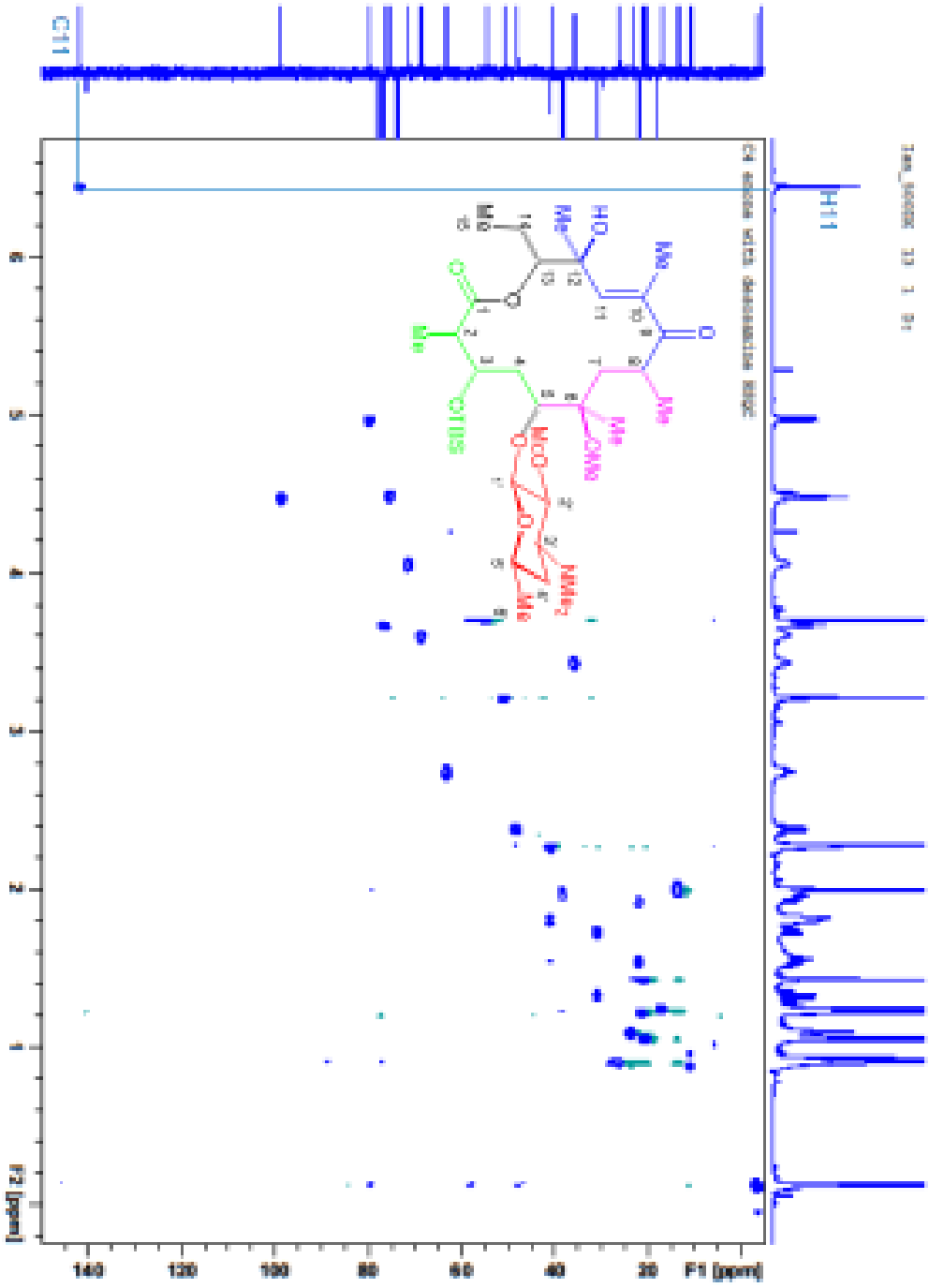

20
TMSO
Et
Me
Me
OTMS
Me
Me
Me
OMe
McO
O
O
NMe2
Me
OTBS
Me
O
O
f1 (ppm)

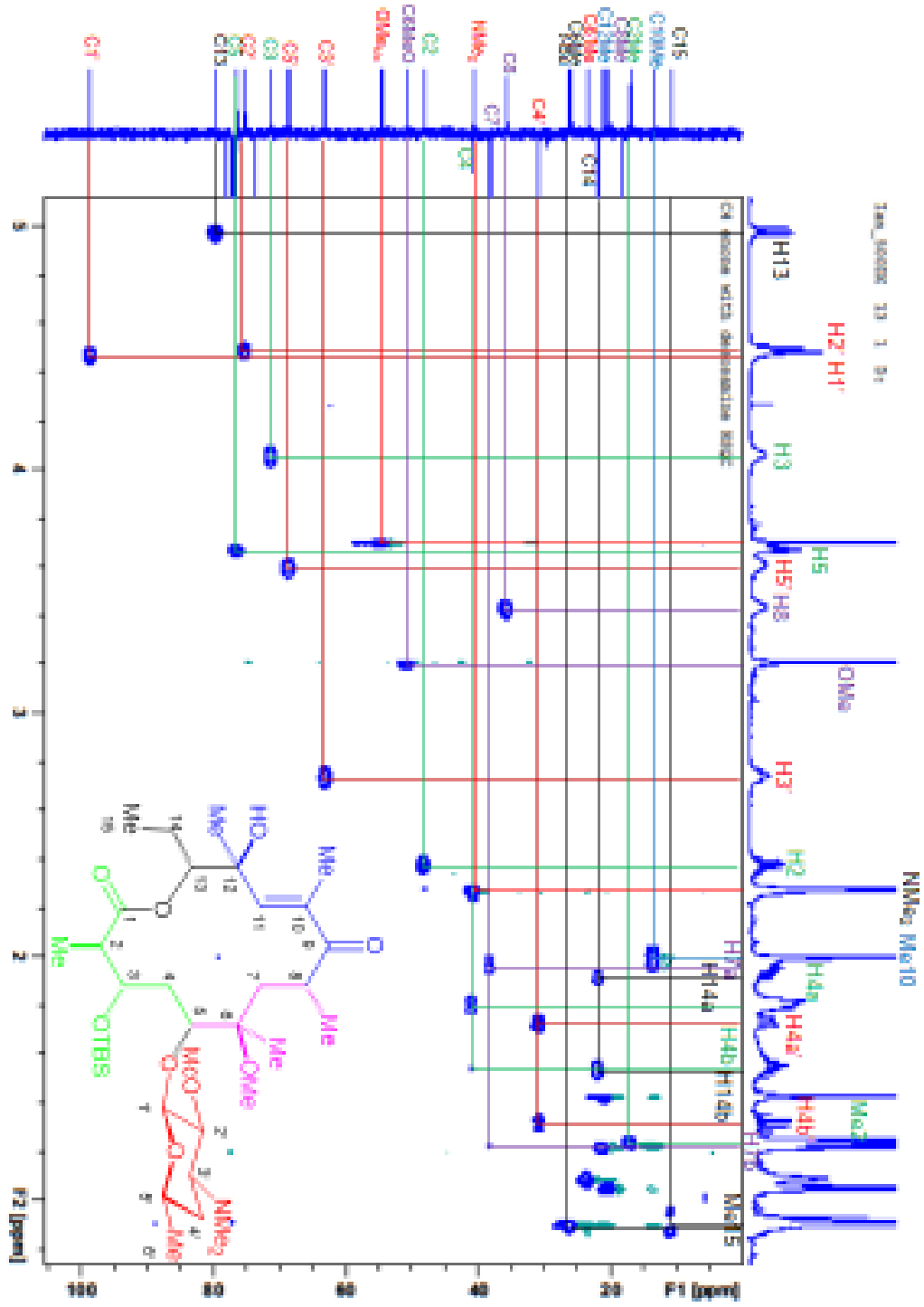

175.87
155.13
141.17
130.60
98.52
81.65
80.39
78.65
77.18
75.87
75.31
71.86
68.66
62.64
54.71
50.57
48.63
40.71
39.36
34.16
31.24
29.69
26.39
23.56
22.75
20.96
20.14
19.65
18.43
16.31
15.45
11.18
2.37
0.47
-2.95
-3.83
f1 (ppm)
TMSO
Me
Et
O
Me
OTMS
Me
Me
OTBS
Me
OMe
McO
O
O
NMe2
Me
20

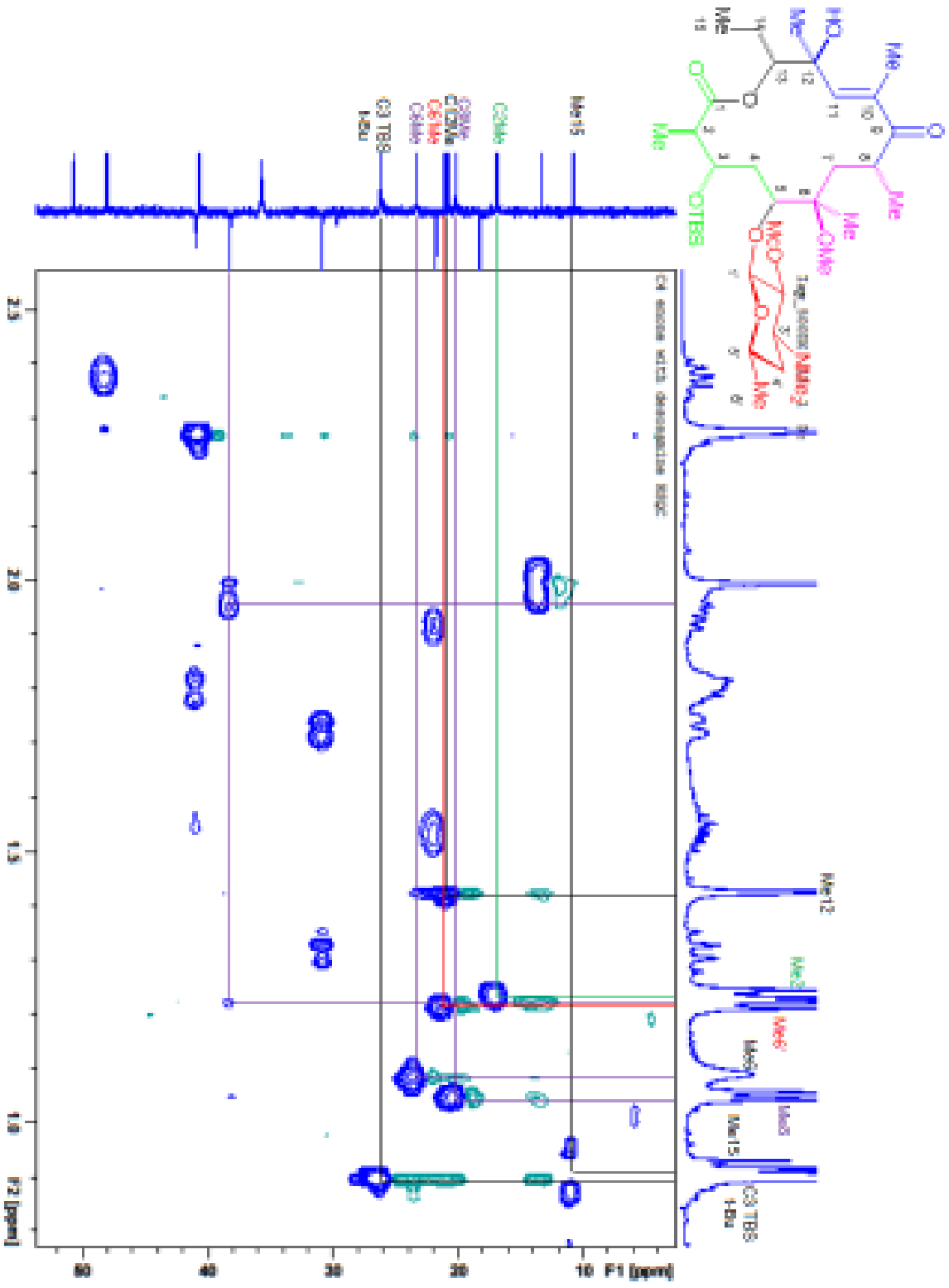

21
Et
Me
HO
Me
O
O
O
Me
Me
OTBS
Me
OMe
McO
O
O
NMe2
Me
f1 (ppm)

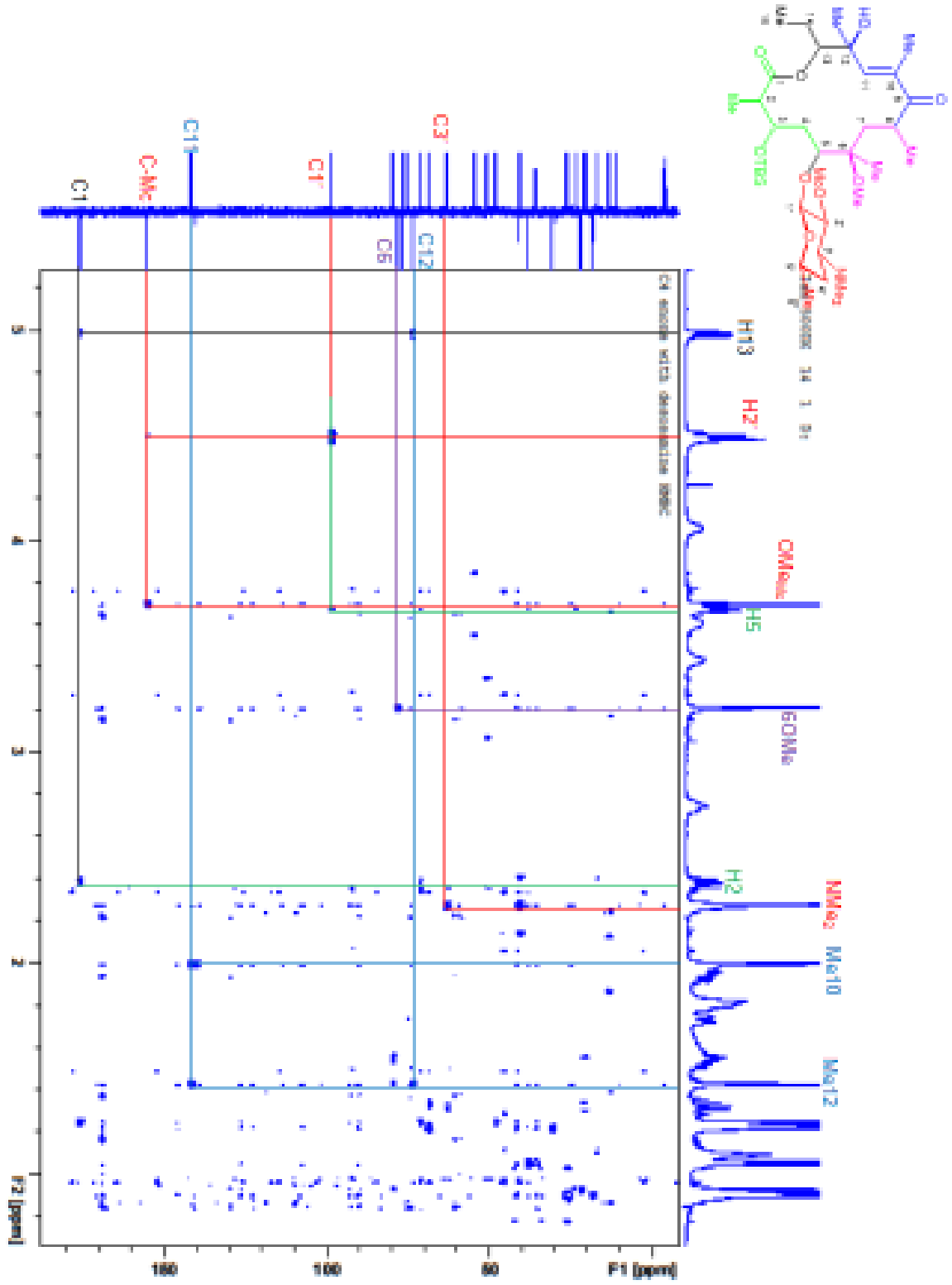

207.94
176.04
155.20
141.83
140.37
98.54
79.55
78.11
77.21
76.31
75.13
73.58
71.26
68.60
63.05
54.55
50.70
48.05
40.66
38.07
35.62
30.60
26.14
23.43
21.68
21.09
20.71
20.21
18.23
16.91
13.36
10.77
-3.41
-4.24
f1 (ppm)
21

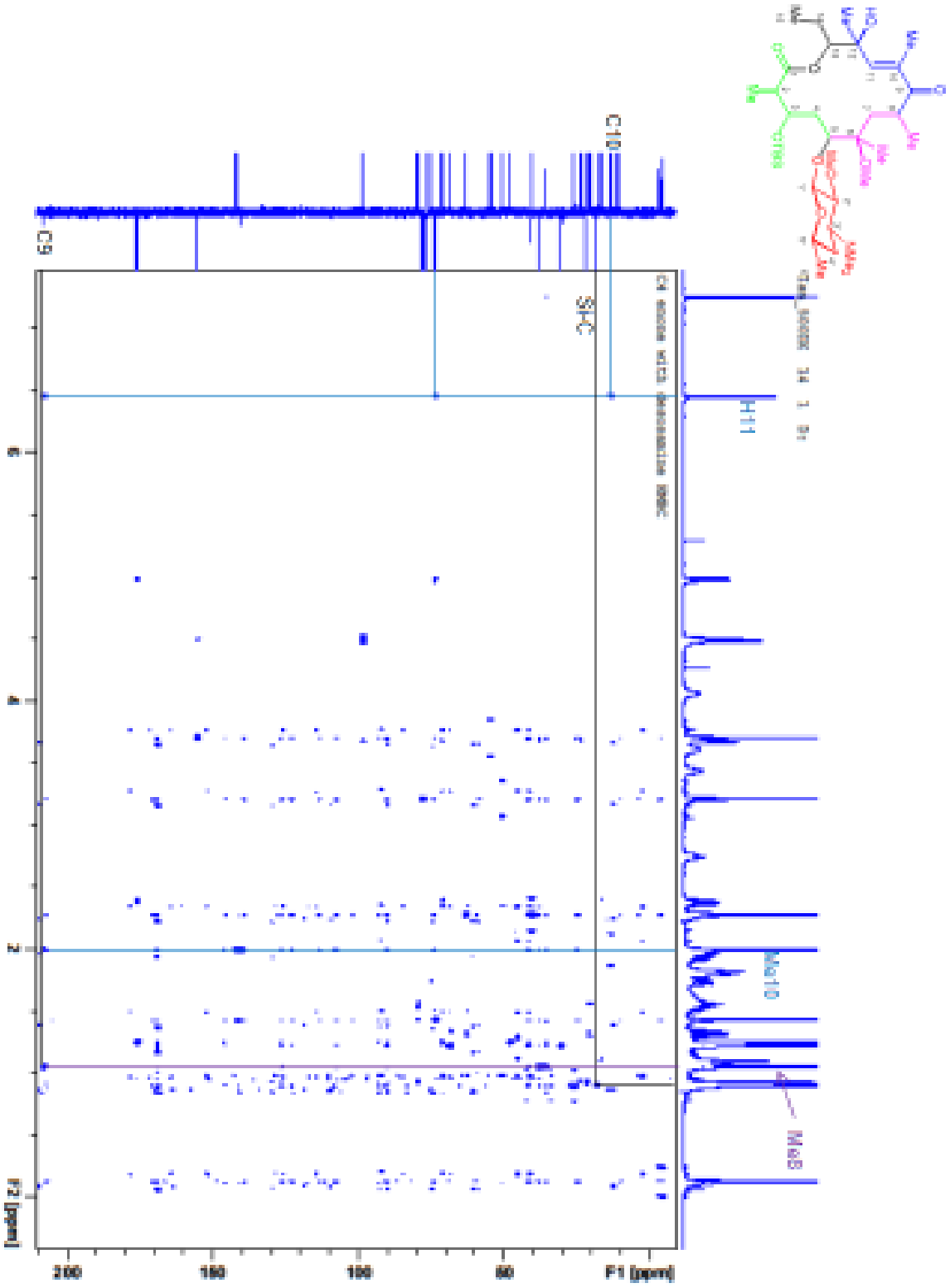

H13
H2'
H1'
H3
H5
H5'
H8'
H3'
H2
H14a
H14b
H4a
H4a'
H4b
H4b'
H6a
H6b
H15
H8
[ppm]
[ppm]

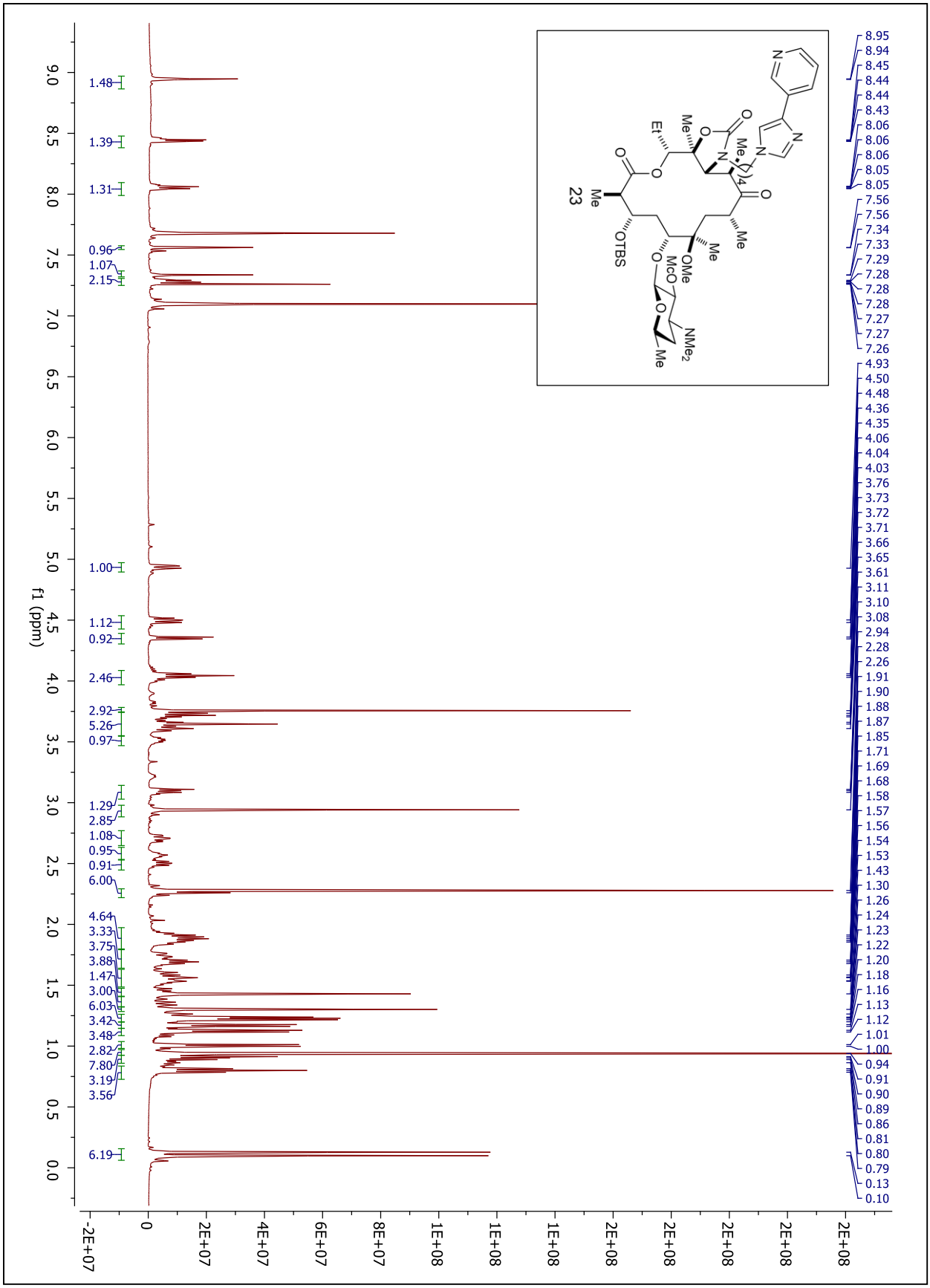

C11
H11
F2 [ppm]
F1 [ppm]
140
120
100
80
60
40
20

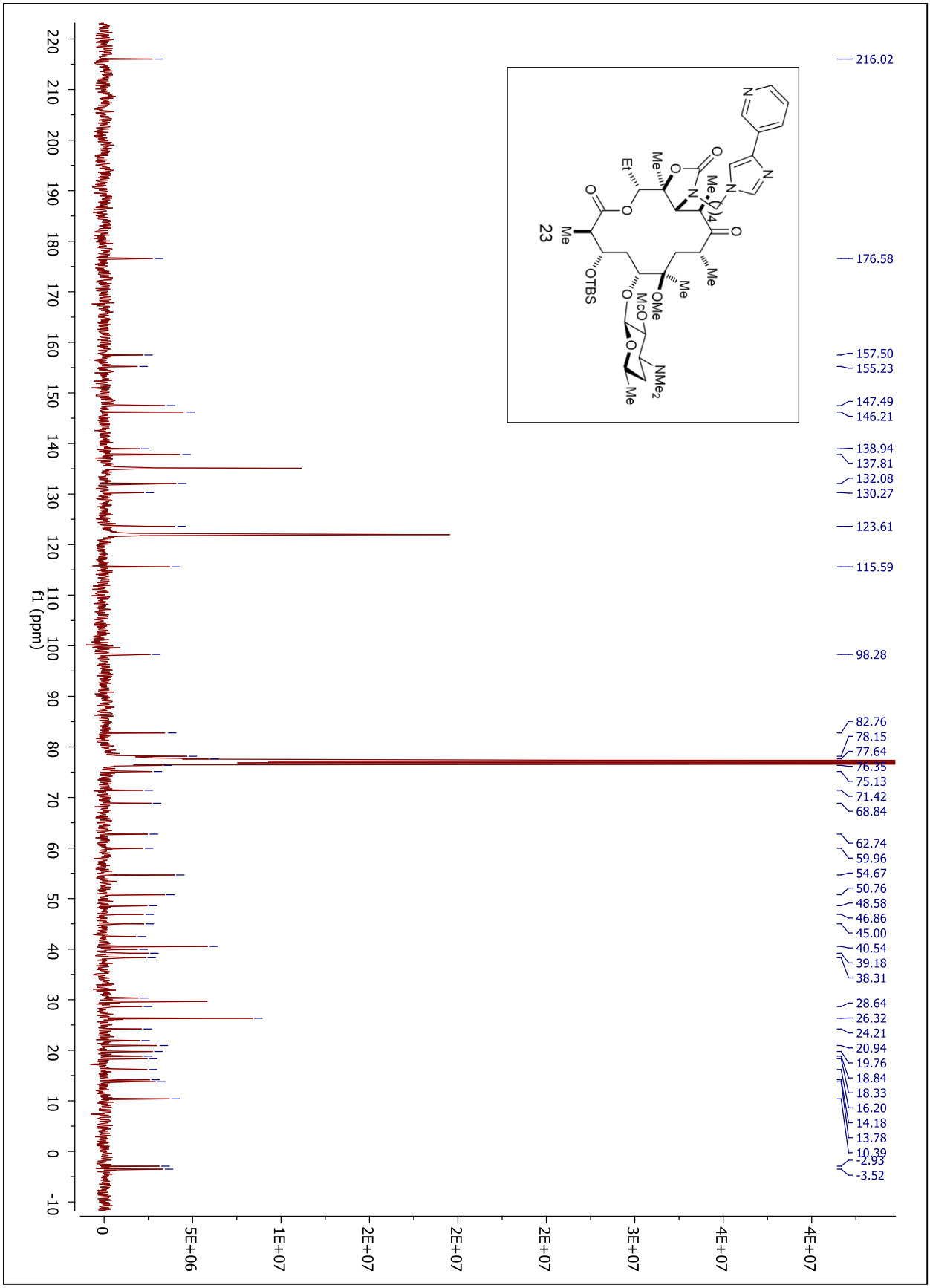

F2 [ppm]
F1 [ppm]
OTBS
OMe
NMe2
Me10

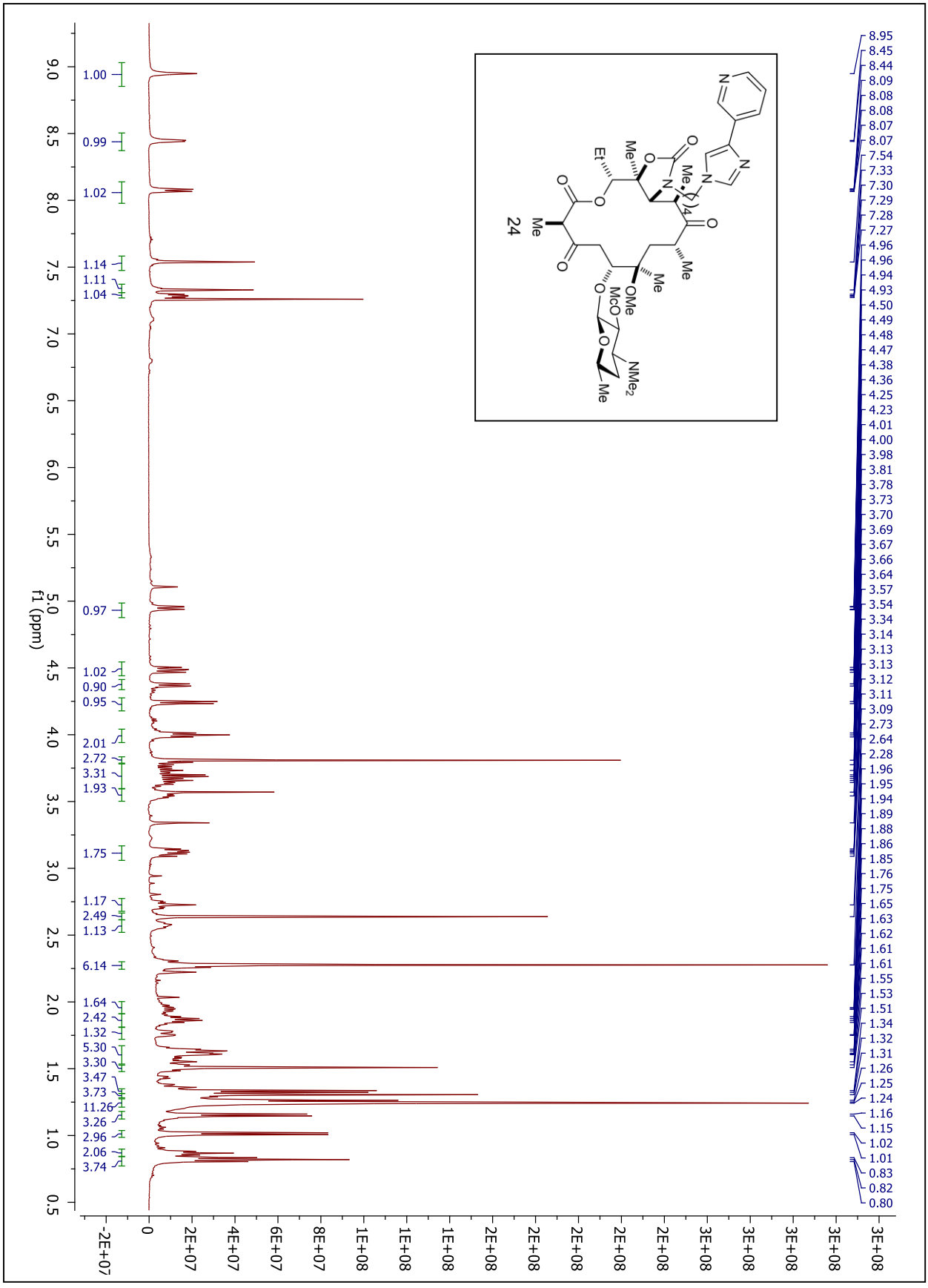


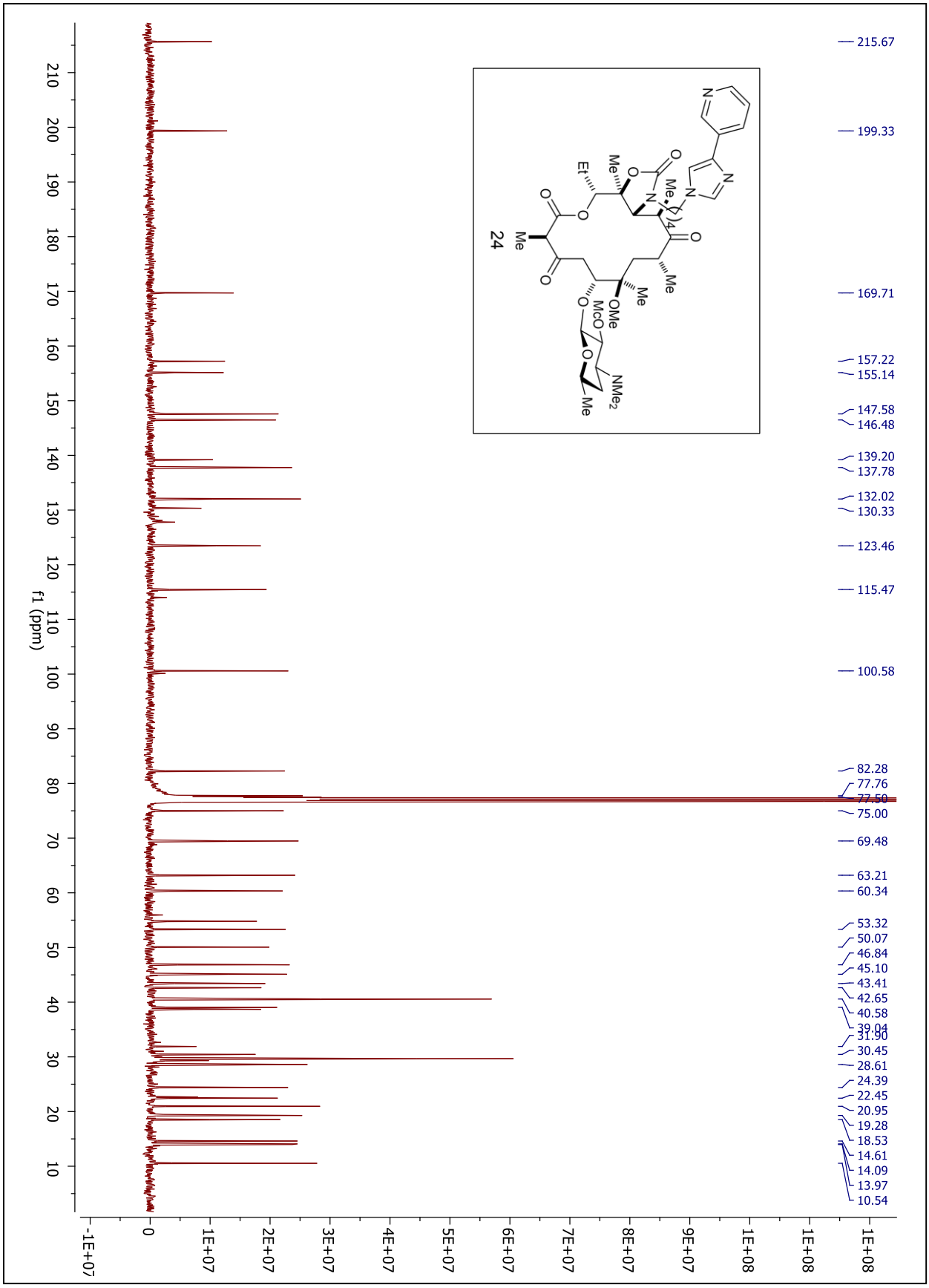

C1
C-Me
C10
C1'
C3'
C6
C12
H13
H2'
OMe
H5
6OMe
H2
NMe
Me10
Me12
F2 [ppm]
F1 [ppm]

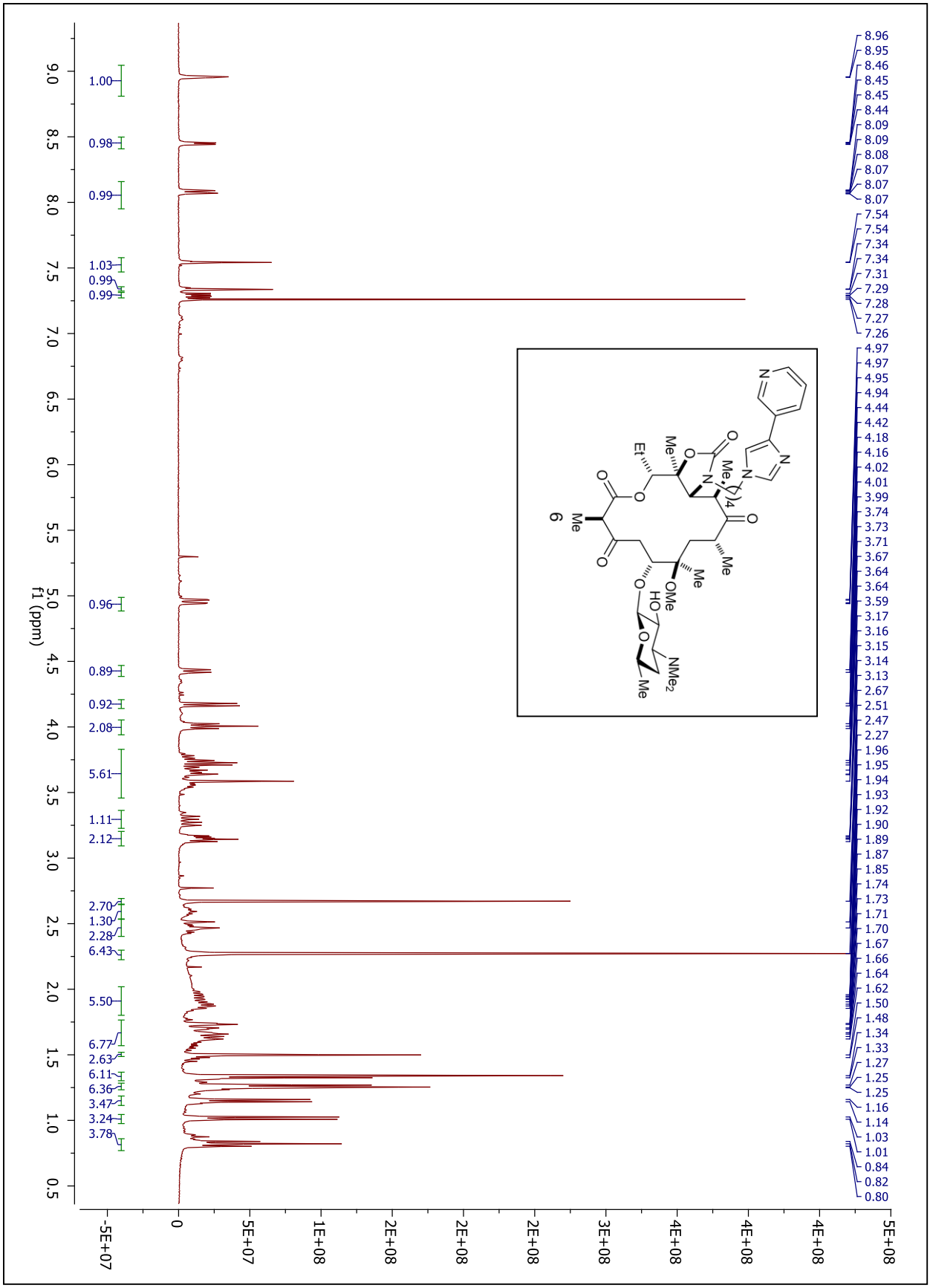

C10
C9
SHC
H1
M10
M9
F2 [ppm]
200
150
100
50
F1 [ppm]


f1 (ppm)
23


216.02
176.58
157.50
155.23
147.49
146.21
138.94
137.81
132.08
130.27
123.61
115.59
98.28
82.76
78.15
77.64
76.35
75.13
71.42
68.84
62.74
59.96
54.67
50.76
48.58
46.86
45.00
40.54
39.18
38.31
28.64
26.32
24.21
20.94
19.76
18.84
18.33
16.20
14.18
13.78
10.39
-2.93
-3.52
f1 (ppm)
23


f1 (ppm)
24


215.67
199.33
169.71
157.22
155.14
147.58
146.48
139.20
137.78
132.02
130.33
123.46
115.47
100.58
82.28
77.76
77.50
75.00
69.48
63.21
60.34
53.32
50.07
46.84
45.10
43.41
42.65
40.58
39.04
31.90
30.45
28.61
24.39
22.45
20.95
19.28
18.53
14.61
14.09
13.97
10.54
f1 (ppm)
24


f1 (ppm)
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
0.98
0.99
1.03
0.99
0.99
0.96
0.89
0.92
2.08
5.61
1.11
2.12
2.70
1.30
2.28
6.43
5.50
6.77
2.63
6.11
6.36
3.47
3.24
3.78
8.96
8.95
8.46
8.45
8.45
8.44
8.09
8.09
8.08
8.07
8.07
8.07
7.54
7.54
7.34
7.34
7.31
7.29
7.28
7.27
7.26
4.97
4.97
4.95
4.94
4.44
4.42
4.18
4.16
4.02
4.01
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3.74
3.73
3.71
3.67
3.64
3.64
3.59
3.17
3.16
3.15
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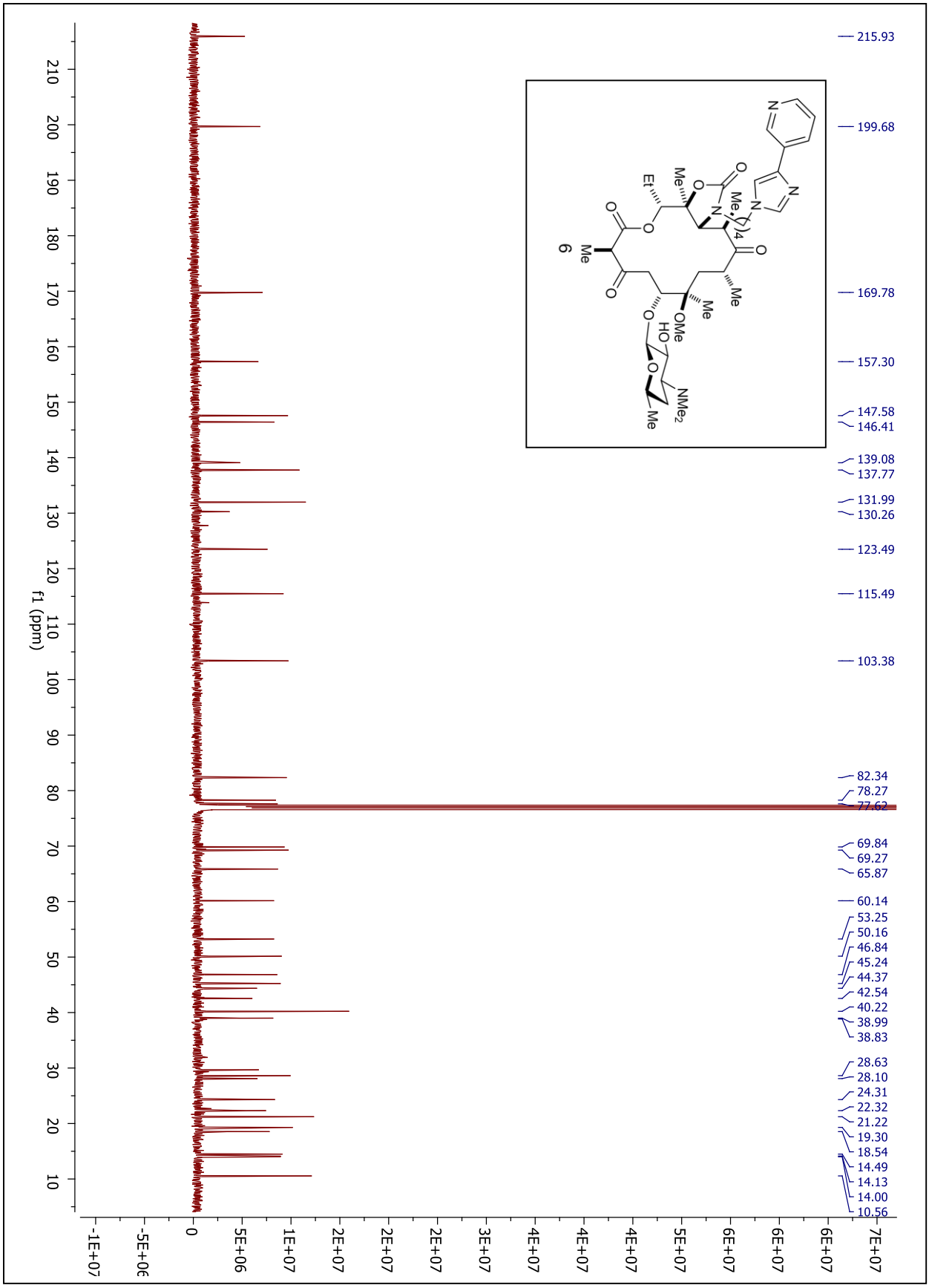
1.95
1.94
1.93
1.92
1.90
1.89
1.87
1.85
1.74
1.73
1.71
1.70
1.67
1.66
1.64
1.62
1.50
1.48
1.34
1.33
1.27
1.25
1.25
1.16
1.14
1.03
1.01
0.84
0.82
0.80
-5E+07
0
5E+07
1E+08
2E+08
2E+08
2E+08
3E+08
4E+08
4E+08
4E+08
5E+08
6
Me
O
O
Et
Me
O
N
N
N
N
Me
4
O
O
Me
Me
OMe
HO
O
O
NMe2
Me


215.93
199.68
169.78
157.30
147.58
146.41
139.08
137.77
131.99
130.26
123.49
115.49
103.38
82.34
78.27
77.62
69.84
69.27
65.87
60.14
53.25
50.16
46.84
45.24
44.37
42.54
40.22
38.99
38.83
28.63
28.10
24.31
22.32
21.22
19.30
18.54
14.49
14.13
14.00
10.56
f1 (ppm)
6

Equations for Saturation and Competitive Binding[7-9]

Saturation Binding[7, 9]

A = [(Amax - Amin) / [L]t] x {[([R]t + [L]t + K_d)/2] –{[([R]t + [L]t + K_d)/2]2 – [L]t[R]t}0.5} + Amin

A = Anisotropy, [L]t = concentration of labeled ligand, [R]t = concentration of receptor and K_d = dissociation constant of the ligand-receptor complex.

Competitive Binding[8, 9]

A = (Amax - Amin) / [L]t x (([L]t x ((2x((Klig + Kcomp + [L]t + [I]t - [R]t)^2 - 3x(Kcomp x ([L]t - [R]t) + Klig x ([I]t - [R]t) + Klig x Kcomp))^0.5x COS(ARCCOS((-2x(Klig + Kcomp + [L]t + [I]t - [R]t)^3 + 9x(Klig + Kcomp + [L]t + [I]t - [R]t) x (Kcomp x ([L]t - [R]t)+ Klig x ([I]t - [R]t) + Klig x Kcomp) - 27x(-1x Klig x Kcomp x [R]t)) / (2x (((Klig + Kcomp + [L]t + [I]t - [R]t)^2 - 3x (Kcomp x ([L]t - [R]t) + Klig x ([I]t - [R]t) + Klig x Kcomp))^3)^0.5))) / 3)) - (Klig + Kcomp + [L]t + [I]t - [R]t))) / ((3x Klig) + ((2x((Klig + Kcomp + [L]t + [I]t - [R]t)^2 - 3x(Kcomp x ([L]t - [R]t) + Klig x ([I]t - [R]t) + Klig x Kcomp))^0.5xCOS(ARCCOS((-2x(Klig + Kcomp + [L]t + [I]t - [R]t)^3 + 9x(Klig + Kcomp + [L]t + [I]t - [R]t) x (Kcomp x ([L]t - [R]t) + Klig x ([I]t - [R]t) + Klig x Kcomp) - 27x(-1x Klig x Kcomp x [R]t)) / (2x((((Klig + Kcomp + [L]t + [I]t - [R]t)^2 - 3x(Kcomp x ([L]t - [R]t) + Klig x ([I]t - [R]t) + Klig x comp))^3)^0.5)))/3)) - (Klig + Kcomp + [L]t + [I]t - [R]t)))) + Amin

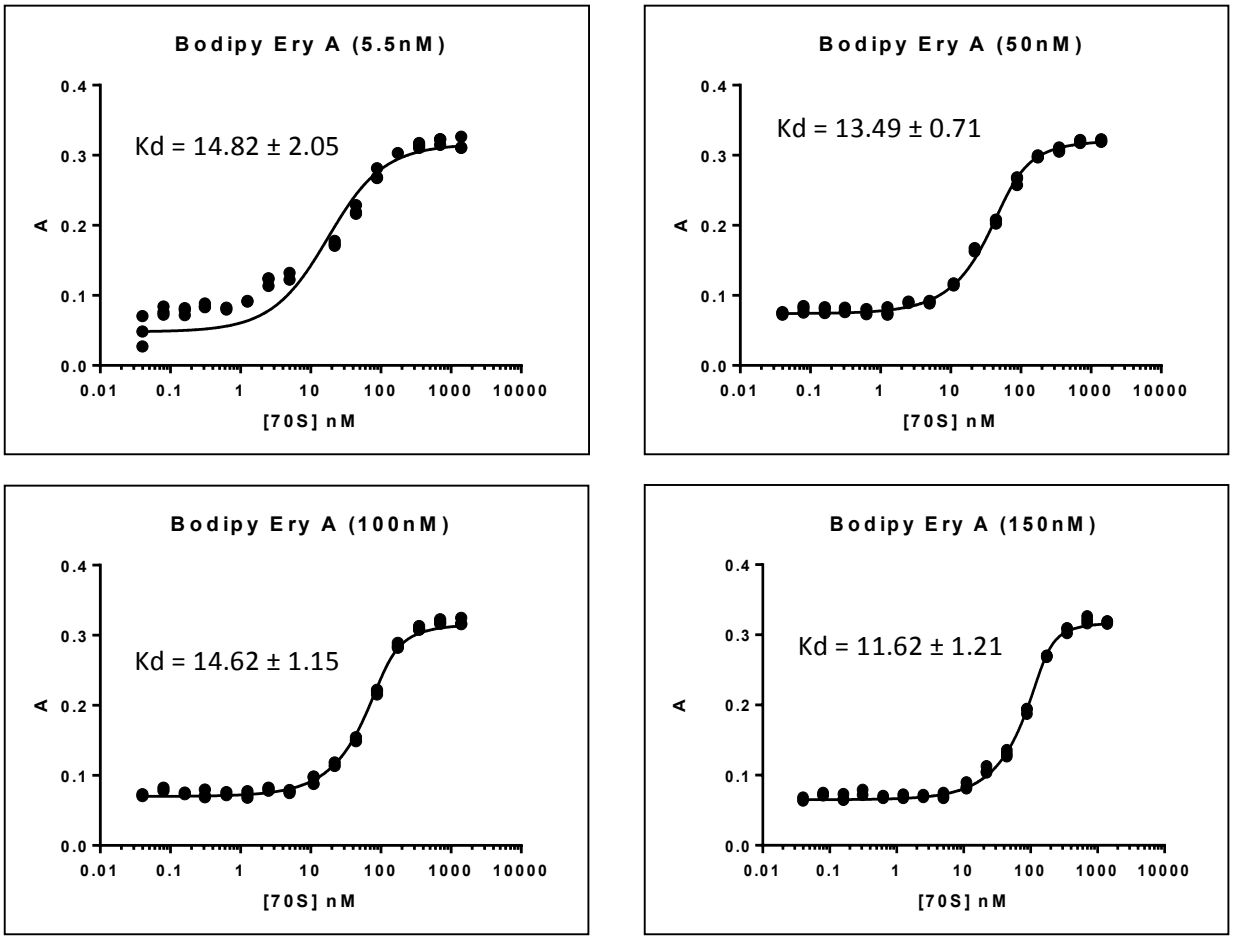
A = Anisotropy, [L]t = concentration of labeled ligand, [R]t = concentration of receptor, [I]t = concentration of the competitive inhibitor, Klig = dissociation of the labeled ligand-receptor complex, Kcomp = dissociation of the competitive inhibitor-receptor complex.

Saturation Binding of BODIPY-Labeled Erythromycin A

Reagents: BODIPY-labeled Erythromycin A was prepared and characterized as described by Yan.[6] *E. coli* 70S Ribosomes were provided by Prof. Barry S. Cooperman, Department of Chemistry, University of Pennsylvania.

Experimental: *E. coli* ribosomes were incubated at 37 °C for 15 minutes and then diluted in binding buffer (20 mM HEPES [pH 7.5], 50 mM NH4Cl, 10 mM MgCl2, 0.05% Tween 20). Ribosomes were added to 96-well plates (Costar flat bottom black) in concentrations from 1400 nM – 0.04 nM. BODIPY Erythromycin A was added to each well at a constant concentration (5.5 nM, 50 nM, 100 nM and 150 nM) and incubated at room temperature for 2 hours before analyzing.

Data Analysis: Fluorescence polarization data were transformed and fit to the above equation. The average K_d (13.64 nM) was used in competition binding experiments.



Competition Binding of BODIPY-labeled erythromycin A with 6:

Reagents: BODIPY-labeled Erythromycin A was prepared and characterized as described by Yan.[6] *E. coli* 70S Ribosomes were provided by Prof. Barry S. Cooperman, Department of Chemistry, University of Pennsylvania.

Experimental: *E. coli* ribosomes were incubated at 37 °C for 15 min and then diluted in binding buffer (20 mM HEPES [pH 7.5], 50 mM NH4Cl, 10 mM $MgCl_2$, 0.05% Tween 20). BODIPY-labeled erythromycin A and Ribosomes were mixed and incubated at room temperature for 30 minutes. 4-desmethyl telithromycin (**6**) was diluted in binding buffer and added to 96-well plates (Costar flat bottom black) in Concentrations from 25600 nM to 0.78 nM. Ribosome/BODIPY Erythromycin A mixture was added to each well for a final concentration of 37.8 nM/5.5 nM, respectively. The plate was incubated at room temperature for 2 h before analyzing.

Data Analysis: Fluorescence polarization data was transformed and fit to the equation above.