Supporting Information

Synthesis and Antibacterial Activity of Propylamycin Derivatives Functionalized at the 5''- and Other Positions with a View to Overcoming Resistance due to Aminoglycoside Modifying Enzymes.

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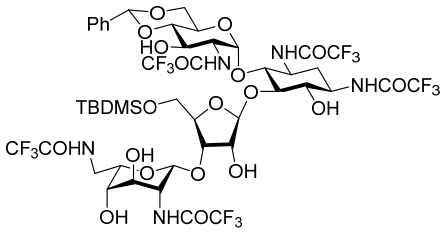
General Experimental

All experiments were carried out under a dry argon atmosphere unless otherwise specified. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Chromatographic purifications were carried over silica gel (230-400 mesh). Thin layer chromatography was performed with precoated glass backed plates (w/UV 254). TLC plates were visualized by UV irradiation (254 nm) and by charring with sulfuric acid in ethanol (20:80, v/v) or with ceric ammonium molybdate solution [$Ce(SO_4)2$: 4 g, $(NH_4)_6Mo_7O_{24}$: 10 g, H_2SO_4: 40 mL, H_2O: 360 mL]. Optical rotations were measured at 589 nm and 21 °C on a digital polarimeter with a path length of 10 cm. ^1H and ^{13}C NMR spectra of all compounds were recorded using at 600 MHz unless otherwise specified and assignments made with the help of COSY, HMBC, and HSQC spectra. ESIHRMS were recorded using a time-of-flight mass spectrometer fitted with an electrospray source.

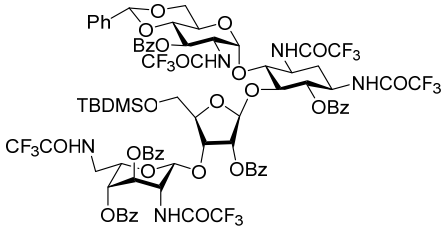


5''-*O*-*tert*-Butyldimethylsilyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (17). An oven-dried 500 mL round-bottom flask was cooled under a stream of argon, equipped with a stirring bar and charged with 4',6'-*O*-benzylidene-penta-*N*-trifluoroacetyl paromomycin **16** (52.6 g, 44.4 mmol, 1.0 equiv), followed by anhydrous DMF (150 mL). Imidazole (7.56 g, 111 mmol, 2.5 equiv) was added to the stirred solution at ambient temperature, after which a solution of *tert*-butyldimethylsilyl chloride (10.4 g, 68.8 mmol, 1.5 equiv) in anhydrous DMF (50 mL) was added dropwise over a period of 15 min. After stirring at ambient temperature for 3 h (the reaction progress was monitored by UPLC-MS assay), water (800 mL) was added to the light yellowish solution and product was back-extracted with EtOAc (3×400 mL). The combined organic extracts were washed with aqueous HCl (0.1 M solution in water, 400 mL), water (400 mL), saturated aqueous $NaHCO_3$ solution (400 mL), then water (400 mL) and, finally brine (300 mL). After drying over anhydrous Na_2SO_4, all volatiles were removed under reduced pressure. The yellow residue was taken up in EtOAc (100 mL) and the solution was slowly poured over 20 min to the stirred hexane (2.0 L) to give a precipitate that was collected by suction filtration. The obtained white powder of **17** (54.9 g, 95 % yield) was dried under reduced pressure and used in subsequent step without further purification. $[\alpha]_D^{23}$ +9.2 (*c* 1.08, MeOH).

^{1}H NMR (400 MHz, CD_3CN) δ: 8.01 (d, *J* = 9.0 Hz, 1H), 7.81 (d, *J* = 9.2 Hz, 1H), 7.66 (d, *J* = 9.0 Hz, 2H), 7.52 – 7.43 (m, 3H), 7.41 – 7.35 (m, 3H), 5.55 (s, 1H), 5.47 (d, *J* = 3.8 Hz, 1H), 5.05 (d, *J* = 1.9 Hz, 1H), 5.00 (d, *J* = 5.4 Hz, 1H), 4.34 (d, *J* = 3.0 Hz, 1H), 4.24 – 4.11 (m, 4H), 4.08 – 3.97 (m, 5H), 3.92 – 3.81 (m, 3H), 3.76 – 3.62 (m, 8H), 3.59 – 3.39 (m, 6H), 2.01 (dt, *J* = 12.9, 4.6 Hz, 1H), 1.64 (q, *J* = 12.9 Hz, 1H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H) ppm.

^{13}C{^{1}H} NMR (101 MHz, CD_3CN) δ: 158.1 – 157.4 (5×$COCF_3$), 138.9, 130.0, 129.2, 129.1, 127.4, 126.3, 115.8 – 115.5 (5×CF_3), 110.4, 102.5, 99.7, 98.2, 86.7, 84.5, 82.3, 79.4, 75.5, 74.1, 72.3, 69.7, 69.1, 68.6, 68.5, 64.6, 63.9, 55.8, 52.3, 50.7, 50.2, 41.3, 32.6, 26.3, 18.9, -5.3, -5.4 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{46}H_{58}N_5O_{19}F_{15}NaSi$ 1320.3153; Found 1320.3129.



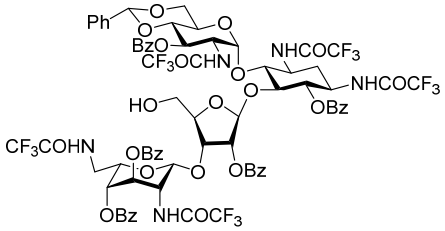
6,3',2'',3''',4'''-Penta-*O*-benzoyl-5''-*O*-*tert*-butyldimethylsilyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (18). To a stirred solution of 5''-*O*-TBDMS paromomycin derivative **17** (54.9 g, 42.3 mmol, 1.0 equiv) in anhydrous pyridine (500 mL) at 0 °C (crushed ice bath) was added DMAP (1.03 g, 8.46 mmol, 0.2 equiv), followed by benzoic anhydride (57.4 g, 254 mmol, 6.0 equiv). The resulting pale yellow solution was heated up to 70 °C and stirred for 40 h, and the reaction progress was monitored by UPLC-MS assay. The resulting dark red suspension was cooled to room temperature, concentrated under reduced pressure and additionally co-evaporated twice with toluene (2×200 mL). The brown oily residue was dissolved in EtOAc (600 mL) and washed with water (400 mL). The aqueous layer was back-extracted with EtOAc (2×200 mL). The combined organic extracts were washed with aqueous HCl (0.1 M solution in water, 200 mL), water (200 mL), saturated aqueous $NaHCO_3$ solution (400 mL), then water (400 mL) and, finally brine (400 mL). Drying over anhydrous Na_2SO_4, filtration and concentration to dryness under reduced pressure afforded brown oil that was filtered through a silica gel pad using gradient elution from 10 % EtOAc in petroleum ether to 50 % EtOAc in petroleum ether. Fractions containing the product (identified by TLC and ESI-MS), were combined and concentrated under reduced pressure. The yellow residue was taken up in DCM (100 mL) and the solution was slowly (within 20 min) poured into well-stirred hexane (2.0 L) to give a precipitate that was collected by suction filtration. The obtained white powder of **18** (70.4 g, 92 % yield) was dried under reduced pressure and used in subsequent step without further purification. An aliquot of **18** (200 mg) was purified by silica gel column chromatography using

gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane; analytical TLC on silica gel, 1:2 EtOAc/petroleum ether, R_f=0.40; $[\alpha]_D^{23}$ +20.9 (*c* 0.96, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.12-8.08 (m, 2H), 8.03-7.99 (m, 2H), 7.97-7.94 (m, 2H), 7.77-7.73 (m, 1H), 7.73 – 7.70 (m, 2H), 7.65 – 7.56 (m, 4H), 7.51 – 7.43 (m, 4H), 7.42 – 7.38 (m, 4H), 7.31 – 7.27 (m, 4H), 7.18 – 7.11 (m, 2H), 7.09-7.05 (m, 1H), 6.99 – 6.90 (m, 2H), 6.19 (d, *J* = 4.2 Hz, 1H), 5.60 – 5.53 (m, 2H), 5.34-5.31 (m, 1H), 5.27 (dd, *J* = 10.6, 9.3 Hz, 1H), 5.11 (dd, *J* = 4.4, 1.6 Hz, 1H), 5.03 (d, *J* = 1.6 Hz, 1H), 5.01 (t, *J* = 3.1 Hz, 1H), 4.65 (dd, *J* = 10.6, 4.2 Hz, 1H), 4.55 (dd, *J* = 6.9, 4.2 Hz, 1H), 4.38 – 4.28 (m, 4H), 4.19-4.15 (m, 1H), 4.04 (dd, *J* = 10.3, 8.3 Hz, 1H), 3.99 – 3.93 (m, 2H), 3.91 – 3.84 (m, 2H), 3.81 (d, *J* = 10.3 Hz, 1H), 3.79 – 3.74 (m, 2H), 3.65-3.61 (m, 2H), 3.58 (dd, *J* = 11.8, 2.3 Hz, 1H), 2.12 (q, *J* = 12.9 Hz, 1H), 1.99 (t, *J* = 4.4 Hz, 1H), 1.03 (s, 9H), 0.20 (s, 3H), 0.17 (s, 3H) ppm, 5H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 168.6, 167.4, 167.3, 166.3, 165.9, 159.6 – 157.4 (5×$COCF_3$), 138.8, 134.9, 134.4, 134.2, 134.1, 131.4, 131.1, 130.9, 130.8, 130.7, 130.7, 130.6, 130.5, 130.4, 130.0, 129.8, 129.6, 129.5, 129.3, 129.0, 128.9, 127.5, 117.7 – 116.5 (5×CF_3), 110.6, 103.2, 98.4, 97.7, 86.7, 83.7, 80.7, 77.8, 77.3, 77.1, 77.0, 76.0, 74.4, 73.8, 72.1, 71.8, 69.4, 67.7, 66.0, 64.6, 62.0, 61.5, 53.3, 50.5, 49.7, 49.5, 41.5, 32.2, 27.0, 19.7, -4.9, -5.3 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{81}H_{78}N_5O_{24}F_{15}NaSi$ 1840.4464; Found 1840.4443.



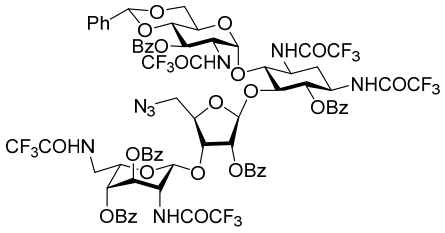
6,3',2'',3''',4'''-Penta-*O*-benzoyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (19). To a stirred solution of penta-*O*-benzoyl paromomycin derivative **18** (70.4 g, 38.7 mmol, 1.0 equiv) in anhydrous THF (350 mL) was dropwise added TBAF (1.0 M solution in THF; 50.3 mL, 50.3 mmol, 1.3 equiv) over 20 min period. Upon completion of the addition, color of the solution changed from light yellow to light brownish. The solution was stirred at ambient temperature for 20 h, and the reaction progress was monitored by UPLC-MS assay. The resulting brown reaction mixture was quenched with saturated aqueous NH_4Cl solution (150 mL), whereupon color changed to orange. The obtained orange suspension was evaporated to dryness under reduced pressure. Water (300 mL) was added to the residue and the solution was back-extracted with EtOAc (3×400 mL). Combined organic layers were washed with brine (2×400 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The yellow residue was taken up in EtOAc (120 mL) and the solution was slowly (over 20 min)

poured into well-stirred hexane (2.0 L) to give a precipitate that was collected by suction filtration. The obtained white powder of **19** (66.0 g, 100 % yield) was dried under reduced pressure and used in subsequent step without further purification. An aliquot of **7** (200 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH; analytical TLC on silica gel, 1:2 EtOAc/petroleum ether, R_f=0.21. $[\alpha]_D^{23}$ +17.8 (*c* 1.07, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.27 (dd, *J* = 8.4, 1.4 Hz, 2H), 8.11 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.97 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.80 – 7.76 (m, 1H), 7.74 – 7.72 (m, 2H), 7.71 – 7.67 (m, 2H), 7.65 – 7.62 (m, 1H), 7.60 – 7.56 (m, 1H), 7.49 – 7.47 (m, 2H), 7.47 – 7.43 (m, 3H), 7.42 – 7.38 (m, 4H), 7.32 – 7.28 (m, 3H), 7.24 – 7.20 (m, 2H), 7.00 – 6.94 (m, 1H), 6.91 (dd, *J* = 8.4, 6.8 Hz, 2H), 6.21 (d, *J* = 4.1 Hz, 1H), 5.62 – 5.56 (m, 2H), 5.36 (s, 1H), 5.33 – 5.29 (m, 1H), 5.29 – 5.26 (m, 2H), 5.24 – 5.22 (m, 1H), 5.08 (d, *J* = 4.4 Hz, 1H), 4.73 (dd, *J* = 10.6, 4.1 Hz, 1H), 4.51 (dd, *J* = 8.4, 4.4 Hz, 1H), 4.45 (ddd, *J* = 8.9, 4.4, 2.0 Hz, 1H), 4.40 – 4.29 (m, 4H), 4.08 (dd, *J* = 10.3, 8.4 Hz, 1H), 4.01 – 3.93 (m, 3H), 3.91 – 3.84 (m, 2H), 3.83 – 3.79 (m, 1H), 3.75 – 3.67 (m, 2H), 3.54 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.17 (q, *J* = 12.9 Hz, 1H), 2.03 (dt, *J* = 12.9, 4.4 Hz, 1H) ppm, 6H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.3, 167.2, 166.5, 166.1, 165.7, 160.0 – 158.3 (5×$COCF_3$), 138.9, 135.0, 135.0, 134.4, 134.4, 134.3, 131.3, 131.0, 130.9, 130.8, 130.7, 130.6, 130.5, 130.0, 129.9, 129.9, 129.7, 129.5, 129.3, 129.2, 129.1, 129.0, 127.5, 118.5 – 115.9 (5×CF_3), 110.3, 103.1, 98.4, 97.8, 86.8, 82.4, 80.5, 77.4, 77.2, 76.6, 75.7, 74.5, 71.7, 69.8, 69.4, 67.9, 64.7, 61.7, 53.3, 50.5, 50.1, 49.6, 49.5, 40.8, 32.1 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{75}H_{64}N_5O_{24}F_{15}Na$ 1726.3599; Found 1726.3567.



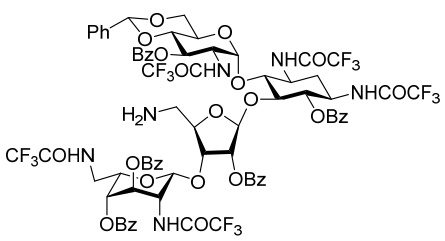
5''-Deoxy-5''-azido-6,3',2'',3''',4'''-penta-*O*-benzoyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (20). A stirred solution of 5''-hydroxy paromomycin derivative **19** (40.0 g, 23.5 mmol, 1.0 equiv) in anhydrous DCM (500 mL) was cooled to -10 °C (crushed ice/NaCl bath) and treated with 2,6-lutidine (13.6 mL, 117 mmol, 5.0 equiv). Trifluoromethanesulfonic anhydride (11.8 mL, 70.4 mmol, 3.0 equiv) was then added dropwise at -10 °C over a period of 20 min. Upon completion of the addition the yellow solution turned brown and was stirred at -10 °C for 1 h. The resulting mixture was washed with

water (300 mL) and organic layer was back-extracted with DCM (2×300 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residual brownish oil was dissolved in MeCN (200 mL) and sodium azide (7.79 g, 120 mmol, 5.0 equiv) was added, followed by 18-crown-6 (3.17 g, 12.0 mmol, 0.5 equiv). The resulting suspension was stirred at ambient temperature for 3 h, and the reaction progress was monitored by UPLC-MS assay. The obtained orange suspension was concentrated under the reduced pressure, and the residue was partitioned between EtOAc (300 mL) and water (400 mL). The aqueous layer was back-extracted with EtOAc (2×300 mL) and combined organic extracts were washed with brine (2×200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography using gradient elution from 20 % EtOAc in petroleum ether to 100 % EtOAc. Fractions containing the product (detected by TLC and ESI-MS) were combined and concentrated under reduced pressure. The yellow oily residue was taken up in DCM (30 mL) and the solution was slowly (over 20 min) poured into well-stirred hexane (1.0 L) to give a precipitate that was collected by suction filtration. The obtained pale yellow powder of **20** (31.7 g, 77 % yield) was dried under reduced pressure and used in subsequent step. An aliquot of **20** (200 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:2 EtOAc/petroleum ether, R_f=0.30. $[\alpha]_D^{23}$ +41.9 (*c* 1.17, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.29 (dd, *J* = 8.4, 1.4 Hz, 2H), 8.11 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.98 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.81 – 7.78 (m, 1H), 7.74 – 7.68 (m, 4H), 7.66 – 7.62 (m, 1H), 7.60 – 7.57 (m, 1H), 7.50 – 7.47 (m, 2H), 7.47 – 7.42 (m, 3H), 7.41 – 7.37 (m, 4H), 7.31 – 7.27 (m, 3H), 7.24 – 7.19 (m, 2H), 6.97 – 6.92 (m, 1H), 6.91 – 6.86 (m, 2H), 6.30 (d, *J* = 4.1 Hz, 1H), 5.63 – 5.55 (m, 2H), 5.36 (s, 1H), 5.32 – 5.30 (m, 1H), 5.29 – 5.26 (m, 2H), 5.25 – 5.21 (m, 1H), 5.06 (d, *J* = 4.4 Hz, 1H), 4.69 (dd, *J* = 10.6, 4.1 Hz, 1H), 4.53 (dd, *J* = 8.6, 4.4 Hz, 1H), 4.49 (ddd, *J* = 9.3, 4.4, 1.9 Hz, 1H), 4.42 – 4.27 (m, 4H), 4.10 (dd, *J* = 10.3, 8.4 Hz, 1H), 3.98 (td, *J* = 9.7, 4.7 Hz, 1H), 3.95 (d, *J* = 9.3 Hz, 1H), 3.93 – 3.88 (m, 2H), 3.88 – 3.82 (m, 2H), 3.80 (dt, *J* = 8.6, 2.6 Hz, 1H), 3.67 (dd, *J* = 13.5, 2.6 Hz, 1H), 3.43 (dd, *J* = 14.1, 4.4 Hz, 1H), 2.18 (q, *J* = 12.9 Hz, 1H), 2.04 (dt, *J* = 12.9, 4.4 Hz, 1H) ppm, 5H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.3, 167.2, 166.5, 166.0, 165.7, 159.8 – 158.4 (5×$COCF_3$), 138.8, 135.0, 135.0, 134.4, 134.4, 134.3, 131.3, 131.0, 130.9, 130.8, 130.7, 130.6, 130.5, 130.0, 129.9, 129.9, 129.7, 129.5, 129.4, 129.2, 129.1, 129.0, 127.5, 118.7 – 115.7 (5×CF_3), 110.6, 103.1, 98.3, 98.0, 87.1, 80.8, 79.5, 77.3, 77.1, 76.4, 75.1, 74.3, 71.6, 69.8, 69.5, 67.8, 64.8, 53.3, 50.6, 50.4, 50.1, 49.6, 49.5, 40.7, 32.1 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{75}H_{63}N_8O_{23}F_{15}Na$ 1751.3664; Found 1751.3624.



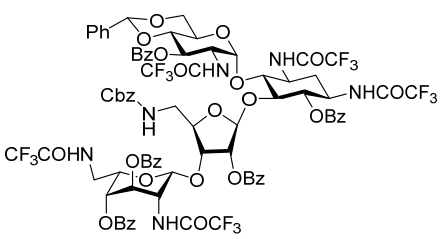
5''-Deoxy-5''-amino-6,3',2'',3''',4'''-penta-*O*-benzoyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (21). To a stirred solution of 5''-deoxy-5''-azido paromomycin derivative **20** (31.7 g, 18.3 mmol, 1.0 equiv) in a mixture of degassed THF (300 mL, bubbling a vigorously stirred THF with argon for 15 min) and water (206 mL) was dropwise added PMe_3 (1.0 M solution in THF; 18.3 mL, 18.3 mmol, 1.0 equiv) over 20 min at 0 °C (crushed ice bath). After stirring at ambient temperature for 10 h (the reaction progress was monitored by UPLC-MS assay), the yellowish mixture was concentrated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 100 % EtOAc. Fractions containing the product (detected by TLC and ESI-MS) were combined and concentrated under reduced pressure to give product **21** (12.6 g, 40 % yield) as a white powder. An aliquot of **21** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 10 % to 90 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.22; $[\alpha]_D^{23}$ +18.0 (*c* 1.00, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.35 – 8.30 (m, 2H), 8.14 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.97 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.83 – 7.78 (m, 1H), 7.75 – 7.72 (m, 2H), 7.72 – 7.69 (m, 2H), 7.67 – 7.65 (m, 1H), 7.62 – 7.58 (m, 1H), 7.52 – 7.48 (m, 2H), 7.48 – 7.42 (m, 4H), 7.42 – 7.38 (m, 3H), 7.32 – 7.28 (m, 3H), 7.23 (dd, *J* = 8.4, 7.4 Hz, 2H), 6.97 – 6.92 (m, 1H), 6.89 (dd, *J* = 8.4, 6.7 Hz, 2H), 5.99 (d, *J* = 4.2 Hz, 1H), 5.64 – 5.58 (m, 2H), 5.40 (s, 1H), 5.35 – 5.32 (m, 1H), 5.30 (t, *J* = 2.9 Hz, 1H), 5.28 – 5.26 (m, 1H), 5.13 (d, *J* = 4.2 Hz, 1H), 4.74 (dd, *J* = 10.6, 4.2 Hz, 1H), 4.46 – 4.42 (m, 1H), 4.41 (d, *J* = 8.8 Hz, 1H), 4.37 (d, *J* = 4.4 Hz, 1H), 4.36 – 4.34 (m, 1H), 4.34 – 4.28 (m, 2H), 4.28 – 4.25 (m, 1H), 4.25 – 4.22 (m, 1H), 4.15 – 4.11 (m, 1H), 4.01 – 3.96 (m, 3H), 3.93 – 3.89 (m, 1H), 3.86 (t, *J* = 9.9 Hz, 1H), 3.34 (dd, *J* = 14.1, 3.4 Hz, 1H), 3.27 (dd, *J* = 13.7, 2.8 Hz, 1H), 2.92 (dd, *J* = 13.7, 8.4 Hz, 1H), 2.25 (q, *J* = 12.9 Hz, 1H), 2.05 (dt, *J* = 12.9, 4.4 Hz, 1H) ppm, 7H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.3, 167.2, 166.4, 165.8, 165.6, 160.2 – 158.4 (5×$COCF_3$), 138.7, 135.0, 135.0, 134.5, 134.5, 134.5, 131.3, 131.0, 131.0, 130.7, 130.6, 130.6, 130.5, 130.0, 130.0, 129.9,

129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 127.5, 117.8 – 116.5 (5×CF_3), 110.5, 103.1, 98.0, 97.6, 87.3, 80.5, 77.5, 77.2, 77.1, 75.8, 75.6, 71.2, 69.9, 69.4, 67.7, 64.8, 53.6, 50.5, 49.9, 49.6, 49.4, 44.4, 40.7, 31.8 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{75}H_{66}N_6O_{23}F_{15}$ 1703.3940; Found 1703.3945.



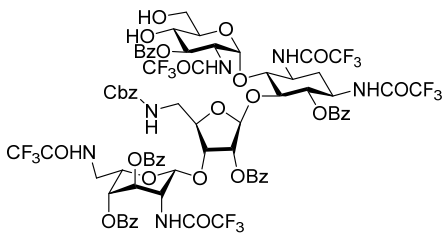
5''-Deoxy-5''-(*O*-benzylcarbamoyl)-6,3',2'',3''',4'''-penta-*O*-benzoyl-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (22). A stirred solution of 5''-deoxy-5''-amino paromomycin derivative **21** (3.59 g, 2.11 mmol, 1.0 equiv) in anhydrous DCM (30 mL) was cooled to 0 °C (crushed ice bath) and Cbz-Cl (1.2 mL, 8.43 mmol, 4.0 equiv) was added dropwise over 5 min. The resulting yellowish solution was stirred at 0 °C for 1 h followed by dropwise addition (over 5 min) of pyridine (0.34 mL, 4.21 mmol, 2.0 equiv). The resulting yellow solution was stirred at 0 °C for 1 h, then warmed up to room temperature and stirring was continued for additional 2 h. The reaction progress was monitored by UPLC-MS assay. The yellowish solution was diluted with water (50 mL) and back-extracted with DCM (2×30 mL). Combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude yellow oil was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane. Fractions containing the product (detected with TLC and ESI-MS) were combined and concentrated under reduced pressure. The residue was dissolved in DCM (10 mL) and hexane (100 mL) was added. The formed white precipitate was collected by suction filtration and dried under reduced pressure to give product **22** (3.45 g, 89 % yield) as a white powder. An aliquot of **22** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 10 % to 90 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:2 EtOAc/petroleum ether, R_f=0.24. $[\alpha]_D^{23}$ +6.5 (*c* 1.74, MeOH).

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.66 (t, J = 5.9 Hz, 1H), 8.12 – 8.02 (m, 2H), 7.97 – 7.93 (m, 4H), 7.93 – 7.87 (m, 1H), 7.87 – 7.83 (m, 2H), 7.81 – 7.74 (m, 2H), 7.69 – 7.65 (m, 2H), 7.63 – 7.60 (m, 1H), 7.59 – 7.57 (m, 1H), 7.53 – 7.46 (m, 5H), 7.43 – 7.40 (m, 2H), 7.38 – 7.36 (m, 2H), 7.33 – 7.28 (m, 11H), 7.21 – 7.15 (m, 2H), 7.09 – 7.03 (m, 2H), 7.01 – 6.89 (m, 1H), 6.08 – 5.95 (m, 1H), 5.70 – 5.61 (m, 1H), 5.59 – 5.53 (m, 1H), 5.46 – 5.40 (m, 2H), 5.34 – 5.24 (m, 3H), 5.14 (d, J = 5.1 Hz, 1H), 5.11 (t, J = 4.5 Hz, 1H), 5.08 – 5.02 (m, 2H), 4.95 – 4.82 (m, 1H), 4.57 – 4.50 (m, 1H), 4.33 – 4.27 (m, 3H), 4.25 – 4.18 (m, 2H), 4.15 – 4.07 (m, 2H), 4.00

– 3.97 (m, 2H), 3.90 – 3.81 (m, 2H), 3.71 – 3.63 (m, 2H), 3.45 – 3.28 (m, 1H), 3.27 – 3.13 (m, 1H), 2.39 – 2.29 (m, 1H) ppm.

$^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ: 167.3, 167.2, 165.8, 165.1, 164.2, 157.7, 158.4 – 157.0 (5×$COCF_3$), 136.8, 136.3, 134.4, 134.3, 134.2, 133.8, 133.7, 130.3, 130.0, 129.9, 129.8, 129.8, 129.4, 128.9, 128.9, 128.8, 128.7, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.4, 126.4, 126.3, 117.1 – 114.3 (5×CF_3), 107.0, 102.0, 98.8, 97.6, 81.1, 78.7, 77.9, 75.5, 74.2, 72.7, 69.9, 68.9, 68.4, 67.1, 64.1, 53.9, 49.9, 49.4, 49.2, 48.7, 42.3, 40.9, 31.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{71}N_6O_{25}F_{15}Na$ 1859.4127; Found 1859.4125.



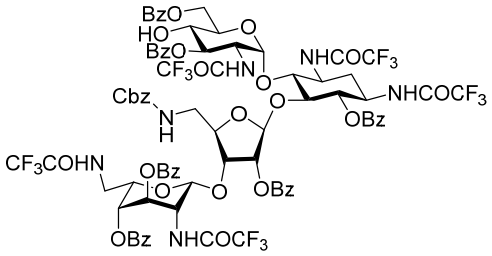
5′′-Deoxy-5′′-(*O*-benzylcarbamoyl)-6,3′,2′′,3′′′,4′′′-penta-*O*-benzoyl-1,3,2′,2′′′,6′′′-penta-*N*-trifluoroacetyl paromomycin (23). A solution of 5′′-deoxy-5′′-carboxybenzylamino paromomycin derivative **22** (2.64 g, 1.43 mmol, 1.0 equiv) in 80 % aqueous acetic acid (12.4 mL, prepared by mixing 10.1 mL of glacial acetic acid and 2.3 mL of water) was heated to 65 °C for 18 h, and the reaction progress was monitored by UPLC-MS assay. After cooling to room temperature, the colorless solution was concentrated under reduced pressure and the residue was co-evaporated with toluene (50 mL). The crude colorless oil was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 100 % EtOAc. Fractions containing the product (detected with TLC and ESI-MS) were combined and concentrated under reduced pressure. The residue was dissolved in DCM (10 mL) and hexane (100 mL) was added. The formed white precipitate was collected by suction filtration and dried under reduced pressure to give product **23** (2.16 g, 86 % yield) as a white powder. An aliquot of **23** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.32. $[\alpha]_D^{23}$ +27.4 (*c* 1.02, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.26 – 8.23 (m, 2H), 8.10 – 8.06 (m, 2H), 8.00 – 7.97 (m, 2H), 7.85 – 7.80 (m, 2H), 7.76 – 7.72 (m, 1H), 7.64 – 7.58 (m, 6H), 7.48 – 7.42 (m, 7H), 7.37 – 7.33 (m, 2H), 7.32 – 7.29 (m, 1H), 7.29 – 7.24 (m, 2H), 7.15 (dd, J =7.7, 7.5 Hz, 1H), 7.01 – 6.97 (m, 2H), 5.88 (d, J = 4.0 Hz, 1H), 5.38 (dd, J = 11.0, 8.8 Hz, 1H), 5.34 (s, 1H), 5.33 – 5.30 (m, 1H), 5.29 (t, J = 3.0 Hz, 1H), 5.25 (d, J = 12.6 Hz, 1H), 5.22 (d, J = 4.6 Hz, 1H), 5.20 – 5.17 (m, 2H), 5.17 – 5.13 (m, 1H), 4.54 (dd, J = 11.0, 4.0 Hz, 1H), 4.51 – 4.47 (m, 1H),

4.36 – 4.31 (m, 2H), 4.25 (dd, J = 8.1, 3.6 Hz, 1H), 4.12 (dd, J = 10.4, 8.6 Hz, 1H), 3.95 (ddd, J = 8.4, 6.1, 3.0 Hz, 1H), 3.90 (d, J = 10.5 Hz, 1H), 3.81 – 3.79 (m, 2H), 3.78 – 3.75 (m, 1H), 3.62 (dd, J = 14.2, 3.2 Hz, 1H), 3.57 (d, J = 6.5 Hz, 2H), 3.40 (dd, J = 14.2, 6.2 Hz, 1H), 2.11 (q, J = 12.6 Hz, 1H), 2.03 (dt, J = 13.1, 4.6 Hz, 1H) ppm, 8H exchangeable protons merge with H_2O.

$^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ: 167.8, 167.2, 166.5, 166.4, 165.5, 159.2, 159.9 – 158.2 (5×$COCF_3$), 138.3, 135.1, 134.9, 134.7, 134.4, 134.3, 134.2, 131.3, 131.1, 131.0, 130.9, 130.8, 130.7, 130.5, 129.9, 129.8, 129.6, 129.5, 129.5, 129.4, 129.4, 129.2, 129.0, 128.8, 118.8 – 115.5 (5×CF_3), 109.7, 98.9, 97.5, 85.1, 81.9, 78.5, 77.7, 76.8, 76.5, 74.9, 74.2, 73.8, 69.6, 69.4, 67.8, 61.9, 53.4, 50.4, 50.3, 49.7, 49.6, 43.8, 41.0, 32.3 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{76}H_{65}N_6O_{25}F_{15}Na$ 1771.3814; Found 1771.3860



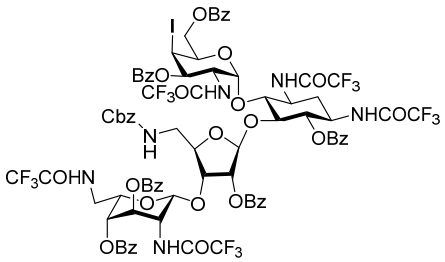
5''-Deoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (24). A stirred solution of paromomycin derivative **23** (2.06 g, 1.18 mmol, 1.0 equiv) in anhydrous MeCN (25 mL) was cooled to 0 °C (crushed ice bath). NEt_3 (0.25 mL, 1.76 mmol, 1.5 equiv) was added, followed by dropwise addition of a benzoyl cyanide (170 mg, 1.29 mmol, 1.1 equiv) solution in anhydrous MeCN (5 mL) over 30 min period. After stirring for additional 2 h at 0 °C, the clear colorless solution was quenched by addition of MeOH (0.5 mL) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane. Fractions containing the product (detected with TLC and ESI-MS), were combined and concentrated under reduced pressure. The residue was dissolved in DCM (10 mL) and hexane (100 mL) was added to the well-stirred solution. The formed white precipitate was collected by suction filtration and dried under reduced pressure to give product **24** (1.98 g, 91 % yield) as a white powder. An aliquot of **24** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.44. $[\alpha]_D^{23}$ +39.3 (*c* 1.09, MeOH).

^1H NMR (600 MHz, CD_3OD) δ: 8.30 – 8.17 (m, 2H), 8.14 – 8.03 (m, 4H), 8.04 – 7.98 (m, 2H), 7.84 – 7.76 (m, 2H), 7.74 (dd, J = 7.7, 7.5 Hz, 1H), 7.68 – 7.56 (m, 5H), 7.56 – 7.48 (m, 2H), 7.48 – 7.40 (m, 7H), 7.36 – 7.27 (m, 4H), 7.27 – 7.18 (m, 3H), 7.11 (dd, J = 7.7, 7.5 Hz, 1H), 7.01 – 6.93 (m, 2H), 5.93 (d, J = 4.0 Hz, 1H), 5.49

– 5.44 (m, 1H), 5.37 (s, 1H), 5.33 (t, *J* = 9.8 Hz, 1H), 5.27 (t, *J* = 3.0 Hz, 1H), 5.23-5.12 (m, 3H), 5.11 (d, *J* = 12.6 Hz, 1H), 5.06 (d, *J* = 12.6 Hz, 1H), 4.67 (d, *J* = 12.6 Hz, 1H), 4.64 – 4.55 (m, 2H), 4.47 – 4.42 (m, 1H), 4.39 (t, *J* = 9.0 Hz, 1H), 4.36 – 4.28 (m, 2H), 4.26 (dd, *J* = 8.4, 4.7 Hz, 1H), 4.12 (dd, *J* = 10.3, 8.4 Hz, 1H), 4.09 – 4.02 (m, 2H), 3.96 – 3.88 (m, 1H), 3.85 – 3.80 (m, 1H), 3.62 (dd, *J* = 14.6, 3.2 Hz, 1H), 3.55 – 3.44 (m, 2H), 3.37 (dd, *J* = 14.6, 5.7 Hz, 1H), 2.15 (q, *J* = 12.9 Hz, 1H), 2.05 (dt, *J* = 12.9, 4.4 Hz, 1H) ppm, 7H exchangeable protons merge with H_2O.

$^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ: 168.1, 167.8, 167.2, 166.4, 166.3, 165.5, 159.0, 160.1 – 157.7 (5×$COCF_3$), 138.3, 135.0, 134.9, 134.7, 134.4, 134.3, 131.3, 131.2, 131.0, 130.9, 130.8, 130.7, 130.6, 130.5, 129.9, 129.7, 129.6, 129.6, 129.5, 129.4, 129.4, 129.2, 128.9, 128.5, 118.5 – 116.0 (5×CF_3), 109.6, 98.8, 97.6, 85.3, 81.5, 78.1, 78.1, 76.7, 76.6, 74.9, 73.7, 72.0, 69.6, 69.3, 67.7, 67.7, 63.7, 53.5, 50.6, 50.3, 49.8, 43.4, 40.8, 32.2 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{71}N_6O_{26}F_{15}Na$ 1875.4076; Found 1875.4088.



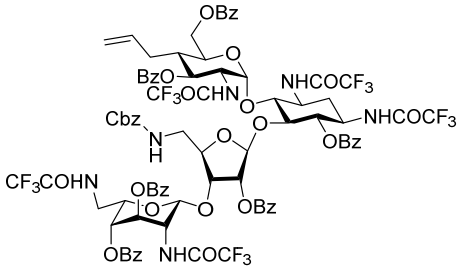
4',5''-Dideoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-4'-iodo-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl 4'-*epi*-paromomycin (25). A stirred solution of hexa-*O*-benzoyl paromomycin derivative **24** (6.39 g, 3.45 mmol, 1.0 equiv) in anhydrous DCM (70 mL) was cooled to 0 °C (crushed ice bath) and treated with anhydrous pyridine (2.1 mL, 25.9 mmol, 7.5 equiv). Trifluoromethanesulfonic anhydride (1.7 mL, 10.3 mmol, 3.0 equiv) was then added dropwise over a period of 15 min. Upon completion of the addition, the yellow solution turned light brown. After stirring at 0 °C for 1 h, the solution was washed with water (120 mL) and organic layer was back-extracted with DCM (2×60 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The obtained yellow oil was dissolved in anhydrous acetone (70 mL) and potassium iodide (5.73 g, 34.5 mmol, 10.0 equiv) was added. The resulting well-stirred suspension was heated at 55 °C for 6 h, and the reaction progress was monitored by UPLC-MS assay. After cooling to room temperature, the orange suspension was filtered through the pad of *Celite*®. The filter plug was washed with DCM, the combined filtrates were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane. Fractions containing the product (detected with TLC and ESI-MS) were combined and concentrated under reduced pressure. The residue was dissolved in DCM (20 mL) and hexane (200 mL) was added to the well-stirred solution. The formed

white precipitate was collected by suction filtration and dried under reduced pressure to give product **25** (5.64 g, 83 % yield) as a white powder. An aliquot of **25** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.56; $[\alpha]_D^{23}$ +28.8 (*c* 1.04, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.31-8.23 (m, 2H), 8.13-8.06 (m, 2H), 8.06 – 7.98 (m, 4H), 7.82-7.73 (m, 3H), 7.70 – 7.57 (m, 5H), 7.56 – 7.37 (m, 11H), 7.35 – 7.30 (m, 2H), 7.29 – 7.20 (m, 3H), 7.05 (dd, *J* = 7.6, 7.4 Hz, 1H), 7.00-6.90 (m, 2H), 6.11 (d, *J* = 3.8 Hz, 1H), 5.40 (s, 1H), 5.35 – 5.29 (m, 2H), 5.27 – 5.25 (m, 1H), 5.24 – 5.11 (m, 5H), 5.03 (dd, *J* = 11.0, 3.8 Hz, 1H), 4.66 (dd, *J* = 11.0, 3.8 Hz, 1H), 4.61 (dd, *J* = 10.7, 5.4 Hz, 1H), 4.52 – 4.47 (m, 1H), 4.41 – 4.28 (m, 5H), 4.07 (dd, *J* = 10.3, 8.5 Hz, 1H), 3.94 (ddd, *J* = 8.9, 5.8, 3.5 Hz, 1H), 3.90 – 3.86 (m, 1H), 3.75 – 3.64 (m, 2H), 3.61 – 3.49 (m, 2H), 3.42 (dd, *J* = 14.7, 5.8 Hz, 1H), 2.14 (q, *J* = 12.8 Hz, 1H), 2.04 (dt, *J* = 12.8, 4.4 Hz, 1H) ppm, 6H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.1, 167.0, 166.6, 166.5, 166.2, 165.6, 159.2, 160.2 – 158.1 (5×$COCF_3$), 138.4, 135.0, 135.0, 134.9, 134.7, 134.6, 134.3, 131.3, 131.1, 130.9, 130.9, 130.7, 130.6, 130.5, 130.2, 129.9, 129.8, 129.6, 129.5, 129.4, 129.3, 129.1, 128.9, 128.8, 118.5 – 115.7 (5×CF_3), 109.8, 98.8, 97.3, 85.4, 81.7, 77.9, 77.6, 76.7, 76.7, 73.7, 70.2, 69.7, 67.8, 67.7, 67.6, 67.5, 52.0, 50.3, 50.3, 49.7, 43.4, 40.9, 37.8, 32.0 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{70}N_6O_{25}F_{15}NaI$ 1985.3093; Found 1985.3112.



4'-Allyl-4',5''-dideoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (26). To a stirred solution of 4'-deoxy-4'-iodo-4'-*epi*-paromomycin **25** (1.01 g, 0.51 mmol, 1.0 equiv) in anhydrous 1,2-dichlorobenzene (35 mL) under argon atmosphere was added allyl phenyl sulfone (0.79 mL, 5.13 mmol, 10.0 equiv). After stirring at ambient temperature for 5 min, the resulting colorless clear solution was cooled to 0 °C (crushed ice bath) and BEt_3 (1.0 M solution in hexanes; 1.54 mL, 1.54 mmol, 3.0 equiv) was then added over a period of 1 min with gentle stirring keeping the needle under the surface of the solution. The septa was removed and the colorless reaction solution was stirred under air at 0 °C for 1 h, whereupon another portion of BEt_3 (1.0 M solution in hexanes; 1.54 mL, 1.54 mmol, 3.0 equiv)

was added by the same technique and stirring continued for another 1 hour. Altogether, five portions of BEt_3 (5×1.54 mL) were added after every 1-2 h to achieve full conversion (the reaction progress was monitored by UPLC-MS assay). Them, the reaction mixture was concentrated under reduced pressure, the residue was dissolved in DCM (20 mL) and filtered through a silica gel pad (45×45 mm). The filter plug was first washed with DCM (300 mL) to separate the excess of allyl phenyl sulfone, and then washed with EtOAc (250 mL) to collect the product). The product-containing filtrate was evaporated under reduced pressure and the residue was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 70 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Fractions containing the product (detected by TLC and ESI-MS), were combined and concentrated under reduced pressure. The residue was dissolved in DCM (5 mL) and hexane (50 mL) was added to the solution. The formed white precipitate was dried under reduced pressure to give product **26** (498 mg, 40 % yield) as a white powder; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.60; $[\alpha]_D^{23}$ +28.7 (*c* 1.21, MeOH).

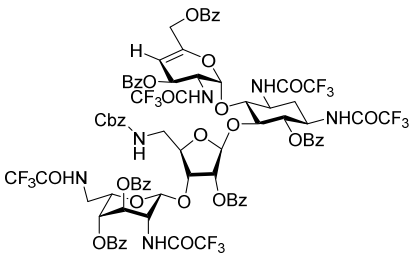
^{1}H NMR (400 MHz, CD_3OD) δ: 8.29 – 8.21 (m, 2H), 8.10-8.02 (m, 4H), 8.01-7.95 (m, 2H), 7.83 – 7.74 (m, 3H), 7.70 – 7.58 (m, 5H), 7.55 – 7.42 (m, 9H), 7.36 – 7.22 (m, 7H), 7.08 (dd, *J* = 7.7, 7.5 Hz, 1H), 7.00-6.93 (m, 2H), 6.02 (d, *J* = 4.0 Hz, 1H), 5.88 – 5.72 (m, 1H), 5.50 (t, *J* = 10.7 Hz, 1H), 5.38 (s, 1H), 5.33 (dd, *J* = 10.5, 9.3 Hz, 1H), 5.28 (t, *J* = 2.9 Hz, 1H), 5.24 – 5.17 (m, 3H), 5.16 – 5.07 (m, 2H), 5.06 – 4.97 (m, 1H), 4.91 – 4.88 (m, 1H), 4.67 – 4.58 (m, 3H), 4.49 – 4.45 (m, 1H), 4.41 (t, *J* = 8.8 Hz, 1H), 4.38 – 4.26 (m, 3H), 4.15 – 4.07 (m, 2H), 3.98 – 3.89 (m, 1H), 3.88 – 3.85 (m, 1H), 3.72 – 3.63 (m, 1H), 3.58 – 3.48 (m, 2H), 3.42 – 3.34 (m, 1H), 2.50 – 2.40 (m, 1H), 2.31 – 2.24 (m, 2H), 2.14 (q, *J* = 12.7 Hz, 1H), 2.07-2.00 (m, 1H) ppm, 6H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ: 67.8, 167.6, 167.2, 166.4, 166.2, 165.6, 160.2 – 158.3 (5×$COCF_3$), 159.0, 138.3, 135.4, 135.0, 134.9, 134.6, 134.5, 134.5, 134.3, 131.3, 131.1, 131.0, 130.9, 130.8, 130.6, 130.5, 129.9, 129.7, 129.6, 129.5, 129.4, 129.4, 129.1, 128.9, 128.5, 117.9, 119.2 – 115.5 (5×CF_3), 109.8, 98.8, 97.8, 85.7, 81.4, 78.2, 78.1, 76.8, 76.7, 73.7, 72.4, 71.3, 69.7, 67.7, 67.7, 64.7, 54.4, 50.7, 50.3, 49.7, 43.5, 42.7, 40.8, 32.6, 32.3 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{86}H_{75}N_6O_{25}F_{15}Na$ 1899.4440; Found 1899.4436.

General Procedure A for the photochemically initiated radical allylation of iodo paromomycin derivatives 25 and 29

An oven-dried Biotage microwave pressure vial (10 mL) was cooled under a stream of argon, equipped with a stirring bar and charged with iodide (1.0 equiv) and 2,4,5,6-tetra(9*H*-carbazol-9-yl)isophthalonitrile (4CzIPN) (0.05 equiv). The vial was sealed and flushed with argon. Anhydrous degassed DMSO was added, followed by NEt_3 (3.0 equiv) and allyl phenyl sulfone (2.0-5.0 equiv). The well-stirred yellow reaction mixture was irradiated at ambient temperature with blue LED light for 1-18 h. After dilution with water (15 mL) and addition of EtOAc (15 mL), layers were separated, and the aqueous layer was back-extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure.



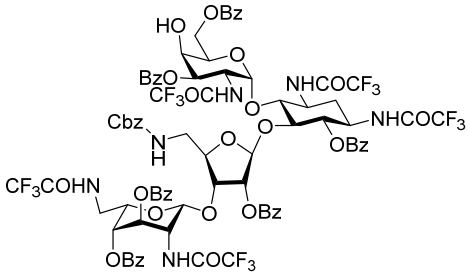
4',5''-Dideoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin-4'-ene (27). The title compound was obtained from 4'-*epi*-iodo-paromomycin **25** (50 mg, 0.026 mmol, 1.0 equiv), 4CzIPN (1 mg, 0.0013 mmol, 0.05 equiv), allyl phenyl sulfone (8 µL, 0.051 mmol, 2.0 equiv) and NEt_3 (11 µL, 0.076 mmol, 3.0 equiv) in degassed DMSO (1 mL) by following General Procedure A. Pure product **27** was obtained by silica gel column chromatography using gradient elution from 25 % EtOAc in heptane to 35 % EtOAc in heptane; white powder (30 mg, 64 % yield), analytical TLC on silica gel, 1:2 EtOAc/petroleum ether, R_f=0.26. $[\alpha]_D^{23}$ +55.7 (*c* 0.93, MeOH).

^{1}H NMR (400 MHz, CD_3OD) δ: 8.28 – 8.21 (m, 2H), 8.13 – 8.08 (m, 2H), 8.08 – 8.03 (m, 2H), 8.01 – 7.96 (m, 2H), 7.93 – 7.86 (m, 2H), 7.78 – 7.72 (m, 1H), 7.70 – 7.56 (m, 7H), 7.53 – 7.44 (m, 7H) 7.42 – 7.37 (m, 2H), 7.36 – 7.22 (m, 6H), 7.11 – 7.01 (m, 1H), 6.07 – 5.98 (m, 1H) 5.73 – 5.64 (m, 1H), 5.41 – 5.33 (m, 2H), 5.31 – 5.27 (m, 2H), 5.25 (dd, *J* = 9.0, 3.6 Hz, 2H), 5.19 – 5.15 (m, 1H), 5.14 – 5.09 (m, 1H), 5.08 – 5.04 (m, 1H), 4.97 – 4.90 (m, 1H), 4.83 – 4.74 (m, 2H), 4.47 (t, *J* = 6.6 Hz, 1H), 4.39 – 4.24 (m, 3H), 4.17 – 4.05 (m, 2H), 4.04 – 3.96 (m, 1H), 3.72 – 3.66 (m, 1H), 3.60 – 3.49 (m, 4H), 2.16 (q, *J* = 12.7 Hz, 1H), 2.09 – 2.01 (m, 1H) ppm, 6H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ: 167.4, 167.2, 167.1, 166.6, 166.4, 165.5, 160.2 – 158.2 (5×$COCF_3$), 149.5, 138.3, 135.1, 134.9, 134.9, 134.6, 134.4, 131.3, 131.1, 131.0, 130.9, 130.8, 130.7, 130.7, 130.6, 130.5, 129.9, 129.9, 129.7, 129.6, 129.5, 129.5, 129.3, 128.9, 128.7, 119.0 – 115.2 (5×CF_3), 109.3, 100.0,

99.0, 97.8, 83.3, 82.2, 78.8, 78.4, 76.7, 75.9, 73.7, 69.5, 68.0, 67.8, 67.7, 63.8, 51.3, 50.4, 49.9, 49.5, 43.1, 41.1, 31.9 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{69}N_6O_{25}F_{15}Na$ 1857.3971; Found 1857.4004.

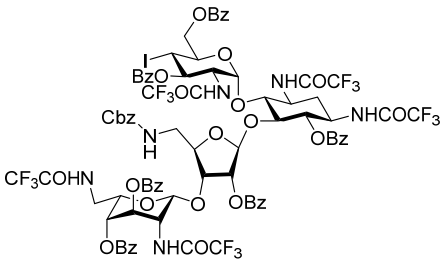


5''-Deoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl 4'-*epi*-paromomycin (28). A stirred solution of 4'-hydroxy paromomycin derivative **24** (600 mg, 0.32 mmol, 1.0 equiv) in anhydrous DCM (12 mL) was cooled to 0 °C (crushed ice bath) and treated with anhydrous pyridine (0.2 mL, 2.4 mmol, 7.5 equiv). Trifluoromethanesulfonic anhydride (0.16 mL, 0.97 mmol, 3.0 equiv) was then added dropwise over a period of 5 min, whereupon the light yellow solution turned brownish. After stirring at 0 °C for 1 h, the reaction mixture was washed with aqueous 5 % $KHSO_4$ solution (15 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ solution (15 mL) and water (20 mL). Organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The brownish viscous oily residue was dissolved in anhydrous DMF (8 mL) and sodium nitrite (134 mg, 1.94 mmol, 6.0 equiv) was added, whereupon the color turned dark red. The reaction mixture was stirred at ambient temperature for 18 h, and the reaction color gradually changed back to the light brown. Water (20 mL) was then added to the brownish reaction mixture and the solution was back-extracted with EtOAc (3×20 mL). Combined organic layers were washed with water (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 filtered and concentrated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography using gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane. Fractions containing the product (detected by TLC and ESI-MS), were combined and concentrated under reduced pressure. The residue was dissolved in DCM (5 mL) and hexane (50 mL) was poured to the solution. The formed white precipitate was collected by suction filtration and dried under reduced pressure to give product **28** (427 mg, 71 % yield) as a white powder. An aliquot of **28** (60 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 50 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.56. $[\alpha]_D^{23}$ +22.0 (*c* 1.08, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.30-8.22 (m, 2H), 8.12-8.07 (m, 2H), 8.07 – 8.00 (m, 5H), 7.82 – 7.78 (m, 2H), 7.78-7.72 (m, 1H), 7.69 – 7.54 (m, 7H), 7.53 – 7.40 (m, 9H), 7.37 – 7.31 (m, 2H), 7.31 – 7.24 (m, 3H), 7.14 – 7.08 (m, 1H), 7.01 – 6.94 (m, 2H), 6.05 (d, *J* = 3.8 Hz, 1H), 5.44 – 4.98 (m, 10H), 4.57 – 4.52 (m, 1H), 4.51 – 4.46 (m, 2H), 4.39 – 4.26 (m, 5H), 4.10 – 4.01 (m, 1H), 3.95 (ddd, *J* = 8.4, 5.4, 3.4 Hz, 1H), 3.86 – 3.79 (m, 1H), 3.69 – 3.62 (m, 1H), 3.60 (dd, *J* = 14.0, 5.4 Hz, 1H), 3.53 (dd, *J* = 14.3, 7.2 Hz, 1H), 3.44 (dd, *J* = 14.3, 5.4 Hz, 1H), 2.14 (q, *J* = 12.8 Hz, 1H), 2.03 (dt, *J* = 12.8, 4.3 Hz, 1H) ppm, 7H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.5, 167.4, 167.2, 166.5, 166.3, 165.5, 160.0 – 158.1 (5×$COCF_3$), 138.4, 135.0, 134.9, 134.7, 134.5, 134.4, 134.3, 134.2, 131.3, 131.1, 131.0, 130.9, 130.9, 130.8, 130.7, 130.6, 130.5, 130.5, 129.9, 129.7, 129.6, 129.6, 129.5, 129.5, 129.4, 129.2, 128.9, 128.7, 118.7 – 115.7 (5×CF_3), 109.7, 98.9, 97.7, 85.3, 82.0, 78.1, 77.4, 76.8, 76.5, 73.7, 72.6, 69.7, 69.6, 67.8, 67.8, 66.4, 62.7, 50.4, 50.3, 49.8, 49.6, 43.3, 40.9, 32.1 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{71}N_6O_{26}F_{15}Na$ 1875.4076; Found 1875.4097.



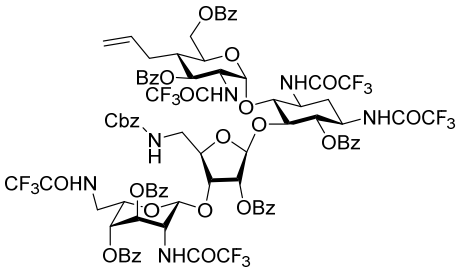
4',5''-Dideoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-4'-iodo-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (29). A stirred solution of 4'-*epi*-hydroxy paromomycin derivative **28** (368 mg, 0.20 mmol, 1.0 equiv) in anhydrous DCM (10 mL) was cooled to 0 °C (crushed ice bath) and treated with anhydrous pyridine (0.12 mL, 1.5 mmol, 7.5 equiv). Trifluoromethanesulfonic anhydride (0.10 mL, 0.60 mmol, 3.0 equiv) was then added dropwise over a period of 5 min. Upon completion of the addition the yellow solution turned brownish. After stirring at 0 °C for 1 h, the resulting mixture was washed with aqueous 5 % $KHSO_4$ solution (15 mL). Organic layer was washed with saturated aqueous $NaHCO_3$ solution (15 mL) and water (20 mL). Organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The brownish oily residue was dissolved in anhydrous acetone (10 mL) and potassium iodide (330 mg, 2.0 mmol, 10.0 equiv) was added. The resulting well-stirred suspension was heated at at 55 °C for 6 h, and the reaction progress was monitored by UPLC-MS assay. After cooling to room temperature, the brown suspension was filtered through the pad of *Celite*®. The filter plug was washed with DCM, the filtrate was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel column

chromatography using gradient elution from 25 % EtOAc in hexane to 50 % EtOAc in hexane. Product-containing fractions (detected with TLC and ESI-MS) were combined and concentrated under reduced pressure. The residue was dissolved in DCM (5 mL) and hexane (50 mL) was added. The formed white precipitate was collected by suction filtration and dried under reduced pressure to give product **29** (297 mg, 76 % yield) as a white powder. An aliquot of **29** (60 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 50 % to 95 % MeCN in 0.1 % aqueous AcOH solution; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.62. $[\alpha]_D^{23}$ +21.5 (*c* 1.21, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.28-8.21 (m, 2H), 8.11-8.05 (m, 4H), 8.03-7.99 (m, 2H), 7.80 – 7.73 (m, 3H), 7.66 – 7.58 (m, 5H), 7.52 – 7.41 (m, 9H), 7.32 – 7.22 (m, 7H), 7.12-7.05 (m, 1H), 6.99 – 6.93 (m, 2H), 6.14 – 6.06 (m, 1H), 5.72 – 5.64 (m, 1H), 5.36 – 5.29 (m, 2H), 5.26 (t, *J* = 3.0 Hz, 1H), 5.23 – 5.12 (m, 3H), 5.12 – 5.06 (m, 2H), 4.84 – 4.80 (m, 2H), 4.69 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.51 – 4.39 (m, 4H), 4.37 – 4.29 (m, 2H), 4.25 (dd, *J* = 8.4, 4.7 Hz, 1H), 4.15 – 4.07 (m, 1H), 3.92 – 3.85 (m, 1H), 3.84 – 3.79 (m, 1H), 3.66 – 3.57 (m, 1H), 3.55 – 3.45 (m, 2H), 3.36 (dd, *J* = 14.4, 5.7 Hz, 1H), 2.17 (q, *J* = 12.8 Hz, 1H), 2.05 (dt, *J* = 12.8, 4.3 Hz, 1H) ppm, 6H exchangeable protons merge with H_2O.

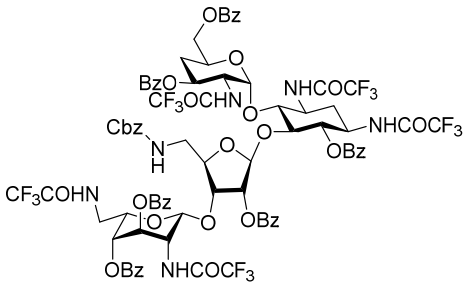
^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ: 167.6, 167.2, 166.8, 166.4, 166.2, 165.5, 159.8 – 158.4 (5×$COCF_3$), 138.2, 135.0, 134.9, 134.7, 134.6, 134.4, 134.2, 131.3, 131.0, 131.0, 130.9, 130.9, 130.7, 130.6, 130.5, 130.5, 129.9, 129.9, 129.8, 129.6, 129.6, 129.6, 129.5, 129.4, 129.1, 129.0, 128.9, 128.6, 118.6 – 115.9 (5×CF_3), 110.0, 98.7, 97.7, 85.6, 81.4, 78.2, 78.0, 76.7, 76.7, 74.6, 73.8, 72.6, 69.7, 67.7, 67.7, 66.7, 54.2, 50.6, 50.3, 49.7, 49.6, 43.3, 40.8, 32.2, 25.0 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{70}N_6O_{25}F_{15}NaI$ 1985.3093; Found 1985.3099.



4’-Allyl-4’,5’’-dideoxy-5’’-(*O*-benzylcarbamoyl)-6,3’,6’,2’’,3’’’,4’’’-hexa-*O*-benzoyl-1,3,2’,2’’’,6’’’-penta-*N*-trifluoroacetyl paromomycin (26). The title compound was obtained from 4’-iodo-paromomycin **29** (100 mg, 0.050 mmol, 1.0 equiv), 4CzIPN (2 mg, 0.0025mmol, 0.05 equiv), allylphenyl sulfone (40 µL, 0.255 mmol, 5.0 equiv) and NEt_3 (25 µL, 0.155 mmol, 3.0 equiv) in degassed DMSO (2 mL) by following General Procedure A. Pure product **26** was

obtained as a white powder (54 mg, 58 % yield) after purification by reversed-phase preparative HPLC. Compound **26** had spectra data identical to the above isolated sample.

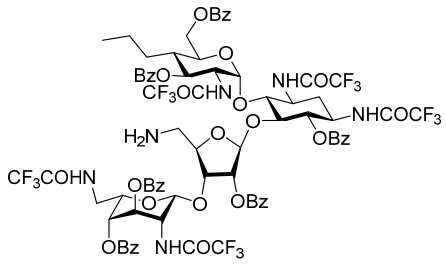


4',5''-Dideoxy-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (30-H). The title compound was formed as a side-product in the photochemical allylation of 4'-iodo-paromomycin **29** (see above). Purification of crude product mixture by reversed-phase preparative HPLC afforded **30-H** as a white powder (17 mg, 19 % yield); analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.30; $[\alpha]_D^{23}$ +38.3 (*c* 0.34, MeOH).

^{1}H NMR (400 MHz, CD_3CN) δ: 8.35 – 8.14 (m, 3H), 8.09 – 7.92 (m, 8H), 7.88 – 7.71 (m, 4H), 7.71 – 7.56 (m, 7H), 7.54 – 7.39 (m, 10H), 7.36 – 7.24 (m, 7H), 7.10 – 7.06 (m, 1H), 6.99 – 6.95 (m, 1H), 6.25 – 6.06 (m, 1H), 6.01 – 5.84 (m, 1H), 5.39 – 5.22 (m, 4H), 5.21 – 5.17 (m, 1H), 5.15 – 5.04 (m, 3H), 5.02 – 4.67 (m, 2H), 4.54 – 4.38 (m, 3H), 4.09 – 3.84 (m, 3H), 3.74 – 3.59 (m, 2H), 3.49 – 3.35 (m, 1H), 3.27 – 3.11 (m, 1H), 2.46 – 2.37 (m, 1H), 2.14 – 2.04 (m, 2H).

^{13}C{^{1}H} NMR (101 MHz, CD_3CN) δ: 166.9, 166.6, 166.6, 166.5, 165.8, 165.6, 165.0, 157.9-157.1 (5x$COCF_3$), 137.9, 134.9, 134.8, 134.7, 134.4, 134.4, 134.3, 134.2, 134.1, 134.0, 130.9, 130.8, 130.8, 130.6, 130.5, 130.4, 130.3, 130.3, 130.2, 129.7, 129.7, 129.5, 129.5, 129.3, 129.1, 129.0, 128.9, 128.8, 128.8, 128.5, 128.4, 118.5 – 116.0 (5×CF_3, overlapped with MeCN), 115.5, 109.4, 98.2, 97.9, 97.0, 84.7, 82.7, 81.5, 81.0, 78.3, 78.0, 76.9, 76.6, 76.3, 75.7, 72.9, 69.5, 69.2, 69.1, 67.6, 67.2, 67.1, 66.3, 63.3, 53.5, 50.8, 49.9, 49.7, 49.6, 49.4, 49.1, 44.1, 40.7, 33.5, 31.6.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{83}H_{71}N_6O_{25}F_{15}Na$ 1859.4127; Found 1859.4147.



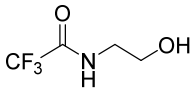
4',5''-Dideoxy-4'-propyl-5''-amino-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (31). 5 % Pd on carbon (1.69 g, 0.79 mmol, 3.0 equiv) was added at ambient temperature to a stirred solution of alkene **26** (498 mg, 0.26 mmol, 1.0 equiv) in 80 % aqueous acetic acid (12.4 mL, prepared by mixing 10.1 mL of glacial acetic acid and 2.3 mL

of water) and the mixture was stirred under 5 atm of hydrogen at ambient temperature for 18 h. The reaction progress was monitored by UPLC-MS assay. The black suspension was filtered through a silica gel pad, the filter plug was washed with 80 % aqueous acetic acid and the filtrate was evaporated under reduced pressure. The yellowish residue was dissolved in DCM (5 mL) and hexane (50 mL) was added to the solution. The formed beige precipitate was collected by suction filtration and dried under reduced pressure to give product **31** (463 mg, 100 % yield) as a white powder. An aliquot of **31** (100 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 5 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Product-containing fractions were detected by TLC and ESI-MS (visualization of TLC spots was done by immersing the TLC plate into a solution of sulfuric acid in ethanol (1:15 v:v) and subsequent drying the TLC plate with heat-gun). Combined fractions were concentrated under reduced pressure to afford pure material; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.16. $[\alpha]_D^{23}$ +34.9 (*c* 1.26, MeOH).

^{1}H NMR (400 MHz, CD_3OD) δ: 8.32 – 8.28 (m, 2H), 8.16-8.06 (m, 4H), 8.02 – 7.99 (m, 2H), 7.80 – 7.75 (m, 1H), 7.74 – 7.68 (m, 4H), 7.67 – 7.60 (m, 3H), 7.57 – 7.50 (m, 3H), 7.50 – 7.43 (m, 4H), 7.43 – 7.38 (m, 2H), 7.25-7.18 (m, 2H), 6.99 – 6.88 (m, 3H), 6.02 (d, *J* = 4.0 Hz, 1H), 5.50 (t, *J* = 10.7 Hz, 1H), 5.37 (s, 1H), 5.34 – 5.26 (m, 3H), 5.24 – 5.21 (m, 1H), 5.11 (d, *J* = 4.0 Hz, 1H), 4.68 (dd, *J* = 12.5, 2.4 Hz, 1H), 4.56 – 4.49 (m, 2H), 4.47 – 4.39 (m, 2H), 4.37 – 4.25 (m, 2H), 4.25 – 4.20 (m, 1H), 4.14 – 4.06 (m, 2H), 3.96 (t, *J* = 2.4 Hz, 1H), 3.86 – 3.75 (m, 2H), 3.36 (dd, *J* = 14.1, 3.9 Hz, 1H), 3.06 (dd, *J* = 13.7, 3.6 Hz, 1H), 2.83 (dd, *J* = 13.7, 7.4 Hz, 1H), 2.32 (tt, *J* = 10.9, 3.6 Hz, 1H), 2.19 (q, *J* = 12.9 Hz, 1H), 2.03 (dt, *J* = 12.9, 4.4 Hz, 1H), 1.54 – 1.37 (m, 3H), 1.33 – 1.24 (m, 1H), 0.79 (t, *J* = 6.8 Hz, 3H) ppm, 7H exchangeable protons merge with H_2O.

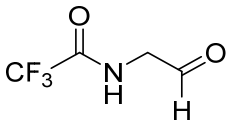
^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ: 167.8, 167.6, 167.3, 166.4, 165.9, 165.6, 160.2 – 157.9 (5×$COCF_3$), 158.8, 135.0, 134.6, 134.5, 134.5, 134.4, 131.3, 131.1, 131.1, 131.0, 130.7, 130.7, 130.6, 130.6, 130.5, 130.5, 130.0, 129.8, 129.7, 129.7, 129.6, 129.3, 129.2, 129.2, 129.0, 119.0 – 115.2 (5×CF_3), 110.0, 98.2, 97.5, 86.8, 81.3, 77.7, 77.7, 77.1, 76.1, 75.0, 73.0, 71.2, 69.9, 67.6, 65.0, 54.4, 50.7, 50.0, 49.8, 49.5, 49.3, 44.8, 42.8, 40.6, 32.1, 30.4, 20.5, 14.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{78}H_{72}N_6O_{23}F_{15}$ 1745.4409; Found 1745.4438.

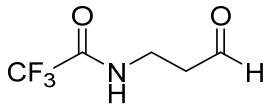


2,2,2-Trifluoro-*N*-(2-hydroxyethyl)acetamide (S1). The title compound was prepared according to the literature procedure.[2] Thus, to a stirred solution of ethyl-2,2,2-trifluoroacetate (15.7 g, 111 mmol, 1.35 equiv) in anhydrous DCM (10 mL) at 0 °C (crushed ice bath) was

added 2-amino-1-ethanol (5.0 mL, 82 mmol, 1.0 equiv). After warming up to room temperature, the colorless solution was stirred at ambient temperature for 2 h and then concentrated to dryness under reduced pressure to give product **S1** as a colorless viscous oil (12.3 g, 96 % yield). The oil solidified as a white solid after standing for several days. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.83 (br s, 1H), 3.81 (t, *J* = 5.2 Hz, 2H), 3.55 (dt, *J* = 5.5, 5.2 Hz, 2H), 1.94 (br s, 1H) ppm, which was identical to that from the literature.[1]

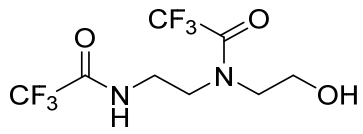


2,2,2-Trifluoro-*N*-(2-oxoethyl)acetamide (32). An oven-dried pressure tube (100 mL) was cooled under a stream of argon and charged with 2,2,2-trifluoro-*N*-(2-hydroxyethyl)acetamide **S1**, 1.50 g, 9.55 mmol, 1.0 equiv), IBX (2.94 g, 10.5 mmol, 1.1 equiv) and anhydrous THF (25 mL). After heating at 60 °C for 18 h, the orange suspension was cooled to room temperature and filtered through the pad of *Celite*®. The filter plug was washed with THF and filtrate was evaporated to dryness under reduced pressure to give product **32** as a brownish solid (1.48 g, 100 % yield) which was used further without additional purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.72 (s, 1H), 7.02 (br s, 1H), 4.35 (dd, *J* = 4.8, 0.6 Hz, 2H) ppm.

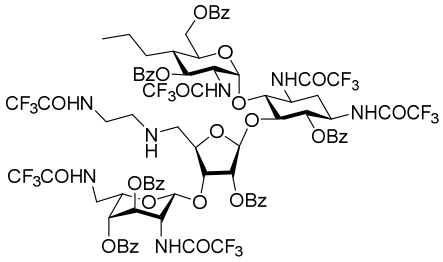


2,2,2-Trifluoro-*N*-(3-oxopropyl)acetamide (33). To a cooled (0 °C, crushed ice bath) neat ethyl-2,2,2-trifluoroacetate (1.78 g, 12.6 mmol, 1.35 equiv) was added neat 3,3-diethoxypropan-1-amine (1.5 mL, 9.3 mmol, 1.0 equiv). The yellowish solution was warmed up to room temperature, stirred at ambient temperature for 3 h and then concentrated to dryness under reduced pressure to give *N*-(3,3-diethoxypropyl)-2,2,2-trifluoroacetamide as a yellow oil (2.26 g, 100 % yield).[2]

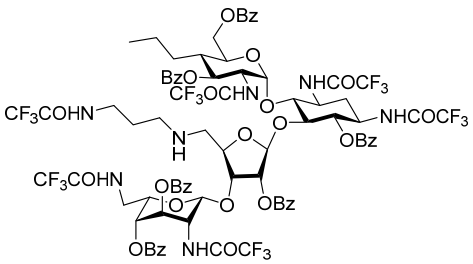
The obtained *N*-(3,3-diethoxypropyl)-2,2,2-trifluoroacetamide (2.26 g, 9.3 mmol, 1.0 equiv) was added dropwise to aqueous HCl (1.0 M solution in water, 30 mL) over a period of 15 min at 0 °C (crushed ice bath). After warming to ambient temperature and stirring for 1 h, the yellowish solution was extracted with Et_2O (3×40 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure to give product **33** as a colorless oil (1.27 g, 81 % yield) which was used in further steps without additional purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.83 (s, 1H), 6.84 (br s, 1H), 3.66 (dt, *J* = 5.9, 5.6 Hz, 2H), 2.85 (t, *J* = 5.6 Hz, 2H) ppm, which was identical to that from the literature.[3]



General Procedure B for reductive amination of amine 31 with aldehydes 32 and 33. An oven-dried 50 mL round-bottom flask was cooled under an argon atmosphere and charged with amine **31** (1.0 equiv) and aldehydes **32** or **33** (2.0 equiv), followed by anhydrous THF (5 mL/0.1 mmol of the amine). The clear solution was stirred at ambient temperature for 10 min, then $Na(OAc)_3BH$ (4.0 equiv) was added. The resulting pale yellow suspension was stirred at ambient temperature for 18 h and the reaction progress was monitoring by UPLC-MS assay. Upon complete conversion, the yellowish suspension was evaporated under reduced pressure. The crude residue was diluted with water (20 mL) and extracted with EtOAc (3×20 mL). Combined organic extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was dissolved in DCM (5 mL) and hexane (50 mL) was added. The formed pale yellow precipitate was filtered off, washed with hexane (30 mL), dried under reduced pressure and used in the next step without further purification.



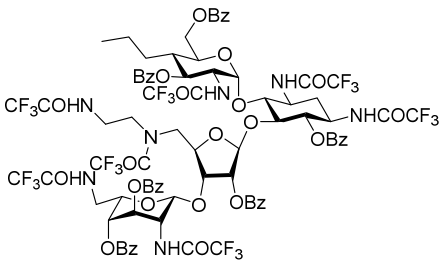
4',5''-Dideoxy-4'-propyl-5''-(2-trifluoroacetamidoethylamino)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (34). The title compound was obtained as a yellowish solid (648 mg, 100 % yield) from amine **31** (600 mg, 0.34 mmol, 1.0 equiv), aldehyde **32** (107 mg, 0.69 mmol, 2.0 equiv) and $Na(OAc)_3BH$ (292 mg, 1.38 mmol, 4.0 equiv) by following General Procedure B. The crude product **34** was used in further without additional purification.



4',5''-Dideoxy-4'-propyl-5''-(3-trifluoroacetamidopropylamino)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (35). The title compound was obtained as a yellowish solid (653 mg, 100 % yield) from amine **31** (600 mg, 0.34 mmol, 1.0 equiv), aldehyde **33** (116 mg, 0.69 mmol, 2.0 equiv) and $Na(OAc)_3BH$ (292 mg, 1.38 mmol, 4.0 equiv) by following General Procedure B. The crude product **35** was used in further steps without additional purification.

General Procedure C for trifluoroacetylation of amines 34 and 35.

An oven-dried 50 mL round-bottom flask was cooled under an argon atmosphere and charged with amine **34** and **35** (1.0 equiv), followed by anhydrous DCM (5 mL/0.1 mmol of the amine). The clear solution was cooled to 0 °C (crushed ice) and trifluoroacetic anhydride (4.0–5.0 equiv) was added dropwise within 5 min. The resulting yellowish solution was stirred at 0 °C for 30 min, then pyridine (2.0-2.5 equiv) was added dropwise within 5 min at 0 °C temperature. The yellowish solution was stirred at ambient temperature for 2 h and the reaction progress was monitored by UPLC-MS assay. Upon complete conversion water (1 mL) was added to the dark brownish solution and all volatiles were removed under reduced pressure. The crude product was diluted with aqueous HCl (0.5 M solution in water, 50 mL) and back-extracted with EtOAc (3×30 mL). Combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered through a silica gel pad and evaporated under reduced pressure. The residue was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 70 % to 95 % MeCN in 0.1 % aqueous AcOH solution. The obtained product after chromatography and evaporation was dissolved in DCM (5 mL) and hexane (50 mL) was added. The formed pale yellow precipitate was filtered off, washed with hexane (30 mL) and dried under reduced pressure.



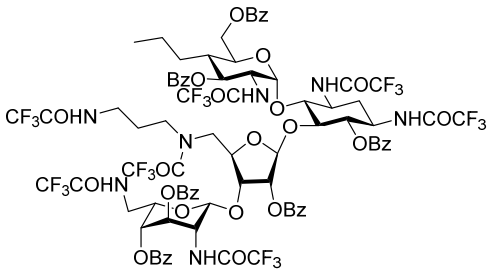
4',5''-Dideoxy-4'-propyl-5''-(2-aminoethylamino)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (36). The title compound was obtained from amine **34** (648 mg, 0.34 mmol, 1.0 equiv), trifluoroacetic anhydride (0.19 mL, 1.38 mmol, 4.0 equiv) and pyridine (55 µL, 0.69 mmol, 2.0 equiv) by following General Procedure C. Purification by reversed-phase preparative HPLC afforded **36** as a white powder (224 mg, 33 % yield); analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.31.

^{1}H NMR (600 MHz, CD_3OD) δ: 8.29 – 8.24 (m, 2H), 8.13 – 8.10 (m, 2H), 8.08 – 7.98 (m, 4H), 7.80 – 7.77 (m, 1H), 7.74 – 7.59 (m, 7H), 7.55 – 7.44 (m, 9H), 7.28 – 7.21 (m, 2H), 7.00-6.95 (m, 1H), 6.94-6.90 (m, 1H), 6.90-6.86 (m, 1H), 6.01-5.97 (m, 1H), 5.47 – 5.40 (m, 1H), 5.29 (dd, *J* = 10.5, 9.3 Hz, 1H), 5.25-5.23 (m, 1H), 5.22 – 5.17 (m, 1H), 5.16 (d, *J* = 4.4 Hz, 1H), 5.10 – 5.07 (m, 1H), 4.69 – 4.63 (m, 1H), 4.61 – 4.54 (m, 2H), 4.47 – 4.43 (m, 1H), 4.42 – 4.36 (m, 2H), 4.36 – 4.29 (m, 2H), 4.18 – 4.03 (m, 4H), 4.01 – 3.93 (m, 1H), 3.79 (dt, *J* = 13.9, 5.2 Hz, 2H), 3.75 – 3.68 (m, 1H), 3.66 – 3.58 (m, 2H), 3.56 – 3.47 (m, 2H), 3.44 (dd, *J* = 13.9, 8.4 Hz, 1H), 2.38 – 2.26 (m, 1H), 2.12 (q, *J* = 12.8 Hz, 1H), 2.05 (dt, *J* = 12.8, 4.4 Hz, 1H), 1.48 – 1.35 (m, 3H),

1.31 – 1.23 (m, 1H), 0.78 (dt, *J* = 10.5, 6.9 Hz, 3H) ppm, 6H exchangeable protons merge with H_2O. $[\alpha]_D^{23}$ +34.0 (*c* 1.21, MeOH).

$^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ: 167.9, 167.6, 167.2, 166.2, 166.1, 165.7, 159.8 – 157.6 (7×$COCF_3$), 135.1, 135.0, 134.6, 134.5, 134.4, 131.3, 131.1, 131.0, 130.8, 130.8, 130.7, 130.7, 130.6, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.5, 129.3, 129.2, 129.0, 118.6 – 115.7 (7×CF_3), 110.2, 98.4, 97.8, 85.9, 79.5, 78.4, 78.0, 77.8, 77.6, 76.8, 76.2, 75.7, 74.2, 73.5, 73.1, 71.4, 69.9, 68.0, 65.0, 54.1, 50.6, 49.6, 48.3, 46.9, 42.7, 40.5, 37.7, 32.3, 30.4, 20.8, 14.6 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{84}H_{74}N_7O_{25}F_{21}Na$ 2002.4297; Found 2002.4412.



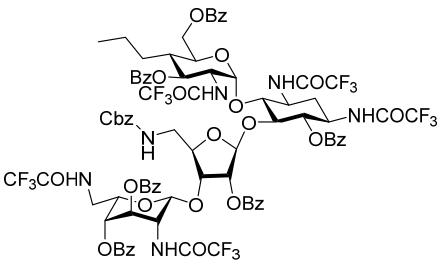
4',5''-Dideoxy-4'-propyl-5''-(3-aminoethylamino)-1,3-diaminopropane)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (37). The title compound was obtained from amine **35** (653 mg, 0.34 mmol, 1.0 equiv), trifluoroacetic anhydride (0.19 mL, 1.38 mmol, 4.0 equiv) and pyridine (55 µL, 0.69 mmol, 2.0 equiv) by following General Procedure C. Purification by reversed-phase preparative HPLC afforded **37** as a white powder (232 mg, 34 % yield); analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.31. $[\alpha]_D^{23}$ +29.0 (*c* 1.14, MeOH).

^1H NMR (600 MHz, $CDCl_3$) δ: 8.63 (dd, *J* = 9.0, 3.6 Hz, 1H), 8.27 – 8.18 (m, 2H), 8.14 – 8.07 (m, 2H), 7.96 – 7.91 (m, 4H), 7.77 – 7.70 (m, 2H), 7.68 – 7.61 (m, 4H), 7.60 – 7.49 (m, 6H), 7.49 – 7.40 (m, 8H), 7.32 (t, *J* = 6.2 Hz, 1H), 7.25 – 7.22 (m, 1H, overlapped with $CHCl_3$), 6.84 (d, *J* = 7.6 Hz, 1H), 6.79 (t, 1H, J=7.0 Hz), 6.74 (t, *J* = 7.6 Hz, 2H), 6.60 (d, *J* = 9.7 Hz, 1H), 5.94 (d, *J* = 4.0 Hz, 1H), 5.42 (s, 1H), 5.37 – 5.30 (m, 2H), 5.20 (t, *J* = 9.9 Hz, 1H), 5.15 (t, *J* = 2.7 Hz, 1H), 5.04 – 4.97 (m, 2H), 4.66 (dd, *J* = 12.1, 3.6 Hz, 1H), 4.53 (dd, *J* = 12.1, 5.1 Hz, 1H), 4.46 (dt, *J* = 10.2, 4.0 Hz, 1H), 4.39 (dd, *J* = 8.0, 4.3 Hz, 1H), 4.33 – 4.24 (m, 2H), 4.24 – 4.15 (m, 4H), 4.13-4.07 (m, 2H), 3.99 – 3.91 (m, 2H), 3.62 (p, *J* = 7.6 Hz, 1H), 3.57 – 3.50 (m, 1H), 3.36 (dd, *J* = 13.9, 3.6 Hz, 1H), 3.23 – 3.09 (m, 3H), 2.35 (dt, *J* = 13.2, 4.4 Hz, 1H), 2.25-2.17 (m, 1H), 2.00 – 1.94 (m, 1H), 1.87 – 1.82 (m, 1H), 1.67 (q, *J* = 12.7 Hz, 1H), 1.45 – 1.37 (m, 3H), 1.35-1.27 (m, 1H), 0.80 (t, *J* = 6.8 Hz, 3H) ppm, 6H exchangeable protons merge with H_2O.

$^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ: 167.8, 166.8, 166.7, 164.7, 164.6, 164.4, 158.8 – 155.8 (7×$COCF_3$), 134.4, 134.1, 134.0, 133.8, 133.7, 133.6, 130.4, 130.0, 129.9, 129.7, 129.7, 129.6, 129.6, 129.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.4, 128.1, 128.0, 127.8, 126.9, 117.4 – 114.2 (7×CF_3), 109.0, 97.6, 96.0, 85.2, 79.0,

75.1, 74.9, 74.7, 73.7, 71.8, 70.2, 69.6, 66.6, 65.6, 53.4, 49.5, 48.9, 47.4, 45.2, 43.7, 42.7, 41.5, 39.5, 36.4, 32.1, 29.2, 29.0, 26.1, 20.4, 14.4 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{85}H_{76}N_7O_{25}F_{21}Na$ 2016.4453; Found 2016.4429.



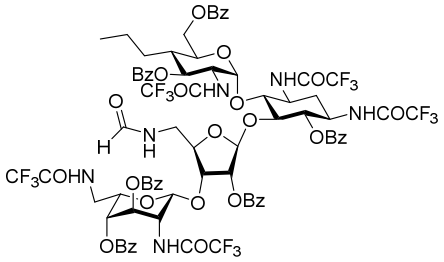
4',5''-Dideoxy-4'-propyl-5''-(*O*-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (38). A stirred solution of 5''-deoxy-5''-amino paromomycin derivative **31** (515 mg, 0.30 mmol, 1.0 equiv) in anhydrous DCM (20 mL) was cooled to 0 °C (crushed ice bath) and Cbz-Cl (126 µL, 0.89 mmol, 3.0 equiv) was added dropwise over 5 min. The resulting yellowish solution was stirred at 0 °C for 1 h followed by dropwise addition (over 5 min) of pyridine (71 µL, 0.89 mmol, 3.0 equiv). The resulting yellow solution was stirred at 0 °C for 1 h, then warmed up to room temperature and stirring was continued for additional 2 h. The reaction progress was monitored by UPLC-MS assay. The yellowish solution was diluted with water (50 mL) and back-extracted with DCM (2×30 mL). Combined organic extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude yellow oil was dissolved in DCM (5 mL) and hexane (50 mL) was added to the solution. The formed beige precipitate was collected by suction filtration and dried under reduced pressure to give product **38** (520 mg, 94 % yield) as a white powder. An aliquot of **38** (50 mg) was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 60 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Product-containing fractions were detected by TLC and ESI-MS. Combined fractions were concentrated under reduced pressure to afford pure material; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.62. $[\alpha]_D^{23}$ +75.7 (*c* 0.53, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.66 (t, J = 5.6 Hz, 1H), 8.13 – 8.03 (m, 4H), 7.99 – 7.95 (m, 2H), 7.94 – 7.91 (m, 2H), 7.91 – 7.83 (m, 2H), 7.74 – 7.67 (m, 2H), 7.65 – 7.60 (m, 2H), 7.60 – 7.56 (m, 2H), 7.51 – 7.45 (m, 5H), 7.44 – 7.39 (m, 4H), 7.37 – 7.29 (m, 5H), 7.28 – 7.27 (m, 1H), 7.26 – 7.20 (m, 3H), 7.14 – 7.04 (m, 2H), 6.87 – 6.81 (m, 1H), 6.81 – 6.73 (m, 1H), 6.02 – 5.91 (m, 1H), 5.67 (d, J = 3.7 Hz, 1H), 5.39 – 5.31 (m, 2H), 5.29 – 5.21 (m, 2H), 5.15 – 5.08 (m, 3H), 5.01 (t, J = 10.0 Hz, 1H), 4.81 (s, 1H), 4.59 – 4.50 (m, 2H), 4.42 – 4.37 (m, 1H), 4.33 – 4.28 (m, 1H), 4.19 – 4.15 (m, 1H), 4.14 – 4.09 (m, 2H), 4.09 – 4.05 (m, 2H), 4.01 – 3.94 (m, 2H), 3.78 – 3.71 (m, 1H), 3.62 (ddd, J = 14.2, 6.1, 2.9 Hz, 1H), 3.37 (ddd, J = 14.5, 8.8, 5.4 Hz, 1H), 3.33

– 3.24 (m, 1H), 2.30 – 2.22 (m, 1H), 2.09 – 2.02 (m, 1H), 1.58 – 1.51 (m, 1H), 1.44 – 1.38 (m, 2H), 0.78 (t, J = 7.0 Hz, 3H).

$^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ: 167.2, 166.9, 165.7, 164.9, 164.1, 163.1, 157.8, 157.7-156.5 (5×$COCF_3$), 136.4, 134.4, 134.2, 134.2, 134.0, 133.9, 133.7, 130.3, 129.9, 129.9, 129.8, 129.6, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.4, 128.3, 128.3, 128.2, 127.9, 127.4, 117.0-114.8 (5×CF_3), 99.1, 96.2, 81.3, 78.2, 77.4, 77.2, 76.9, 75.5, 74.2, 72.7, 70.8, 70.7, 69.0, 67.3, 67.1, 64.8, 53.8, 49.7, 49.5, 48.7, 42.0, 41.0, 31.5, 29.4, 19.8, 19.6, 14.4 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{86}H_{77}N_6O_{25}F_{15}Na$ 1901.4597; Found 1901.4622.

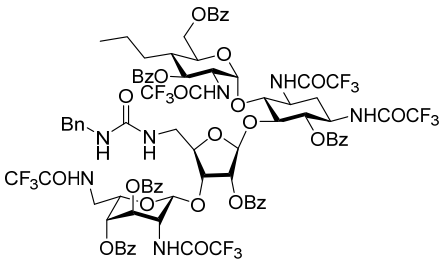


4',5''-Dideoxy-4'-propyl-5''-formamido-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (39). A stirred solution of 5''-deoxy-5''-amino paromomycin derivative **31** (500 mg, 0.29 mmol, 1.0 equiv) in anhydrous DCM (20 mL) was treated with formyl acetate (113 µL, 1.43 mmol, 5 equiv) at ambient temperature. The resulting yellowish solution was stirred at ambient temperature overnight. The reaction progress was monitored by UPLC-MS assay. The yellowish solution was evaporated under reduced pressure. The crude yellow oil was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 60 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Product-containing fractions were detected by TLC and ESI-MS. Combined fractions were concentrated under reduced pressure to give product **39** (290 mg, 57 % yield) as a white powder; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.56. $[\alpha]_D^{23}$ +60.4 (*c* 0.49, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.02 (s, 1H), 8.19 (s, 1H), 8.15 – 8.05 (m, 4H), 8.02 – 7.96 (m, 2H), 7.94 – 7.90 (m, 2H), 7.88 – 7.81 (m, 2H), 7.74 – 7.68 (m, 2H), 7.68 – 7.64 (m, 2H), 7.64 – 7.57 (m, 3H), 7.57 – 7.39 (m, 9H), 7.35 – 7.30 (m, 1H), 7.29 – 7.26 (m, 2H), 7.16 (t, J = 7.4 Hz, 1H), 7.13 – 7.04 (m, 2H), 6.91 – 6.75 (m, 3H), 5.72 (d, J = 3.6 Hz, 1H), 5.42 – 5.33 (m, 2H), 5.30 – 5.25 (m, 1H), 5.23 (t, J = 3.0 Hz, 1H), 5.10 (d, J = 4.6 Hz, 1H), 5.02 (t, J = 9.9 Hz, 1H), 4.78 – 4.71 (m, 1H), 4.64 (dd, J = 12.2, 6.1 Hz, 1H), 4.58 – 4.52 (m, 1H), 4.42 – 4.34 (m, 1H), 4.23 – 3.95 (m, 9H), 3.77 – 3.67 (m, 2H), 3.53 – 3.46 (m, 1H), 3.41 – 3.34 (m, 1H), 2.30 – 2.22 (m, 1H), 2.15 – 2.09 (m, 1H), 1.64 – 1.56 (m, 1H), 0.80 (t, J = 7.0 Hz, 3H).

$^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ: 167.1, 166.8, 166.8, 165.5, 164.8, 164.1, 162.5, 158.2-156.6 (5×$COCF_3$), 134.3, 134.1, 134.0, 133.9, 133.6, 130.2, 129.7, 129.7, 129.7, 129.4, 129.0, 128.8, 128.7, 128.7, 128.7, 128.6, 128.2, 128.0, 128.0, 127.3, 116.9-114.4 (5×CF_3), 107.3, 98.0, 95.8, 80.6, 77.2, 77.0, 76.8, 74.8, 74.0, 72.9, 70.9, 70.6, 69.0, 67.0, 64.6, 53.7, 49.3, 48.3, 41.9, 40.8, 38.2, 31.4, 29.4, 19.8, 14.3 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{79}H_{72}N_6O_{24}F_{15}$ 1773.4358; Found 1773.4353.



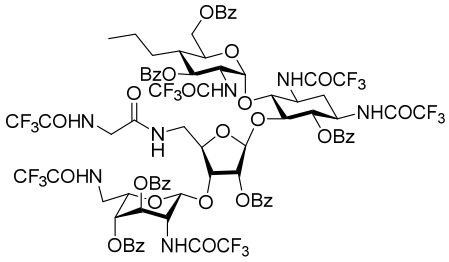
4',5''-Dideoxy-4'-propyl-5''-(*N*-benzylureido)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (40). A stirred solution of 5''-deoxy-5''-amino paromomycin derivative **31** (720 mg, 0.41 mmol, 1.0 equiv) in anhydrous DCM (25 mL) was treated with benzyl isocyanate (130 µL, 1.03 mmol, 2.5 equiv) at ambient temperature. The resulting yellowish solution was stirred at ambient temperature overnight. The reaction progress was monitored by UPLC-MS assay. The yellowish solution was diluted with water (50 mL) and back-extracted with DCM (3×30 mL). Combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude yellow oil was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 60 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Product-containing fractions were detected by TLC and ESI-MS. Combined fractions were concentrated under reduced pressure to give product **40** (350 mg, 45 % yield) as a white powder; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.59. $[\alpha]_D^{23}$ +44.5 (*c* 0.31, $CHCl_3$).

^1H NMR (600 MHz, $CDCl_3$) δ: 9.78 (s, 1H), 8.12 – 8.08 (m, 2H), 8.06 – 8.03 (m, 2H), 7.96 – 7.92 (m, 2H), 7.92 – 7.89 (m, 2H), 7.88 – 7.82 (m, 2H), 7.71 – 7.65 (m, 3H), 7.63 (t, J = 7.5 Hz, 1H), 7.60 – 7.54 (m, 4H), 7.50 – 7.46 (m, 3H), 7.44 – 7.35 (m, 6H), 7.32 – 7.26 (m, 7H), 7.26 – 7.21 (m, 3H), 7.10 – 7.03 (m, 2H), 6.81 – 6.61 (m, 2H), 5.84 (d, J = 3.7 Hz, 1H), 5.62 (dd, J = 8.5, 3.8 Hz, 1H), 5.44 (t, J = 6.0 Hz, 1H), 5.33 – 5.27 (m, 2H), 5.26 – 5.22 (m, 2H), 5.11 (d, J = 5.0 Hz, 1H), 4.86 (t, J = 9.9 Hz, 1H), 4.78 – 4.73 (m, 1H), 4.69 – 4.63 (m, 1H), 4.55 – 4.48 (m, 2H), 4.41 – 4.33 (m, 3H), 4.20 – 4.14 (m, 2H), 4.09 – 4.03 (m, 3H), 3.99 – 3.93 (m, 2H), 3.85 (t, J = 9.5 Hz, 1H), 3.68 (dd, J = 13.9, 5.7 Hz, 1H), 3.39 – 3.32 (m, 1H), 3.13 – 3.01 (m, 1H), 2.29 (dt, J = 13.6, 4.6 Hz, 1H), 2.03 – 1.99 (m, 1H), 0.87 – 0.83 (m, 3H).

$^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ: 167.6, 167.2, 166.7, 165.3, 164.9, 164.0, 159.1, 158.7-157.0 (5×$COCF_3$), 139.5, 134.5, 134.3, 134.1, 134.0, 133.6, 130.2, 129.9, 129.9, 129.8, 129.8, 129.6, 129.2, 128.9, 128.8,

128.8, 128.8, 128.7, 128.7, 128.3, 128.2, 128.2, 127.4, 127.3, 127.2, 117.1-114.4 (5×CF_3), 107.2, 99.7, 95.1, 81.9, 81.4, 79.5, 77.4, 77.2, 76.9, 75.8, 74.4, 73.0, 70.9, 70.8, 68.8, 65.1, 53.6, 49.7, 48.9, 48.7, 44.3, 42.4, 42.1, 41.5, 31.7, 29.5, 29.2, 20.5, 19.6, 14.4 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{86}H_{79}N_7O_{24}F_{15}$ 1878.4937; Found 1878.4929.



4',5''-Dideoxy-4'-propyl-5''-(*N*-trifluoroacetylglycinamido)-6,3',6',2'',3''',4'''-hexa-*O*-benzoyl-1,3,2',2''',6'''-penta-*N*-trifluoroacetyl paromomycin (41). A stirred solution of (2,2,2-trifluoroacetyl)glycine (34 mg, 0.20 mmol, 1.4 equiv), HATU (82 mg, 0.22 mmol, 1.5 equiv) in anhydrous DMF (3 mL) at ambient temperature was treated with 4-methylmorpholine (40 µL, 0.36 mmol, 2.5 equiv). The resulting yellowish solution was stirred at ambient temperature for 1 h followed by addition of 5''-deoxy-5''-amino paromomycin **31** (250 mg, 0.14 mmol, 1.0 equiv) solution in anhydrous DMF (2 mL). The resulting yellowish solution was stirred at ambient temperature overnight. The reaction progress was monitored by UPLC-MS assay. The yellowish solution was diluted with saturated aqueous $NaHCO_3$ solution (50 mL) and back-extracted with DCM (3×30 mL). Combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude yellow oil was purified by reversed-phase preparative HPLC (XBridge® Prep C18 OBD™ column) using gradient elution from 60 % to 95 % MeCN in 0.1 % aqueous AcOH solution. Product-containing fractions were detected by TLC and ESI-MS. Combined fractions were concentrated under reduced pressure to give product **41** (145 mg, 53 % yield) as a white powder; analytical TLC on silica gel, 1:1 EtOAc/petroleum ether, R_f=0.54. $[\alpha]_D^{23}$ +49.9 (*c* 0.33, MeOH).

^{1}H NMR (600 MHz, CD_3OD) δ: 8.30 – 8.23 (m, 2H), 8.13 – 8.05 (m, 4H), 8.05 – 7.99 (m, 2H), 7.80 – 7.73 (m, 3H), 7.71 – 7.66 (m, 2H), 7.66 – 7.59 (m, 3H), 7.56 – 7.51 (m, 2H), 7.51 – 7.42 (m, 7H), 7.27 – 7.21 (m, 2H), 7.06 – 7.01 (m, 1H), 6.98 – 6.91 (m, 2H), 6.03 (d, J = 4.0 Hz, 1H), 5.48 (t, J = 10.7 Hz, 1H), 5.34 (s, 1H), 5.33 – 5.26 (m, 2H), 5.20 – 5.12 (m, 3H), 4.66 – 4.53 (m, 3H), 4.47 – 4.43 (m, 1H), 4.39 – 4.28 (m, 3H), 4.23 (dd, J = 8.5, 4.5 Hz, 1H), 4.16 – 4.03 (m, 4H), 3.98 (dd, J = 14.2, 2.8 Hz, 1H), 3.91 – 3.83 (m, 2H), 3.57 (d, J = 6.5 Hz, 2H), 3.19 (dd, J = 14.2, 7.6 Hz, 1H), 2.44 – 2.36 (m, 1H), 2.20 – 2.12 (m, 1H), 2.07 – 2.01 (m, 1H), 1.47 – 1.40 (m, 2H), 1.35 – 1.22 (m, 2H), 0.78 (t, J = 6.9 Hz, 3H) ppm, 7H exchangeable protons merge with H_2O.

$^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ: 170.4, 167.9, 167.6, 167.3, 166.5, 166.1, 165.6, 160.2-158.0 (6x$COCF_3$), 135.0, 134.9, 134.6, 134.6, 134.5, 134.3, 131.3, 131.1, 131.1, 131.0, 130.8, 130.7, 130.7, 130.5, 130.0, 129.9, 129.8, 129.6, 129.6, 129.6, 129.3, 129.3, 129.1, 118.9-115.6 (6×CF_3), 110.0, 98.9, 97.6, 85.9, 81.3, 78.8, 77.8, 76.9, 76.5, 73.9, 72.9, 71.4, 69.8, 67.8, 64.9, 54.5, 50.6, 50.2, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 43.6, 43.0, 42.6, 40.9, 32.3, 30.5, 20.8, 14.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{82}H_{73}N_7O_{25}F_{18}Na$ 1920.4266; Found 1920.4257.

General Procedure D for global deprotection of trifluoroacetamides 36, 37, 39 and 41.

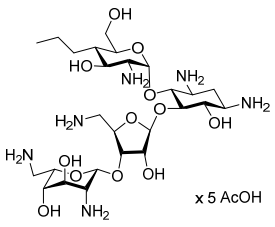
An oven-dried 20 mL pressure vial was cooled under an argon atmosphere and charged with trifluoroacetamides **36**, **37**, **39**, or **41** (1.0 equiv). Anhydrous MeOH (4 mL/0.1 mmol of the starting material) was added and the resulting clear solution was treated under argon atmosphere with $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 23.0-30.0 equiv). After stirring for 24 h at 65 °C temperature (the reaction progress was monitored by UPLC-MS assay), the resulting white slurry was concentrated to dryness under reduced pressure. Anhydrous Et_2O (10 mL) was added to the white residue and the resulting white suspension was sonicated for 2 min in an ultrasonic bath, whereupon the resulting white suspension was centrifuged for 10 min at 2150 rpm. The colorless clear supernatant was decanted and fresh anhydrous Et_2O (10 mL) was added to the white residue. The sonication/centrifugation sequence was repeated two more times. The resulting white precipitate was dried under reduced pressure. The residue was taken up in water (10 mL/0.1 mmol of the starting material), stirred and treated with $Ba(OH)_2$ (20.0 equiv) at ambient temperature. The resulting white slurry was stirred and heated to 80 °C temperature for 18 h and the reaction progress was monitored by UPLC-MS assay. The white reaction suspension was cooled to 0 °C (crushed ice bath) and dry ice was added portionwise until pH 7–8. The resulting mixture was centrifuged for 10 min at 2150 rpm. The clear supernatant was decanted and fresh MeOH (10 mL) was added to the white precipitate. The mixture was sonicated for 2 min in an ultrasonic bath. Fresh MeOH (10 mL) was added once again and the sonication/centrifugation sequence was repeated two more times. The combined supernatant solutions were concentrated under reduced pressure. The crude product was diluted with MeOH (15 mL), the resulting suspension was filtered, and the filter cake was washed with MeOH. The combined filtrates were concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC (XBridge® BEH Prep OBD™ Amide column) using gradient elution from 95:5 A:B to 10:90 A:B (eluent A - 0.05 % solution of AcOH in MeCN;

eluent B - 0.05 % solution of AcOH in water). Product-containing fractions (identified by ninhydrin stain test and ESI-MS) were combined and concentrated. The residue was treated with glacial acetic acid (0.5 mL), followed by addition of MeCN (5 mL). The formed white solid was dried under reduced pressure.

General Procedure E for global deprotection of trifluoroacetamides 38 and 40.

An oven-dried 20 mL pressure vial was cooled under an argon atmosphere and charged with trifluoroacetamides **38** or ,**40** (1.0 equiv). Anhydrous MeOH (4 mL/0.1 mmol of the starting material) was added and the resulting clear solution was treated under argon atmosphere with $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 23.0-30.0 equiv). After stirring for 24 h at 65 °C temperature (the reaction progress was monitored by UPLC-MS assay), the resulting white slurry was concentrated to dryness under reduced pressure. Anhydrous Et_2O (10 mL) was added to the white residue and the resulting white suspension was sonicated for 2 min in an ultrasonic bath, whereupon the resulting white suspension was centrifuged for 10 min at 2150 rpm. The colorless clear supernatant was decanted and fresh anhydrous Et_2O (10 mL) was added to the white residue. The sonication/centrifugation sequence was repeated two more times. The resulting white precipitate was dried under reduced pressure. The residue was taken up in water (10 mL/0.1 mmol of the starting material), stirred and treated with $Ba(OH)_2$ (20.0 equiv) at ambient temperature. The resulting white slurry was stirred and heated to 80 °C temperature for 18 h and the reaction progress was monitored by UPLC-MS assay. The white reaction suspension was cooled to 0 °C (crushed ice bath) and dry ice was added portionwise until pH 7–8. The resulting mixture was centrifuged for 10 min at 2150 rpm. The clear supernatant was decanted and fresh MeOH (10 mL) was added to the white precipitate. The mixture was sonicated for 2 min in an ultrasonic bath. Fresh MeOH (10 mL) was added once again and the sonication/centrifugation sequence was repeated two more times. The combined supernatant solutions were concentrated under reduced pressure. The crude product was diluted with MeOH (15 mL), the resulting suspension was filtered, and the filter cake was washed with MeOH. The combined filtrates were concentrated under reduced pressure. The residue was diluted with AcOH (16 mL) and water (4 mL), and to the well-stirred solution was added 10 % Pd on carbon (1.0 equiv) at ambient temperature to a stirred solution. The reaction mixture was stirred under 5 atm of hydrogen at ambient temperature for 18 h, and progress of the reaction was monitored by UPLC-MS assay. The black suspension was filtered through the pad of *Celite*®, the filter plug was washed with 50% AcOH in water and the filtrate was evaporated under reduced pressure. The residue was purified by reversed-phase preparative HPLC (XBridge® BEH Prep OBD™ Amide

column) using gradient elution from 95:5 A:B to 10:90 A:B (eluent A - 0.05 % solution of AcOH in MeCN; eluent B - 0.05 % solution of AcOH in water). Product-containing fractions (identified by ninhydrin stain test and ESI-MS) were combined and concentrated. The residue was treated with glacial acetic acid (0.5 mL), followed by addition of MeCN (5 mL). The formed white solid was dried under reduced pressure.



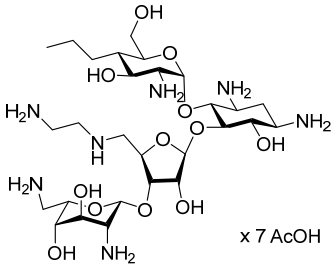
5''-Amino-4',5''-dideoxy-4'-propyl paromomycin pentaacetate (8). The title compound was obtained from paromomycin derivative **38** (550 mg, 0.29 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 10.0 mL, 7.02 mmol, 24.0 equiv), barium hydroxide (1.0 g, 5.85 mmol, 20.0 equiv) and Pd/C (10 mol% on carbon; 247 mg, o,23 mmol, 1 equiv) by following General Procedure E. Purification by reversed-phase preparative HPLC, followed by treatment with glacial acetic acid afforded the hexaacetate salt of **8** as a white amorphous solid (75.0 mg, 32 % yield). $[\alpha]_D^{23}$ +39.1 (*c* 0.81, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.78 (1H, d, J=3.8 Hz) 5.47 (1H, s) 5.33 (1H, s) 4.56 (1H, t, J=5.9 Hz) 4.48 (1H, d, J=5.2 Hz) 4.35 (1H, t, J=5.2 Hz) 4.34-4.30 (1H, m) 4.26 (1H, t, J=3.1 Hz) 4.04-3.93 (3H, m) 3.93-3.84 (3H, m) 3.78-3.72 (2H, m) 3.64-3.60 (1H, m) 3.52-3.41 (4H, m) 3.41-3.27 (3H, m) 2.44 (1H, dt, J=12.1, 3.4 Hz) 1.97 (15H, s) 1.82 (1H, q, J=12.7 Hz) 1.73-1.67 (1H, m) 1.56-1.47 (2H, m) 1.41-1.27 (2H, m) 0.91 (3H, t, J=7.2 Hz) ppm, 24H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 108.8, 95.3, 95.1, 83.0, 77.4, 77.0, 76.8, 74.0, 73.0, 72.1, 70.2, 67.6, 67.4, 67.1, 61.1, 55.2, 50.8, 50.2, 50.0, 49.2, 42.1, 41.7, 40.4, 28.5, 27.9, 22.5, 18.6, 13.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{26}H_{53}N_6O_{12}$ 641.3721; Found 641.3732.

Elemental analysis: Anal. Calcd for $C_{28}H_{57}N_7O_{12}$•5AcOH•4H_2O•4H_2CO_3: C, 38.10; H, 7,03; N, 6.66; Found C, 38.25; H, 6.80; N, 6.67.



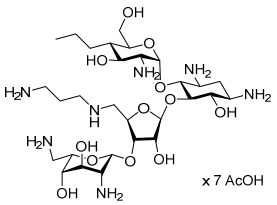
5''-(2-Aminoethylamino)-4',5''-dideoxy-4'-propyl paromomycin heptaacetate (9). The title compound was obtained from paromomycin derivative **36** (223 mg, 0.11 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 3.7 mL, 2.6 mmol, 23.0 equiv) and barium hydroxide (386 mg, 2.25 mmol, 20.0 equiv) by following General Procedure D. Purification by reversed-phase preparative HPLC, followed by treatment with glacial acetic acid afforded the heptaacetate salt of **9** as a white amorphous solid (25.0 mg, 20 % yield). $[\alpha]_D^{23}$ +38.5 (*c* 1.50, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.71 (d, *J* = 3.9 Hz, 1H), 5.39 (d, *J* = 3.3 Hz, 1H), 5.32 (d, *J* = 1.8 Hz, 1H), 4.45 (t, *J* = 5.3 Hz, 1H), 4.38 (dd, *J* = 5.3, 3.3 Hz, 1H), 4.34 – 4.30 (m, 1H), 4.27 – 4.21 (m, 2H), 3.99 – 3.86 (m, 5H), 3.85 – 3.83 (m, 1H), 3.76 – 3.68 (m, 2H), 3.62 – 3.59 (m, 1H), 3.49 – 3.38 (m, 3H), 3.37 – 3.29 (m, 2H), 3.18 (t, *J* = 6.9 Hz, 2H), 3.10 (dd, *J* = 12.9, 3.8 Hz, 1H), 3.05 – 3.00 (m, 2H), 2.94 (dd, *J* = 12.9, 8.5 Hz, 1H), 2.43 (dt, *J* = 12.7, 4.2 Hz, 1H), 1.80 (q, *J* = 12.7 Hz, 1H), 1.71 – 1.64 (m, 1H), 1.55 – 1.45 (m, 2H), 1.41 – 1.26 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H) ppm, 26H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 109.3, 96.1, 95.4, 83.9, 79.7, 78.0, 77.4, 74.0, 73.2, 72.1, 70.3, 67.6, 67.3, 67.0, 61.2, 55.3, 50.9, 50.8, 49.7, 49.2, 45.5, 42.3, 40.4, 37.9, 28.7, 28.0, 18.6, 13.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{28}H_{58}N_7O_{12}$ 684,4143; Found 684,4145.

Elemental analysis: Anal. Calcd for $C_{28}H_{57}N_7O_{12}$•7AcOH•$2H_2O$: C, 44,24; H, 7,87; N, 8,60; Found C, 44,24; H, 7,68; N, 8,36.



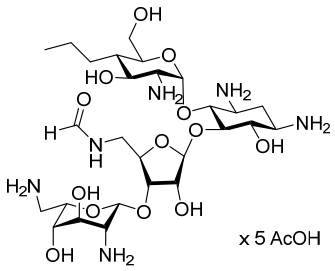
5''-(3-Aminopropylamino)-4',5''-dideoxy-4'-propyl paromomycin heptaacetate (10). The title compound was obtained from paromomycin derivative **37** (231 mg, 0.12 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 4.4 mL, 3.1 mmol, 27.0 equiv) and barium hydroxide (397 mg, 2.31 mmol, 20.0 equiv) by following General Procedure D. Purification by reversed-phase preparative HPLC was followed by treatment with glacial acetic acid to afford the heptaacetate salt of **10** as a white amorphous solid (28.9 mg, 22 % yield). $[\alpha]_D^{23}$ +49.8 (*c* 1.52, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.67 (d, *J* = 3.8 Hz, 1H), 5.44 (d, *J* = 2.6 Hz, 1H), 5.30 (d, *J* = 1.9 Hz, 1H), 4.51 (t, *J* = 5.6 Hz, 1H), 4.43 (dd, *J* = 5.1, 2.6 Hz, 1H), 4.35 – 4.30 (m, 2H), 4.24 (t, *J* = 3.1 Hz, 1H), 3.94 – 3.82 (m, 6H), 3.75 – 3.69 (m, 2H), 3.61 – 3.57 (m, 1H), 3.47 – 3.35 (m, 4H), 3.34 – 3.28 (m, 1H), 3.26 – 3.19 (m, 2H), 3.13 – 3.05 (m, 4H), 2.38 (dt, *J* = 12.7, 4.2 Hz, 1H), 2.12 – 2.01 (m, 2H), 1.74 (q, *J* = 12.7 Hz, 1H), 1.69 – 1.63 (m, 1H), 1.57 – 1.44 (m, 2H), 1.41 – 1.25 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H) ppm, 26H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 108.8, 96.2, 95.3, 83.2, 77.8, 77.4, 77.2, 73.8, 72.9, 72.2, 70.4, 67.7, 67.4, 67.3, 61.2, 55.5, 50.8, 50.4, 50.1, 49.3, 45.2, 42.3, 40.4, 36.8, 29.2, 27.9, 24.5, 18.4, 13.8 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{29}H_{60}N_7O_{12}$ 698,4300; Found 698,4333.

Elemental analysis: Anal. Calcd for $C_{29}H_{59}N_7O_{12}$•7AcOH•2H_2O: C, 44,75; H, 7,95; N, 8,49; Found C, 44,79; H, 8,07; N, 8,40.



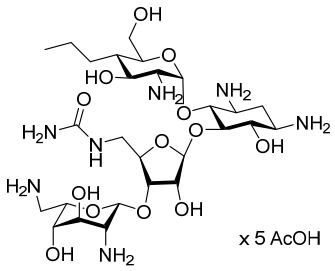
4',5''-Dideoxy-5''-formamido-4'-propyl paromomycin pentaacetate (11). The title compound was obtained from paromomycin derivative **39** (330 mg, 0.19 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 6.5 mL, 4.6 mmol, 24.0 equiv) and barium hydroxide (651 mg, 3.8 mmol, 20.0 equiv) by following General Procedure D. Purification by reversed-phase preparative HPLC was followed by treatment with glacial acetic acid to afforded the pentaacetate salt of **11** as a white amorphous solid (45 mg, 27 % yield). $[\alpha]_D^{23}$ +36.5 (*c* 0.33, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 8.19 (1H, s) 5.69 (1H, d, J=3.9 Hz) 5.34 (1H, d, J=3.5 Hz) 5.31-5.29 (1H, m) 4.44 (1H, t, J=5.6 Hz) 4.36-4.31 (2H, m) 4.28-4.24 (2H, m) 3.95-3.84 (6H, m) 3.73-3.67 (3H, m) 3.63-3.60 (1H, m) 3.56 (1H, dd, J=14.6, 6.0 Hz) 3.49-3.39 (4H, m) 3.37-3.31 (1H, m) 2.44 (1H, dt, J=12.3, 3.9 Hz) 1.80 (1H, q, J=12.6 Hz) 1.72-1.66 (1H, m) 1.56-1.48 (2H, m) 1.41-1.29 (2H, m) 0.91 (3H, t, J=7.2 Hz) ppm, 23H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 164.9 (folded signal), 109.7, 96.2, 95.6, 84.1, 79.9, 79.4, 79.1, 76.8, 74.3, 73.0, 72.2, 70.3, 70.2, 67.6, 67.4, 61.2, 55.4, 50.8, 49.7, 49.1, 42.3, 40.5, 39.4, 28.8, 27.9, 22.6, 18.5, 13.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{27}H_{53}N_6O_{13}$ 669.3671; Found 669.3654.

Elemental analysis: Anal. Calcd for $C_{27}H_{52}N_6O_{13}$•6AcOH•4H_2O: C, 42,54; H, 7,69; N, 7,63; Found C, 42,44; H, 7,27; N, 7,56.



4',5''-Dideoxy-4'-propyl-5''-ureido-paromomycin pentaacetate (12). The title compound was obtained from paromomycin derivative **40** (350 mg, 0.19 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 6.3 mL, 4.4 mmol, 24 equiv), barium hydroxide (638 mg, 3.7 mmol, 20.0 equiv) and Pd/C (10 mol% on carbon; 193 mg, 0.18 mmol, 1 equiv) by following General Procedure E. Purification by reversed-phase preparative

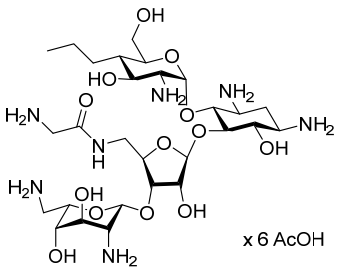
HPLC was followed by treatment with glacial acetic acid to afforded the pentaacetate salt of **12** as a white amorphous solid (55 mg, 31 % yield). $[\alpha]_D^{23}$ +227.8 (*c* 0.48, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.67 (1H, d, J=4.0 Hz) 5.34 (1H, d, J=3.9 Hz) 5.31 (1H, d, J=1.8 Hz) 4.45 (1H, t, J=5.3 Hz) 4.36-4.31 (2H, m) 4.26-4.22 (2H, m) 3.97-3.89 (4H, m) 3.88 (1H, d, J=8.7 Hz) 3.85 (1H, t, J=1.8 Hz) 3.75-3.68 (2H, m) 3.63-3.61 (1H, m) 3.53 (1H, dd, J=14.6, 4.9 Hz) 3.50-3.44 (2H, m) 3.44-3.32 (4H, m) 2.45 (1H, dt, J=12.8, 4.3 Hz) 1.81 (1H, q, J=12.8 Hz) 1.73-1.66 (1H, m) 1.56-1.47 (2H, m) 1.42-1.26 (2H, m) 0.91 (3H, t, J=7.2 Hz) ppm, 24H exchangeable protons merge with H_2O.

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 151.4, 109.7, 96.5, 95.7, 84.2, 80.6, 79.7, 77.1, 74.4, 72.8, 72.1, 67.6, 67.4, 67.2, 67.0, 61.2, 55.5, 50.8, 49.6, 49.2, 42.3, 41.5, 40.5, 28.7, 27.9, 27.7, 22.5, 18.5, 13.8 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{27}H_{54}N_7O_{13}$ 684.3780; Found 684.3764.

Elemental analysis: Anal. Calcd for $C_{27}H_{53}N_7O_{13}$•6AcOH•5H_2O: C, 41,04; H, 7.73; N, 8.65; Found C, 41.26; H, 7.07; N, 8,44.



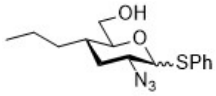
4',5''-Dideoxy-5''-glycinamido-4'-propyl paromomycin hexacetate (13). The title compound was obtained from paromomycin derivative **41** (295 mg, 0.16 mmol, 1.0 equiv), $Mg(OMe)_2$ (7-8 % solution in anhydrous methanol; 5.2 mL, 3.8 mmol, 24.0 equiv) and barium hydroxide (532 mg, 3.11 mmol, 20.0 equiv) by following General Procedure D. Purification by reversed-phase preparative HPLC was followed by treatment with glacial acetic acid to afford the hexaacetate salt of **13** as a white amorphous solid (75 mg, 43 % yield). $[\alpha]_D^{23}$ +42.6 (*c* 0.50, D_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.77 (0.40H, d, J=3.8 Hz) 5.67 (0.6H, d, J=3.8 Hz) 5.46 (0.40H, d, J=2.0 Hz) 5.36 (0.60H, d, J=3.8 Hz) 5.32 (1H, dd, J=5.8, 2.0 Hz) 4.56 (0.40H, dd, J=6.8, 5.1 Hz) 4.47 (0.40H, dd, J=5.0, 2.0 Hz) 4.39 (0.60H, t, J=5.6 Hz) 4.36-4.33 (1H, m) 4.33-4.27 (1H, m) 4.27-4.22 (1.60H, m) 3.97-3.94 (1H, m) 3.93-3.89 (3H, m) 3.88-3.84 (3H, m) 3.79-3.69 (3H, m) 3.63-3.60 (1H, m) 3.55-3.48 (1H, m) 3.48-3.39 (4H, m) 3.37-3.26 (2H, m) 2.42 (1H, ddt, J=12.4, 8.2, 4.2 Hz) 1.96 (20H, s) 1.77 (1H, q, J=12.4 Hz) 1.73-1.66 (1H, m) 1.57-1.46 (2H, m) 1.41-1.29 (2H, m) 0.91 (3H, t, J=7.1 Hz) ppm, 25H exchangeable protons merge with H_2O.

$^{13}C\{^1H\}$ NMR (151 MHz, D_2O) δ: 156.7, 109.4, 108.9, 96.5, 95.8, 95.6, 95.2, 84.1, 83.1, 80.4, 79.1, 77.4, 77.0, 74.1, 73.9, 73.1, 72.3, 70.2, 67.6, 67.5, 67.2, 61.2, 55.4, 55.3, 50.8, 49.9, 49.2, 42.2, 41.7, 41.5, 40.4, 29.0, 27.9, 22.7, 18.5, 13.7 ppm.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{28}H_{56}N_7O_{13}$ 698.3936; Found found 698.3942.

Elemental analysis: Anal. Calcd for $C_{28}H_{55}N_7O_{13}$•7AcOH•$3H_2O$: C, 43.04; H, 7.65; N, 8.36; Found C, 43.16; H, 7.37; N, 8.17.



43

Phenyl 2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (**43**). To a solution of **42** (218 mg, 1.10 mmol) in 1,2-dichloroethane (6.5 ml) were added trimethyl(phenylthio)silane (630 μl, 3.31 mmol) and ZnI_2 (1.06 g, 3.31 mmol) and the mixture was stirred for 45 h at room temperature. After diluting with 1,2-dichloroethane (11 ml), the mixuture was passed through a Celite pad and the cake was washed with 1,2-dichloroethane (6.5 ml). The combined filtrate was washed with saturated $NaHCO_3$ solution and saturated NaCl solution (1×20 ml, each), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was taken in a solution of methanol/water (10:1, 6 ml) and added K_2CO_3 (305 mg, 2.21 mmol). The mixture was stirred for 1 h at room temperature and then concentrated *in vacuo*. The residue was taken in chloroform (10 ml) and washed with water (1×10 ml). The aqueous phase was extracted with chloroform (2×5 ml). The organic phase was combined, washed with saturated NaCl solution (1×20 ml), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by Flash column chromatography on silica gel (hexane:ethyl acetate = 12:1 to 6:1) to afford **43** (295 mg, 87%) as an anomeric mixture (α:β = 2.8:1); analytical TLC on silica gel, 3:7 EtOAc/hexanes, R_f=0.60.

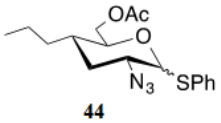
Analytical data for **43** (α anomer): ^1H NMR (500 MHz, $CDCl_3$) δ: 7.58 – 7.48 (m, 2H), 7.37 – 7.24 (m, 3H), 5.55 (dd, *J* = 4.9, 1.3 Hz, 1H), 4.04 (ddd, *J* = 10.5, 5.8, 2.5 Hz, 1H), 3.84 (dt, *J* = 12.5, 4.6 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.67 – 3.61 (m, 1H), 2.07 (dtd, *J* = 13.0, 4.3, 1.5 Hz, 1H), 1.82 (t, *J* = 6.2 Hz, 1H), 1.78 – 1.68 (m, 1H), 1.54 (q, *J* = 12.5 Hz, 1H), 1.50 – 1.15 (m, 4H), 0.94 (t, *J* = 7.1 Hz, 3H).

$^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ: 133.6, 132.6, 129.1, 127.7, 88.6, 73.8, 62.8, 59.3, 35.2, 33.1, 29.8, 19.1, 14.2.

Analytical data for **43** (β anomer): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.58 – 7.48 (m, 2H), 7.37 – 7.24 (m, 3H), 4.50 (d, *J* = 9.9 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.61 – 3.55 (m, 1H), 3.31 (ddd, *J* = 11.5, 9.9, 4.8 Hz, 1H), 3.24 (ddd, *J* = 9.6, 6.6, 2.6 Hz, 1H), 2.33 (dt, *J* = 13.0, 4.4 Hz, 1H), 2.16 (t, *J* = 6.6 Hz, 1H), 1.68 – 1.58 (m, 1H), 1.50 – 1.15 (m, 5H), 1.12 – 1.00 (m, 1H), 0.90 (t, *J* = 7.1 Hz, 1H).

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ: 132.7, 132.2, 129.0, 128.0, 88.2, 83.3, 63.1, 59.7, 35.6, 35.3, 32.7, 19.2, 14.2.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{15}H_{21}N_3O_2SNa$ $[M+Na]^+$ 330.1252, found 330.1252.



44

Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (**44**). To a solution of **43** (203 mg, 660 μmol) in pyridine (2.1 ml) was added acetic anhydride (187 μl, 1.98 mmol) and the mixture was stirred for 8 h at room temperature. After completion, the reaction mixture was concentration *in vacuo*, co-evaporated with toluene, and purified by Flash column chromatography on silica gel (hexane:ethyl acetate = 7:1 to 4:1) to afford **44** (223 mg, 97%) as an anomeric mixture (α:β = 2.7:1); analytical TLC on silica gel, 1:4 EtOAc/hexane, R_f=0.85.

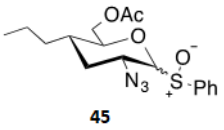
Analytical data for **44** (α anomer): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.57 – 7.51 (m, 2H), 7.35 – 7.23 (m, 3H), 5.58 (dd, *J* = 4.9, 1.4 Hz, 1H), 4.31 – 4.17 (m, 3H), 3.90 (dt, *J* = 12.5, 4.6 Hz, 1H), 2.13 – 2.06 (m, 1H), 2.04 (s, 3H), 1.78 – 1.70 (m, 1H), 1.55 (q, *J* = 12.5 Hz, 1H), 1.51 – 1.18 (m, 4H), 0.93 (t, *J* = 6.9 Hz, 3H).

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ: 170.8, 133.8, 132.7, 132.1, 129.0, 127.5, 88.4, 71.3, 64.2, 59.0, 35.7, 33.2, 30.0, 20.8, 19.0, 14.1.

Analytical data for **44** (β anomer): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.63 – 7.57 (m, 2H), 7.35 – 7.23 (m, 3H), 4.47 (d, *J* = 9.9 Hz, 1H), 4.35 (dd, *J* = 11.9, 2.3 Hz, 1H), 4.13 (dd, *J* = 12.0, 6.6 Hz, 1H), 3.39 (ddd, *J* = 10.2, 6.6, 2.3 Hz, 1H), 3.34 (ddd, *J* = 11.6, 9.9, 4.9 Hz, 1H), 2.34 (dt, *J* = 13.1, 4.4 Hz, 1H), 2.09 (s, 3H), 1.78 – 1.61 (m, 2H, H4), 1.48 – 1.37 (m, 2H), 1.32 – 1.20 (m, 1H), 1.17 – 1.07 (m, 1H), 0.90 (t, *J* = 7.0 Hz, 3H).

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ: 170.9, 133.8, 132.7, 132.6, 128.8, 127.8, 88.4, 80.5, 64.4, 59.4, 35.7, 35.7, 32.9, 20.9, 19.1, 14.1.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{17}H_{23}N_3O_3SNa$ 372.1358, found 372.1352.



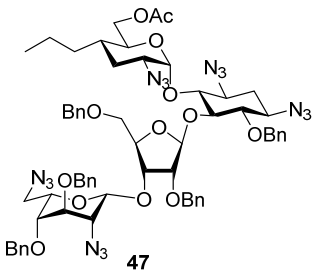
45

Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranosyl sulfoxide (45). To a solution of **44** (173 mg, 495 μmol) in mixture of acetonitrile/water (10:1, 4.4 ml) were added, $NaHCO_3$ (208 mg, 2.47 mmol) and a solution of Selectfluor (190 mg, 594 μmol) in acetonitrile/water (10:1, 4.4 ml). The reaction mixture was stirred at room temperature for 1 h. After completion, solvents were evaporated under reduced pressure, the residue was taken up in dicloromethane (120 ml) and washed with saturated $NaHCO_3$ solution:water (1:9), brine solution (1×120 ml, each), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **45** (177 mg, 98%) as an anomeric mixtures (α:β = 2.7:1) which are also a mixture of diastereomer on sulfur. The sulfur of α anomer is most likely with configuration S_S. These compounds were used to the next reaction without purification; analytical TLC on silica gel, 1:4 EtOAc/hexane, R_f=0.20.

Analytical data for **45** (α anomer with S_S diastereomer): **^{1}H NMR** (500 MHz, $CDCl_3$) δ: 7.77 – 7.46 (m, 5H), 4.76 (ddd, *J* = 10.7, 5.3, 2.3 Hz, 1H), 4.30 (d, *J* = 5.9 Hz, 1H), 4.15 – 4.08 (m, 1H), 4.06 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.02 (dd, *J* = 12.3, 5.3 Hz, 1H), 2.34 (q, *J* = 12.3 Hz, 1H), 2.22 (dt, *J* = 12.6, 4.4 Hz, 1H), 1.99 (s, 3H), 1.79 – 1.60 (m, 1H), 1.51 – 1.43 (m, 1H), 1.43 – 1.33 (m, 3H), 1.33 – 1.26 (m, 2H), 0.91 (t, *J* = 6.9 Hz, 3H).

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ: 170.7, 141.3 130.9, 129.1, 125.5, 91.6, 77.1, 64.4, 58.8, 35.1, 33.1, 29.5, 20.8, 18.9, 14.1.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{17}H_{23}N_3O_4SNa$ 388.1307, found 388.1311.



47

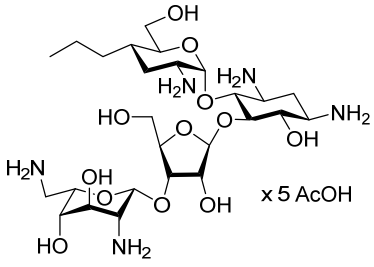
1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47). Donor **45** (100 mg, 0.27 mmol), acceptor **46** (248 mg, 0.25 mmol), and TTBP (203 mg, 0.82 mmol) were charged to a round-bottom flask, co-evaporated with toluene three times, and dried in *vacuo* overnight. The flask was purged with argon, mixed with activated 4 Å MS and the mixture was dissolved in dry dichloromethane (4 mL) and stirred at room temperature for 1 h. The reaction mixture was cooled to −70 °C, treated with Tf_2O (48 μL, 0.29 mmol) for 30 min, warmed slowly to −50 °C and continued stirring for 3 h, at −50 °C before the reaction was quenched with triethylamine (0.2 mL). The reaction mixture was diluted with

EtOAc (15 mL), filtered through Celite®, and the filtrate was washed with saturated aqueous solution of $NaHCO_3$ followed by brine. Solvents were evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel (hexanes: ethyl acetate 20:1 to 4:1) to give **47** (147 mg, α:β, 12:1, 48%) as a white foam; analytical TLC on silica gel, 1:4 EtOAc/hexanes, R_f=0.55; $[\alpha]_D^{23}$ +37.5 (*c* 1, $CHCl_3$).

Analytical data for **47** (α anomer): **^{1}H NMR** (600 MHz, $CDCl_3$) δ: 7.64 – 6.91 (m, 25H), 6.09 (d, *J* = 3.5 Hz, 1H), 5.69 (d, *J* = 5.6 Hz, 1H), 5.01 (d, *J* = 10.7 Hz, 1H), 4.90 (d, *J* = 1.9 Hz, 1H), 4.74 (d, *J* = 10.7 Hz, 1H), 4.64 (dd, *J* = 21.6, 11.9 Hz, 2H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.53 – 4.43 (m, 3H), 4.39 – 4.26 (m, 5H), 4.21 – 4.15 (m, 1H), 4.01 (t, *J* = 5.2 Hz, 1H), 3.99 – 3.94 (m, 2H), 3.87 (dd, *J* = 10.3, 2.3 Hz, 1H), 3.80 (dq, *J* = 4.9, 2.9, 2.0 Hz, 2H), 3.75 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.67 (dd, *J* = 13.0, 8.5 Hz, 1H), 3.63 – 3.58 (m, 1H), 3.52 – 3.41 (m, 2H), 3.40 – 3.36 (m, 1H), 3.32 (t, *J* = 9.3 Hz, 1H), 3.18 – 3.14 (m, 1H), 2.94 (dd, *J* = 12.9, 4.0 Hz, 1H), 2.87 (ddd, *J* = 12.6, 4.6, 3.5 Hz, 1H), 2.27 (dt, *J* = 13.2, 4.6 Hz, 1H), 2.11 (s, 3H), 1.92 (dt, *J* = 12.4, 4.3 Hz, 1H), 1.83 (q, *J* = 12.3 Hz, 1H), 1.59 (qd, *J* = 14.7, 14.1, 5.2 Hz, 1H), 1.51 – 1.35 (m, 3H), 1.33 – 1.16 (m, 2H), 0.93 (t, *J* = 7.1 Hz, 3H).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ: 171.0, 138.5, 138.0, 137.7, 137.1, 137.0, 128.7, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.5, 127.5, 127.4, 106.0, 98.6, 95.4, 84.4, 82.4, 82.0, 81.9, 75.6, 75.0, 74.8, 74.3, 73.3, 73.2, 72.9, 72.4, 71.8, 71.6, 70.9, 70.4, 64.7, 60.6, 60.5, 57.3, 57.2, 51.1, 35.4, 33.3, 32.8, 27.4, 21.0, 19.0, 14.2.

HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{63}H_{73}N_{15}O_{13}Na$ 1270.5404, found 1270.5409.



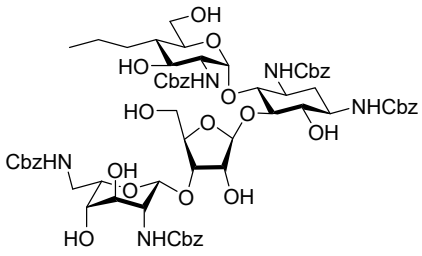
3',4'-Dideoxy-4'-propyl paromomycin pentaacetate (14). A solution of compound **47** (90 mg, 72.1 μmol) in dioxane: 10% aq. acetic acid in deionized water (1:1, 3 mL) was treated with $Pd(OH)_2$/C (150 mg) at room temperature under 48 atm of hydrogen for 24 h. After completion as indicated by LRMS, the reaction mixture was filtered through Celite®, filtrate was concentrated to dryness. The crude reaction mixture was taken up in 2.5 mL saturated aqueous barium hydroxide solution and heated to 65 °C for 3 hr. After completion as indicated by LRMS, barium hydroxide was quenched by addition of dry ice (pH =7), the white precipitate was filtered off and filtrate was concentrated to dryness. The crude product was taken up in 10% aqueous acetic acid and

loaded on a CM Sephadex C-25 column, eluted with water followed by a gradient of 0.1% - 1.0% ammonium hydroxide in deionized water. The fractions containing the product were combined and lyophilized with glacial acetic acid to afford **14** (6.6 mg, 41%) as pentaacetate salt in the form of off white solid. $[\alpha]_D^{23}$ +50.8 (*c* 0.26, H_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.38 (d, *J* = 3.7 Hz, 1H), 5.17 (d, *J* = 3.0 Hz, 1H), 5.12 (d, *J* = 1.8 Hz, 1H), 4.32 (t, *J* = 5.8 Hz, 1H), 4.15 (dd, *J* = 5.1, 3.1 Hz, 1H), 4.13 (t, *J* = 4.7 Hz, 1H), 4.05 (t, *J* = 3.1 Hz, 1H), 4.03 – 3.99 (m, 1H), 3.80 (t, *J* = 9.6 Hz, 1H), 3.75 – 3.65 (m, 3H), 3.64 (dt, *J* = 2.8, 1.3 Hz, 1H), 3.58 (dd, *J* = 12.3, 5.0 Hz, 1H), 3.55 – 3.49 (m, 2H), 3.46 (dd, *J* = 12.1, 6.8 Hz, 1H), 3.42 – 3.40 (m, 1H), 3.39 – 3.32 (m, 1H), 3.25 (dd, *J* = 13.7, 6.6 Hz, 1H), 3.21 – 3.14 (m, 2H), 2.30 (dt, *J* = 12.6, 4.3 Hz, 1H), 1.93 – 1.86 (m, 1H) 1.79 (s, 15H), 1.66 (q, *J* = 12.7 Hz, 1H), 1.54 (q, *J* = 11.2, 10.4 Hz, 1H), 1.42 (q, *J* = 12.3 Hz, 1H), 1.24 – 1.19 (m, 2H), 1.09 – 1.04 (m, 1H), 0.99 (q, *J* = 9.6 Hz, 1H), 0.68 (t, *J* = 6.9 Hz, 3H).

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 179.3, 109.9, 95.4, 95.3, 84.0, 81.3, 78.0, 75.2, 73.2, 72.1, 70.1, 67.5, 67.2, 61.1, 60.2, 50.7, 49.6, 49.2, 49.0, 40.3, 33.8, 32.0, 28.1, 26.3, 22.0, 18.3, 13.2.

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{26}H_{52}N_5O_{12}$ 626.3612, found 626.3618.



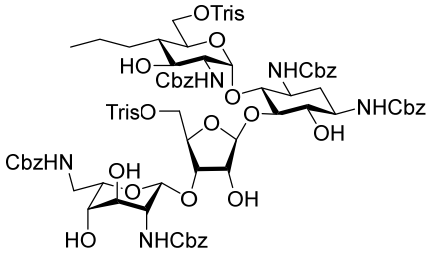
48

1,3,2′,2‴,6‴-Penta-*N*-benzyloxycarbonyl paromomycin (48). A solution of Propylamycin pentaacetate **1** (500 mg, 0.53 mmol) and *N*-(benzyloxycarbonyloxy)succinimide (1.16 g, 4.68 mmol) in DMF (2 mL) was cooled to 0 °C in an ice bath, added DIPEA (1.63 mL, 9.35 mmol) and stirred at 0 °C for 3 h. After completion, the reaction mixture was quenched by addition of 0.5 mL butylamine, diluted with ethyl acetate (50 mL) and washed sequentially with 0.5 N aq. NaOH solution (10 mL), 1 N HCl solution (10 mL) followed by saturated aqueous $NaHCO_3$ solution. The aqueous layer was re-extracted in ethyl acetate and the combined organic layer was washed with brine, dried on Na_2SO_4, evaporated, and purified by column chromatography on silica gel (1:3 hexane: ethyl acetate to 20:1 ethyl acetate: methanol) to obtain compound **48** as a white foam (596 mg, 86%); analytical TLC on silica gel, 3:17 Methanol/ethyl acetate, R_f=0.70; $[\alpha]_D^{23}$ +29.4 (*c* 1, MeOH).

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{66}H_{81}N_5O_{23}Na$ 1334.5214; Found 1334.5215.

6′,5″-Bis-*O*-(2,4,6-triisopropylbenzenesulfonyl)-1,3,2′,2‴, 6‴-penta-*N*-benzyloxycarbonyl paromomycin (49). A solution of compound **48** (530 mg, 0.40 mmol), in pyridine (4 mL) was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (367 mg, 1.21 mmol) at room temperature for 18 h. After complete consumption f starting material, Ac_2O (315μL, 3.33 mmol) was added to the reaction mixture and continued stirring at room temperature for another 5 h. The reaction mixture was diluted with ethyl acetate (100 mL), washed with 1 N HCl solution (2x10 mL), saturated aqueous $NaHCO_3$ and brine solution (1 x15 ml, each), dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by column chromatography on silica gel (hexnes: ethyl acetate 3:2 to 1:3) to obtain acetylated intermediate (525 mg, 63%) as off white foam; analytical TLC on silica gel, 1:1 EtOAc/Hexane, R_f=0.55; $[\alpha]_D^{23}$ +19.7 (*c* 1, $CHCl_3$).

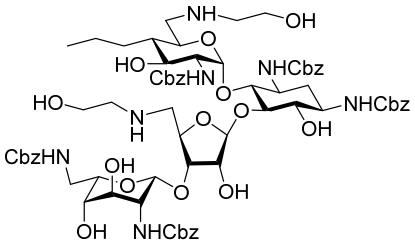
HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{106}H_{135}N_5O_{32}S_2Na$ 2076.8429, found 2076.8469.



49

The acetylated ditrisyl intermediate obtained in previous step (190 mg, 92.4 μmol) was treated with 0.2 M solution of NaOMe in methanol (5 mL) at room temperature for 8 h. After complete deacetylation, the reaction mixture was neutralized with amberlyst® 15, filtered and purified by column chromatography on silica gel (methanol: dichloromethane 0:1 to 1:4) to obtain compound **49** in 45% yield (136 mg) over 3 steps; analytical TLC on silica gel, 1:19 Methanol/EtOAc, R_f=0.75; $[\alpha]_D^{23}$ = +30.2 (*c* 1, $CHCl_3$).

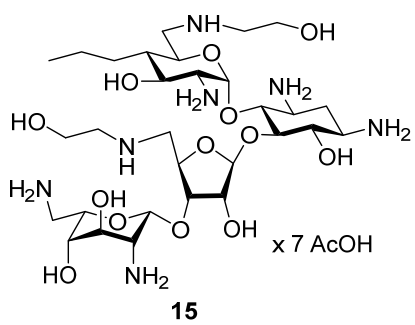
HRMS (ESI/Q-TOF): *m/z* $[M+Na]^+$ Calcd for $C_{96}H_{125}N_5O_{27}S_2Na$ 1866.7896, found 1866.7882.



50

6′,5″-Dideoxy-bis-*N*-(2-hydroxyethyl)-1,3,2′,2‴,6‴-penta-*N*-benzyloxycarbonyl paromomycin (50). A solution of compound **49** (70 mg, 37.9 μmol) in 8 mL of ethanolamine was stirred at room temperature for 12 h. After complete conversion as indicated by LRMS, the reaction mixture was cooled diluted with dichloromethane (25 mL) and washed with brine (2x 4 mL). The aqueous layer was extracted in dichloromethane (4x10 mL), and the combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure and purified by column chromatography on silica gel (methanol: dichloromethane 1:25 to 1:5) to afford compound **49** (19 mg, 40%); analytical TLC on silica gel, 3:17 Methanol/$CHCl_3$, R_f=0.8; $[\alpha]_D^{23}$ +24.1 (*c* 0.9, MeOH)

HRMS (ESI/Q-TOF): *m/z* $[M+H]^+$ Calcd for $C_{70}H_{92}N_7O_{23}$ 1398.6245, found 1398.6238.



15

6′,5″-Dideoxy-bis-*N*-(2-hydroxyethyl) paromomycin (15). A solution of compound **50** (18 mg, 12.9 μmol) in dioxane: deionized water (1:1, 1.5 mL) was treated with Pd/C (30 mg) at room temperature under 1 atm of hydrogen (balloon) for 18 h. After completion, as indicated by LRMS, the reaction mixture was filtered through Celite®, concentrated to dryness and purified by passing through a CM Sephadex C-25 column, loading in 10% aqueous acetic acid and eluting with deionized water followed by a gradient of 0.1% - 1.0% ammonium hydroxide in deionized water. The fractions containing the product were combined and lyophilized with glacial acetic acid to afford **15** (6.6 mg, 44%) as heptaacetate salt in the form of a white solid. $[\alpha]_D^{23}$ +26.7 (*c* 0.44, H_2O).

^{1}H NMR (600 MHz, D_2O) δ: 5.79 (d, *J* = 3.8 Hz, 1H), 5.34 (d, *J* = 2.2 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 4.36 (t, *J* = 5.8 Hz, 1H), 4.27 (dd, *J* = 5.2, 2.3 Hz, 1H), 4.21 (ddd, *J* = 9.6, 6.6, 3.1 Hz, 1H), 4.16 (d, *J* = 5.4 Hz, 1H), 4.07 (t, *J* = 3.1 Hz, 1H), 3.97 (t, *J* = 10.5 Hz, 1H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.83 (td, *J* = 9.8, 9.2, 4.0 Hz, 2H), 3.71 (q, *J* = 6.1, 5.3 Hz, 4H), 3.67 (d, *J* = 3.2 Hz, 1H), 3.66 – 3.56 (m, 1H), 3.43 (d, *J* = 3.0 Hz, 1H), 3.38 (dd, *J* = 13.1, 3.0 Hz, 1H), 3.32 (dd, *J* = 13.1, 2.6 Hz, 1H), 3.29 – 3.12 (m, 6H), 3.09 (dt, *J* = 10.3, 4.6 Hz, 4H), 2.24 – 2.19 (m, 1H), 1.61 (q, *J* = 12.5 Hz, 1H), 1.47 (td, *J* = 10.4, 5.0 Hz, 1H), 1.34 (td, *J* = 11.1, 10.7, 5.5 Hz, 2H), 1.26 – 1.05 (m, 2H), 0.73 (t, *J* = 7.2 Hz, 3H).

^{13}C{^{1}H} NMR (151 MHz, D_2O) δ: 106.9, 94.0, 93.2, 82.1, 75.9, 75.6, 71.7, 70.6, 68.8, 67.7, 66.2, 66.0, 64.8, 60.0, 56.1, 55.2, 54.9, 53.5, 49.4, 48.9, 48.3, 48.2, 48.0, 47.5, 42.6, 39.8, 39.0, 26.5, 21.4, 17.2, 12.3.

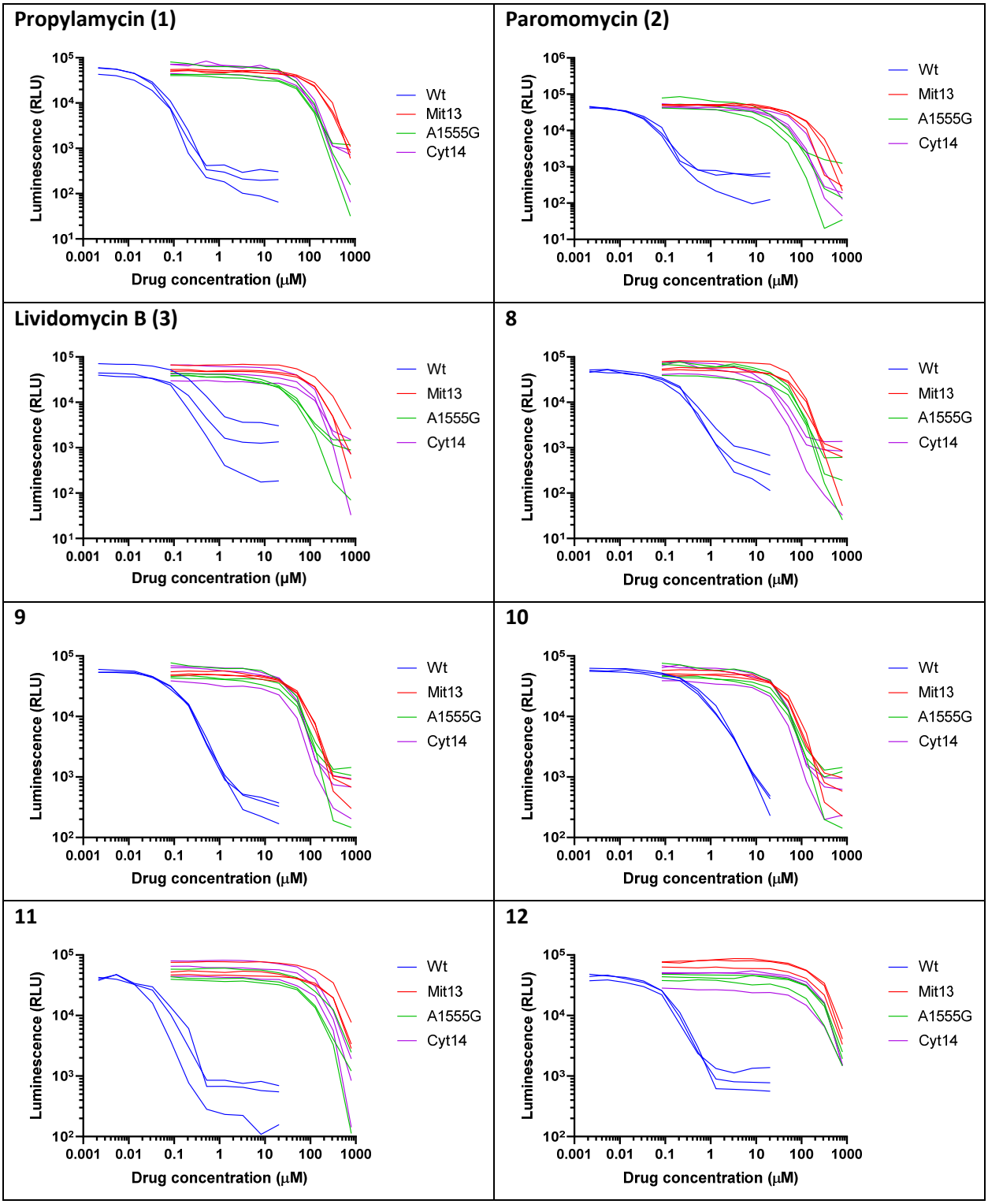
HRMS (ESI/Q-TOF): m/z [M+Na]+ Calcd for $C_{30}H_{61}N_7O_{13}Na$ 750.4203, found 750.4225.

Table S1. Bacterial strains used in this study

No	Bacterial species	Aminoglycoside Resistance Genes	Source	Reference
AG001	*Escherichia coli*	None	Clinical isolate, IMM	[4]
AG003	*Escherichia coli*	*aac(3)-II*	Clinical isolate, IMM	[4]
AG038	*Staphylococcus aureus*	None	Clinical isolate, IMM	[4]
AG163	*Escherichia coli*	*aph(3')-I*	Clinical isolate, IMM	[5]
AG166	*Escherichia coli*	*aph(3')-IIa*	Clinical isolate, IMM	[6]
AG173	*Escherichia coli*	*aac(3)-IV*	Clinical isolate, IMM	[6]
AG175	*Escherichia coli*	*aac(6')-I*	Clinical isolate, IMM	[7]
AG215	*Klebsiella pneumoniae*	None	Clinical isolate, IMM	[8]
AG220	*Pseudomonas aeruginosa*	*aph(3')-IIb*	ATCC 27853	
AG225	*Acinetobacter baumannii*	None	Clinical isolate, IMM	[4]
AG290	*Enterobacter cloacae*	None	Clinical isolate, IMM	[4]
EC026	*Escherichia coli*	None	Laboratory strain DH5α	[5]
EC102	*Escherichia coli*	*armA*	Engineered strain, IMM	[5]
EC103	*Escherichia coli*	*rmtB*	Engineered strain, IMM	[5]
EC118	*Escherichia coli*	*aac(3)-IV*	Engineered strain, IMM	[5]
EC125	*Escherichia coli*	*aph(3')-IIb*	Engineered strain, IMM	[5]
EC141	*Escherichia coli*	*aph(3')-VI*	Engineered strain, IMM	[5]
EC189	*Escherichia coli*	*aph(3')-Ia*	Engineered strain, IMM	This study
EC191	*Escherichia coli*	*aph(3')-IIa*	Engineered strain, IMM	This study
Strains used as source of hybrid ribosomes for in-vitro translation assays				
SZ380	*Mycobacterium smegmatis*	*None*	Engineered strain, IMM	[9]
SZ480	*M. smegmatis* Cyt14	*None*	Engineered strain, IMM	[9]
SZ485	*M. smegmatis* Mit13	*None*	Engineered strain, IMM	[10]
SZ496	*M. smegmatis* Mit A1555G	*None*	Engineered strain, IMM	[10]

IMM, Institute of Medical Microbiology, University of Zurich

Cell-free translation inhibition graphs



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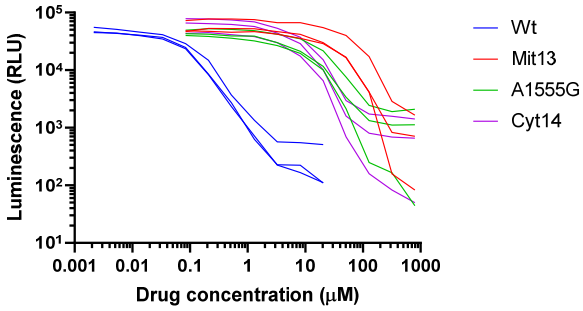

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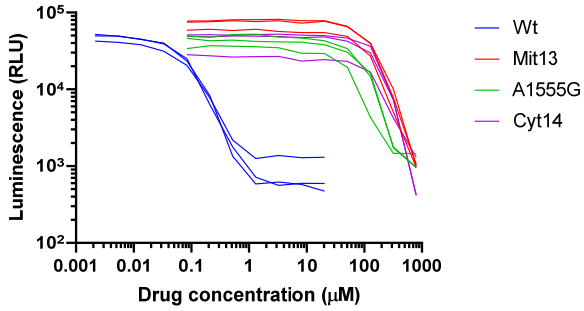

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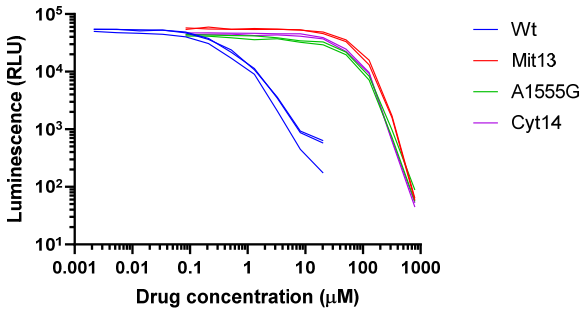
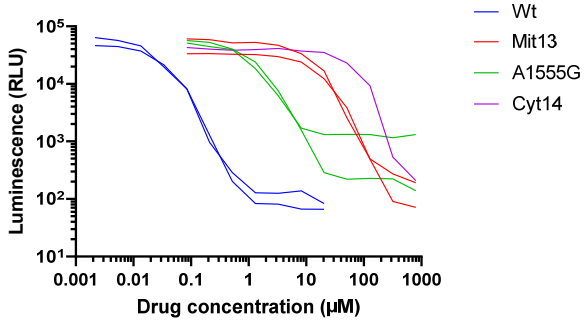
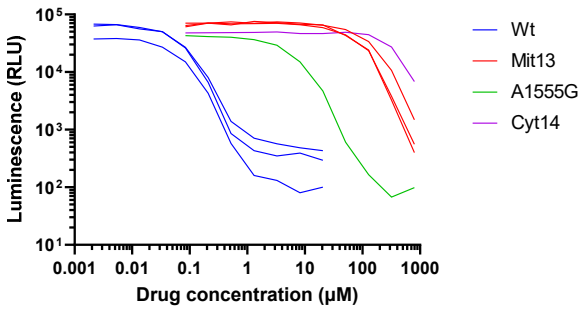


Gentamicin



Plazomicin



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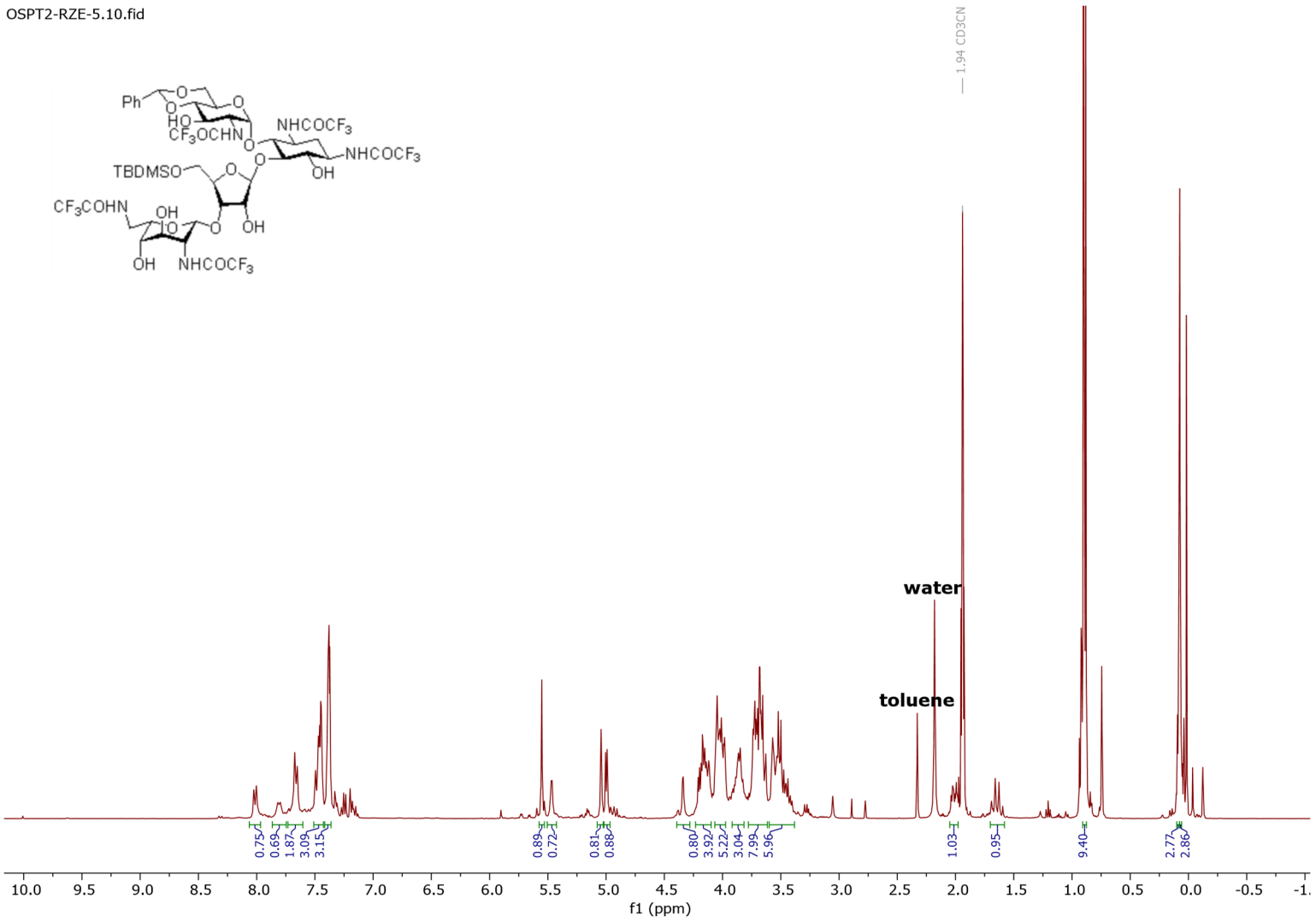
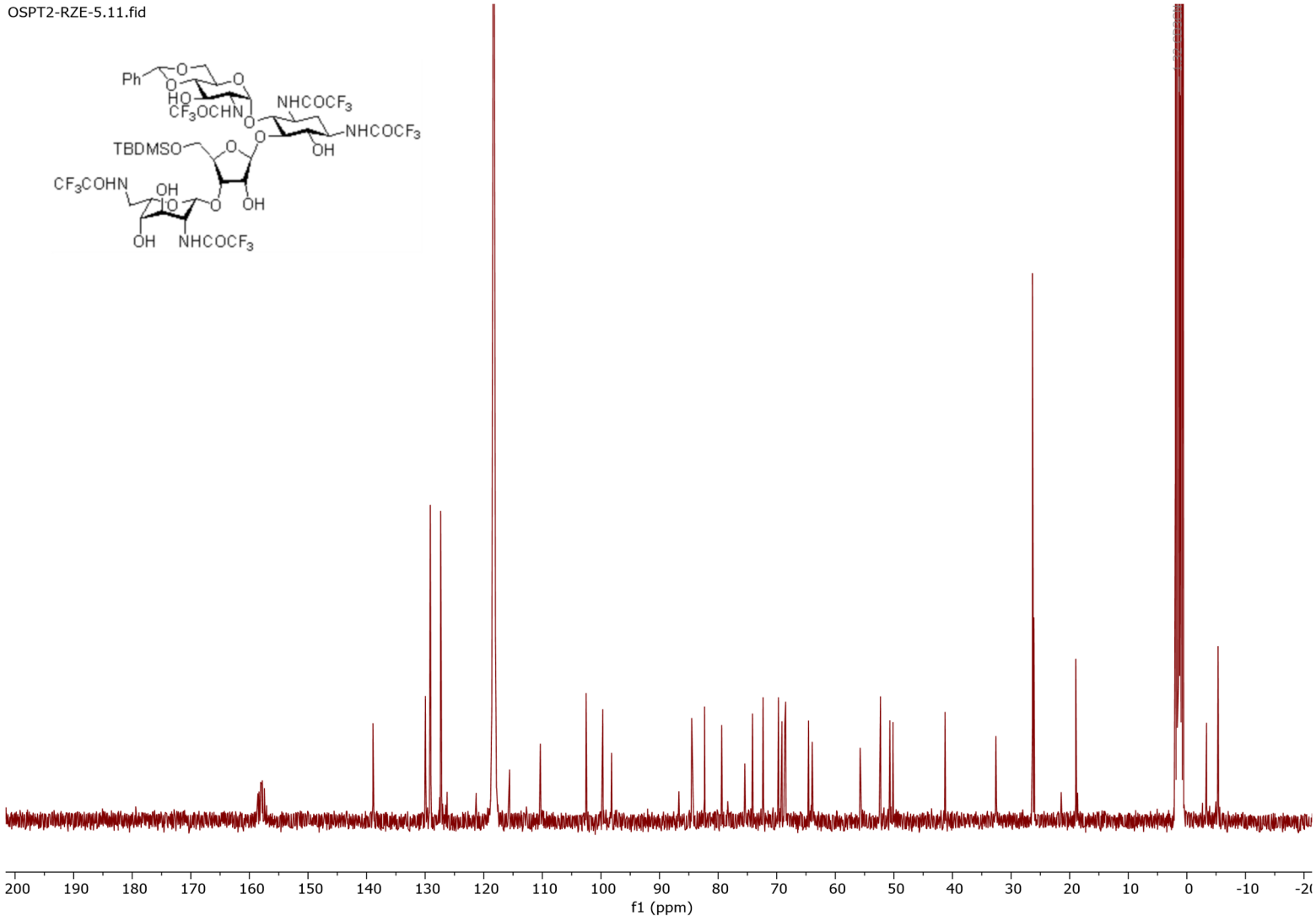
5″-*O*-*tert*-Butyldimethylsilyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (17)

[^{1}H-NMR, 400 MHz, CD_3CN]



5″-*O*-*tert*-Butyldimethylsilyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (17)

[^{13}C-NMR, 100.6 MHz, CD_3CN]



5″-*O*-*tert*-Butyldimethylsilyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (17)

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MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_406 1000 Zogota RZE-6
MS_POS_2300_RES_7min ACN_Form_5-98_040_4min 1:C,4 1.000000 MS_Tune Col#43

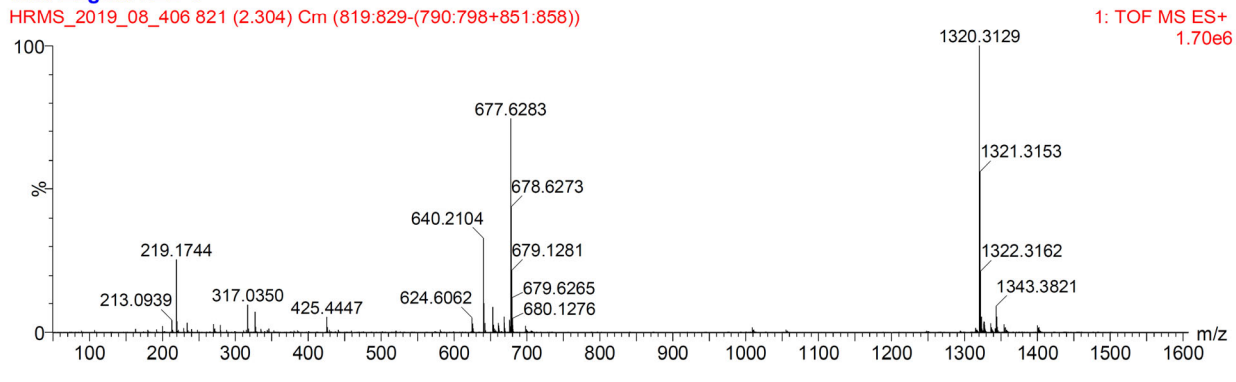
Elemental Composition Report:

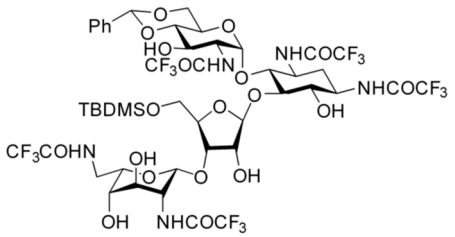
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
526 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 1-110 N: 1-10 O: 0-20 F: 15-15 Na: 1-1 Si: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1320.3129	100.00	1320.3153	-2.4	-1.8	13.5	521.8	0.079	92.45	C46 H58 N5 O19 F15 Na Si
		1320.3167	-3.8	-2.9	18.5	524.3	2.583	7.55	C47 H54 N9 O15 F15 Na Si

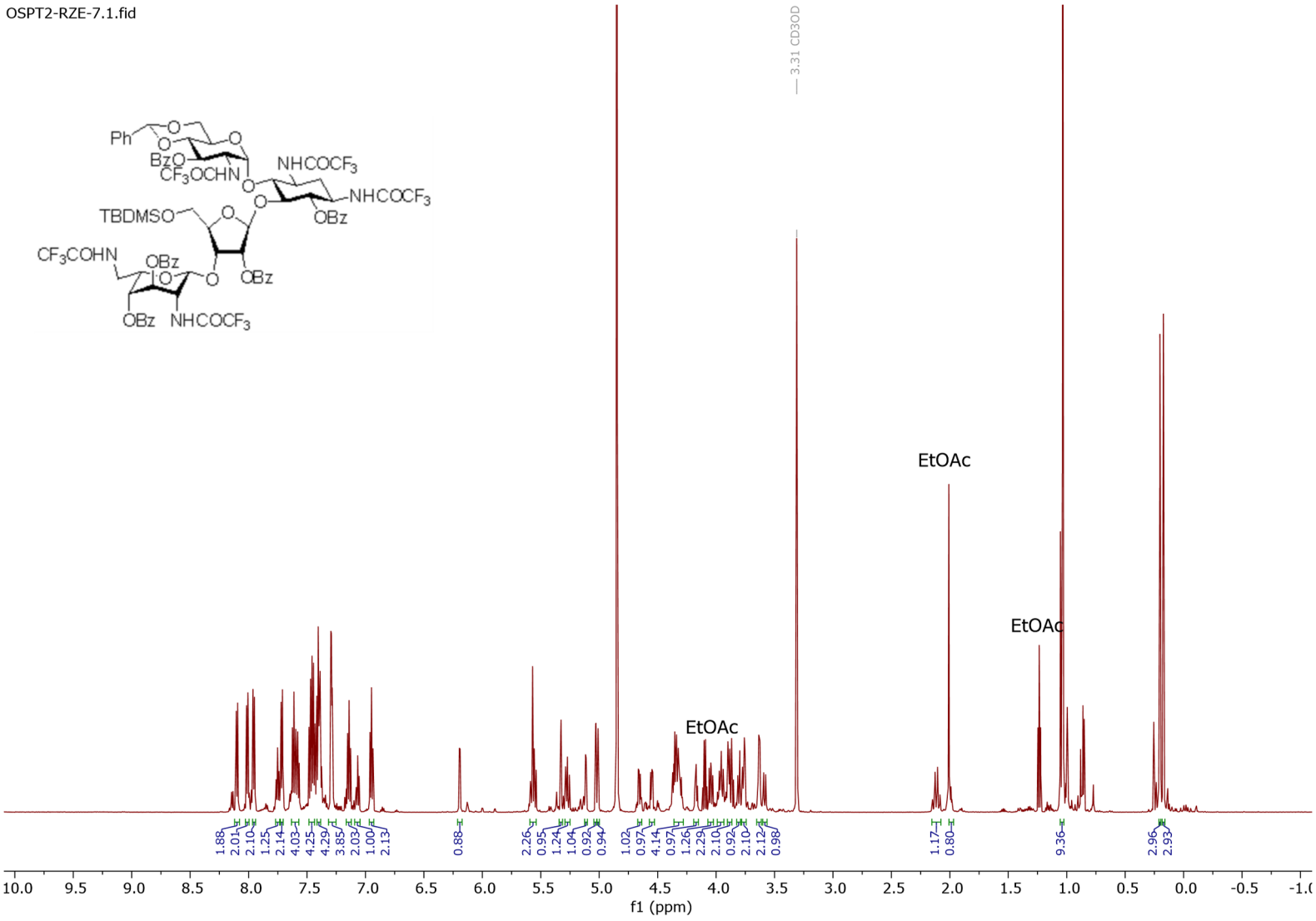
1000 Zogota RZE-6





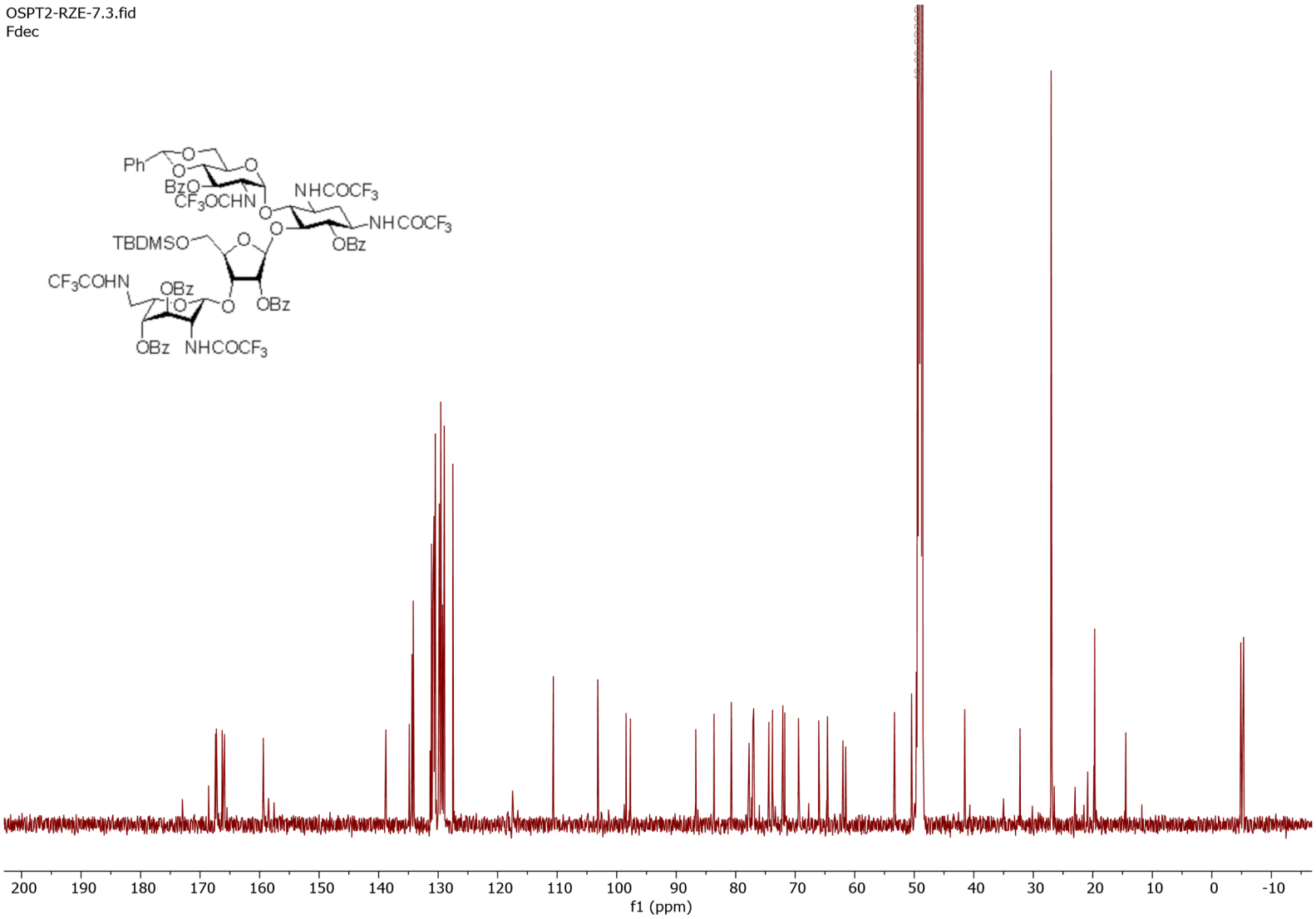
6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-5″-*O*-tert-butyldimethylsilyl-4′,6′-O-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (18)

[^{1}H-NMR, 600 MHz, CD_3OD]



6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-5″-*O*-tert-butyldimethylsilyl-4′,6′-O-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (18)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-5″-*O*-tert-butyldimethylsilyl-4′,6′-O-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (18)

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MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_431 1001 Zogota RZE-7
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:C,5 10.000000 MS_Tune Col#43

Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
349 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-81 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1 Si: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
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1001 Zogota RZE-7
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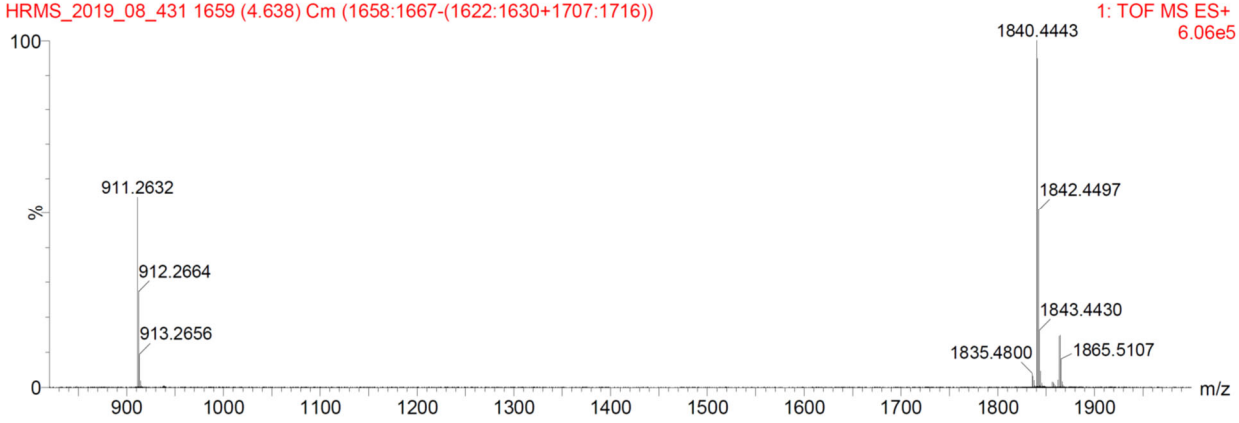
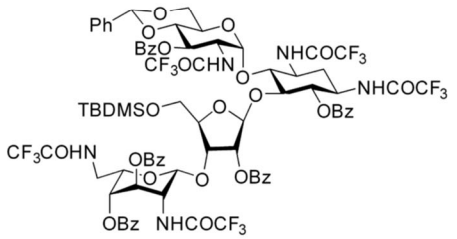
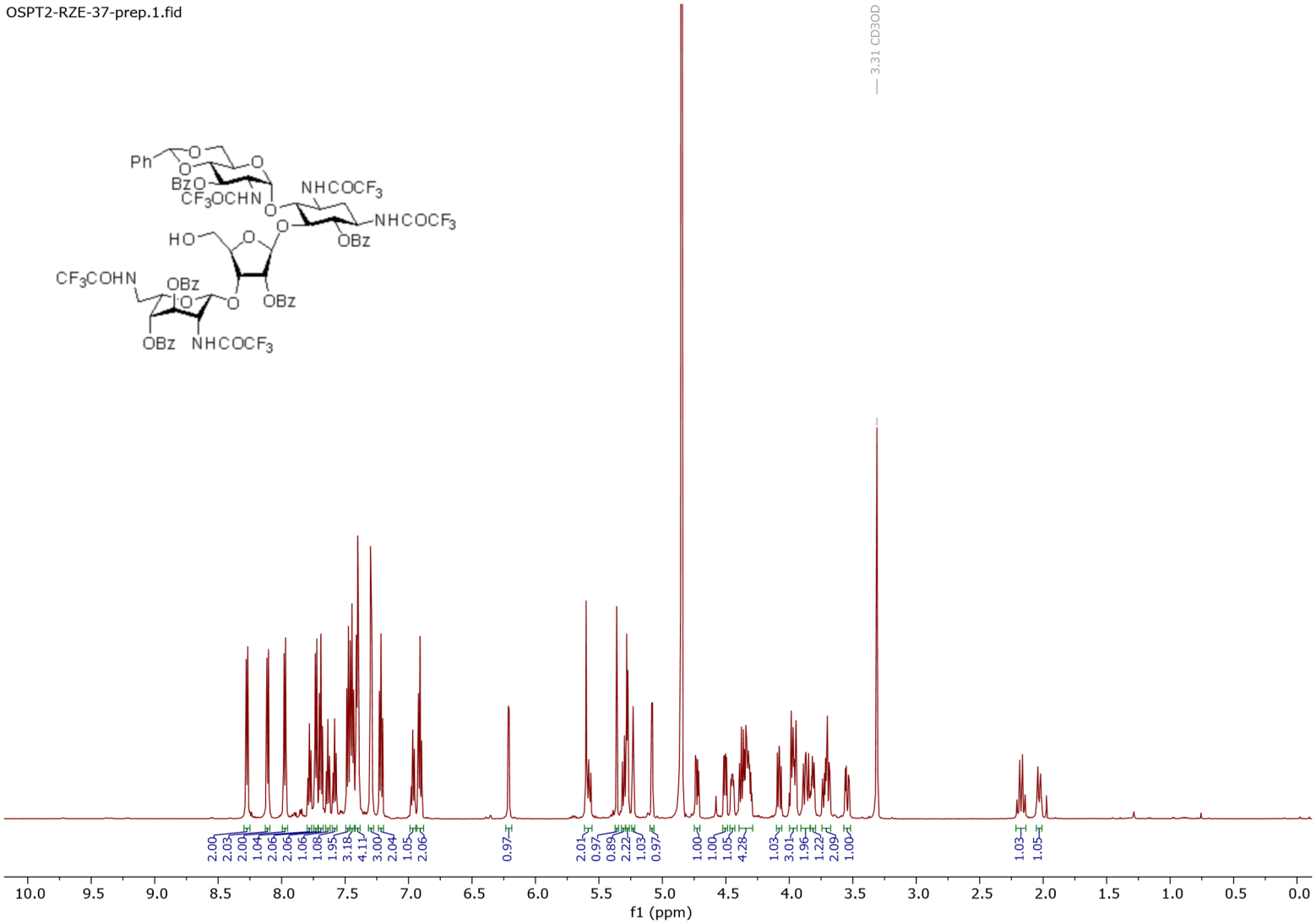
1: TOF MS ES+
6.06e5





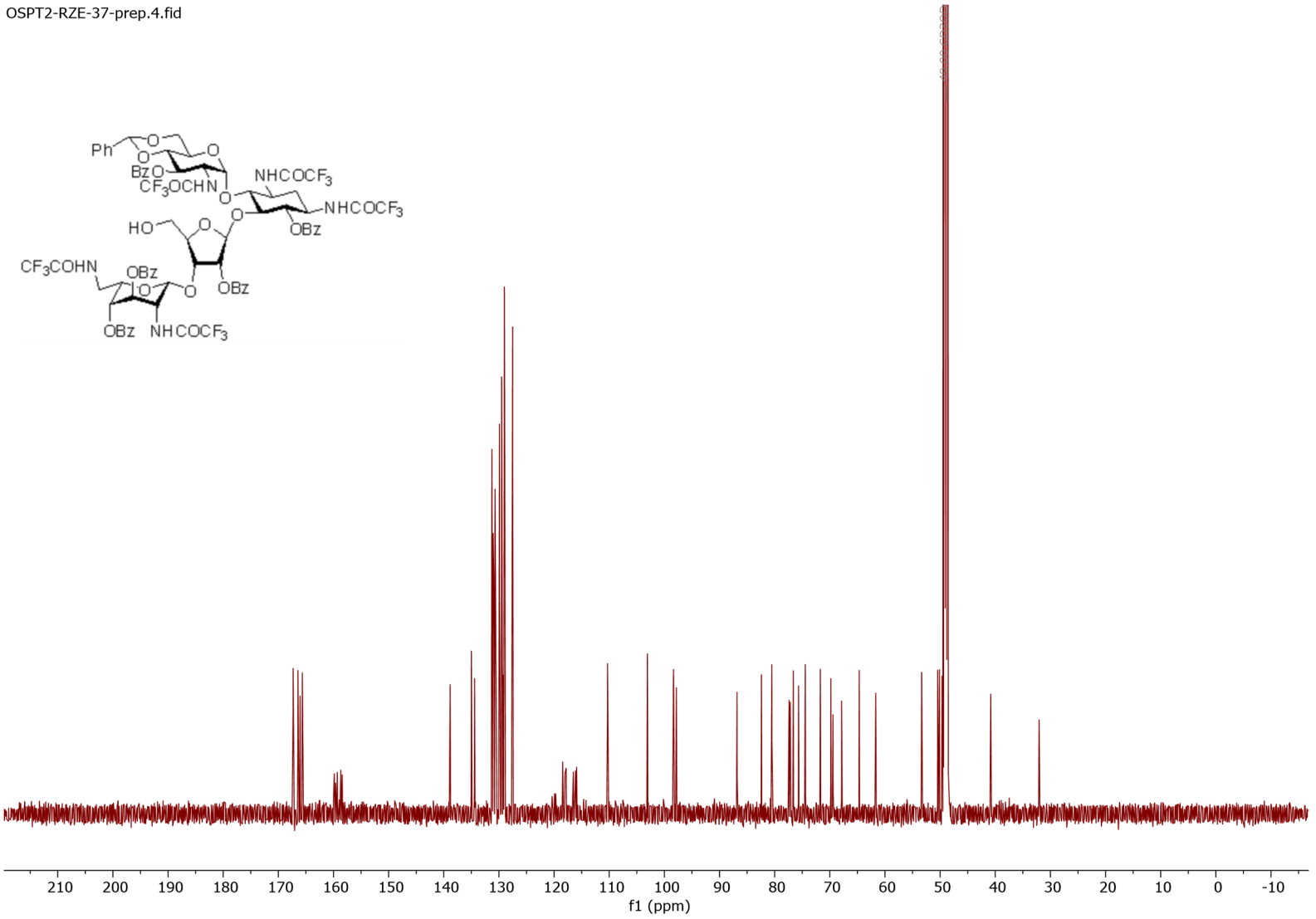
6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (19)

[^{1}H-NMR, 600 MHz, CD_3OD]



6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (19)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



6,3′,2″,3‴,4‴-Penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (19)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_433 1002 Zogota RZE-9
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:C,6 10.000000 MS_Tune Col#43

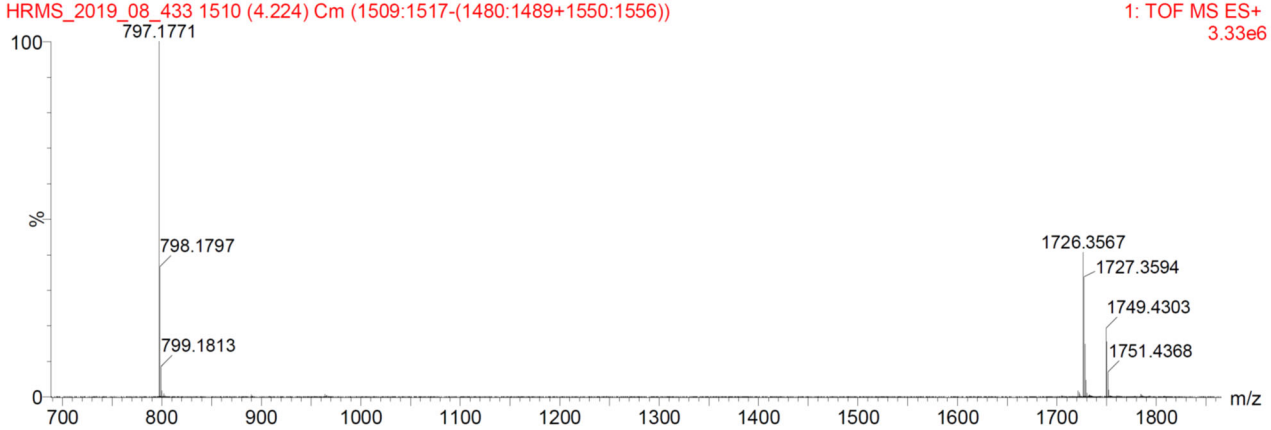
Elemental Composition Report:

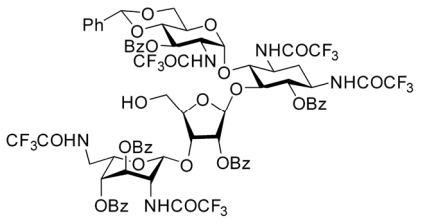
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
402 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-75 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1726.3567	100.00	1726.3599	-3.2	-1.9	38.5	420.6	n/a	n/a	C75 H64 N5 O24 F15 Na

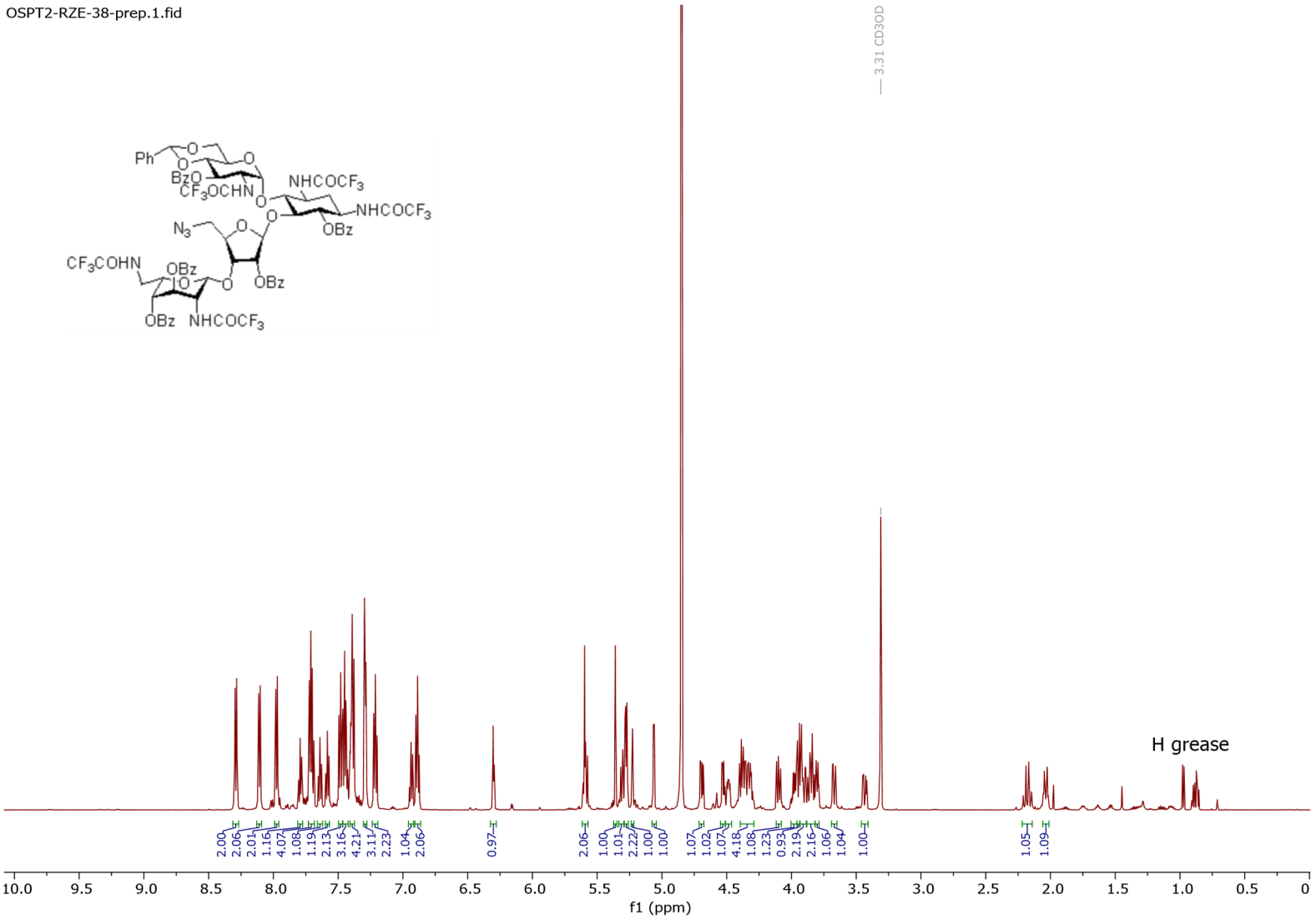
1002 Zogota RZE-9
HRMS_2019_08_433 1510 (4.224) Cm (1509:1517-(1480:1489+1550:1556))
1: TOF MS ES+
3.33e6





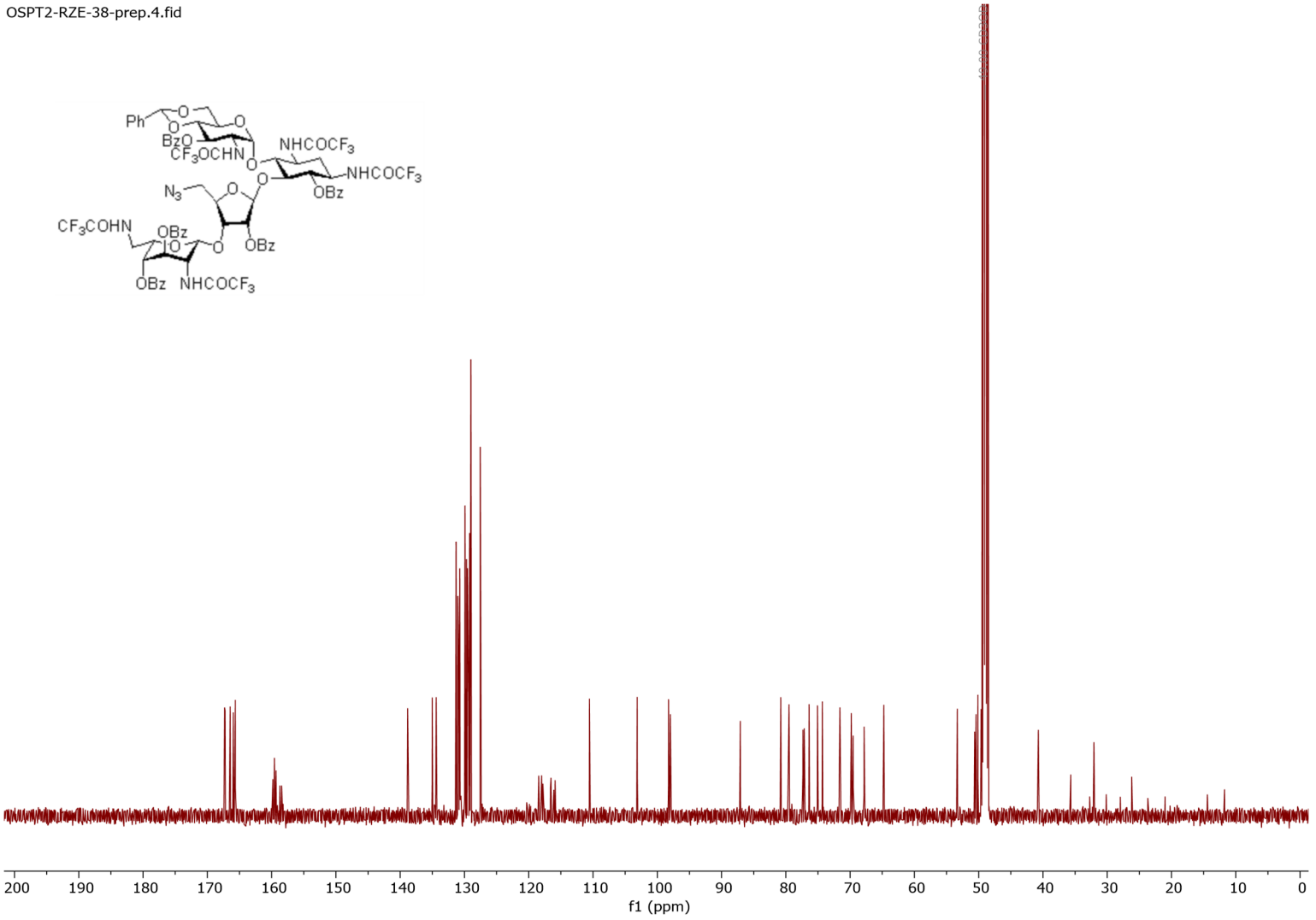
5″-Deoxy-5″-azido-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (20)

[^{1}H-NMR, 600 MHz, CD_3OD]



5″-Deoxy-5″-azido-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (20)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



5″-Deoxy-5″-azido-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (20)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_435 1003 Zogota RZE-38
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:C,7 10.000000 MS_Tune Col#43

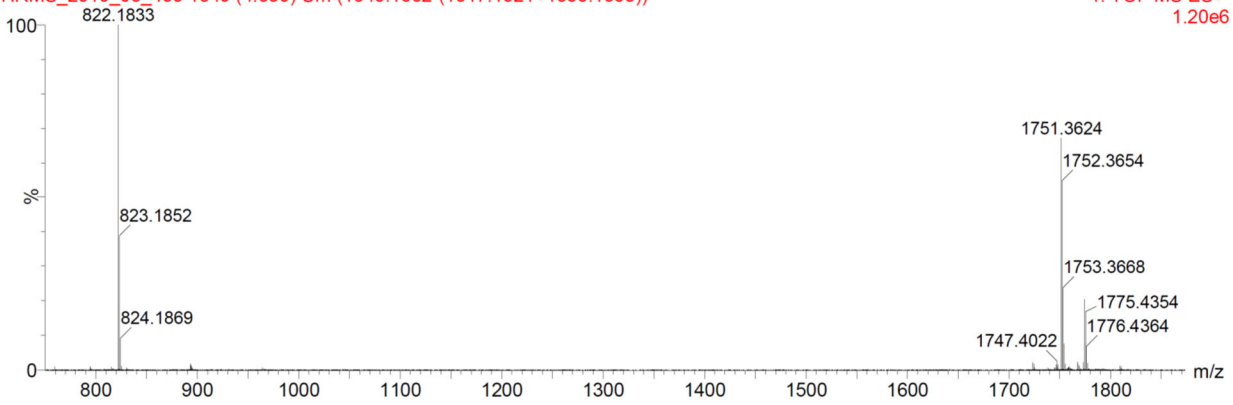
Elemental Composition Report:

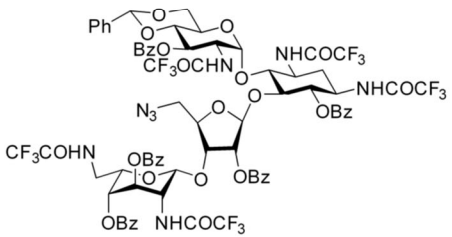
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
252 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-75 H: 1-110 N: 1-10 O: 0-23 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1751.3624	100.00	1751.3664	-4.0	-2.3	40.5	463.4	n/a	n/a	C75 H63 N8 O23 F15 Na

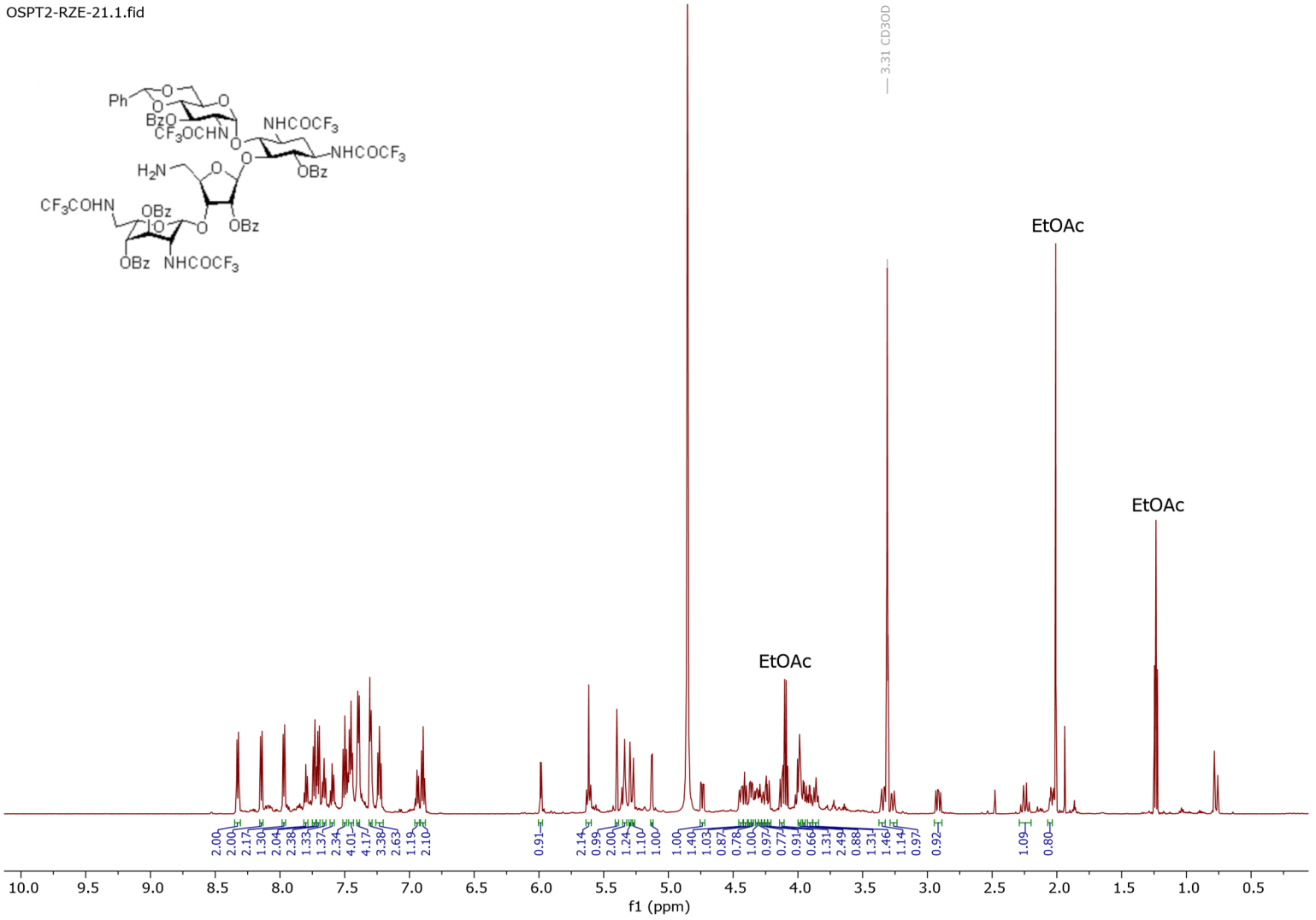
1003 Zogota RZE-38
HRMS_2019_08_435 1549 (4.330) Cm (1545:1552-(1517:1521+1583:1588))
1: TOF MS ES+
1.20e6





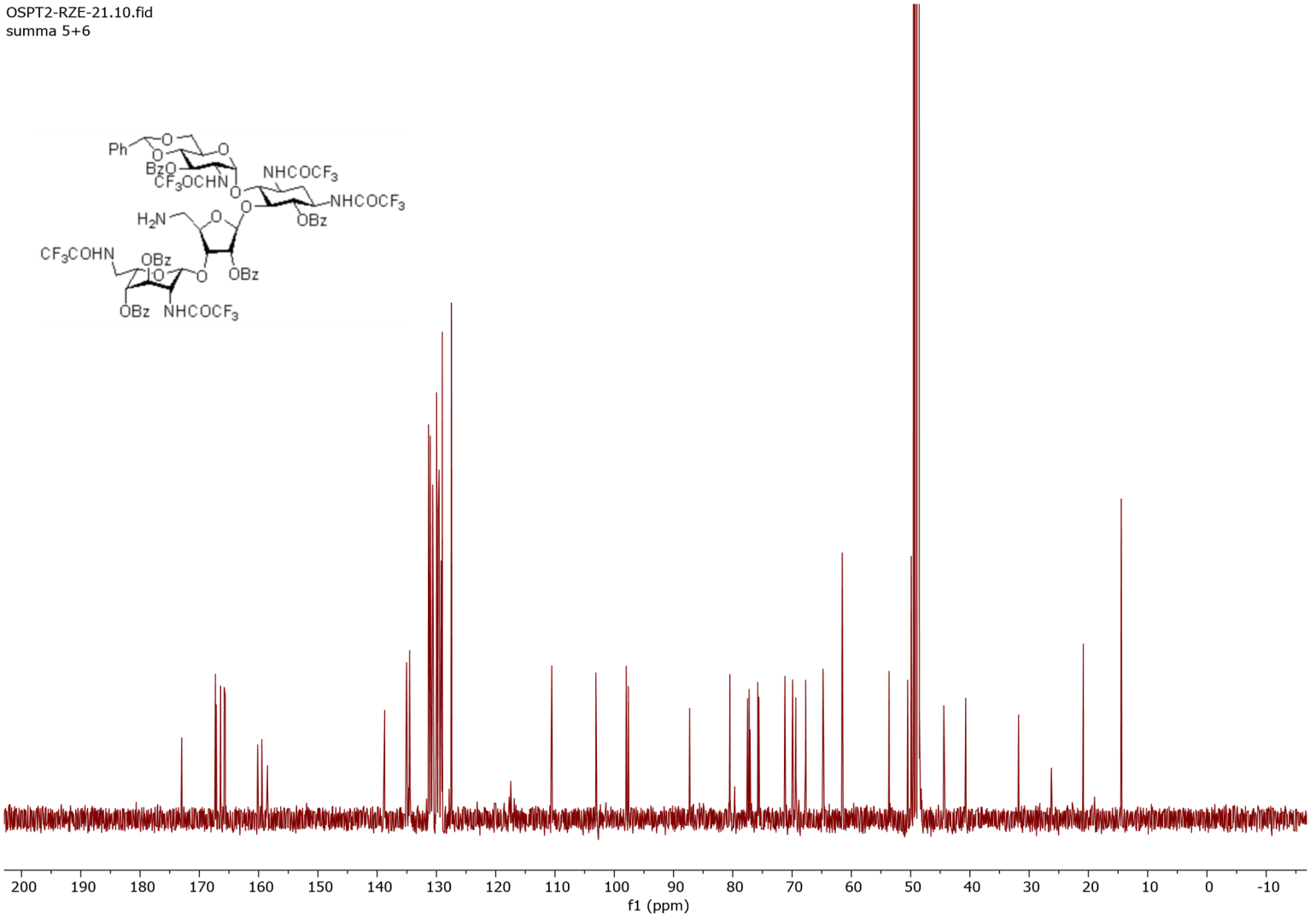
5″-Deoxy-5″-amino-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (21)

[^{1}H-NMR, 600 MHz, CD_3OD]



5″-Deoxy-5″-amino-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (21)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



5″-Deoxy-5″-amino-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (21)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_437 1004 Zogota RZE-16
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:C,8 10.000000 MS_Tune Col#43

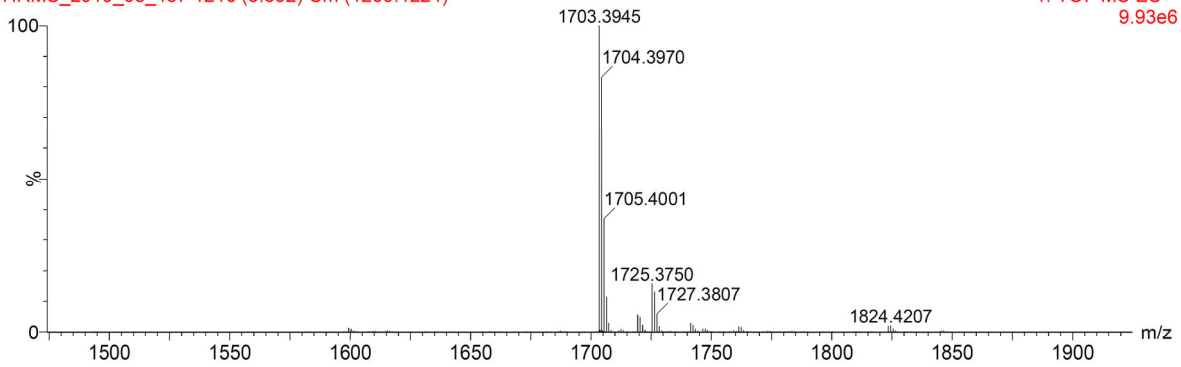
Elemental Composition Report:

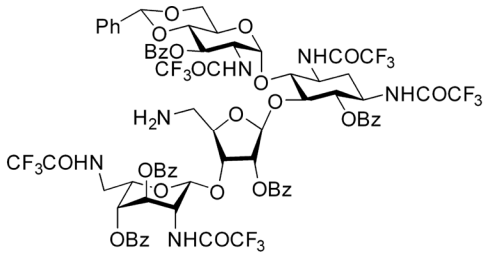
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
293 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-75 H: 1-110 N: 1-10 O: 0-23 F: 15-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1703.3945	100.00	1703.3940	0.5	0.3	38.5	672.3	n/a	n/a	C75 H66 N6 O23 F15

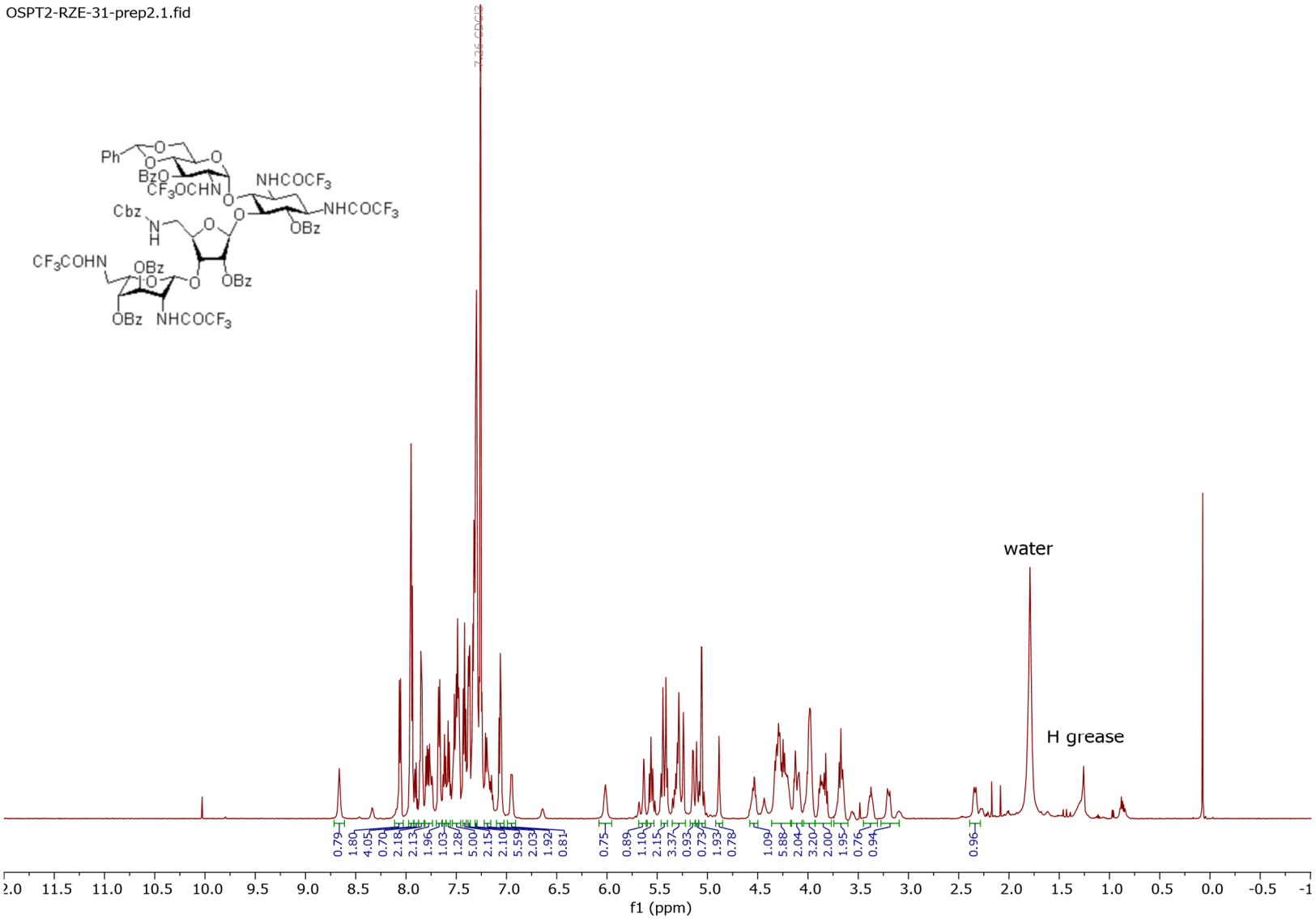
1004 Zogota RZE-16
HRMS_2019_08_437 1210 (3.392) Cm (1209:1224)
1: TOF MS ES+
9.93e6





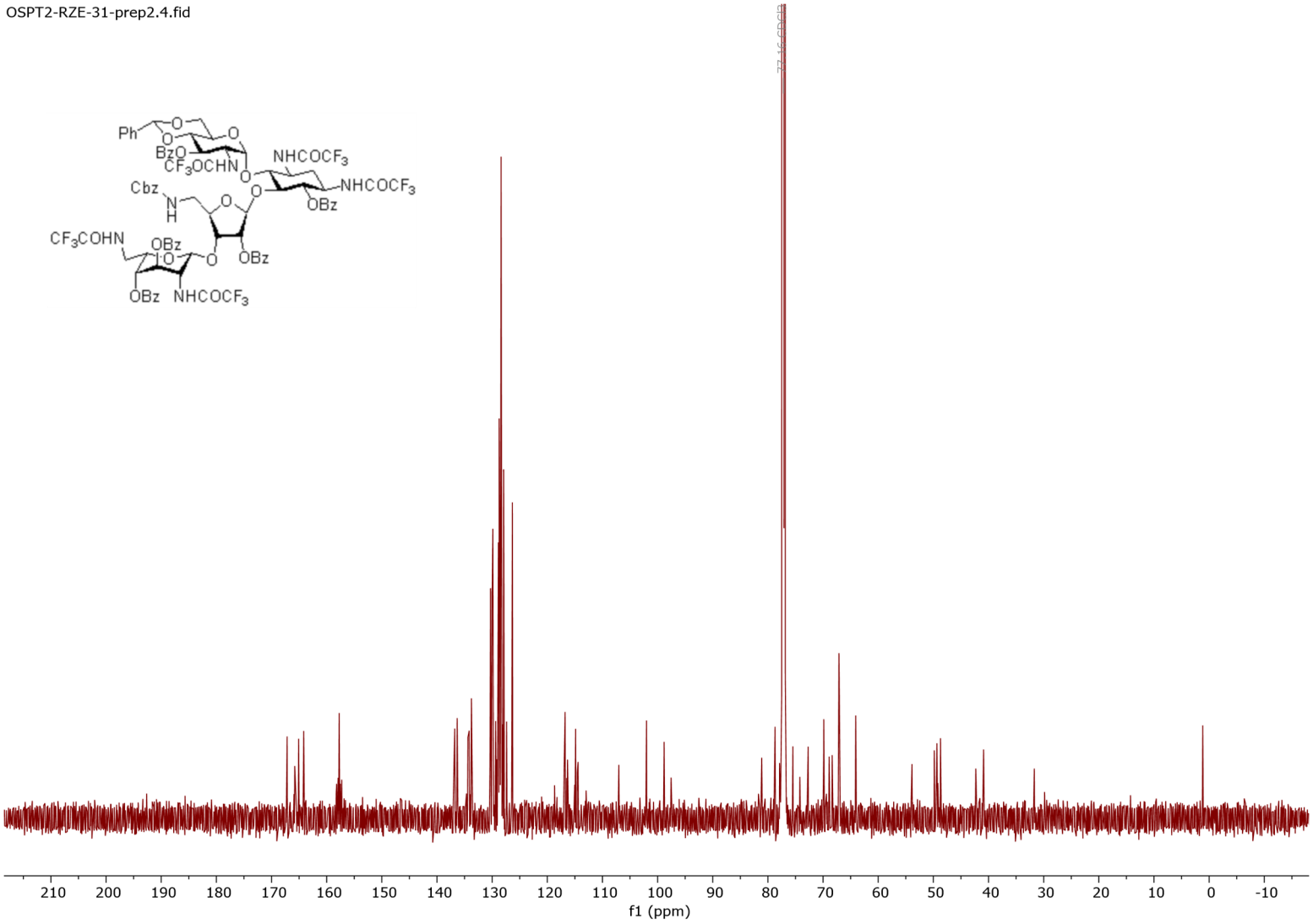
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (22)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (22)

[^{13}C-NMR, 150.9 MHz, $CDCl_3$]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (22)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_439 1005 Zogota RZE-31
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,1 10.000000 MS_Tune Col#43

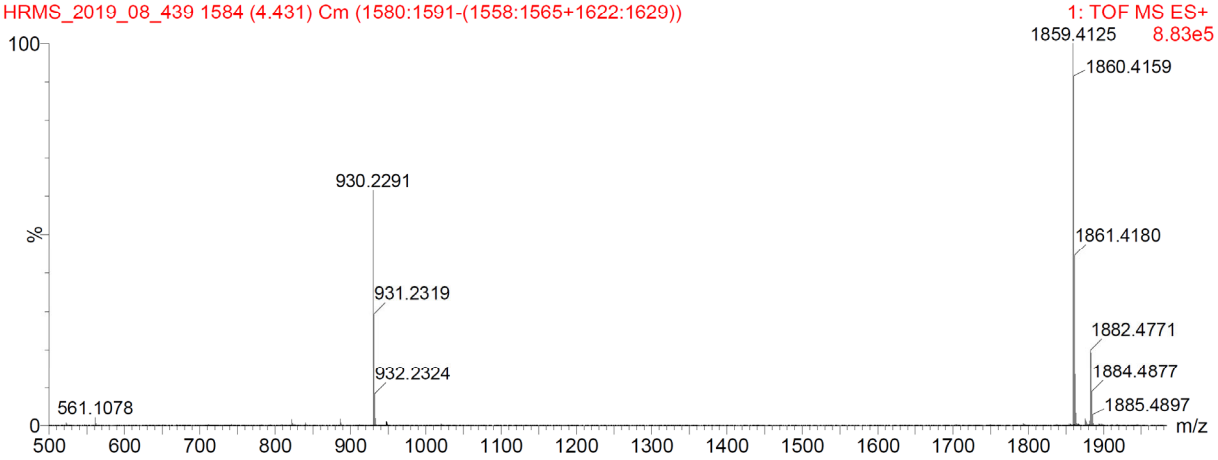
Elemental Composition Report:

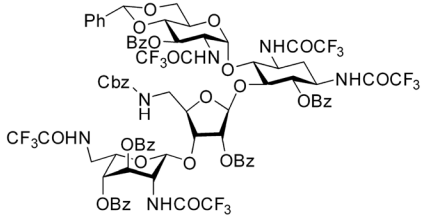
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
290 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-83 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1859.4125	100.00	1859.4127	-0.2	-0.1	43.5	485.1	n/a	n/a	C83 H71 N6 O25 F15 Na

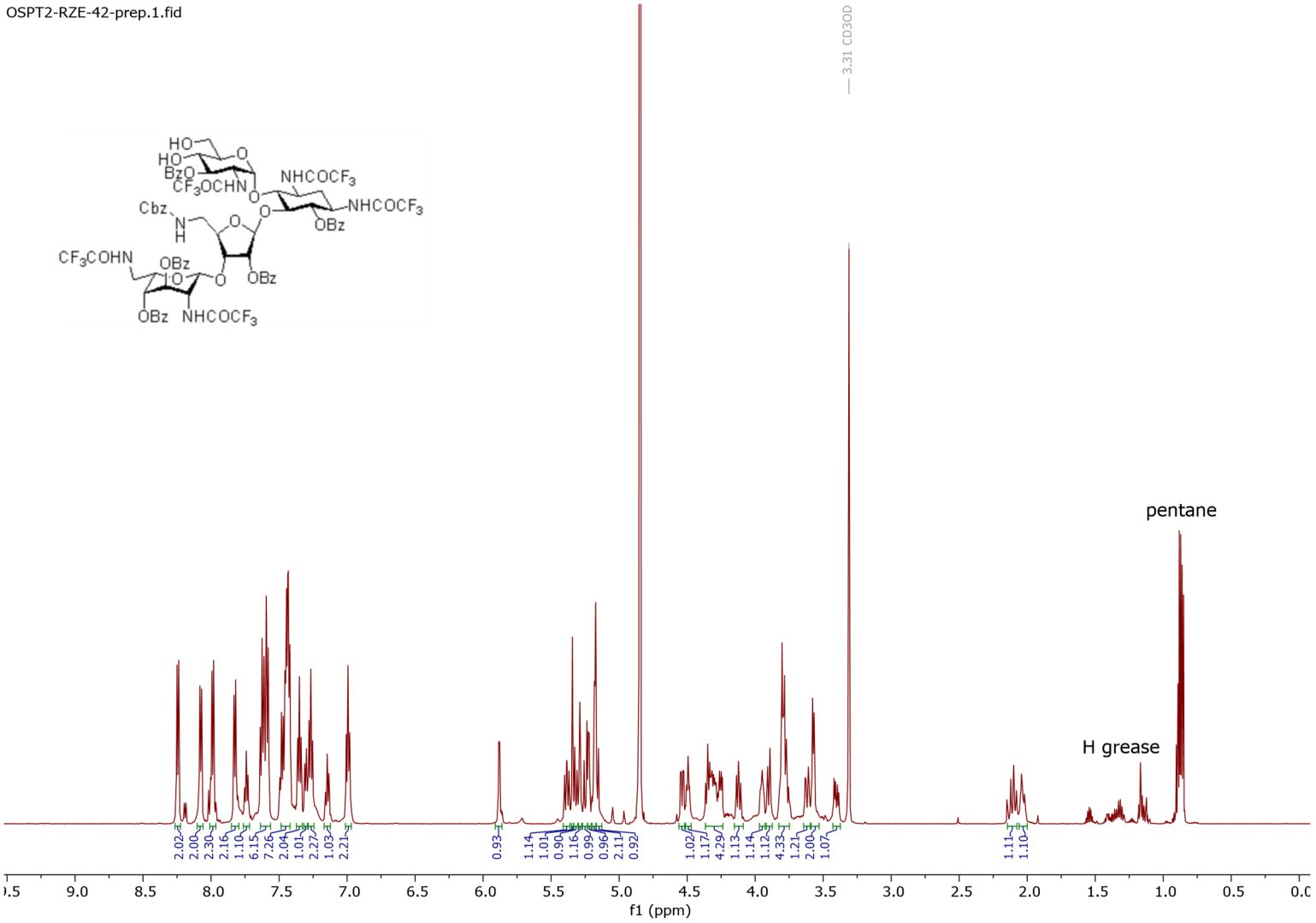
1005 Zogota RZE-31
HRMS_2019_08_439 1584 (4.431) Cm (1580:1591-(1558:1565+1622:1629))





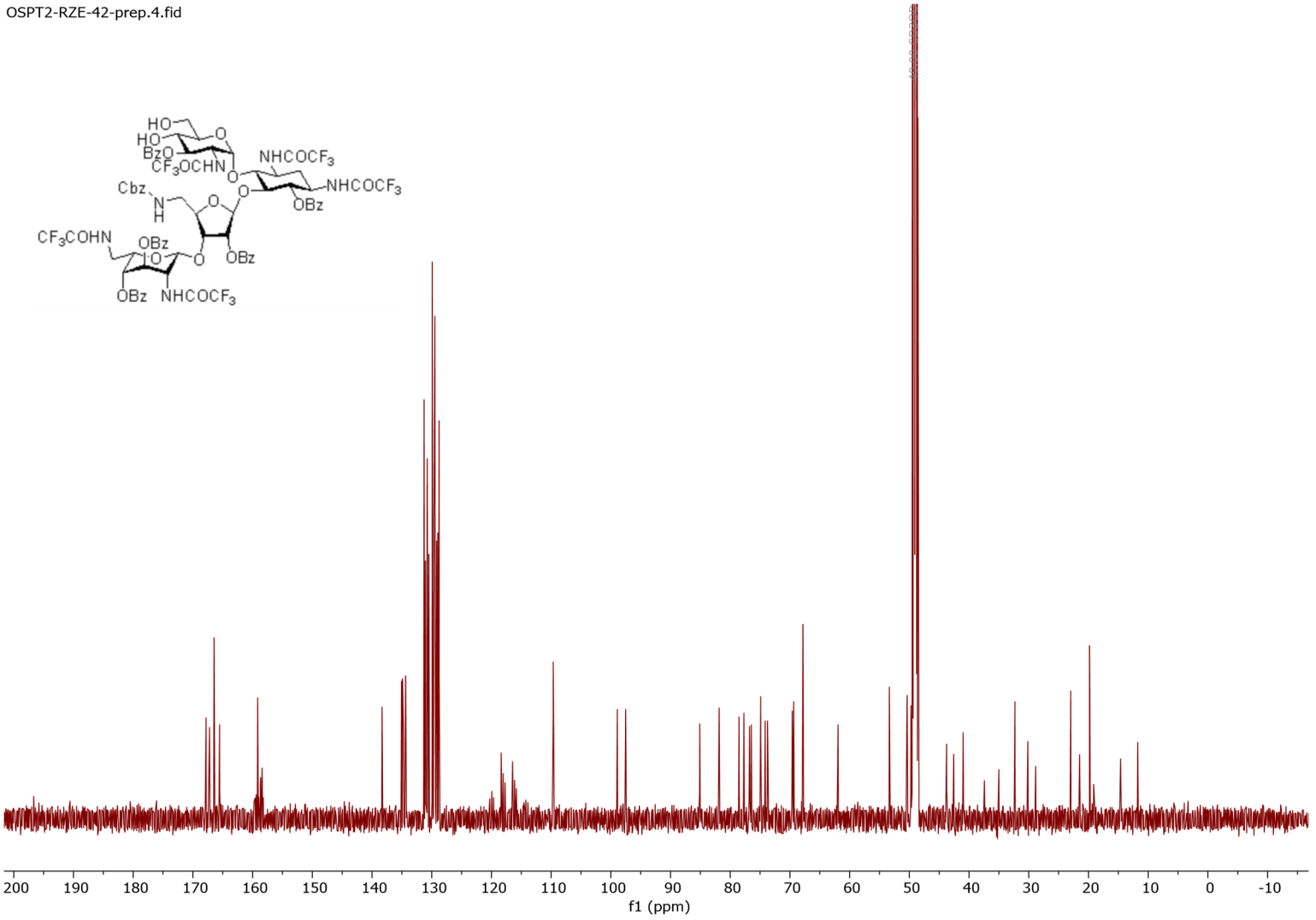
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (23)

[^{1}H-NMR, 600 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (23)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,2″,3‴,4‴-penta-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (23)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-S*i*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_441 1006 Zogota RZE-42
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,2 10.000000 MS_Tune Col#43

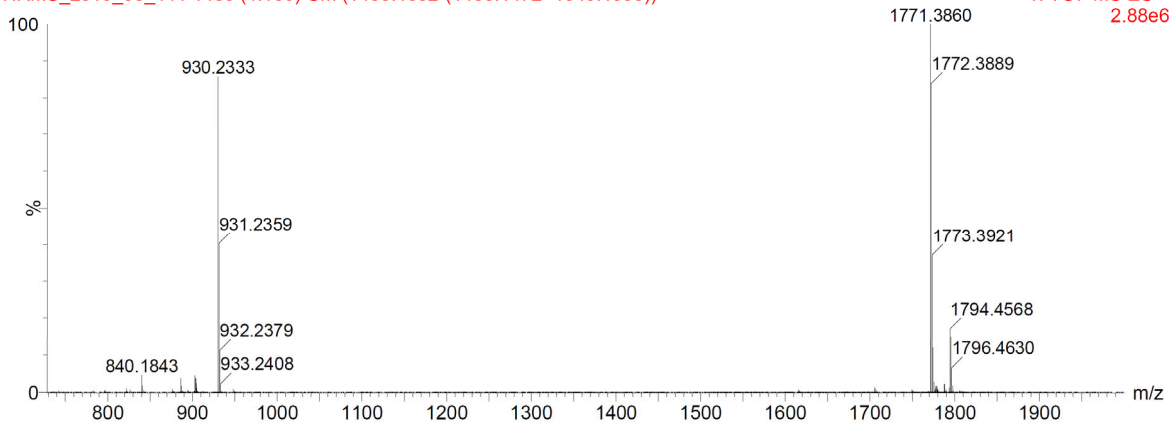
Elemental Composition Report:

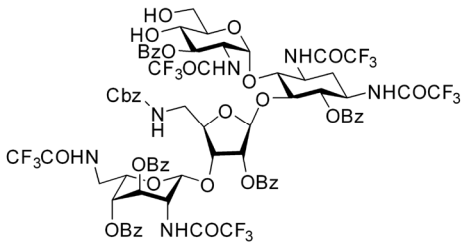
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
307 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-76 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1771.3860	100.00	1771.3814	4.6	2.6	38.5	533.3	0.427	65.26	C76 H67 N6 O25 F15 Na
		1771.3926	-6.6	-3.7	38.5	533.9	1.057	34.74	C75 H67 N8 O24 F15 Na

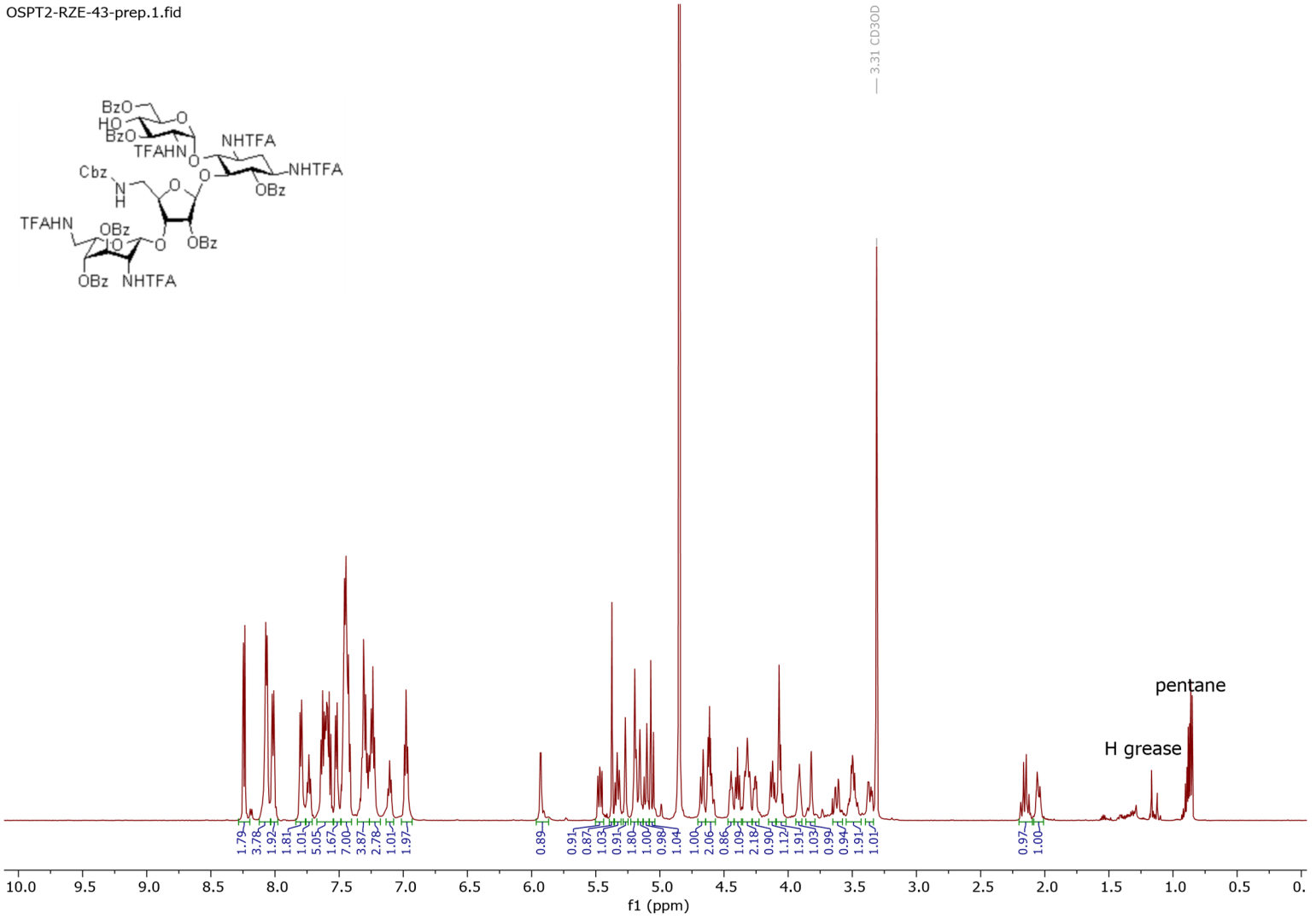
1006 Zogota RZE-42
HRMS_2019_08_441 1489 (4.166) Cm (1488:1502-(1458:1472+1549:1558))
1: TOF MS ES+
2.88e6





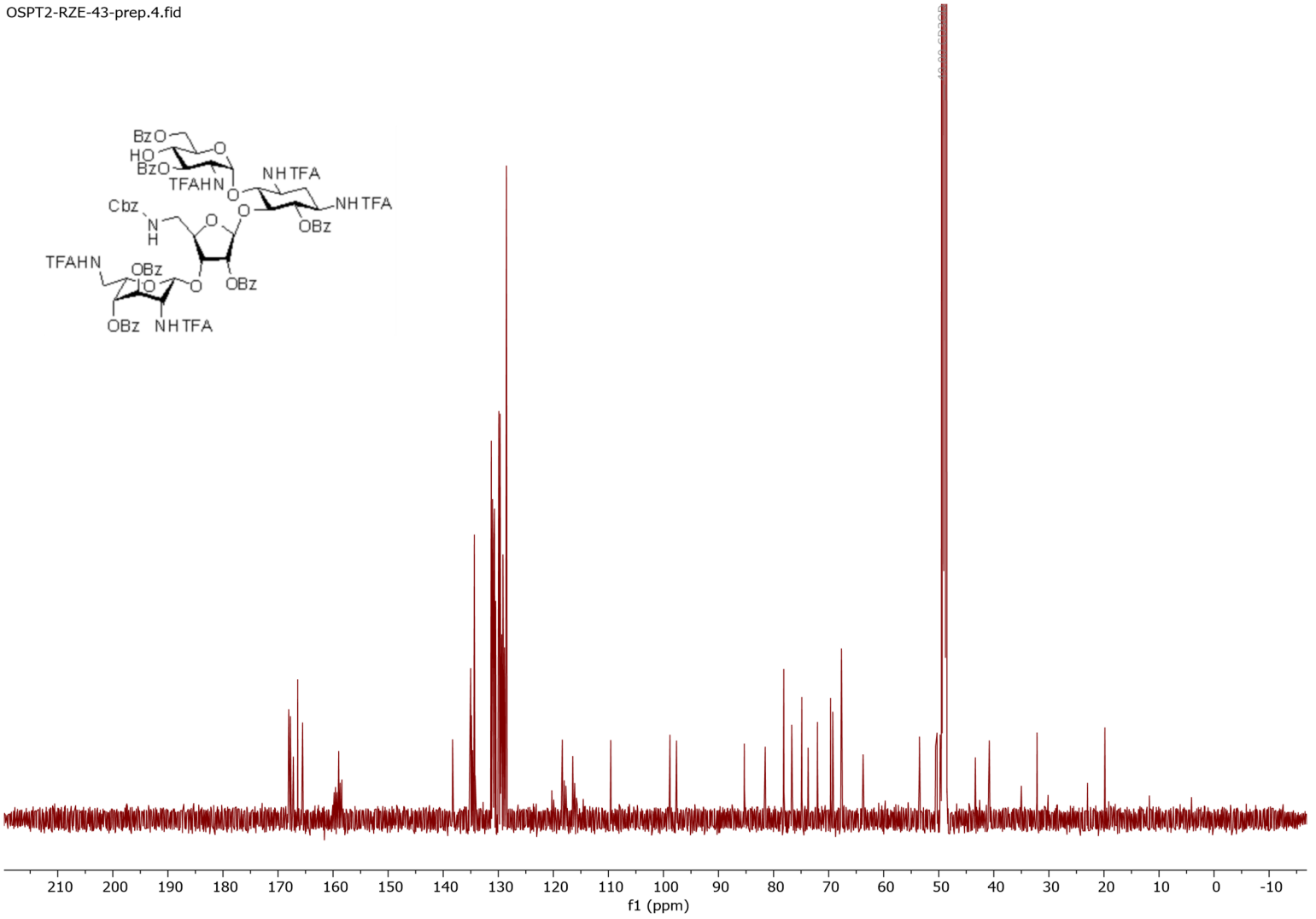
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H-NMR, 600 MHz, CD_3OD]



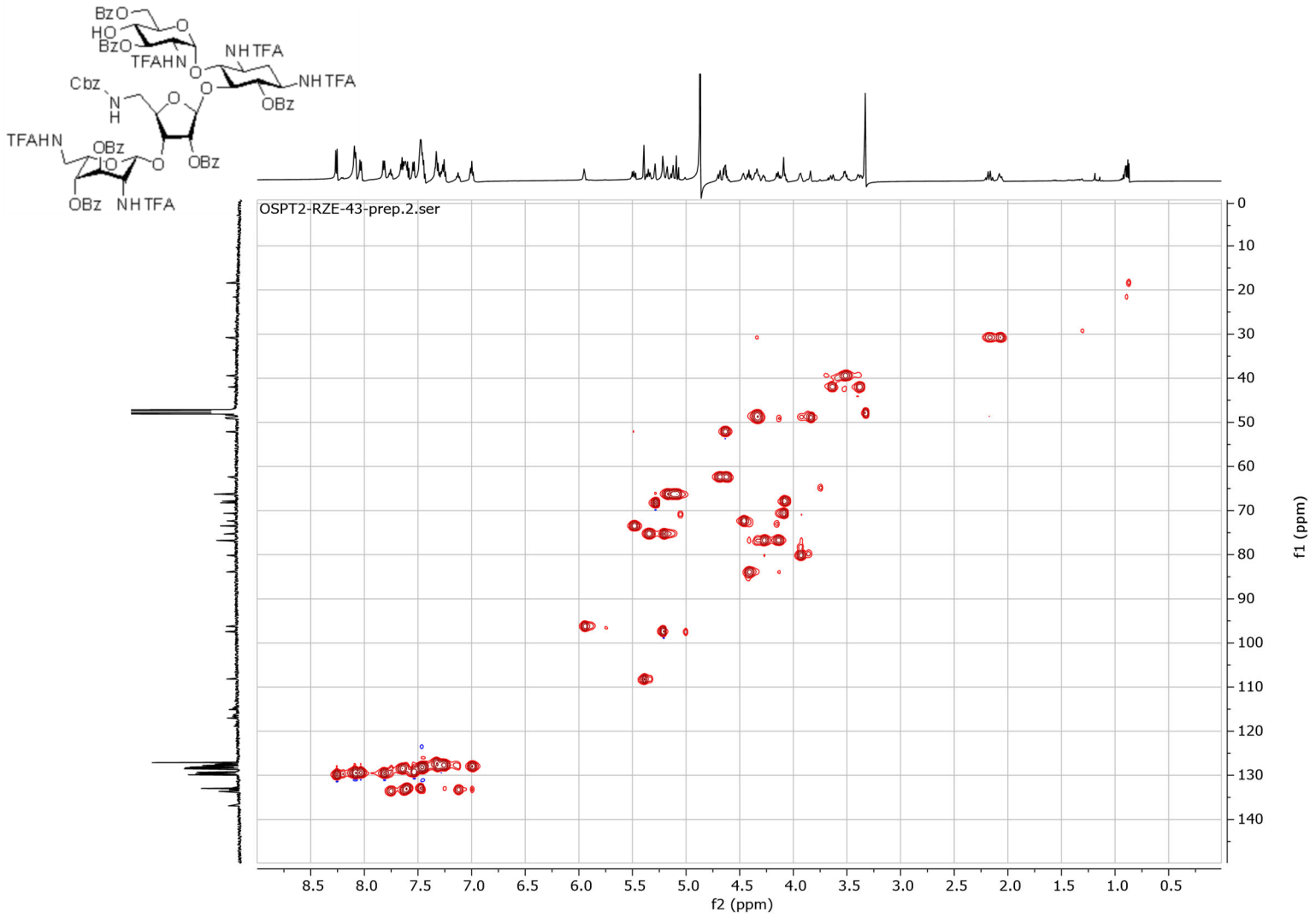
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



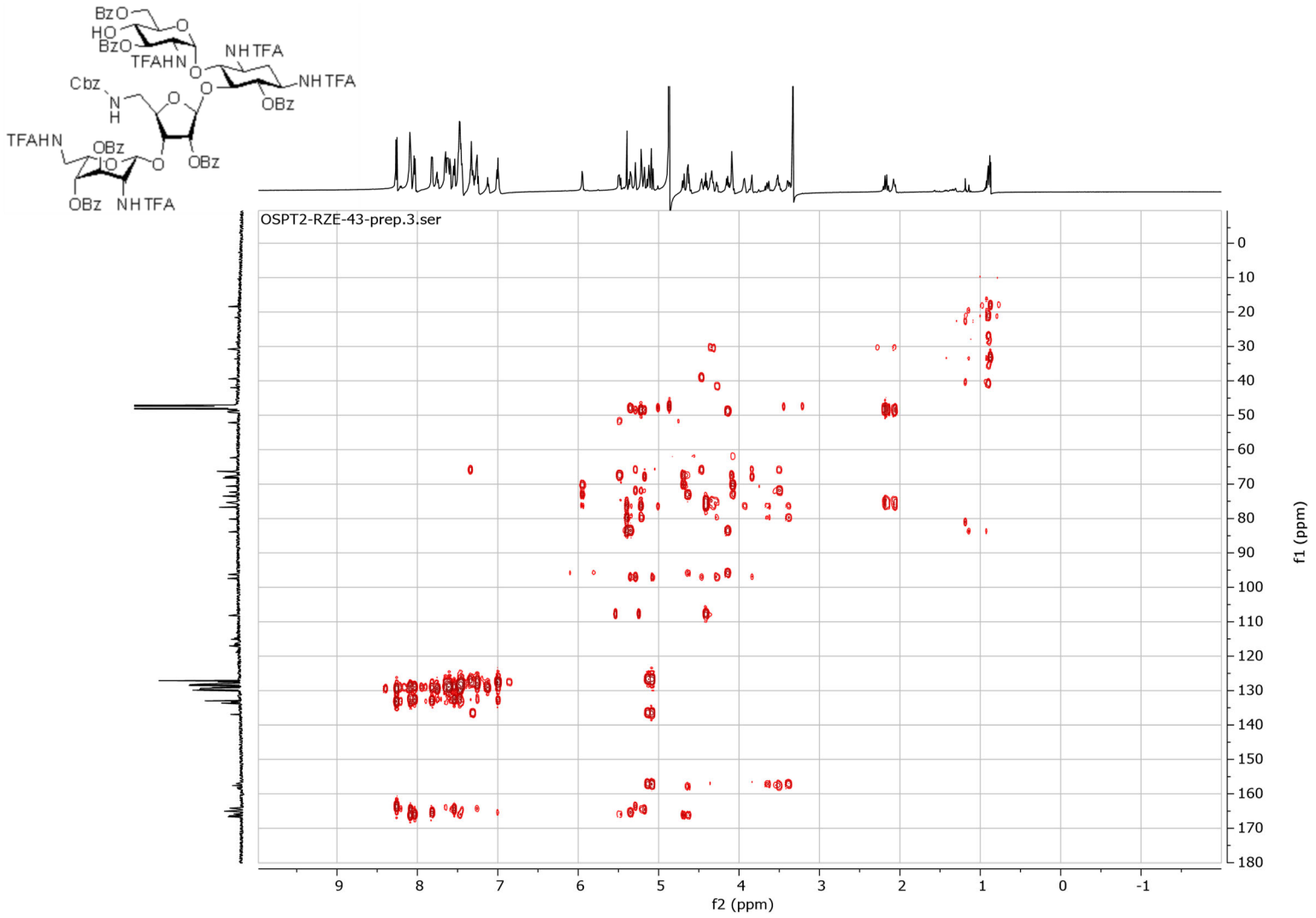
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, CD_3OD]



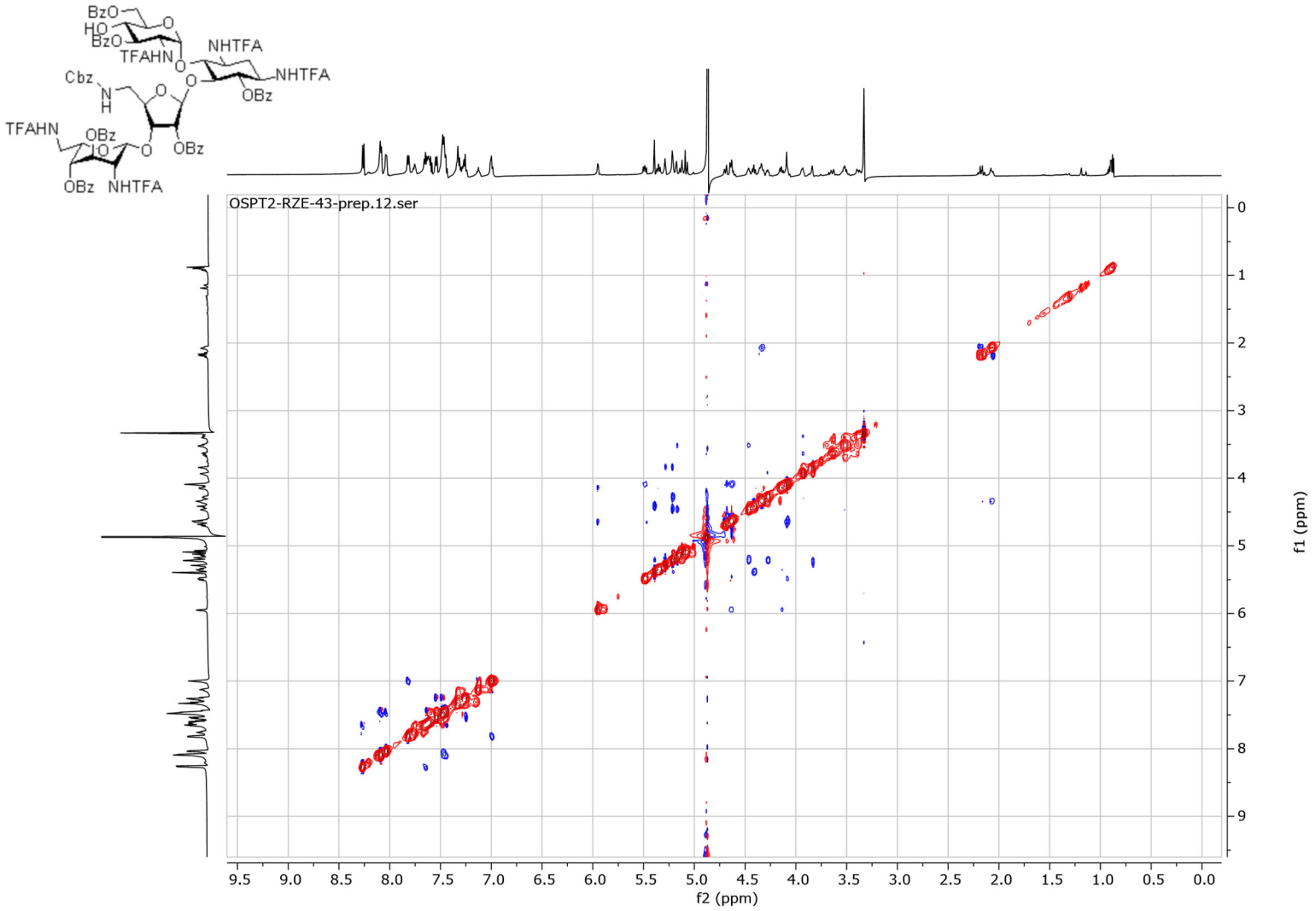
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, CD_3OD]



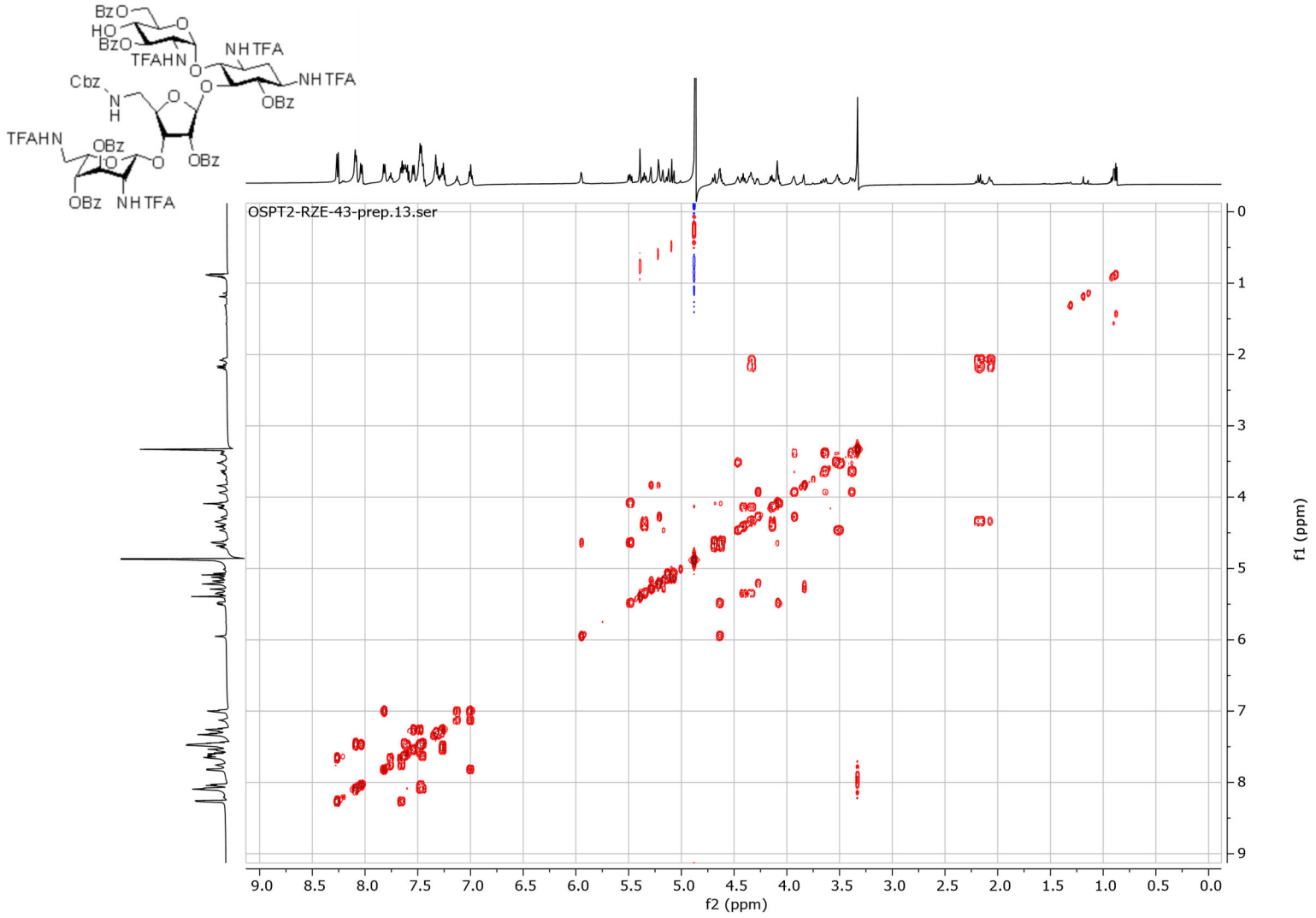
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H, ^{1}H ROESY, 600 MHz, CD_3OD]



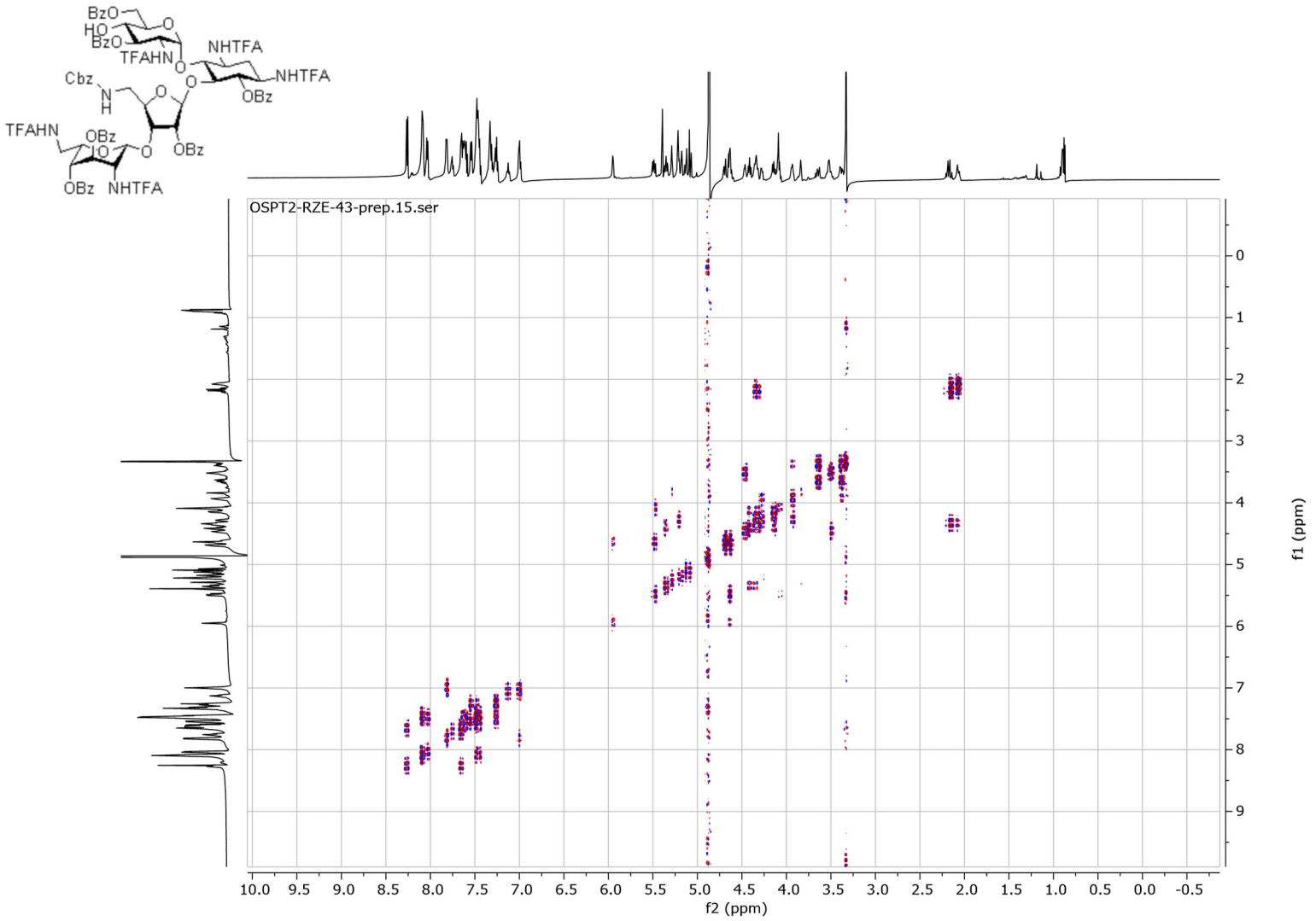
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H, ^{1}H COSY, 600 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

[^{1}H, ^{1}H DQF-COSY, 600 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (24)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_443 1007 Zogota RZE-43
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,3 10.000000 MS_Tune Col#43

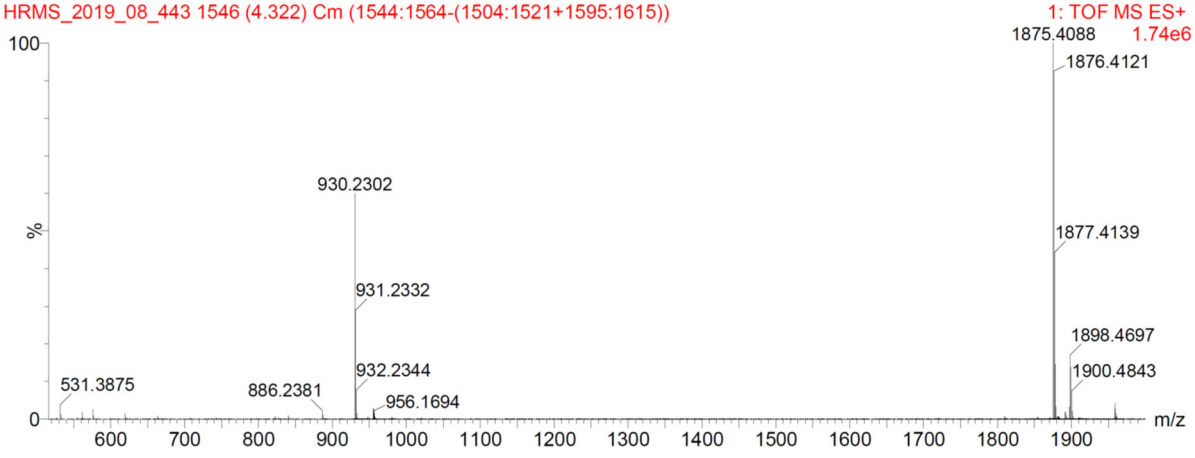
Elemental Composition Report:

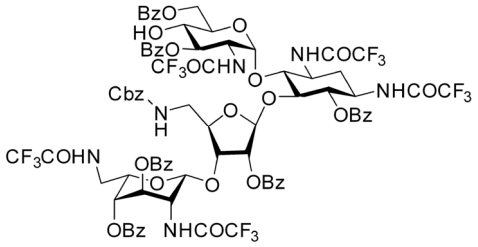
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
300 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-83 H: 1-110 N: 1-10 O: 0-26 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1875.4088	100.00	1875.4076	1.2	0.6	43.5	525.9	n/a	n/a	C83 H71 N6 O26 F15 Na

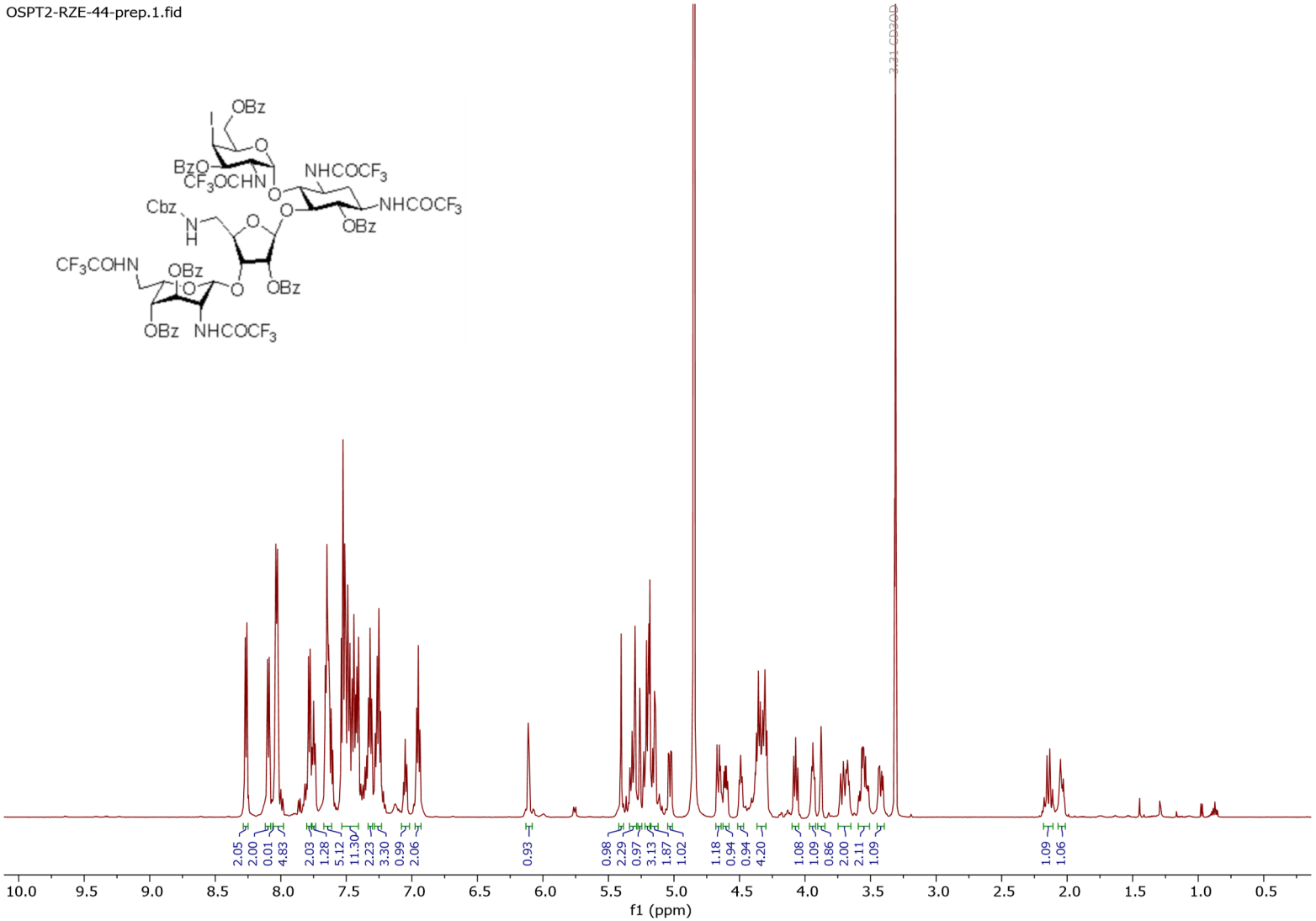
1007 Zogota RZE-43
HRMS_2019_08_443 1546 (4.322) Cm (1544:1564-(1504:1521+1595:1615))





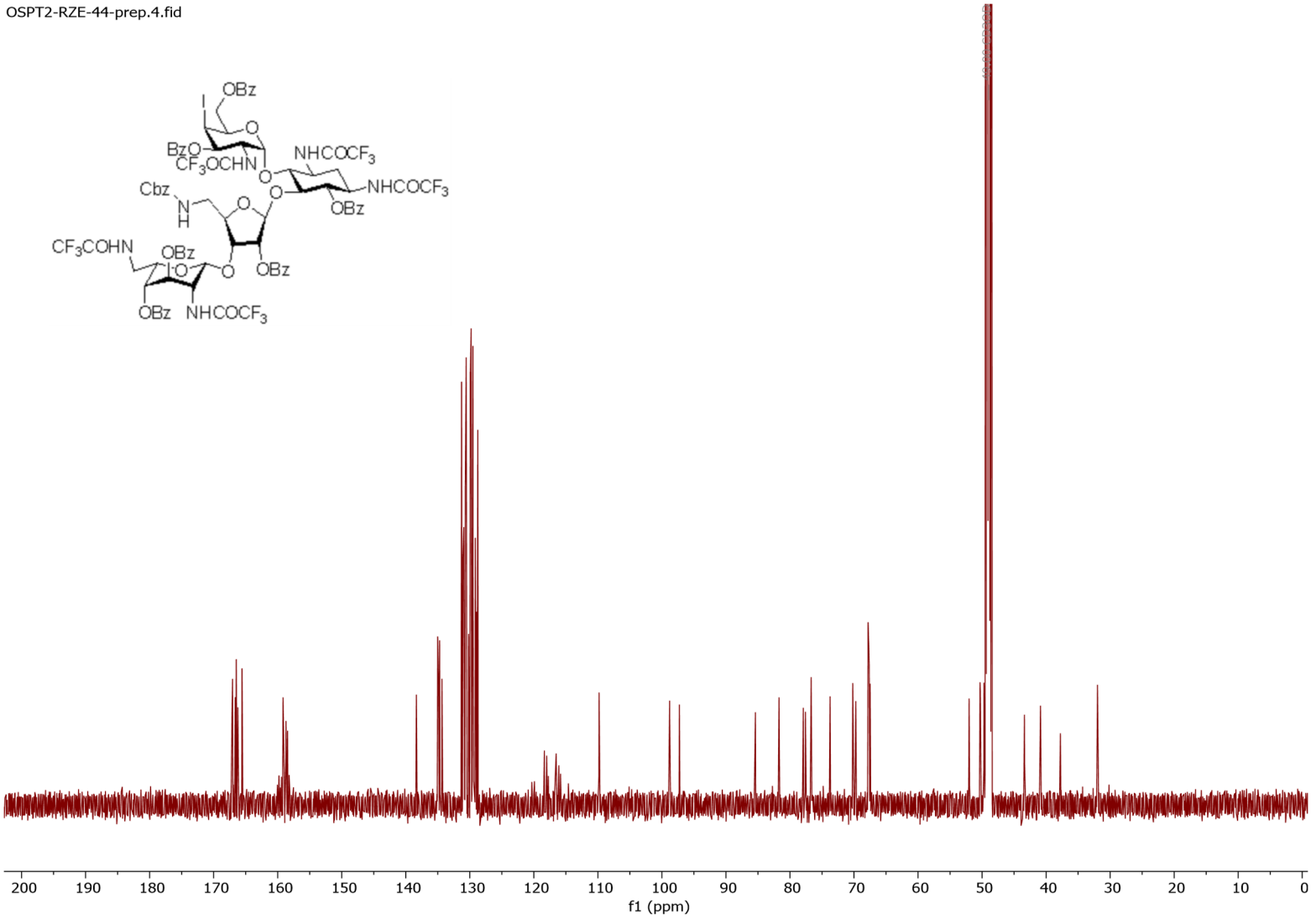
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H-NMR, 600 MHz, CD_3OD]



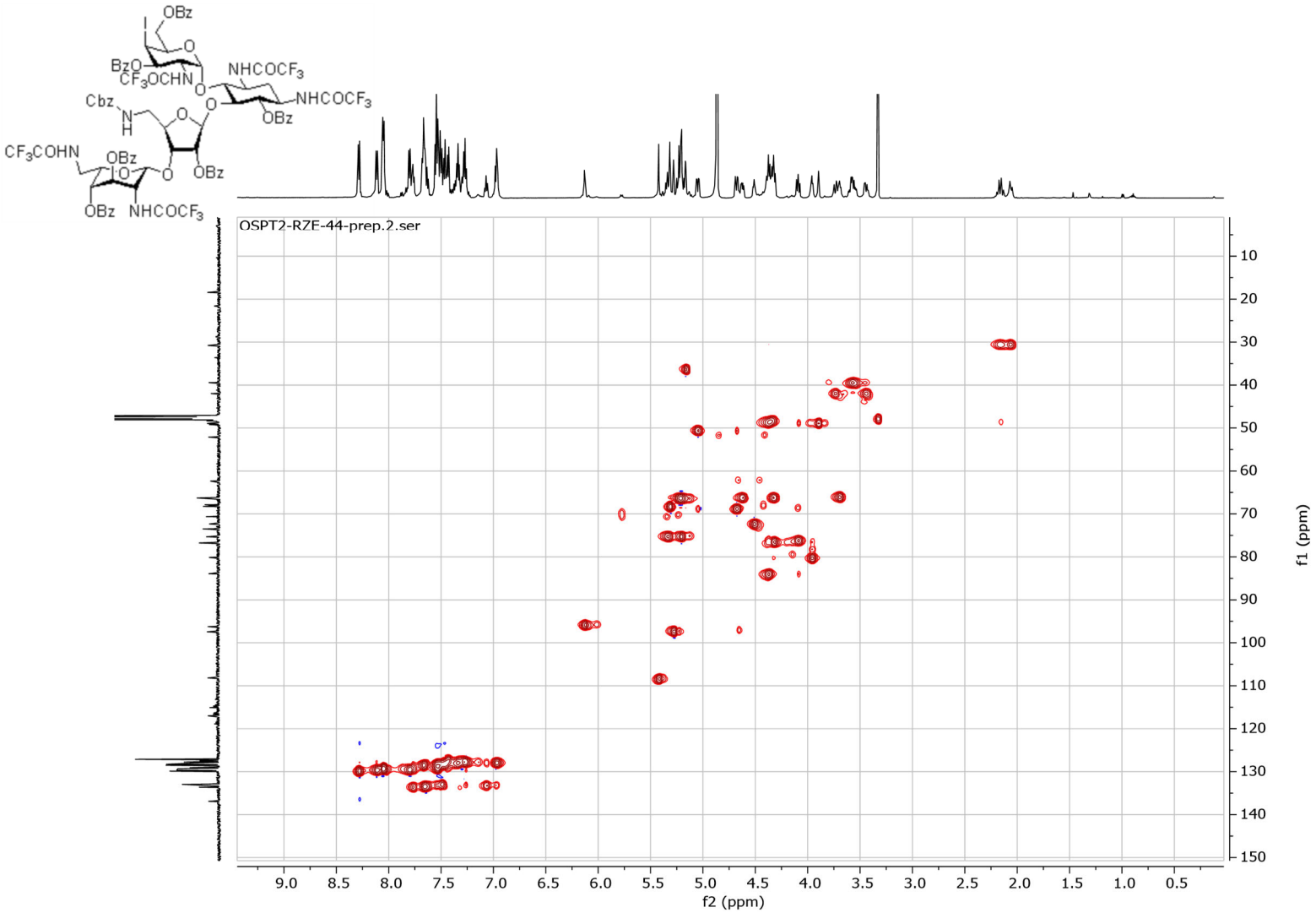
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



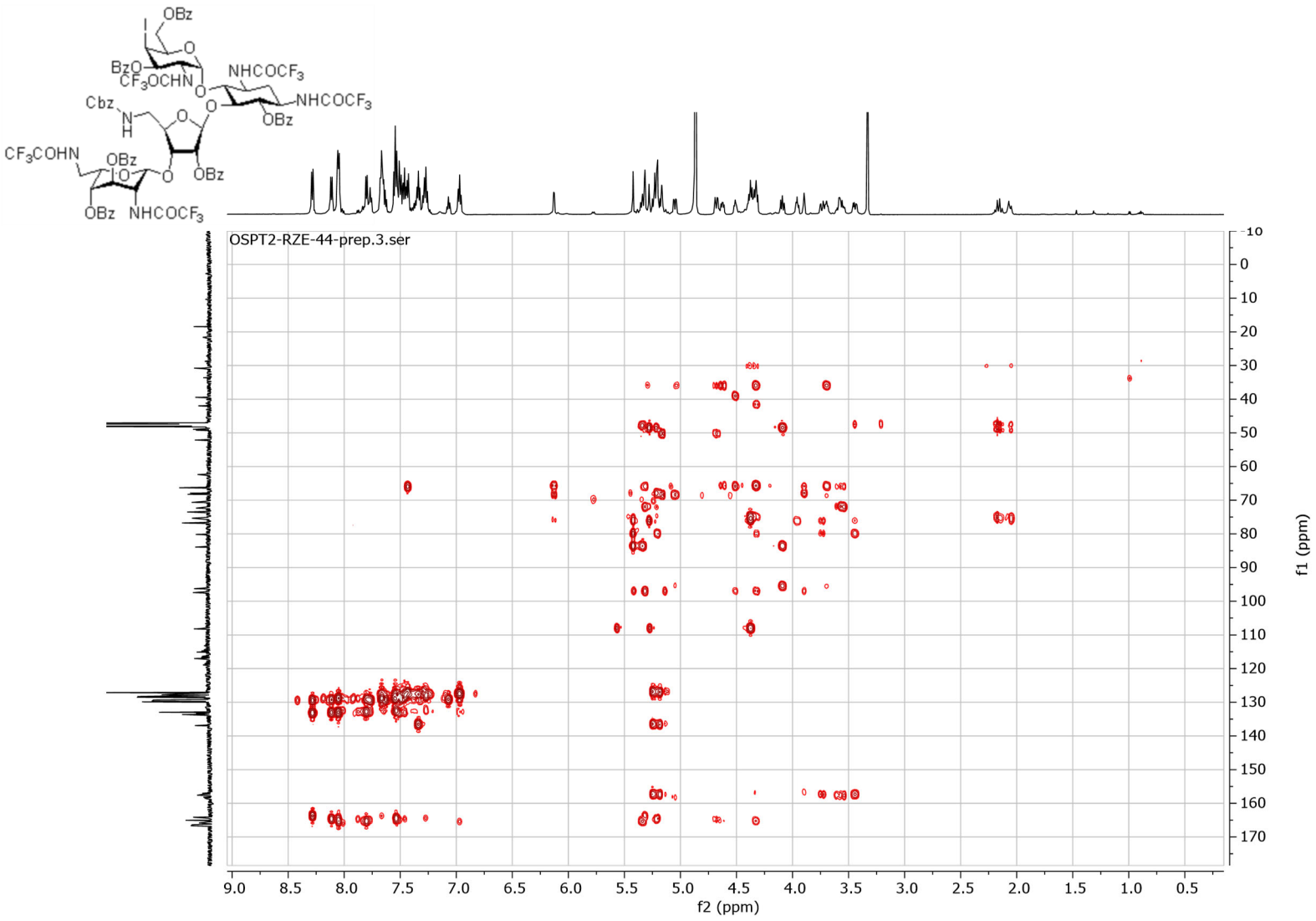
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, CD_3OD]



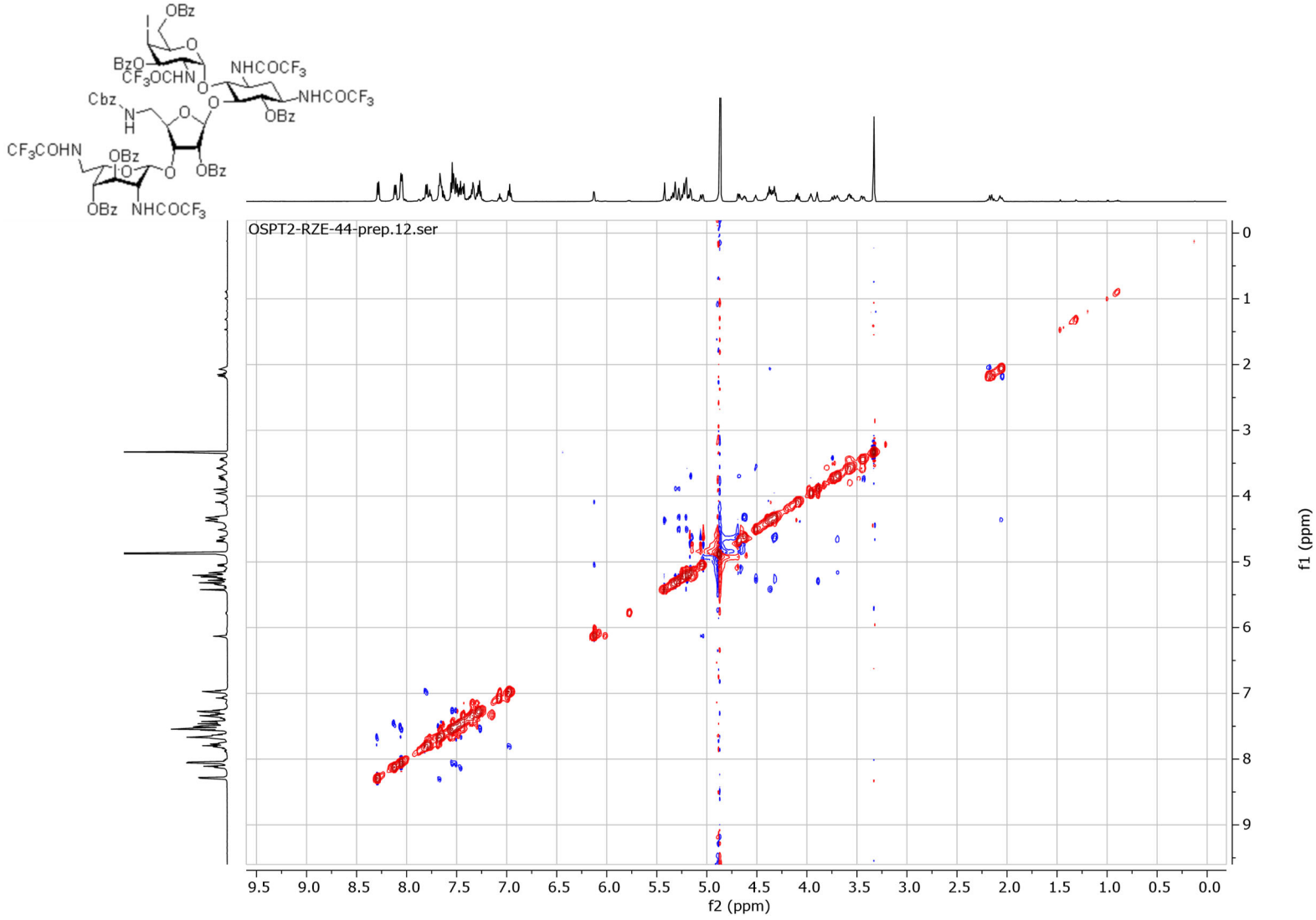
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, CD_3OD]



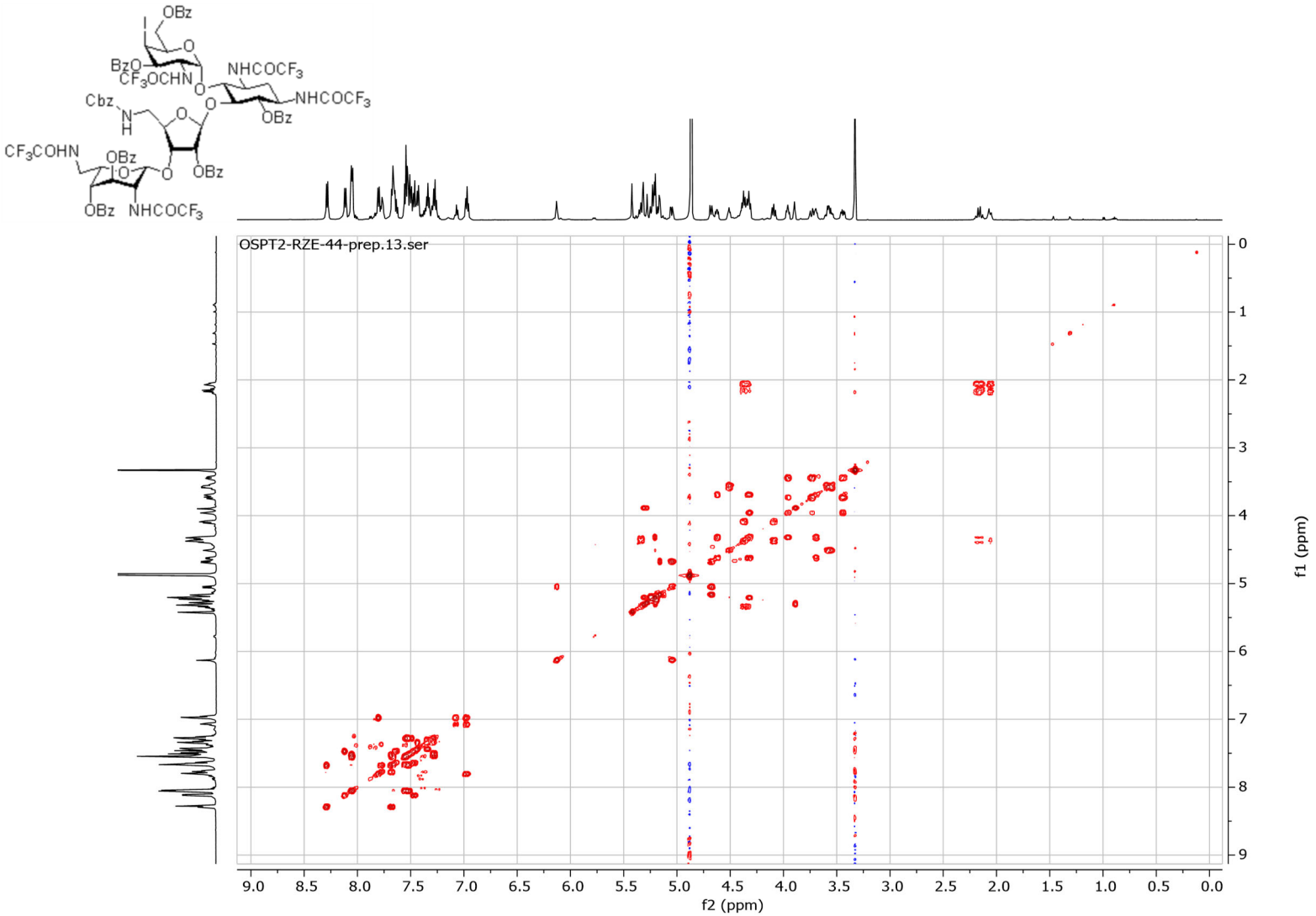
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H, ^{1}H ROESY, 600 MHz, CD_3OD]



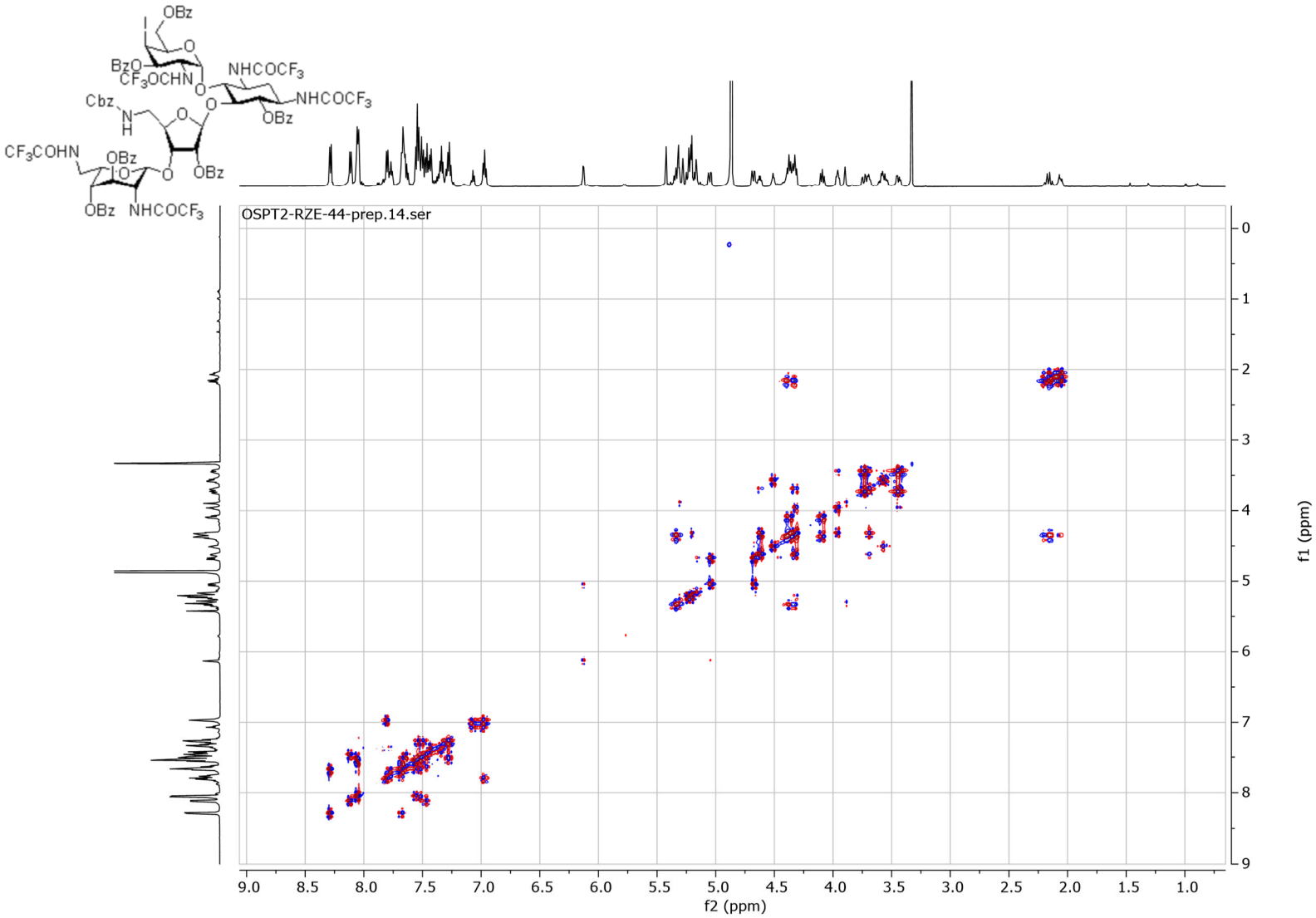
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H, ^{1}H COSY, 600 MHz, CD_3OD]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

[^{1}H, ^{1}H DQF-COSY, 600 MHz, CD_3OD]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (25)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_445 1008 Zogota RZE-44
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,4 10.000000 MS_Tune Col#43

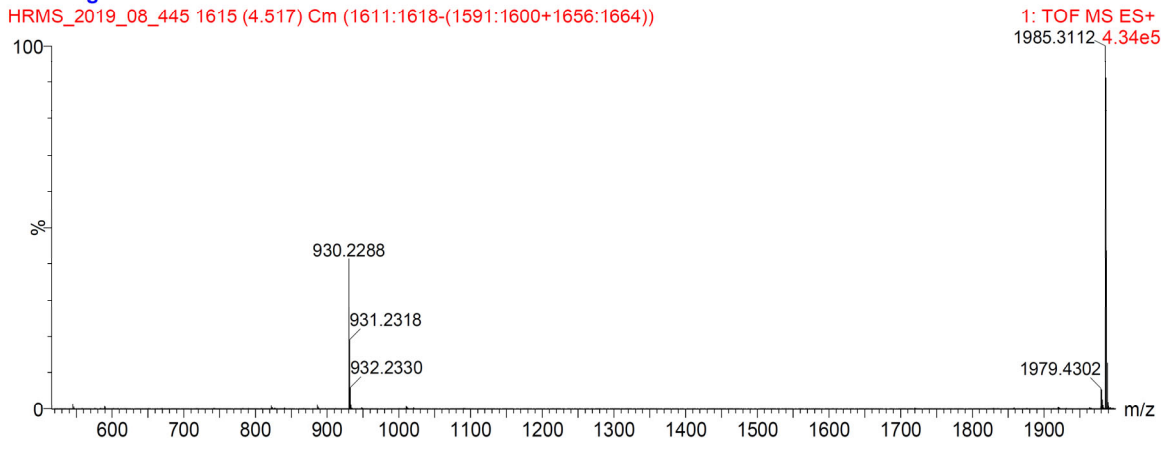
Elemental Composition Report:

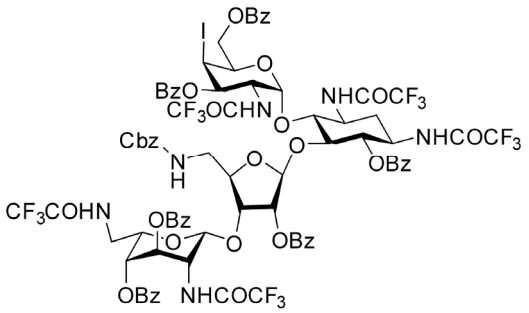
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
294 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-83 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1 I: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1985.3112	100.00	1985.3093	1.9	1.0	43.5	398.8	0.082	92.11	C83 H70 N6 O25 F15 Na I
		1985.3206	-9.4	-4.7	43.5	401.2	2.539	7.89	C82 H70 N8 O24 F15 Na I

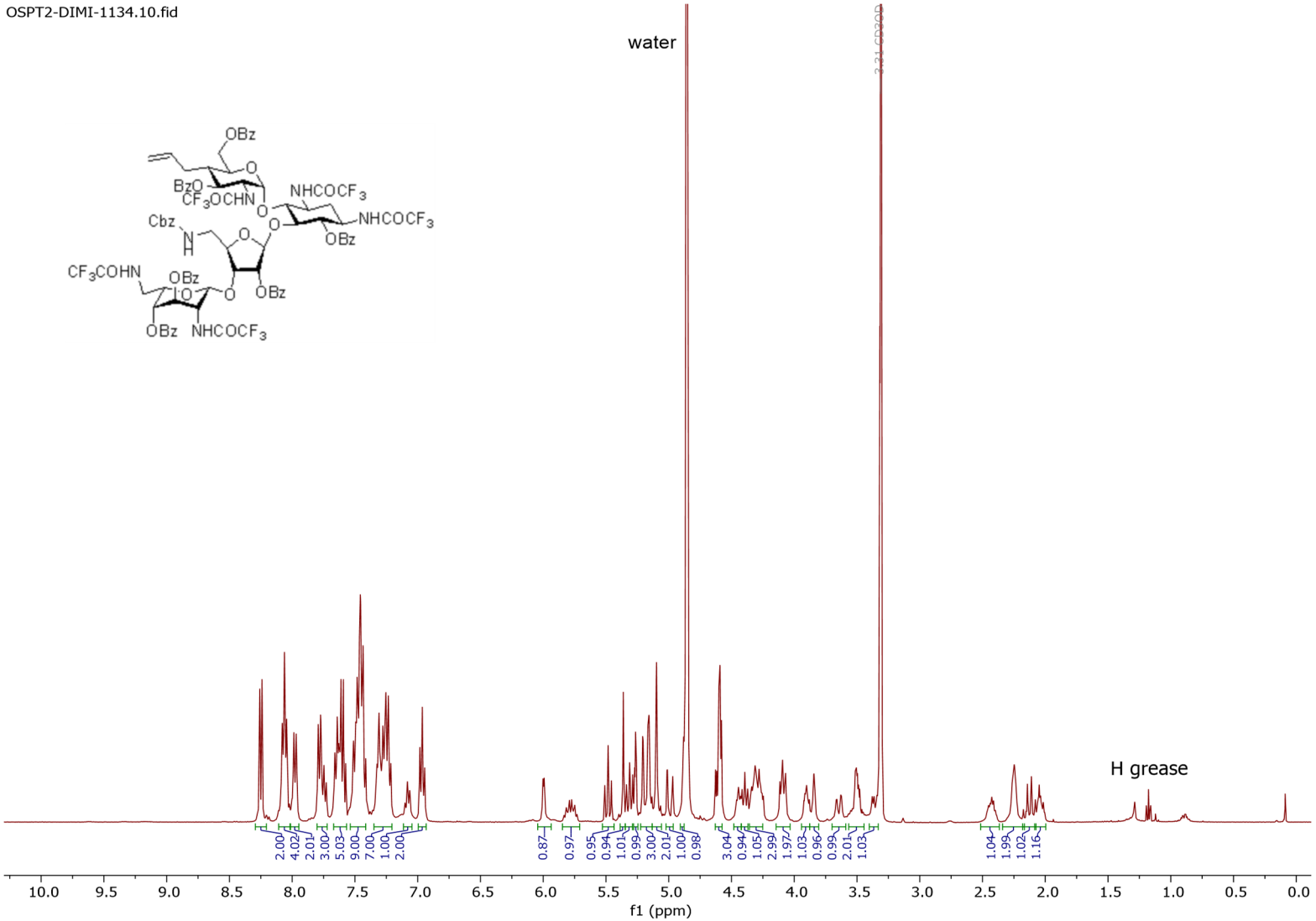
1008 Zogota RZE-44





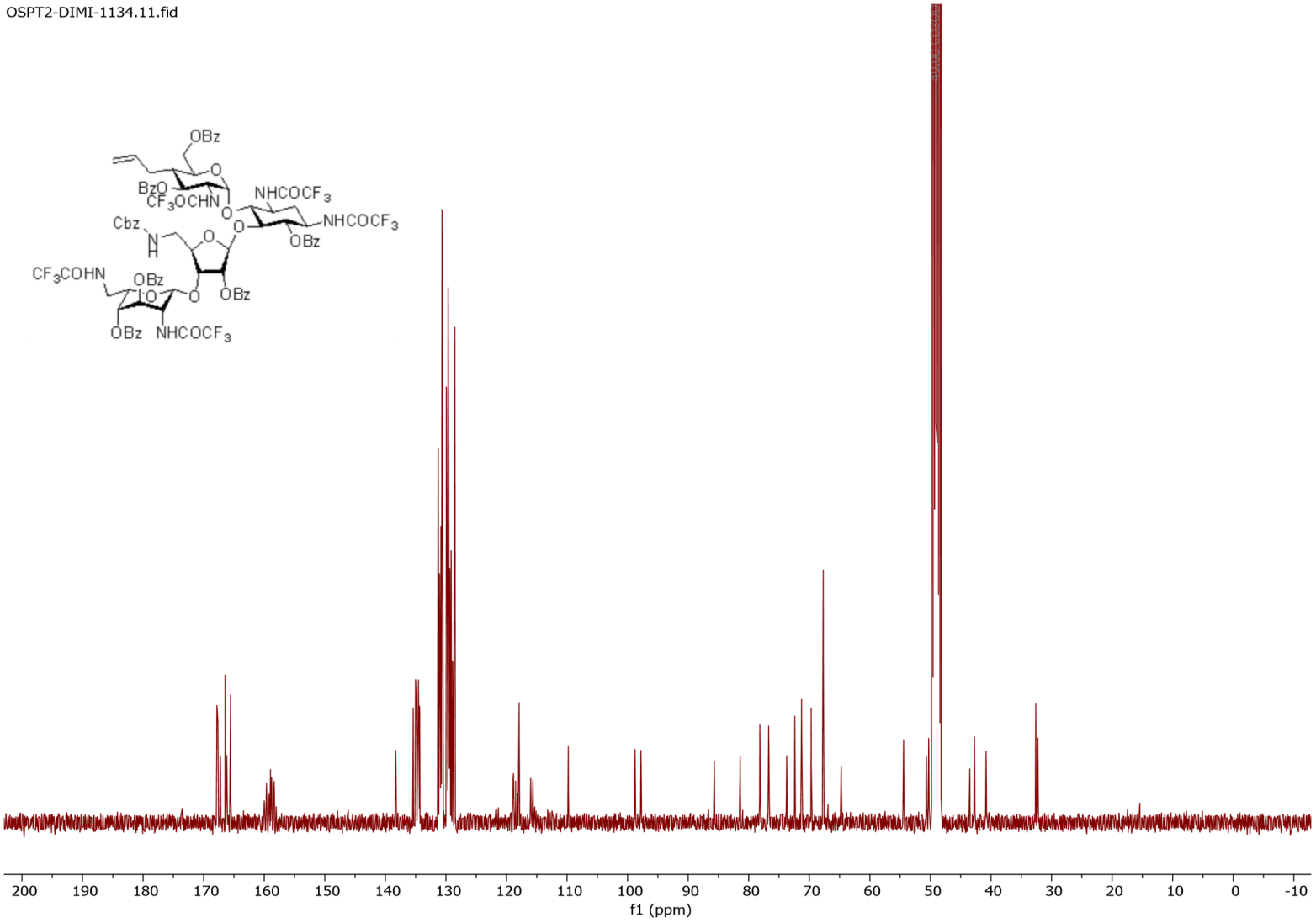
4′-Allyl-4′,5″-dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (26)

[^{1}H-NMR, 400 MHz, CD_3OD]



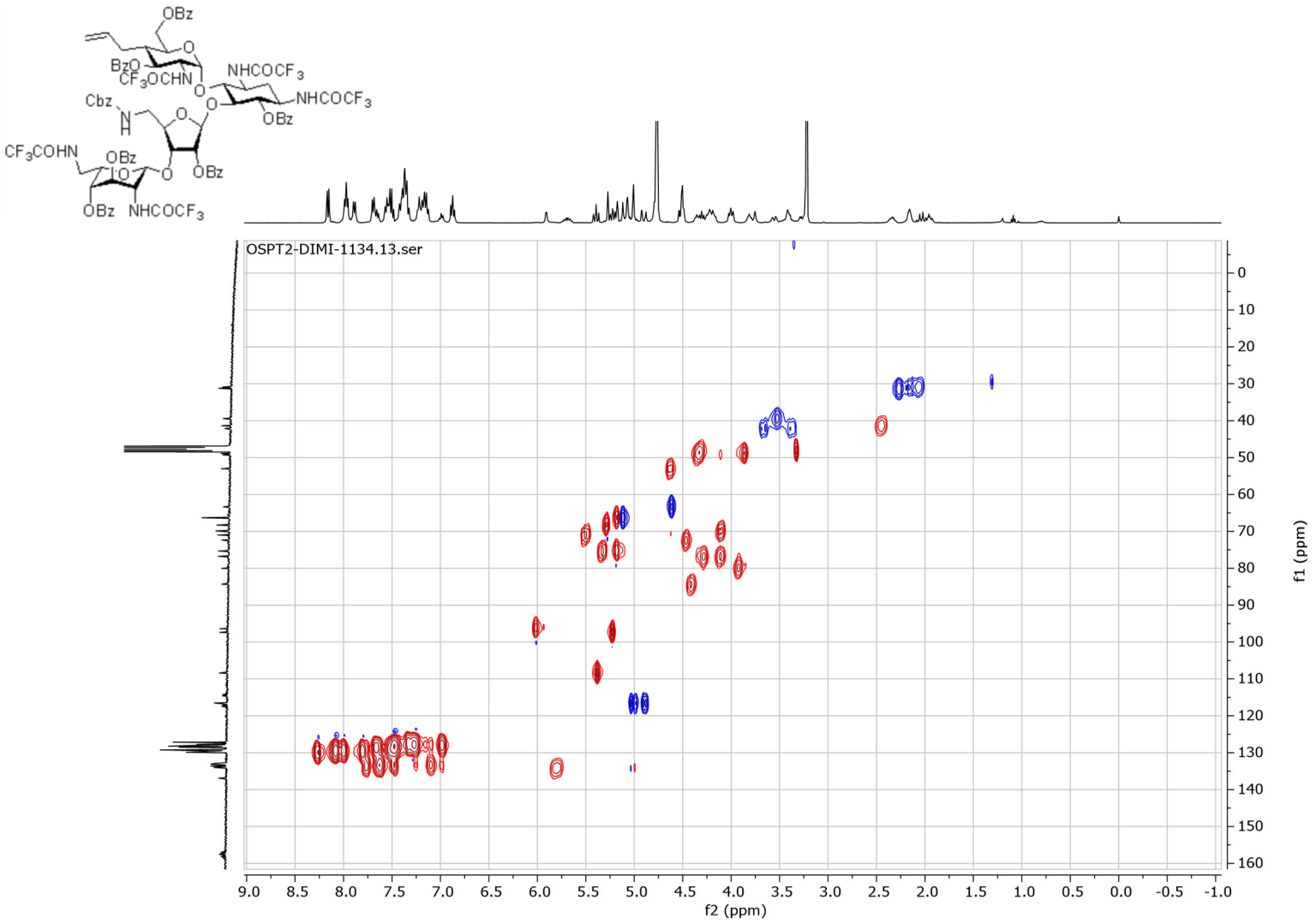
4′-Allyl-4′,5″-dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (26)

[^{13}C-NMR, 100.6 MHz, CD_3OD]



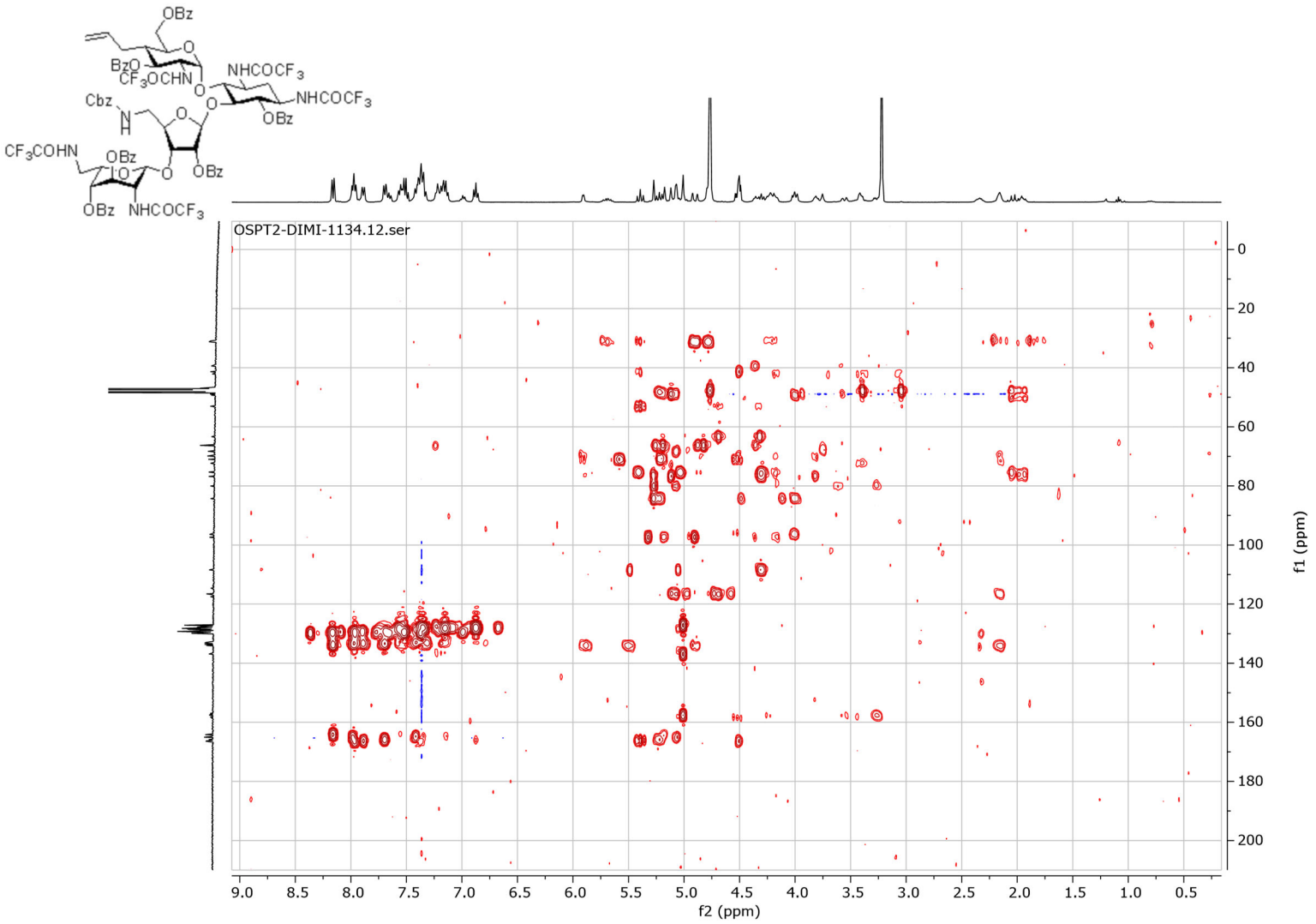
4′-Allyl-4′,5″-dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (26)

[^{1}H, ^{13}C HSQC, 400 MHz, 100.6 MHz, CD_3OD]



4′-Allyl-4′,5″-dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (26)

[^{1}H, ^{13}C HMBC, 400 MHz, 100.6 MHz, CD_3OD]



4′-Allyl-4′,5″-dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (26)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_447 1009 Zogota RZE-47
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,5 10.000000 MS_Tune Col#43

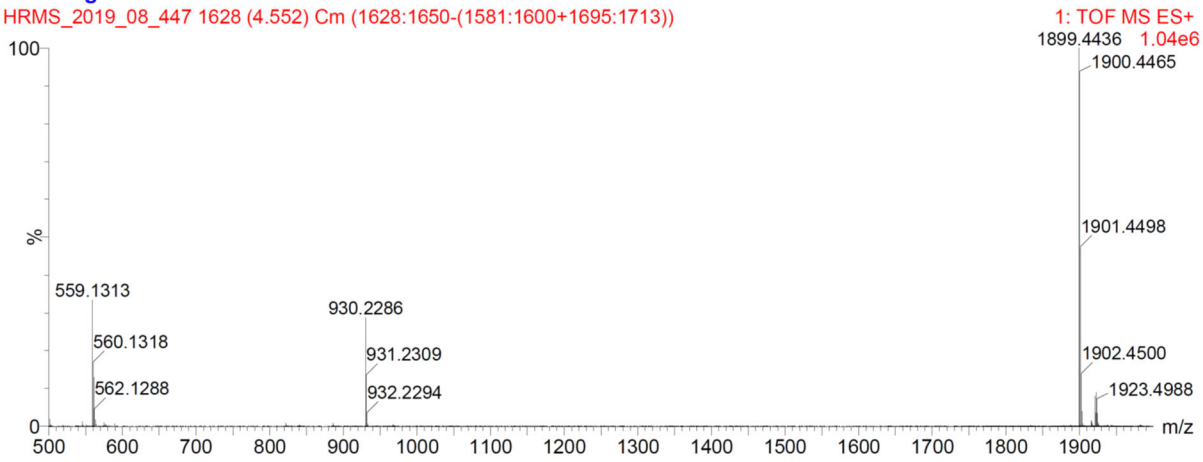
Elemental Composition Report:

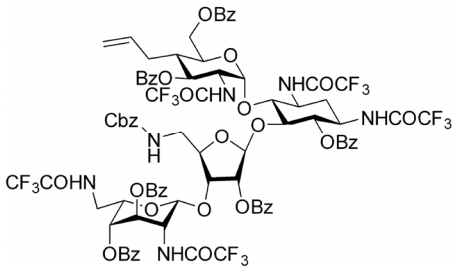
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
279 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-86 H: 1-110 N: 1-10 O: 0-25 F: 15-15 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1899.4436	100.00	1899.4440	-0.4	-0.2	44.5	484.0	n/a	n/a	C86 H75 N6 O25 F15 Na

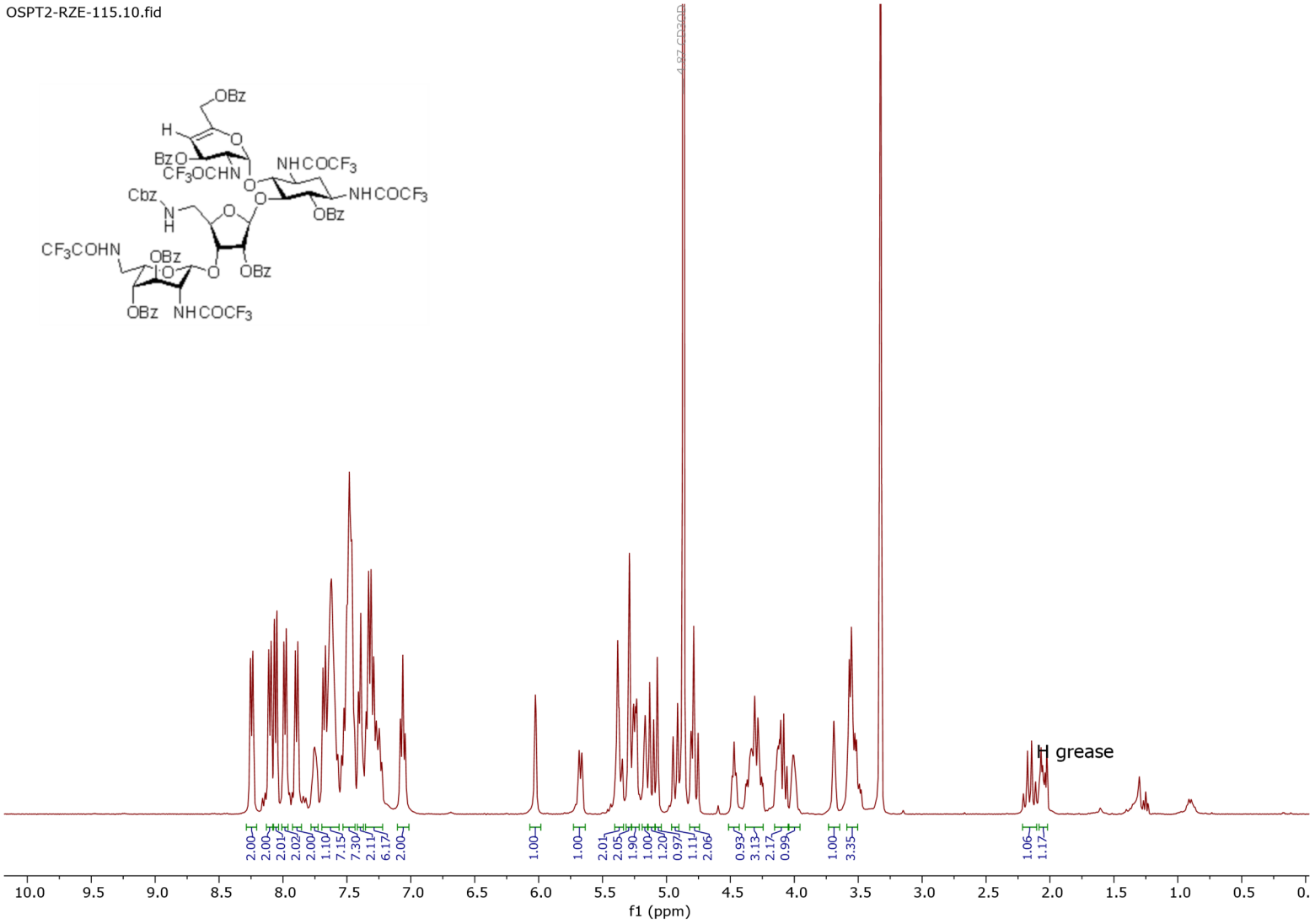
1009 Zogota RZE-47
HRMS_2019_08_447 1628 (4.552) Cm (1628:1650-(1581:1600+1695:1713))





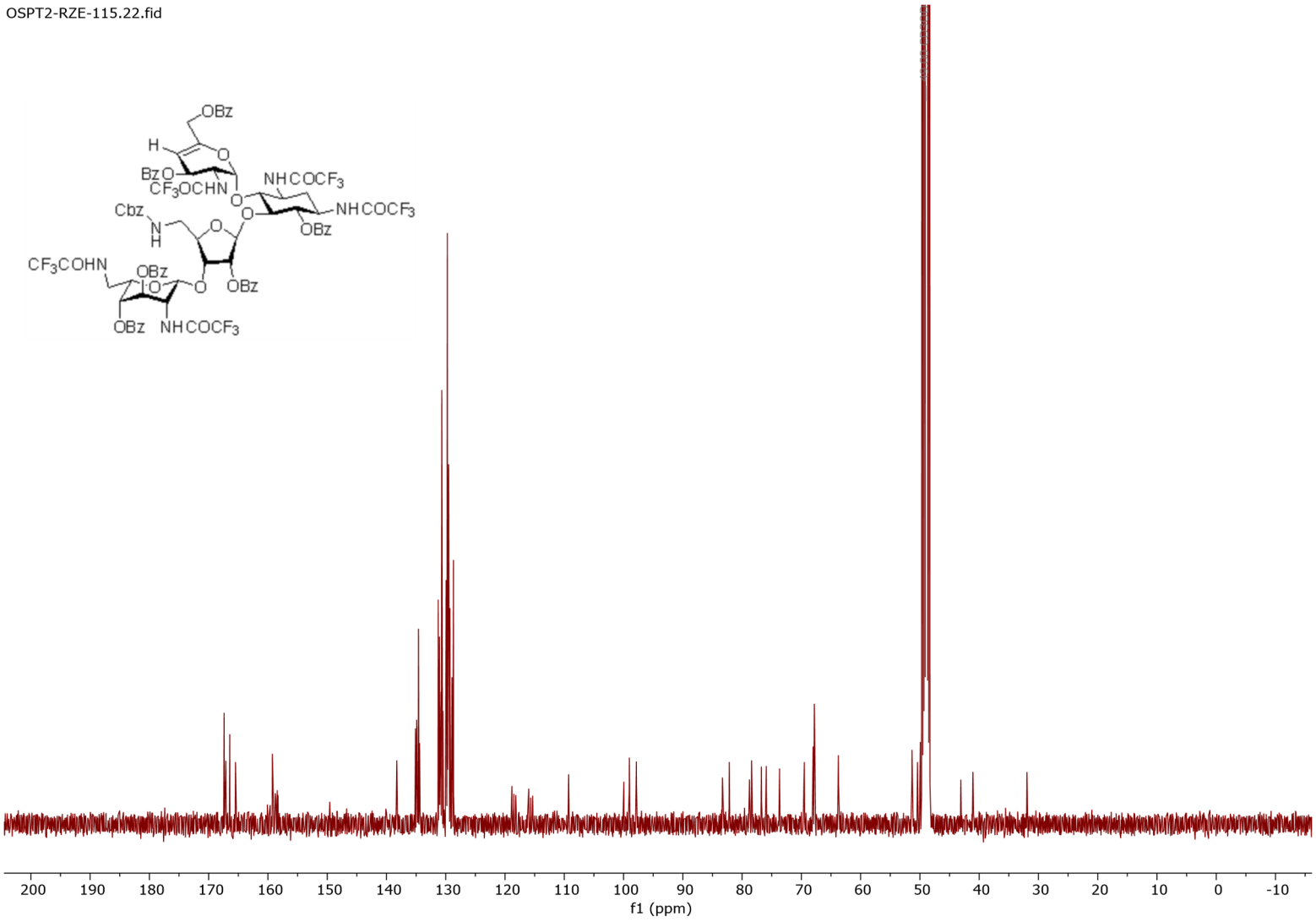
4',5''-Dideoxy-5''-(O-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-O-benzoyl-1,3,2',2''',6'''-penta-N-trifluoroacetyl paromomycin-4'-ene (27)

[^{1}H-NMR, 400 MHz, CD_3OD]



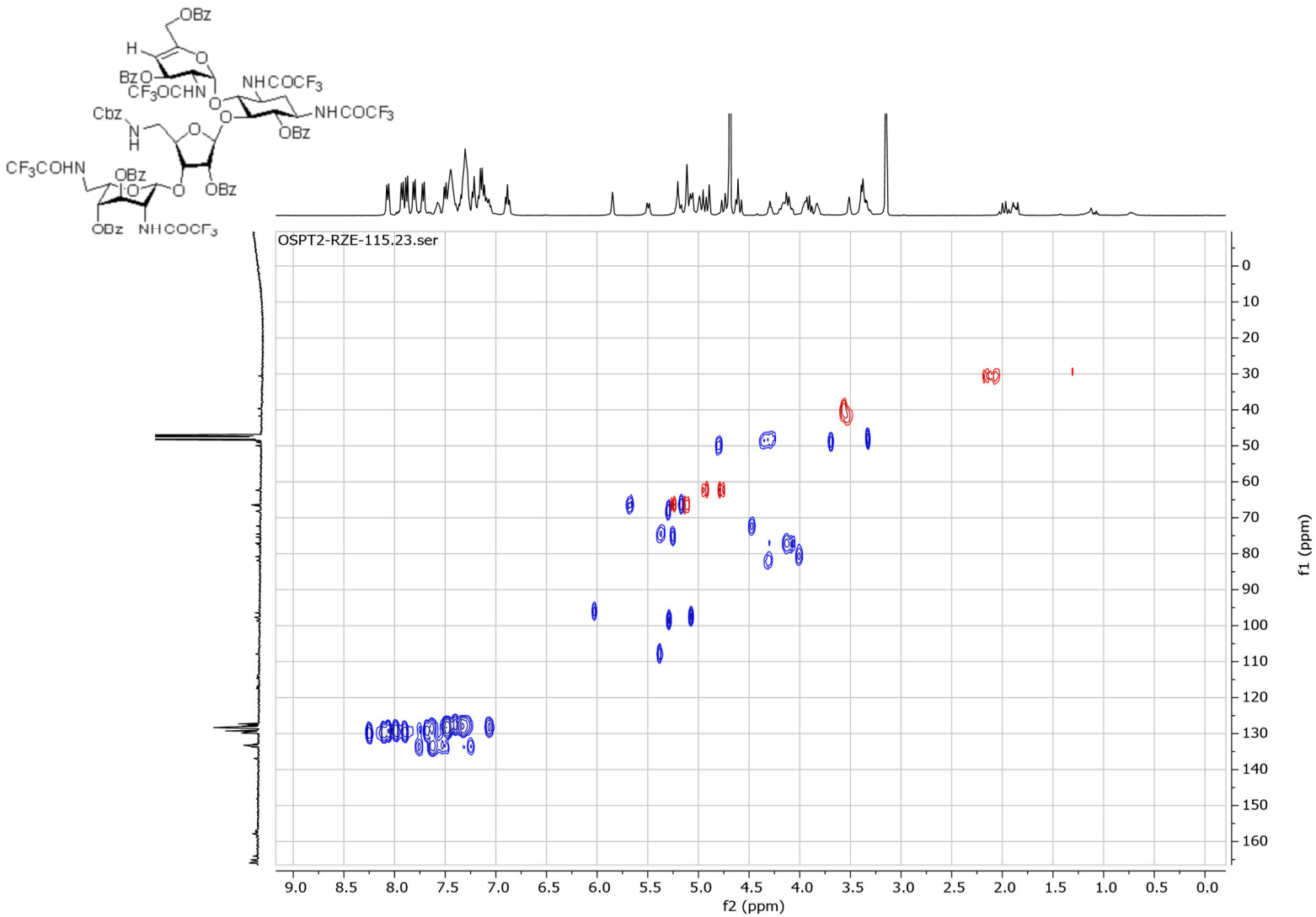
4′,5″-Dideoxy-5″-(O-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-O-benzoyl-1,3,2′,2‴,6‴-penta-N-trifluoroacetyl paromomycin-4′-ene (27)

[^{13}C-NMR, 100.6 MHz, CD_3OD]



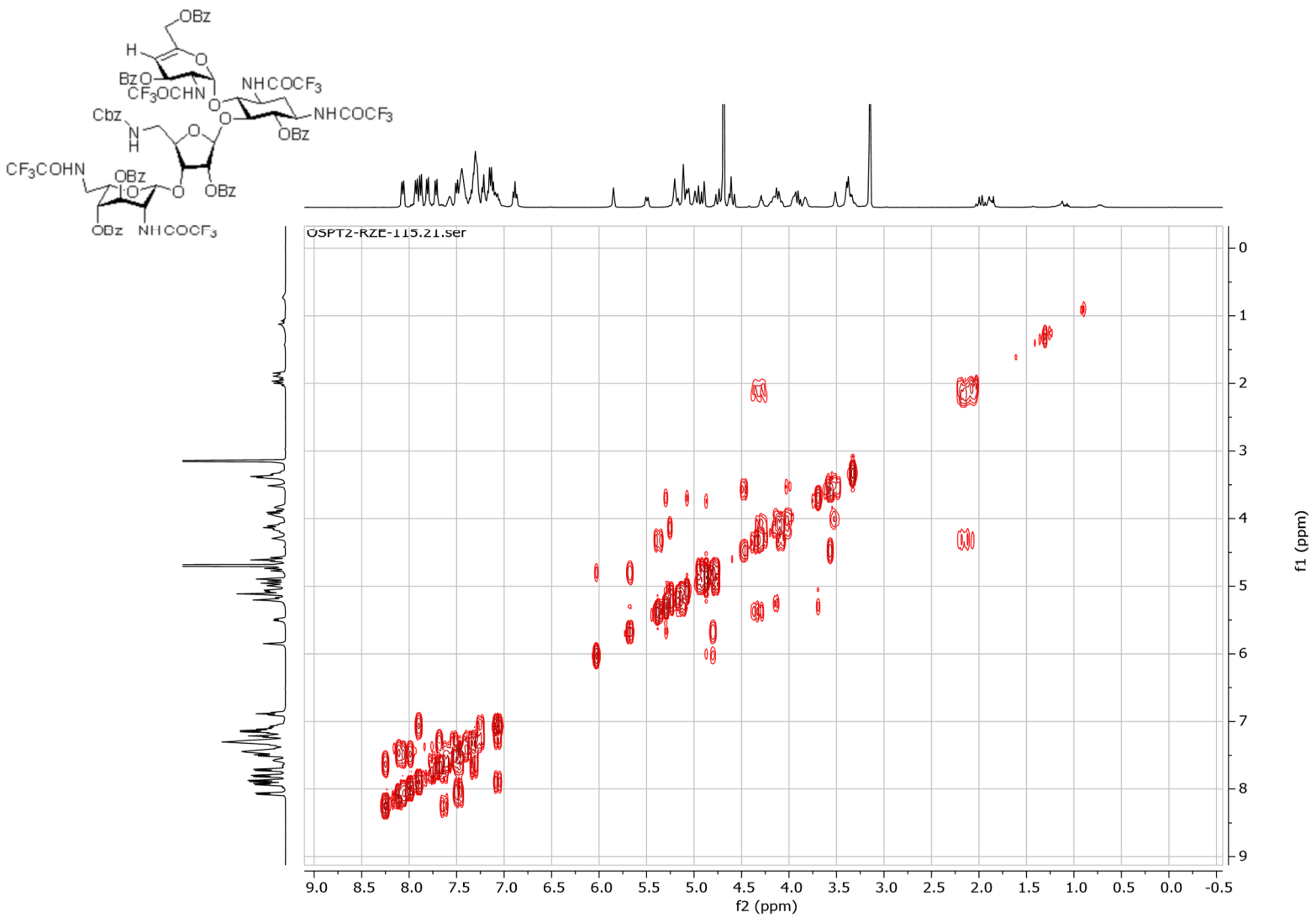
4′,5″-Dideoxy-5″-(O-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-O-benzoyl-1,3,2′,2‴,6‴-penta-N-trifluoroacetyl paromomycin-4′-ene (27)

[^{1}H, ^{13}C HSQC, 400 MHz, 100.6 MHz, CD_3OD]



4',5''-Dideoxy-5''-(O-benzylcarbamoyl)-6,3',6',2'',3''',4'''-hexa-O-benzoyl-1,3,2',2''',6'''-penta-N-trifluoroacetyl paromomycin-4'-ene (27)

[^{1}H, ^{1}H COSY, 400 MHz, CD_3OD]



4′,5″-Dideoxy-5″-(O-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-O-benzoyl-1,3,2′,2‴,6‴-penta-N-trifluoroacetyl paromomycin-4′-ene (27)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-S*i*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_05_212 2374 Zogota RZE-115
MS_POS_2300_RES_7min ACN_Form_5-98_040_7min 1:D,8 5.000000 MS_Tune Col#66

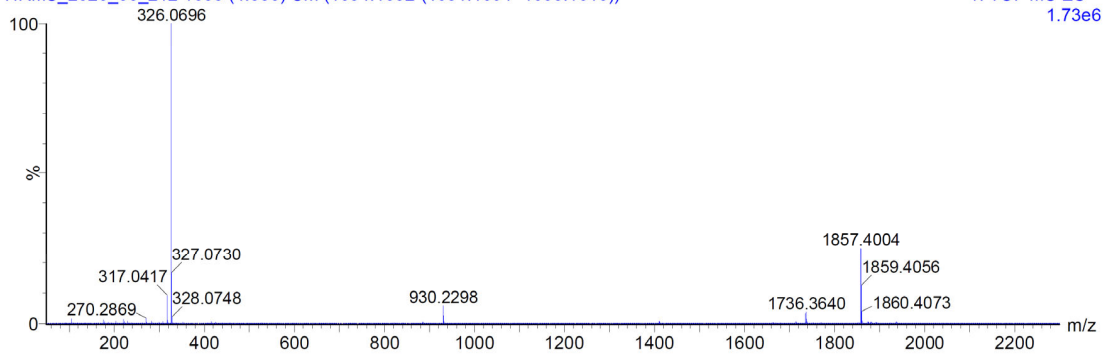
Elemental Composition Report:

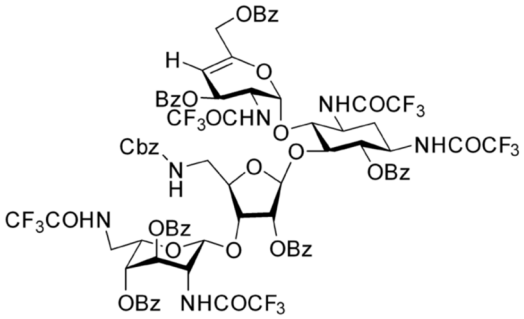
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
1127 formula(e) evaluated with 4 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-25 F: 15-15 Na: 1-1

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1857.4004	1857.4011	-0.7	-0.4	48.5	947.8	2.200	11.08	C88 H69 N4 O23 F15 Na
	1857.3984	2.0	1.1	49.5	946.6	1.000	36.80	C84 H65 N10 O21 F15 Na
	1857.3971	3.3	1.8	44.5	946.9	1.290	27.52	C83 H69 N6 O25 F15 Na
	1857.4083	-7.9	-4.3	44.5	947.0	1.403	24.59	C82 H69 N8 O24 F15 Na

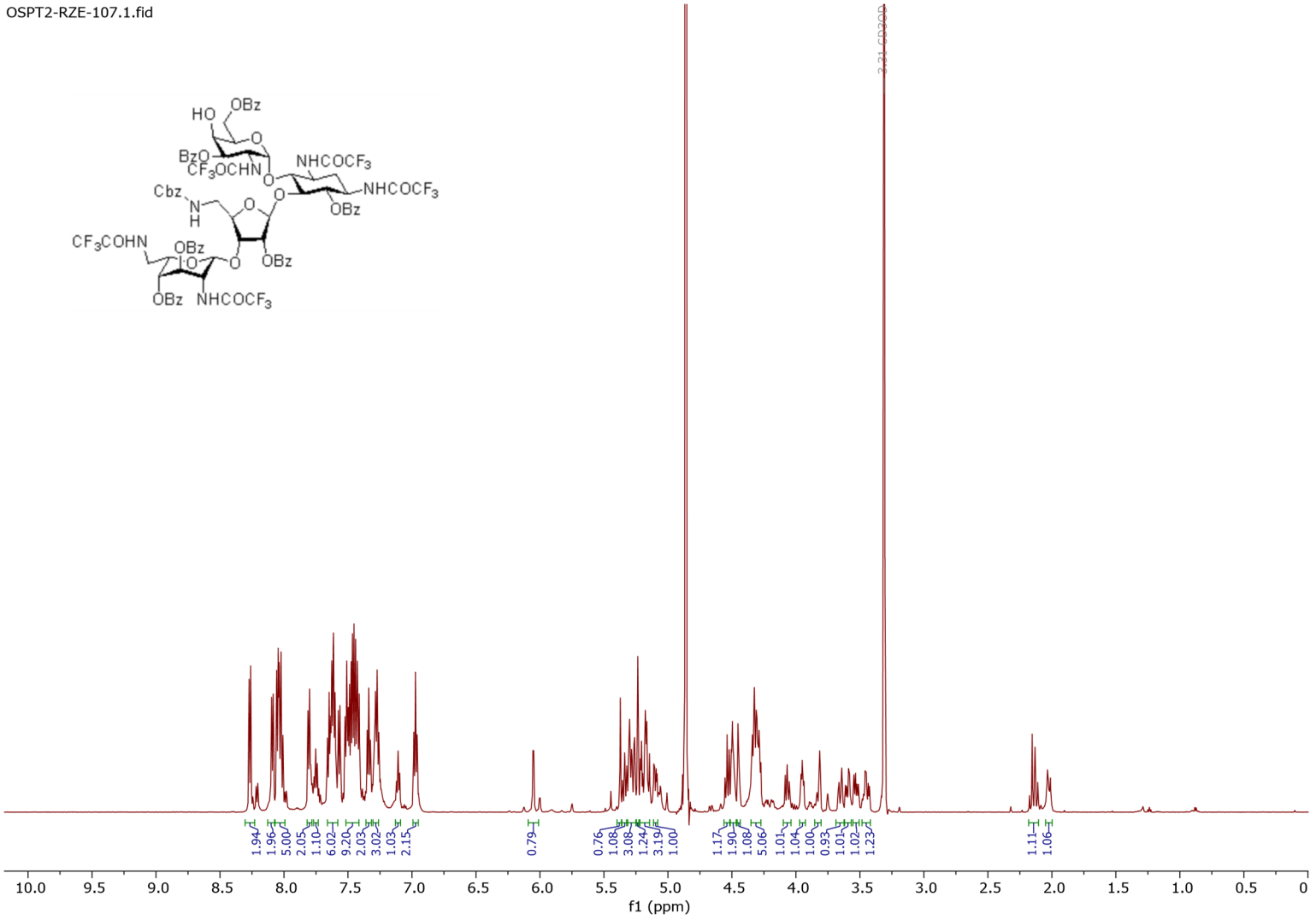
2374 Zogota RZE-115
HRMS_2020_05_212 1638 (4.580) Cm (1634:1652-(1681:1694+1603:1616))
1: TOF MS ES+
1.73e6





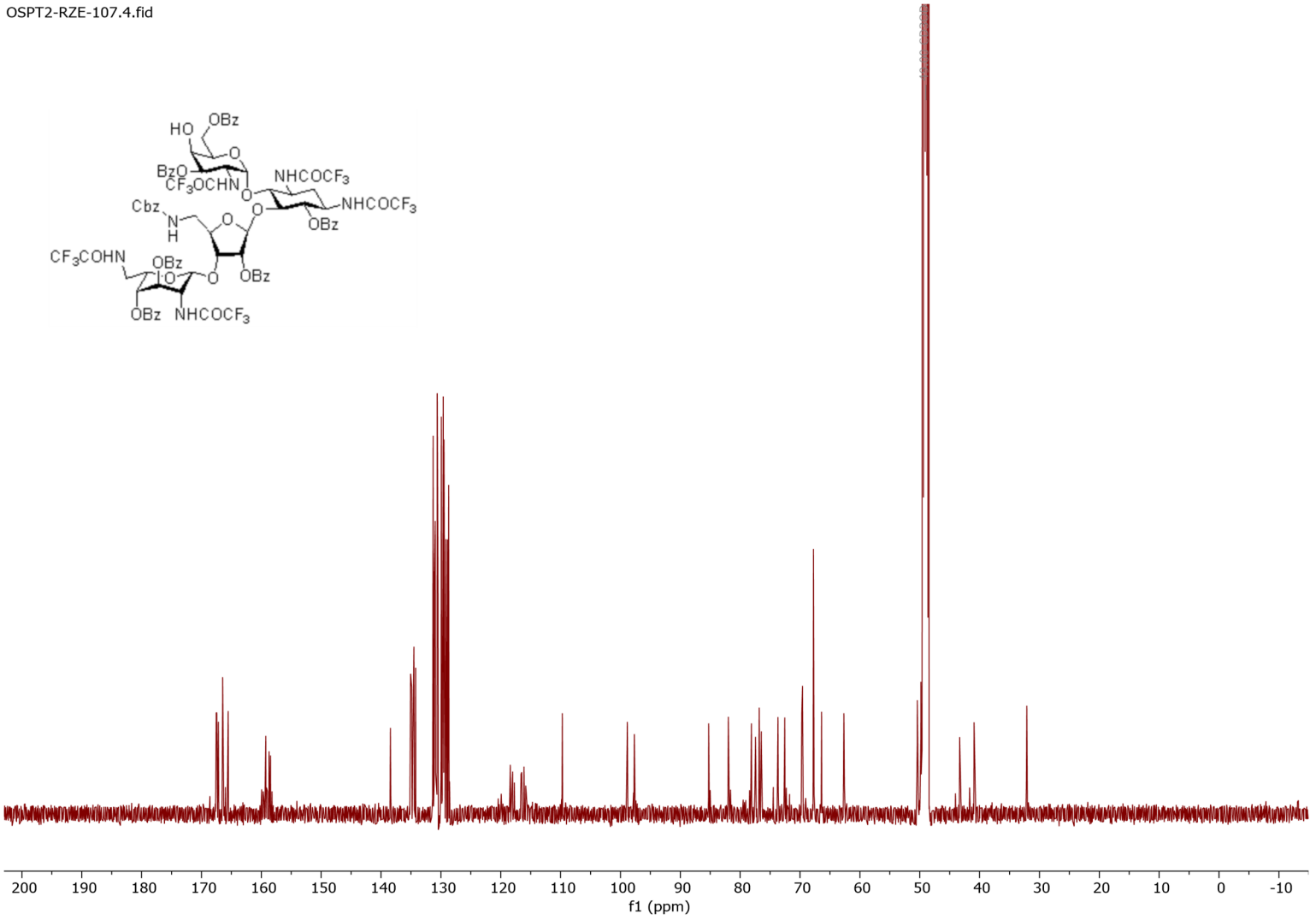
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H-NMR, 600 MHz, CD_3OD]



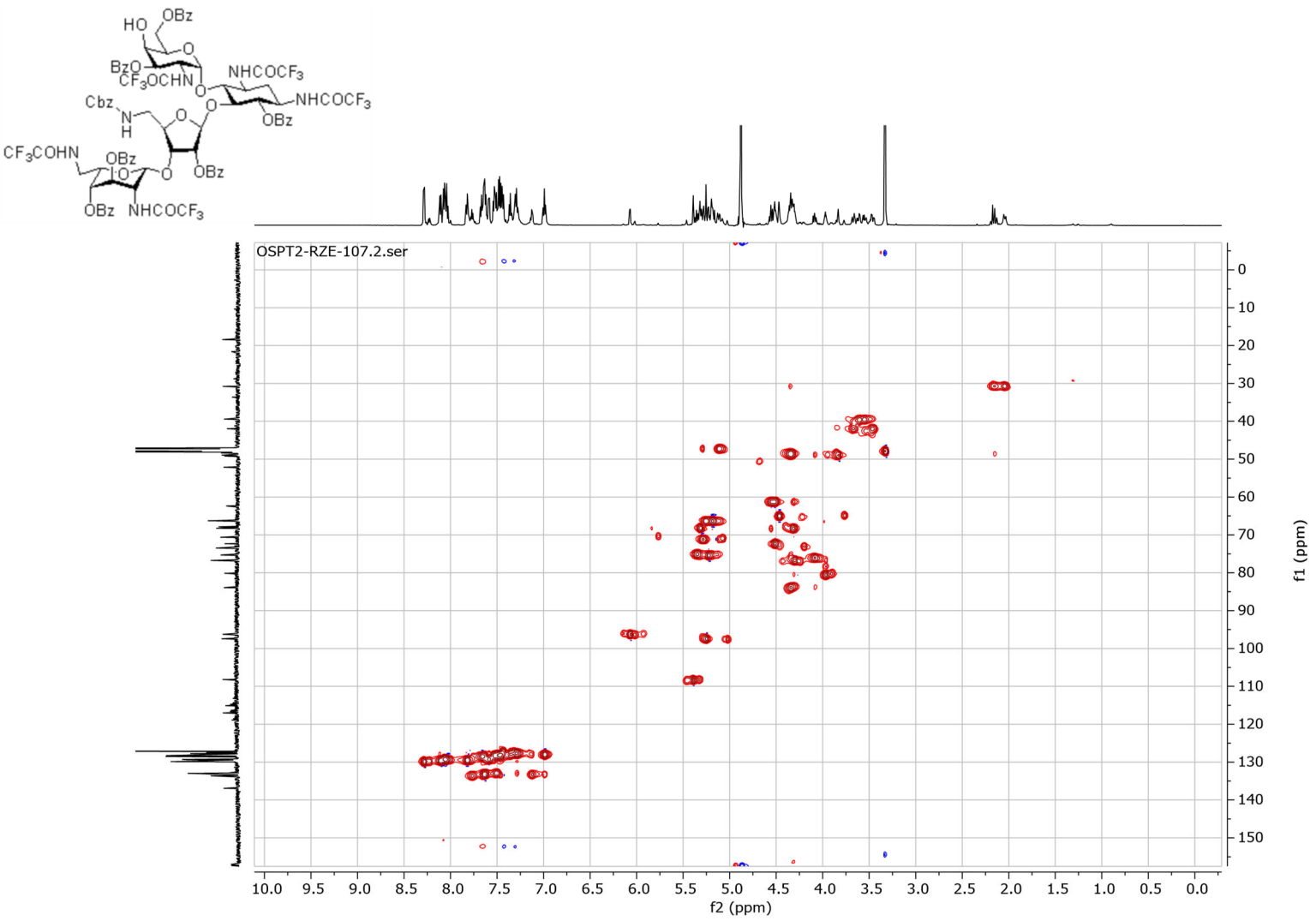
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



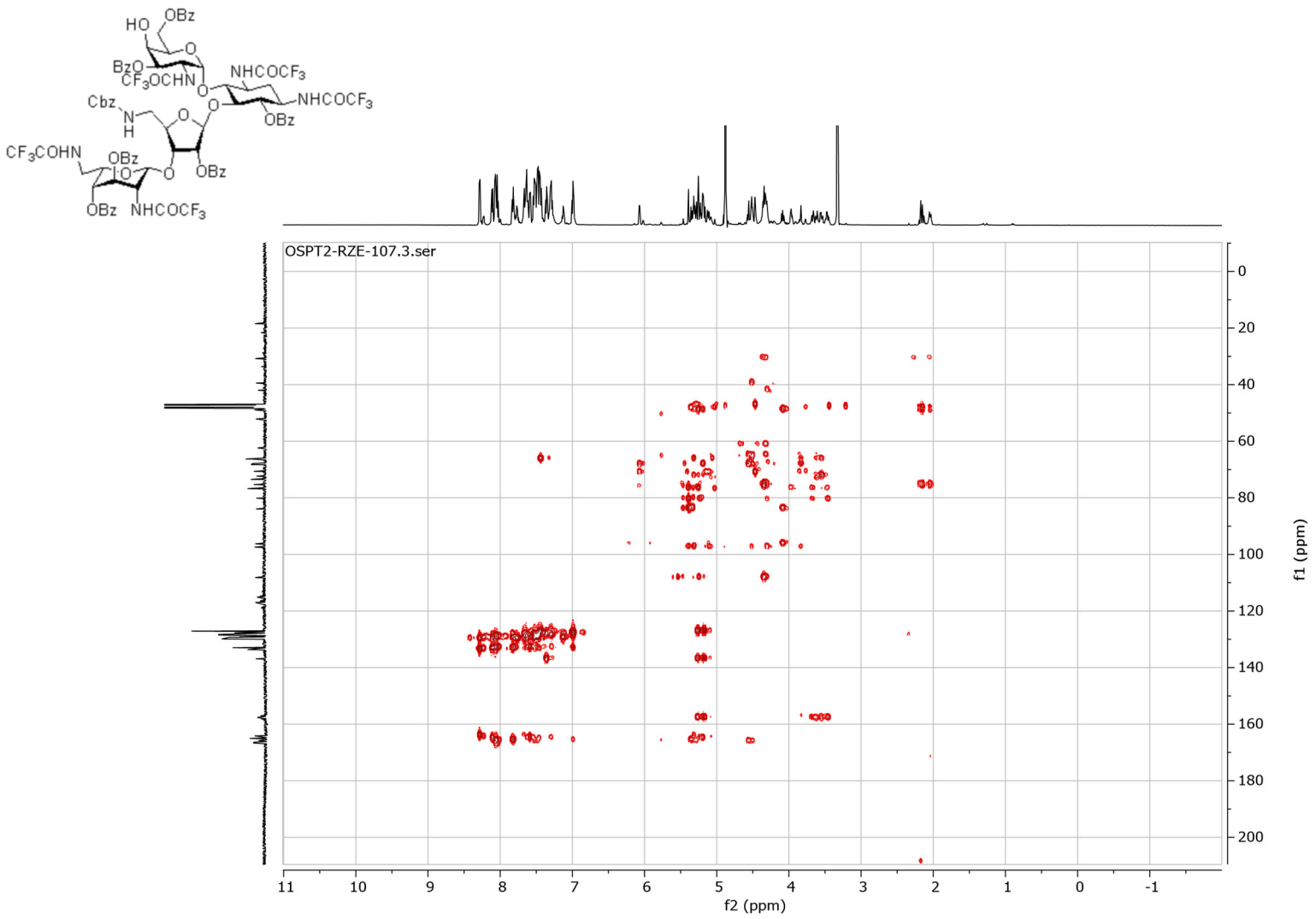
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, CD_3OD]



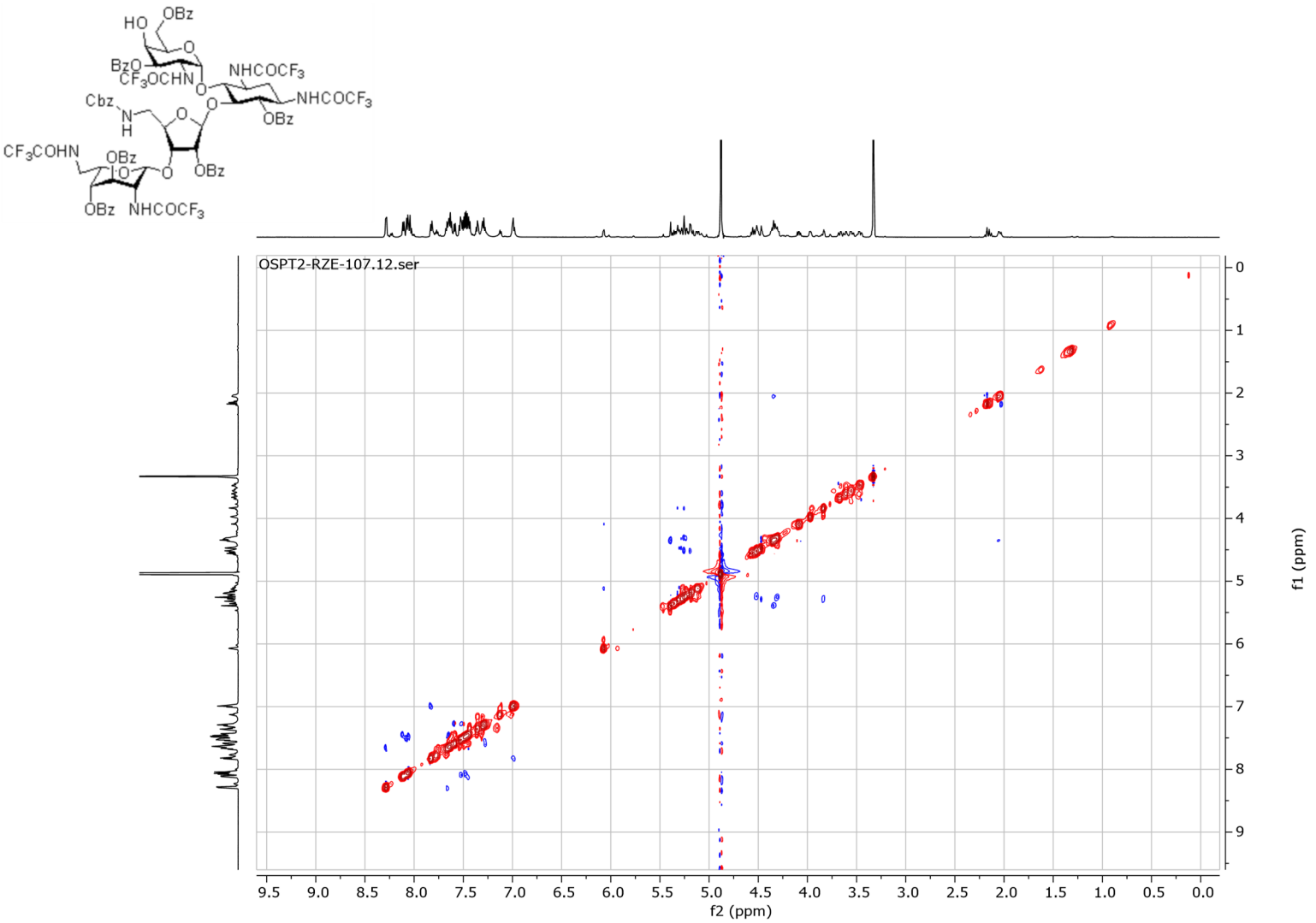
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, CD_3OD]



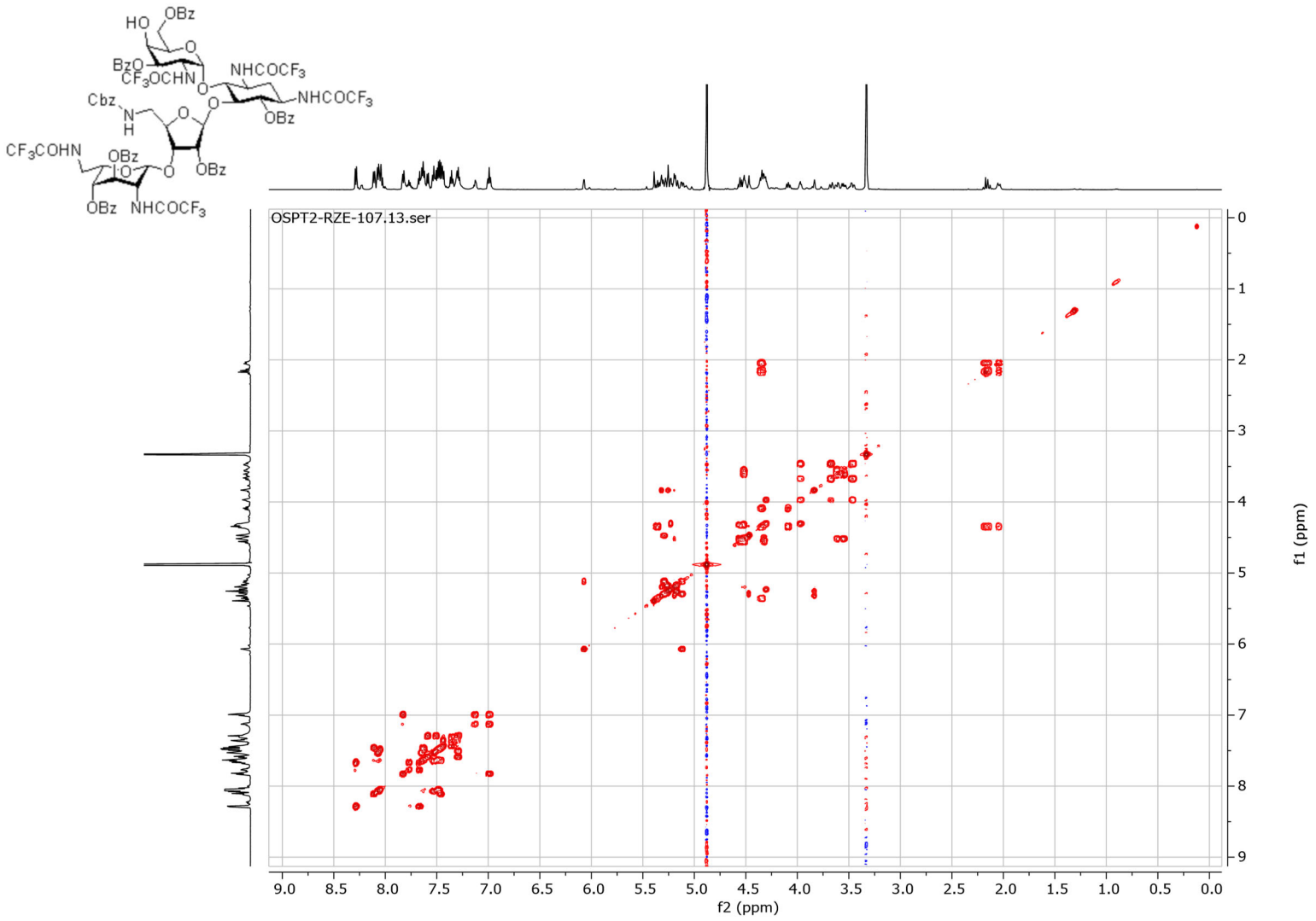
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H, ^{1}H ROESY, 600 MHz, CD_3OD]



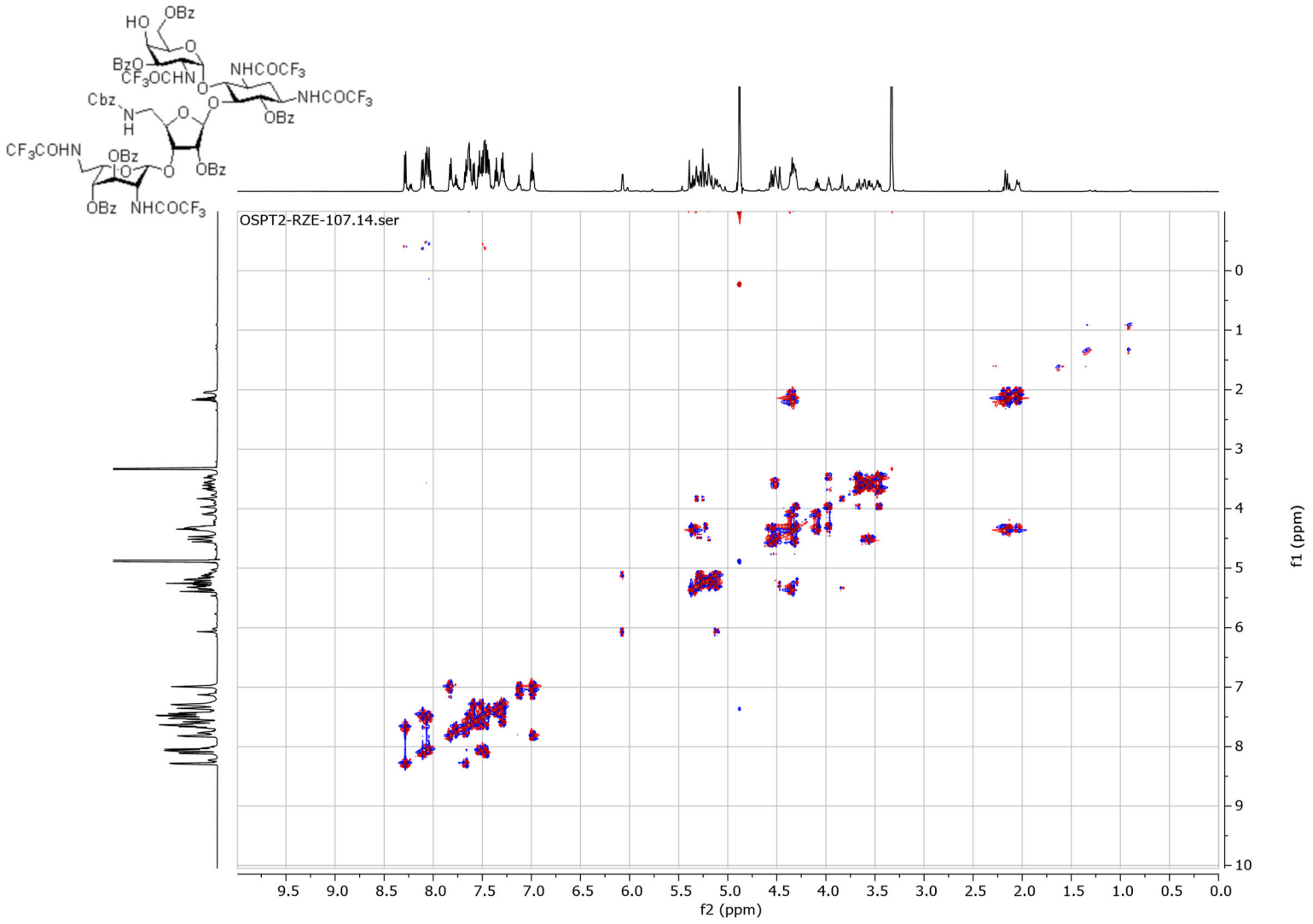
5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H, ^{1}H COSY, 600 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

[^{1}H, ^{1}H DQF-COSY, 600 MHz, CD_3OD]



5″-Deoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl 4′-*epi*-paromomycin (28)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_04_322 2182 Zogota RZE-107
MS_POS_500-2300_RES_7min ACN_Form_5-98_040_7min 2:A,1 1.000000 MS_Tune Col#66

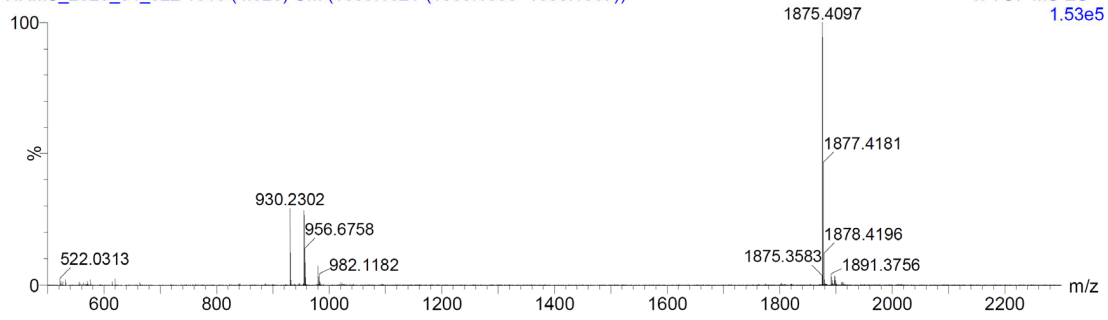
Elemental Composition Report:

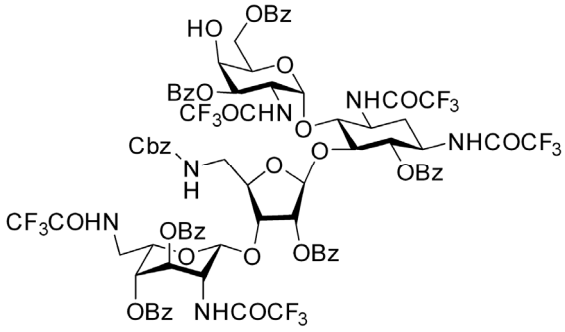
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
1508 formula(e) evaluated with 10 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-30 Na: 1-1 F: 15-15

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1875.4097	1875.4103	-0.6	-0.3	42.5	771.1	2.217	10.90	C87 H75 O28 Na F15
	1875.4090	0.7	0.4	48.5	770.1	1.261	28.35	C84 H67 N10 O22 Na F15
	1875.4116	-1.9	-1.0	47.5	771.2	2.265	10.38	C88 H71 N4 O24 Na F15
	1875.4076	2.1	1.1	43.5	770.2	1.343	26.10	C83 H71 N6 O26 Na F15
	1875.4063	3.4	1.8	38.5	770.3	1.416	24.27	C82 H75 N2 O30 Na F15

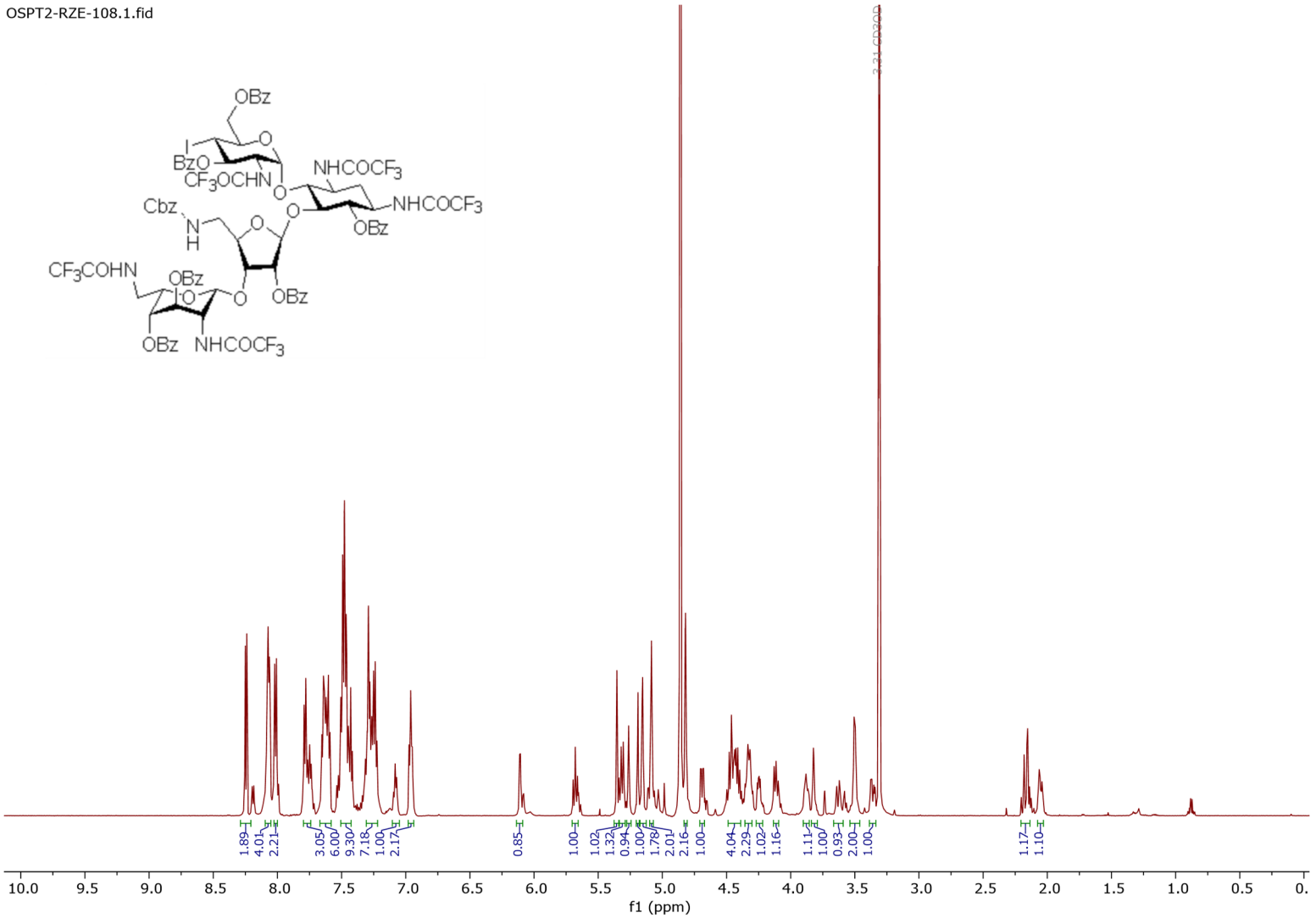
2182 Zogota RZE-107
HRMS_2020_04_322 1616 (4.520) Cm (1609:1621-(1583:1600+1655:1667))
1: TOF MS ES+
1.53e5





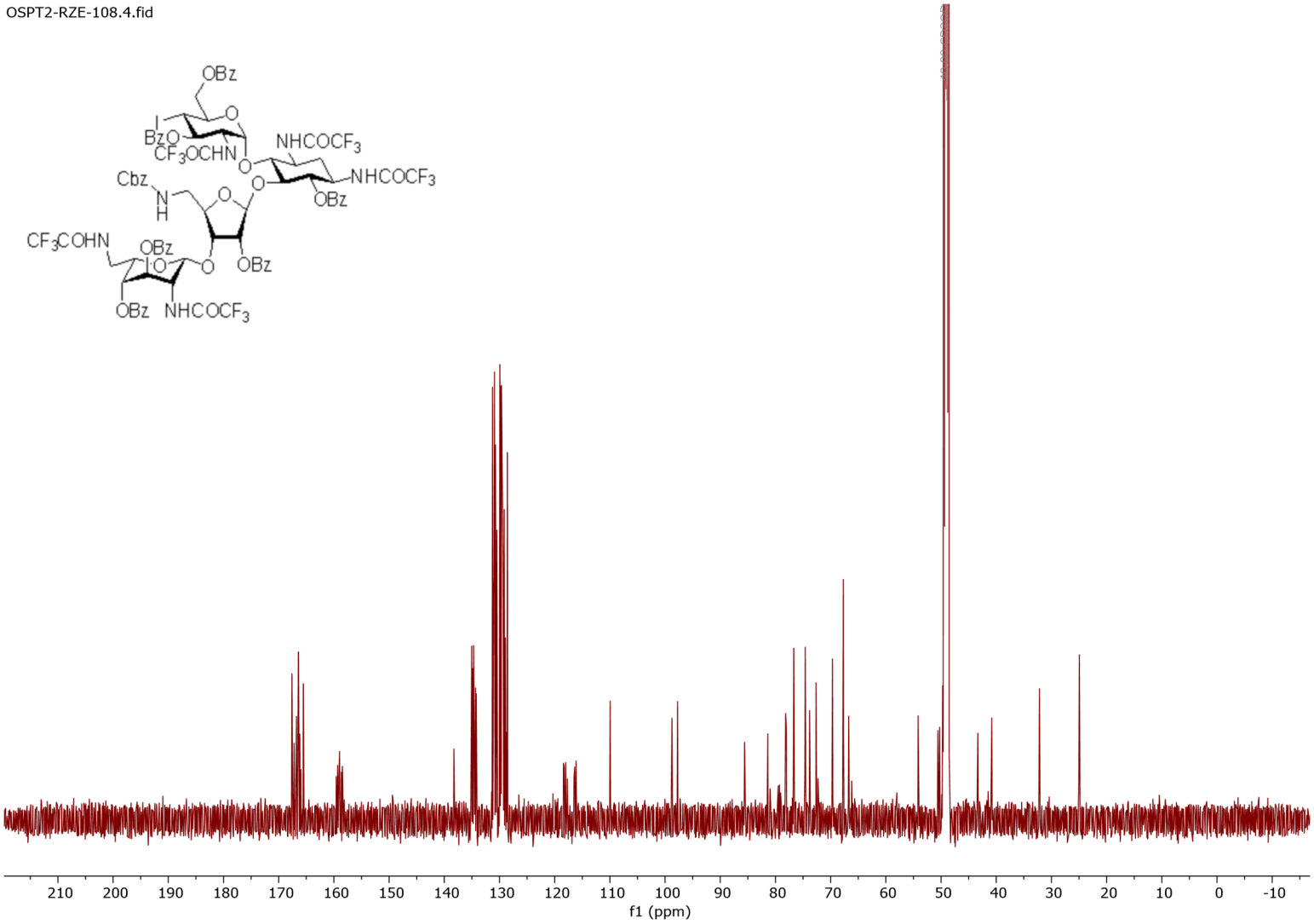
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H-NMR, 600 MHz, CD_3OD]



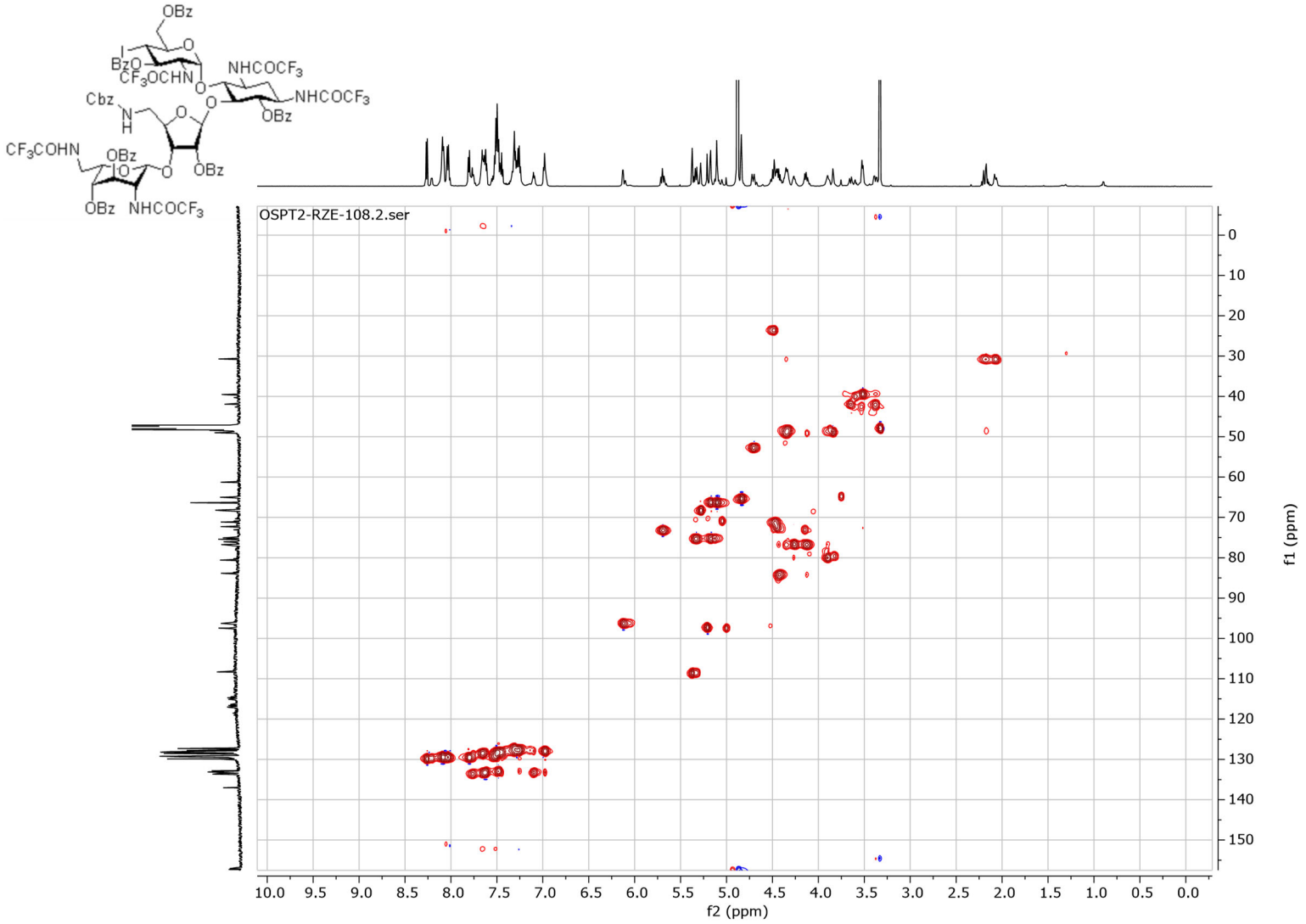
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



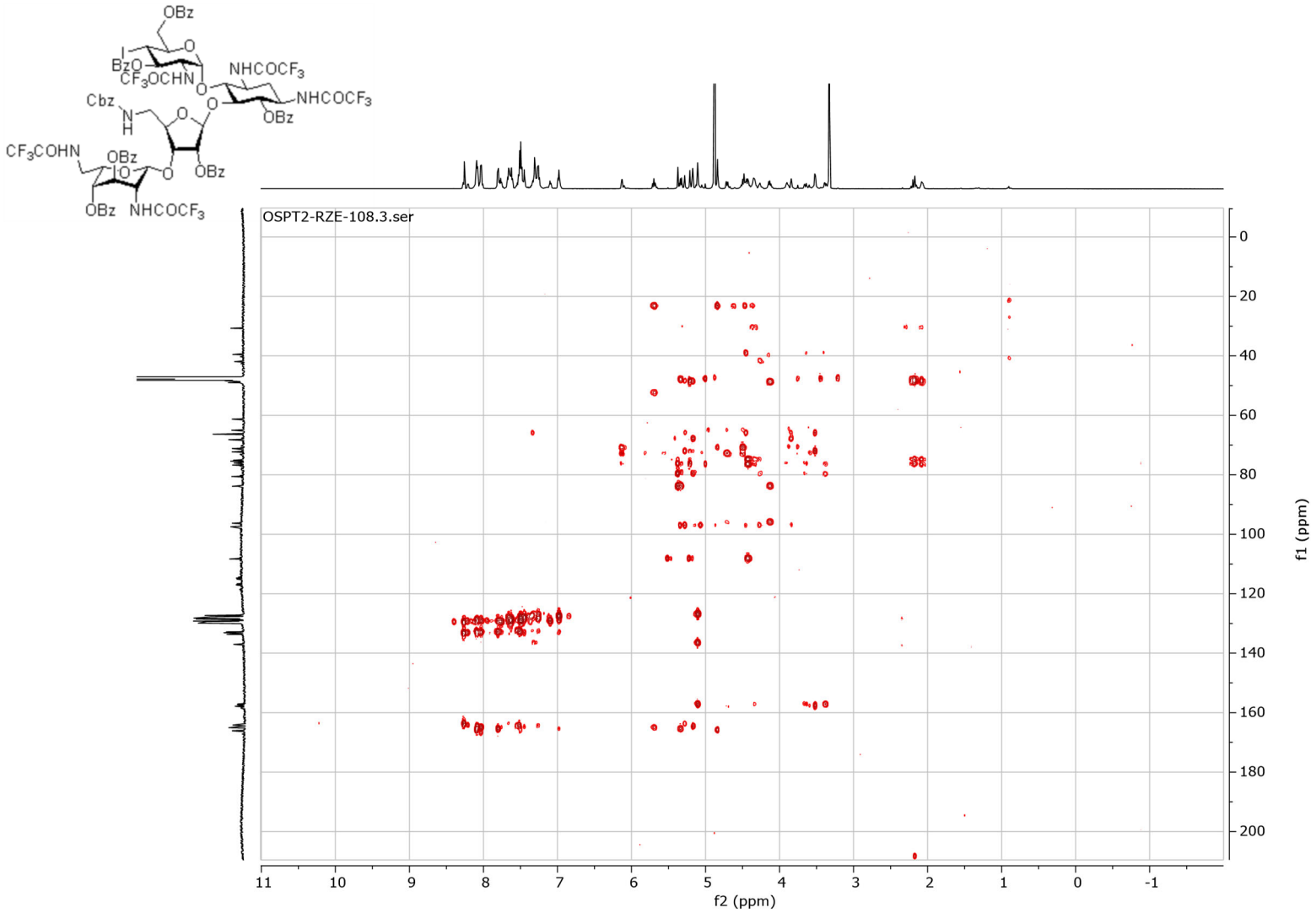
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, CD_3OD]



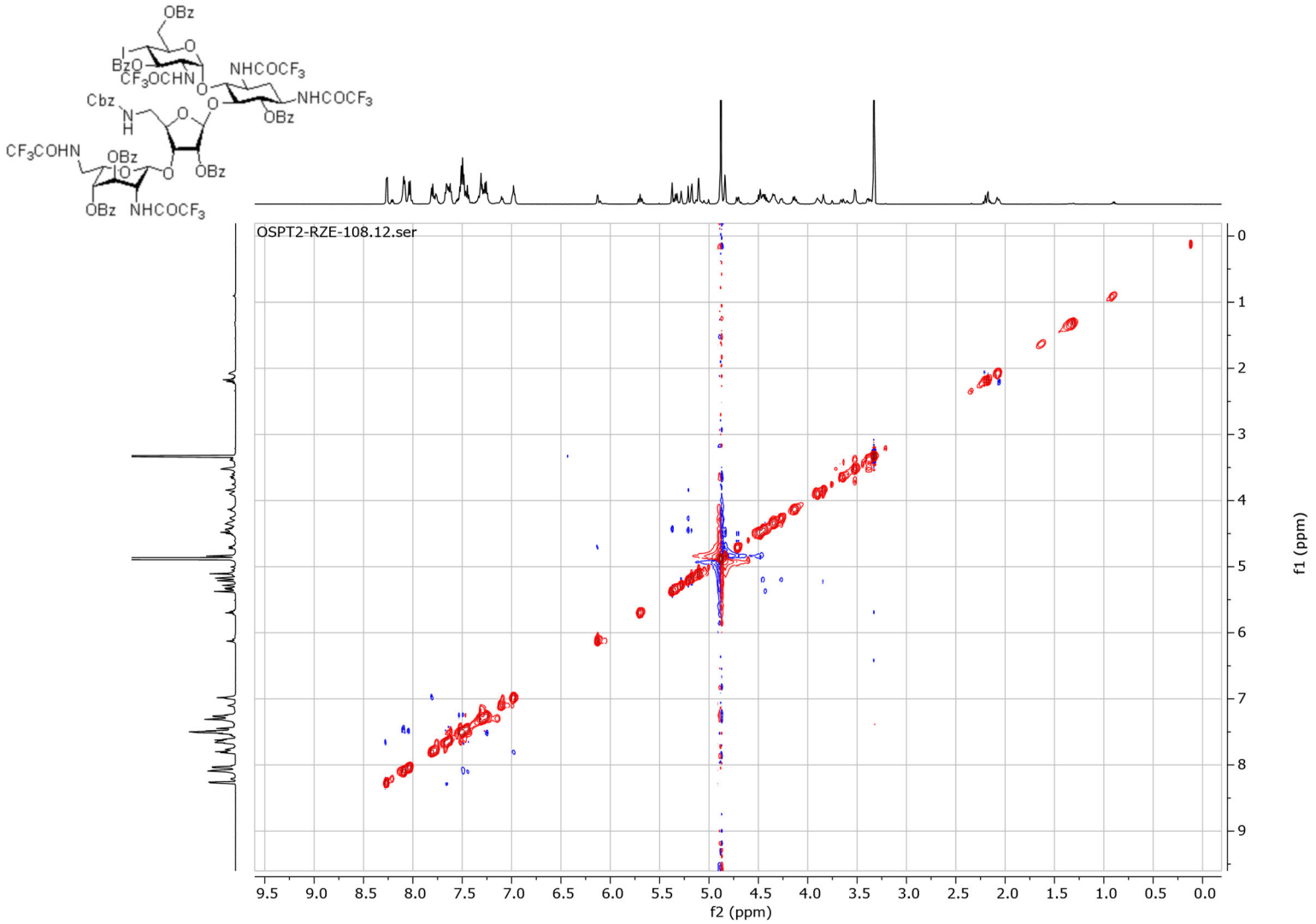
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, CD_3OD]



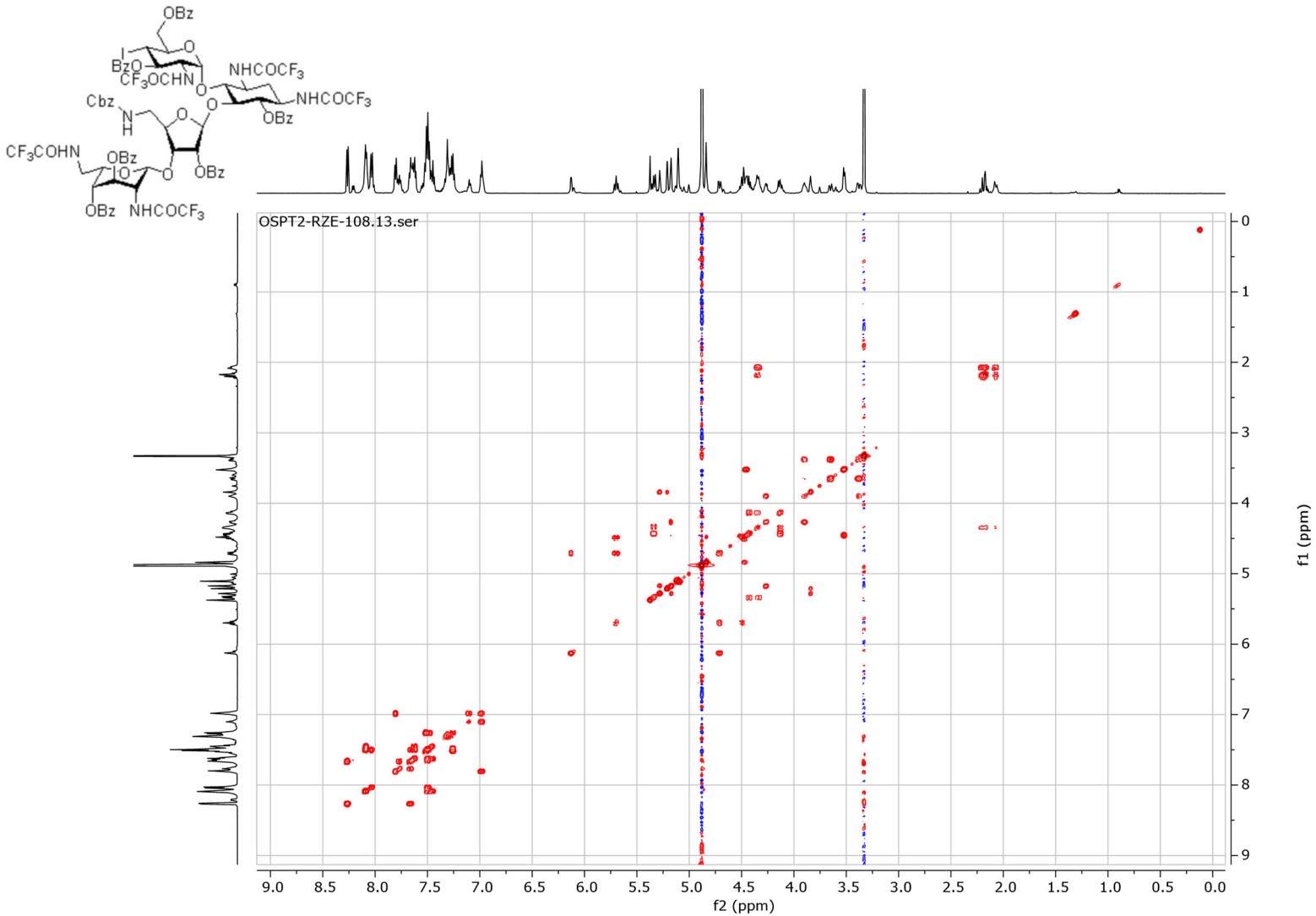
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H, ^{1}H ROESY, 600 MHz, CD_3OD]



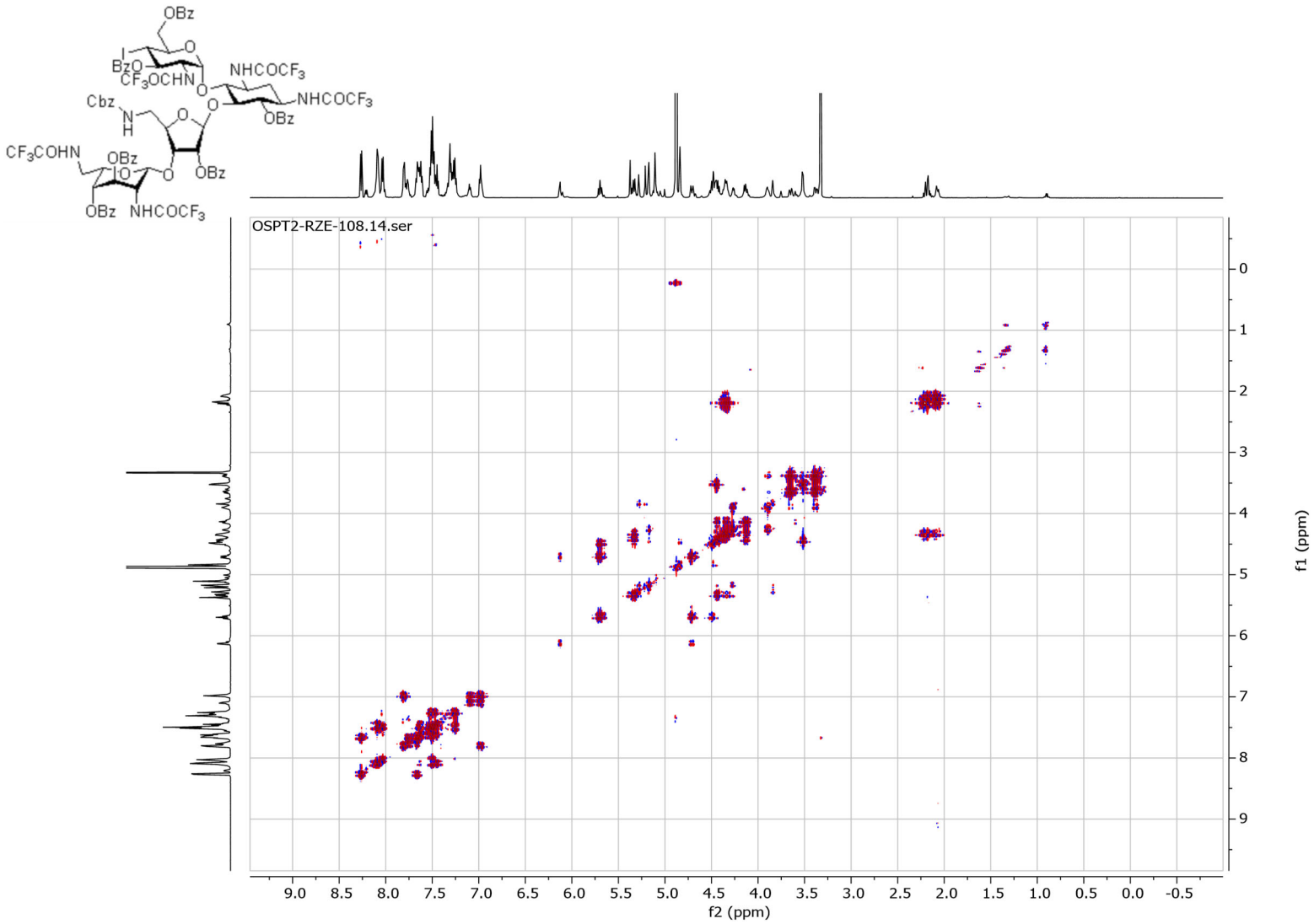
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H, ^{1}H COSY, 600 MHz, CD_3OD]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

[^{1}H, ^{1}H DQF-COSY, 600 MHz, CD_3OD]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-4′-iodo-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (29)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_04_324 2183 Zogota RZE-108
MS_POS_500-2300_RES_7min ACN_Form_5-98_040_7min 2:A,2 1.000000 MS_Tune Col#66

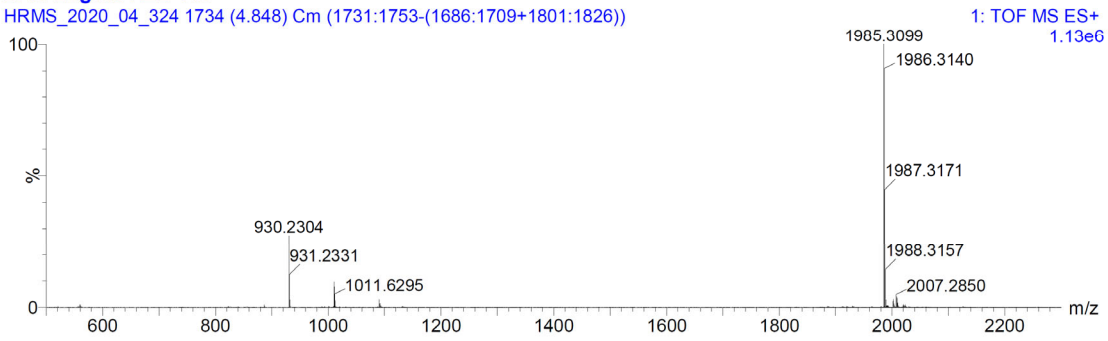
Elemental Composition Report:

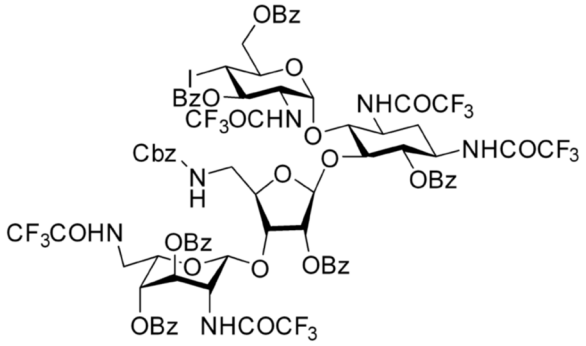
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
1619 formula(e) evaluated with 11 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-30 F: 15-15 Na: 1-1 I: 1-1

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1985.3099	1985.3093	0.6	0.3	43.5	992.7	1.145	31.84	C83 H70 N6 O25 F15 Na I
	1985.3107	-0.8	-0.4	48.5	992.8	1.231	29.19	C84 H66 N10 O21 F15 Na I
	1985.3080	1.9	1.0	38.5	992.8	1.161	31.33	C82 H74 N2 O29 F15 Na I
	1985.3120	-2.1	-1.1	42.5	994.7	3.093	4.54	C87 H74 O27 F15 Na I
	1985.3134	-3.5	-1.8	47.5	995.1	3.472	3.11	C88 H70 N4 O23 F15 Na I

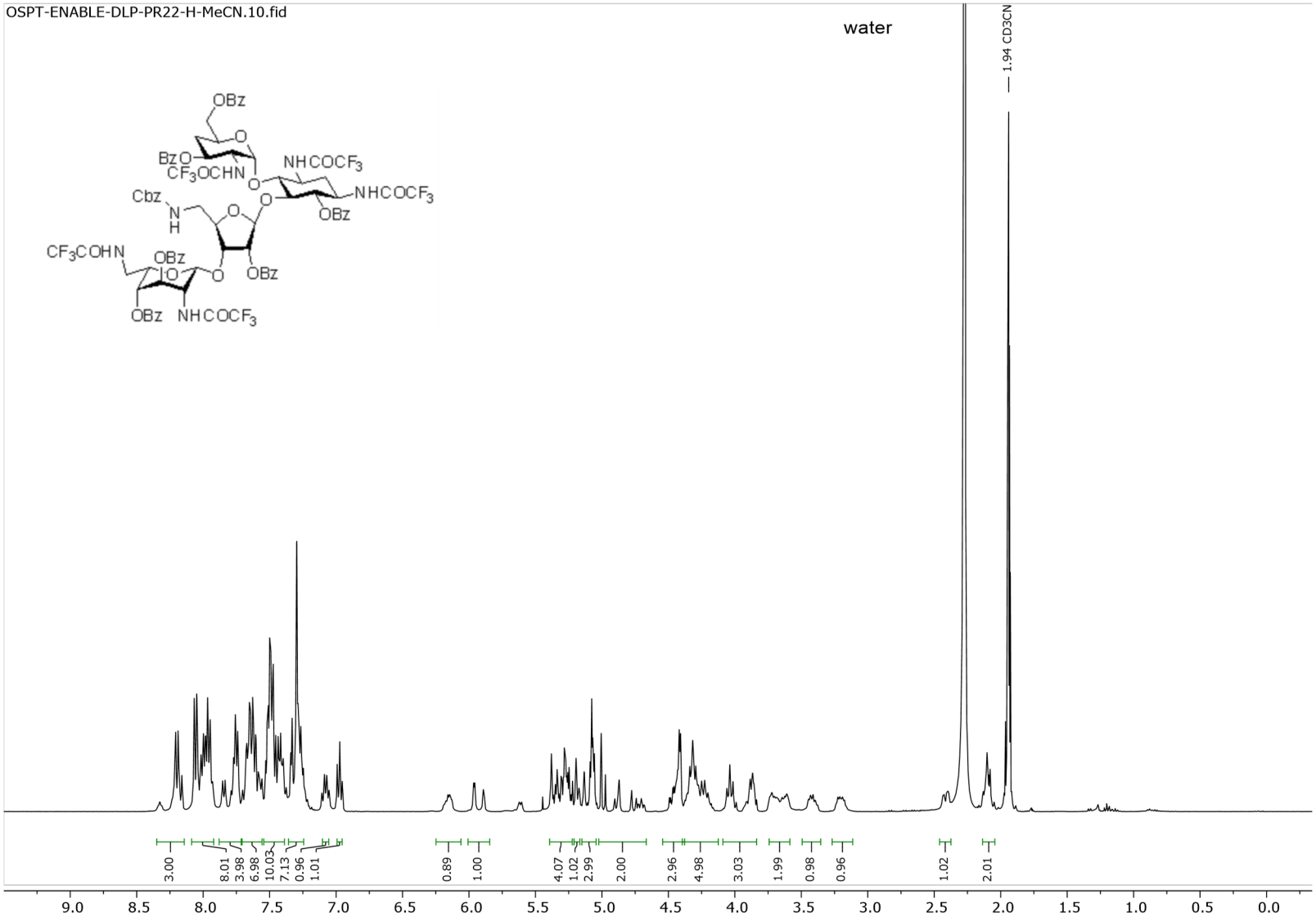
2183 Zogota RZE-108





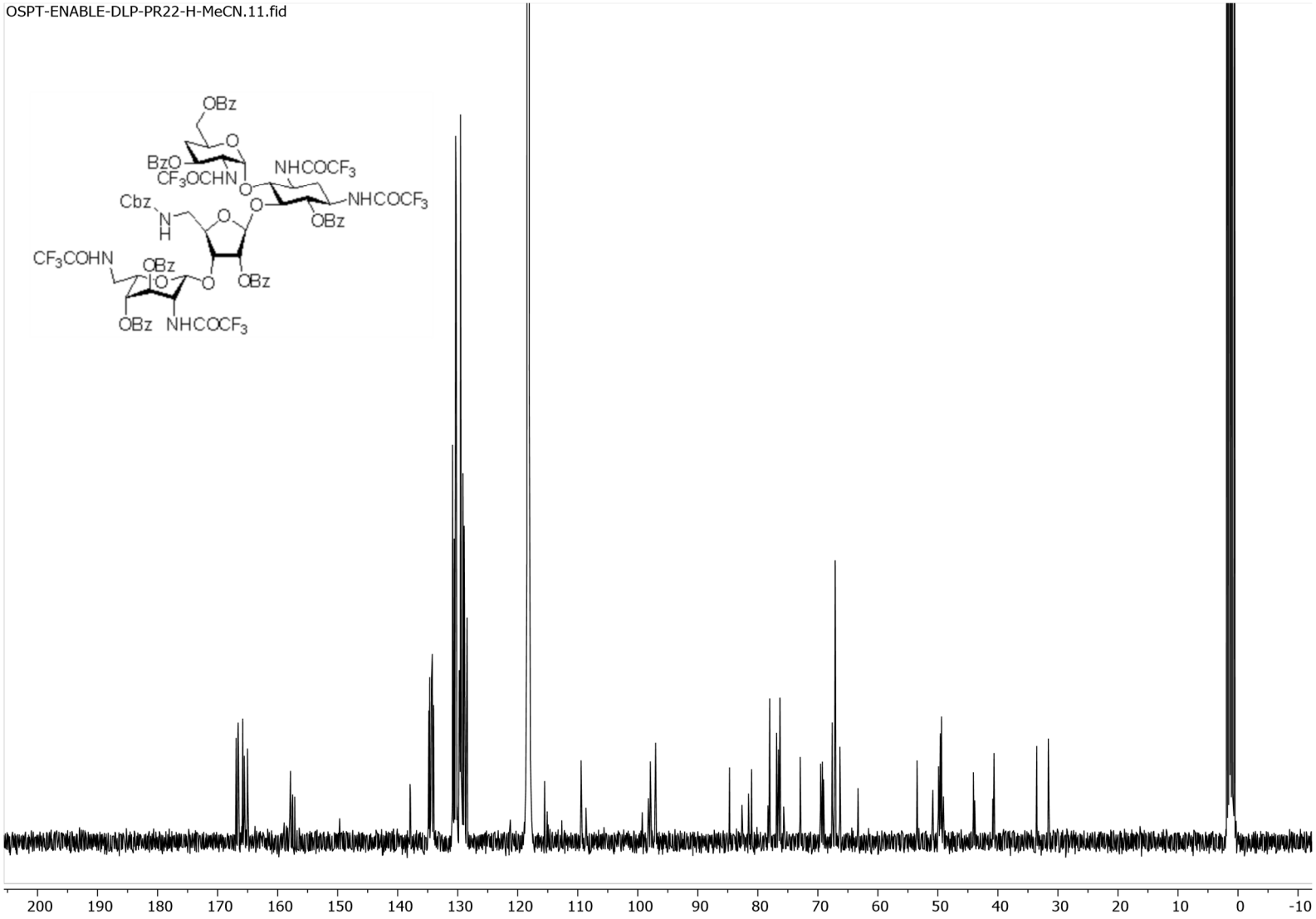
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (30-H)

[^{1}H-NMR, 400 MHz, CD_3CN]



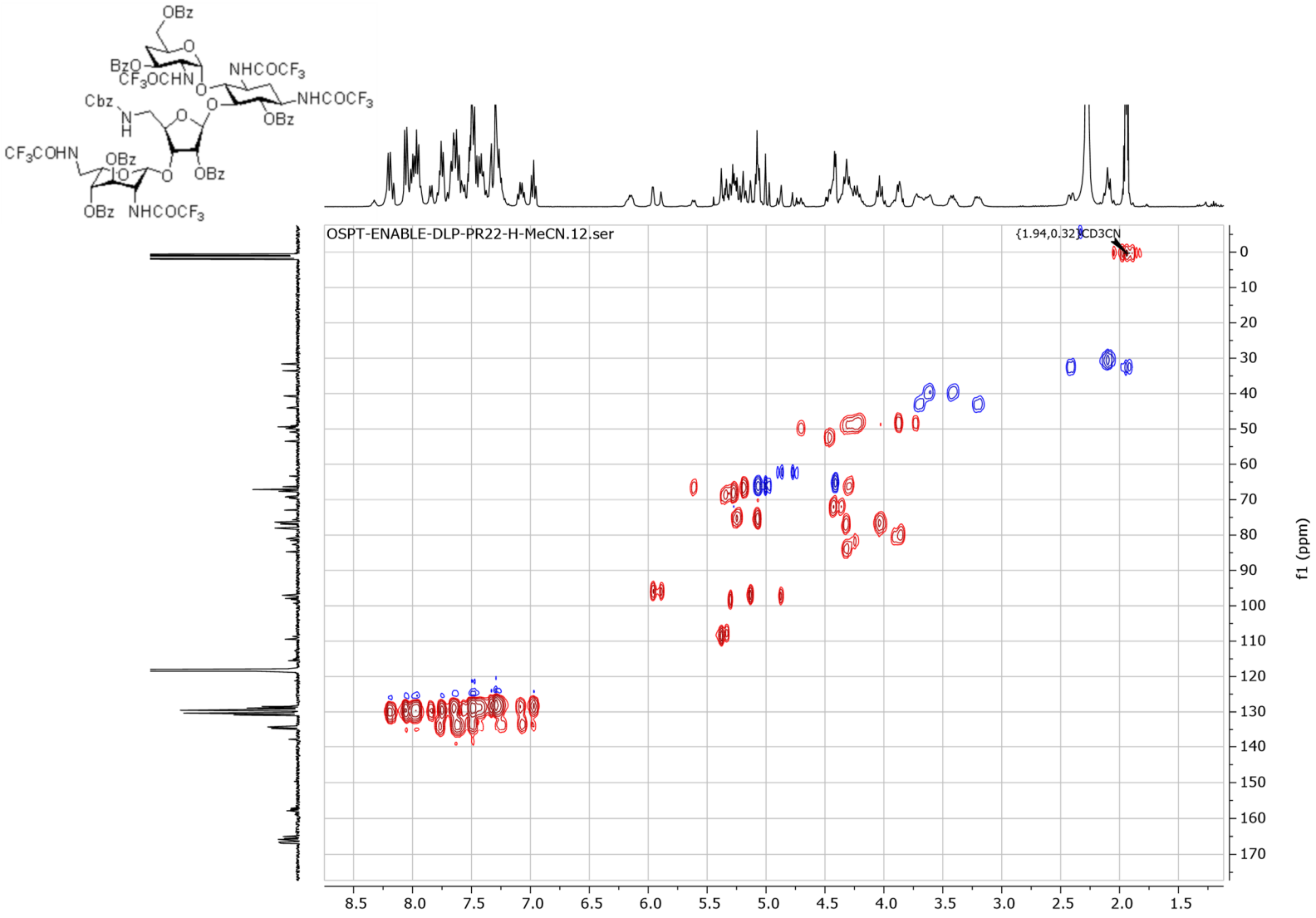
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (30-H)

[^{13}C-NMR, 100.6 MHz, CD_3CN]



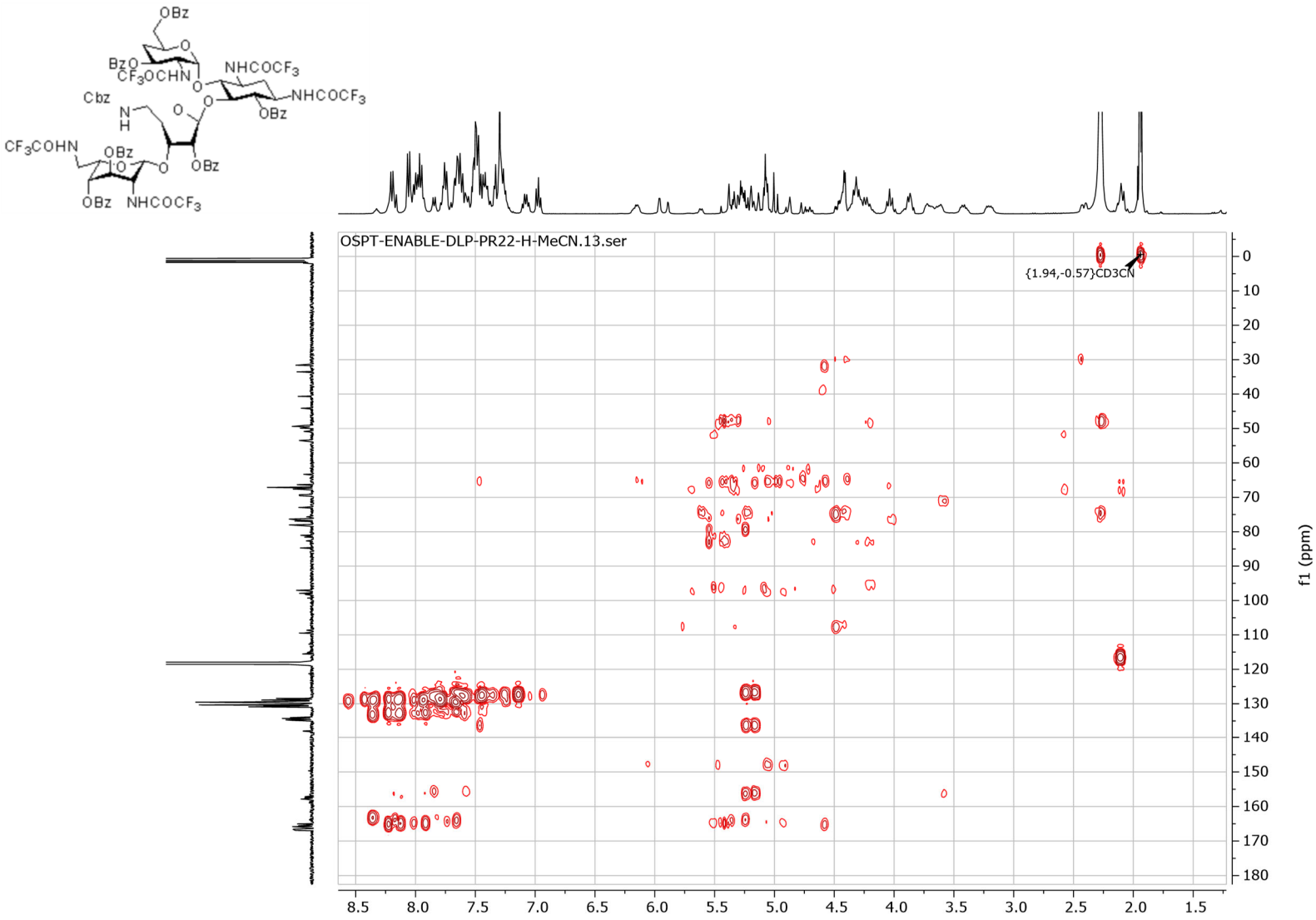
4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (30-H)

[^{1}H, ^{13}C HSQC, 400 MHz, 100.6 MHz, CD_3CN]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (30-H)

[^{1}H, ^{13}C HMBC, 400 MHz, 100.6 MHz, CD_3CN]



4′,5″-Dideoxy-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (30-H)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

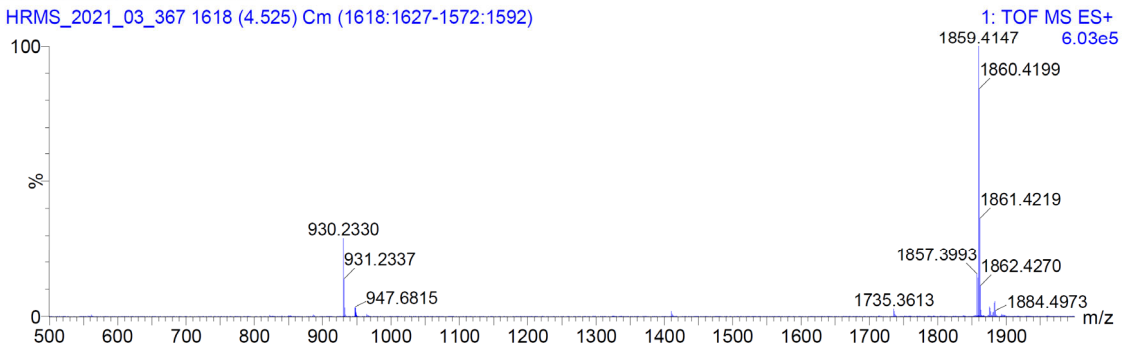
Sample:
HRMS_2021_03_367 484 Lubriks DLP-PR22-H
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:C,7 1.000000 MS_Tune Col#66

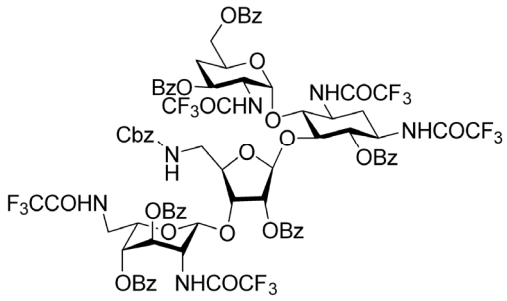
Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
3951 formula(e) evaluated with 15 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-25 O: 0-25 Na: 1-1 F: 15-15

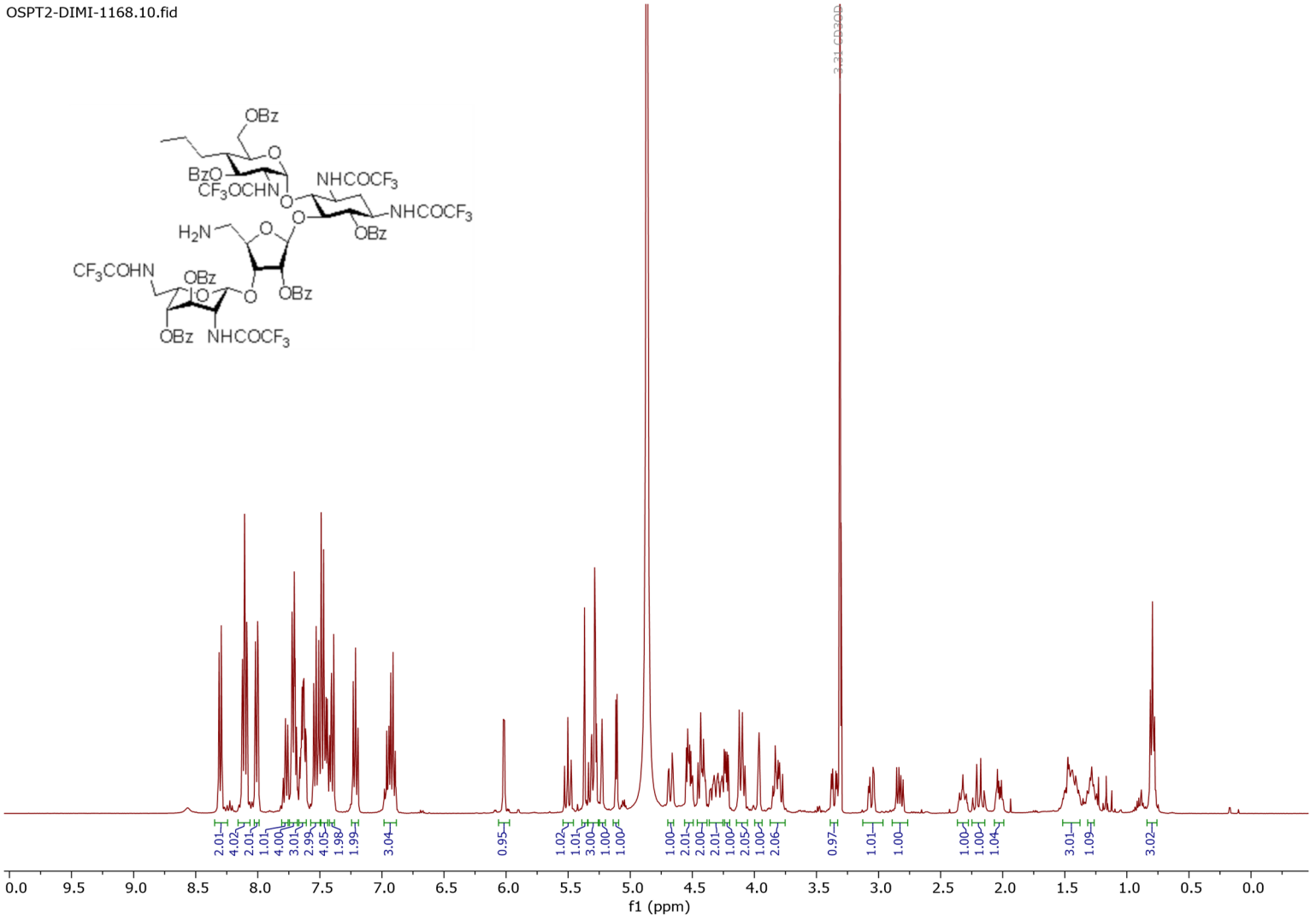
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1859.4147	100.00	1859.4145	0.2	0.1	41.5	852.6	0.258	77.26	C69 H63 N22 O22 Na F15
		1859.4140	0.7	0.4	48.5	857.2	4.817	0.81	C84 H67 N10 O21 Na F15
		1859.4167	-2.0	-1.1	47.5	857.3	4.952	0.71	C88 H71 N4 O23 Na F15
		1859.4127	2.0	1.1	43.5	857.1	4.743	0.87	C83 H71 N6 O25 Na F15
		1859.4172	-2.5	-1.3	40.5	854.0	1.592	20.35	C73 H67 N16 O24 Na F15





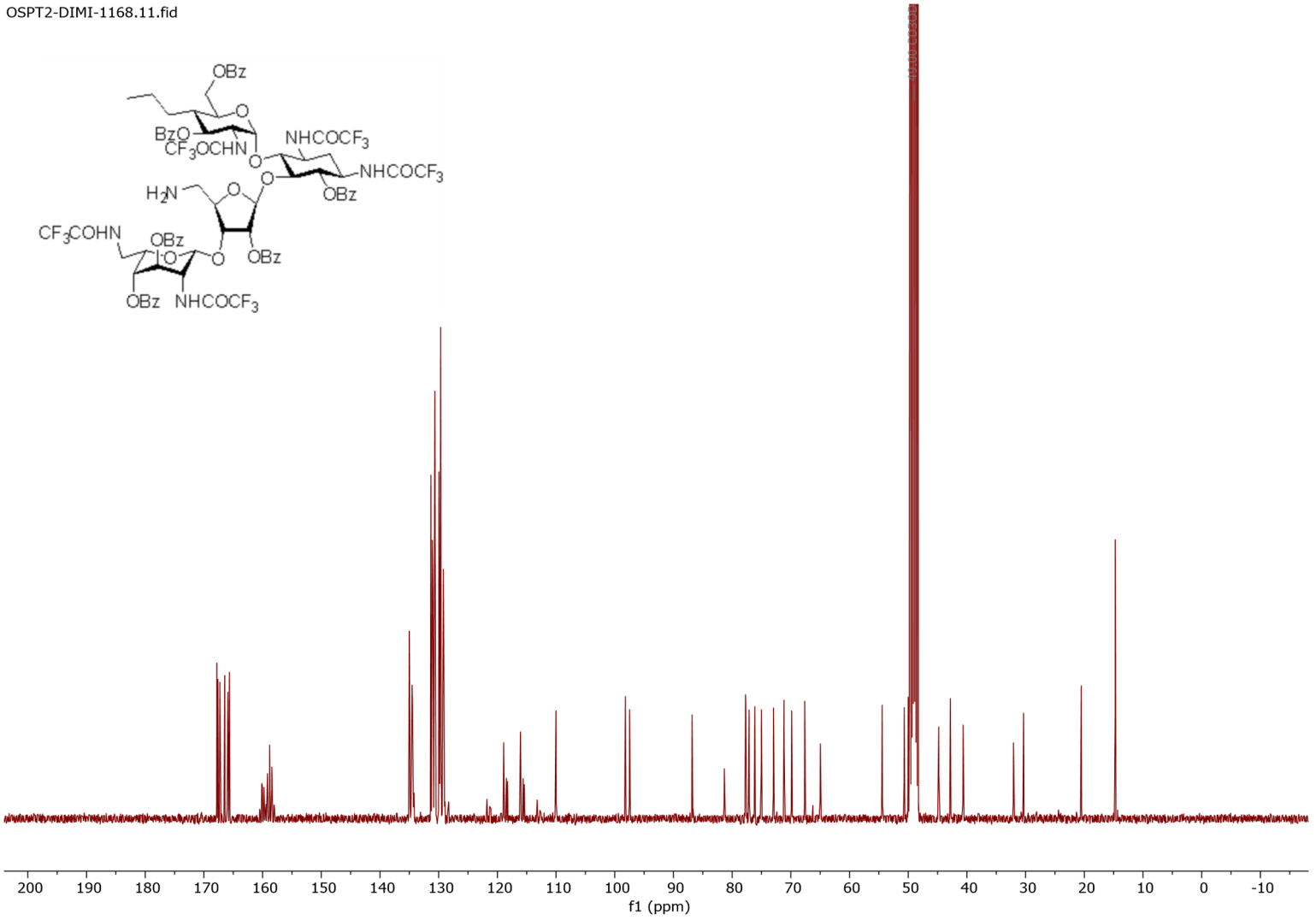
4′,5″-Dideoxy-4′-propyl-5″-amino-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (31)

[^{1}H-NMR, 400 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-amino-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (31)

[^{13}C-NMR, 100.6 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-amino-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (31)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

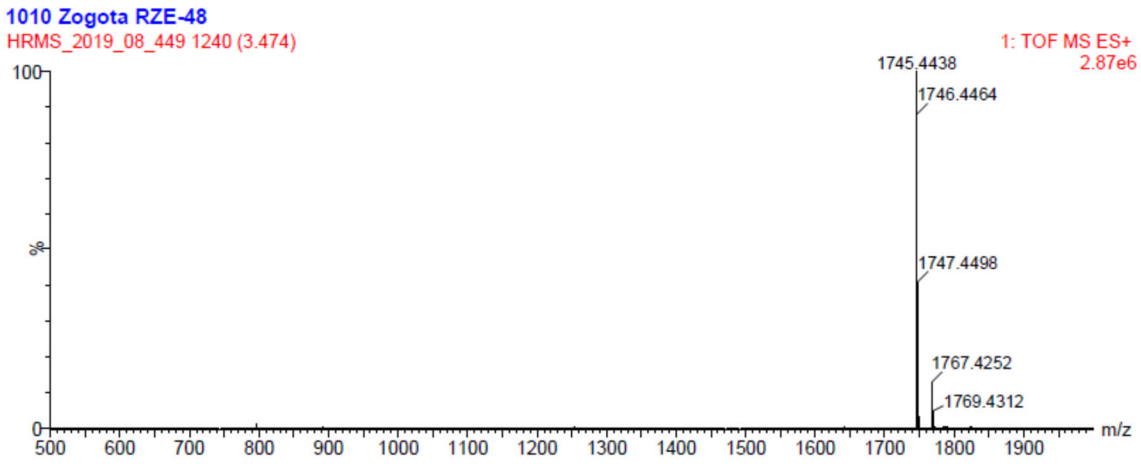
Sample:
HRMS_2019_08_449 1010 Zogota RZE-48
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:D,6 10.000000 MS_Tune Col#43

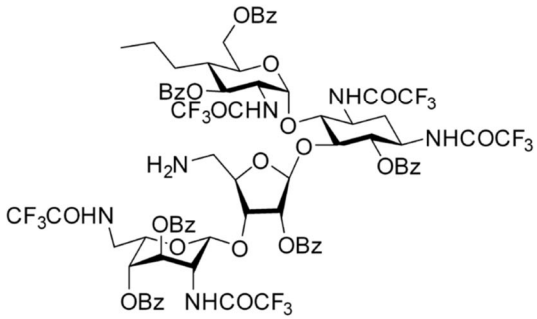
Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
276 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-78 H: 1-110 N: 1-10 O: 0-23 F: 15-15

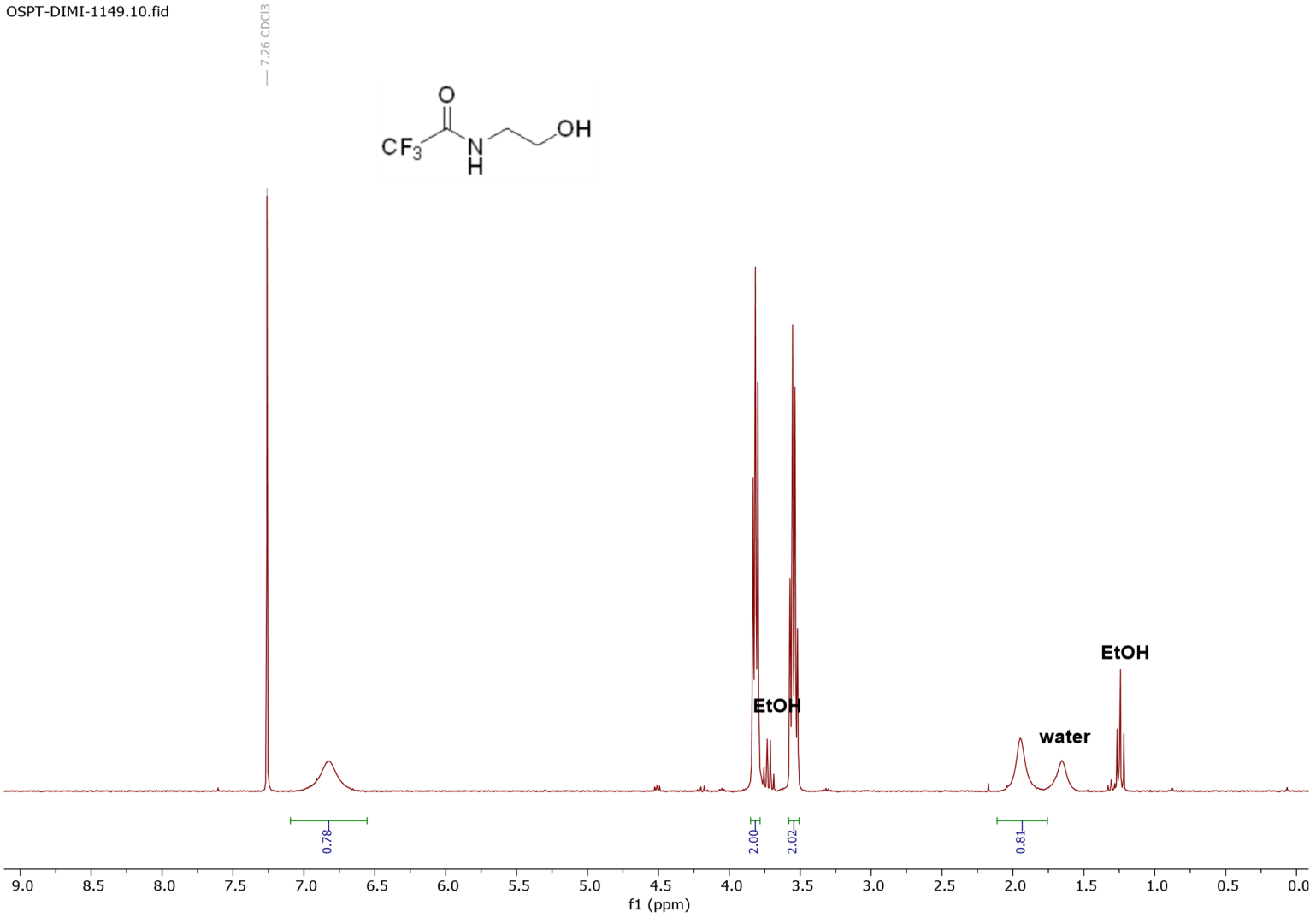
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1745.4438	100.00	1745.4409	2.9	1.7	38.5	271.4	0.132	87.59	C78 H72 N6 O23 F15
		1745.4522	-8.4	-4.8	38.5	273.4	2.087	12.41	C77 H72 N8 O22 F15





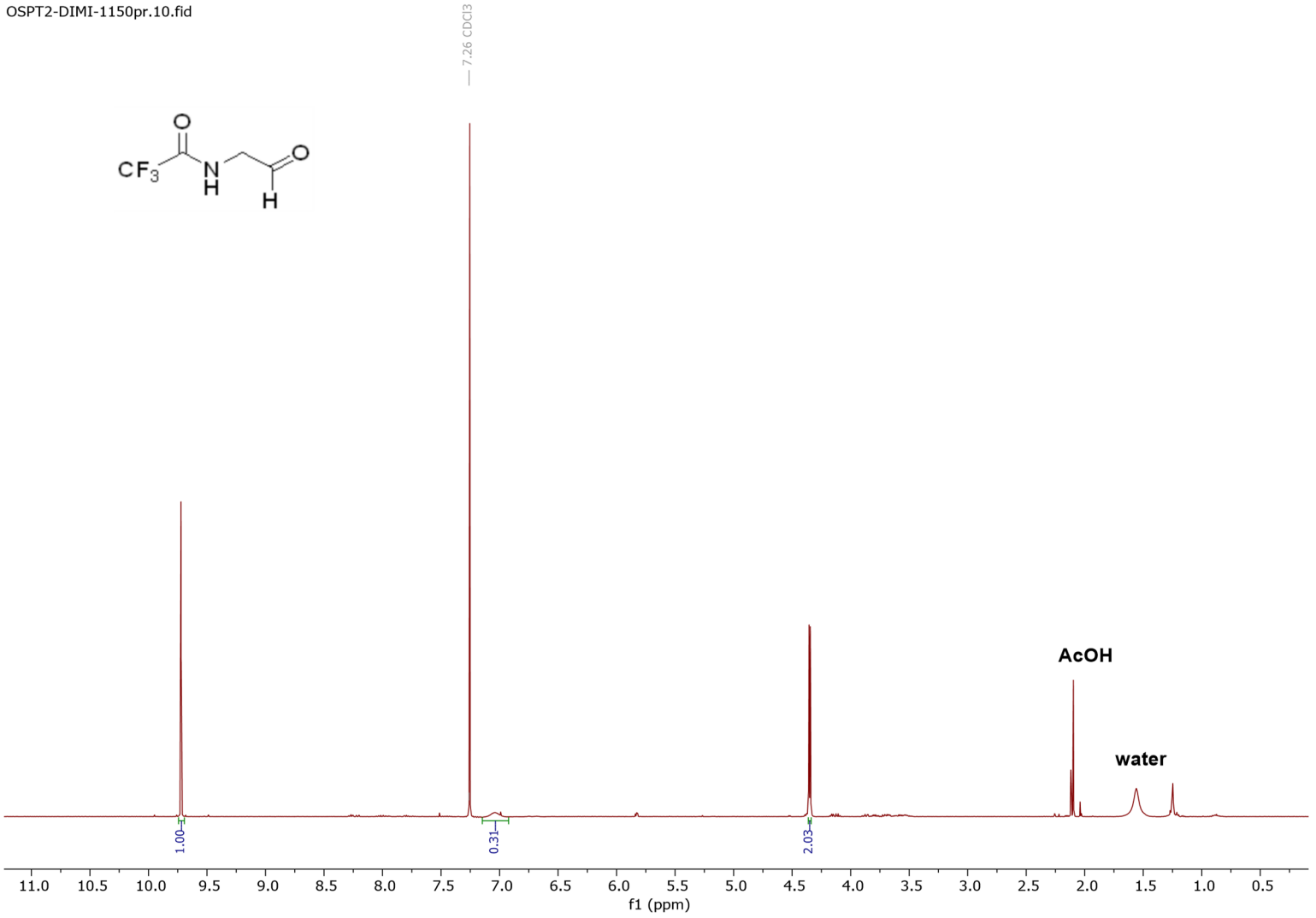
2,2,2-Trifluoro-*N*-(2-hydroxyethyl)acetamide (S1)

[^{1}H-NMR, 300 MHz, $CDCl_3$]



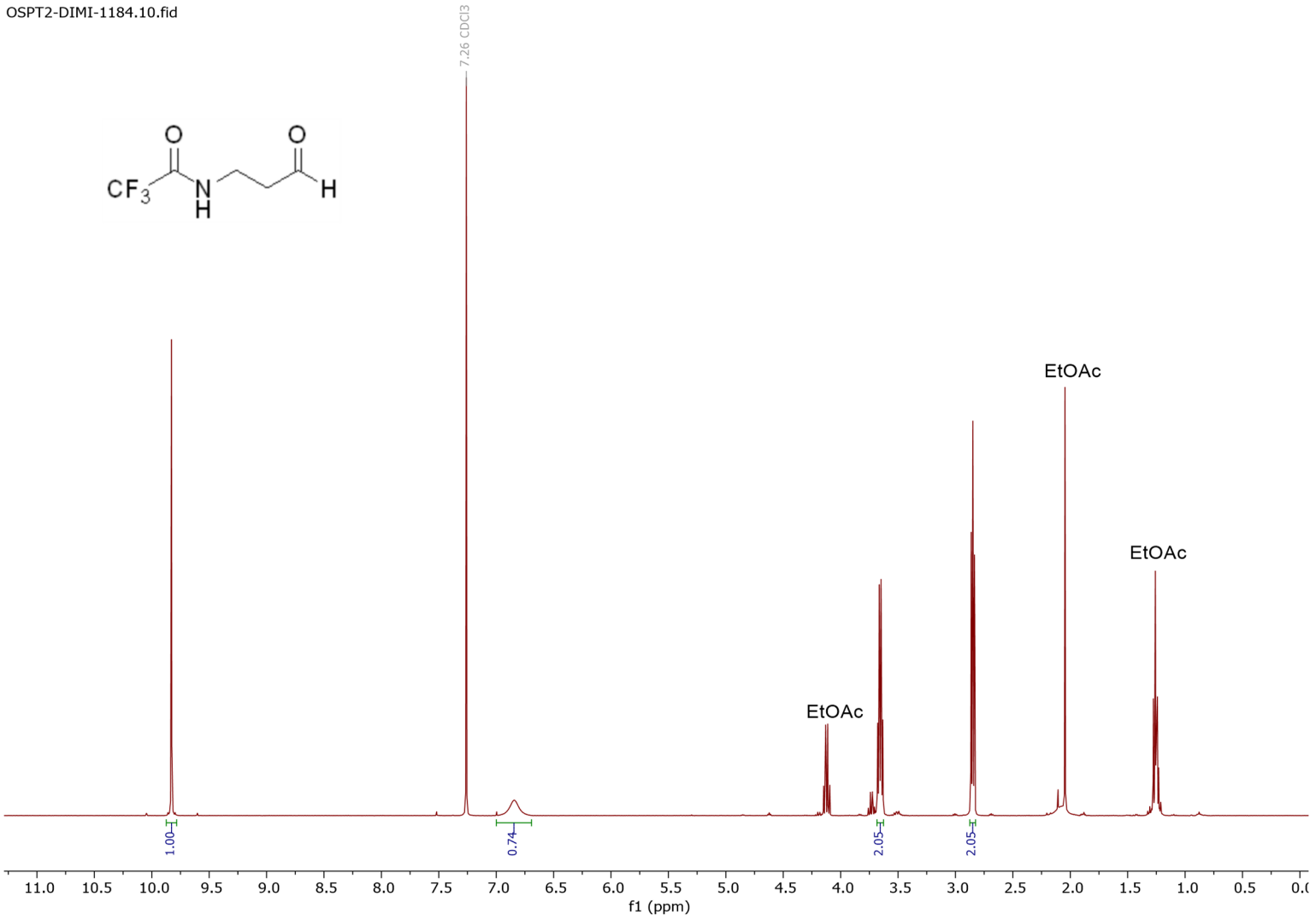
2,2,2-Trifluoro-*N*-(2-oxoethyl)acetamide (32)

[^{1}H-NMR, 400 MHz, $CDCl_3$]



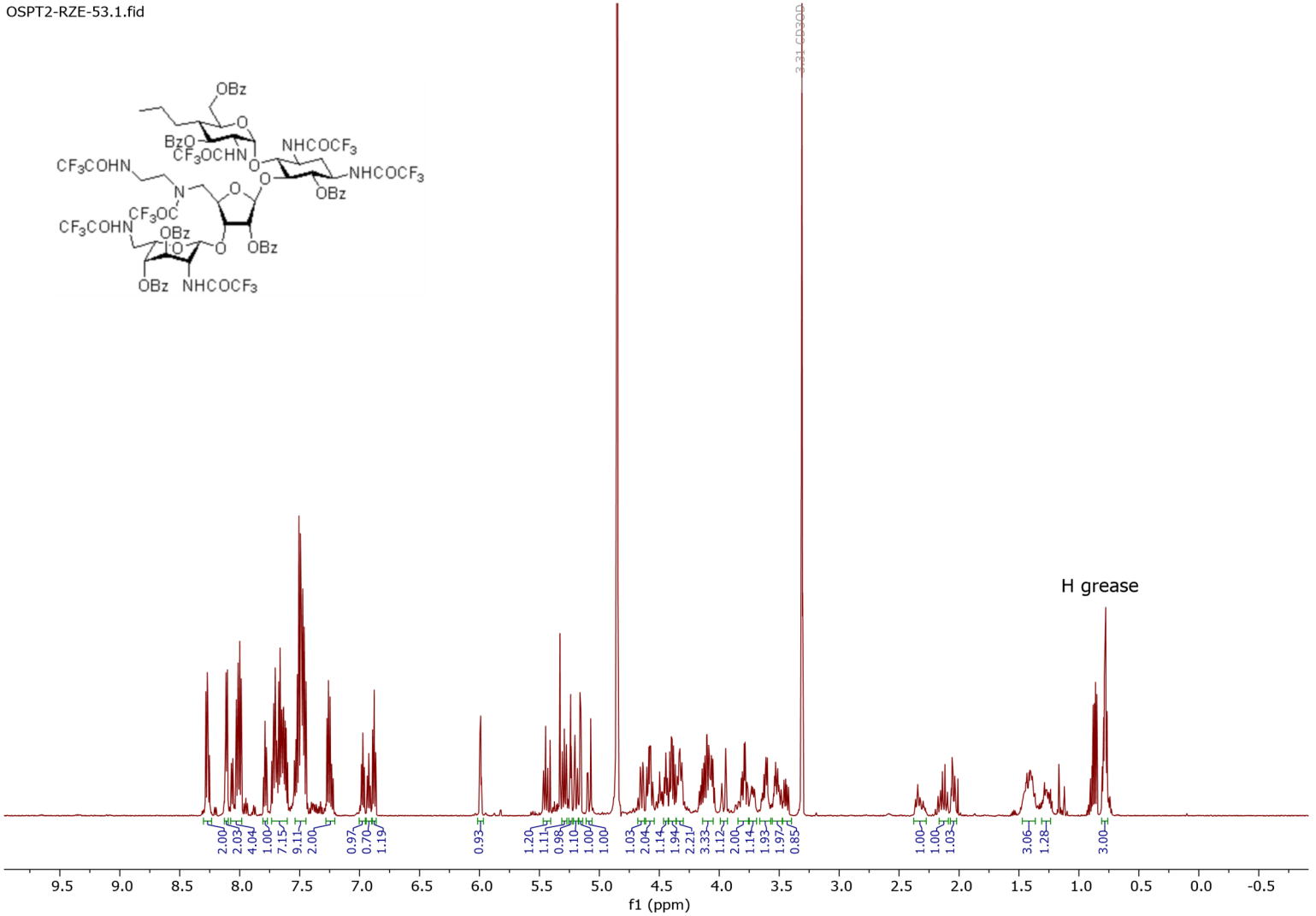
2,2,2-Trifluoro-N-(3-oxopropyl)acetamide (33)

[^{1}H-NMR, 400 MHz, $CDCl_3$]



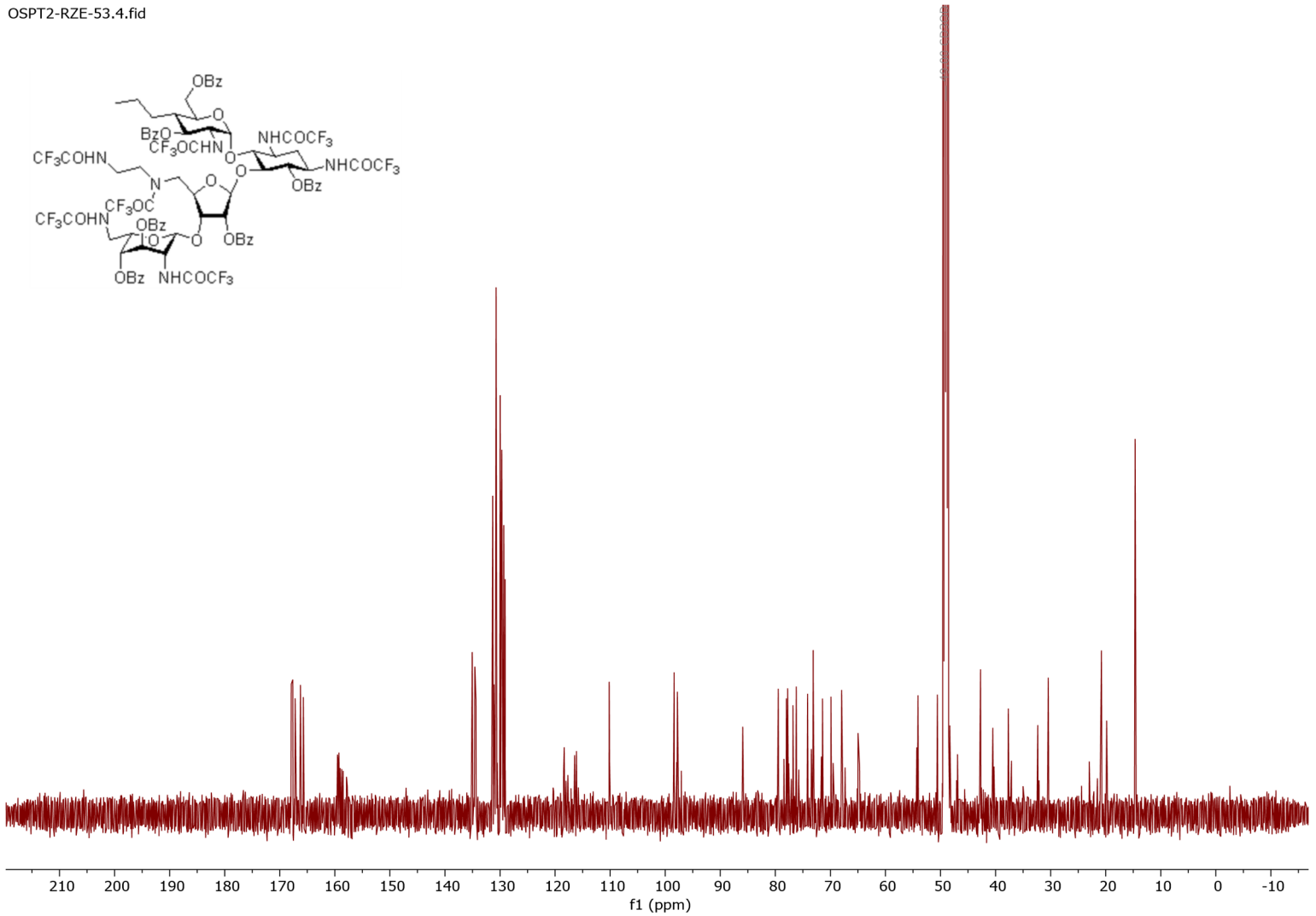
4′,5″-Dideoxy-4′-propyl-5″-(2-aminoethylamino)-1,2-ethanediamine)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (36)

[^{1}H-NMR, 600 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-(2-aminoethylamino)-1,2-ethanediamine)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (36)

[^{13}C-NMR, 150.9 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-(2-aminoethylamino)-1,2-ethanediamine)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (36)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2019_08_460 1011 Zogota RZE-53
MS_POS_500-2300_RES_7min ACN_Form_5-98_040_7min 1:F,1 10.000000 MS_Tune Col#43

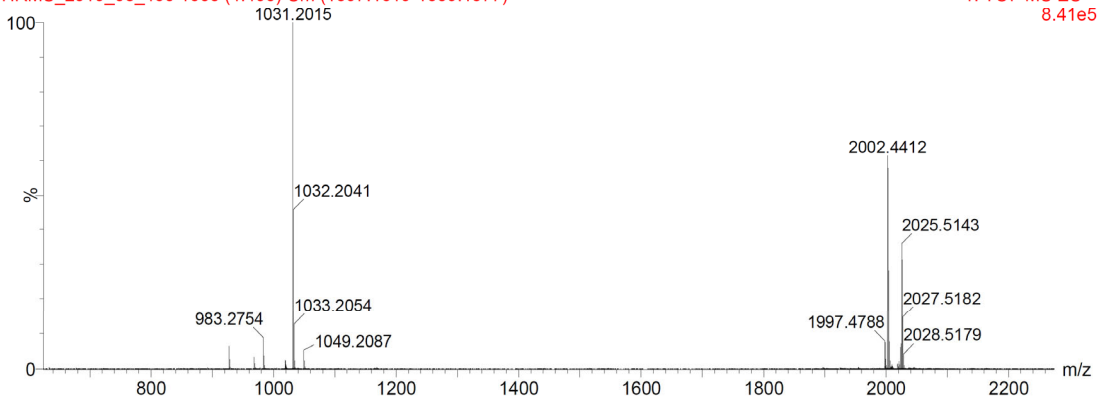
Elemental Composition Report:

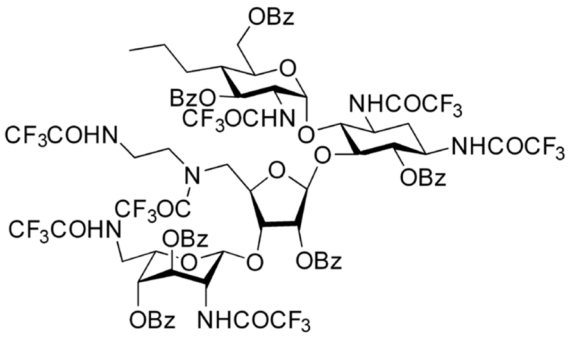
Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
264 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-84 H: 1-110 N: 1-10 O: 0-25 F: 21-21 Na: 1-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
2002.4412	100.00	2002.4409	0.3	0.1	40.5	486.5	0.273	76.11	C83 H74 N9 O24 F21 Na
		2002.4297	11.5	5.7	40.5	487.6	1.432	23.89	C84 H74 N7 O25 F21 Na

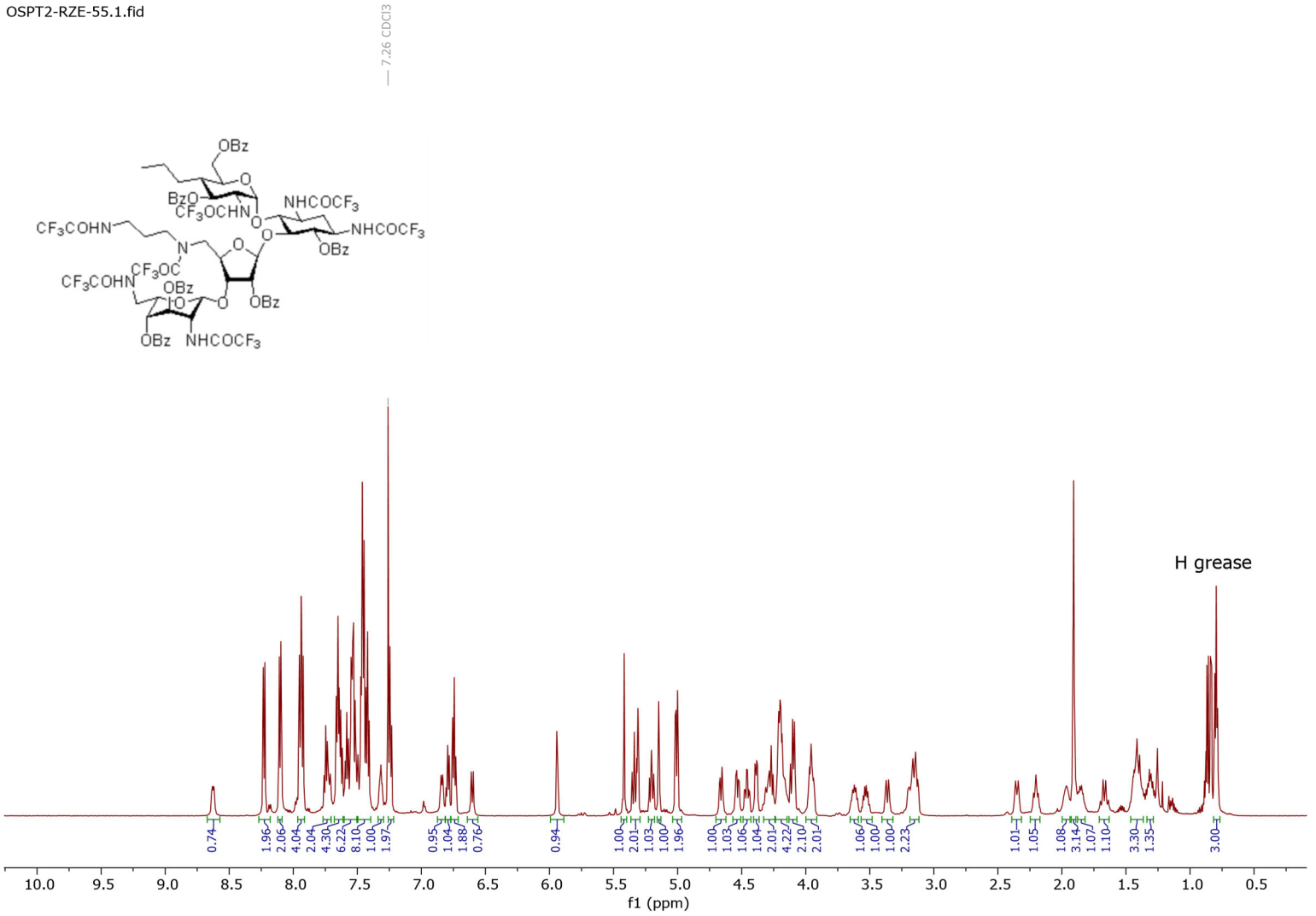
1011 Zogota RZE-53
HRMS_2019_08_460 1605 (4.490) Cm (1597:1619-1559:1577) 1: TOF MS ES+ 8.41e5





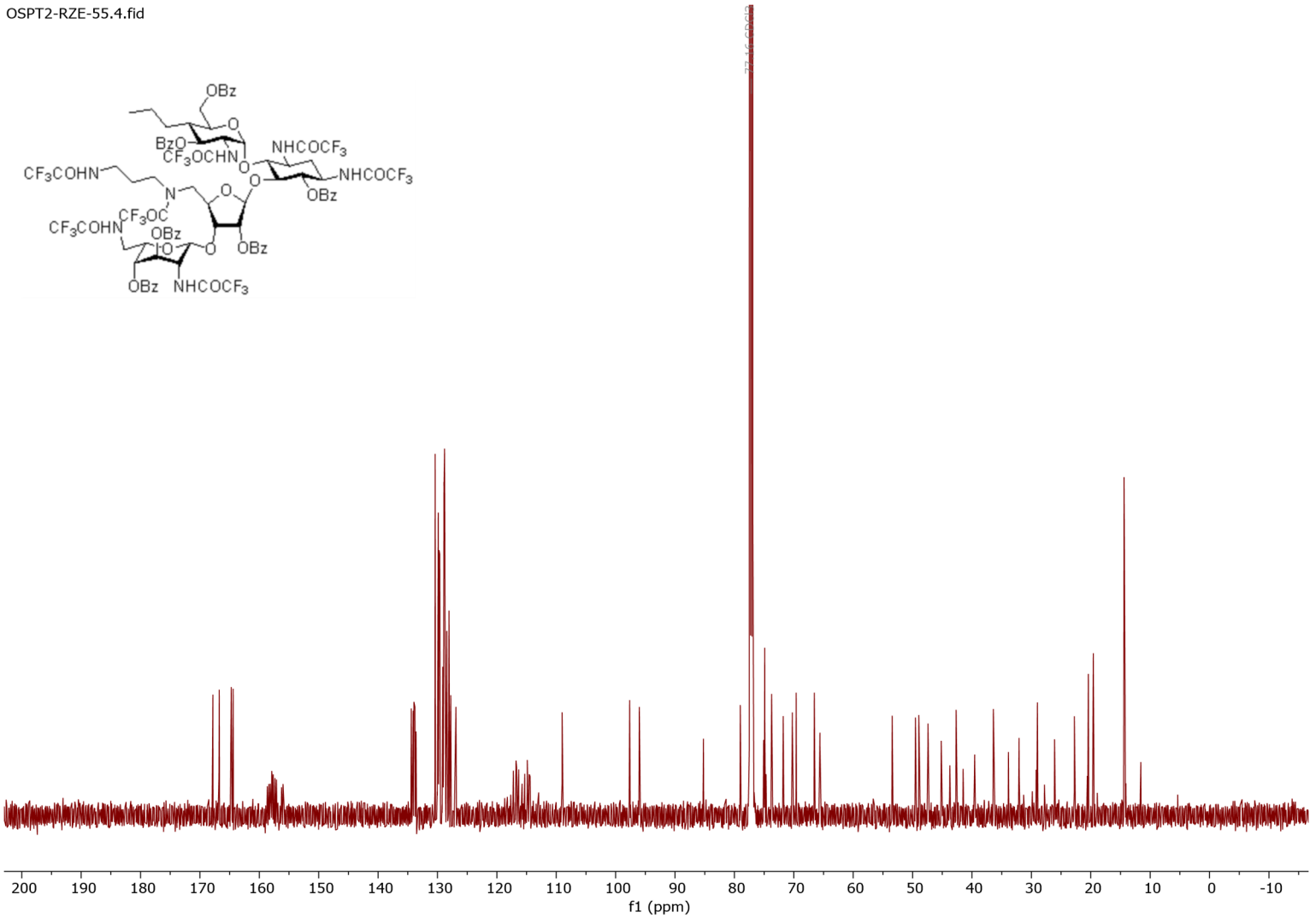
4′,5″-Dideoxy-4′-propyl-5″-(3-aminoethylamino)-1,3-diaminopropane)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (37)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(3-aminoethylamino)-1,3-diaminopropane)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (37)

[^{13}C-NMR, 150.9 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(3-aminoethylamino)-1,3-diaminopropane)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-hepta-*N*-trifluoroacetyl paromomycin (37)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_02_160 1864 Zogota RZE-55
MS_POS_500-2300_RES_7min ACN_Form_5-98_040_7min 2:C,7 5.000000 MS_Tune Col#43

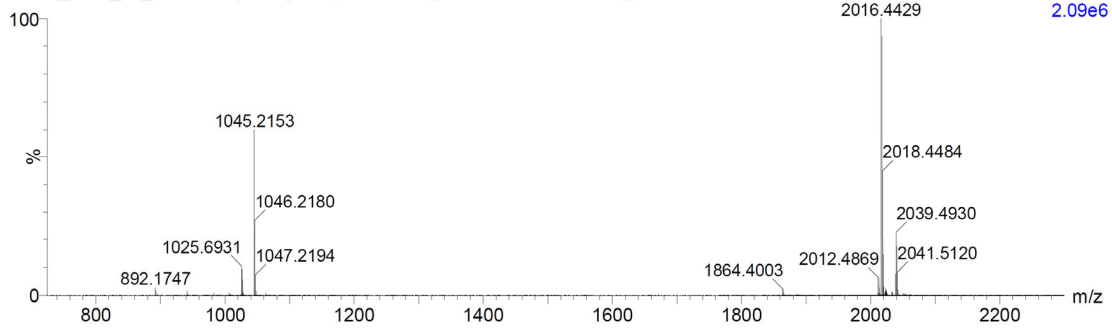
Elemental Composition Report:

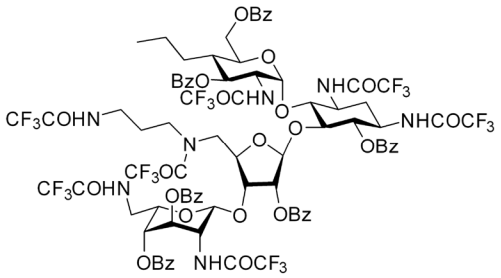
Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
17545 formula(e) evaluated with 8 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-90 H: 0-80 N: 0-7 O: 0-25 F: 0-21 Na: 0-1

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
2016.4429	100.00	2016.4453	-2.4	-1.2	40.5	586.5	0.376	68.65	C85 H76 N7 O25 F21 Na
		2016.4442	-1.3	-0.6	44.5	587.7	1.559	21.03	C88 H75 N7 O24 F20 Na
		2016.4477	-4.8	-2.4	43.5	588.4	2.271	10.32	C87 H75 N7 O25 F21
2017.4457	93.39	2017.4446	1.1	0.5	44.5	607.3	0.103	90.22	C89 H75 N6 O23 F21 Na
		2017.4533	-7.6	-3.8	43.5	609.5	2.325	9.78	C90 H78 N4 O25 F20 Na

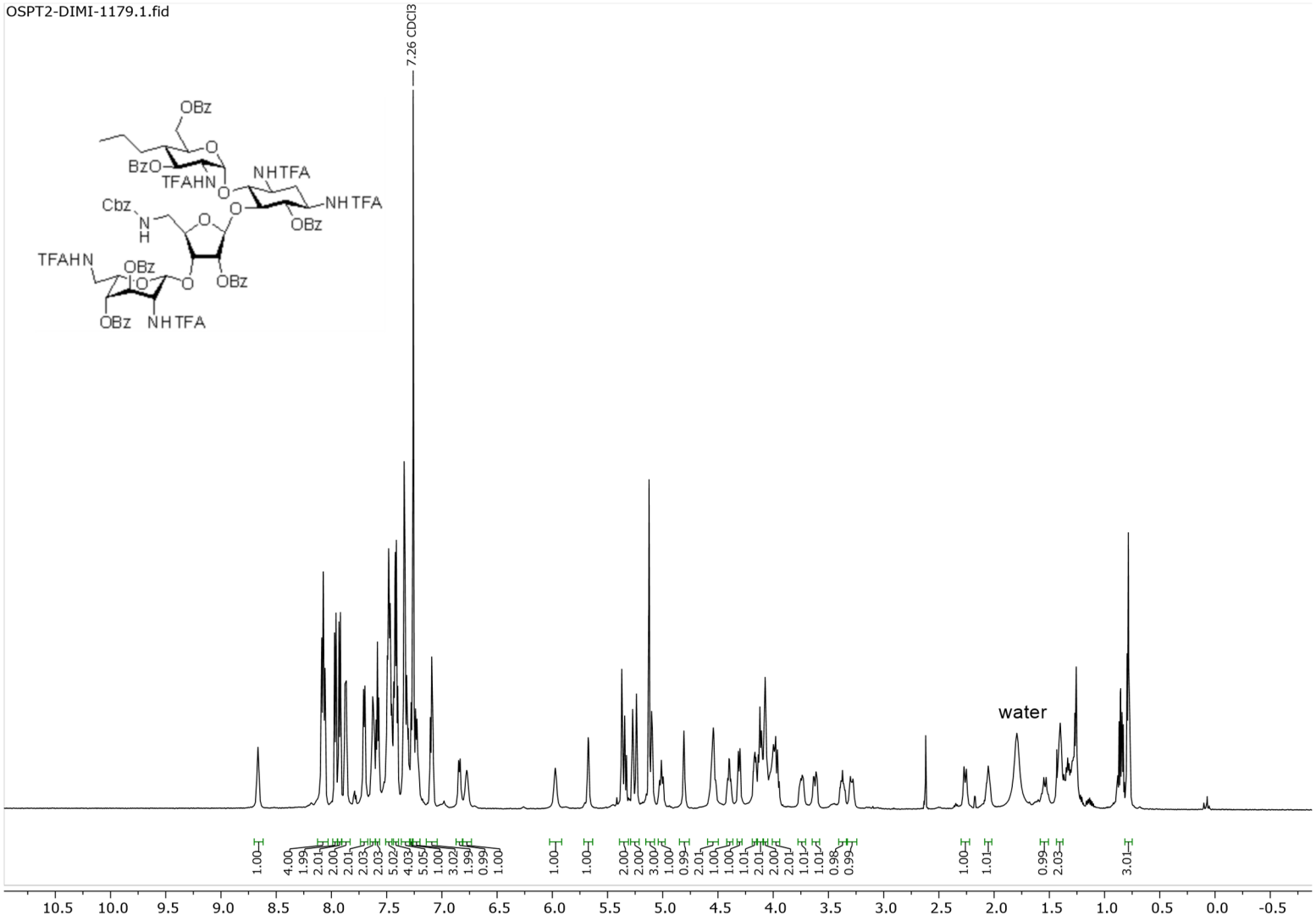
1864 Zogota RZE-55
HRMS_2020_02_160 1718 (4.804) Cm (1715:1744-(1592:1620+1864:1884)) 1: TOF MS ES+ 2.09e6





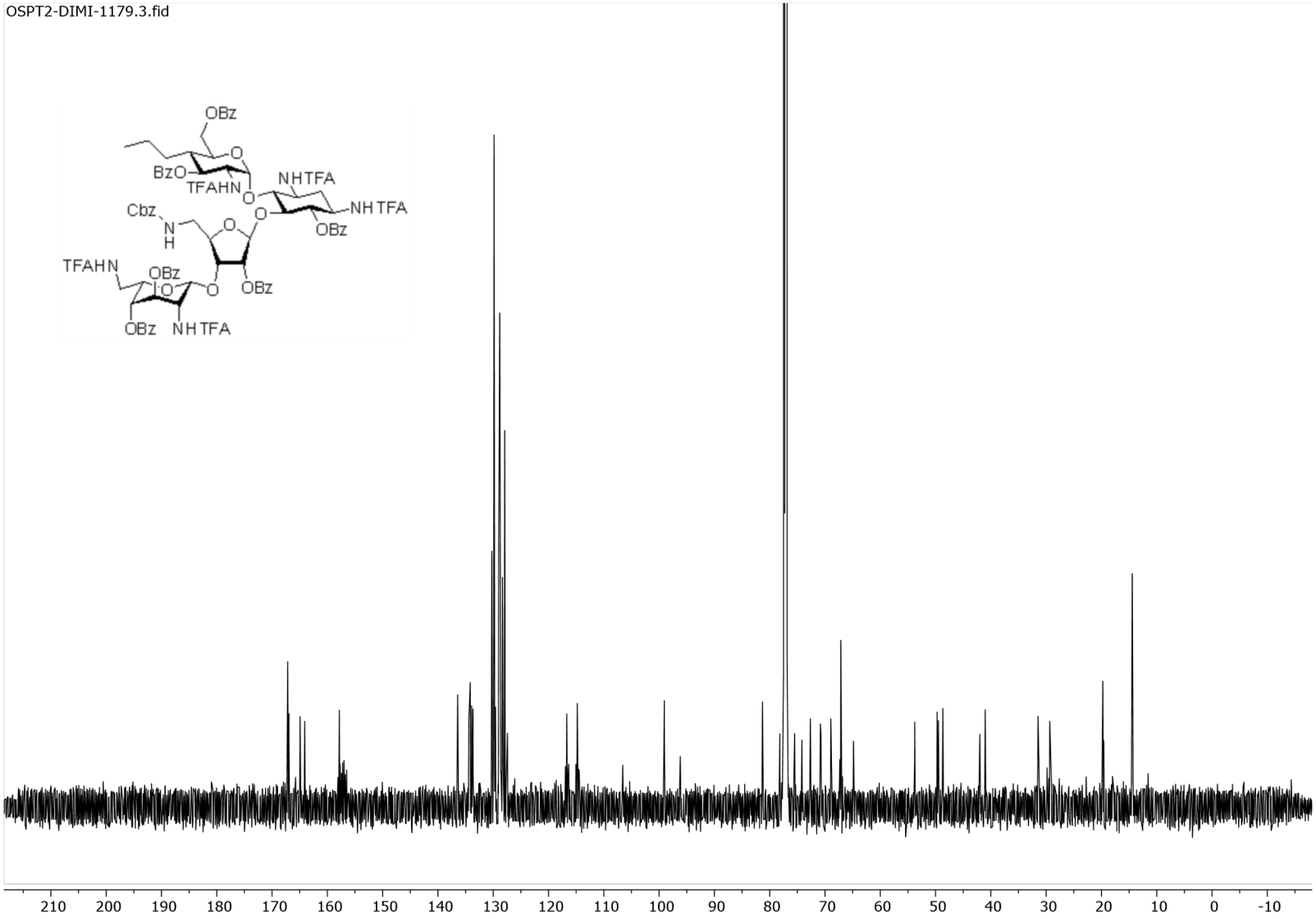
4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



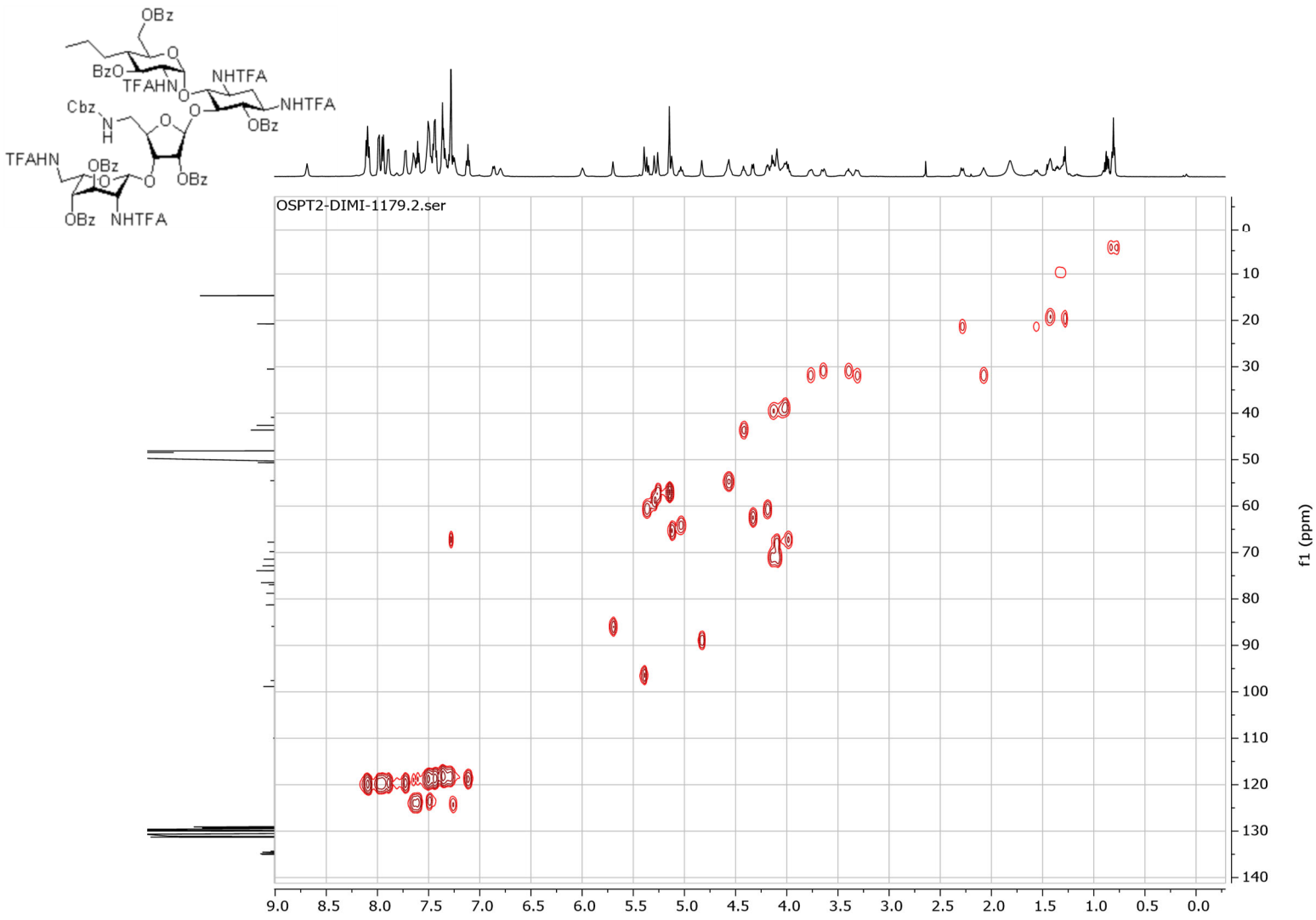
4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

[^{13}C-NMR, 150.9 MHz, $CDCl_3$]



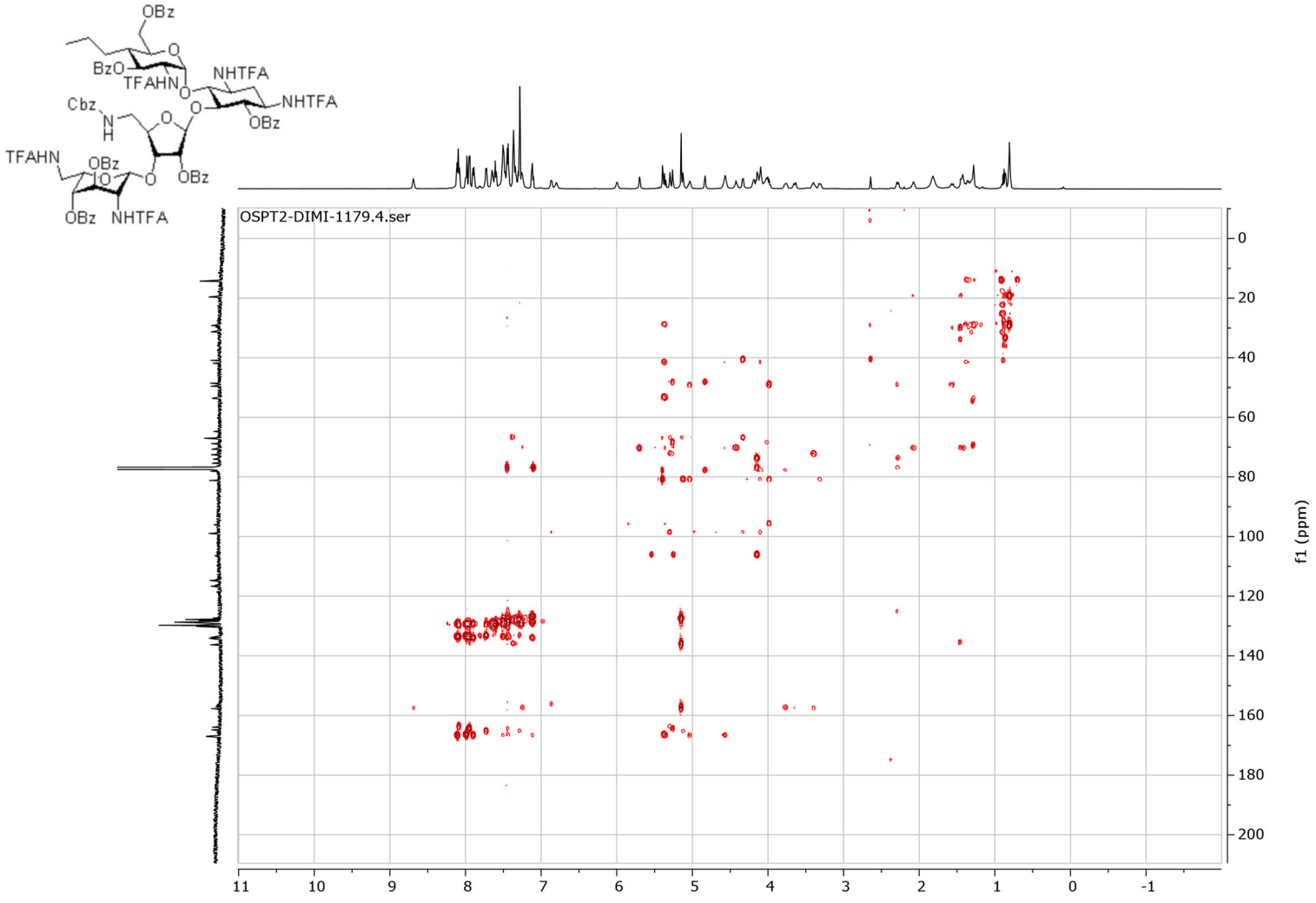
4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, $CDCl_3$]



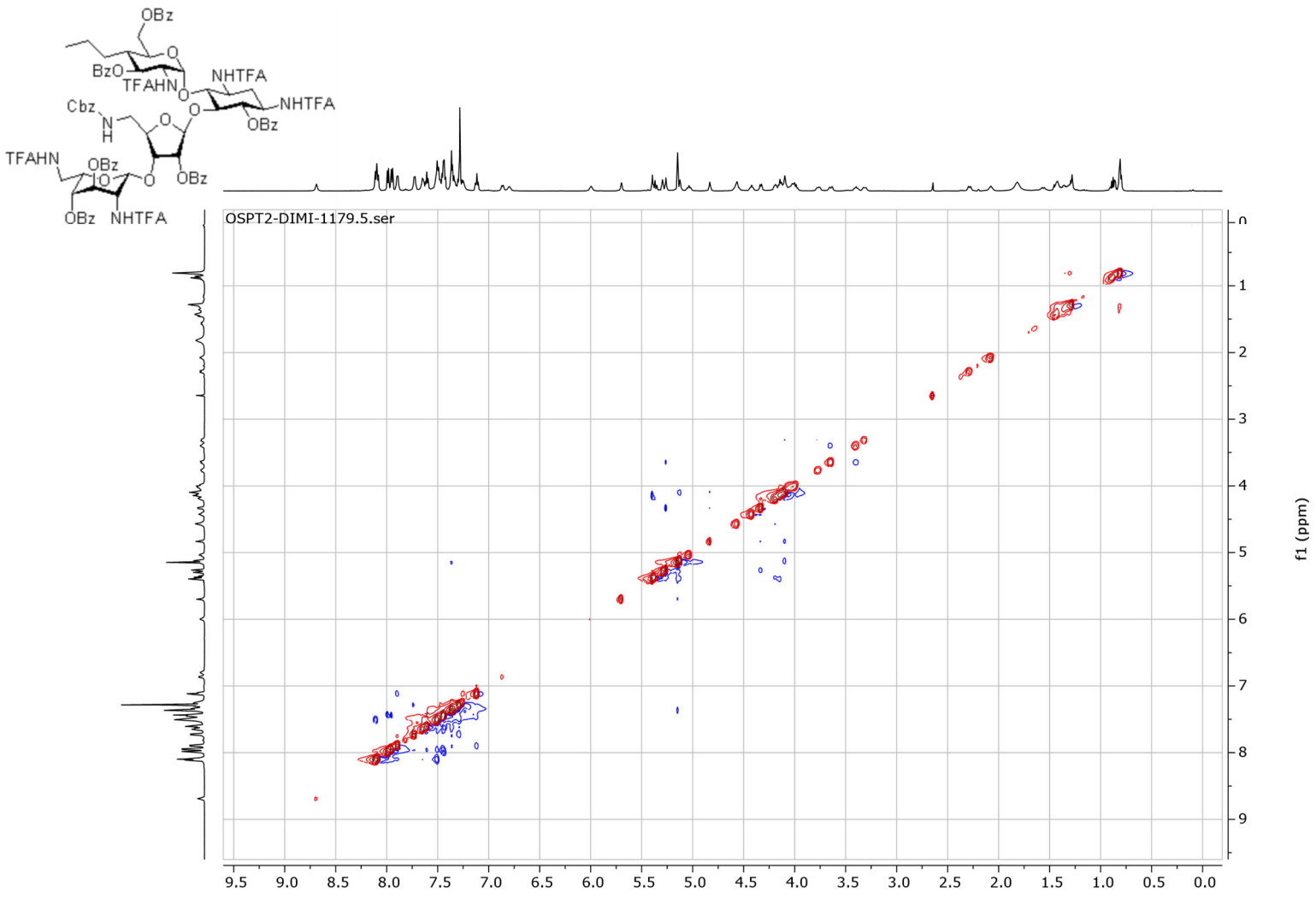
4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

[^{1}H, ^{1}H ROESY, 600 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(*O*-benzylcarbamoyl)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (38)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

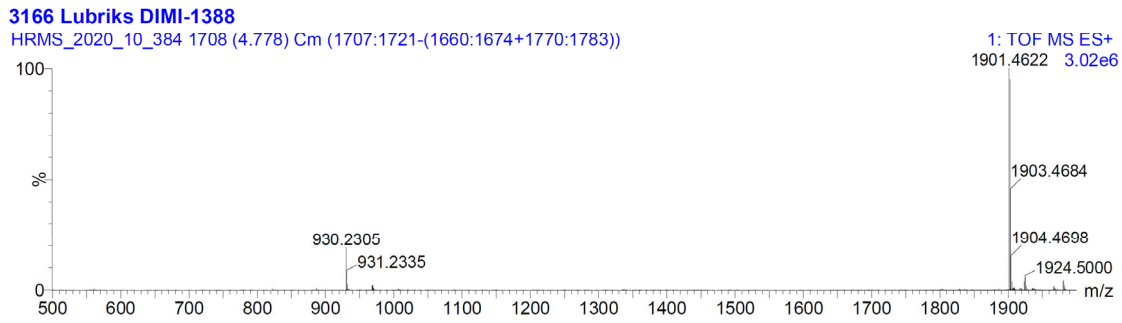
Sample:
HRMS_2020_10_384 3166 Lubriks DIMI-1388
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:E,6 1.000000 MS_Tune Col#66

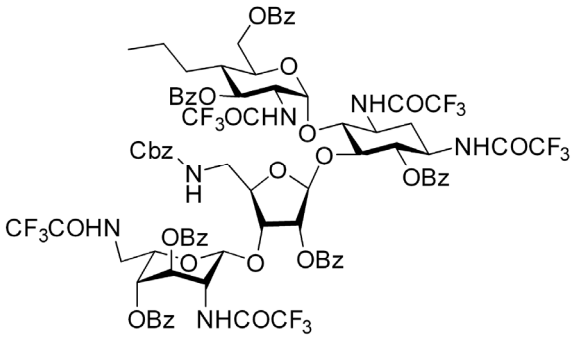
Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions
2256 formula(e) evaluated with 15 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-30 F: 15-15 Na: 1-1

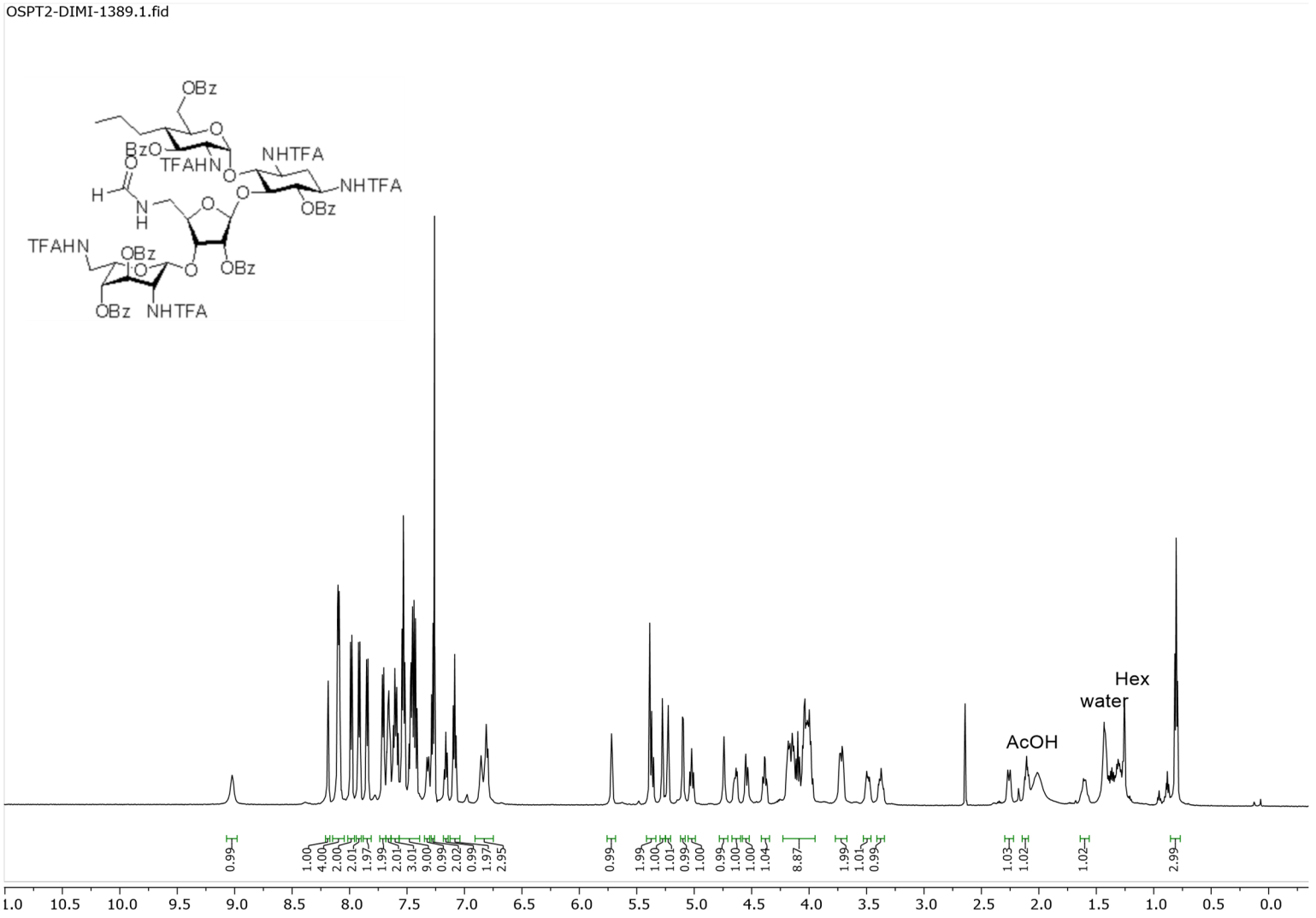
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1901.4622	100.00	1901.4623	-0.1	-0.1	42.5	1624.2	6.156	0.21	C90 H81 O27 F15 Na
		1901.4628	-0.6	-0.3	35.5	1618.1	0.031	96.92	C75 H77 N12 O28 F15 Na
		1901.4610	1.2	0.6	48.5	1622.4	4.359	1.28	C87 H73 N10 O21 F15 Na
		1901.4637	-1.5	-0.8	47.5	1624.3	6.260	0.19	C91 H77 N4 O23 F15 Na
		1901.4597	2.5	1.3	43.5	1622.3	4.274	1.39	C86 H77 N6 O25 F15 Na





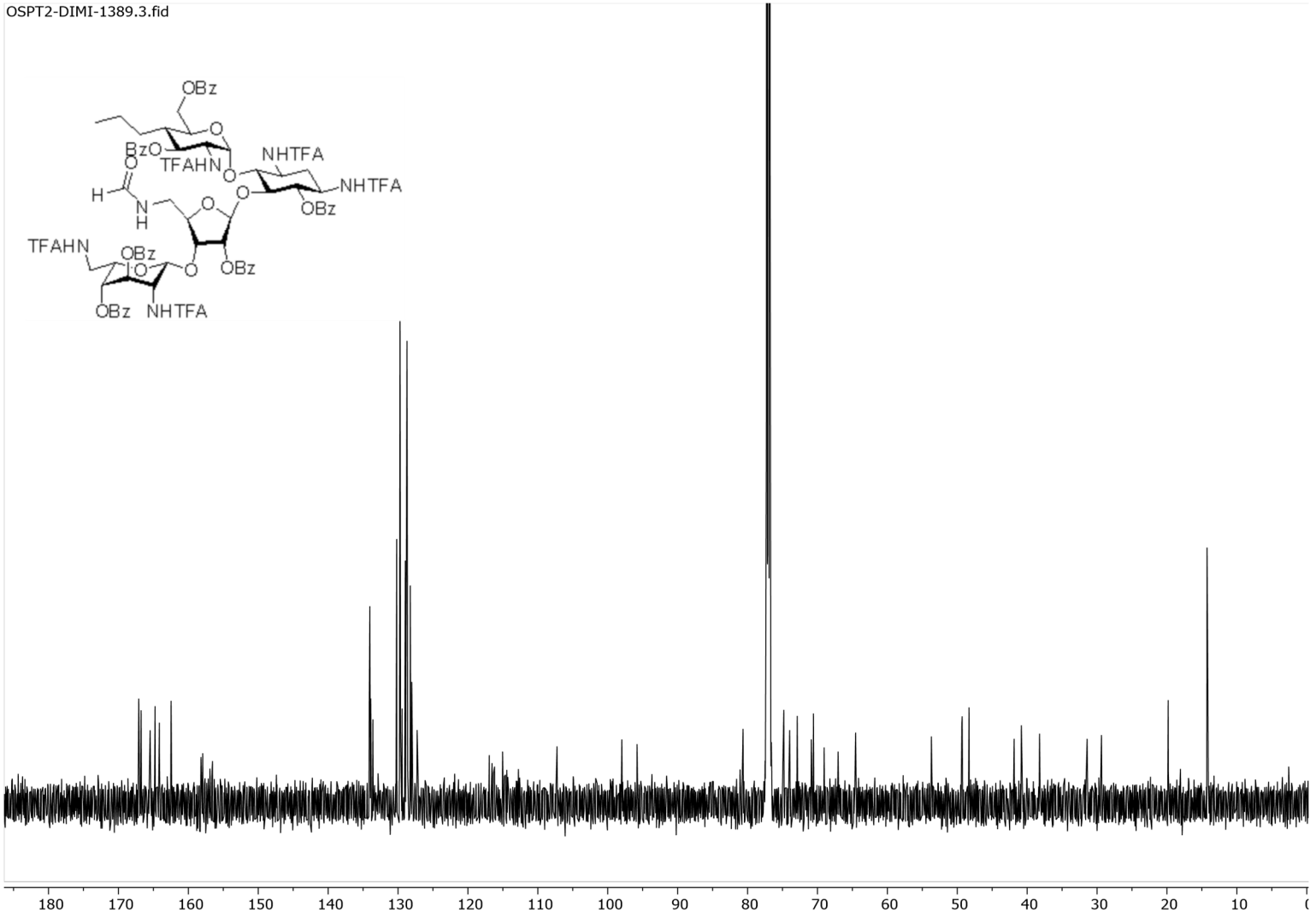
4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



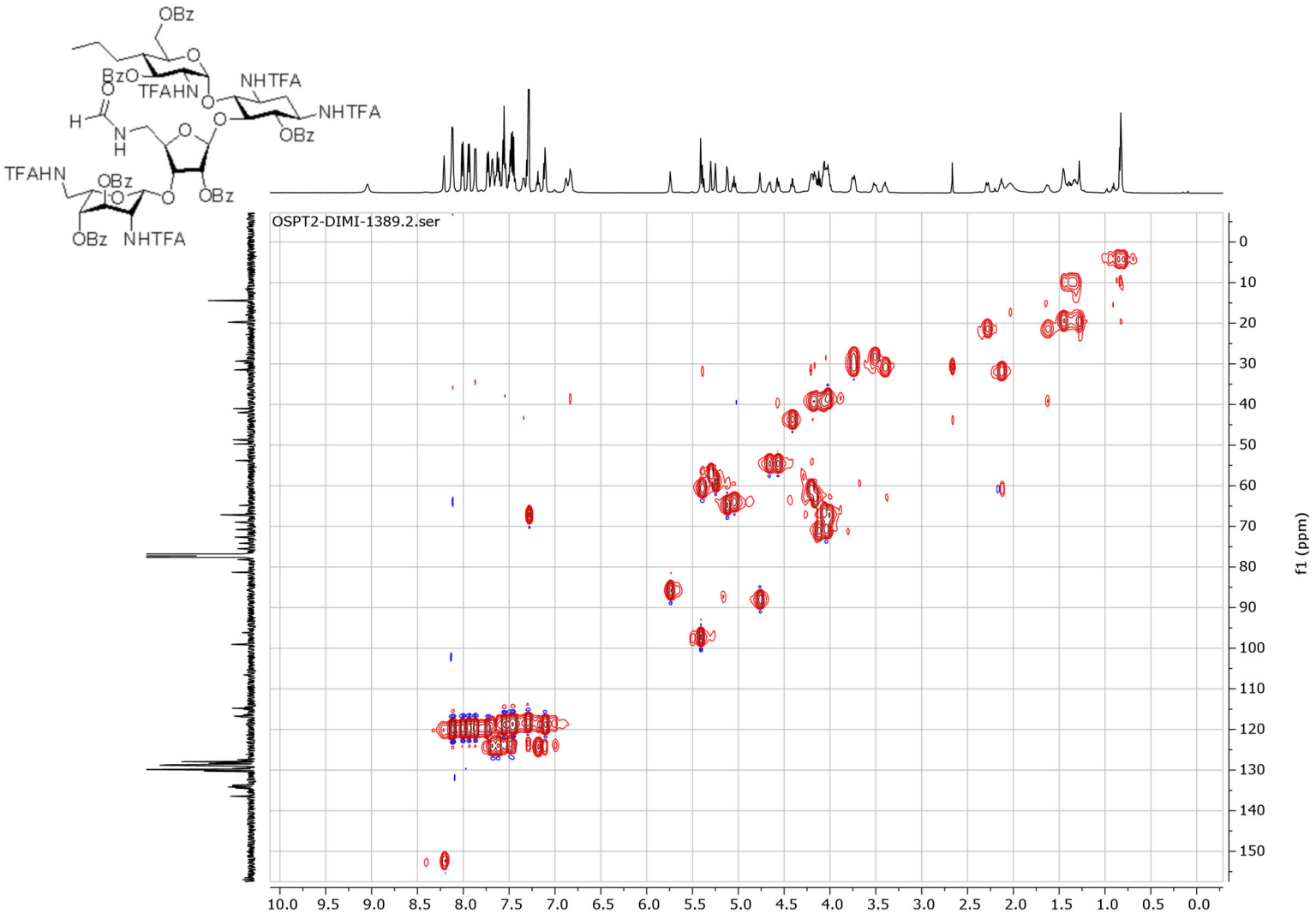
4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

[^{13}C-NMR, 150.9 MHz, $CDCl_3$]



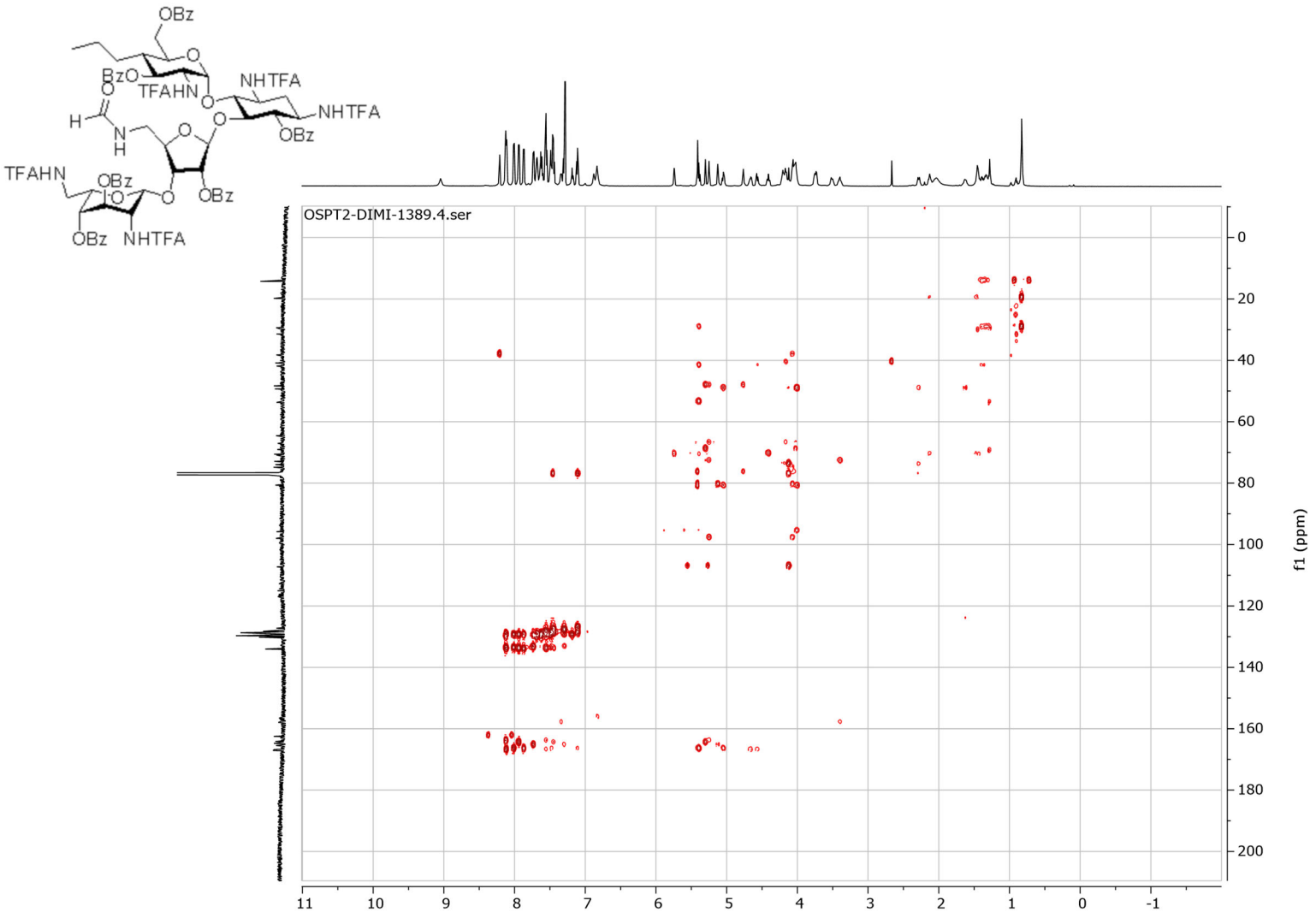
4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, $CDCl_3$]



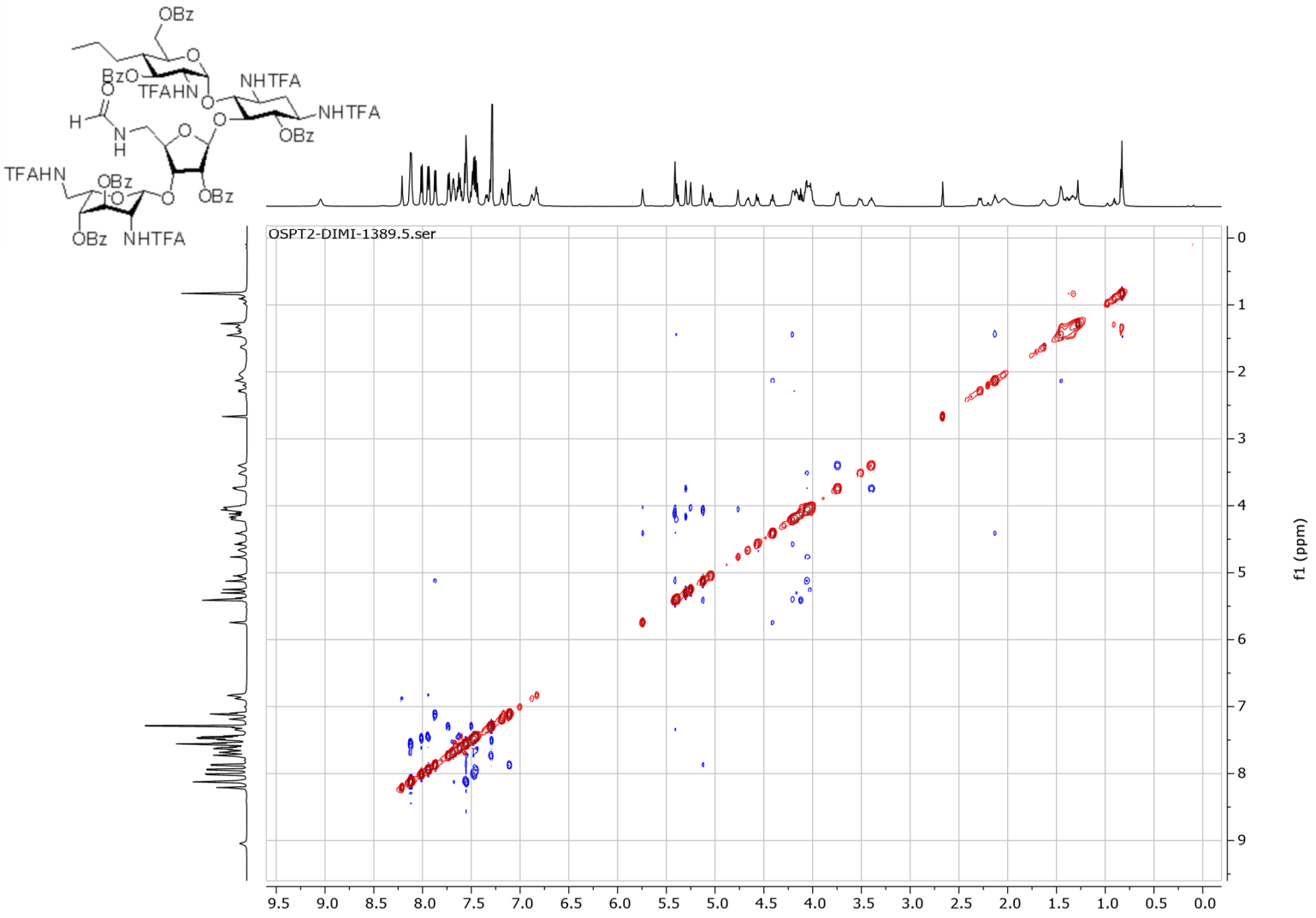
4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

[^{1}H, ^{1}H ROESY, 600 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-formamido-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (39)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_10_386 3167 Lubriks DIMI-1389
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:E,7 1.000000 MS_Tune Col#66

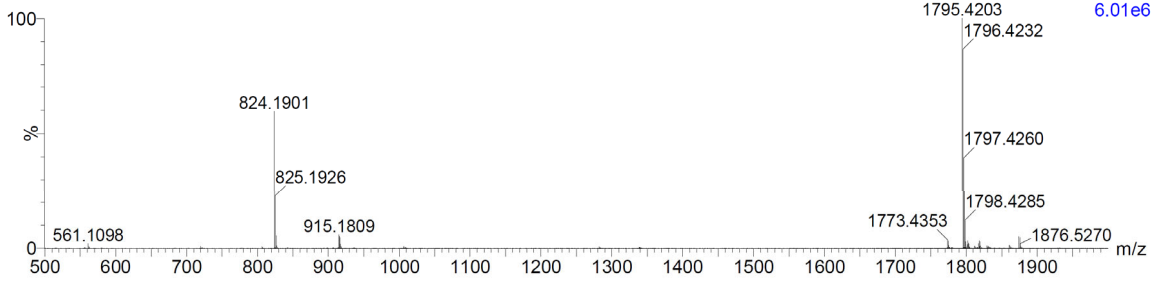
Elemental Composition Report:

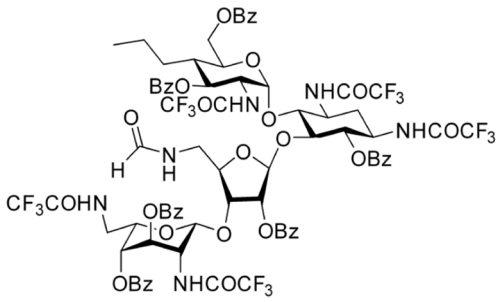
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions
3130 formula(e) evaluated with 21 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-30 F: 15-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1773.4353	100.00	1773.4350	0.3	0.2	27.5	1282.4	0.317	72.80	C63 H72 N14 O29 F15
		1773.4358	-0.5	-0.3	39.5	1285.1	2.957	5.20	C79 H72 N6 O24 F15
		1773.4345	0.8	0.5	34.5	1284.9	2.823	5.94	C78 H76 N2 O28 F15
		1773.4372	-1.9	-1.1	44.5	1285.2	3.107	4.48	C80 H68 N10 O20 F15
		1773.4332	2.1	1.2	40.5	1284.3	2.155	11.59	C75 H68 N12 O22 F15

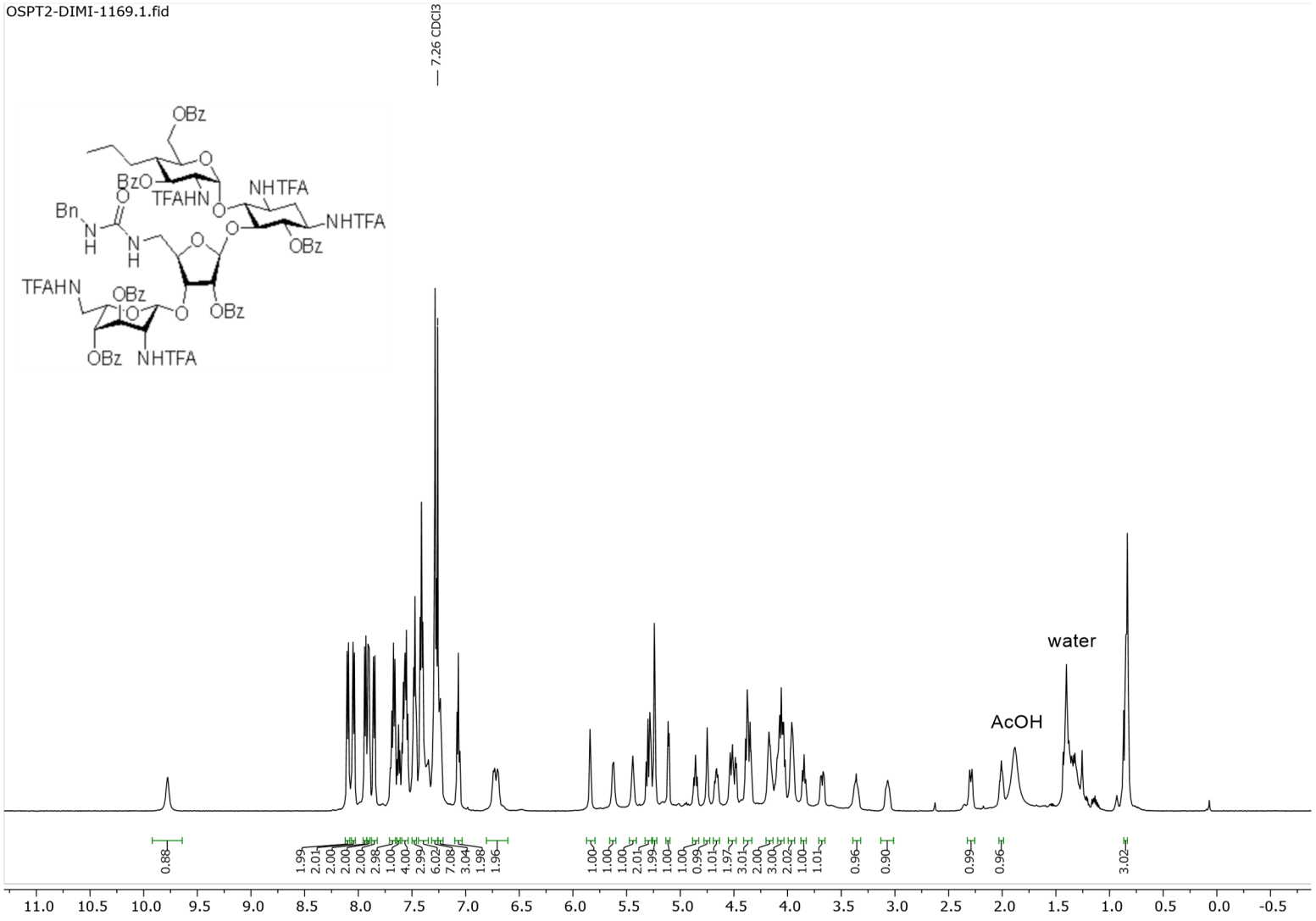
3167 Lubriks DIMI-1389
HRMS_2020_10_386 1612 (4.509) Cm (1603:1627-(1569:1585+1655:1679))
1: TOF MS ES+
6.01e6





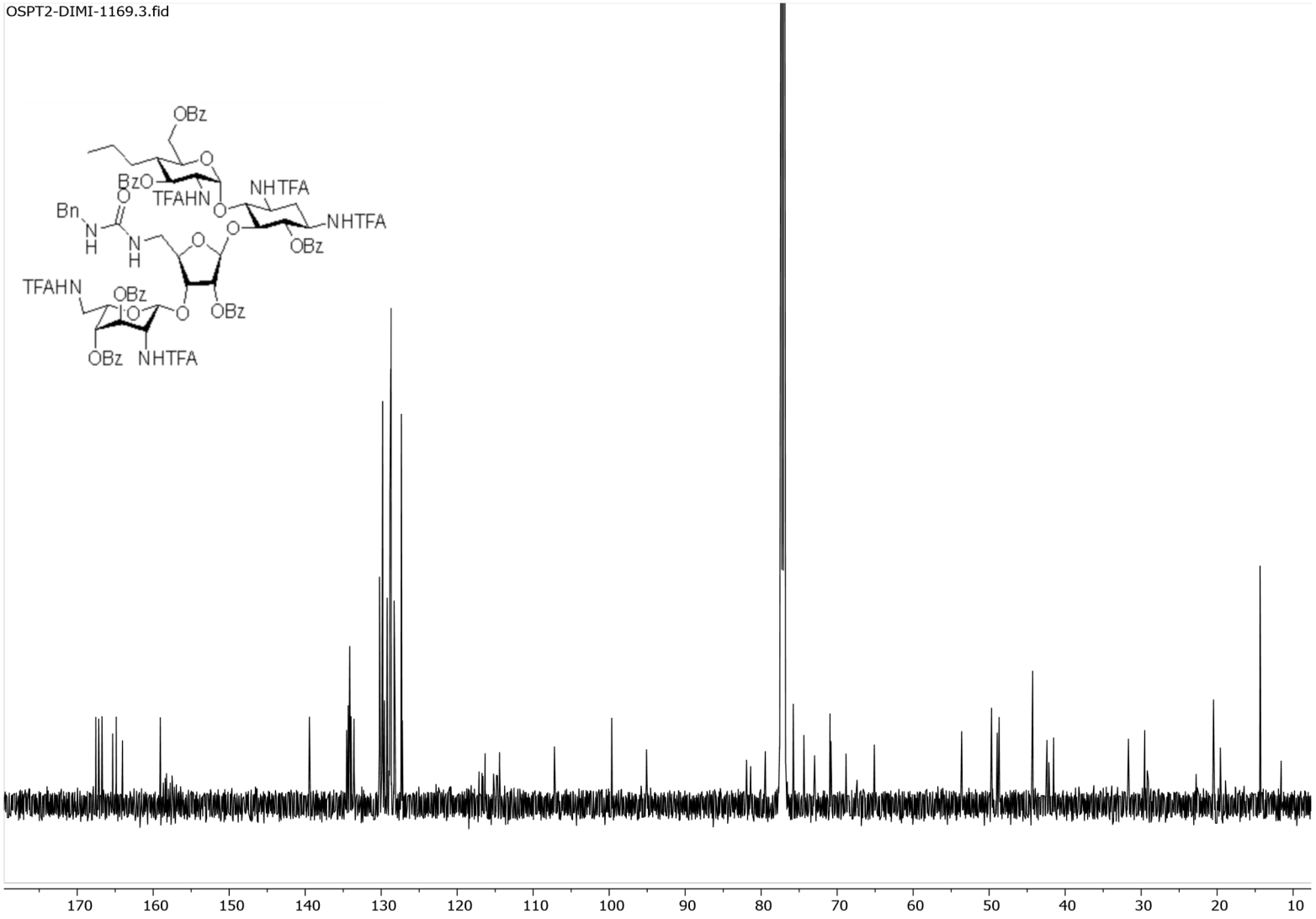
4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



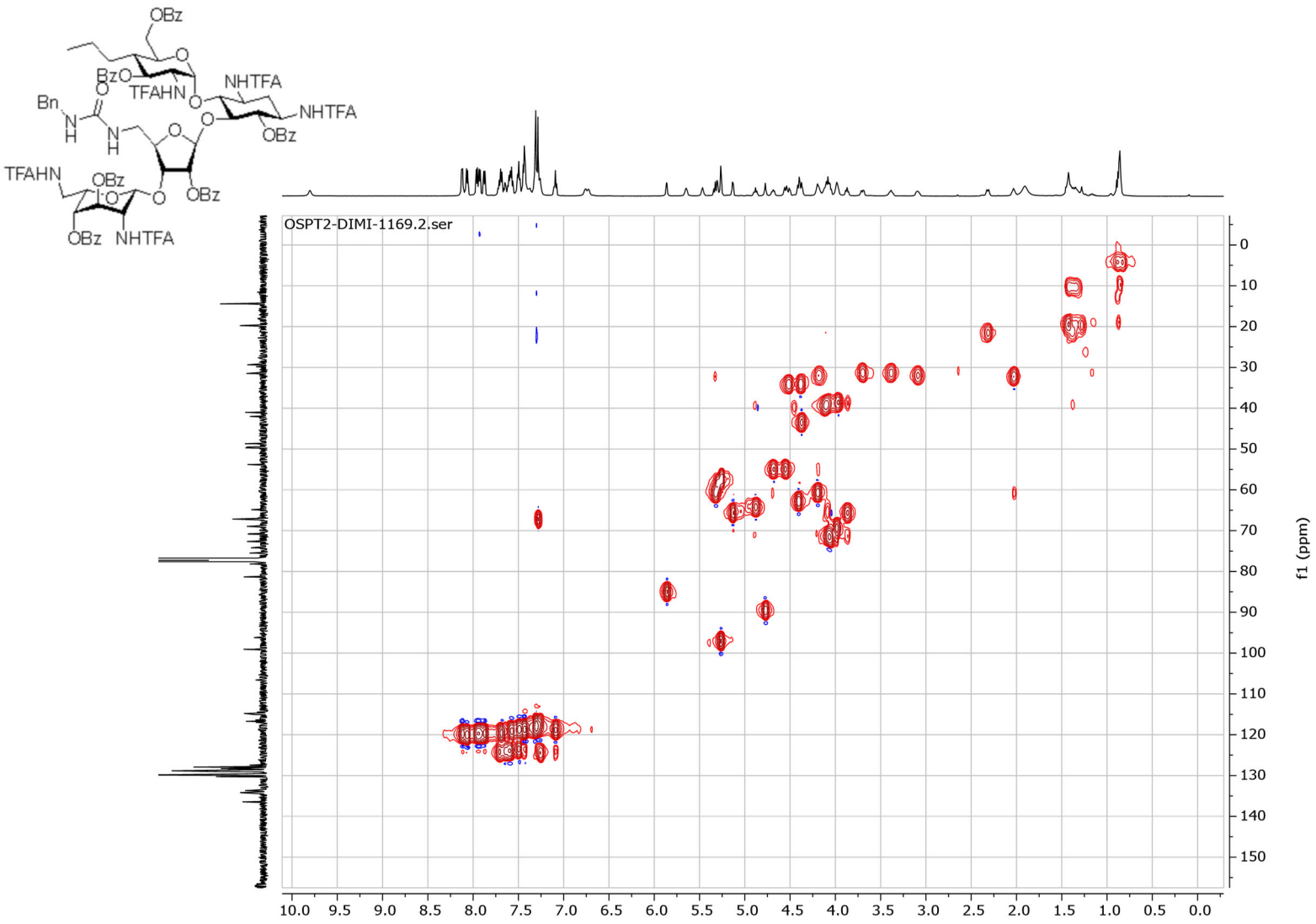
4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

[^{13}C-NMR, 150.9 MHz, $CDCl_3$]



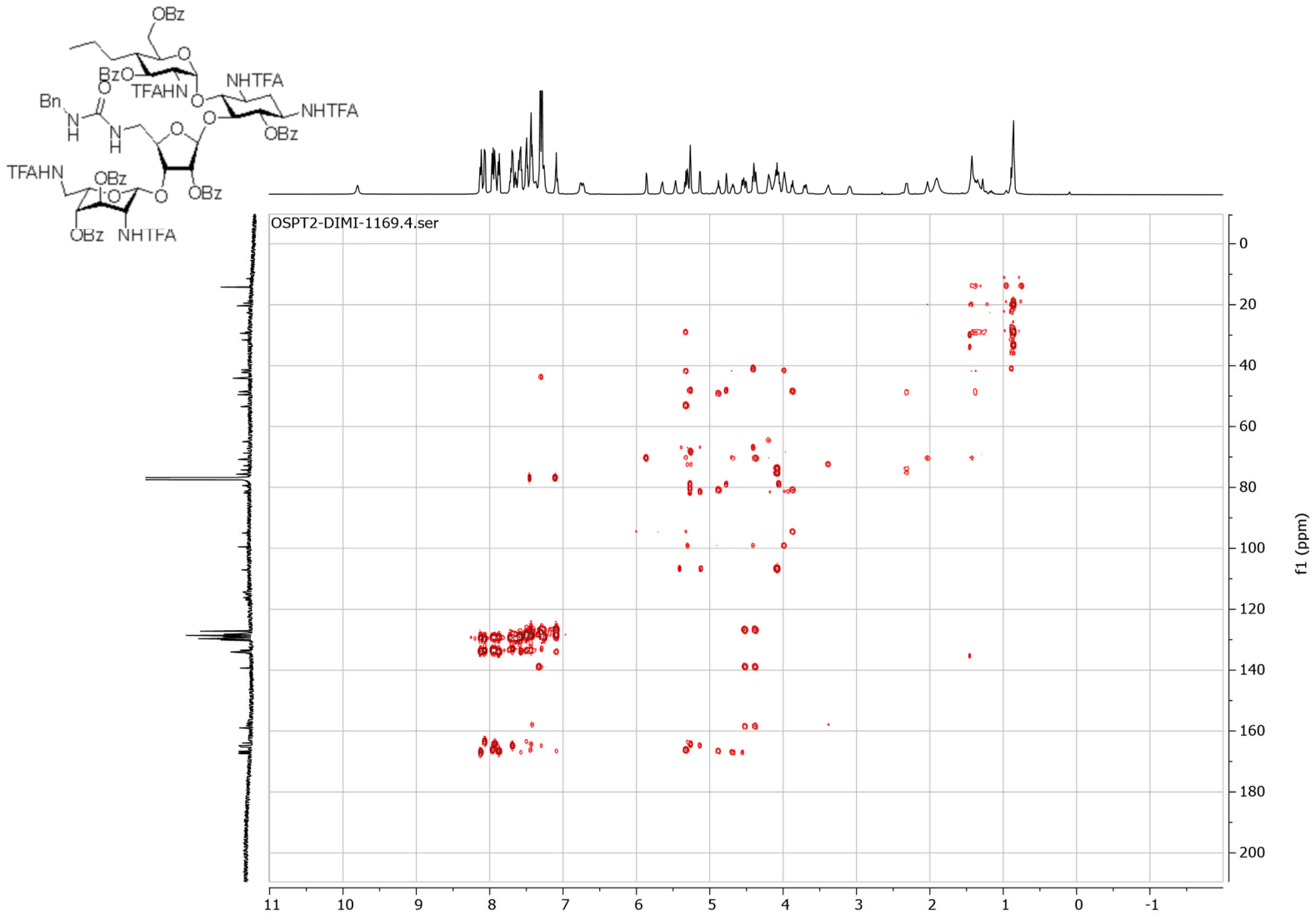
4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, $CDCl_3$]



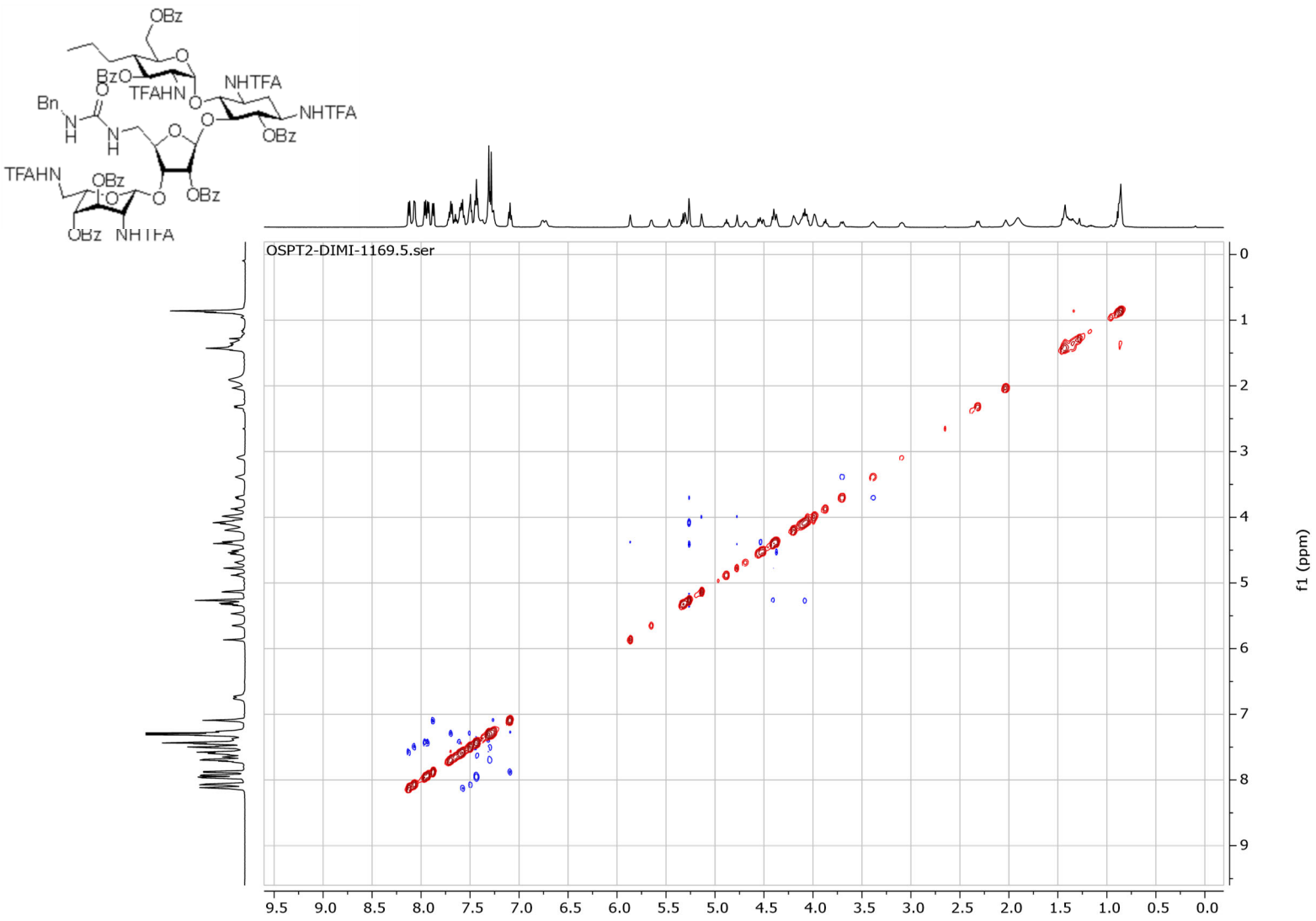
4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

[^{1}H, ^{1}H ROESY, 600 MHz, $CDCl_3$]



4′,5″-Dideoxy-4′-propyl-5″-(*N*-benzylureido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (40)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-S*i*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:
HRMS_2020_10_382 3165 Lubriks DIMI-1387
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:E,5 1.000000 MS_Tune Col#66

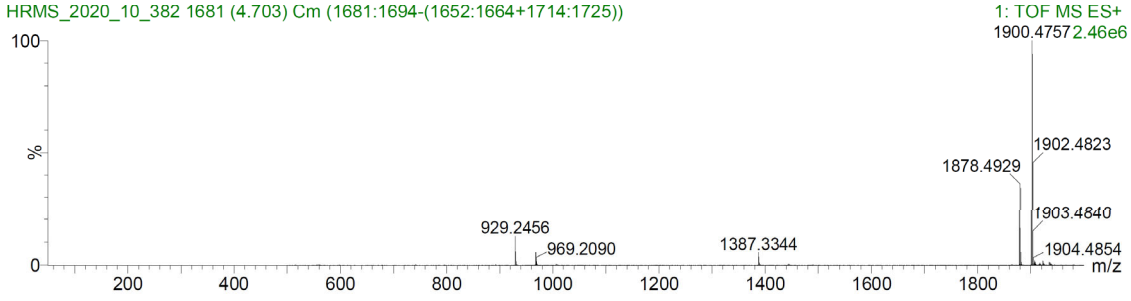
Elemental Composition Report:

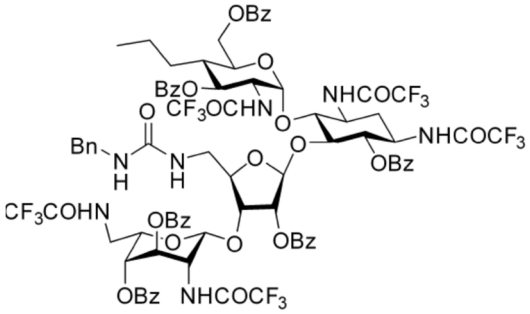
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions
2255 formula(e) evaluated with 17 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-30 F: 15-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1878.4929	100.00	1878.4929	0.0	0.0	31.5	1339.8	0.151	85.98	C70 H79 N15 O29 F15
		1878.4924	0.5	0.3	38.5	1344.1	4.476	1.14	C85 H83 N3 O28 F15
		1878.4937	-0.8	-0.4	43.5	1344.1	4.452	1.17	C86 H79 N7 O24 F15
		1878.4910	1.9	1.0	44.5	1341.8	2.244	10.60	C82 H75 N13 O22 F15
		1878.4950	-2.1	-1.1	48.5	1344.1	4.496	1.12	C87 H75 N11 O20 F15

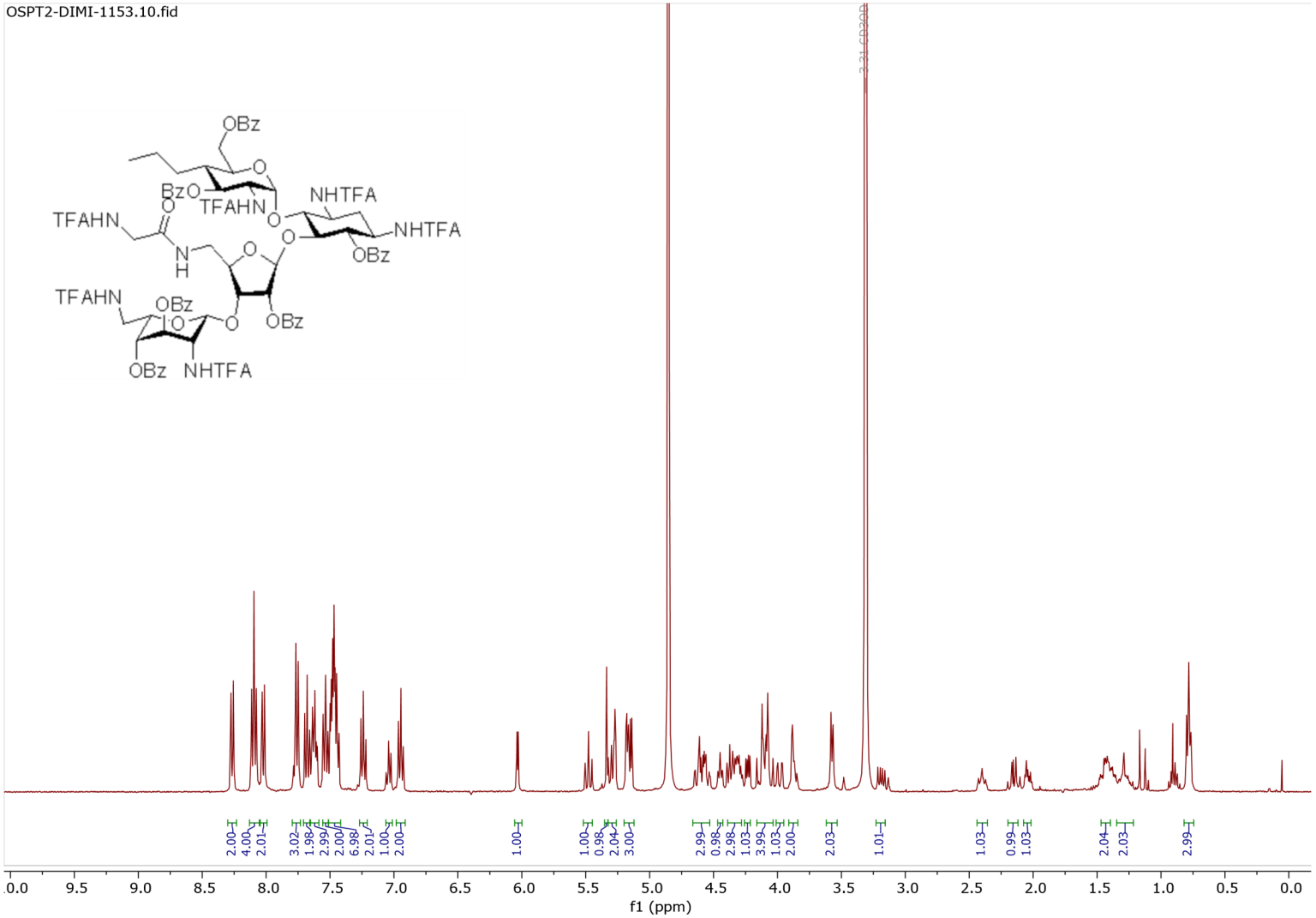
3165 Lubriks DIMI-1387





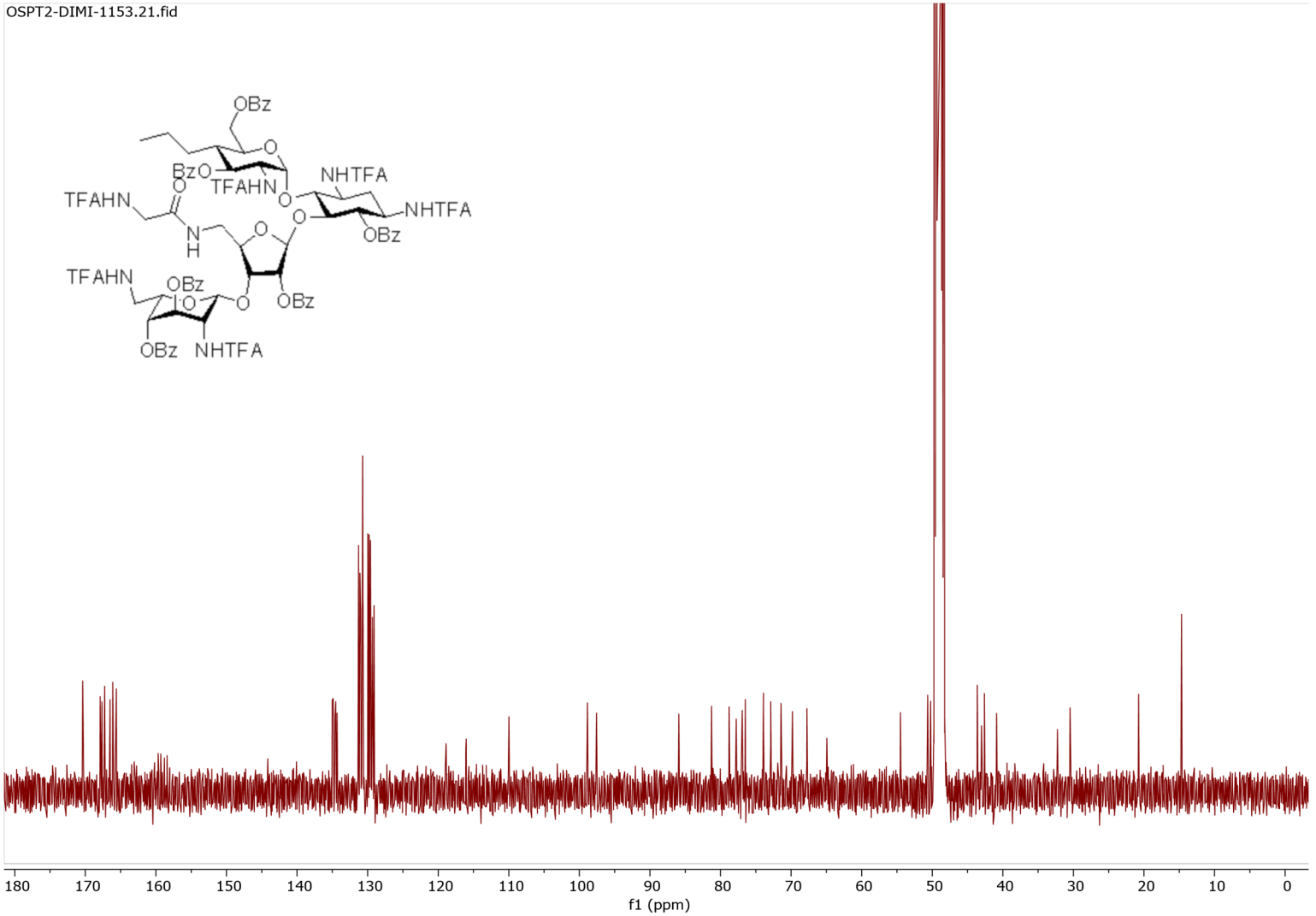
4′,5″-Dideoxy-4′-propyl-5″-(*N*-trifluoroacetylglycinamido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (41)

[^{1}H-NMR, 400 MHz, CD_3OD]



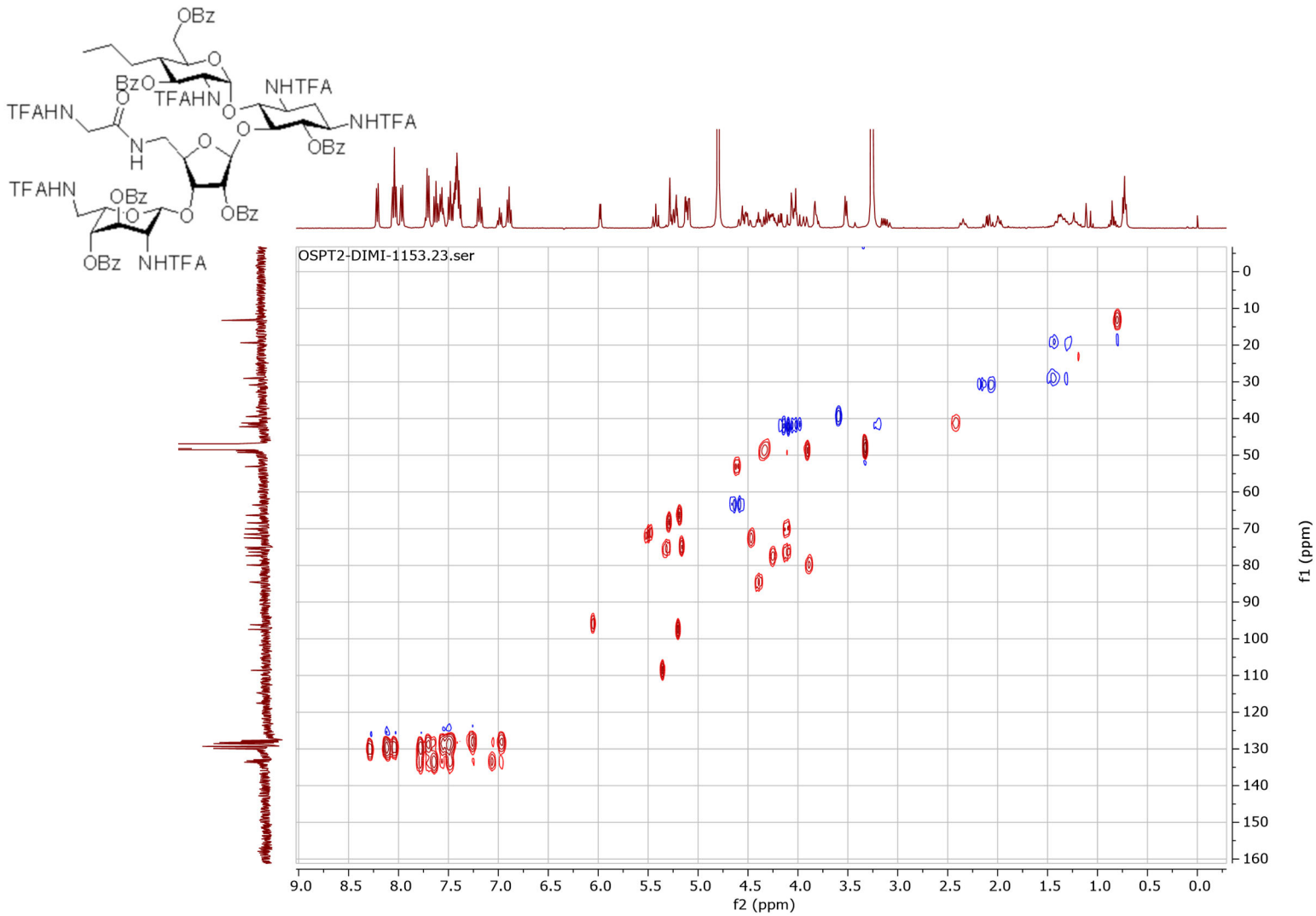
4′,5″-Dideoxy-4′-propyl-5″-(*N*-trifluoroacetylglycinamido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (41)

[^{13}C-NMR, 100.6 MHz, CD_3OD]



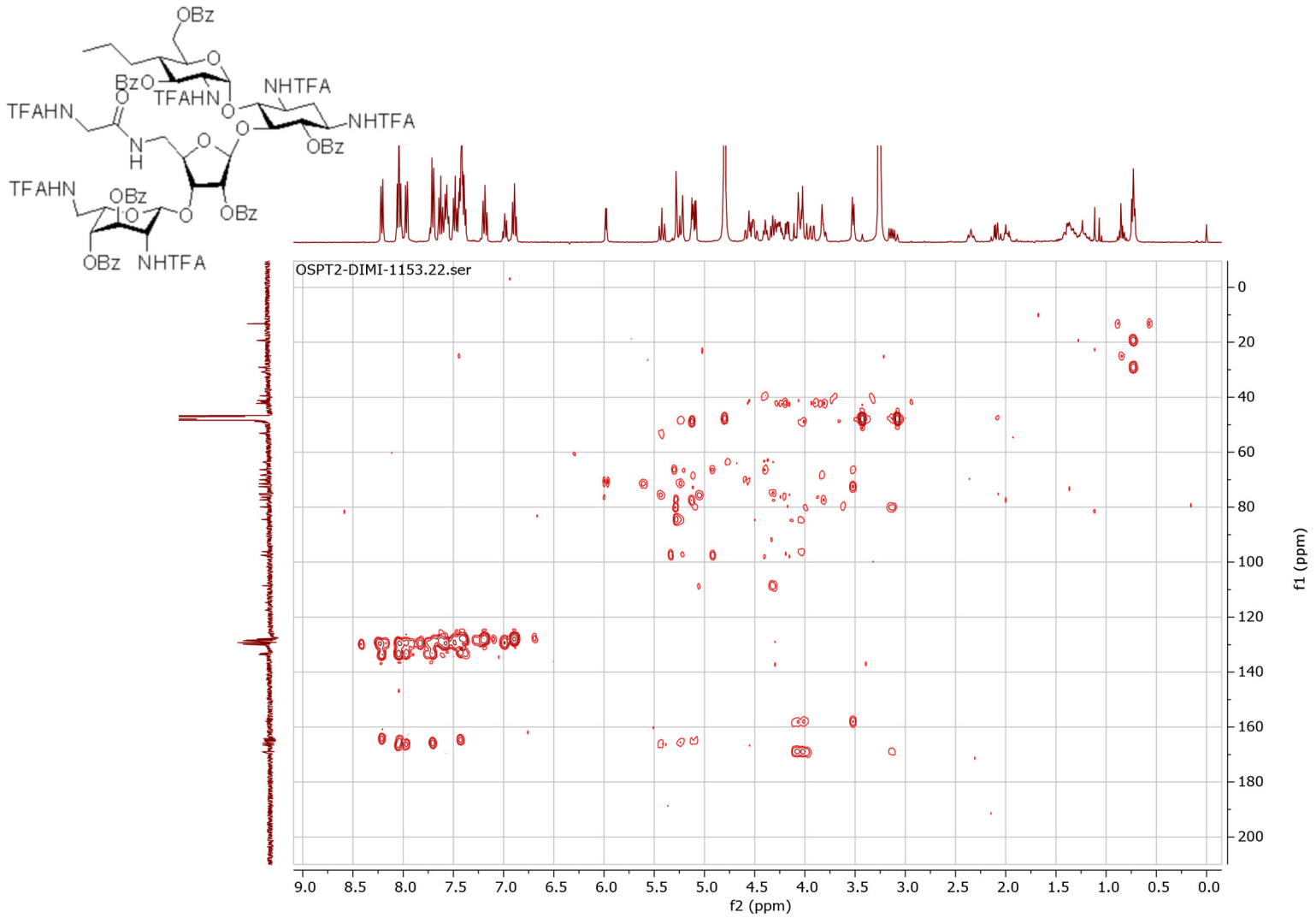
4′,5″-Dideoxy-4′-propyl-5″-(*N*-trifluoroacetylglycinamido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (41)

[^{1}H, ^{13}C HSQC, 400 MHz, 100.6 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-(*N*-trifluoroacetylglycinamido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (41)

[^{1}H, ^{13}C HMBC, 400 MHz, 100.6 MHz, CD_3OD]



4′,5″-Dideoxy-4′-propyl-5″-(*N*-trifluoroacetylglycinamido)-6,3′,6′,2″,3‴,4‴-hexa-*O*-benzoyl-1,3,2′,2‴,6‴-penta-*N*-trifluoroacetyl paromomycin (41)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

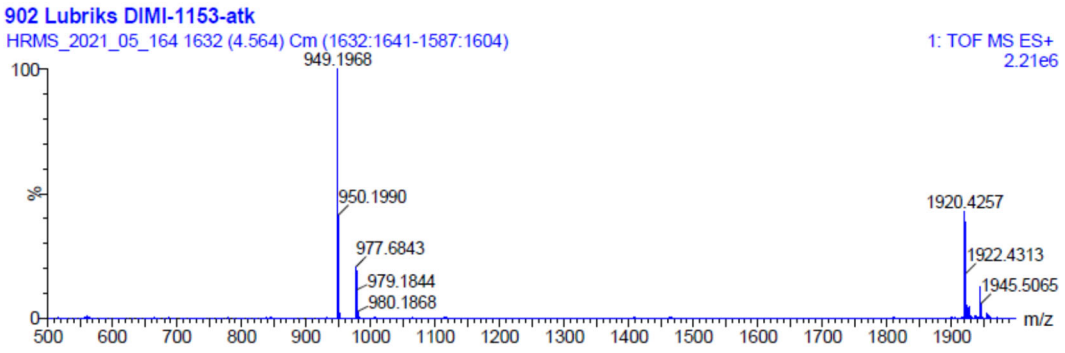
Sample:
HRMS_2021_05_164 902 Lubriks DIMI-1153-atk
MS_POS_500-2000_RES_7min ACN_Form_5-98_040_7min 1:B,1 1.000000 MS_Tune Col#66

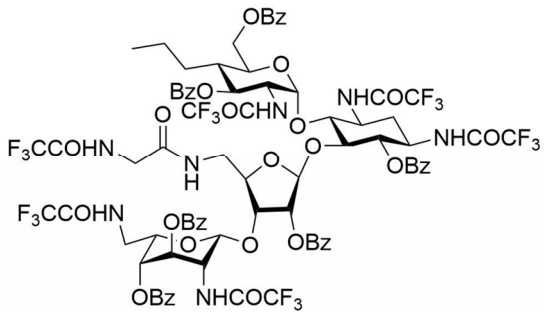
Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
2094 formula(e) evaluated with 12 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-110 N: 0-15 O: 0-25 F: 18-18 Na: 1-1

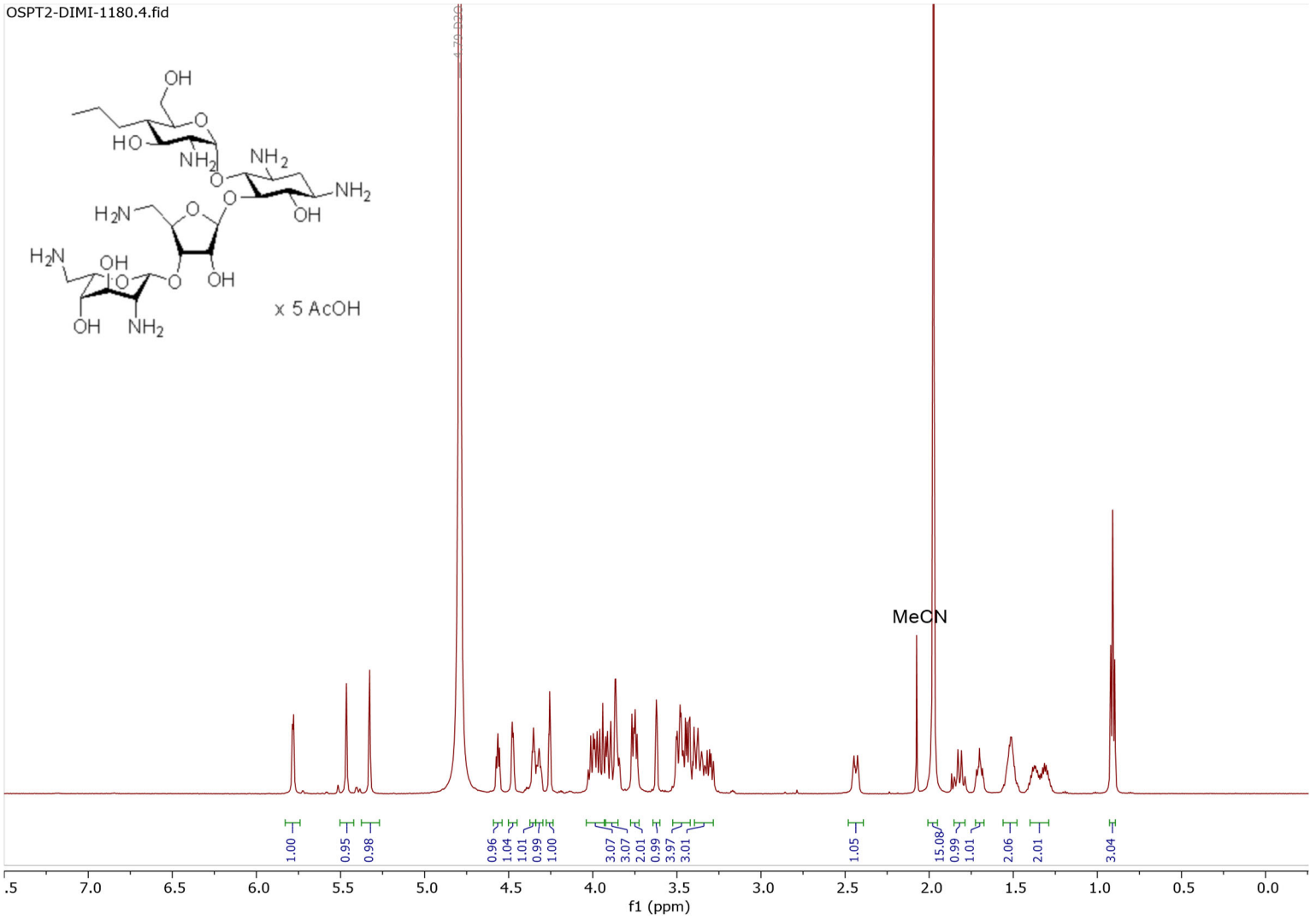
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1920.4257	100.00	1920.4266	-0.9	-0.5	40.5	990.4	1.666	18.91	C82 H73 N7 O25 F18 Na
		1920.4240	1.7	0.9	41.5	989.4	0.625	53.52	C78 H69 N13 O23 F18 Na
		1920.4235	2.2	1.1	48.5	992.3	3.541	2.90	C93 H73 N O22 F18 Na
		1920.4280	-2.3	-1.2	45.5	990.4	1.693	18.40	C83 H69 N11 O21 F18 Na
		1920.4208	4.9	2.6	49.5	991.5	2.769	6.27	C89 H69 N7 O20 F18 Na





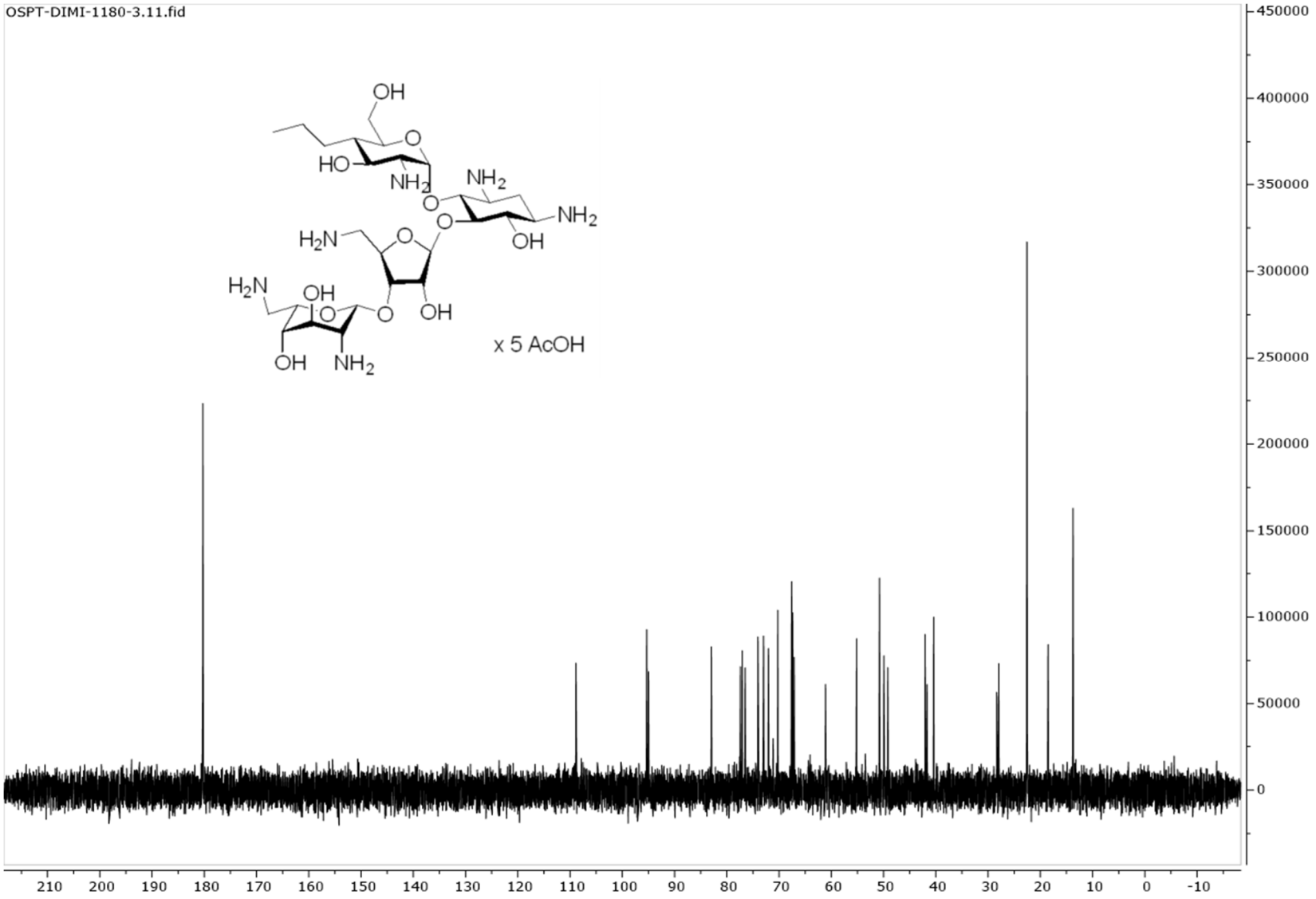
5″-Amino-4′,5″-dideoxy-4′-propyl paromomycin pentaacetate (8)

[^{1}H-NMR, 600 MHz, D_2O]



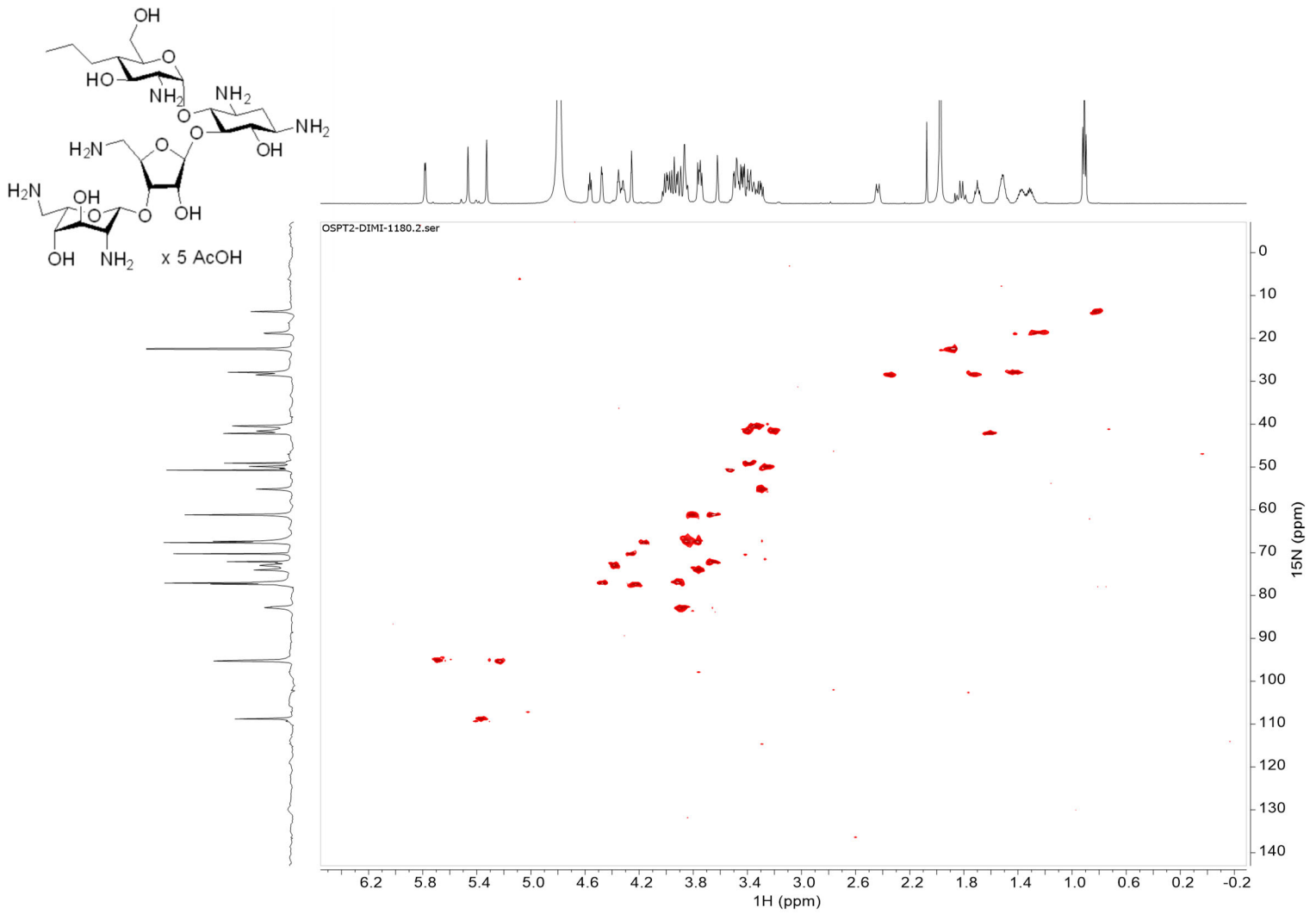
5″-Amino-4′,5″-dideoxy-4′-propyl paromomycin pentaacetate (8)

[^{13}C-NMR, 150.9 MHz, D_2O]



5″-Amino-4′,5″-dideoxy-4′-propyl paromomycin pentaacetate (8)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



5″-Amino-4′,5″-dideoxy-4′-propyl paromomycin pentaacetate (8)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2019_06_031	787 Lubriks DIMI-1180				
MS_POS_RES_4min	ACN_Form_5-98_040_4min	1:C,5	5.000000	MS_Tune	Col#43

Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

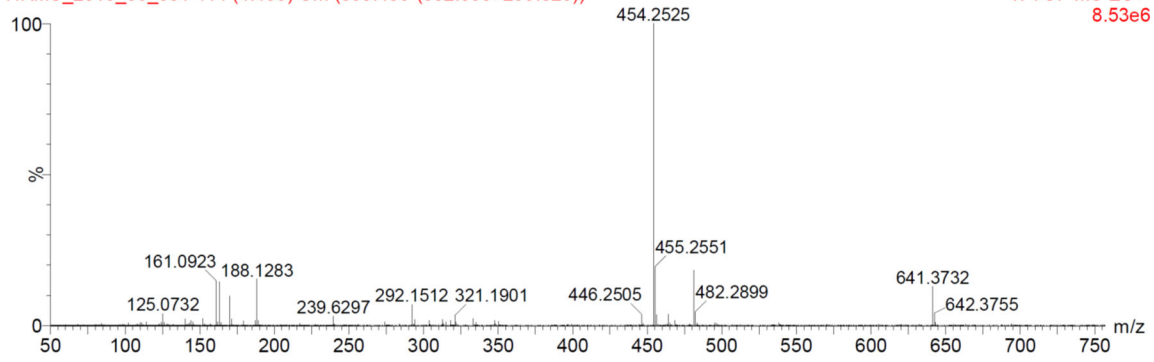
Monoisotopic Mass, Even Electron Ions
1165 formula(e) evaluated with 7 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 1-100 H: 1-110 N: 0-10 O: 0-15

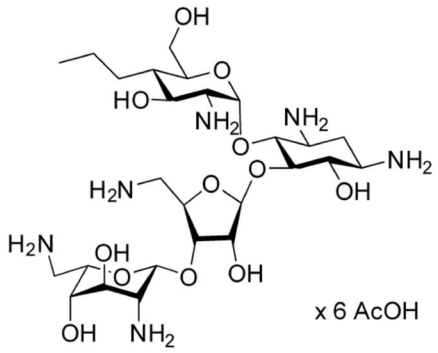
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
641.3732	100.00	641.3721	1.1	1.7	3.5	143.3	0.000	99.99	C26 H53 N6 O12
		641.3735	-0.3	-0.5	8.5	152.5	9.206	0.01	C27 H49 N10 O8
		641.3743	-1.1	-1.7	20.5	162.8	19.429	0.00	C43 H49 N2 O3

787 Lubriks DIMI-1180

HRMS_2019_06_031 414 (1.195) Cm (399:436-(552:590+293:325))

1: TOF MS ES+
8.53e6

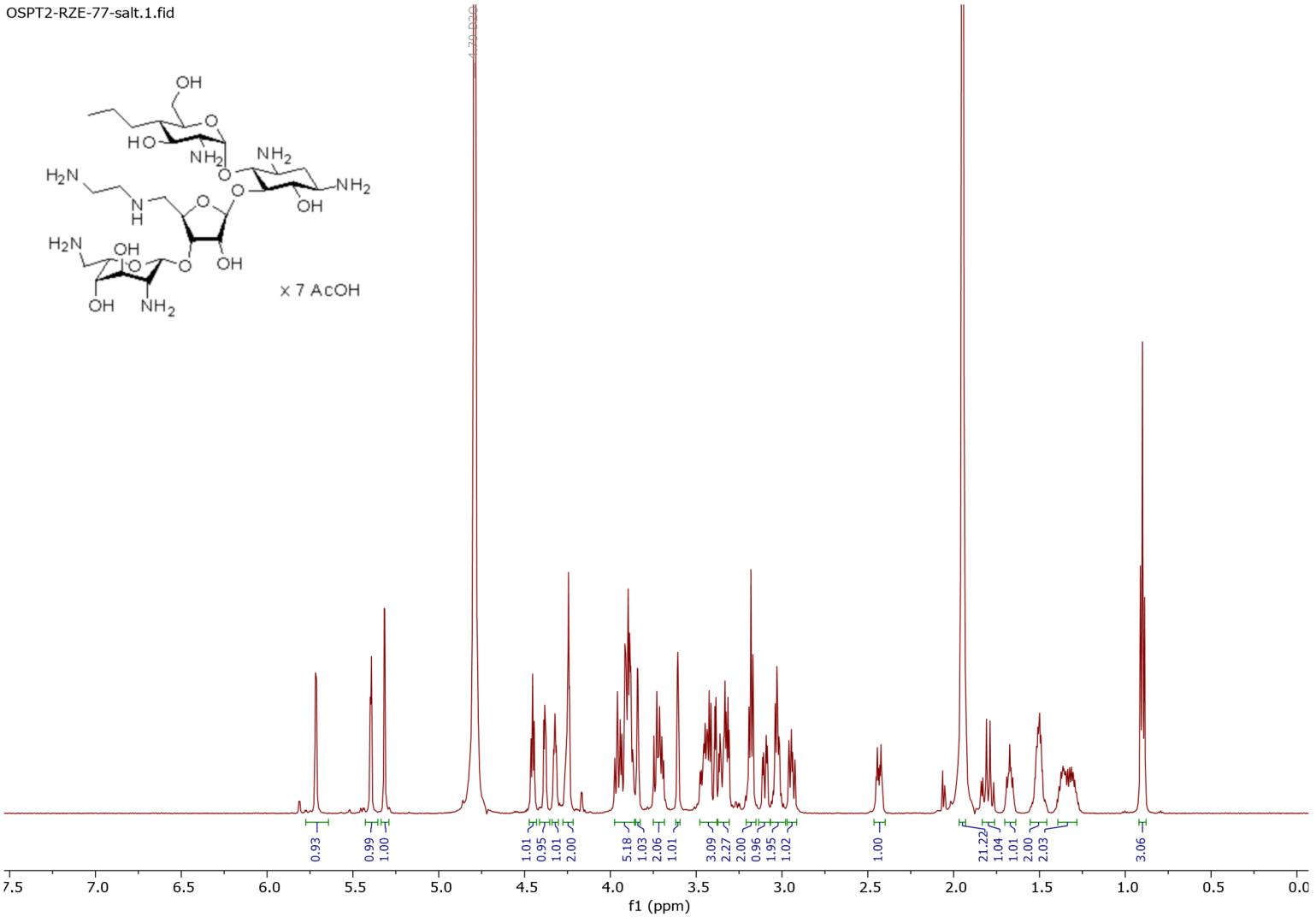




x 6 AcOH

5″-(2-Aminoethylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (9)

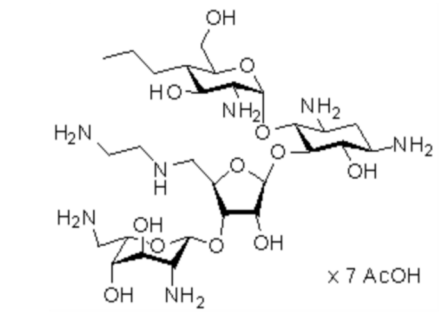
[^{1}H-NMR, 600 MHz, D_2O]



5″-(2-Aminoethylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (9)

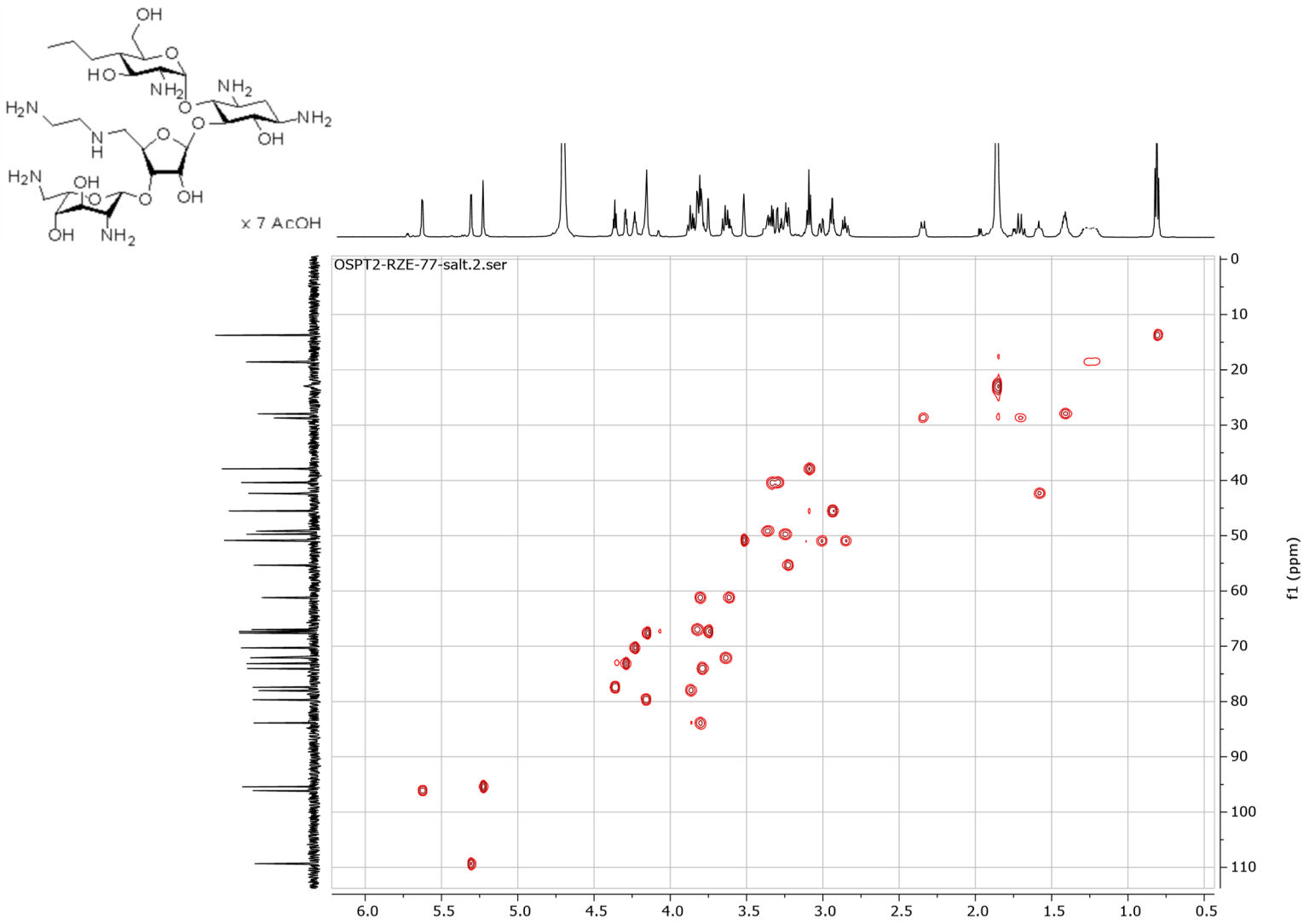
[^{13}C-NMR, 150.9 MHz, D_2O]

OSPT2-RZE-77-salt.4.fid



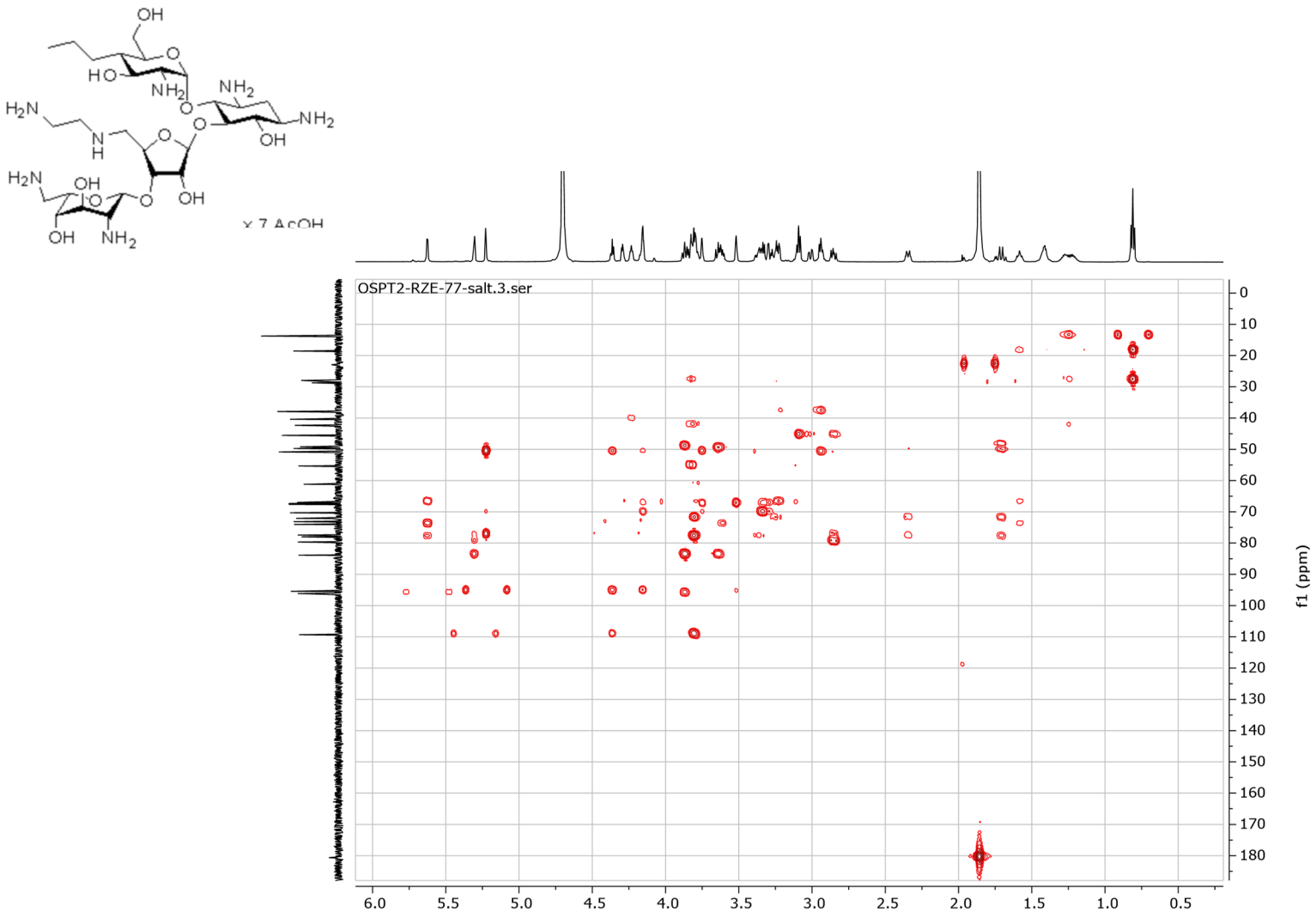
5″-(2-Aminoethylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (9)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



5″-(2-Aminoethylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (9)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, D_2O]



5″-(2-Aminoethylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (9)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2020_02_181	1867 Zogota RZE-77				
MS_POS_RES_7min	ACN_Form_5-98_040_7min	2:D,7	10.000000	MS_Tune	Col#43

Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

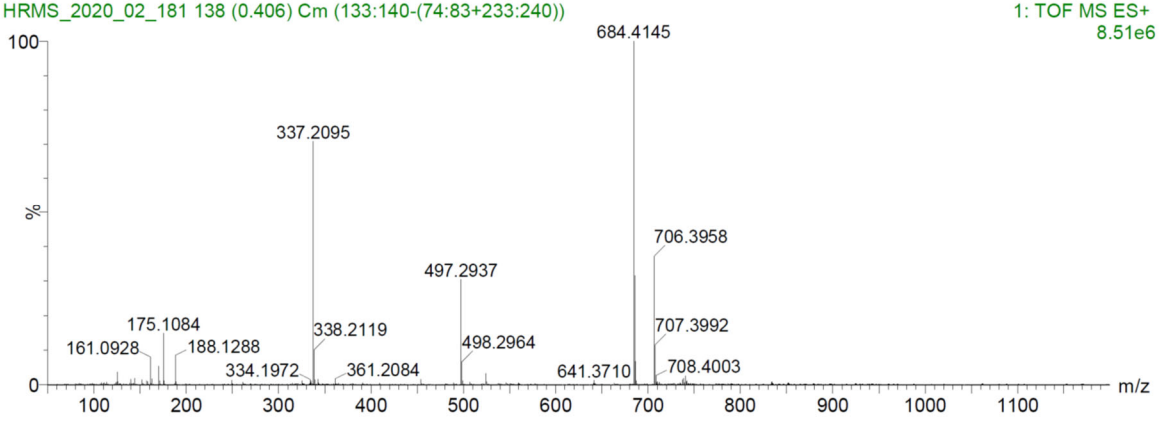
Monoisotopic Mass, Even Electron Ions
1114 formula(e) evaluated with 6 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-80 N: 0-10 O: 0-15

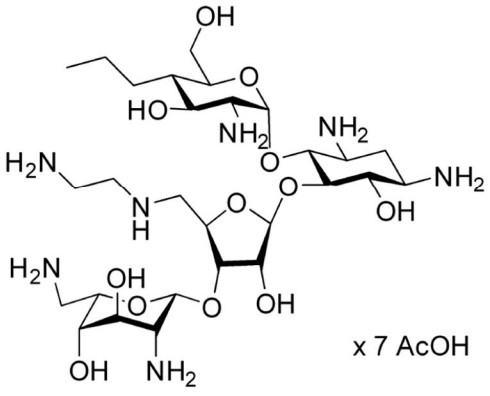
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
684.4145	100.00	684.4143	0.2	0.3	3.5	623.3	0.007	99.32	C28 H58 N7 O12
		684.4170	-2.5	-3.7	2.5	628.4	5.148	0.58	C32 H62 N O14
		684.4138	0.7	1.0	21.5	630.1	6.870	0.10	C41 H50 N9 O

1867 Zogota RZE-77

HRMS_2020_02_181 138 (0.406) Cm (133:140-(74:83+233:240))

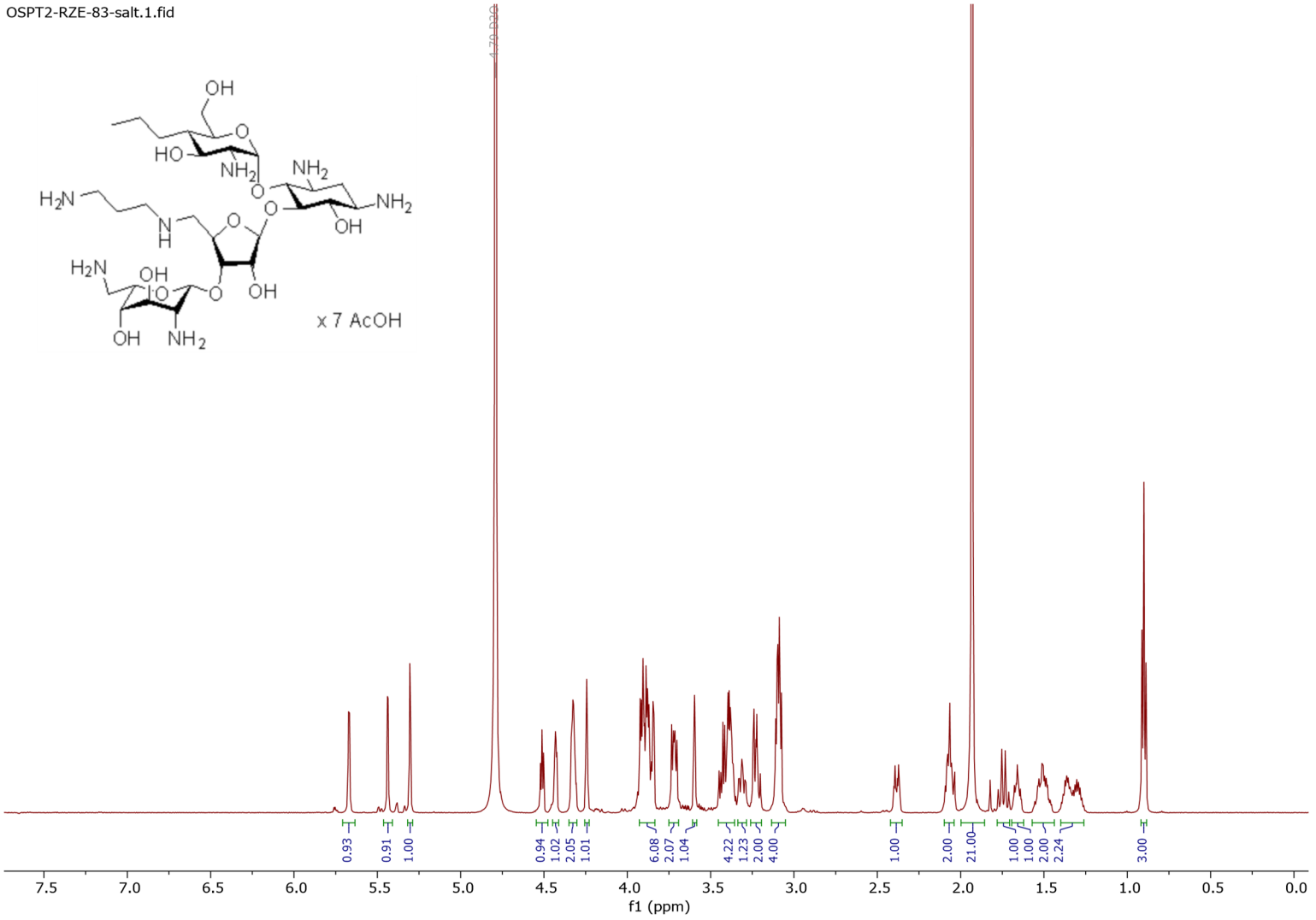
1: TOF MS ES+
8.51e6





5''-(3-Aminopropylamino)-4',5''-dideoxy-4'-propyl paromomycin heptaacetate (10)

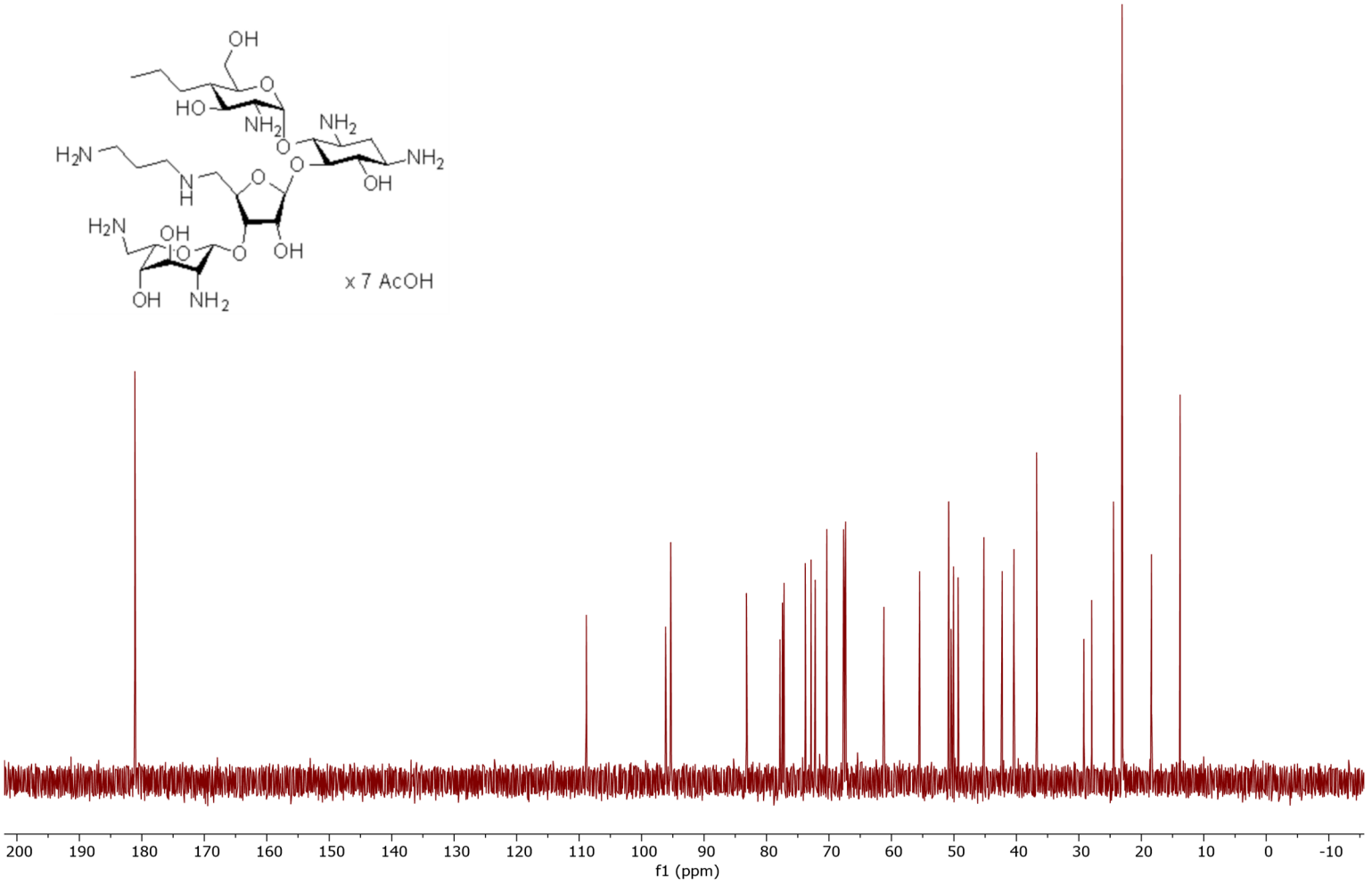
[^{1}H-NMR, 600 MHz, D_2O]



5″-(3-Aminopropylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (10)

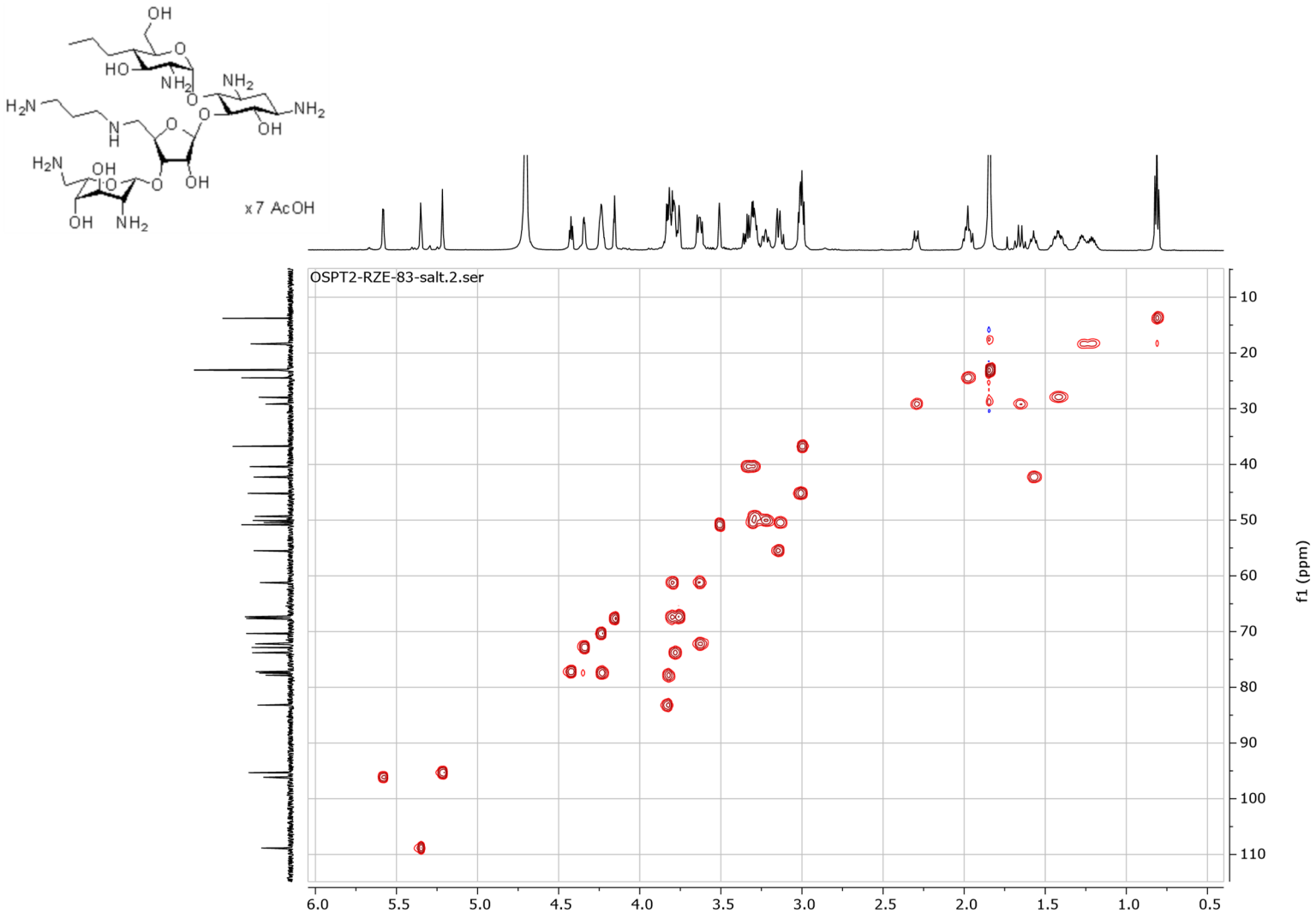
[^{13}C-NMR, 150.9 MHz, D_2O]

OSPT2-RZE-83-salt.4.fid



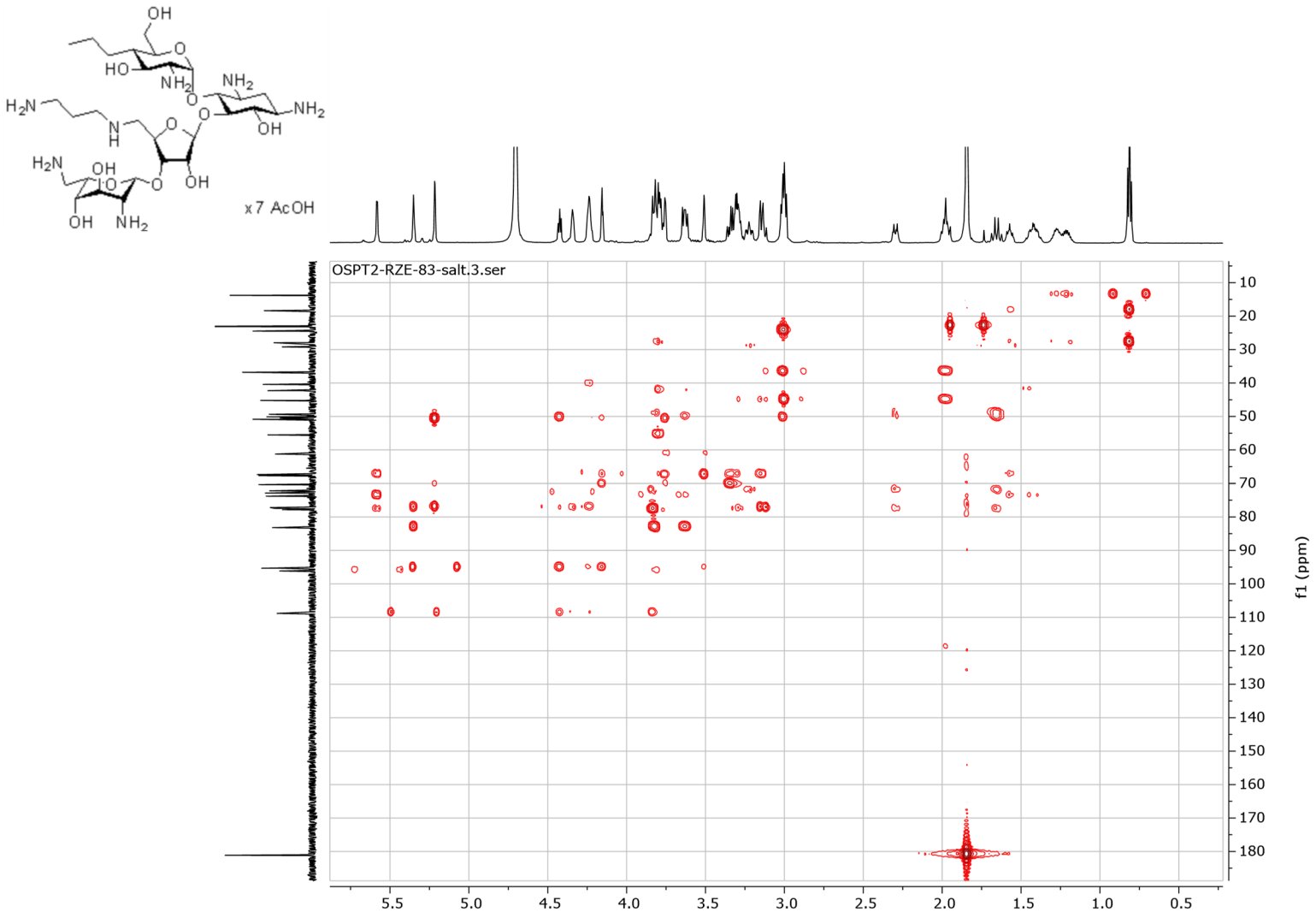
5″-(3-Aminopropylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (10)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



5″-(3-Aminopropylamino)-4′,5″-dideoxy-4′-propyl paromomycin heptaacetate (10)

[^{1}H, ^{13}C HMBC, 600 MHz, 150.9 MHz, D_2O]



5''-(3-Aminopropylamino)-4',5''-dideoxy-4'-propyl paromomycin heptaacetate (10)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2020_02_463	1967 Zogota RZE-83				
MS_POS_RES_4min	ACN_Form_5-98_040_4min	1:C,3	5.000000	MS_Tune	Col#66

Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

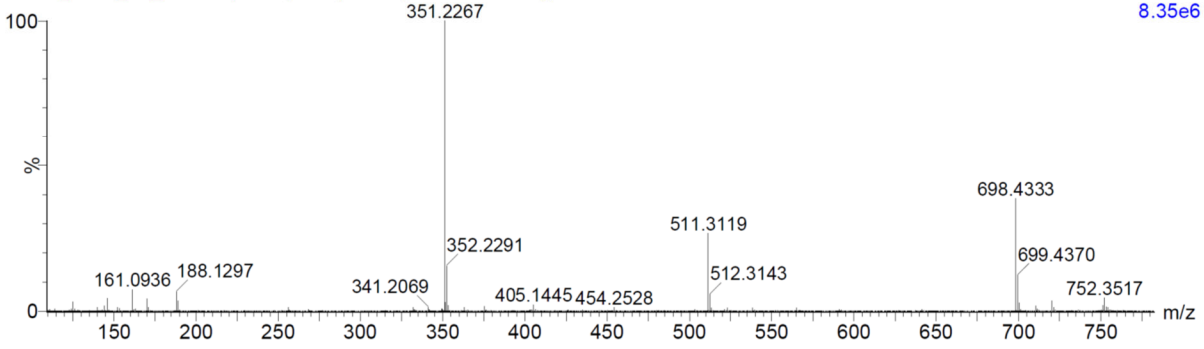
Monoisotopic Mass, Even Electron Ions
1515 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-10 O: 0-20

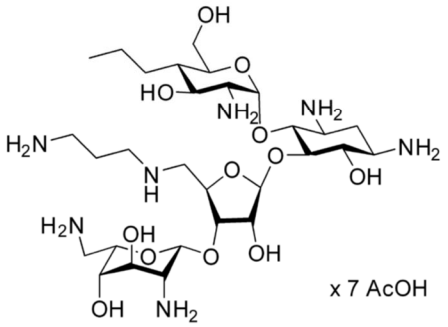
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
698.4333	100.00	698.4300	3.3	4.7	3.5	465.3	0.475	62.20	C29 H60 N7 O12
		698.4327	0.6	0.9	2.5	466.2	1.413	24.35	C33 H64 N O14
		698.4340	-0.7	-1.0	7.5	467.1	2.271	10.32	C34 H60 N5 O10

1967 Zogota RZE-83

HRMS_2020_02_463 89 (0.272) Cm (88:93-(66:73+127:137))

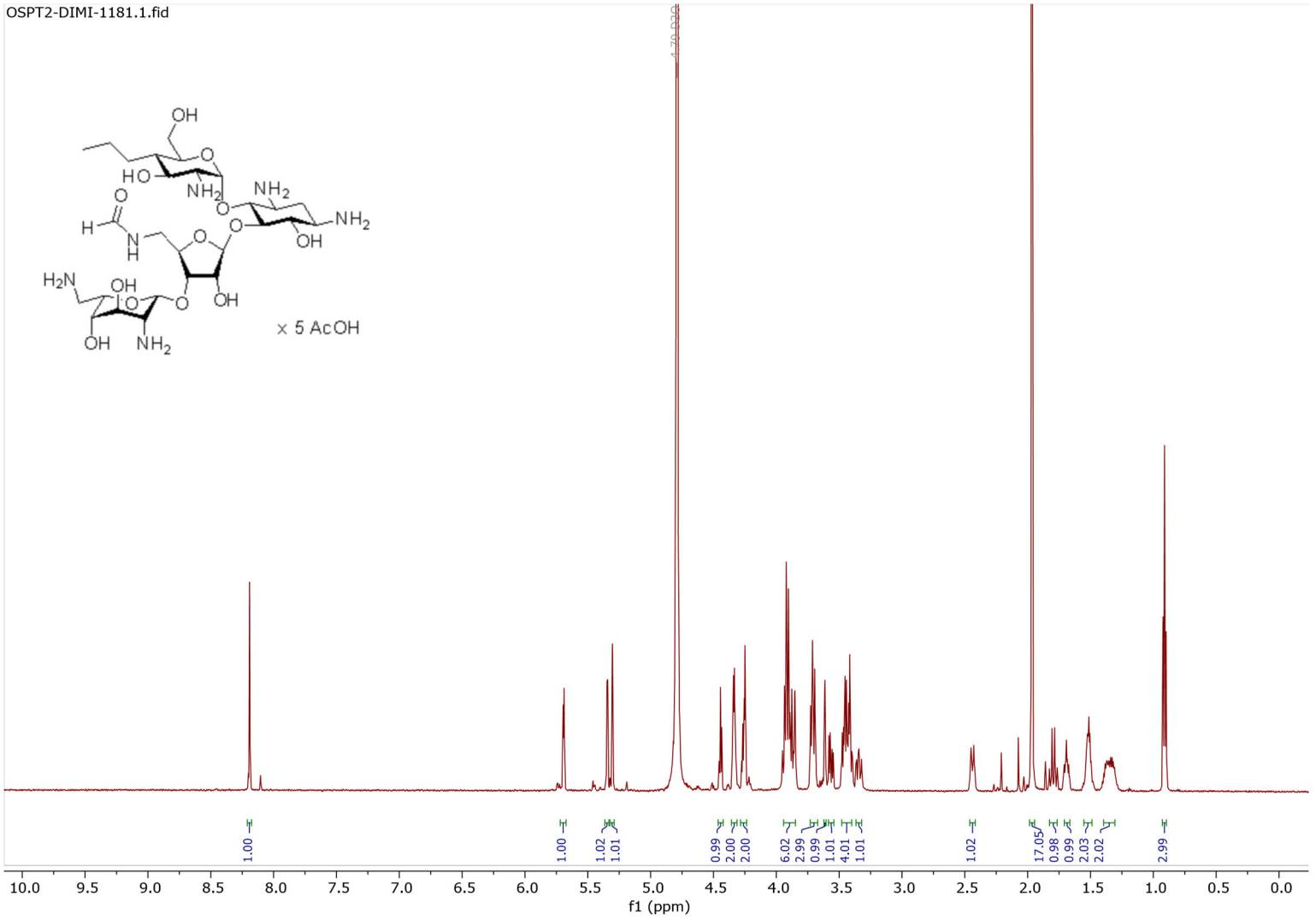
1: TOF MS ES+
8.35e6





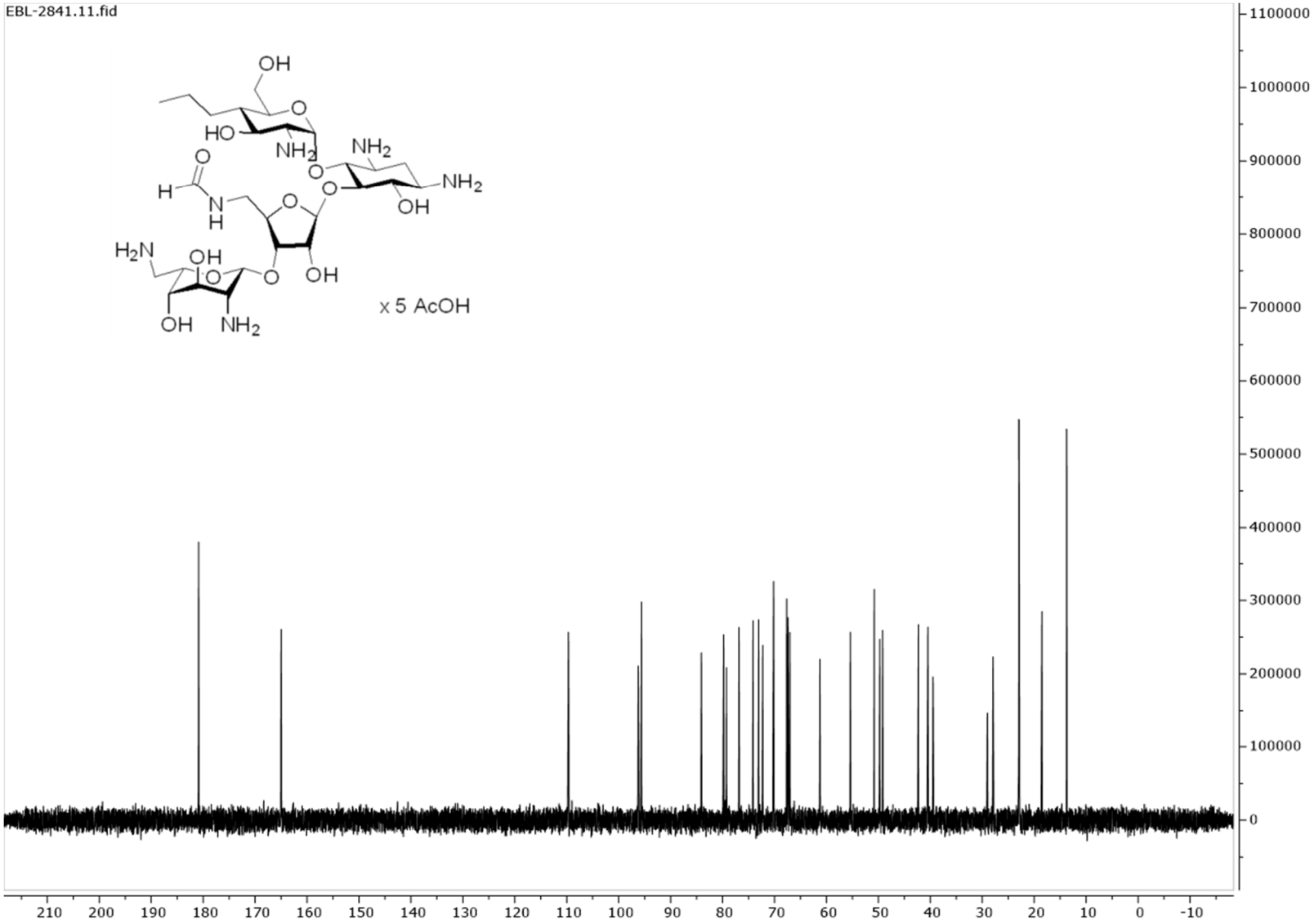
4',5''-Dideoxy-5''-formamido-4'-propyl paromomycin pentaacetate (11)

[^{1}H-NMR, 600 MHz, D_2O]



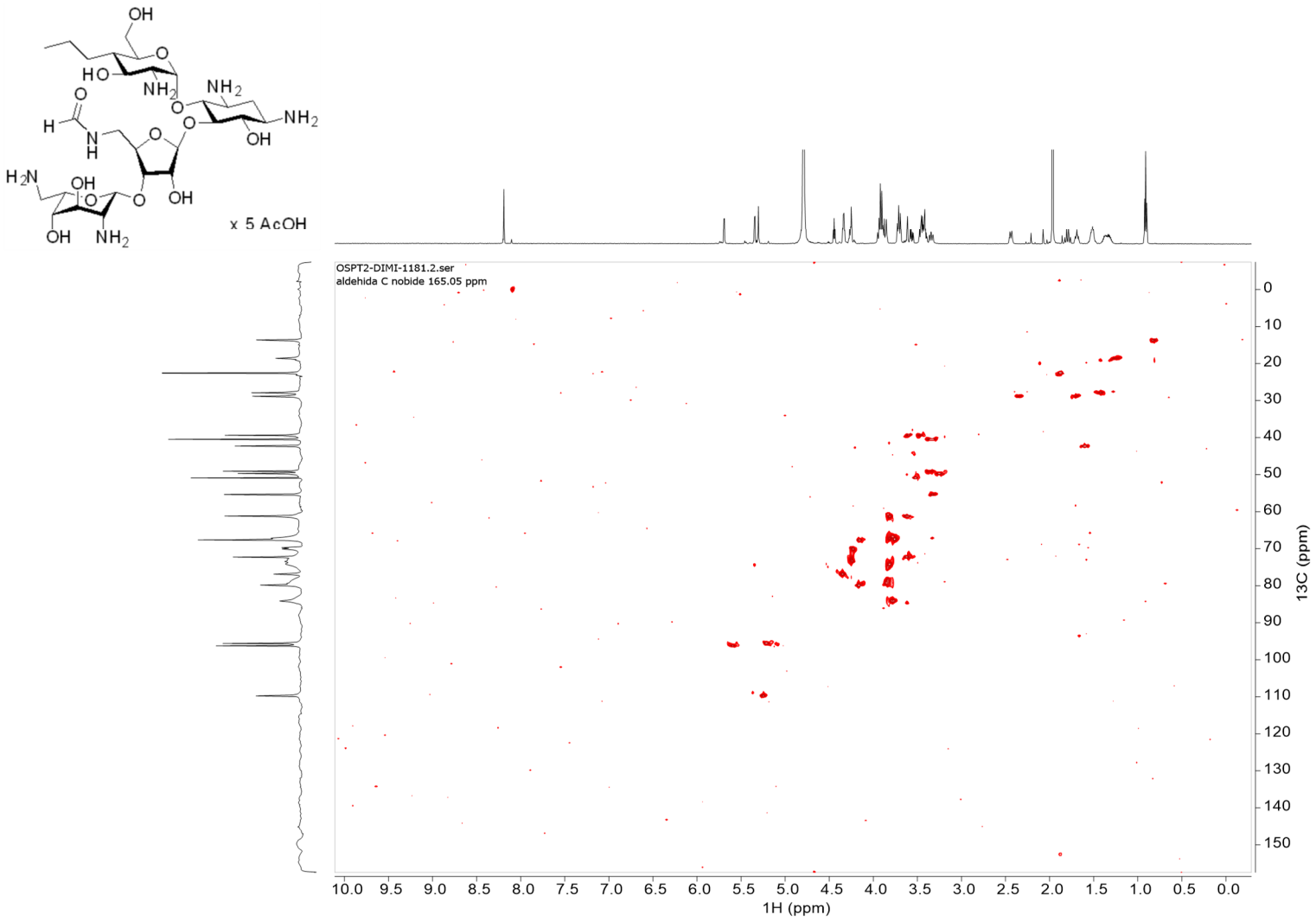
4',5''-Dideoxy-5''-formamido-4'-propyl paromomycin pentaacetate (11)

[^{13}C-NMR, 150.9 MHz, D_2O]



4',5''-Dideoxy-5''-formamido-4'-propyl paromomycin pentaacetate (11)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



4′,5″-Dideoxy-5″-formamido-4′-propyl paromomycin pentaacetate (11)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-S*i*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2019_06_033	788 Lubriks DIMI-1181				
MS_POS_RES_4min	ACN_Form_5-98_040_4min	1:C,6	1.000000	MS_Tune	Col#43

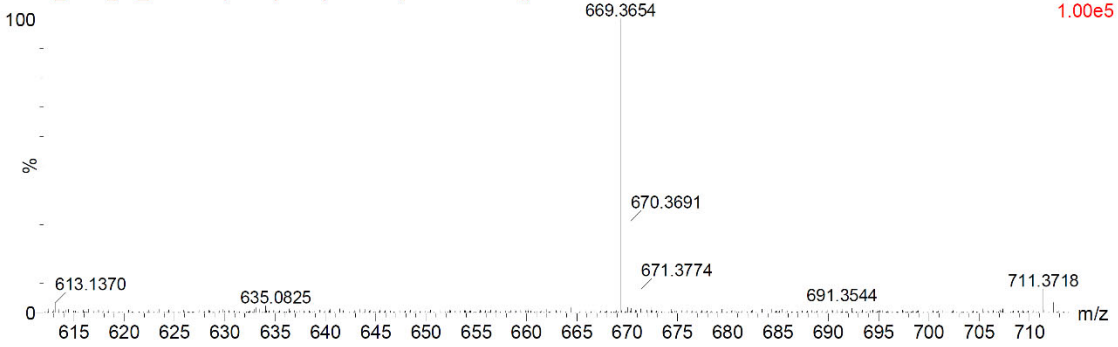
Elemental Composition Report:

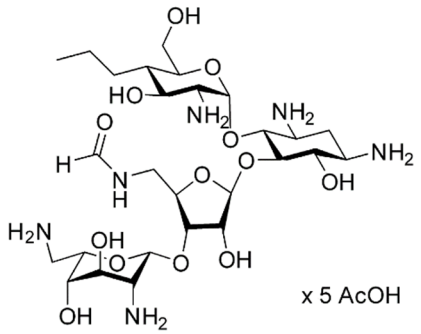
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1224 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-100 H: 1-110 N: 0-10 O: 0-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
669.3654	100.00	669.3671	-1.7	-2.5	4.5	271.3	0.001	99.93	C27 H53 N6 O13
		669.3639	1.5	2.2	12.5	279.3	8.003	0.03	C38 H53 O10
		669.3652	0.2	0.3	17.5	279.7	8.324	0.02	C39 H49 N4 O6
		669.3665	-1.1	-1.6	22.5	280.1	8.744	0.02	C40 H45 N8 O2

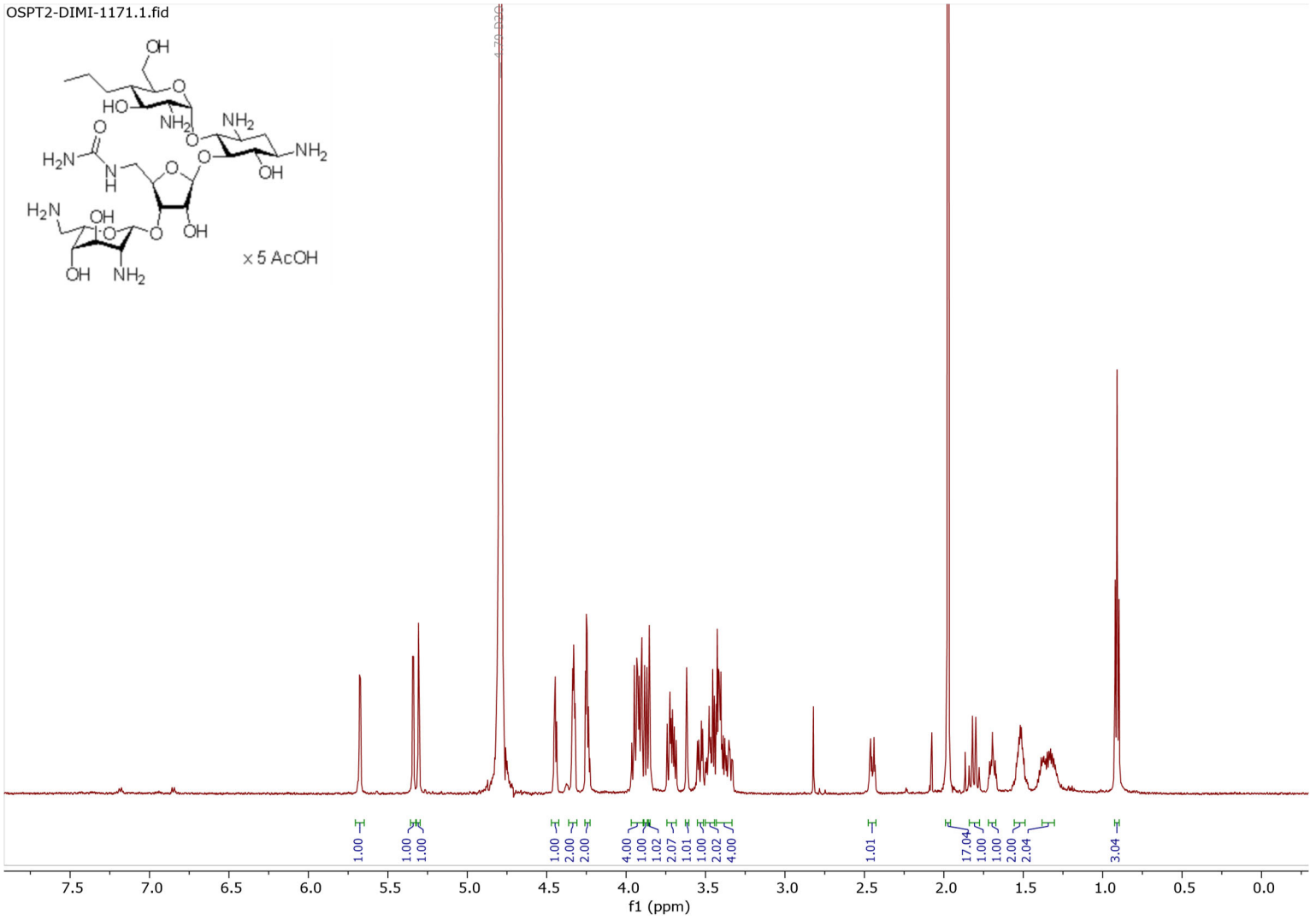
788 Lubriks DIMI-1181
HRMS_2019_06_033 146 (0.434) Cm (137:183-(438:473+9:42))
1: TOF MS ES+
1.00e5





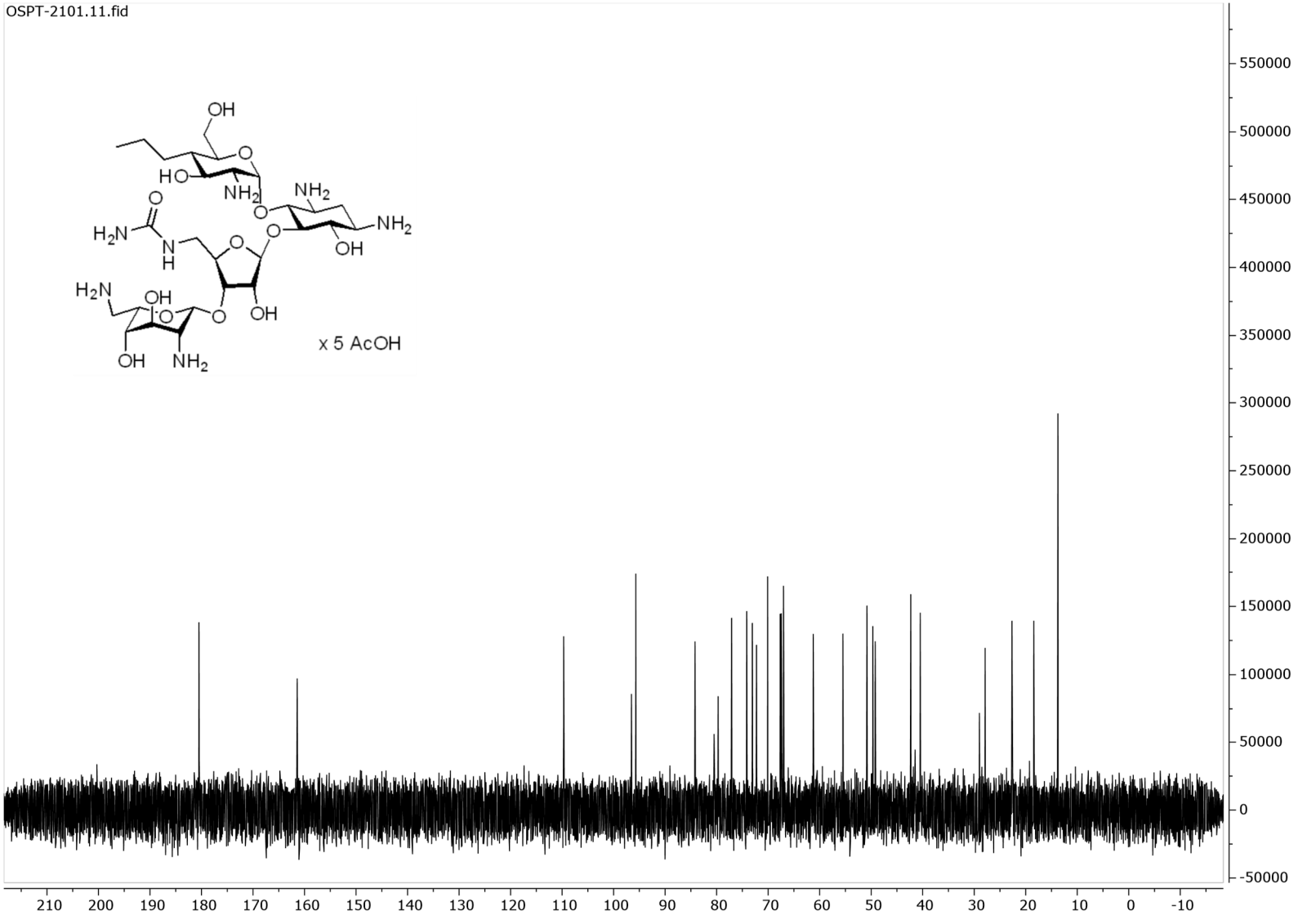
4',5''-Dideoxy-4'-propyl-5''-ureido-paromomycin pentaacetate (12)

[^{1}H-NMR, 600 MHz, D_2O]



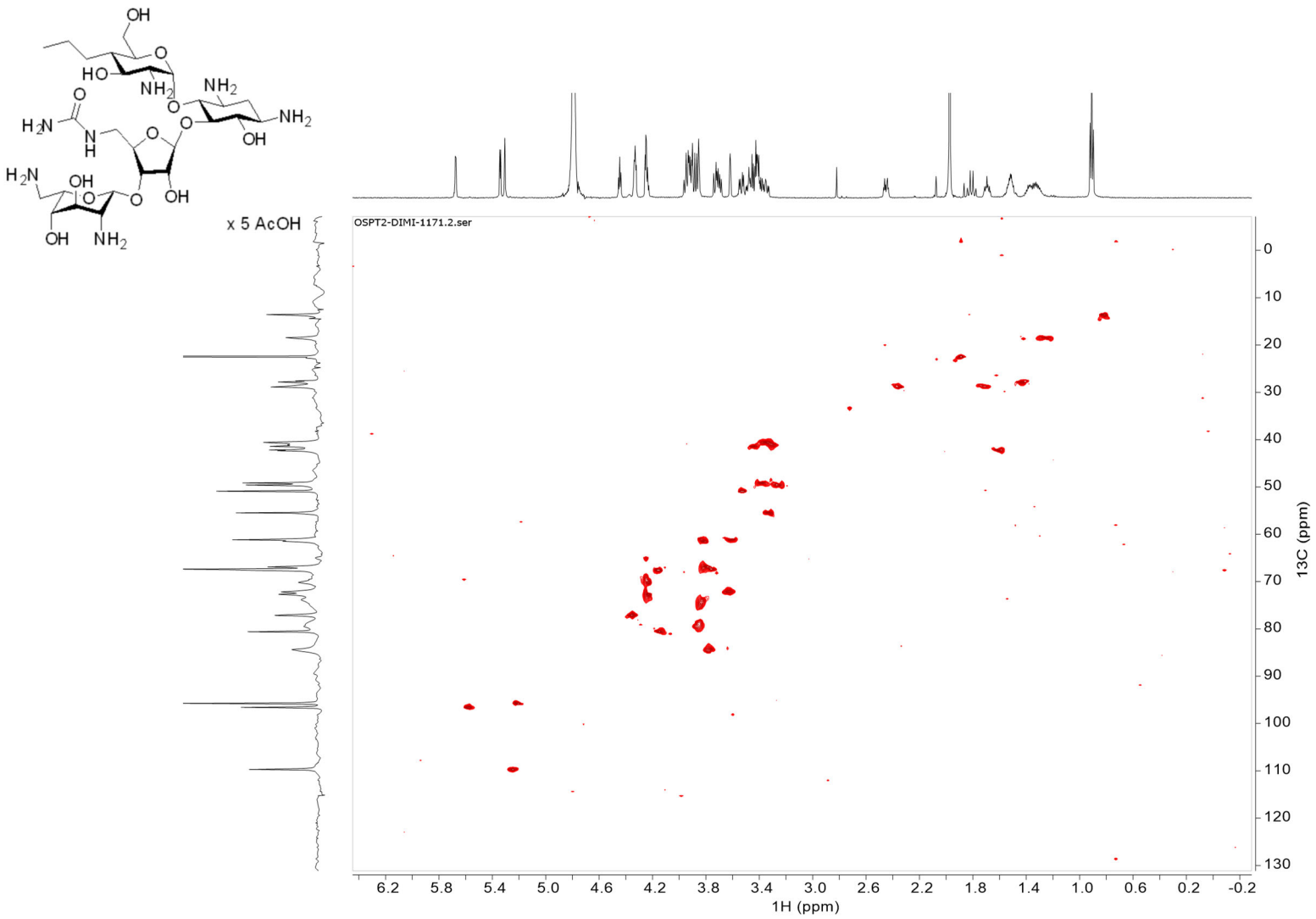
4′,5″-Dideoxy-4′-propyl-5″-ureido-paromomycin pentaacetate (12)

[^{13}C-NMR, 150.9 MHz, D_2O]



4',5''-Dideoxy-4'-propyl-5''-ureido-paromomycin pentaacetate (12)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



4′,5″-Dideoxy-4′-propyl-5″-ureido-paromomycin pentaacetate (12)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2019_06_017	786 Lubriks DIMI-1171				
MS_POS_RES_4min	ACN_Form_5-98_040_4min	2:B,7	5.000000	MS_Tune	Col#43

Elemental Composition Report:

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

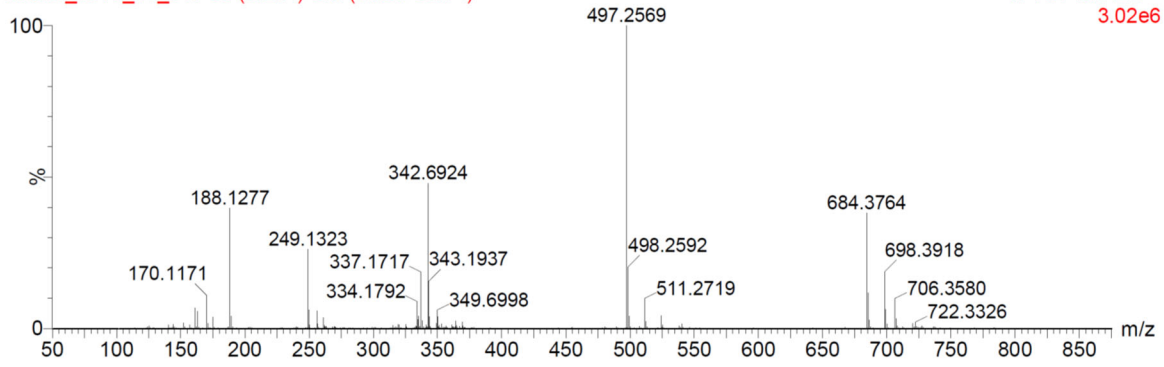
Monoisotopic Mass, Even Electron Ions
1240 formula(e) evaluated with 5 results within limits (up to 3 closest results for each mass)
Elements Used:
C: 1-100 H: 1-110 N: 0-10 O: 0-15

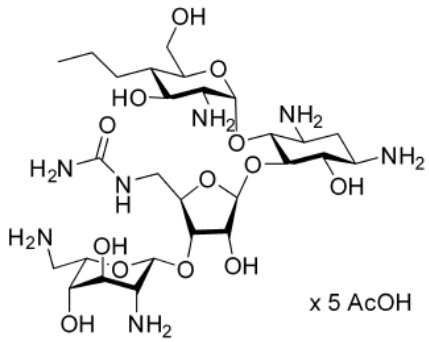
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
684.3764	100.00	684.3780	-1.6	-2.3	4.5	528.4	0.001	99.89	C27 H54 N7 O13
		684.3761	0.3	0.4	17.5	535.5	7.116	0.08	C39 H50 N5 O6
		684.3774	-1.0	-1.5	22.5	536.4	8.000	0.03	C40 H46 N9 O2

786 Lubriks DIMI-1171

HRMS_2019_06_017 86 (0.264) Cm (84:93-69:74)

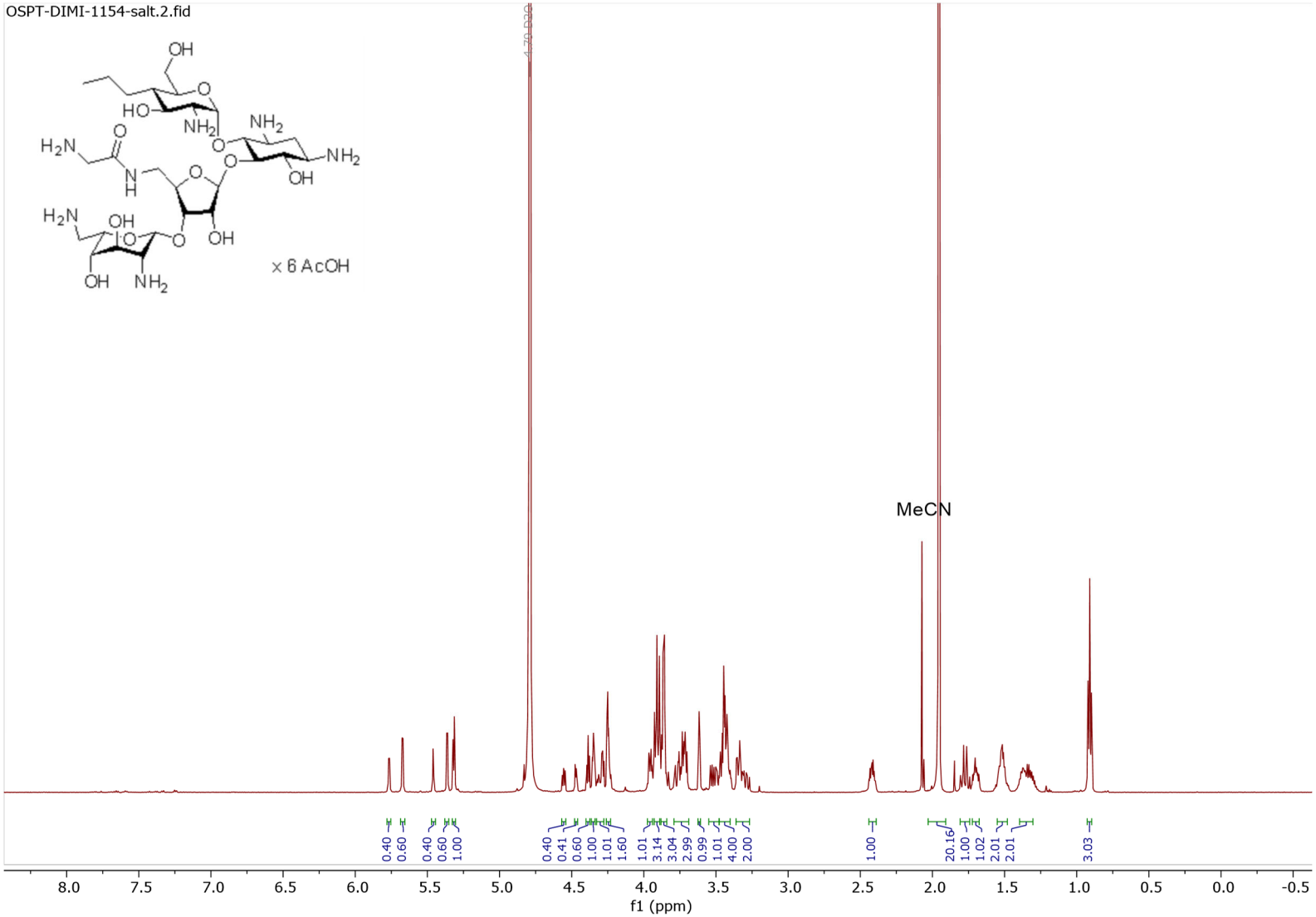
1: TOF MS ES+
3.02e6





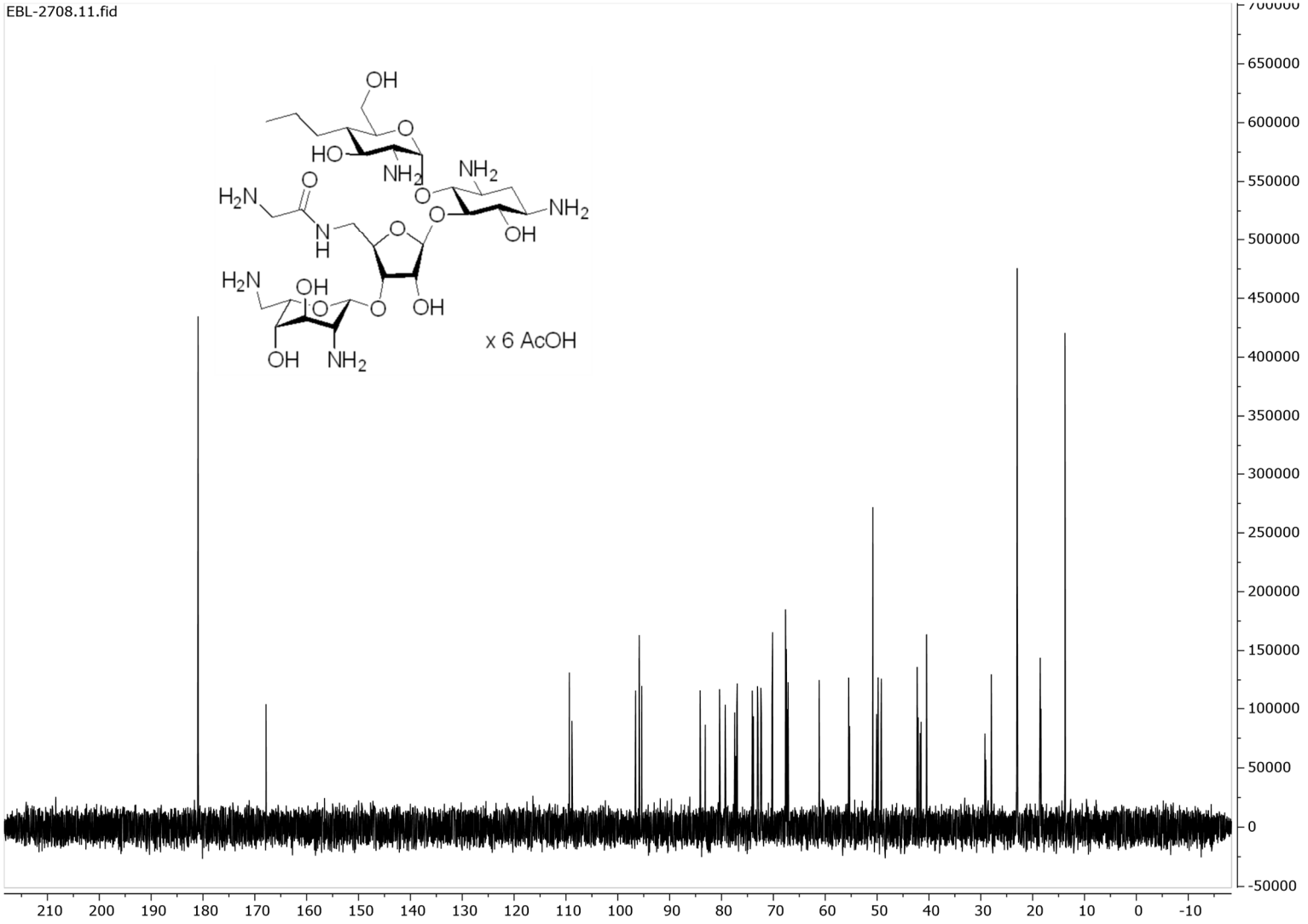
4′,5″-Dideoxy-5″-glycinamido-4′-propyl paromomycin hexacetate (13)

[^{1}H-NMR, 600 MHz, D_2O]



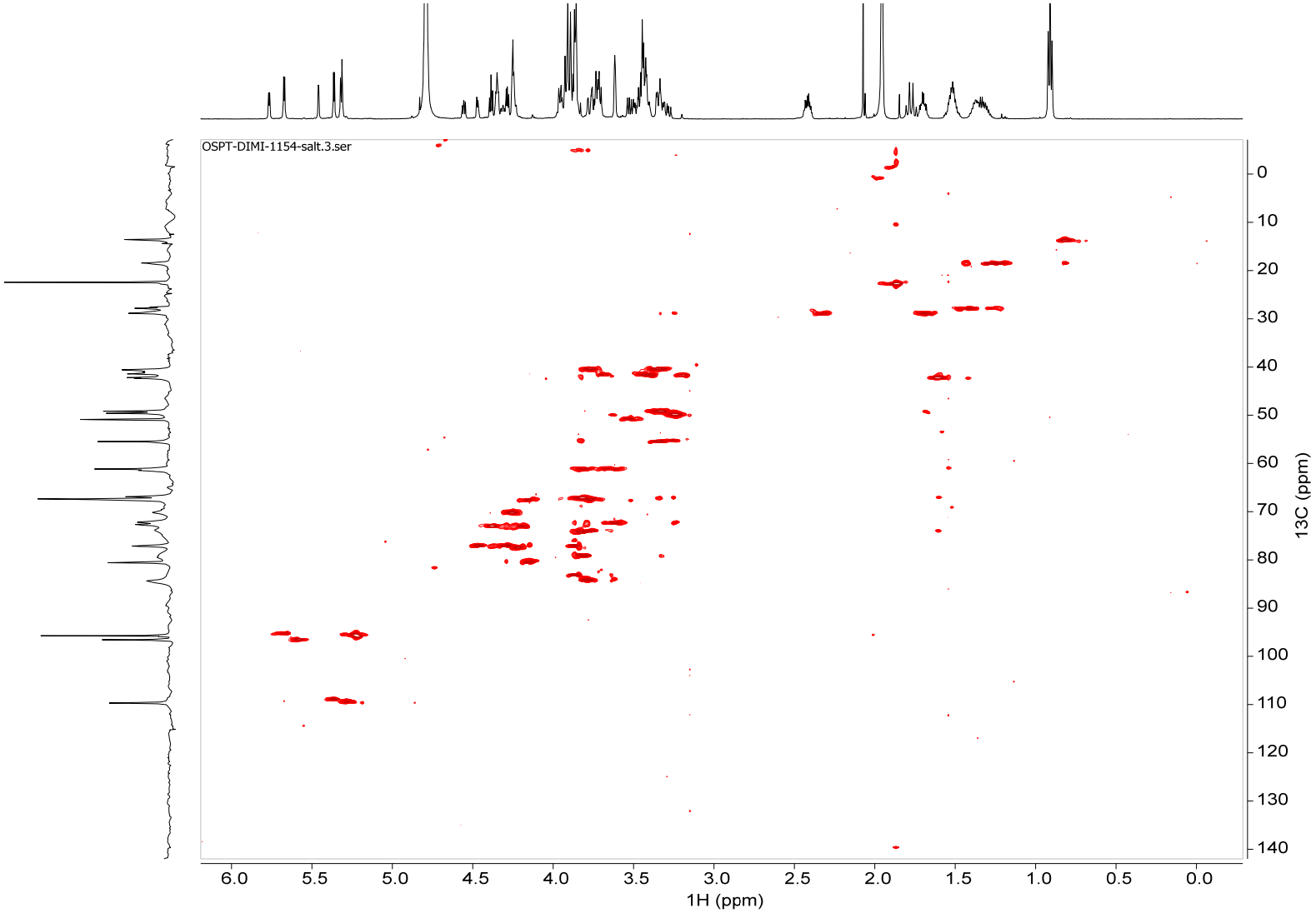
4′,5″-Dideoxy-5″-glycinamido-4′-propyl paromomycin hexacetate (13)

[^{13}C-NMR, 150.9 MHz, D_2O]



4',5''-Dideoxy-5''-glycinamido-4'-propyl paromomycin hexacetate (13)

[^{1}H, ^{13}C HSQC, 600 MHz, 150.9 MHz, D_2O]



4′,5″-Dideoxy-5″-glycinamido-4′-propyl paromomycin hexacetate (13)

Latvijas Organiskās sintēzes institūts
Fizikāli organiskās ķīmijas laboratorija

MS: Waters Synapt G2-*Si*	Capillary, kV:	0.7	**LC: Acquity UPLC H-Class**	Column:	Acquity UPLC BEH C18
ESI+	Cone, V:	40			2.1x50mm, 1.7µm

Sample:

HRMS_2019_04_226	623 DIMI-1154				
MS_POS_RES_4min	ACN_Form_5-98_040_4min	MS_Tune	1:E,6	0.300000	Col#43

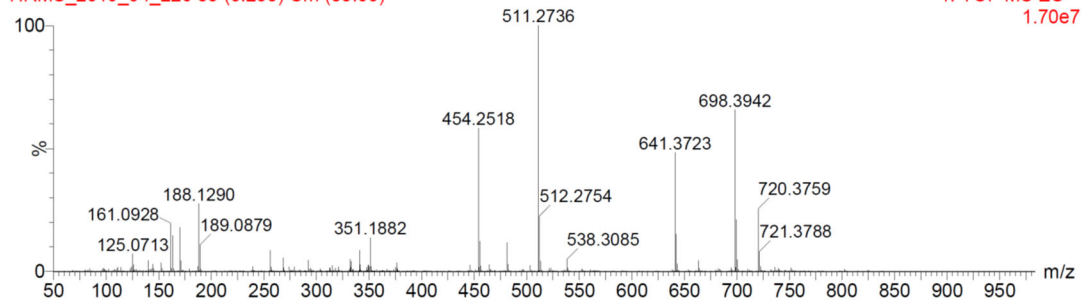
Elemental Composition Report:

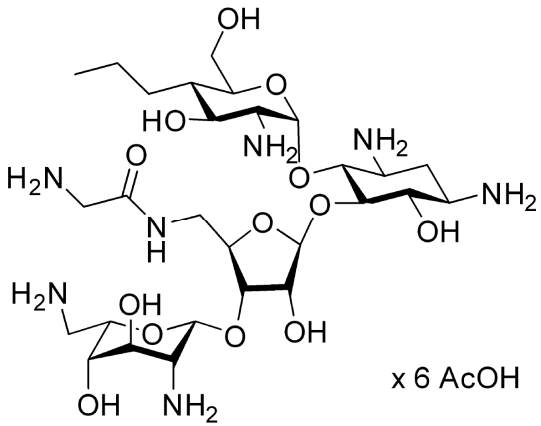
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5251 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-100 H: 1-110 N: 0-50 O: 0-50

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
698.3942	100.00	698.3936	0.6	0.9	4.5	745.2	0.194	82.40	C28 H56 N7 O13
		698.3949	-0.7	-1.0	9.5	747.1	2.128	11.91	C29 H52 N11 O9
		698.3936	0.6	0.9	15.5	747.9	2.918	5.40	C26 H44 N21 O3
		698.3954	-1.2	-1.7	2.5	751.0	6.036	0.24	C14 H48 N23 O10
		698.3931	1.1	1.6	22.5	752.7	7.779	0.04	C41 H48 N9 O2

623 DIMI-1154

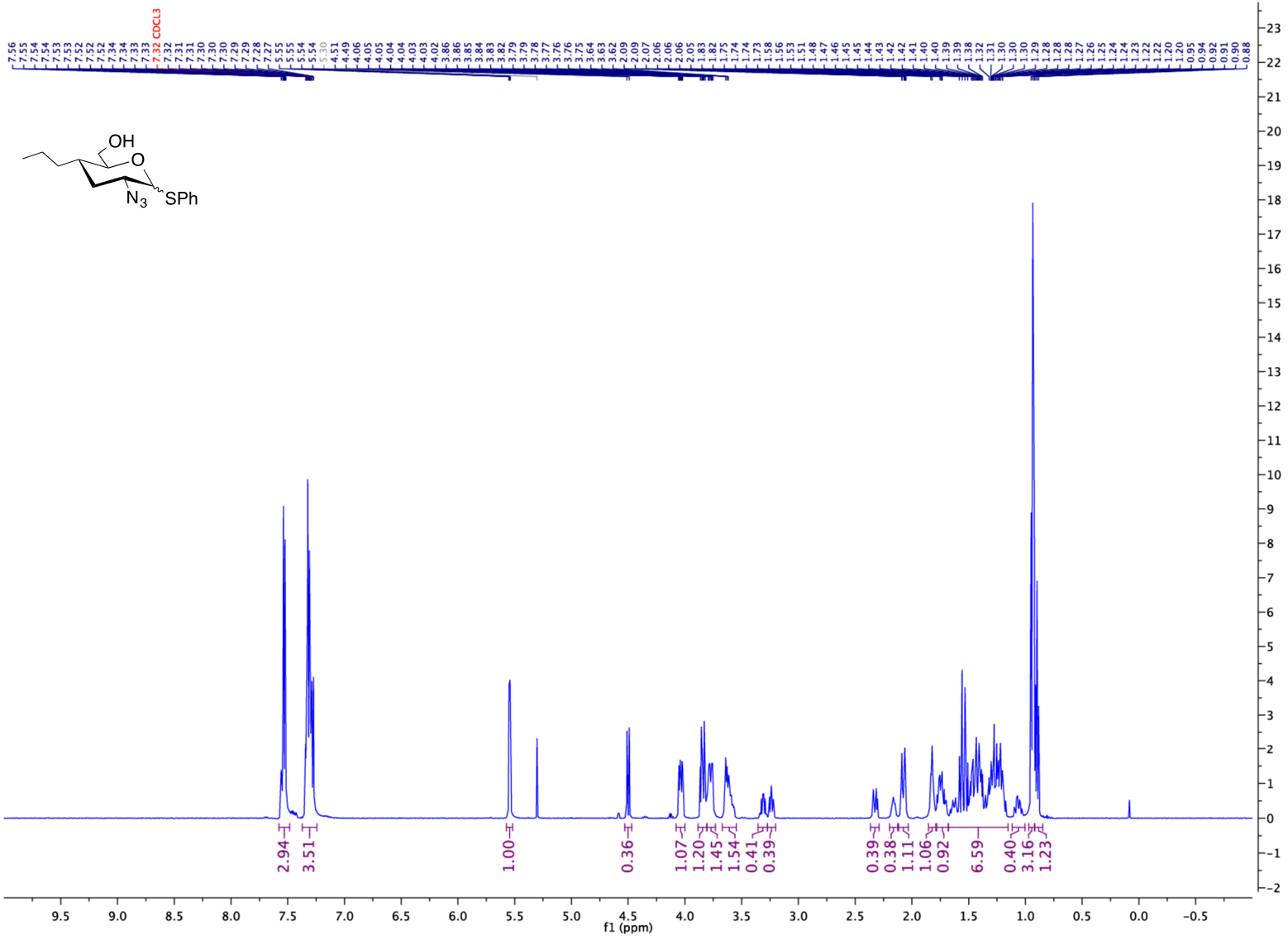




x 6 AcOH

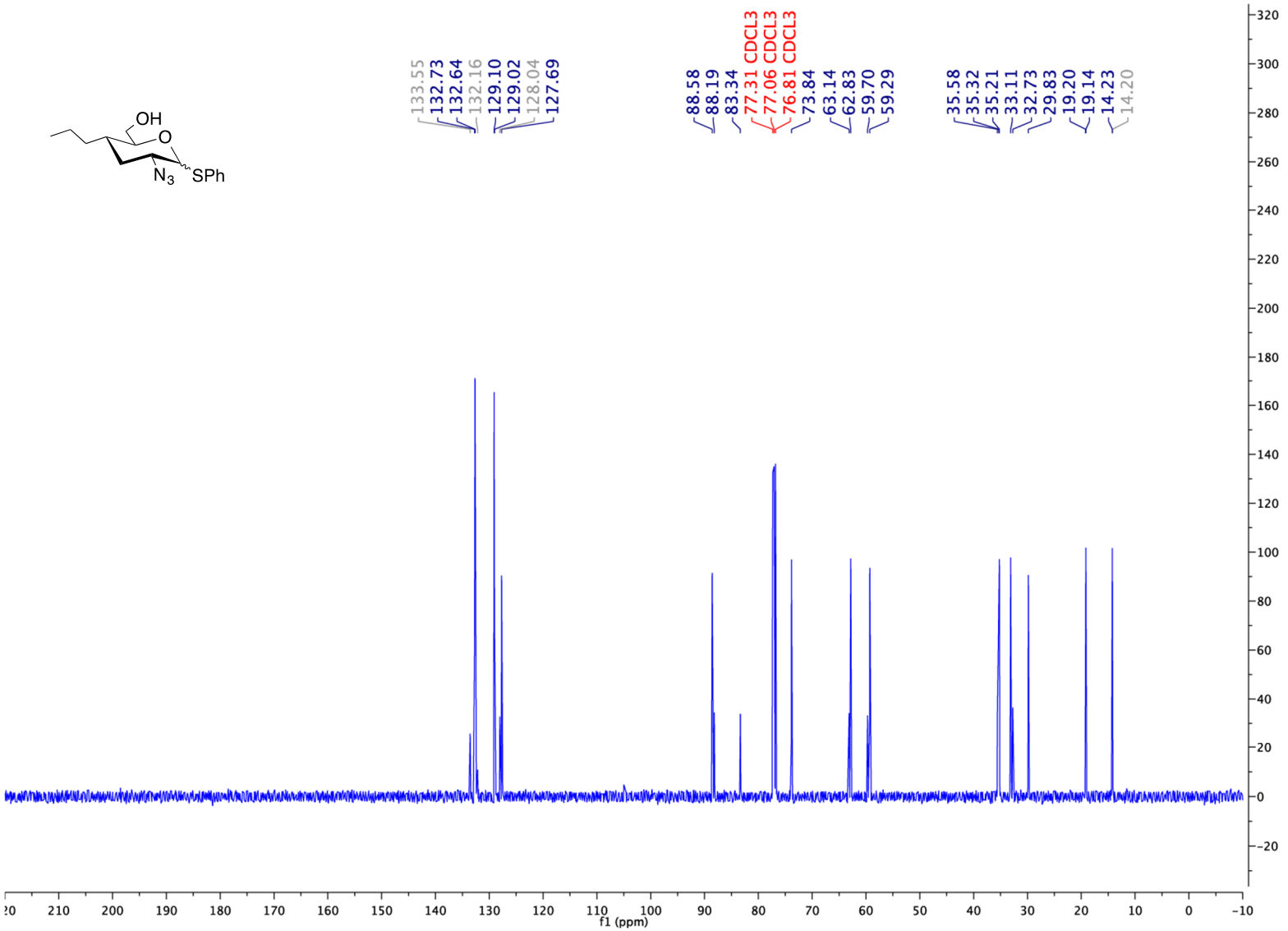
Phenyl 2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (43)

^{1}H NMR (500 MHz, $CDCl_3$)



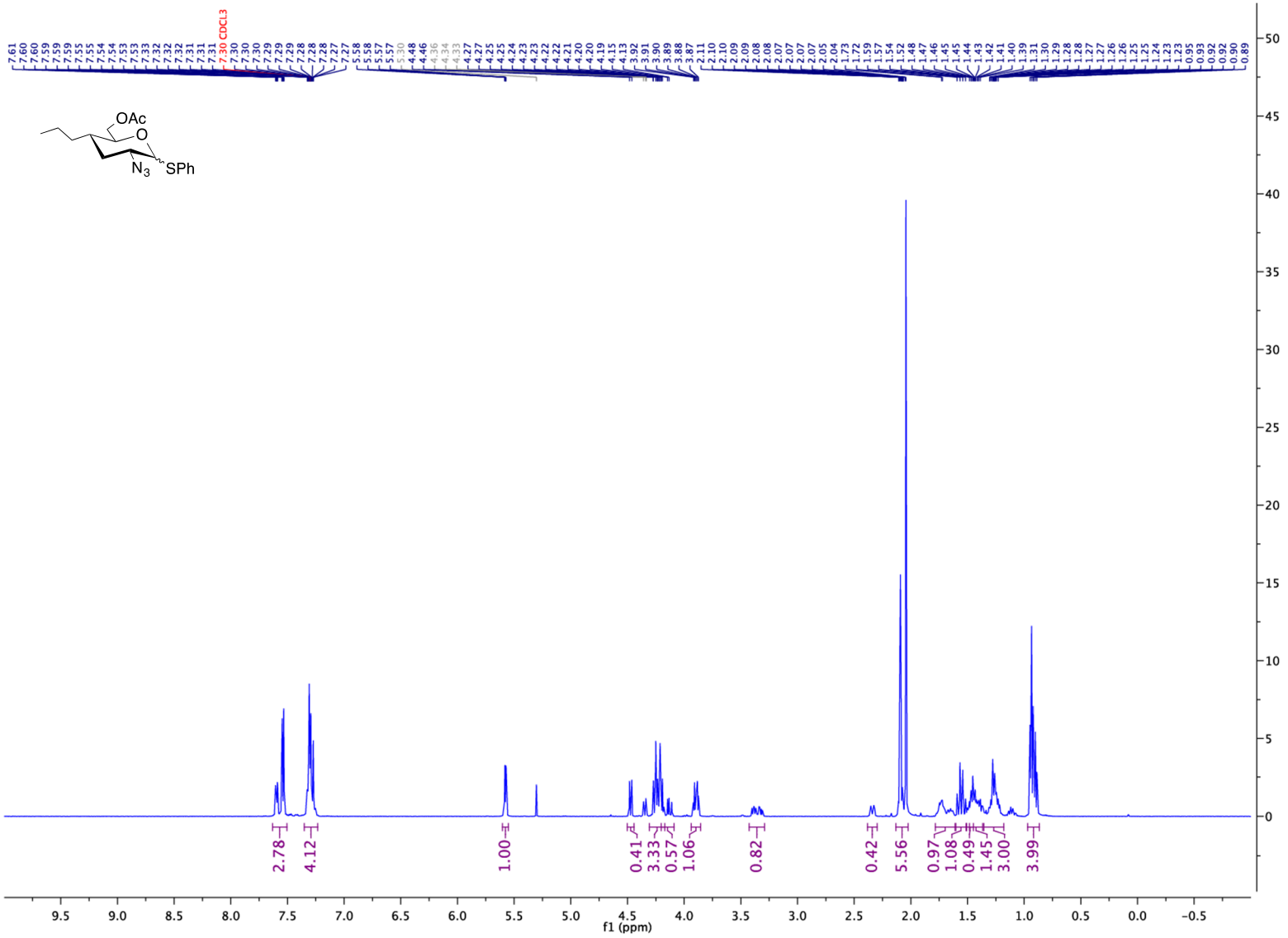
Phenyl 2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (43)

[^{13}C-NMR, 125 MHz, D_2O]



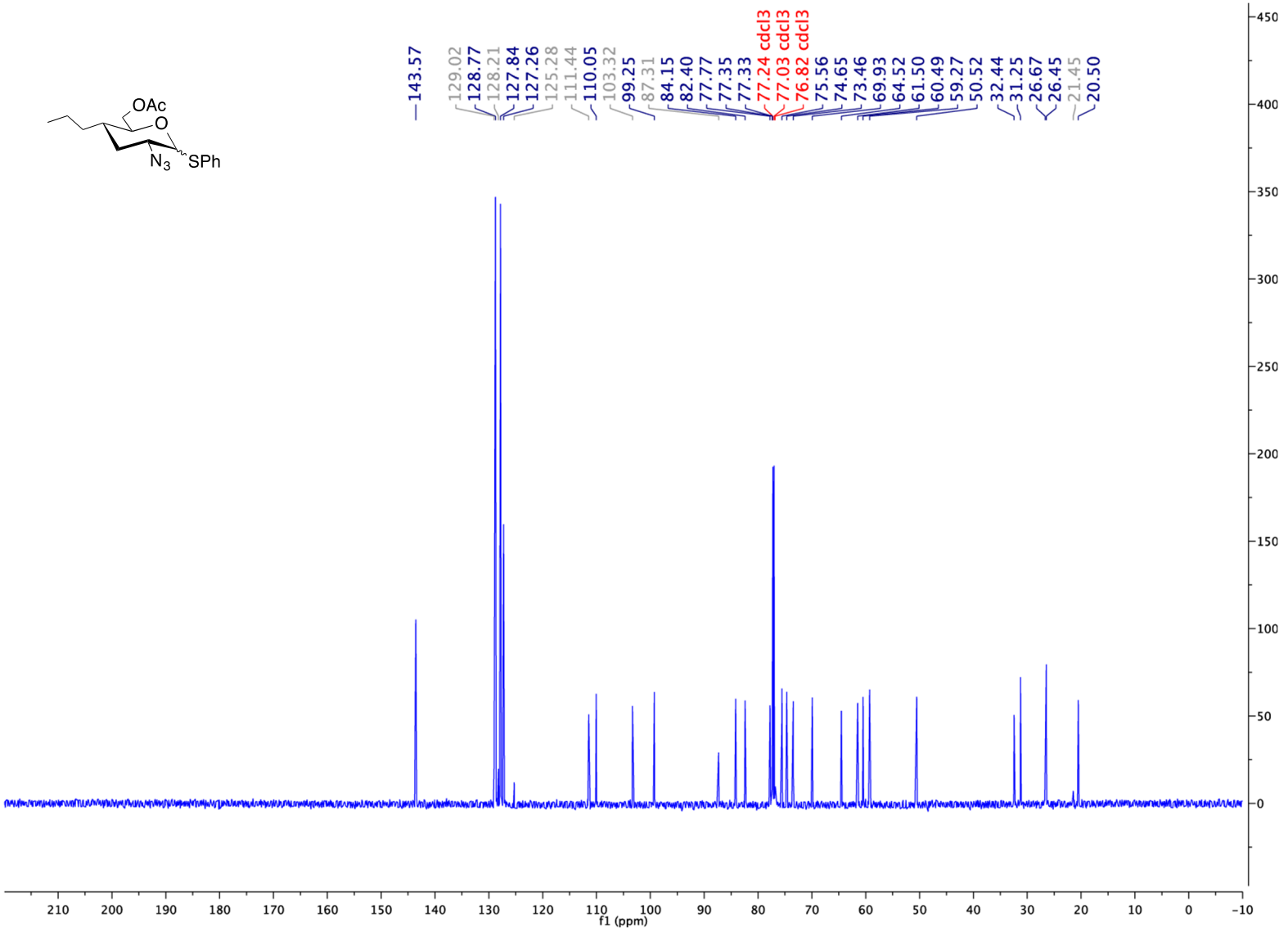
Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (44)

^{1}H NMR (500 MHz, $CDCl_3$)

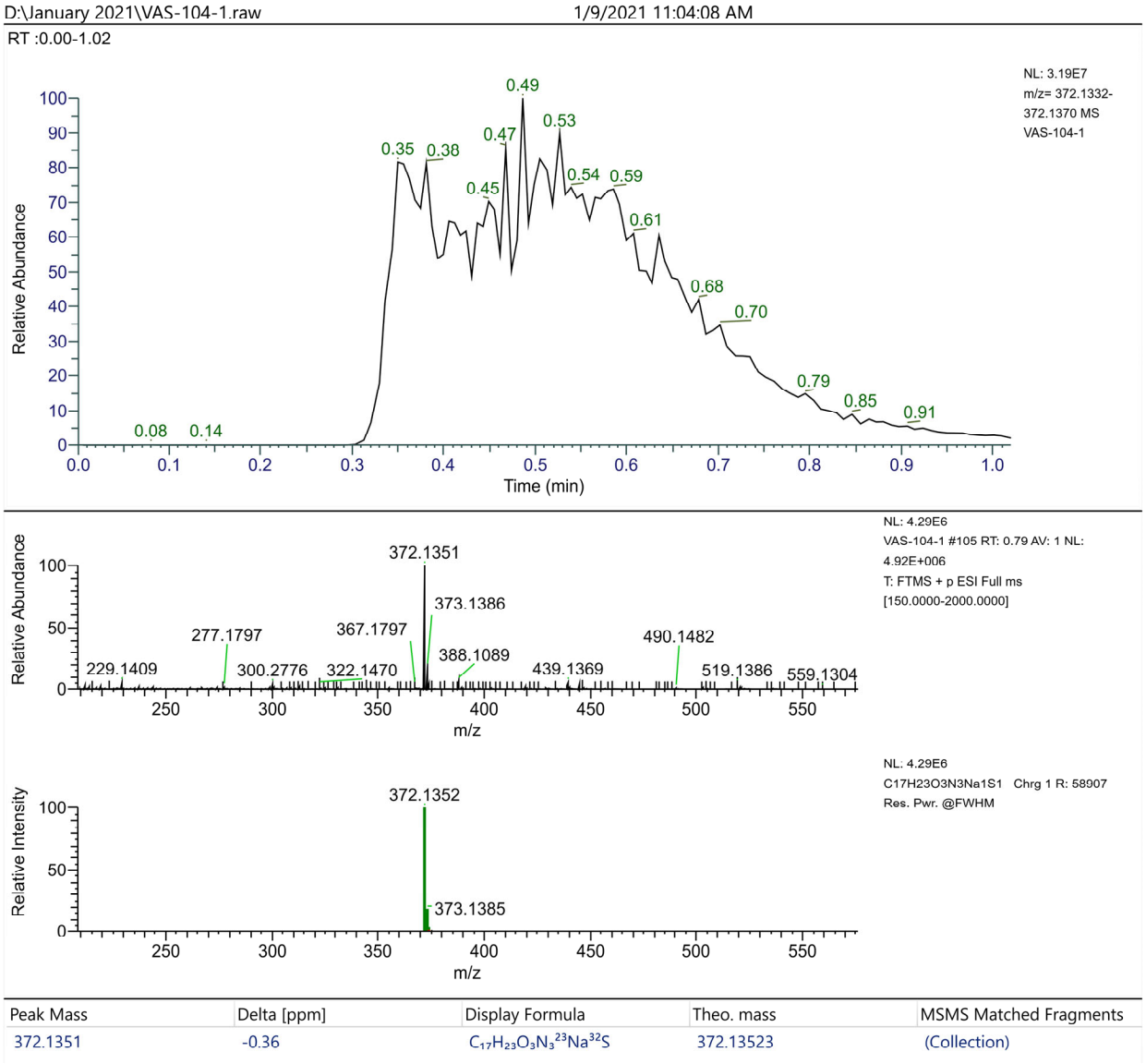


Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (44)

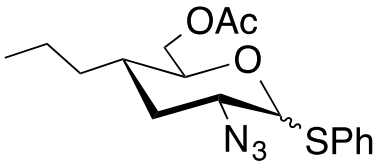
[^{13}C-NMR, 125 MHz, $CDCl_3$]



Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranoside (44)

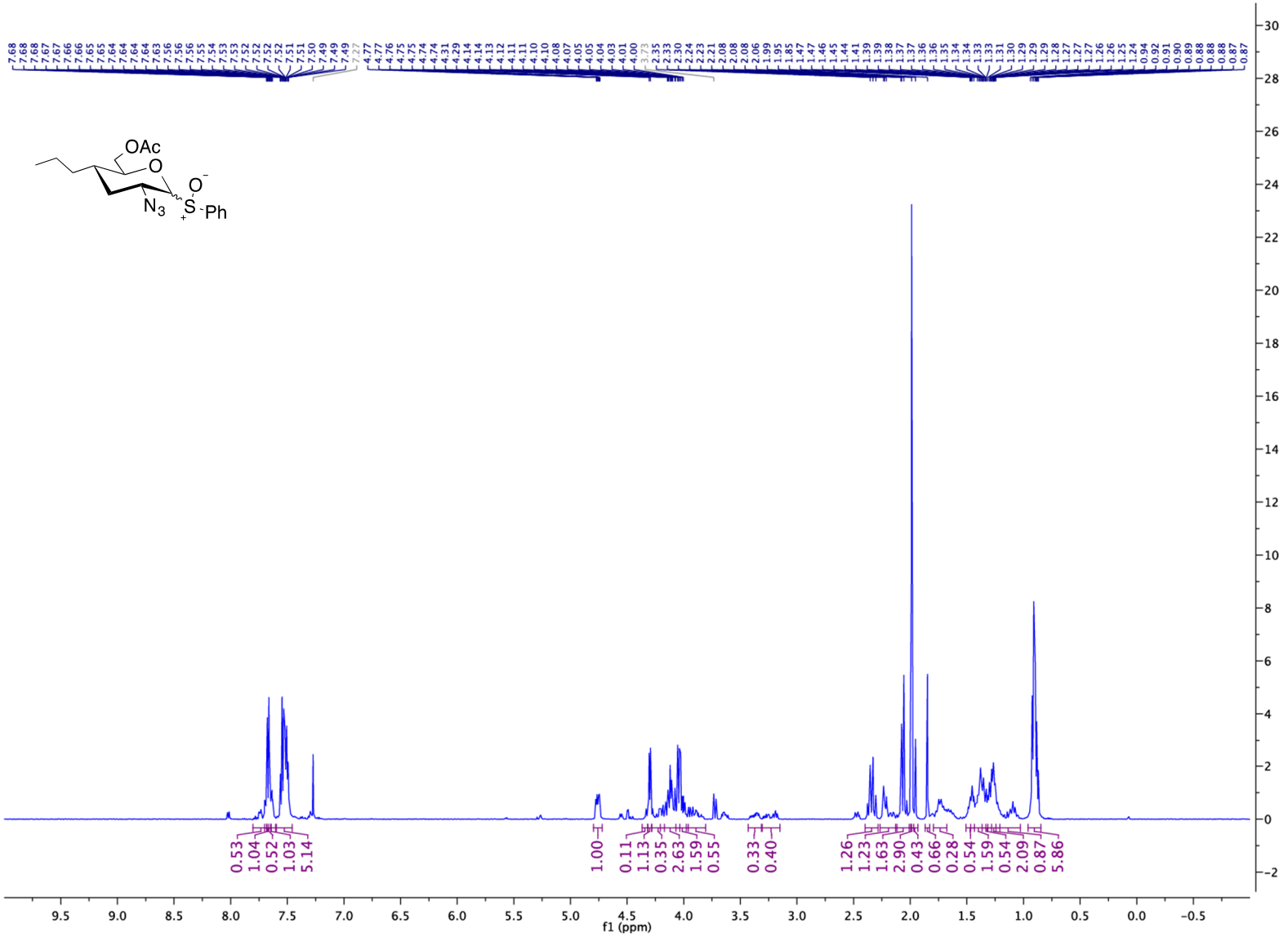


Peak Mass	Delta [ppm]	Display Formula	Theo. mass	MSMS Matched Fragments
372.1351	-0.36	$C_{17}H_{23}O_3N_3{}^{23}Na^{32}S$	372.13523	(Collection)



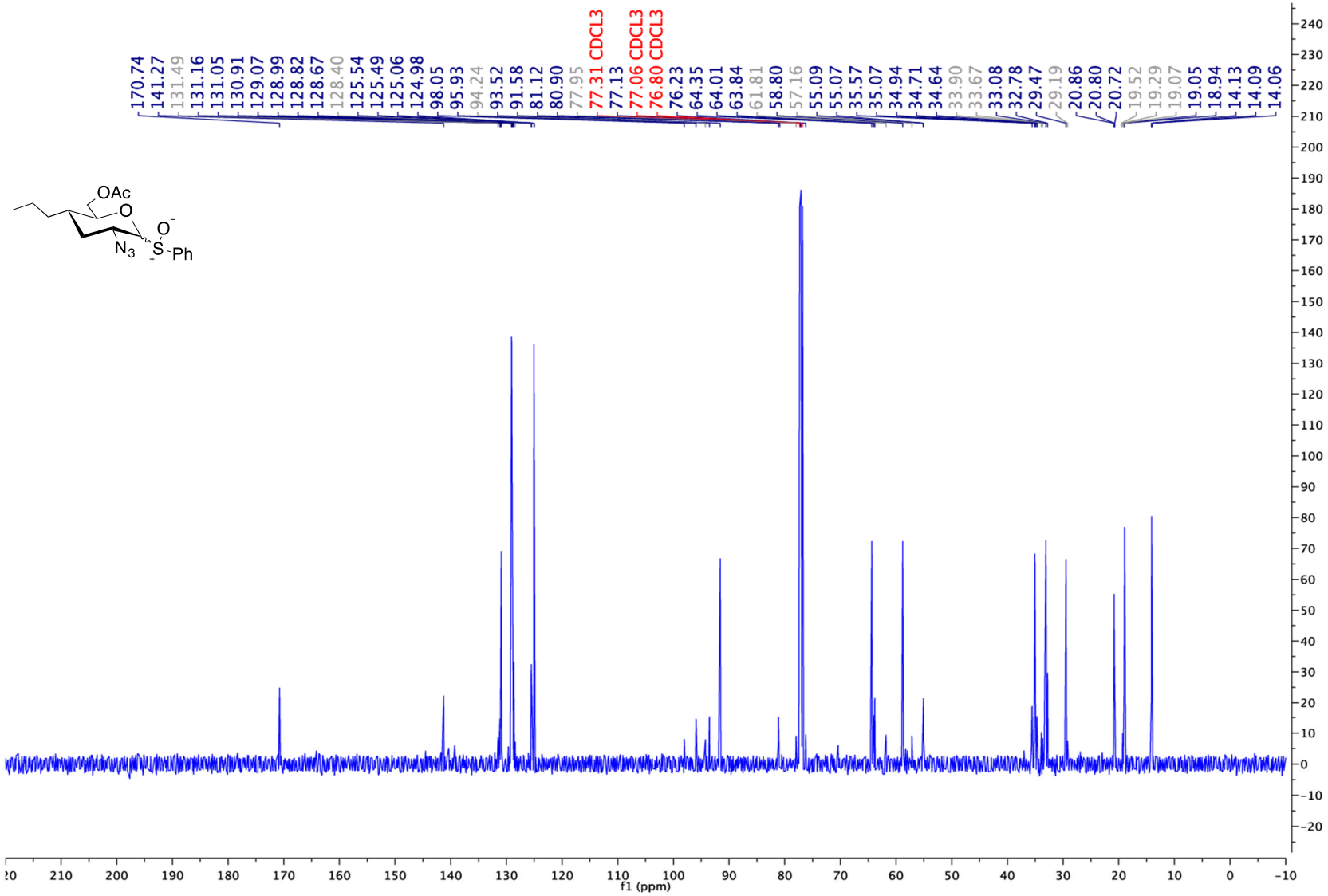
Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranosyl sulfoxide (45)

^{1}H NMR (500 MHz, $CDCl_3$)

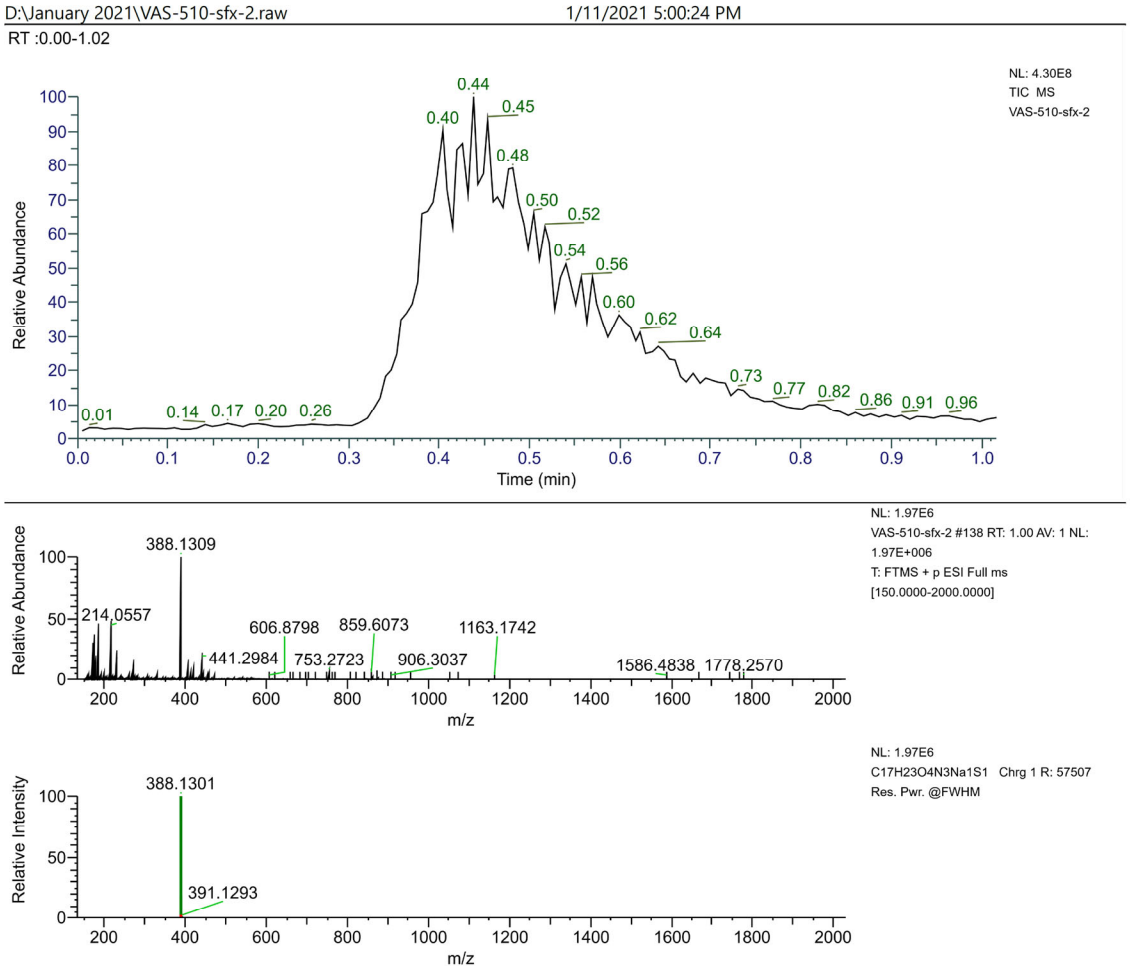


Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranosyl sulfoxide (45)

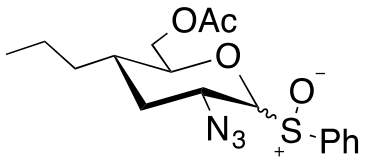
[^{13}C-NMR, 125 MHz, D_2O]



Phenyl 6-*O*-acetyl-2-azido-2,3,4-trideoxy-4-*C*-ethyl-1-thio-β-D-glucopyranosyl sulfoxide (45)

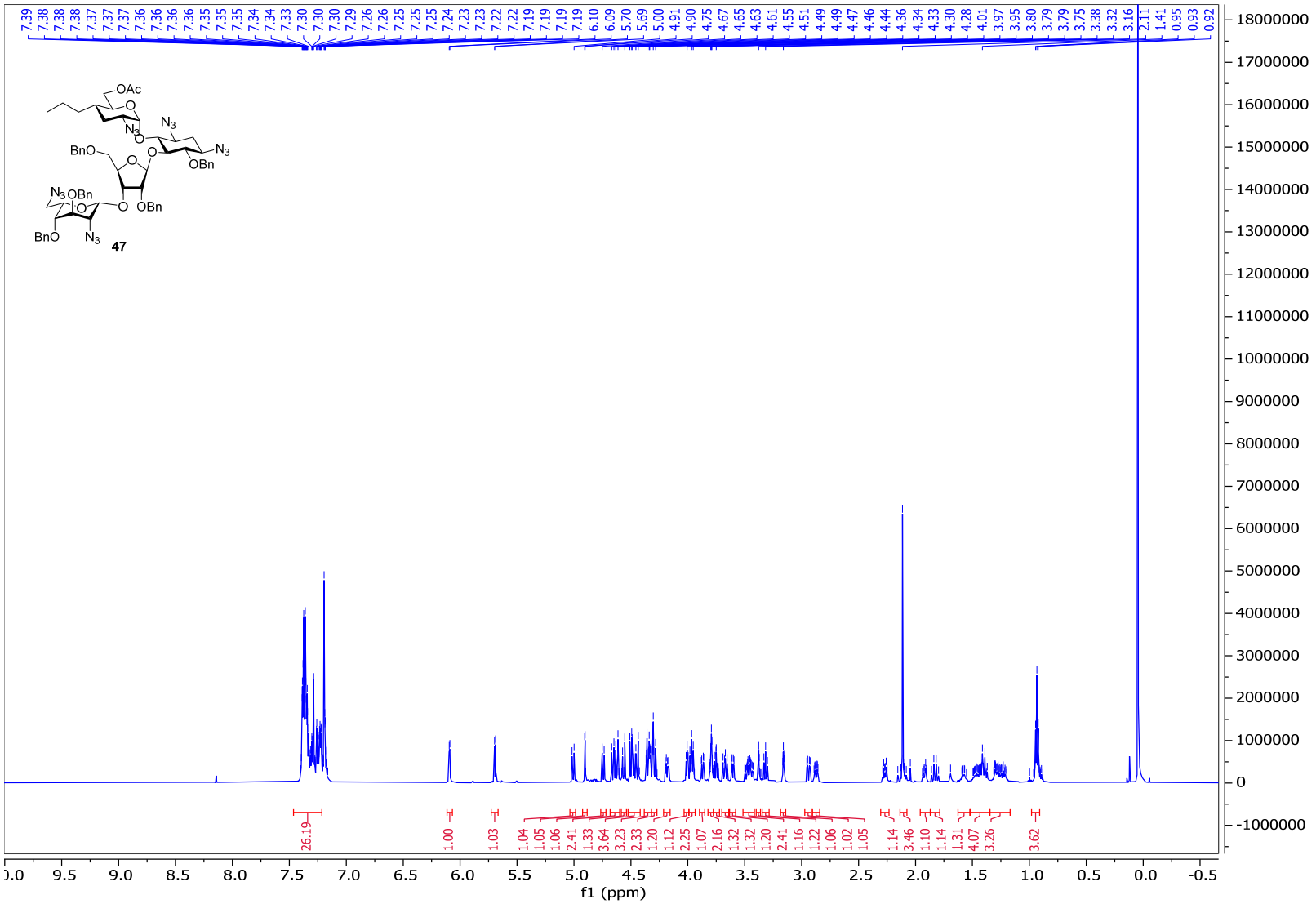


Peak Mass	Delta [ppm]	Display Formula	Theo. mass	MSMS Matched Fragments
388.1309	1.99	$C_{17}H_{23}O_4N_3{}^{23}Na^{32}S$	388.13015	(Collection)
388.1309	-4.21	$C_{19}H_{22}O_4N_3{}^{32}S$	388.13255	(Collection)
388.1309	3.92	$C_{15}H_{26}ON_5{}^{32}S_3$	388.12940	(Collection)
388.1309	0.31	$C_{25}H_{19}O_2N^{23}Na$	388.13080	(Collection)
388.1309	4.48	$C_{22}H_{18}O_4N_3$	388.12918	(Collection)



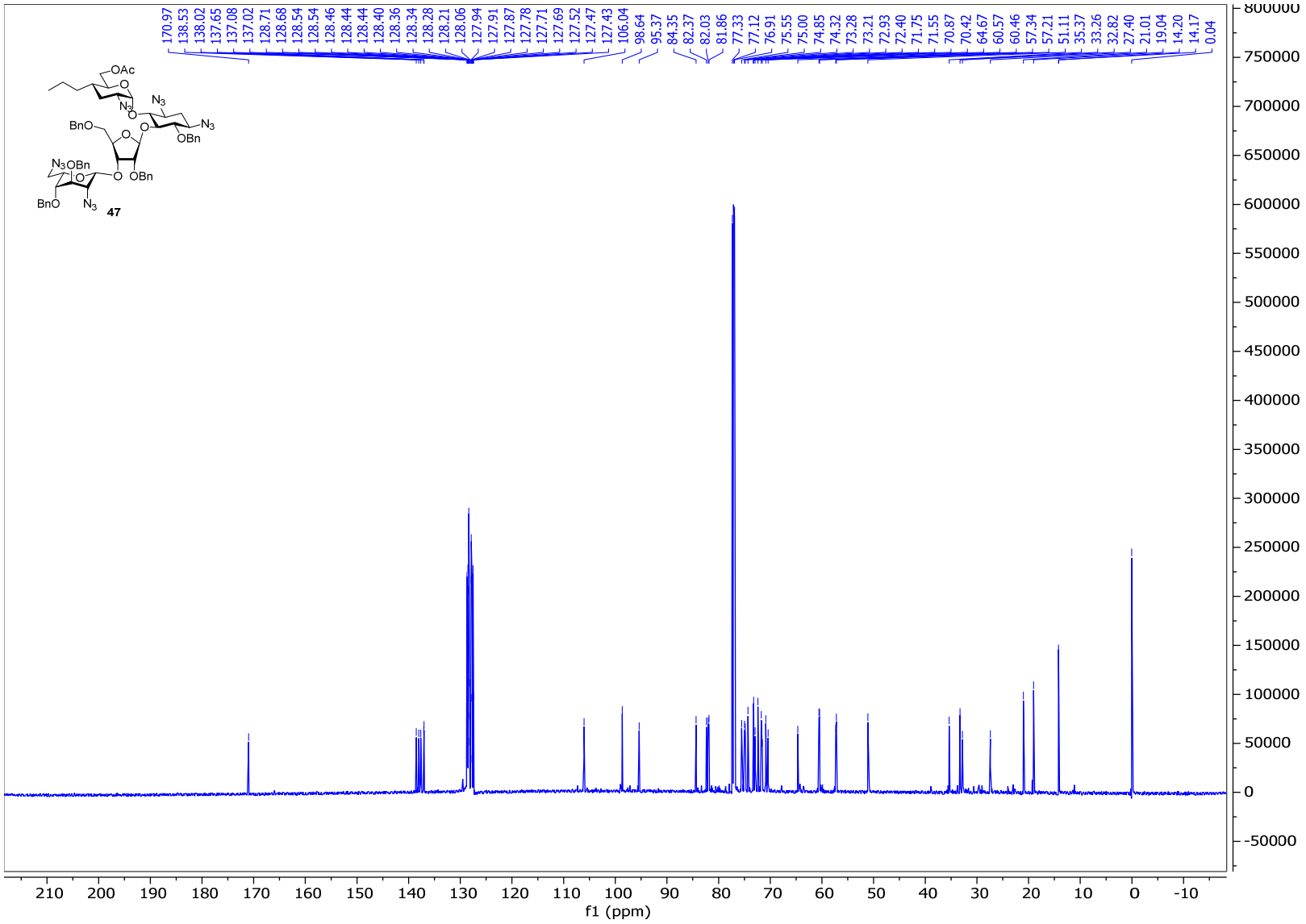
1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)

[^{1}H-NMR, 600 MHz, $CDCl_3$]



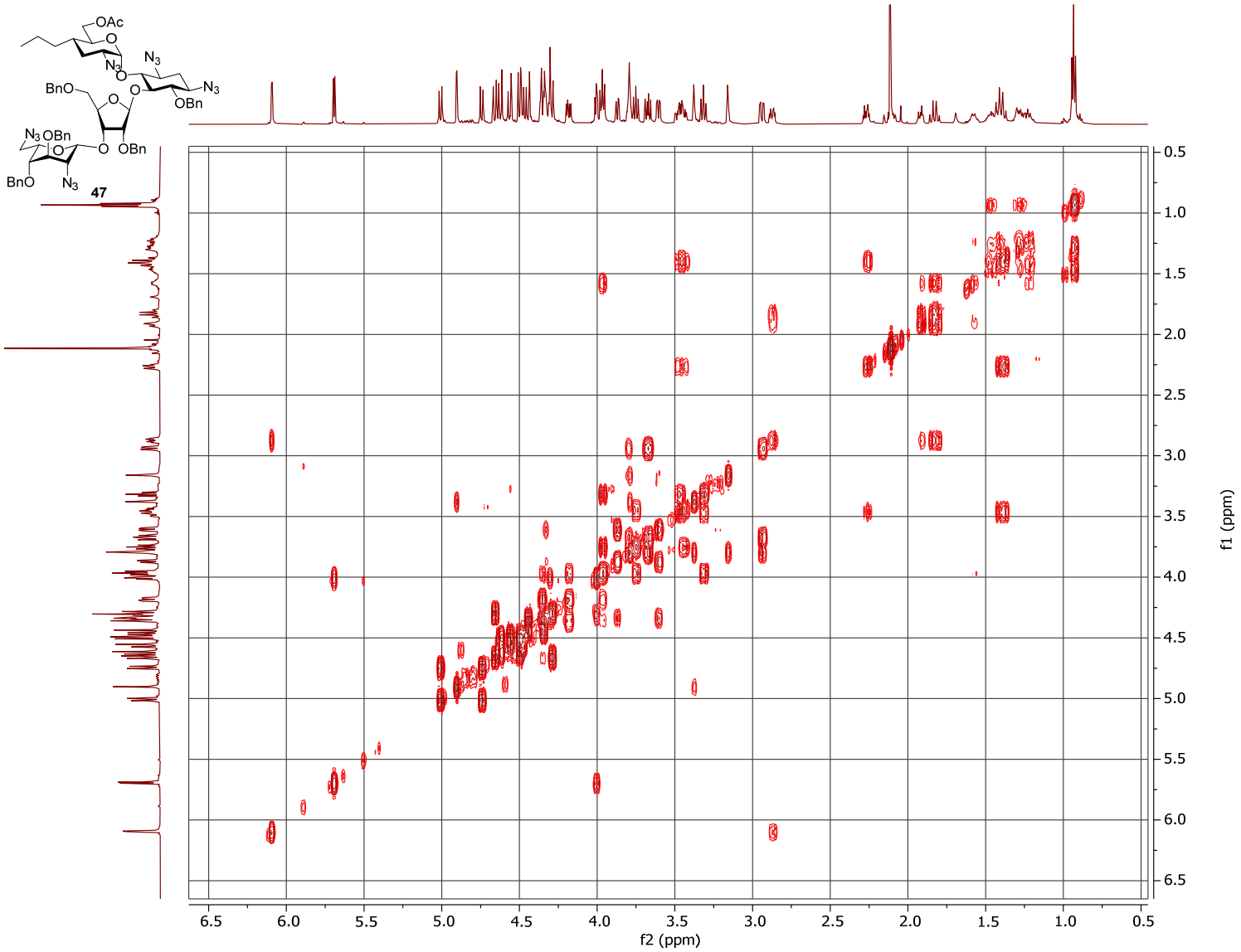
1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)

[^{13}C-NMR, 151 MHz, $CDCl_3$]



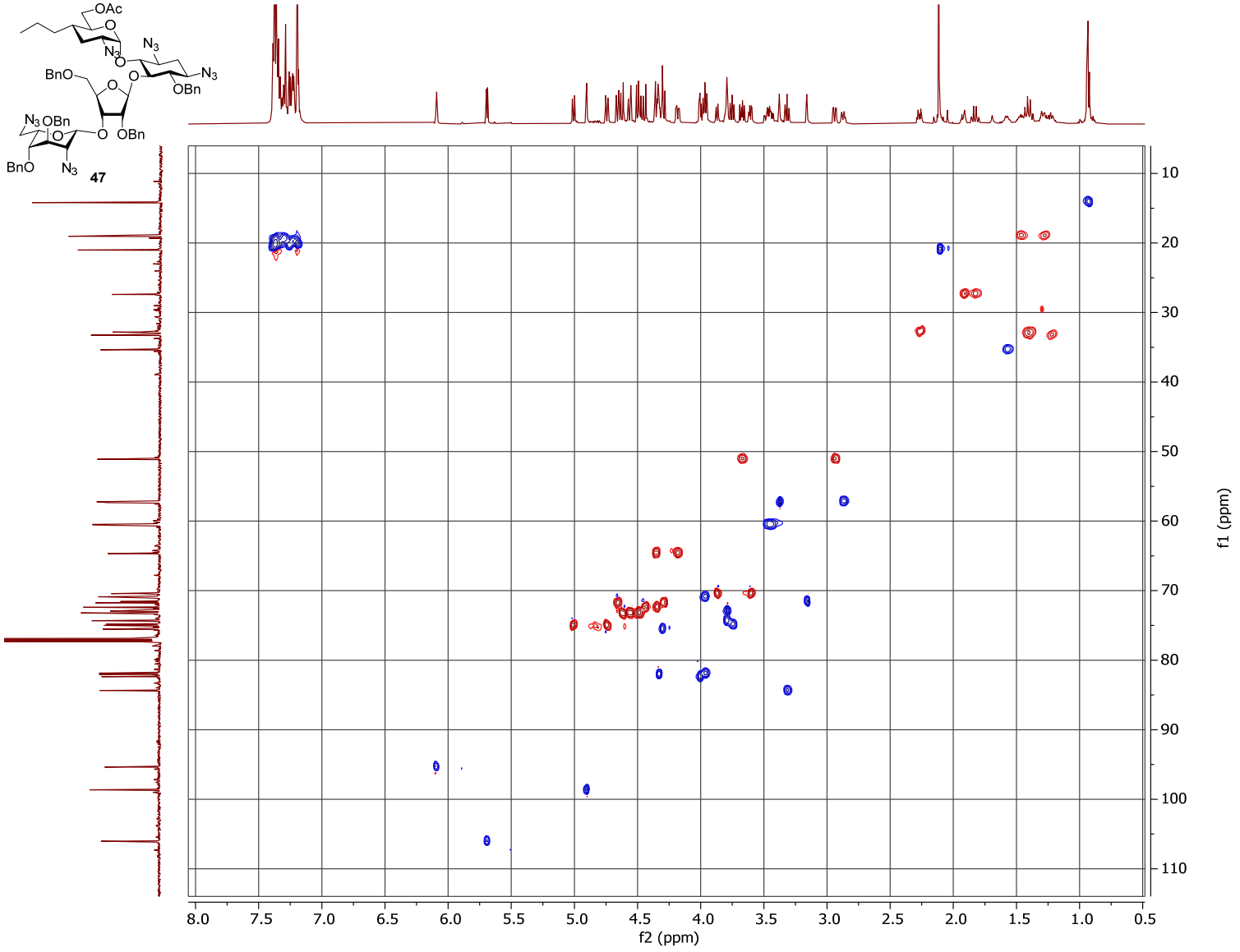
1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)

[^{1}H, ^{1}H COSY, 600 MHz, $CDCl_3$]



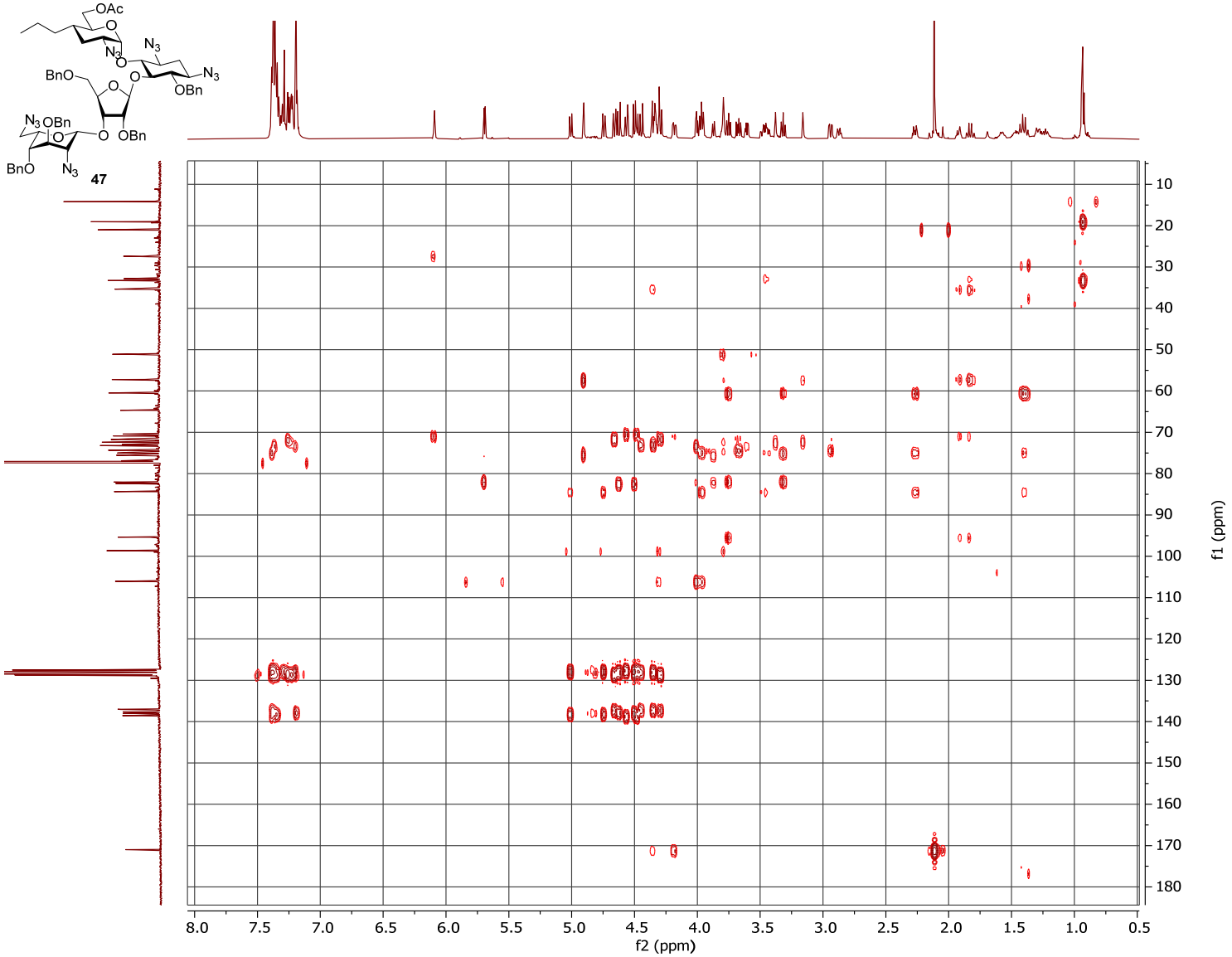
1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)

[^{1}H, ^{13}C HSQC, 600 MHz, 151 MHz, $CDCl_3$]

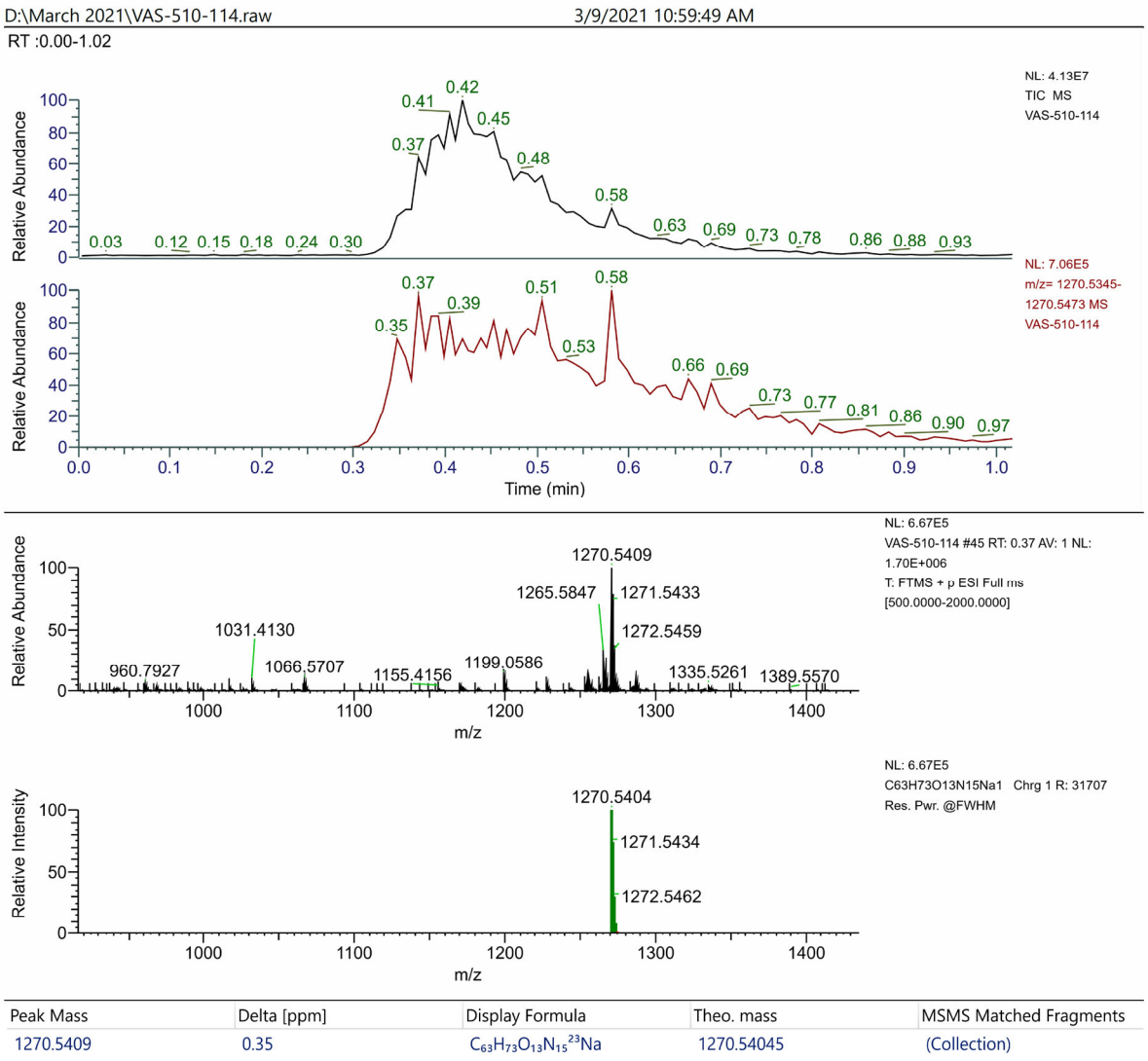


1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)

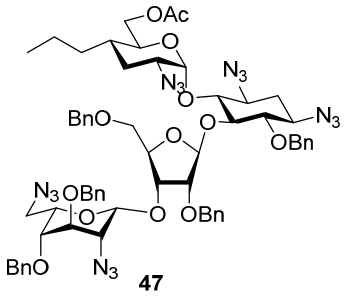
[^{1}H, ^{13}C HMBC, 600 MHz, 151 MHz, $CDCl_3$]



1,3,2′,3′,4′,2‴,6‴-Heptadeoxy-4′-propyl-6′-acetyl-1,3,2′,2‴,6‴-pentaazido-6,2″,5″,3‴,4‴-penta-*O*-benzyl paromomycin (47)



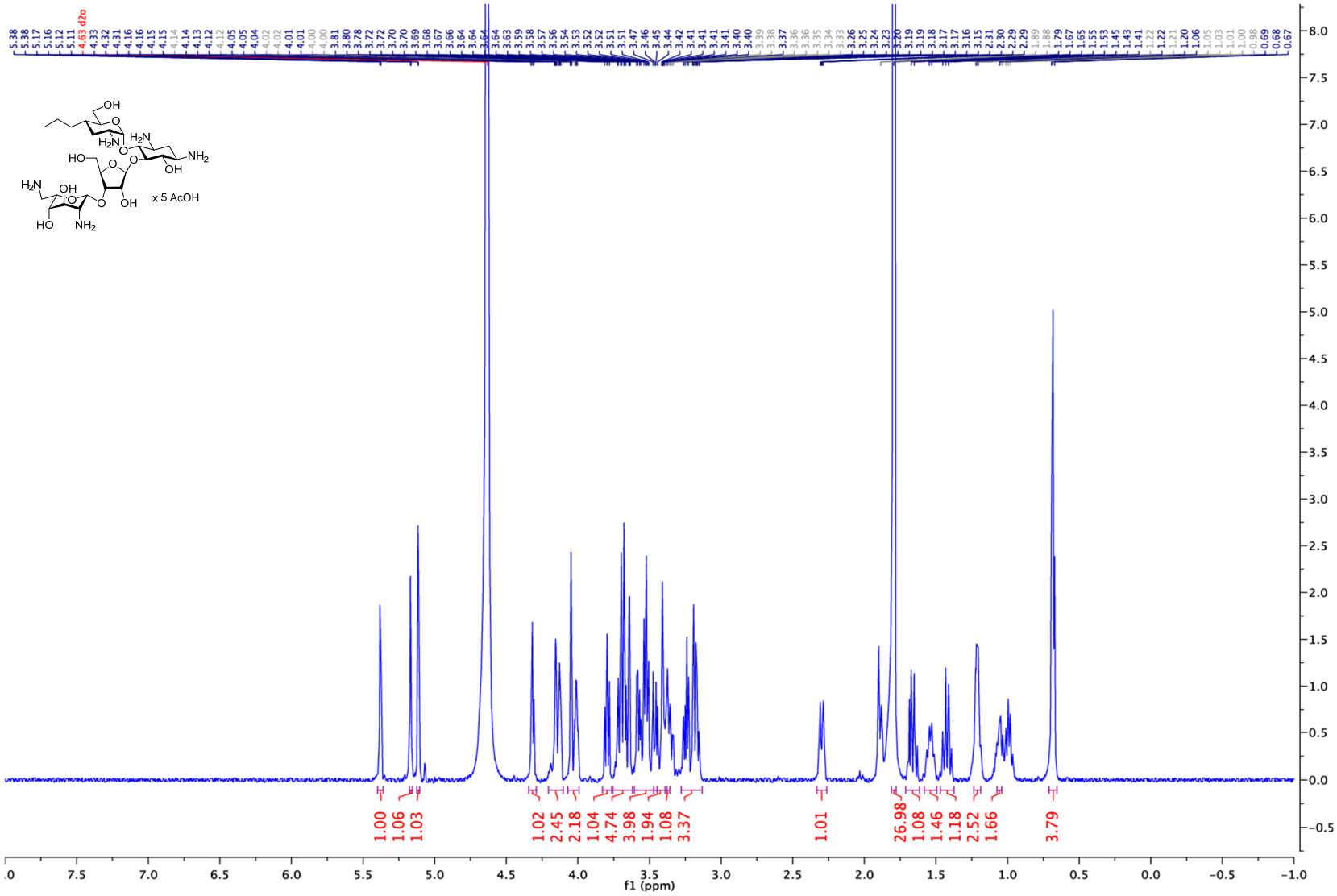
Peak Mass	Delta [ppm]	Display Formula	Theo. mass	MSMS Matched Fragments
1270.5409	0.35	$C_{63}H_{73}O_{13}N_{15}{}^{23}Na$	1270.54045	(Collection)



47

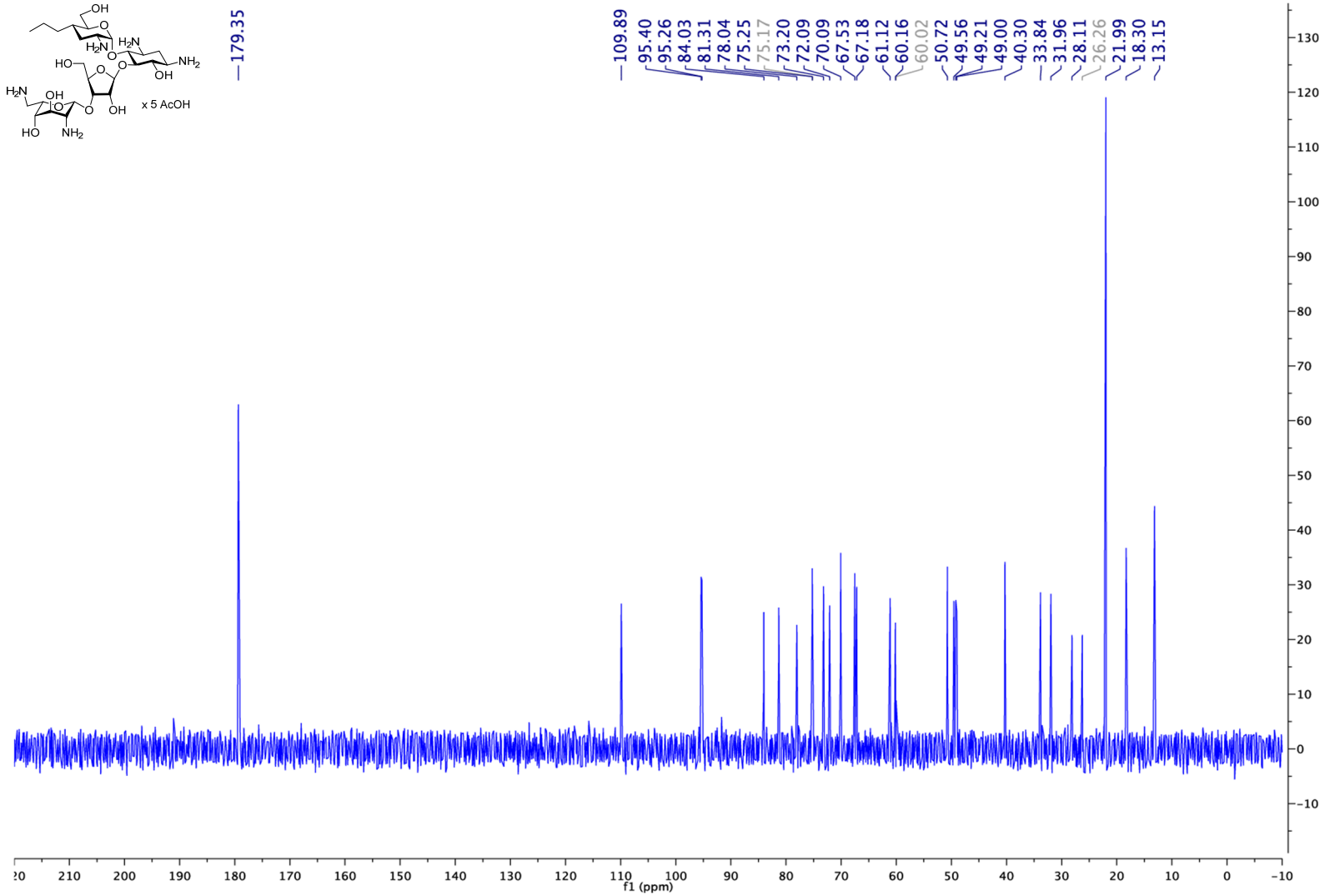
3′,4′-Dideoxy-4′-propyl paromomycin pentaacetate (14)

[^{1}H-NMR, 600 MHz, D_2O]



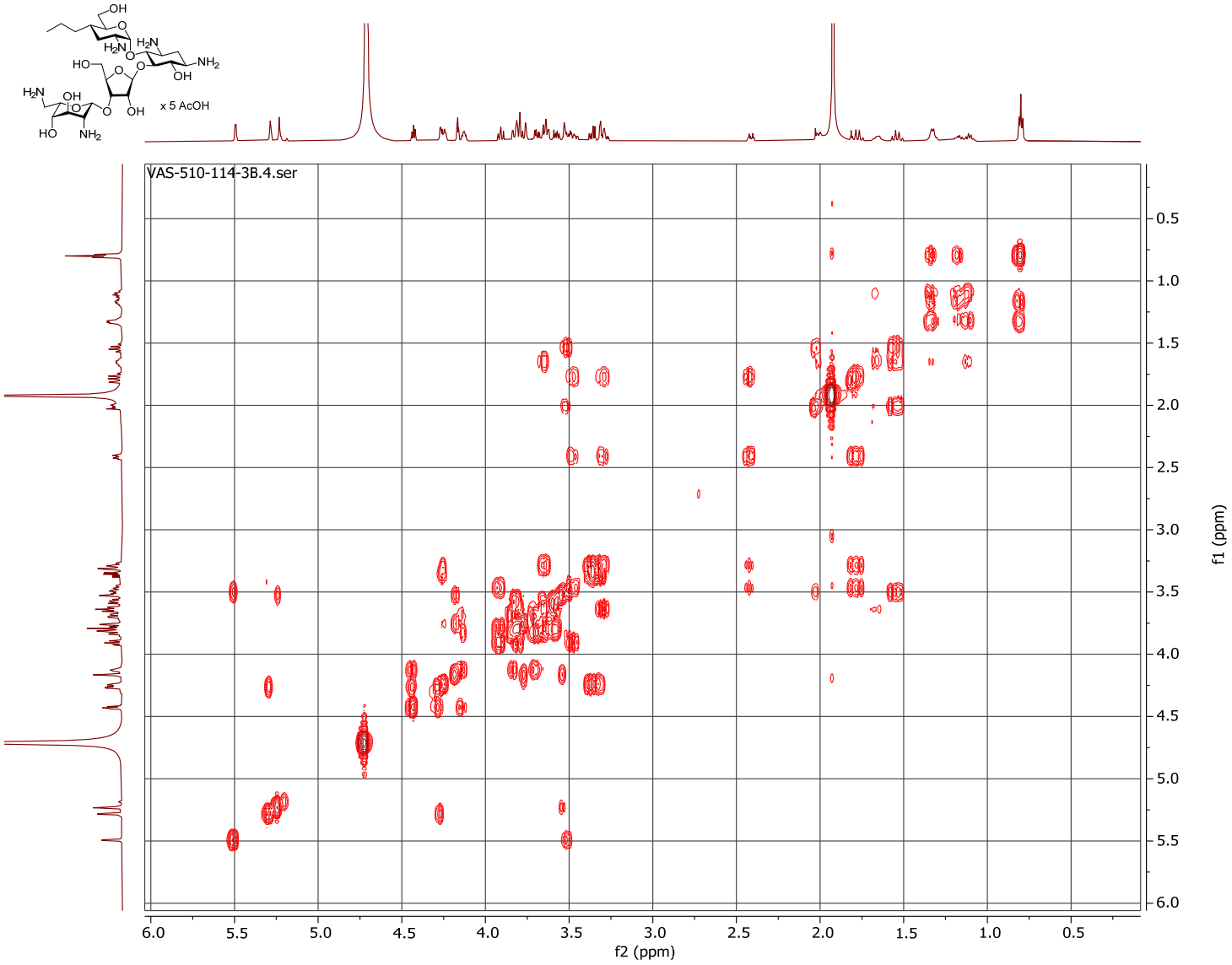
3′,4′-Dideoxy-4′-propyl paromomycin pentaacetate (14)

[^{13}C-NMR, 151 MHz, D_2O]



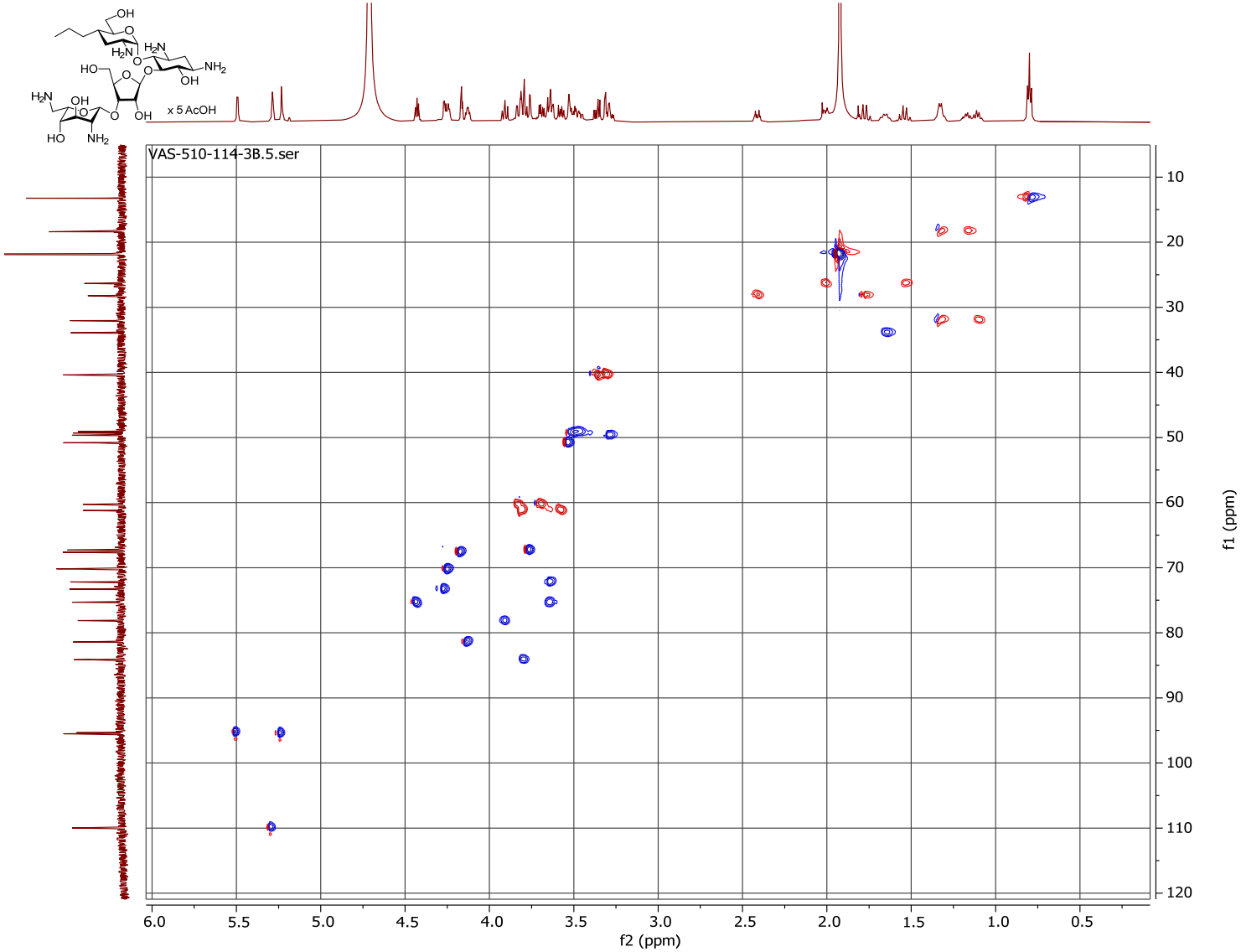
3′,4′-Dideoxy-4′-propyl paromomycin pentaacetate (14)

[^{1}H, ^{1}H COSY, 600 MHz, $CDCl_3$]



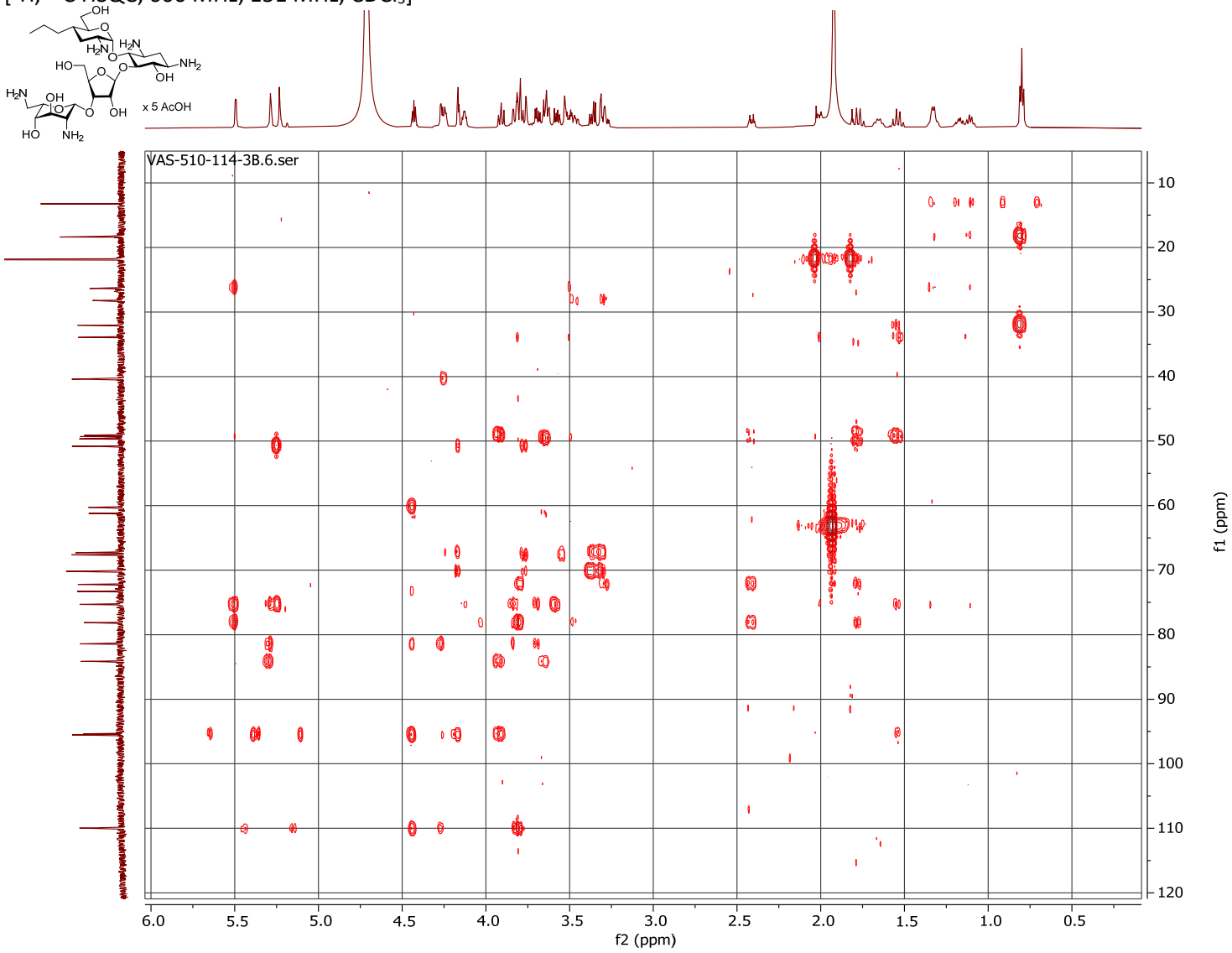
3′,4′-Dideoxy-4′-propyl paromomycin pentaacetate (14)

[^{1}H, ^{13}C HSQC, 600 MHz, 151 MHz, $CDCl_3$]

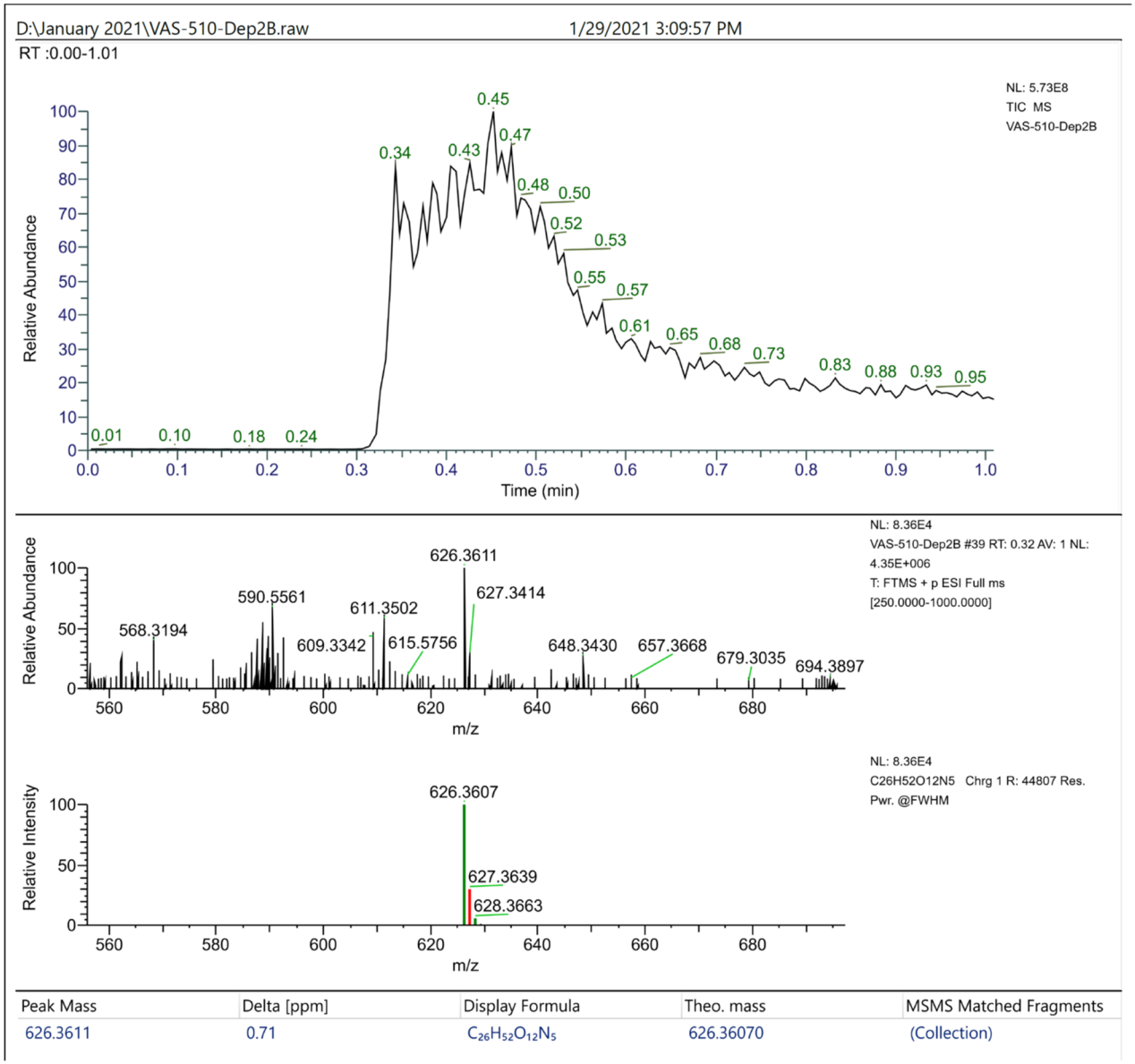


3',4'-Dideoxy-4'-propyl paromomycin pentaacetate (14)

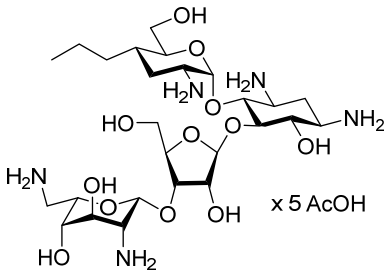
[^{1}H, ^{13}C HSQC, 600 MHz, 151 MHz, $CDCl_3$]



3′,4′-Dideoxy-4′-propyl paromomycin pentaacetate (14)

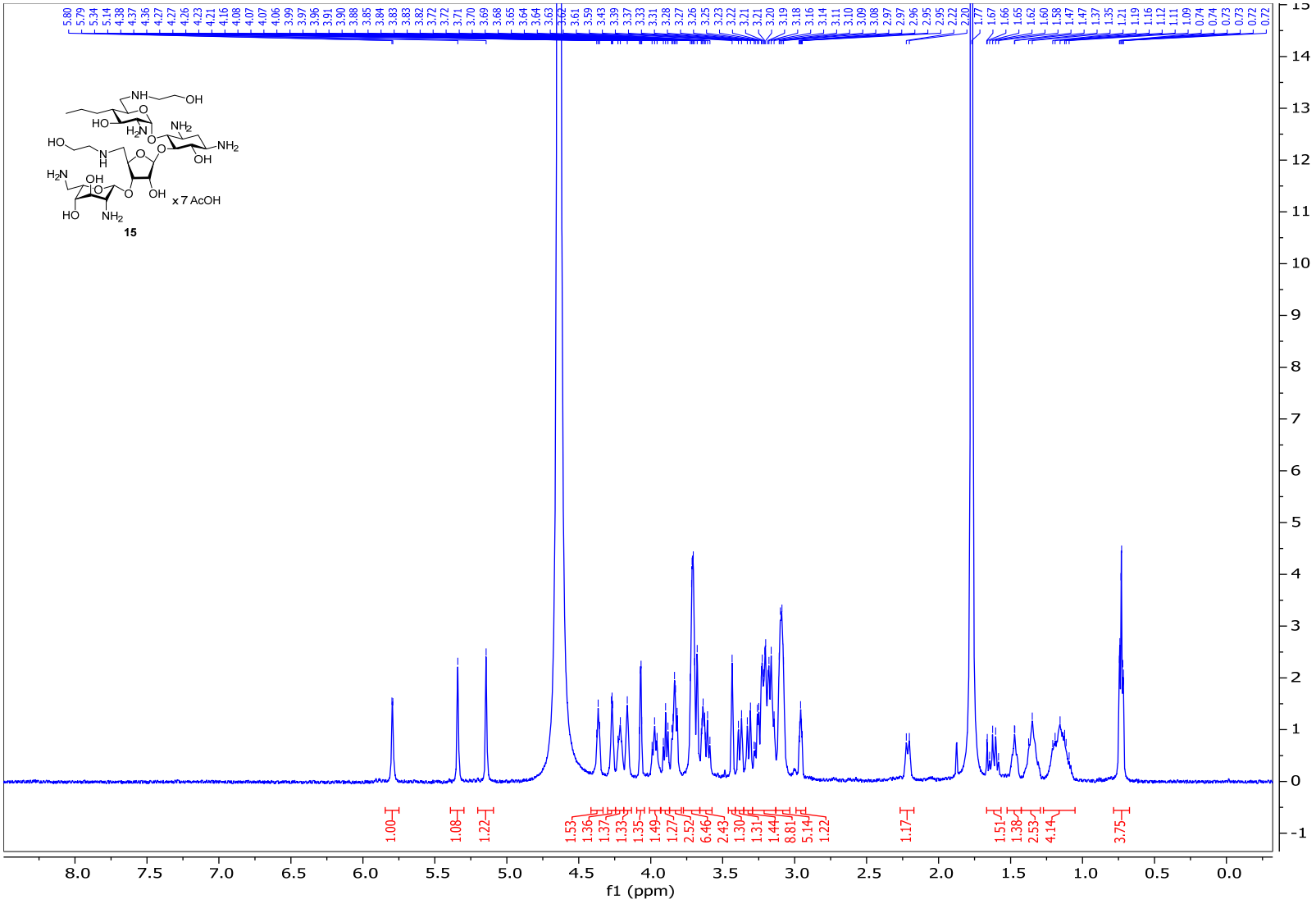


Peak Mass	Delta [ppm]	Display Formula	Theo. mass	MSMS Matched Fragments
626.3611	0.71	$C_{26}H_{52}O_{12}N_5$	626.36070	(Collection)



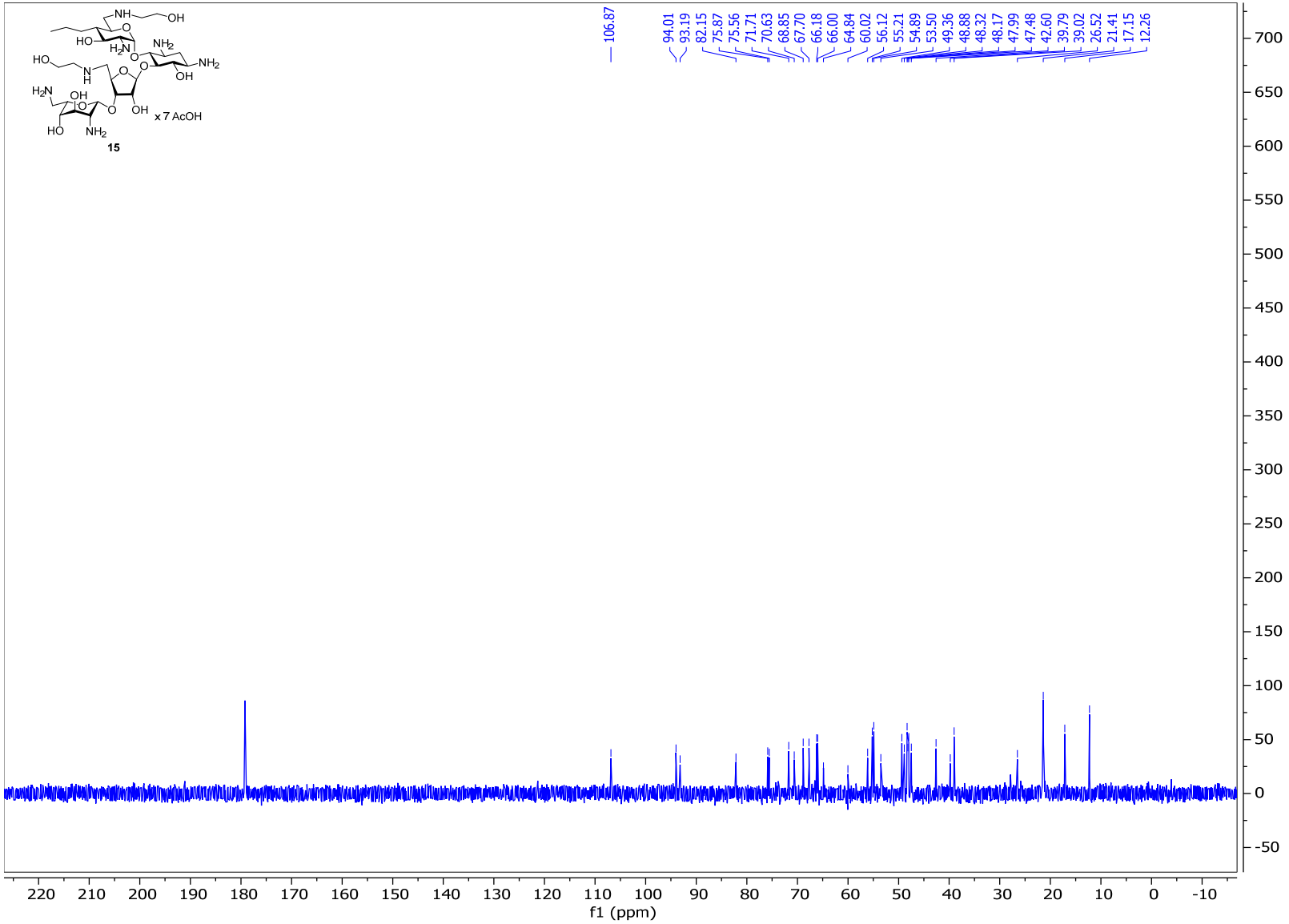
4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

[^{1}H-NMR, 600 MHz, D_2O]



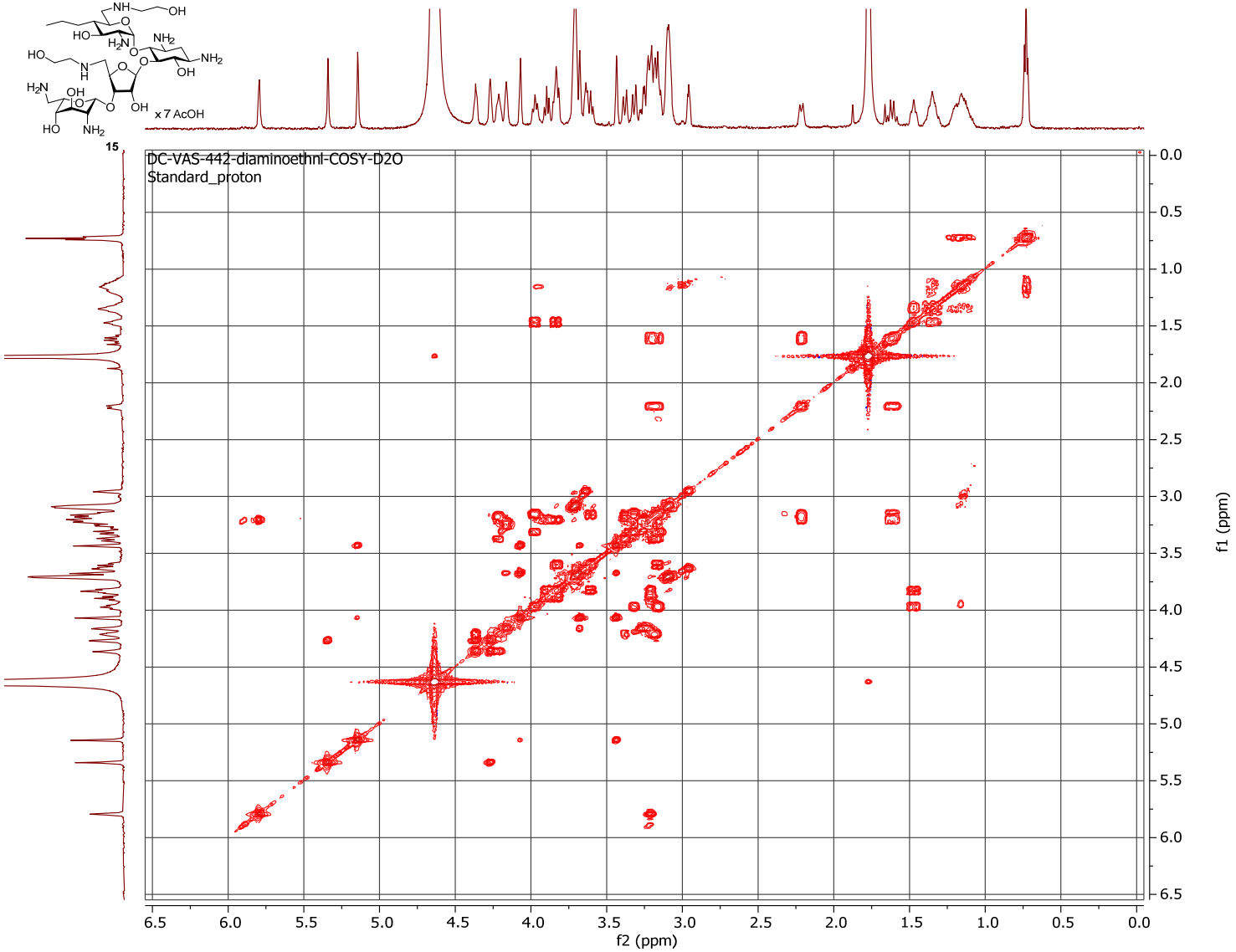
4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

[^{13}C-NMR, 151 MHz, D_2O]



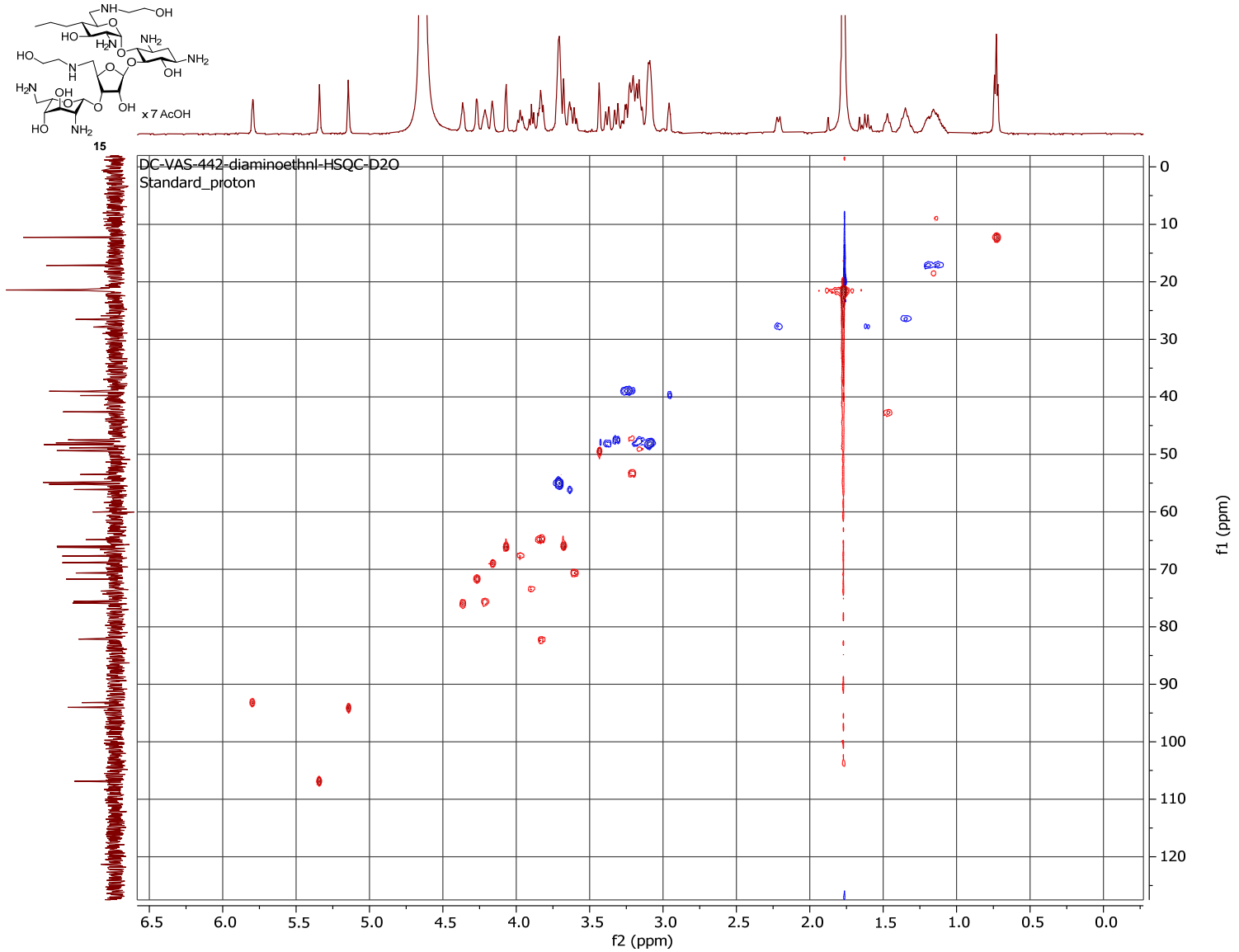
4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

[^{1}H, ^{1}H COSY, 600 MHz, $CDCl_3$]



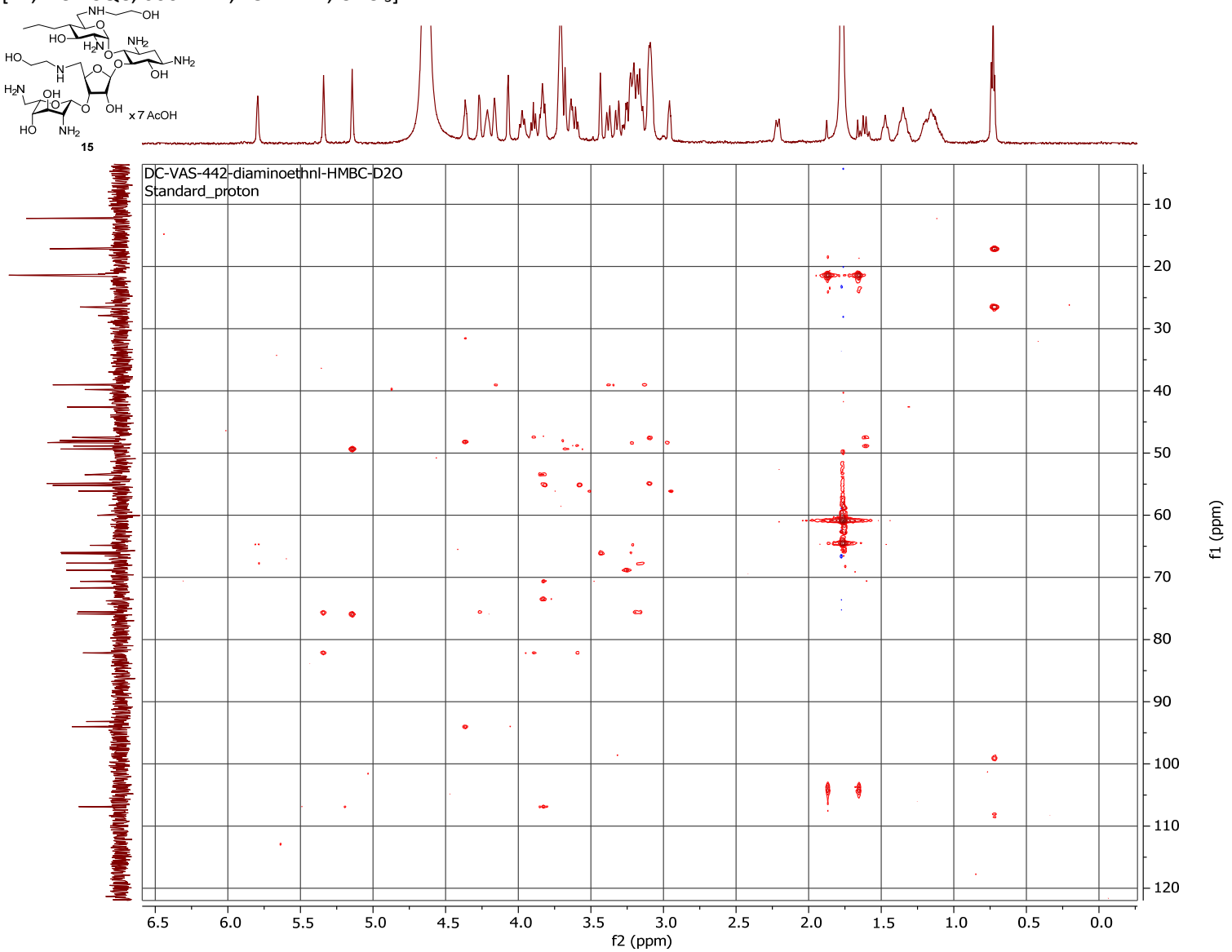
4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

[^{1}H, ^{13}C HSQC, 600 MHz, 151 MHz, $CDCl_3$]



4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

[^{1}H, ^{13}C HSQC, 600 MHz, 151 MHz, $CDCl_3$]



4′,6′,5″-Trideoxy-6′,5″-[bis-*N*-(2-hydroxyethyl)]-4′-propyl paromomycin heptaacetate (15)

Elemental Composition Report

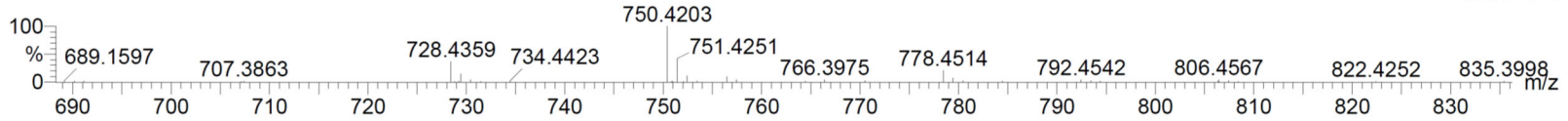
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

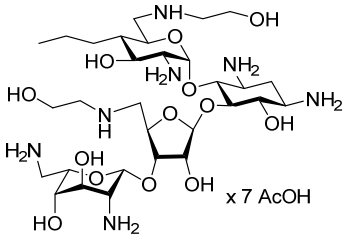
Monoisotopic Mass, Even Electron Ions
4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 29-31 H: 59-62 N: 5-8 O: 12-14 Na: 0-1
vas-diethanolamin-final

2017_0422_24 11 (0.213)

LCT Premier
1: TOF MS ES+
1.27e+003



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
750.4203	750.4225	-2.2	-2.9	3.5	26.2	0.0	C30 H61 N7 O13 Na



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