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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 23 ºC 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.

Synthesis and Characterization

6,3',4',2'',3''',4'''-Hexa-*O*-acetyl -5''-carboxy-1,3,2',6',2''',6'''-hexadesamino-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomomycin (24). To a stirred solution of **23** (1.13 g, 0.95 mmol) in pyridine (9.5 mL) was added trityl chloride (560 mg, 1.9 mmol). Additional trityl chloride (2.04 g, 7.3 mmol) was added over the course of the reaction as progress slowed. After 52 h, Ac_2O (5 mL) and DMAP (30 mg, 0.25 mmol) were added. After 16 h, the reaction was quenched with H_2O, diluted with EtOAc, and washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness, after which the crude product was purified through silica gel chromatography (0-10% MeOH in DCM). The resulting solid (1.31 g) was dissolved in DCM (14 mL) and $FeCl_3{\cdot}6H_2O$ (418 mg, 1.55 mmol) was added. After 2 h, the reaction was diluted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to dryness, after which the crude product was purified through silica gel chromatography (0-10% MeOH in DCM). A portion (246 mg) of the resulting solid (624 mg) was dissolved in MeCN:H_2O (1.7 mL, 1:1) and cooled to 0 °C, after which $NaHCO_3$ (28 mg, 0.33 mmol), BAIB (150 mg, 0.47 mmol) and TEMPO (14 mg, 0.09 mmol) were added. After 3 h, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous $Na_2S_2O_3$. The reaction mixture was diluted with EtOAc and washed with brine, after which the organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified via silica gel chromatography (0-20% MeOH in DCM, R_f = 0.1 in 6% MeOH in DCM) to give **24** (181 mg, 33% over four steps) as a white solid. $[\alpha]_D^{23}$ = +9.3 (*c* = 1.0, EtOAc); ^1H NMR (900 MHz, MeOD) δ 6.45 (s, 1H), 5.43 (d, *J* = 5.2 Hz, 1H), 5.20 (dd, *J* = 11.0, 9.2 Hz), 5.02 – 5.00 (m, 2H), 5.00 – 4.98 (m, 1H), 4.88 – 4.84 (m, 2H), 4.82 – 4.80 (m, 1H), 4.57 (dd, *J* = 4.6, 2.9 Hz, 1H), 4.54 (d, *J* = 2.9 Hz, 1H), 4.31 (dd, *J* = 11.0, 3.9 Hz, 1H), 4.26 – 4.22 (m, 2H), 4.22 – 4.20 (m, 1H), 4.18 (ddd, *J* = 12.6, 10.5, 4.4 Hz, 1H), 4.10 (t, *J* = 8.8 Hz, 1H), 4.02 (dt, *J* = 10.3, 3.4 Hz, 1H), 3.90 (dd, *J* = 10.2, 8.4 Hz, 1H), 3.77 (dd, *J* = 14.6, 3.7 Hz, 1H), 3.65 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.44 – 3.38 (m, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (q, *J* = 13.0 Hz, 1H), 1.99 (s, 3H), 1.97 (dt, *J* = 13.0, 4.5 Hz, 1H), 1.93 (s, 3H); ^{13}C NMR (226 MHz, MeOD) δ 172.1, 170.6, 170.4, 170.3, 169.9, 169.2, 168.7, 162.2 – 153.8 (m), 118.4 – 113.8 (m), 107.1, 98.0, 95.2, 83.5, 80.9, 79.1, 75.8, 75.5, 75.0, 71.6, 70.6, 68.7, 68.0, 67.6, 65.6, 51.4, 49.0, 48.22, 48.15, 39.2, 38.8, 30.6, 19.9, 19.2, 19.12, 19.09, 19.07, 19.0. ESI-HRMS: *m/z* calc for $C_{47}H_{50}N_6O_{26}F_{18}Na$ $[M+Na]^+$ 1479.23795, found 1479.2375.

6,3',4',2'',3''',4'''-Hexa-*O*-acetyl -1,3,2',6',2''',6'''-hexadesamino-4''-deshydroxymethyl-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomycin (26). To a stirred solution of **24** (505 mg, 0.35 mmol) in THF (3.4 mL) at 0 °C covered in foil was added Et_3N (0.06 mL, 0.42 mmol) followed by 1-oxa-2-oxo-3-thiaindolizinium chloride **25** (132 mg, 0.69 mmol). After 40 mins, *tert*-dodecyl thiol (0.41 mL, 1.99 mmol) was added and the reaction was exposed to white light. After 1 h, the reaction was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified via silica gel chromatography (25-70% EtOAc in hexanes, R_f = 0.6 in 6% MeOH in DCM) to give **26** (205 mg, 42%) as an off-white solid. $[\alpha]_D^{23}$ = +16.9 (*c* = 1.0, EtOAc); ^{1}H NMR (900 MHz, MeOD) δ 5.65 (d, *J* = 3.8 Hz, 1H), 5.29 (d, *J* = 3.4 Hz, 1H), 5.24 (dd, *J* = 11.0, 9.1 Hz, 1H), 5.03 – 4.97 (m, 2H), 4.93 (dd, *J* = 10.2, 9.2 Hz, 1H), 4.91 (d, *J* = 2.0 Hz, 1H), 4.86 – 4.84 (m, 1H), 4.83 (dd, *J* = 5.2, 3.4 Hz, 1H), 4.51 (q, *J* = 5.4 Hz, 1H), 4.41 (dd, *J* = 10.9, 3.8 Hz, 1H), 4.30 – 4.24 (m, 1H), 4.22 (td, *J* = 6.8, 1.7 Hz, 1H), 4.20 – 4.17 (m, 1H), 4.17 – 4.15 (m, 1H), 4.10 – 4.07 (m, 3H), 3.83 (dd, *J* = 10.2, 8.7 Hz, 1H), 3.77 (dd, *J* = 10.0, 5.0 Hz, 1H), 3.63 (dd, *J* = 14.7, 4.1 Hz, 1H), 3.53 – 3.48 (m, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 2.01 – 1.97 (m, 2H), 1.96 (s, 3H); ^{13}C NMR (226 MHz, MeOD) δ 170.6, 170.3, 170.2, 169.9, 169.3, 168.7, 158.4 – 156.8 (m), 118.4 – 113.8 (m), 107.3, 97.7, 95.7, 82.3, 76.8, 75.7, 75.4, 74.9, 71.7, 70.3, 70.2, 69.1, 68.1, 67.9, 65.8, 51.6, 48.9, 48.2, 48.1, 39.2, 39.1, 30.9, 19.6, 19.2, 19.1, 19.1, 19.0. ESI-HRMS: *m/z* calc for $C_{46}H_{50}N_6O_{24}F_{18}Na$ $[M+Na]^+$ 1435.24812, found 1435.2456.

4''-Deshydroxymethylneomomycin hexaacetate salt (27). To a stirred solution of **26** (83 mg, 0.059 mmol) in MeOH (0.6 mL) was added $Mg(OMe)_2$ solution (0.6 mL, 6-10% by wt). After 17 h, the reaction was concentrated to dryness and redissolved in 0.5 mL dioxane and 0.5 mL 1 N NaOH. After 6 h, the reaction mixture was neutralized with AcOH and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **27** (30.7 mg, 55% over 3 steps); $[\alpha]_D^{23}$ = +16.1 (*c* = 0.4, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.81 (d, *J* = 4.0 Hz, 1H, H1'), 5.37 (d, *J* = 4.2 Hz, 1H, H1''), 5.25 (d, *J* = 1.8 Hz, 1H), 4.57 (td, *J* = 4.9, 3.5 Hz, 1H), 4.30 (m, 2H), 4.24 (ddd, *J* = 6.4, 4.1, 1.5 Hz, 1H), 4.16 (t, *J* = 3.2 Hz, 1H), 4.00 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.96 (ddd, *J* = 10.3, 7.2, 3.5 Hz, 1H), 3.88 (dd, *J* = 10.7, 9.0 Hz, 1H), 3.79 (t, *J* = 9.0 Hz, 1H), 3.77 – 3.71 (m, 2H), 3.60 (dd, *J* = 10.6, 8.9 Hz, 1H), 3.53 (dt, *J* = 3.1, 1.3 Hz, 1H), 3.43 – 3.27 (m, 5H), 3.26 – 3.15 (m, 3H), 2.26 (dt, *J* = 12.7, 4.3 Hz, 1H), 1.85 (s, 18H), 1.61 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 181.1, 109.6, 96.3, 95.3, 84.7, 78.3, 77.4, 74.8, 73.0, 70.7, 70.6, 70.2, 69.1, 69.0, 67.6, 67.3, 53.5, 50.9, 50.0, 48.8, 40.3, 40.1, 30.2, 23.1; ESI-HRMS: *m/z* calc for $C_{22}H_{44}N_6O_{12}Na$ $[M+Na]^+$ 607.29094, found 607.2903.

1,3,2',6',2''',6'''-Hexaazido-1,3,2',6',2''',6'''-hexadesamino-5''-*O*-(2,4,6- triisopropylbenzenesulfonyl)neomycin (**29**). To a stirred solution of **28** (1.93 g, 2.50 mmol) in pyridine (11 mL) was added trisyl chloride (1.35 g, 4.45 mmol). Additional trisyl chloride (0.63 g, 2.08 mmol) was added as reaction progress was sluggish. After 40h, the reaction was quenched with water and diluted with EtOAc. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated to dryness. The crude mixture was purified via flash chromatography (0-20% MeOH in DCM, R_f = 0.4 in 10% MeOH/DCM) to give the desired product **29** (1.58 g, 61%). $[\alpha]_D^{23}$ = +67.3 (*c* = 1.0, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 7.30 (s, 2H), 5.99 (d, *J* = 3.9 Hz, 1H), 5.41 (d, *J* = 1.8 Hz, 1H), 5.07 (d, *J* = 1.8 Hz, 1H), 4.34 (dd, *J* = 4.4, 1.8 Hz, 1H), 4.33 – 4.31 (m, 1H), 4.31 – 4.27 (m, 2H), 4.25 (dd, *J* = 10.8, 8.0 Hz, 1H), 4.19 (p, *J* = 6.9 Hz, 2H), 4.16 (ddd, *J* = 9.3, 6.4, 2.3 Hz, 1H), 3.98 (ddd, *J* = 8.4, 4.7, 2.0 Hz, 1H), 3.91 (t, *J* = 3.4 Hz, 1H), 3.89

(dd, *J* = 10.5, 8.9 Hz, 1H), 3.69 (t, *J* = 8.9 Hz, 1H), 3.65 – 3.62 (m, 2H), 3.56 (ddd, *J* = 12.4, 9.9, 4.5 Hz, 1H), 3.53 – 3.43 (m, 4H), 3.41 (t, *J* = 2.6 Hz, 1H), 3.39 – 3.34 (m, 3H), 3.28 (dd, *J* = 12.9, 4.7 Hz, 1H), 2.97 (p, *J* = 6.9 Hz, 1H), 2.23 (dt, *J* = 12.9, 4.5 Hz, 1H), 1.36 (q, *J* = 12.6 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H), 1.30 – 1.29 (m, 6H), 1.29 – 1.29 (m, 6H); ^{13}C NMR (226 MHz, MeOD) δ 154.0, 150.9, 128.9, 123.7, 110.3, 98.3, 96.0, 84.4, 78.9, 76.6, 76.3, 75.7, 74.1, 73.0, 71.9, 71.4, 71.2, 70.9, 69.6, 68.1, 63.2, 60.5, 60.2, 60.0, 51.4, 50.8, 34.1, 31.7, 29.4, 23.8, 22.5. ESI-HRMS: *m/z* calc for $C_{38}H_{56}N_{18}O_{15}SNa$ $[M+Na]^+$ 1059.37854, found 1059.3806.

1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-iodoneomycin (**30**). To a stirred solution of **29** (372 mg, 0.36 mmol) in DMF (2.8 mL) was added NaI (593 mg, 3.96 mmol) and the temperature was raised to 80 °C. After 45 h, the reaction was diluted with EtOAc and washed with water. The aqueous layer was washed twice with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude solid was purified via flash chromatography (0-10% MeOH in DCM, R_f = 0.2 in 6% MeOH in DCM) to give the desired compound **30** (234 mg, 76%) as a white solid. $[\alpha]_D^{23}$ = +123.1 (*c* = 0.78, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 6.14 (d, *J* = 3.9 Hz, 1H), 5.36 (d, *J* = 1.9 Hz, 1H), 5.12 (d, *J* = 1.8 Hz, 1H), 4.43 (dd, *J* = 4.6, 2.0 Hz, 1H), 4.25 (dd, *J* = 6.6, 4.6 Hz, 1H), 4.17 – 4.12 (m, 2H), 4.04 (ddd, *J* = 8.6, 4.4, 1.9 Hz, 1H), 3.96 (t, *J* = 3.3 Hz, 1H), 3.90 (dd, *J* = 10.4, 8.8 Hz, 1H), 3.71 – 3.68 (m, 3H), 3.66 (dd, *J* = 10.7, 2.9 Hz, 1H), 3.63 (dd, *J* = 9.9, 8.8 Hz, 1H), 3.59 – 3.55 (m, 1H), 3.53 (dd, *J* = 13.2, 2.3 Hz, 1H), 3.48 – 3.43 (m, 2H), 3.43 – 3.40 (m, 2H), 3.39 – 3.34 (m, 4H), 2.23 (dt, *J* = 12.9, 4.4 Hz, 1H), 1.36 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 110.2, 98.6, 95.9, 85.2, 81.1, 79.7, 76.8, 75.7, 74.3, 74.3, 71.9, 71.4, 71.3, 70.0, 68.3, 63.6, 60.6, 60.4, 60.1, 51.3, 51.2, 31.8, 6.9; ESI-HRMS: *m/z* calc for $C_{23}H_{33}N_{18}O_{12}INa$ $[M+Na]^+$ 903.14622, found 903.1454.

5''-Deoxyneomycin hexaacetate salt (31). To a stirred solution of **30** (55 mg, 0.062 mmol) in dioxane/water (1:1, 2.4 mL) was added Pd/C (109 mg). The reaction was purged with H_2 and pressurized to 50 psi. After 17 h, the reaction was filtered through Celite® and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.1-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **31** (19.5 mg, 33%) as a white solid. $[\alpha]_D^{23}$ = +39.2 (*c* = 0.27, H_2O); ^{1}H NMR (900 MHz, D_2O) δ 5.83 (d, *J* = 4.0 Hz, 1H), 5.27 (d, *J* = 3.4 Hz, 1H), 5.21 (d, *J* = 1.8 Hz, 1H), 4.30 (t, *J* = 4.1 Hz, 1H), 4.27 – 4.22 (m, 1H), 4.18 (m, 2H), 4.15 (t, *J* = 3.2 Hz, 1H), 3.97 (ddd, *J* = 10.4, 7.4, 3.5 Hz, 1H), 3.85 (dd, *J* = 10.7, 9.1 Hz, 1H), 3.78 – 3.72 (m, 2H), 3.64 (t, *J* = 9.5 Hz, 1H), 3.55 (t, *J* = 9.8 Hz, 1H), 3.50 (t, *J* = 2.1 Hz, 1H), 3.40 – 3.33 (m, 4H), 3.31 (dd, *J* = 13.7, 4.2 Hz, 1H), 3.22 – 3.18 (m, 1H), 3.18 (s, 1H), 3.09 (ddd, *J* = 13.5, 9.9, 4.2 Hz, 1H), 2.20 (dt, *J* = 12.8, 4.3 Hz, 1H), 1.85 (s, 18H), 1.53 (q, *J* = 12.6 Hz, 1H), 1.33 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (226 MHz, D_2O) δ 181.0, 110.2, 95.8, 95.4, 85.4, 80.7, 79.3, 78.1, 73.5, 73.1, 70.8, 70.1, 69.1, 69.0, 67.7, 67.5, 53.6, 51.0, 50.3, 48.9, 40.4, 40.2, 30.9, 23.0, 18.4. ESI-HRMS: *m/z* calc for $C_{23}H_{46}N_6O_{12}Na$ $[M+Na]^+$ 621.30659, found 621.3065.

1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-pthtalimidoneomycin (**32).** To a stirred solution of **29** (562 mg, 0.54 mmol) in DMF (5.4 mL) was added 18-crown-6 (2.87 g, 10.9 mmol) and potassium phthalimide (1.069 g, 5.77 mmol) and the temperature was raised to 95 °C. After 41 h, the reaction was diluted with EtOAc and washed with water. The aqueous layer was washed twice with EtOAc and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated to

dryness. The crude solid was purified via flash chromatography (0-10% MeOH in DCM, R_f = 0.2 in 6% MeOH in DCM) to give the desired compound **32** (243 mg, 50%) as a pale yellow solid. $[\alpha]_D^{23}$ = +75.8 (*c* = 1.0, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 7.95 – 7.89 (m, 2H), 7.82 (m, 2H), 5.71 (d, *J* = 3.9 Hz, 1H), 5.34 (d, *J* = 1.9 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 4.53 (dd, *J* = 6.3, 4.6 Hz, 1H), 4.43 (q, *J* = 5.8 Hz, 1H), 4.36 (dd, *J* = 4.6, 1.9 Hz, 1H), 4.20 (ddd, *J* = 9.9, 6.0, 2.3 Hz, 1H), 4.11 (dd, *J* = 14.2, 5.5 Hz, 1H), 4.08 (dd, *J* = 14.2, 5.8 Hz, 1H), 4.03 (ddd, *J* = 7.6, 5.3, 1.9 Hz, 1H), 3.94 (t, *J* = 3.5 Hz, 1H), 3.80 (dd, *J* = 10.5, 8.9 Hz, 1H), 3.69 – 3.64 (m, 2H), 3.58 (dd, *J* = 13.2, 2.3 Hz, 1H), 3.54 (t, *J* = 9.1 Hz, 1H), 3.51 – 3.46 (m, 3H), 3.44 (t, *J* = 2.6 Hz, 1H), 3.39 – 3.35 (m, 2H), 3.32 – 3.29 (m, 1H), 3.24 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.03 (t, *J* = 9.5 Hz, 1H), 2.20 (dt, *J* = 12.8, 4.5 Hz, 1H), 1.24 (q, *J* = 12.5 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 168.6, 133.9, 132.2, 123.0, 109.5, 98.5, 96.8, 83.0, 78.9, 78.4, 76.6, 75.9, 73.8, 73.2, 71.7, 71.4, 71.3, 69.6, 68.3, 63.7, 60.8, 60.6, 59.5, 51.5, 50.9, 39.7, 31.5; ESI-HRMS: *m/z* calc for $C_{31}H_{37}N_{19}O_{14}Na$ $[M+Na]^+$ 922.26596, found 922.2670.

5''-Amino-1,3,2',6',2''',6'''-hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesaminoneomycin (33). To a stirred solution of **32** (217 mg, 0.24 mmol) in MeOH (2.4 mL) was added hydrazine hydrate (0.2 mL). After 3 h, the reaction was concentrated to dryness and purified via flash chromatography (0-40% MeOH in DCM, R_f = 0.18 in 40% MeOH in DCM) to give the desired compound **33** (136 mg, 73%) as a pale yellow solid. $[\alpha]_D^{23}$ = +103.5 (*c* = 0.45, MeOH); ^{1}H NMR (600 MHz, MeOD) δ 5.81 (d, *J* = 3.8 Hz, 1H), 5.40 (d, *J* = 1.2 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 4.39 – 4.35 (m, 2H), 4.21 (ddd, *J* = 10.0, 5.8, 2.4 Hz, 1H), 4.11 (td, *J* = 7.1, 3.8 Hz, 1H), 4.06 (ddd, *J* = 8.8, 4.1, 1.9 Hz, 1H), 3.97 (t, *J* = 3.3 Hz, 1H), 3.93 (dd, *J* = 10.5, 8.8 Hz, 1H), 3.73 – 3.68 (m, 3H), 3.63 (dd, *J* = 9.9, 8.9 Hz, 1H), 3.59 – 3.51 (m, 2H), 3.49 – 3.44 (m, 3H), 3.43 – 3.38 (m, 1H), 3.38 – 3.34 (m, 2H), 3.08 – 3.00 (m, 2H), 2.87 (dd, *J* = 13.4, 7.4 Hz, 1H), 2.26 (dt, *J* = 12.8, 4.4 Hz, 1H), 1.40 (q, *J* = 12.4 Hz, 1H); ^{13}C NMR (151 MHz, MeOD) δ 109.4, 98.1, 97.1, 83.9, 80.9, 77.0, 76.3, 74.5, 73.5, 71.9, 71.3, 70.4, 69.8, 68.2, 62.8, 60.6, 60.2, 59.8, 51.2, 44.0, 31.6; ESI-HRMS: *m/z* calc for $C_{23}H_{36}N_{19}O_{12}$ $[M+H]^+$ 770.27853, found 770.2783.

5''-Amino-5''-deoxyneomycin heptaacetate salt (34). To a stirred solution of **33** (17 mg, 0.022 mmol) in dioxane/water (1:1, 1.2 mL) was added $Pd(OH)_2$/C. (32 mg). The reaction was purged with H_2 and pressurized to 50 psi. After 26 h, the reaction was filtered through Celite® and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.1-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **34** (15.7 mg, 70%) as a white solid. $[\alpha]_D^{23}$ = +22.8 (*c* = 0.25, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.70 (d, *J* = 3.7 Hz, 1H), 5.40 (d, *J* = 2.9 Hz, 1H), 5.25 (d, *J* = 1.8 Hz, 1H), 4.43 (t, *J* = 5.7 Hz, 1H), 4.33 (dd, *J* = 5.3, 3.0 Hz, 1H), 4.25 (m, 2H), 4.17 (t, *J* = 3.1 Hz, 1H), 3.96 (ddd, *J* = 10.2, 7.0, 3.6 Hz, 1H), 3.87 (t, *J* = 9.2 Hz, 1H), 3.82 – 3.76 (m, 3H), 3.64 (dd, *J* = 10.5, 9.2 Hz, 1H), 3.53 (p, *J* = 1.3 Hz, 1H), 3.42 – 3.31 (m, 5H), 3.27 – 3.18 (m, 2H), 3.17 – 3.12 (m, 2H), 2.25 (dt, *J* = 12.7, 4.2 Hz, 1H), 1.87 (s, 21H), 1.60 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 180.9, 108.6, 95.9, 95.7, 83.5, 77.7, 77.6, 77.2, 73.2, 72.5, 70.8, 70.25, 70.16, 69.4, 67.7, 67.5, 53.9, 50.8, 50.4, 49.1, 41.7, 40.4, 40.2, 30.2, 22.9; ESI-HRMS: *m/z* calc for $C_{23}H_{47}N_7O_{12}Na$ $[M+Na]^+$ 636.31749, found 636.3177.

5''-Deoxy-5''-formamidoneomycin hexaacetate salt (35). To a stirred solution of **33** (29 mg, 0.035 mmol) in DCM (1 mL) was added formic acetic anhydride (1 mL). After 1.5 h, the reaction was concentrated to dryness. The crude product was traken up in MeOH (1.5 mL) and saturated aqueous $NaHCO_3$ (0.4 mL) was added. After 3 h, the reaction was concentrated to dryness,

redissolved in EtOAc and washed with water, followed by concentration of the organic layer to dryness and coevaporation of the crude product three times with toluene. The crude product was taken up in dioxane:water (1:1, 1 mL) and $Pd(OH)_2$ on carbon (65 mg) was added. The reaction mixture was stirred under H_2 at 50 psi for 17h, filtered through Celite®, and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **35** (15.6 mg, 41% over 3 steps) as a white solid. $[\alpha]_D^{23}$ = +32.0 (*c* = 0.20, H_2O); ^{1}H NMR (900 MHz, D_2O) δ 8.09 (s, 1H), 5.72 (d, *J* = 3.8 Hz, 1H), 5.28 (d, *J* = 3.7 Hz, 1H), 5.21 (d, *J* = 1.7 Hz, 1H), 4.34 (td, *J* = 5.3, 1.3 Hz, 1H), 4.26 – 4.22 (m, 2H), 4.19 (q, *J* = 5.3 Hz, 1H), 4.15 (t, *J* = 3.0 Hz, 1H), 3.98 (ddd, *J* = 10.3, 7.3, 3.4 Hz, 1H), 3.82 (dd, *J* = 10.6, 9.0 Hz, 1H), 3.79 – 3.73 (m, 2H), 3.66 (t, *J* = 9.4 Hz, 1H), 3.60 (dd, *J* = 14.5, 4.2 Hz, 1H), 3.57 (dd, *J* = 10.6, 9.0 Hz, 1H), 3.50 (dt, *J* = 2.8, 1.4 Hz, 1H), 3.44 (dd, *J* = 14.5, 6.3 Hz, 1H), 3.41 – 3.34 (m, 3H), 3.31 (ddd, *J* = 13.7, 4.0, 1.4 Hz, 1H), 3.27 (dd, *J* = 10.7, 3.9 Hz, 1H), 3.21 – 3.17 (m, 2H), 3.09 (ddd, *J* = 12.1, 9.9, 4.3 Hz, 1H), 2.19 (dt, *J* = 12.8, 4.3 Hz, 1H), 1.84 (s, 18H) 1.53 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (226 MHz, D_2O) δ 181.2, 165.0, 109.6, 95.9, 95.5, 85.2, 80.0, 79.2, 77.2, 73.2, 73.0, 70.8, 70.2, 69.4, 69.1, 67.7, 67.4, 53.8, 50.8, 50.2, 48.9, 40.5, 40.1, 39.8, 30.9, 23.1. ESI-HRMS: *m/z* calc for $C_{24}H_{47}N_7O_{13}Na$ $[M+Na]^+$ 664.31241, found 664.3121.

5''-Acetamido-5''-deoxyneomycin hexaacetate salt (36). To a stirred solution of **33** (73 mg, 0.095 mmol) in THF (1 mL) and Et_3N (0.19 mL, 1.33 mmol) was added acetic anhydride (0.04 mL, 0.4 mmol). After 2h, the reaction was quenched with methanol and concentrated to dryness. The crude product was taken up in methanol (1.5 mL) and sodium methoxide (32 mg, 0.59 mmol) was added. After 30 mins, Amberlyst-15H was added and the solution was filtered and evaporated to dryness after coevaporation with toluene. The crude product was taken up in dioxane:water (1:1, 3 mL) and $Pd(OH)_2$ on carbon (166 mg) was added. The reaction mixture was stirred under H_2 at 50 psi for 14h, filtered through Celite®, and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **36** (23.2 mg, 24% over 3 steps) as a white solid. $[\alpha]_D^{23}$ = +41.1 (*c* = 0.61, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.73 (d, *J* = 3.8 Hz, 1H), 5.30 (d, *J* = 3.7 Hz, 1H), 5.22 (d, *J* = 1.8 Hz, 1H), 4.34 (t, *J* = 5.3 Hz, 1H), 4.25 (m, 2H), 4.18 (m, 2H), 4.00 (ddd, *J* = 10.3, 7.2, 3.5 Hz, 1H), 3.84 (dd, *J* = 10.7, 9.0 Hz, 1H), 3.80 – 3.76 (m, 2H), 3.69 (t, *J* = 9.4 Hz, 1H), 3.62 – 3.54 (m, 2H), 3.52 (dt, *J* = 3.0, 1.3 Hz, 1H), 3.43 – 3.35 (m, 4H), 3.32 (dd, *J* = 13.7, 4.1 Hz, 1H), 3.29 – 3.25 (m, 1H), 3.25 – 3.19 (m, 2H), 3.16 – 3.07 (m, 1H), 2.22 (dt, *J* = 12.7, 4.2 Hz, 1H), 1.98 (s, 3H), 1.87 (s, 18H) 1.56 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 180.9, 174.7, 109.6, 95.8, 95.5, 85.2, 80.2, 79.1, 77.5, 73.3, 73.0, 70.8, 70.2, 69.3, 69.1, 67.7, 67.4, 53.9, 50.9, 50.2, 48.9, 41.4, 40.5, 40.1, 30.8, 22.9, 22.1; ESI-HRMS: *m/z* calc for $C_{25}H_{49}N_7O_{13}Na$ $[M+Na]^+$ 678.32806, found 678.3290.

6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene-5''-carboxy-1,3,2',2''',6'''-pentadesamino-1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (38). To a stirred solution of **37** (1.23 g, 1.04 mmol) in pyridine (10 mL) was added trityl chloride (2.88 g, 10.4 mmol). After 40 h, acetic anhydride (3 mL) and DMAP (31 mg, 0.25 mmol) were added. After 24 h, the reaction was quenched with H_2O, diluted with EtOAc, and washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness, after which the crude product was purified through silica gel chromatography (0-10% MeOH in DCM). A portion (661 mg) of the resulting solid (1.52 g) was dissolved in DCM (4 mL) and $FeCl_3{\cdot}6H_2O$ (219 mg, 0.81

mmol) was added. After 20 mins, the reaction was diluted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to dryness, after which the crude product was purified through silica gel chromatography (0-10% MeOH in DCM). The resulting solid (392 mg) was dissolved in DCM (2 mL) and cooled to 0 °C, after which 2 mL H_2O, BAIB (239 mg, 0.70 mmol) and TEMPO (9.5 mg, 0.06 mmol) were added. After 24 h, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous $Na_2S_2O_3$. The reaction mixture was diluted with EtOAc and washed with brine, after which the organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified via silica gel chromatography (0-10% MeOH in DCM, R_f = 0.3 in 10% MeOH in DCM) to give **38** (236 mg, 36% over four steps) as an off-white solid. $[\alpha]_D^{23}$ = -0.5 (*c* = 0.45, MeOH); ^{1}H NMR (600 MHz, MeOD) δ 7.43 (m, 2H), 7.38 – 7.29 (m, 3H), 6.30 (d, *J* = 4.1 Hz, 1H), 5.55 (s, 1H), 5.41 (d, *J* = 4.9 Hz, 1H), 5.31 (t, *J* = 10.1 Hz, 1H), 5.03 – 5.00 (m, 2H), 4.98 (dd, *J* = 10.6, 9.2 Hz, 1H), 4.89 – 4.86 (m, 1H), 4.81 (t, *J* = 4.7 Hz, 1H), 4.58 (dd, *J* = 4.6, 3.2 Hz, 1H), 4.53 (d, *J* = 3.2 Hz, 1H), 4.37 (dd, *J* = 10.6, 4.2 Hz, 1H), 4.30 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.27 – 4.15 (m, 4H), 4.12 (t, *J* = 8.8 Hz, 1H), 3.92 (dd, *J* = 10.2, 8.3 Hz, 1H), 3.86 (td, *J* = 9.8, 4.9 Hz, 1H), 3.79 – 3.71 (m, 2H), 3.67 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.43 – 3.38 (m, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.05 – 1.99 (m, 1H), 1.98 (s, 3H), 1.91 (dt, *J* = 12.8, 4.3 Hz, 1H); ^{13}C NMR (151 MHz, MeOD) δ 172.1, 170.6, 170.6, 170.5, 169.2, 168.8, 158.4 – 156.4, 137.5, 128.7, 127.7, 126.2, 119.4 – 112.4, 107.3, 101.7, 97.9, 96.3, 83.8, 80.7, 79.1, 79.0, 76.4, 75.6, 75.0, 71.7, 69.7, 68.02, 67.96, 65.6, 63.0, 52.1, 49.0, 48.2, 48.1, 39.3, 30.6, 19.9, 19.3, 19.2, 19.1, 19.0; ESI-HRMS: *m/z* calc for $C_{50}H_{52}N_5O_{25}F_{15}Na$ $[M+Na]^+$ 1430.26041, found 1430.2594.

6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene -1,3,2',2''',6'''-pentadesamino-4''-deshydroxymethyl -1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (39). To a stirred solution of **38** (282 mg, 0.20 mmol) in THF (2.3 mL) at 0 °C covered in foil was added Et_3N (0.04 mL, 0.32 mmol) followed by 1-oxa-2-oxo-3-thiaindolizinium chloride **25** (82 mg, 0.43 mmol). After 1 h, *tert*-dodecyl thiol (0.34 mL, 1.4 mmol) was added and the reaction was exposed to white light. After 30 mins, the reaction was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified via silica gel chromatography (30-70% EtOAc in hexanes, R_f = 0.35 in 50% EtOAc in hexanes) to give **39** (128 mg, 47%) as an off-white solid. $[\alpha]_D^{23}$ = +1.0 (*c* = 0.8, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 7.43 (m, 2H), 7.38 – 7.32 (m, 3H), 5.58 (s, 1H), 5.51 (d, *J* = 3.6 Hz, 1H), 5.34 (t, *J* = 10.1 Hz, 1H), 5.28 (d, *J* = 3.1 Hz, 1H), 5.01 – 4.99 (m, 1H), 4.98 (t, *J* = 3.4 Hz, 1H), 4.91 (d, *J* = 2.1 Hz, 1H), 4.86 – 4.83 (m, 2H), 4.52 (q, *J* = 5.8 Hz, 1H), 4.42 (dd, *J* = 10.6, 4.1 Hz, 1H), 4.32 (dd, *J* = 10.3, 5.0 Hz, 1H), 4.27 – 4.17 (m, 3H), 4.14 (t, *J* = 2.5 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.11 – 4.09 (m, 1H), 3.91 (td, *J* = 9.9, 4.9 Hz, 1H), 3.83 (dd, *J* = 10.2, 8.8 Hz, 1H), 3.80 (t, *J* = 9.7 Hz, 1H), 3.78 – 3.71 (m, 2H), 3.55 – 3.48 (m, 2H), 2.17 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.99 – 1.96 (m, 2H); ^{13}C NMR (226 MHz, MeOD) δ 170.7, 170.3, 169.3, 168.7, 158.3 – 156.9, 137.4, 128.7, 127.7, 126.1, 121.5 – 112.6 (m), 107.7, 101.8, 97.6, 97.0, 82.6, 79.0, 77.7, 75.3, 75.1, 75.0, 71.7, 69.9, 69.2, 68.1, 68.0, 65.7, 63.4, 52.4, 48.8, 48.1, 48.0, 39.2, 30.9, 19.6, 19.24, 19.15, 19.1, 19.0. ESI-HRMS: *m/z* calc for $C_{49}H_{52}N_5O_{23}F_{15}Na$ $[M+Na]^+$ 1386.27058, found 1386.2716.

4''-Deshydroxymethylparomomycin pentaacetate salt (40). To a stirred solution of **39** (128 mg, 0.094 mmol) in MeOH (1 mL) was added *p*-toluenesulfonic acid (22 mg, 0.11 mmol). After 3 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified via flash chromatography (50-80% EtOAc in hexanes). The resulting solid (71 mg) was

dissolved in MeOH (0.6 mL) and $Mg(OMe)_2$ solution (0.6 mL, 6-10% by wt) was added. After 25 h, the reaction was concentrated to dryness and redissolved in dioxane (0.6 mL) and NaOH (0.6 mL, 1 N). After 4 h, the reaction mixture was neutralized with AcOH and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **40** (16.8 mg, 83% over 3 steps) as a white solid. $[\alpha]_D^{23}$ = +17.7 (*c* = 0.6, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.58 (d, *J* = 3.9 Hz, 1H), 5.34 (d, *J* = 4.2 Hz, 1H), 5.25 (d, *J* = 1.8 Hz, 1H), 4.58 (td, *J* = 5.0, 3.8 Hz, 1H), 4.28 (ddd, *J* = 8.1, 5.2, 3.2 Hz, 2H), 4.24 (ddd, *J* = 6.8, 4.0, 1.6 Hz, 1H), 4.16 (t, *J* = 3.2 Hz, 1H), 3.98 (dd, *J* = 10.1, 3.7 Hz, 1H), 3.86 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.84 – 3.76 (m, 4H), 3.75 (dt, *J* = 3.1, 1.4 Hz, 1H), 3.69 (dd, *J* = 12.1, 6.6 Hz, 1H), 3.62 (dd, *J* = 10.6, 8.8 Hz, 1H), 3.53 (dt, *J* = 3.1, 1.3 Hz, 1H), 3.48 – 3.41 (m, 1H), 3.40 – 3.32 (m, 3H), 3.31 – 3.21 (m, 2H), 2.34 (dt, *J* = 12.7, 4.3 Hz, 1H), 1.85 (s, 15H) 1.69 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 181.1, 109.7, 96.3, 84.0, 79.4, 77.2, 74.6, 73.9, 72.6, 70.5, 70.2, 69.5, 69.2, 67.6, 67.3, 60.3, 53.9, 50.9, 49.7, 49.2, 40.3, 29.1, 23.0; ESI-HRMS: *m/z* calc for $C_{22}H_{44}N_5O_{13}$ $[M+H]^+$ 586.29301, found 586.2923.

1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-1,3,2',2''',6'''-pentadesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl) paromomycin (**43**). To a stirred solution of **41** (338 mg, 0.41 mmol) in pyridine (2 mL) was added trisyl chloride (259 mg, 0.85 mmol). Additional trisyl chloride (126 mg, 0.41 mmol) was added when progress halted. After 40h, the reaction was quenched with water and diluted with EtOAc. The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated to dryness. The crude mixture was purified via flash chromatography (0-15% MeOH in DCM, R_f = 0.5 in 10% MeOH/DCM) to give **43** as an off-white solid (235 mg, 53%). $[\alpha]_D^{23}$ = +52.9 (*c* = 1.0, EtOAc); ^{1}H NMR (600 MHz, MeOD) δ 7.52 – 7.47 (m, 2H), 7.37 – 7.32 (m, 3H), 7.29 (s, 2H), 5.98 (d, *J* = 4.0 Hz, 1H), 5.59 (s, 1H), 5.38 (d, *J* = 2.0 Hz, 1H), 5.06 (d, *J* = 1.8 Hz, 1H,), 4.34 (dd, *J* = 4.4, 2.0 Hz, 1H), 4.32 – 4.25 (m, 3H), 4.25 – 4.07 (m, 6H), 3.97 (ddd, *J* = 8.8, 4.3, 2.0 Hz, 1H), 3.89 (t, *J* = 3.4 Hz, 1H), 3.78 (t, *J* = 10.2 Hz, 1H), 3.67 (t, *J* = 8.8 Hz, 1H), 3.63 – 3.57 (m, 3H), 3.56 – 3.49 (m, 2H), 3.48 (dd, *J* = 10.1, 4.0 Hz, 1H), 3.43 (ddd, *J* = 12.3, 9.9, 4.3 Hz, 1H), 3.39 (t, *J* = 2.6 Hz, 1H), 3.34 (t, *J* = 9.4 Hz, 1H), 3.24 (dd, *J* = 13.0, 4.2 Hz, 1H), 2.95 (p, *J* = 6.9 Hz, 1H), 2.21 (dt, *J* = 12.9, 4.4 Hz, 1H), 1.37 (q, *J* = 12.5 Hz, 1H), 1.30 (s, 3H), 1.28-1.29 (m, 9H), 1.27 (m, 6H); ^{13}C NMR (151 MHz, MeOD) δ 154.1, 150.9, 137.8, 128.9, 128.6, 127.7, 126.2, 123.7, 110.5, 101.7, 98.4, 97.0, 84.7, 81.5, 79.0, 76.4, 76.24, 76.21, 74.4, 73.0, 70.7, 69.6, 68.7, 68.5, 68.2, 64.0, 63.1, 60.5, 60.15, 60.0, 50.94, 34.1, 31.70, 29.4, 23.81, 23.80, 22.54, 22.53. ESI-HRMS: *m/z* calc for $C_{45}H_{61}N_{15}O_{16}SNa$ $[M+Na]^+$ 1122.40336, found 1122.4009.

4',6'-*O*-Benzylidene-1,3,2',2''',6'''-penta-*N*-benzyloxycarbonyl-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)paromomycin (44). A stirred solution of **42** (2 g, 1.46 mmol) in pyridine (15 mL) was treated with trisyl chloride (4.4 g, 14.56 mmol) (92 mg, 0.48 mmol). After stirring for 72 h the reaction was quenched with MeOH (15 mL) and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/iPrOH (19:1) to give desired product **44** (1.4 g, 58%). $[\alpha]_D^{23}$ +27.7 (*c* 1.2, MeOH). ESIHRMS calculated for $C_{85}H_{101}N_5O_{26}NaS$ $[M+Na]^+$, 1662.6353; found, 1662.6373.[1]

1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesamino-5''-iodoparomomycin (45). To a stirred solution of **43** (582 mg, 0.53 mmol) in acetone (5.6 mL) was added NaI (764 mg, 5.10 mmol), and the reaction was heated to reflux.

Additional NaI (786 mg, 5.24 mmol) was added as progress halted. After 44h, the reaction mixture was concentrated to dryness and purified via flash chromatography (0-20% MeOH in DCM, R_f = 0.5 in 10% MeOH/DCM) to give the desired product **45** (339 mg, 68%) as a white solid. $[\alpha]_D^{23}$ = +69.4 (*c* = 1.0, EtOAc); ^{1}H NMR (900 MHz, MeOD) δ 7.51 (dd, *J* = 7.4, 2.3 Hz, 2H), 7.42 – 7.32 (m, 3H), 6.12 (d, *J* = 4.0 Hz, 1H), 5.62 (s, 1H), 5.36 (d, *J* = 2.0 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 4.45 (dd, *J* = 4.6, 2.0 Hz, 1H), 4.26 (dd, *J* = 6.6, 4.7 Hz, 1H), 4.24 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.17 – 4.11 (m, 3H), 4.05 (ddd, *J* = 8.8, 4.2, 1.9 Hz, 1H), 3.97 (t, *J* = 3.3 Hz, 1H), 3.79 (t, *J* = 10.2 Hz, 1H), 3.74 – 3.69 (m, 3H), 3.68 (dd, *J* = 10.7, 2.9 Hz, 1H), 3.64 (dd, *J* = 9.9, 8.8 Hz, 1H), 3.59 – 3.54 (m, 2H), 3.49 (dd, *J* = 10.1, 4.0 Hz, 1H), 3.47 – 3.44 (m, 2H), 3.42 (dd, *J* = 13.0, 4.2 Hz, 1H), 3.40 – 3.36 (m, 2H), 2.23 (dt, *J* = 12.9, 4.5 Hz, 1H), 1.40 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 137.7, 128.6, 127.7, 126.2, 110.2, 101.7, 98.5, 96.8, 85.1, 81.5, 80.9, 79.4, 76.5, 76.2, 74.3, 74.1, 69.8, 68.7, 68.4, 68.2, 64.2, 63.0, 60.5, 60.2, 60.1, 51.2, 31.7, 6.8. ESI-HRMS: *m/z* calc for $C_{30}H_{38}N_{13}O_{15}INa$ $[M+Na]^+$ 966.17014, found 966.1688.

5''-Deoxyparomomycin pentaacetate salt (46). To a stirred solution of **45** (180 mg, 0.19 mmol) in $CHCl_3$/water (4:1, 2 mL) was added *p*-toluenesulfonic acid (65.2 mg, 0.34 mmol). After 2h, the reaction was quenched with saturated aqueous $NaHCO_3$ and diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to dryness. The crude solid was triturated several times with dichloromethane to remove excess benzaldehyde and give the hydrolyzed product (141 mg). A portion of the crude solid (32 mg) was dissolved in dioxane/water (1:1, 1 mL) and $Pd(OH)_2$ on carbon (76 mg) was added. The reaction mixture was purged with H_2, pressurized to 50 psi, filtered through Celite® after 24h, and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **46** (19.1 mg, 57% over 2 steps). $[\alpha]_D^{23}$ = +32.8 (*c* = 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.67 (d, *J* = 4.0 Hz, 1H), 5.25 (d, *J* = 3.4 Hz, 1H), 5.22 (d, *J* = 1.8 Hz, 1H), 4.29 (dd, *J* = 5.2, 3.5 Hz, 1H), 4.25 (ddd, *J* = 6.0, 4.2, 1.6 Hz, 1H), 4.21 (t, *J* = 5.4 Hz, 1H), 4.19 – 4.15 (m, 2H), 3.89 – 3.81 (m, 3H), 3.80 – 3.74 (m, 3H), 3.69 (dd, *J* = 12.1, 6.5 Hz, 1H), 3.61 (dd, *J* = 10.5, 9.0 Hz, 1H), 3.52 (dt, *J* = 3.0, 1.4 Hz, 1H), 3.43 (t, *J* = 9.4 Hz, 1H), 3.39 – 3.33 (m, 3H), 3.31 (dd, *J* = 13.7, 4.2 Hz, 1H), 3.25 (ddd, *J* = 12.5, 10.5, 4.2 Hz, 1H), 2.35 (dt, *J* = 12.8, 4.3 Hz, 1H), 1.86 (s, 15H), 1.70 (q, *J* = 12.7 Hz, 1H), 1.35 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 181.0, 110.2, 96.0, 95.6, 84.5, 80.5, 79.1, 77.9, 73.8, 73.3, 72.5, 70.1, 69.4, 69.3, 67.7, 67.4, 60.3, 53.8, 50.9, 49.8, 49.1, 40.4, 29.1, 23.0, 18.3. ESI-HRMS: *m/z* calc for $C_{23}H_{45}N_5O_{13}Na$ $[M+Na]^+$ 622.29061, found 622.2912.

1,3,2',5'',2''',6'''-Hexaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesaminoparomomycin (47). To a stirred solution of **43** (164 mg, 0.15 mmol) in DMF (1.5 mL) was added NaN_3 (102 mg, 1.57 mmol) and the reaction was heated to 80 °C. After 15h, the reaction mixture was diluted with EtOAc and washed with water. The aqueous layer was extracted twice with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified via flash chromatography (0-10% MeOH in DCM, R_f = 0.5 in 6% MeOH in DCM) to give the desired compound **47** (99 mg, 78%) as a glassy solid. $[\alpha]_D^{23}$ = +109.3 (*c* = 0.93, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 7.53 – 7.50 (m, 2H), 7.39 – 7.35 (m, 3H), 5.98 (d, *J* = 4.1 Hz, 1H), 5.61 (s, 1H), 5.40 (s, 1H), 5.14 (s, 1H), 4.41 – 4.37 (m, 2H), 4.26 – 4.21 (m, 2H), 4.17 (td, *J* = 10.0, 4.9 Hz, 1H), 4.12 (t, *J* = 9.7 Hz, 1H), 4.07 – 4.03 (m, 1H), 3.96 (t, *J* = 3.4 Hz, 1H), 3.78 (t, *J* = 10.2 Hz, 1H), 3.72 – 3.66 (m, 4H), 3.63 (t, *J* = 9.4 Hz, 1H), 3.58 – 3.50 (m, 3H), 3.48 – 3.43 (m, 2H), 3.42 – 3.37 (m, 2H), 3.31 (dd, *J* = 10.1, 4.0 Hz, 1H), 2.23 (dt, *J* = 13.2, 4.6 Hz, 1H), 1.40 (q, *J* =

12.6 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 137.7, 128.6, 127.7, 126.2, 110.2, 101.7, 98.4, 97.2, 84.3, 81.5, 79.9, 76.8, 76.5, 76.2, 74.3, 73.1, 69.7, 68.6, 68.4, 68.2, 64.2, 63.1, 60.5, 60.2, 59.9, 53.0, 51.2, 31.7; ESI-HRMS: *m/z* calc for $C_{30}H_{38}N_{18}O_{13}Na$ $[M+Na]^+$ 881.27579, found 881.2767.

5''-Azido-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-benzyloxycarbonyl-5''-deoxyparomomycin (48). To a stirred solution of **44** (1.2 g, 0.73 mmol) in DMF (10 mL) was added NaN_3 (951 mg, 14.64 mmol). The reaction mixture was stirred for 2 h at 80 ^{0}C and concentrated under to dryness under reduced pressure. The residue was dissolved in acetone (50 mL) and the precipitate was filtered off. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3$/iPrOH (19:1) to give the desired product **48** (800 mg, 78%). $[\alpha]_D^{25}$ +34.5 (*c* 1.0, MeOH). ESIHRMS calculated for $C_{70}H_{78}N_8O_{23}Na$ $[M+Na]^+$, 1421.5078; found, 1421.5051.[1]

5''-Amino-5''-deoxyparomomycin hexaacetate salt (49). To a solution of **47** (44 mg, 0.051 mmol) in dioxane/water (1:1, 1 mL) was added $Pd(OH)_2$ on carbon. The reaction mixture was purged with H_2 and pressurized to 50 psi. Additional $Pd(OH)_2$ (40 mg) and 10% aqueous AcOH (1.0 mL) were added when progress halted. After 72 h, the reaction mixture was filtered through Celite® and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **49** (27.0 mg, 54%). $[\alpha]_D^{23}$ = +18.7 (*c* = 0.70, H_2O); ^{1}H NMR (900 MHz, D_2O) δ 5.49 (d, *J* = 3.7 Hz, 1H), 5.35 (d, *J* = 2.5 Hz, 1H), 5.21 (d, *J* = 1.9 Hz, 1H), 4.43 (dd, *J* = 6.5, 5.1 Hz, 1H), 4.34 (dd, *J* = 5.1, 2.5 Hz, 1H), 4.25 – 4.20 (m, 2H), 4.14 (t, *J* = 3.2 Hz, 1H), 3.84 – 3.80 (m, 2H), 3.75 (dt, *J* = 2.9, 1.3 Hz, 1H), 3.75 – 3.66 (m, 4H), 3.57 (t, *J* = 9.9 Hz, 1H), 3.48 (dt, *J* = 3.0, 1.3 Hz, 1H), 3.39 (t, *J* = 9.3 Hz, 1H), 3.35 (ddd, *J* = 14.3, 9.8, 4.7 Hz, 2H), 3.30 (dd, *J* = 13.7, 4.1 Hz, 1H), 3.21 (ddd, *J* = 13.2, 9.7, 4.1 Hz, 1H), 3.17 (ddd, *J* = 12.4, 10.4, 4.1 Hz, 1H), 3.13 (dd, *J* = 13.5, 8.9 Hz, 1H), 3.03 (dd, *J* = 10.4, 3.7 Hz, 1H), 2.23 (dt, *J* = 12.9, 4.3 Hz, 1H), 1.83 (s, 16H), 1.57 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (226 MHz, D_2O) δ 181.3, 109.0, 96.4, 95.6, 83.1, 78.6, 77.5, 77.1, 73.7, 73.0, 72.7, 70.9, 70.3, 69.4, 67.8, 67.5, 60.3, 54.2, 50.9, 50.1, 49.4, 41.7, 40.4, 29.8, 23.2; ESI-HRMS: *m/z* calc for $C_{23}H_{46}N_6O_{13}Na$ $[M+Na]^+$ 637.30151, found 637.3020.

5''-Amino-4',6'-*O*-benzylidene-1,3,2',2''',6'''-penta-*N*-benzyloxycarbonyl-5''-deoxyparomomycin (50). To a stirred solution of **48** (600 mg, 0.43 mmol) in a mixture of THF and water (1:1, 12 mL) was PMe_3 (1M in THF, 0.9 mL. 0.9 mmol). After stirring for 2 h at 80 ^{0}C the reaction was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (18:1) to give desired product **50** (500 mg, 85%). $[\alpha]_D^{23}$ +34.5 (*c* 1.0, MeOH). ESIHRMS calculated for $C_{70}H_{81}N_6O_{23}$ $[M+H]^+$, 1373.5353; found, 1373.5349.[1]

5''-Deoxy-5''-formamidoparomomycin pentaacetate salt (51). A stirred solution of **50** (250 mg, 0.18 mmol) in DCM (3 mL) was treated with formic acetic anhydride (3 mL). The reaction mixture was stirred for 2 h and then concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (5 mL) and to this solution was added saturated aqueous $NaHCO_3$ (5 mL). The mixture was stirred for 1 h and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (49:1) to give the desired formylated intermediate (175 mg). A portion of this

solid (80 mg) was dissolved in dioxane (2 mL) and added to a stirred suspension of $Pd(OH)_2/C$ (160 mg, prewashed with glacial acetic acid) in water (2 mL). The reaction mixture was stirred under H_2 (45 psi) for 8 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.6% NH_4OH). The product containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and lyophilized to give the desired product **51** as the peracetate salt (25 mg, 26%). $[\alpha]_D^{23}$ +44.0 (*c* 0.8, H_2O). ^1H NMR (600 MHz, D_2O) δ 7.89 (s, 1H), 5.50 (d, *J* = 2.7 Hz, 1H), 5.07 (d, *J* = 1.5 Hz, 1H), 5.00 (br s, 1H), 4.15 (t, *J* = 4.9 Hz, 1H), 4.08 – 4.05 (m, 1H), 4.05 – 4.01 (m, 1H), 4.00 – 3.92 (m, 2H), 3.78 (t, *J* = 9.0 Hz, 1H), 3.72 – 3.59 (m, 3H), 3.58 – 3.47 (m, 3H), 3.44 (t, *J* = 9.3 Hz, 1H), 3.40 (dd, J_1 = 14.5 Hz, J_2 = 3.5 Hz, 1H), 3.35 – 3.19 (m, 5H), 3.16 (dd, J_1 = 13.5 Hz, J_2 = 6.1 Hz, 1H), 3.13 – 3.04 (m, 2H), 2.21 (dt, J_1 = 12.5 Hz, J_2 = 4.2 Hz, 1H), 1.76 (s, 15H), 1.59 (q, *J* = 12.5 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.4, 164.8, 109.5, 95.3, 95.0, 83.9, 79.7, 77.2, 76.7, 74.1, 72.9, 71.8, 70.0, 68.9, 68.7, 67.4, 67.2, 60.1, 53.3, 50.6, 49.4, 48.8, 40.3, 39.3, 27.8, 20.8. ESIHRMS calculated for $C_{24}H_{47}N_6O_{14}$ $[M+H]^+$, 643.3150; found, 643.3145.

5”-Acetamido-5”-deoxyparomomycin pentaacetate salt (52). A stirred solution of **50** (80 mg, 0.06 mmol) in DCM (1 mL) was treated with an excess of acetic anhydride (1 mL). The reaction mixture was stirred for 24 h, after which pyridine (1 mL) was added and the reaction mixture was stirred for additional 18 hours. The reaction mixture was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (49:1) to give the desired acetylated intermediate (75 mg). The resulting solid was dissolved in MeOH (1 mL) and NaOMe (20 mg, 0.37 mmol) was added. After stirring for 2 h, the reaction mixture was neutralized with Amberlyst-15H, filtered, and concentrated under reduced pressure. The residue was dissolved in dioxane (1.5 ml) and was added to a stirred suspension of Pd/C (75 mg) in 10% AcOH (0.75 mL). The reaction mixture was stirred under a hydrogen atmosphere (45 psi) for 18 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.8% NH_4OH). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and lyophilized to give the desired product **52** as the peracetate salt (26 mg, 49%). $[\alpha]_D^{23}$ +55.7 (*c* 0.87, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.43 (d, *J* = 3.7 Hz, 1H), 5.04 (d, *J* = 3.2 Hz, 1H), 4.97 (br s, 1H), 4.10 (t, *J* = 5.4 Hz, 1H), 4.03 – 3.97 (m, 2H), 3.95 – 3.88 (m, 2H), 3.74 (t, *J* = 9.5 Hz, 1H), 3.66 – 3.56 (m, 3H), 3.55 – 3.44 (m, 3H), 3.42 (t, *J* = 9.8 Hz, 1H), 3.32 (dd, J_1 = 14.5 Hz, J_2 = 3.6 Hz, 1H), 3.29 – 3.22 (m, 2H), 3.20 (t, *J* = 9.1 Hz, 1H), 3.16 (dd, J_1 = 10.6 Hz, J_2 = 4.0 Hz, 1H), 3.15 – 3.09 (m, 2H), 3.09 – 3.01 (m, 2H), 2.18 (dt, J_1 = 12.7 Hz, J_2 = 4.1 Hz, 1H), 1.72 (s, 18H), 1.56 (q, *J* = 12.7 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.9, 174.4, 109.4, 95.4, 95.2, 83.8, 79.9, 77.5, 77.0, 74.1, 73.0, 71.8, 70.0, 68.9, 68.8, 67.4, 67.1, 60.1, 53.4, 50.6, 49.4, 48.8, 41.0, 40.2, 27.8, 21.9, 21.1. ESIHRMS calculated for $C_{25}H_{49}N_6O_{14}$ $[M+H]^+$, 657.3307; found, 657.3273.

5”-Deoxy-5”-ureidoparomomycin pentaacetate salt (53). A stirred solution of **50** (100 mg, 0.07 mmol) in DCM (1 mL) was treated with benzyl isocyanate (50 µL) at RT. The reaction mixture was stirred for 2 h, quenched with MeOH (5 mL), and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (24:1) to give the desired urea intermediate (70 mg, 64%). A portion of this intermediate (30 mg) was dissolved in 80% aqueous AcOH (1 mL) and added to a stirred suspension of Pd/C (90 mg) in 80% AcOH (0.5 mL). The reaction mixture was stirred under a hydrogen atmosphere (45 psi) for 12 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.7% NH_4OH). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and lyophilized to give the desired product **53** as the peracetate salt (8 mg, 42%). $[\alpha]_D^{23}$ +18.5 (*c* 0.27, H_2O). ^1H NMR (600

MHz, D_2O) δ 5.54 (d, *J* = 3.4 Hz, 1H), 5.12 (d, *J* = 3.3 Hz, 1H), 5.05 (br s, 1H), 4.20 (t, *J* = 5.2 Hz, 1H), 4.12 – 4.09 (m, 1H), 4.09 – 4.06 (m, 1H), 4.03 – 3.95 (m, 2H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.74 – 3.64 (m, 3H), 3.60 (br s, 1H), 3.61 – 3.50 (m, 2H), 3.49 (t, *J* = 9.8 Hz, 1H), 3.38 – 3.31 (m, 2H), 3.31 – 3.24 (m, 3H), 3.21 (dd, J_1 = 13.8 Hz, J_2 = 6.2 Hz, 1H), 3.18 – 3.10 (m, 3H), 2.26 (dt, J_1 = 12.7 Hz, J_2 = 4.2 Hz, 1H), 1.82 (s, 15H), 1.64 (q, *J* = 12.7 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.1, 161.2, 109.6, 95.5, 95.4, 84.1, 80.4, 77.7, 77.0, 74.1, 73.0, 71.9, 70.0, 69.0, 68.8, 67.5, 67.3, 60.2, 53.5, 50.7, 49.4, 48.9, 41.4, 40.3, 27.9, 20.6. ESIHRMS calculated for $C_{24}H_{48}N_7O_{14}$ $[M+H]^+$, 658.3259; found, 658.3258.

5-*O*-β-[2''',3'''-Di-*O*-benzoyl-D-erythrofuranosyl]-1,3,2',6'-tetraazido-6,3',4'-tri-*O*-benzylneamine (56). Donor **55** (100 mg, 0.21 mmol), acceptor **54** (70 mg, 0.10 mmol) and activated 4 Å MS were stirred in DCM (2 mL) at rt for 1 h before cooling to -78 °C. $BF_3.OEt_2$ (200 µL, 0.54 mmol) was added and reaction mixture was stirred for 2.5 h at -78 °C. The reaction was quenched at -78 °C with Et_3N (0.5 mL) and filtered through Celite® before it was diluted with EtOAc. The organic layer was washed with $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (5% - 15% EtOAc/hexanes) to give the β anomer **56** (110 mg, >99%) in the form of white solid; $[\alpha]_D^{25}$ = +19.88 (c 6.7, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 – 7.76 (m, 4H), 7.56 – 7.47 (m, 2H), 7.43 – 7.27 (m, 16H), 7.16 – 7.03 (m, 3H), 6.02 (d, *J* = 2.5 Hz, 1H), 5.76 (d, *J* = 3.8 Hz, 1H), 5.70 (q, *J* = 5.0 Hz, 1H), 5.63 (dd, *J* = 4.9, 2.5 Hz, 1H), 4.96 – 4.85 (m, 4H), 4.78 (d, *J* = 11.0 Hz, 1H), 4.69 – 4.64 (m, 1H), 4.63 (t, *J* = 2.8 Hz, 1H), 4.31 (ddd, *J* = 10.0, 5.0, 2.4 Hz, 1H), 4.17 (dd, *J* = 10.0, 4.5 Hz, 1H), 4.11 (dd, *J* = 10.4, 8.8 Hz, 1H), 4.04 (t, *J* = 9.2 Hz, 1H), 3.69 (t, *J* = 9.5 Hz, 1H), 3.60 – 3.46 (m, 4H), 3.46 – 3.34 (m, 3H), 2.33 (dt, *J* = 13.2, 4.6 Hz, 1H), 1.50 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.5, 165.3, 137.7, 137.1, 133.4, 133.3, 129.7, 129.1, 128.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.3, 105.7, 97.1, 84.4, 79.72, 79.75, 78.6, 76.2, 75.7, 75.5, 75.3, 75.0, 71.8, 71.1, 70.4, 63.0, 60.5, 59.7, 51.1, 32.5 ; ESI-HRMS: m/z calcd. for $C_{51}H_{50}N_{12}NaO_{11}$ $[M+Na]^+$ 1029.3620; found, 1029.3656.

5-*O*-β-D-Erythrofuranosylneamine pentaacetate salt (57). A stirred solution of compound **56** (50 mg, 0.05 mmol) in dioxane (0.5 mL) was treated with NaOH (0.5 mL, 3 N) and heated at 50 °C for 1.5 h. PMe_3 (1 M in THF, 0.3 mL) was added to the reaction mixture and stirred at 50 °C for 2 h. The reaction mixture was cooled to rt and neutralized with glacial acetic acid and concentrated in vacuo. The crude product was passed through a silica gel column (50% MeOH/DCM). The product-containing fractions were concentrated, dissolved in dioxane:water:glacial acetic acid (1:2:0.2, 0.6 mL) and 10% Pd/C (40 mg) was added and the reaction mixture was stirred at room temperature under 1 atm of hydrogen (balloon) for 12 h. After completion, the reaction mixture was filtered over Celite®, concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then eluted with a gradient of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **57** (11 mg, 35%) as the peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +17.0 (c 0.4, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.85 (d, *J* = 4.2 Hz, 1H), 5.24 (d, *J* = 3.9 Hz, 1H), 4.19 (q, *J* = 4.4 Hz, 1H), 4.11 – 4.07 (m, 1H), 4.02 (t, *J* = 4.3 Hz, 1H), 3.88 (dd, *J* = 10.3, 9.0 Hz, 1H), 3.83 – 3.77 (m, 1H), 3.77 – 3.70 (m, 2H), 3.68 (dd, *J* = 9.7, 3.6 Hz, 1H), 3.51 (t, *J* = 9.8 Hz, 1H), 3.37 – 3.31 (m, 2H), 3.29 (t, *J* = 9.4 Hz, 1H), 3.26 (dd, *J* = 13.7, 3.6 Hz, 1H), 3.16 (td, *J* = 11.9, 4.2 Hz, 1H), 3.10 (dd, *J* = 13.7, 6.6 Hz, 1H), 2.29 (dt, *J* = 12.6, 4.3 Hz, 1H), 1.68 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 109.8, 94.7, 84.3, 75.6, 74.9, 72.4, 71.5, 70.2, 69.6, 69.3, 68.2, 52.9, 49.3, 48.3, 39.8, 27.8; ESI-HRMS: m/z calcd. for $C_{16}H_{33}N_4O_9$ $[M+H]^+$ 425.2248; found, 425.2231.

1,3,2',6'-Tetraazido-1,3,2',6'-tetradesaminoribostamycin (**58**). To a stirred solution of NaN_3 (12.95 g, 0.20 mol) in H_2O/DCM (1:1, 70 mL) at 0 °C was added Tf_2O (18.5 mL, 0.039 mol) dropwise. After 2 h, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with DCM (60 mL) and the combined organic layers were set aside. To a stirred solution of ribostamycin sulfate (5.05 g, 7.69 mmol) in H_2O (80 mL) at 0 °C were added Et_3N (11 mL) and $CuSO_4{\cdot}5H_2O$ (88 mg, 0.35 mmol). The above TfN_3 solution was added dropwise, followed by MeOH (260 mL). After 16 h, the reaction was concentrated in vacuo until only H_2O remained. The aqueous solution was then acidified to pH 1.5 with 4 N HCl and extracted ten times with EtOAc after addition of NaCl (50 mL increments). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was then purified via silica gel chromatography (0-20% MeOH in DCM, R_f = 0.2 in 6% MeOH in DCM) to give **58** (3.59 g, 54%) as a light brown solid. $[\alpha]_D^{23}$ = +66.6 (*c* = 1.0, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 5.81 (d, *J* = 3.8 Hz, 1H), 5.37 (d, *J* = 1.2 Hz, 1H), 4.19 (ddd, *J* = 10.0, 5.7, 2.4 Hz, 1H), 4.16 – 4.14 (m, 2H), 3.96 (td, *J* = 5.7, 3.3 Hz, 1H), 3.89 (dd, *J* = 10.5, 8.8 Hz, 1H), 3.80 (dd, *J* = 11.8, 3.3 Hz, 1H), 3.71 – 3.65 (m, 3H), 3.57 – 3.52 (m, 2H), 3.48 – 3.42 (m, 3H), 3.38 – 3.35 (m, 1H), 3.12 (dd, *J* = 10.5, 3.8 Hz, 1H), 2.24 (dt, *J* = 12.6, 4.2 Hz, 1H,), 1.41 (q, *J* = 12.3 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 107.9, 97.0, 83.5, 83.2, 76.0, 75.8, 75.5, 71.8, 71.2, 70.7, 70.2, 63.2, 62.2 , 60.5, 59.8, 51.2, 31.6. ESI-HRMS: *m/z* calc for $C_{17}H_{26}N_{12}O_{10}Na$ $[M+Na]^+$ 581.17871, found 581.1787.

1,3,2',6'-Tetraazido-1,3,2',6'-tetradesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)ribostamycin (**60**). To a stirred solution of **58** (1.39 g, 2.49 mmol) in pyridine (13 mL) was added trisyl chloride (1.52 g, 4.98 mmol). After 22 h, the reaction was quenched with water and diluted with EtOAc. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated to dryness. The crude mixture was purified via flash chromatography (0-10% MeOH in DCM, R_f = 0.7 in 10% MeOH/DCM) to give the desired product **60** (834 mg, 41%) as a white solid. $[\alpha]_D^{23}$ = +42.8 (*c* = 0.5, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 7.32 (s, 2H), 5.96 (d, *J* = 3.8 Hz, 1H), 5.36 (d, *J* = 1.3 Hz, 1H), 4.24 – 4.18 (m, 4H), 4.17 – 4.14 (m, 2H), 4.08 (td, *J* = 7.2, 3.1 Hz, 1H), 4.04 (dd, *J* = 7.4, 4.6 Hz, 1H), 3.88 (dd, *J* = 10.4, 8.8 Hz, 1H), 3.66 (t, *J* = 8.9 Hz, 1H), 3.62 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.57 – 3.53 (m, 1H), 3.51 (dd, *J* = 13.3, 2.4 Hz, 1H), 3.48 – 3.42 (m, 2H), 3.37 – 3.35 (m, 1H), 3.32 – 3.28 (m, 2H), 2.97 (hept, *J* = 7.0 Hz, 1H), 2.23 (dt, *J* = 12.9, 4.5 Hz, 1H), 1.38 – 1.32 (m, 1H), 1.32 – 1.28 (m, 18H); ^{13}C NMR (226 MHz, MeOD) δ 154.1, 150.9, 129.0, 123.7, 110.2, 96.0, 83.9, 80.0, 76.5, 75.9, 74.8, 71.9, 71.3, 71.1, 70.8, 70.7, 63.3, 60.6, 60.0, 51.4, 34.1, 31.7, 29.4, 23.73, 23.71, 22.53, 22.5; ESI-HRMS: *m/z* calc for $C_{32}H_{48}N_{12}O_{12}SNa$ $[M+Na]^+$ 847.31276, found 847.3127.

1,2,2',6'Tetra-*N*-benzyloxycarbonyl-5''-*O*-methanesulfonyl ribostamycin (61). A stirred solution of **59** (1 g, 1.01 mmol) in DCM (25 mL) was treated with pyridine (5 mL) and methanesulfonyl chloride (0.1 mL, 14.56 mmol) at 0 ^{0}C. The reaction mixture was allowed to warm to RT and stirring was continued for 3 h before it was quenched with MeOH (5 mL) and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (19:1) to give desired product **61** (280 mg, 26 %). $[\alpha]_D^{23}$ +21.3 (*c* 0.67, MeOH). ESIHRMS calculated for $C_{50}H_{60}N_4O_{20}NaS$ $[M+Na]^+$, 1091.3419; found, 1091.3417.[1]

1,3,2',6',5''-Pentaazido-5''-deoxy-1,3,2',6'-tetradesaminoribostamycin (**62**). To a stirred solution of **60** (383 mg, 0.47 mmol) in DMF (4.7 mL) was added NaN_3 (590 mg, 9.08 mmol) and the reaction was heated to 95 °C. After 21 h, the reaction mixture was

diluted with EtOAc and washed with water. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified via flash chromatography (0-20% MeOH in DCM, R_f = 0.2 in 6% MeOH in DCM) to give **62** (200 mg, 74%) as a white solid. $[\alpha]_D^{23}$ = +90.4 (*c* = 1.0, MeOH); ^{1}H NMR (900 MHz, MeOD) δ 6.00 (d, *J* = 3.9 Hz, 1H), 5.35 (s, 1H), 4.21 – 4.19 (m, 1H), 4.18 (ddd, *J* = 9.9, 5.9, 2.3 Hz, 1H), 4.07 (dd, *J* = 7.3, 4.6 Hz, 1H), 4.02 (td, *J* = 7.1, 3.0 Hz, 1H), 3.88 (dd, *J* = 10.4, 8.8 Hz, 1H), 3.65 (t, *J* = 8.8 Hz, 1H), 3.62 (t, *J* = 9.2 Hz, 1H), 3.60 – 3.51 (m, 3H), 3.48 (dd, *J* = 13.1, 6.9 Hz, 1H), 3.46 – 3.41 (m, 2H, H1), 3.35 (dd, *J* = 18.1, 8.9 Hz, 2H), 3.19 (dd, *J* = 10.4, 3.9 Hz, 1H), 2.23 (dt, *J* = 12.9, 4.5 Hz, 1H), 1.37 (q, *J* = 12.5 Hz, 1H); ^{13}C NMR (226 MHz, MeOD) δ 110.1, 96.2, 84.0, 81.0, 76.4, 76.0, 75.0, 71.8, 71.4, 71.2, 71.1, 63.5, 60.6, 59.9, 53.3, 51.2, 31.7. ESI-HRMS: *m/z* calc for $C_{17}H_{25}N_{15}O_9Na$ $[M+Na]^+$ 616.18519, found 616.1866.

5''-Azido-1,3,2',6'-tetra-*N*-benzyloxycarbonyl-5''-deoxyribostamycin (63). To a stirred solution of **61** (270 mg, 0.25 mmol) in DMF (2.5 mL) was added NaN_3 (329 mg, 5.05 mmol). The reaction mixture was stirred for 4 h at 80 °C and concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (20 mL) and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (20:1) to give the desired product **63** (120 mg, 47 %). $[\alpha]_D^{23}$ +29.8 (*c* 0.67, MeOH). ESIHRMS calculated for $C_{49}H_{57}N_7O_{17}Na$ $[M+Na]^+$, 1038.3709; found, 1038.3727.[1]

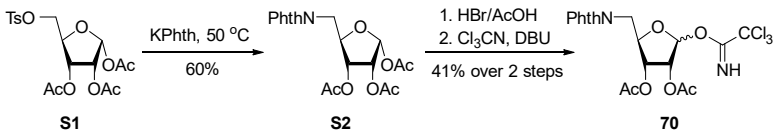
5''-Amino-5''-deoxyribostamycin pentaacetate salt (64). To a solution of **62** (102 mg, 0.17 mmol) in dioxane/water (1:1, 2 mL) was added Pd on carbon (192 mg). The reaction mixture was purged with H_2 and pressurized to 50 psi. After 19 h, the reaction mixture was filtered through Celite and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.2-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **64** (31 mg, 24%). $[\alpha]_D^{23}$ = +31.8 (*c* = 1.0, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.90 (d, *J* = 3.8 Hz, 1H), 5.33 (d, *J* = 1.6 Hz, 1H), 4.13 (dd, *J* = 4.8, 1.6 Hz, 1H), 4.09 (dd, *J* = 7.1, 4.8 Hz, 1H), 4.06 – 4.01 (m, 2H), 3.96 – 3.88 (m, 3H), 3.66 (dd, *J* = 10.5, 9.3 Hz, 1H), 3.43 (t, *J* = 8.9 Hz, 1H), 3.41 – 3.38 (m, 2H), 3.36 (dd, *J* = 13.7, 3.4 Hz, 1H), 3.29 (dd, *J* = 13.5, 3.9 Hz, 1H), 3.27 – 3.22 (m, 2H), 3.11 (dd, *J* = 13.5, 8.1 Hz, 1H), 2.35 (dt, *J* = 12.6, 4.2 Hz, 1H), 1.84 (s, 15H), 1.80 – 1.72 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 180.5, 108.5, 93.7, 83.3, 78.3, 74.8, 74.5, 71.9, 71.3, 70.2, 68.5, 53.1, 50.1, 48.8, 41.9, 39.9, 28.3, 22.7. ESI-HRMS: *m/z* calc for $C_{17}H_{36}N_5O_9$ $[M+H]^+$ 454.25075, found 454.2502.

5''-Amino-1,3,2',6'-tetra-*N*-benzyloxycarbonyl-5''-deoxyribostamycin (65). To a stirred solution of **63** (120 mg, 0.12 mmol) in a mixture of THF and water (1:1, 2 mL) was added trimethylphosphine (1 M in THF, 0.24 mL). After stirring for 2 h at 80 °C the reaction was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (7:3) to give desired product **65** (90 mg, 77 %). $[\alpha]_D^{23}$ +22.7 (*c* 0.67, MeOH). ESIHRMS calculated for $C_{49}H_{60}N_5O_{17}$ $[M+H]^+$, 990.3984; found, 990.4001.[1]

5”-Deoxy-5”-formamidoribostamycin tetraacetate salt (66). A stirred solution of **65** (90 mg, 0.18 mmol) in DCM (2 mL) was treated with formic acetic anhydride (2 mL). The reaction mixture was stirred for 4 h before it was quenched with MeOH (5 mL) and concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (5 mL) and to this solution was added 1N NaOH (1 mL). Then the mixture was stirred for 0.5 h and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (15:1) to give desired intermediate (50 mg). A solution of this intermediate (45 mg, 0.04 mmol) in dioxane (1.5 mL) was added to a stirred suspension of Pd/C (90 mg) in 10 % AcOH (1.5 mL). The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 18 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.7 % NH_4OH). The product containing fractions were concentrated under reduced pressure. The residue was dissolved in 10 % AcOH and lyophilized to give the desired product **66** in the form of its peracetate salt (15 mg, 48 %). $[\alpha]_D^{23}$ +23.0 (*c* 0.5, H_2O). δ^1H NMR (600 MHz, D_2O) δ 7.83 (s, 1H), 5.70 (d, *J* = 3.9 Hz, 1H), 5.03 (d, *J* = 3.1 Hz, 1H), 3.86 (t, *J* = 3.8 Hz, 1H), 3.82 – 3.74 (m, 3H), 3.70 (t, *J* = 9.8 Hz, 1H), 3.67 – 3.62 (m, 1H), 3.60 (t, *J* = 9.1 Hz, 1H), 3.39 (t, *J* = 9.8 Hz, 1H), 3.28 (dd, J_1 = 14.5 Hz, J_2 = 3.6 Hz, 1H), 3.26 – 3.15 (m, 4H), 3.14 (dd, J_1 = 13.5 Hz, J_2 =3.4 Hz, 1H), 3.08 – 3.01 (m, 1H), 3.00 (dd, J_1 = 13.7 Hz, J_2 = 6.8 Hz, 1H), 2.17 (dt, J_1 = 12.7 Hz, J_2 = 4.0 Hz, 1H), 1.73 (s, 12H), 1.59 (q, *J* = 12.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 176.9, 164.5, 109.8, 94.4, 84.9, 81.2, 74.7, 74.5, 72.1, 70.7, 70.2, 69.4, 67.9, 53.0, 49.4, 48.2, 39.9, 39.8, 27.7, 20.5. ESIHRMS calculated for $C_{18}H_{36}N_5O_{10}$ $[M+H]^+$, 482.2462; found, 482.2451.

5”-Acetamido-5”-deoxyribostamycin tetraacetate salt (67). A stirred solution of **65** (150 mg, 0.15 mmol) in MeOH (3.0 mL) was treated with an excess of acetic anhydride (1.5 mL) and stirred for 48 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (12:1) to give the desired intermediate (115 mg). A portion of this solid (45 mg, 0.11 mmol) was dissolved in dioxane (2.5 mL) and was added to a stirred suspension of Pd/C (110 mg) in 10 % AcOH (2.5 mL). The reaction mixture was stirred under a hydrogen atmosphere (40 psi) for 8 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.6 % NH_4OH). The product containing fractions were concentrated under reduced pressure. The residue was dissolved in 10 % AcOH and lyophilized to give the desired product **67** in the form of its tetraacetate salt (50 mg, 64 %). $[\alpha]_D^{25}$ +37.6 (*c* 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.72 (d, *J* = 3.9 Hz, 1H), 5.05 (d, *J* = 2.9 Hz, 1H), 3.88 (dd, J_1 = 4.5 Hz, J_2 = 3.2 Hz, 1H), 3.84 – 3.72 (m, 3H), 3.72 (t, *J* = 9.7 Hz, 1H), 3.69 – 3.64 (m, 1H), 3.62 (t, *J* = 9.0 Hz, 1H), 3.41 (t, *J* = 9.8 Hz, 1H), 3.25 (dd, *J* = 11.3, 4.0 Hz, 1H), 3.23 – 3.17 (m, 2H), 3.15 (t, *J* = 4.1 Hz, 1H), 3.14 – 3.09 (m, 2H), 3.09 – 3.03 (m, 1H), 3.01 (dd, J_1 = 13.6 Hz, J_1 = 6.9 Hz, 1H), 2.19 (dt, J_1 = 12.4, J_1 = 4.0 Hz, 1H), 1.74 (s, 12H), 1.72 (s, 3H), 1.60 (q, *J* = 12.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.1, 173.9, 109.5, 94.1, 84.6, 80.9, 74.4, 74.3, 71.9, 70.7, 70.2, 69.2, 67.6, 52.9, 49.2, 48.1, 41.3, 39.7, 27.5, 21.6, 20.7. ESIHRMS calculated for $C_{19}H_{38}N_5O_{10}$ $[M+H]^+$, 496.2619; found, 496.2621.

Scheme S1. Synthesis of donor 70

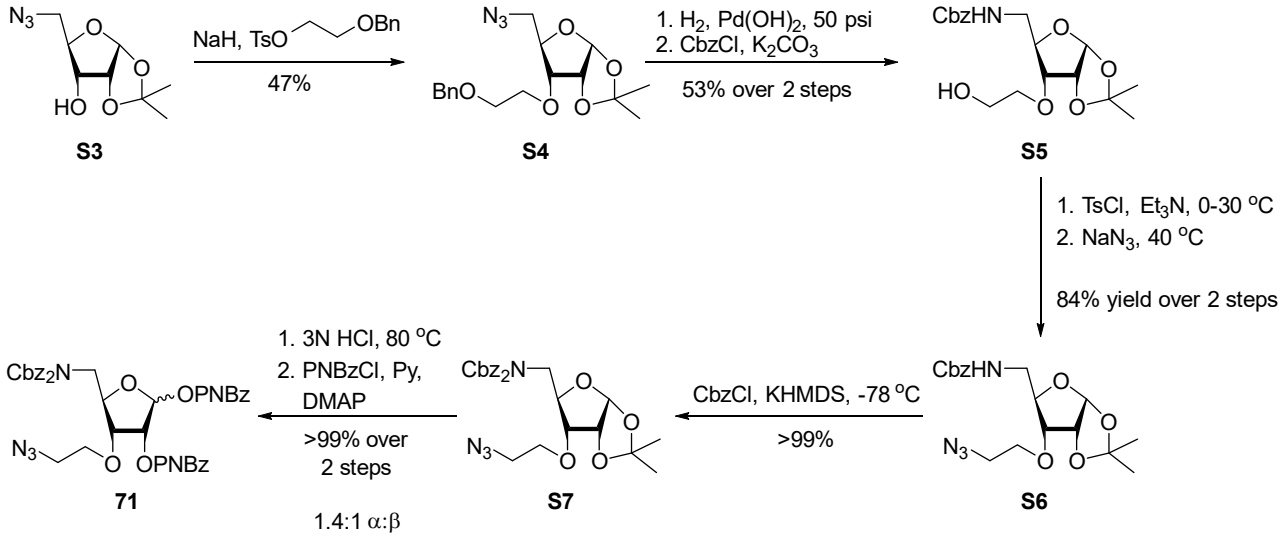


S1 **S2** **70**

1,2,3-Tri-*O*-acetyl-5-deoxy-5-phthalimido-α-D-ribofuranose (S2). 1,2,3-Tri-*O*-acetyl-5-*O*-*p*-tolylsulfonyl-D-ribofuranose **S1**[2] (1.0 g, 2.3 mmol) was dissolved in DMF (20 mL) and treated with potassium phthalimide (1.0 g, 5.4 mmol). The reaction mixture was stirred at 50 °C for 12 h before it was diluted with water and extracted with DCM three times. The organic layer wash then washed with 5% aqueous NaOH and brine, dried over Na_2SO_4, and concentrated. The residue was purified using silica gel column chromatography (eluent: 15% - 35% EtOAc/hexanes) to give **S2** (566 mg, 60%) as a white solid; $[\alpha]_D^{25}$= +49.66 (*c* 1.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 – 7.73 (m, 2H), 7.70 – 7.62 (dd, *J* = 5.4, 3.1 Hz, 2H), 6.34 (d, *J* = 4.5 Hz, 1H), 5.23 (dd, *J* = 6.8, 4.5 Hz, 1H), 5.15 (dd, *J* = 6.7, 3.4 Hz, 1H), 4.48 (td, *J* = 6.8, 3.4 Hz, 1H), 3.86 (dd, *J* = 6.8, 5.2 Hz, 2H), 2.03 – 1.95 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.9, 169.4, 169.2, 168.0, 134.1, 131.8, 123.4, 93.7, 80.4, 70.7, 69.6, 39.3, 20.9, 20.5, 20.2; ESI-HRMS: m/z calcd. for $C_{19}H_{19}NNaO_9$ $[M+Na]^+$ 428.0958; found, 428.0964.

2,3-Di-*O*-acetyl-5-deoxy-5-phthalimido-D-ribofuranose trichloroacetimidate (70). To an ice-cooled solution of **S2** (550 mg, 1.36 mmol) in DCM (5 mL), 33% HBr/acetic acid (0.7 mL, 4.07 mmol) was added followed by stirring for 45 min. After completion, solid $NaHCO_3$ was added to neutralize the reaction, then water was added and the aqueous layer was extracted with DCM three times. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified using silica gel column chromatography (eluent: 20% - 60% EtOAc/hexanes) to give the desired intermediate as a mixture of anomers α:β = 1:3 (200 mg) that was used directly in the next step. A portion of the crude product (190 mg, 0.53 mmol) and trichloroacetonitrile (2 mL) were dissolved in dry DCM (2 mL) and ice-cooled before addition of DBU (2 drops). The reaction mixture was stirred at rt for 5 min and concentrated. The crude product was passed through a silica gel column, basified with 0.5% Et_3N/hexanes, eluting with 0.5% Et_3N in EtOAc/hexanes to give compound **70** (270 mg, 41%), which was used in the next step without further purification.

Scheme S2. Synthesis of donor 71



5-Azido-3-*O*-(2-benzyloxyethyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S4). 5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose **S3**[3] (4.0 g, 18.6 mmol) was dissolved in THF (100 mL) and NaH (100 mg, 24.5 mmol) was added. After stirring for 15

min, 2-benzyloxyethyl tosylate (6.83 g, 22.3 mmol) was added and stirring continued for 36 h. After completion, the reaction was quenched with MeOH, diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 10% to 20% EtOAc/hexanes) to give **S4** (3.08 g, 47%) in the form of a colorless oil; $[\alpha]_D^{25}$= +119.83 (*c* 1.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 – 7.21 (m, 5H), 5.76 (d, *J* = 3.5 Hz, 1H), 4.64 (t, *J* = 3.9 Hz, 1H), 4.56 (s, 2H), 4.14 (dt, *J* = 8.5, 3.2 Hz, 1H), 3.91– 3.71 (m, 2H), 3.76 – 3.64 (m, 4H), 3.32 (dd, *J* = 13.5, 4.0 Hz, 1H), 1.57 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.0, 128.4, 127.7, 113.2, 103.9, 79.5, 77.4, 77.3, 73.3, 70.1, 69.7, 50.6, 26.8, 26.5; ESI-HRMS: m/z calcd. for $C_{17}H_{23}N_3NaO_5$ $[M+Na]^+$ 372.1535; found, 372.1538.

5-Benzyloxycarbonylamino-5-deoxy-3-*O*-(2-hydroxyethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (S5). To a solution of compound **S4** (3.0 g, 8.6 mmol) in dioxane:water (5:1, 30 mL), 20% $Pd(OH)_2$/C (3.0 g, 0.5 equiv) was added and the reaction mixture stirred under 50 psi of hydrogen for 18 h. After completion, the reaction mixture was filtered over Celite®, concentrated to dryness and dissolved in dioxane:water (3:1, 50 mL). K_2CO_3 (6.0 g, 43.5 mmol) and CbzCl (2.5 mL, 17.2 mmol) were added and the reaction mixture was stirred for 4 h. After completion, the reaction mixture was concentrated and purified using silica gel column chromatography (eluent: 0.8% to 1% MeOH/DCM) to give **S5** (1.67 g, 53%) as a colorless oil; $[\alpha]_D^{25}$= +35.47 (*c* 1.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.28 (m, 5H), 5.73 (d, *J* = 3.8 Hz, 1H), 5.10 (d, *J* = 1.4 Hz, 2H), 4.60 (t, *J* = 4.1 Hz, 1H), 4.03 (dt, *J* = 9.0, 3.6 Hz, 1H), 3.77 – 3.61 (m, 5H), 3.55 (dd, *J* = 9.0, 4.4 Hz, 1H), 3.45 (dt, *J* = 14.6, 4.2 Hz, 1H), 3.04 (t, *J* = 5.8 Hz, 1H), 1.56 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.9, 136.3, 128.5, 128.2, 113.1, 104.1, 79.3, 77.1, 77.0, 72.0, 67.0, 61.6, 40.6, 26.6, 26.5; ESI-HRMS: m/z calcd. for $C_{18}H_{25}NNaO_7$ $[M+Na]^+$ 390.1529; found, 390.1537.

3-*O*-(2-Azidoethyl)-5-benzyloxycarbonylamino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S6). To a stirred solution of alcohol **S5** (1.0 g, 2.7 mmol) in THF (5 mL) was added triethylamine (2.8 mL, 20.4 mmol). The reaction mixture was cooled to 0 °C before addition of a solution of p-tolylsulfonyl chloride (975 mg, 5.13 mmol) in THF (5 mL). The reaction mixture was stirred at 30 °C for 48 h before it was concentrated *in vacuo*. The crude product was dissolved in EtOAc and washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The resulting solid was dissolved in DMF (10 mL), treated with NaN_3 (1.05 g, 16.3 mmol) and stirred at 40 °C for 48 h. After completion, the reaction mixture was diluted with acetone and excess NaN_3 was filtered off. The solvent was partially removed under vacuum and the residue was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed under vacuum and the resulting product was purified using silica gel column chromatography (eluent: 10% to 25% EtOAc/hexanes) to give **S6** (900 mg, 84% over two steps) as a viscous oil; $[\alpha]_D^{25}$= +35.38 (*c* 1.9, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 – 7.27 (m, 5H, Ar*H*), 5.73 (d, *J* = 3.7 Hz, 1H), 5.10 (s, 2H), 4.60 (t, *J* = 4.1 Hz, 1H), 4.05 (dt, *J* = 8.6, 4.1 Hz, 1H), 3.82 (ddd, *J* = 10.1, 6.0, 3.8 Hz, 1H), 3.67 (ddd, *J* = 10.3, 6.5, 3.9 Hz, 1H), 3.63 – 3.47 (m, 3H), 3.47 – 3.32 (m, 2H), 1.56 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.5, 136.4, 128.5, 128.1, 113.3, 104.1, 80.0, 77.1, 77.0, 69.4, 66.9, 50.7, 41.1, 26.7, 26.6; ESI-HRMS: m/z calcd. for $C_{18}H_{24}N_4NaO_6$ $[M+Na]^+$ 415.1594; found, 415.1589.

3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S7). A stirred solution of the compound **S6** (200 mg, 0.51 mmol) in THF (8 mL) and HMPA (2 mL) was cooled to -78 °C and KHMDS (0.5 M in toluene, 1.5 mL, 0.66 mmol) and CbzCl (0.3 mL, 2.1 mmol) were added. The reaction mixture was stirred at -78 °C for 2 h before additional

KHMDS (0.5 M in toluene, 3 mL, 1.5 mmol) was added. The reaction was stirred for 30 min and quenched with NH_4Cl, diluted with EtOAc, and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 10% to 25% EtOAc/hexanes) to give **S7** (272 mg, quant) $[\alpha]_D^{25}$= +14.73 (*c* 1.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 – 7.28 (m, 10H), 5.70 (d, *J* = 3.8 Hz, 1H), 5.42 – 5.12 (m, 4H), 4.56 (t, *J* = 4.1 Hz, 1H), 4.20 (dt, *J* = 8.9, 5.4 Hz, 1H), 4.07 (dd, *J* = 5.4, 1.2 Hz, 2H), 3.72 (ddd, *J* = 9.9, 6.0, 4.0 Hz, 1H), 3.57 (dd, *J* = 8.8, 4.4 Hz, 1H), 3.45 (ddd, *J* = 10.1, 6.3, 4.2 Hz, 1H), 3.34 – 3.16 (m, 2H), 1.51 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.6 (C=O), 135.2, 128.5, 128.3, 128.2, 128.1, 113.1, 104.2, 81.3, 77.4, 77.1, 68.9, 68.8, 66.9, 50.5, 47.1, 26.7 ($2CH_3$); ESI-HRMS: m/z calcd. for $C_{26}H_{30}N_4NaO_8$ $[M+Na]^+$ 549.1961; found, 549.1962.

3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-α/β-D-ribofuranose (71). To a stirred solution of compound **S7** (268 mg, 0.51 mmol) in dioxane (10 mL), was added 1 N HCl (4 mL) and the reaction mixture was heated at 80 °C for 2 h. The reaction mixture was cooled, neutralized with solid $NaHCO_3$ and the solvent was evaporated. The residue was dissolved in EtOAc and washed with water and brine, dried with Na_2SO_4 and evaporated. To a solution of the crude mixture in pyridine (10 mL) were added p-nitrobenzoyl chloride (672 mg, 3.6 mmol) and a catalytic amount of DMAP, and the reaction was left to stir overnight. The reaction mixture was diluted with EtOAc and washed with $NaHCO_3$, brine, dried with Na_2SO_4 then concentrated. The crude product was purified using silica gel column chromatography (eluent: 15% - 40% EtOAc/hexanes) to give the α isomer (235 mg, 59%) as a white solid and the β isomer (165 mg, 41%) as a white solid; **α isomer:** $[\alpha]_D^{25}$= +13.85 (*c* 0.4, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 – 8.17 (m, 6H), 8.12 (d, *J* = 8.8 Hz, 2H), 7.48 – 7.17 (m, 10H), 6.64 (d, *J* = 4.4 Hz, 1H), 5.36 (dd, *J* = 6.6, 4.4 Hz, 1H), 5.34 – 5.21 (m, 4H,), 4.61 (td, *J* = 6.2, 3.8 Hz, 1H), 4.18 (dd, *J* = 6.6, 3.8 Hz, 1H), 4.15 – 3.99 (m, 2H), 3.47 (t, *J* = 4.8 Hz, 2H), 3.20 – 3.12 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.6, 163.3, 153.5, 150.9, 150.8, 148.4, 137.3, 134.9, 134.8, 134.1, 131.0, 130.9, 130.8, 128.6, 128.4, 123.7, 123.4, 95.4, 82.6, 77.3, 72.4, 70.1, 69.3, 50.9, 47.7; ESI-HRMS: m/z calcd. for $C_{37}H_{32}N_6NaO_{14}$ $[M+Na]^+$ 807.1874; found, 807.1852; **β isomer:** $[\alpha]_D^{25}$= -32.63 (*c* 0.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 – 7.91 (m, 8H), 7.29 (s, 10H), 6.52 (s, 1H), 5.71 (d, *J* = 4.1 Hz, 1H), 5.19 (q, *J* = 12.3 Hz, 4H), 4.46 (dt, *J* = 8.1, 4.9 Hz, 1H), 4.39 (dd, *J* = 8.0, 4.2 Hz, 1H), 4.30 – 4.14 (m, 2H), 3.73 (ddd, *J* = 9.9, 6.8, 3.3 Hz, 1H), 3.57 (ddd, *J* = 9.5, 6.0, 3.3 Hz, 1H), 3.30 – 3.04 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.5, 162.9, 153.9, 150.9, 150.7, 134.9, 134.4, 134.3, 131.1, 131.0, 128.6, 128.5, 128.2, 128.1, 123.7, 123.6, 99.6, 80.2, 79.2, 74.5, 70.4, 69.2, 50.6, 47.2; ESI-HRMS: m/z calcd. for $C_{37}H_{32}N_6NaO_{14}$ $[M+Na]^+$ 807.1874; found, 807.1877.

5-*O*-β-(2‴,3‴-Di-*O*-acetyl-5‴-deoxy-5‴-phthalimido-D-ribofuranosyl)-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (72). Donor **70** (190 mg, 0.52 mmol), acceptor **68** (701 mg, 0.84 mmol) and activated 4 Å MS were stirred in DCM (3 mL) at RT for 1 h before cooling to 0 °C. $BF_3.OEt_2$ (400 µL, 1.08 mmol) was added and reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched with triethylamine (0.5 mL) and filtered through Celite® before it was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.5% MeOH/DCM) to give the glycoside **72** (470 mg, 76%) as the β anomer in the form of white solid; $[\alpha]_D^{25}$= +131.96 (*c* 5.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 – 7.88 (m, 2H), 7.77 – 7.70 (m, 2H), 5.39 (t, *J* = 9.9 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 2H), 5.30 (s, 1H), 5.12 (d, *J* = 4.9 Hz, 1H), 5.06 (dd, *J* = 7.2, 4.8 Hz, 1H), 4.95 – 4.88 (m, 2H), 4.83 (dd, *J* = 8.2, 2.9 Hz, 1H), 4.66 (dd, *J* = 10.5, 2.9 Hz, 1H), 4.46 (t, *J* = 9.9 Hz, 1H), 4.42 – 4.29 (m, 2H), 4.22 (dd, *J* =

12.2, 5.2 Hz, 1H), 3.96 (d, *J* = 5.0 Hz, 2H), 3.84 – 3.68 (m, 3H), 3.65 – 3.53 (m, 3H), 3.46 – 3.28 (m, 3H), 2.94 (s, 3H), 2.41 (dt, *J* = 12.6, 4.3 Hz, 1H), 2.23 (s, 4H), 2.13 – 1.98 (m, 15H), 1.78 (q, *J* = 11.7 Hz, 1H), 1.43 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3, 170.2, 169.9, 169.8, 169.5, 168.2, 157.2, 134.1, 132.0, 123.7, 106.9, 97.0, 94.8, 93.8, 79.8, 79.1, 78.9, 74.0, 73.4, 72.6, 70.7, 70.3, 69.9, 68.9, 65.4, 65.3, 62.9, 60.21, 60.16, 58.4, 58.2, 57.7, 39.5, 31.4, 31.3, 29.9, 20.9, 20.8, 20.8, 20.7, 20.6, 20.4; ESI-HRMS: m/z calcd. for $C_{47}H_{54}N_{14}NaO_{23}$ $[M+Na]^+$ 1205.3384; found, 1205.3359.

5-*O*-β-[3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-2-*O*-*p*-nitrobenzoyl-D-ribofuranose]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (73). Donor **71β** (165 mg, 0.21 mmol), acceptor **68** (440 mg, 0.52 mmol) and activated 4 Å MS were stirred in DCM (3 mL) at RT for 1 h before cooling to 0 °C. $BF_3.OEt_2$ (300 µL, 0.78 mmol) was added and reaction mixture was stirred for 48 h at 0 °C. The reaction was quenched with triethylamine (0.5 mL) and filtered through Celite® before it was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.5% MeOH/DCM) to give exclusively the β anomer of **73** (136 mg, 45%) in the form of a white solid; $[\alpha]_D^{25}$= +46.26 (*c* 0.9, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.29 – 8.21 (m, 2H), 8.19 – 8.09 (m, 2H), 7.39 – 7.32 (m, 4H), 7.32 – 7.25 (m, 6H), 5.43 – 5.35 (m, 3H), 5.32 (d, *J* = 3.9 Hz, 1H), 5.31 – 5.26 (m, 4H), 5.25 (d, *J* = 4.1 Hz, 1H), 4.89 (dd, *J* = 10.3, 3.9 Hz, 1H), 4.87 (d, *J* = 3.4 Hz, 1H), 4.84 (t, *J* = 9.8 Hz, 1H), 4.78 (dd, *J* = 8.2, 3.2 Hz, 1H), 4.60 (dd, *J* = 10.5, 3.2 Hz, 1H), 4.31 (dd, *J* = 12.3, 2.3 Hz, 1H), 4.28 – 4.18 (m, 2H), 4.18 – 4.10 (m, 2H), 4.08 (dd, *J* = 7.8, 4.5 Hz, 1H), 3.79 (dd, *J* = 8.2, 3.5 Hz, 1H), 3.71 (ddd, *J* = 10.7, 5.3, 2.3 Hz, 1H), 3.66 (td, *J* = 10.9, 4.3 Hz, 1H), 3.63 – 3.52 (m, 4H), 3.49 – 3.43 (m, 2H), 3.39 (ddd, *J* = 12.5, 10.2, 4.2 Hz, 1H), 3.27 (dt, *J* = 12.8, 4.3 Hz, 1H), 3.12 (ddd, *J* = 13.3, 7.3, 3.2 Hz, 1H), 3.02 (ddd, *J* = 13.3, 5.7, 3.2 Hz, 1H), 2.92 (s, 3H), 2.38 (dt, *J* = 12.9, 4.5 Hz, 1H), 2.20 (s, 3H), 2.17 – 2.11 (m, 1H), 2.08 (d, *J* = 4.4 Hz, 6H), 2.04 (s, 3H), 1.77 (q, *J* = 11.8 Hz, 1H), 1.41 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.3, 170.0, 169.9, 169.4, 163.9, 157.0, 153.7, 150.8, 135.3, 134.4, 130.9, 128.5, 128.2, 127.9, 123.7, 106.7, 96.8, 95.4, 94.0, 80.4, 79.5, 79.3, 79.1, 74.8, 74.3, 70.6, 70.3, 70.0, 69.9, 68.9, 65.6, 65.3, 62.9, 60.14, 60.11, 58.3, 58.1, 57.4, 50.6, 48.2, 31.3, 31.0, 29.9, 21.0, 20.9, 20.8, 20.7; ESI-HRMS: m/z calcd. for $C_{60}H_{66}N_{18}NaO_{26}$ $[M+Na]^+$ 1477.4293; found, 1477.4232.

5-*O*-β-(5'''-Deoxy-5'''-formamido-D-ribofuranosyl)apramycin pentaacetate salt (75). To a stirred solution of compound **72** (50 mg, 0.04 mmol) in an iPrOH:water mixture (7:3, 1.5 mL), $NaBH_4$ (90 mg, 2.4 mmol) was added followed by stirring for 2 h. The reaction mixture was diluted with methanol and glacial acetic acid was added dropwise until effervescence stopped. The reaction mixture was concentrated in vacuo followed by the addition of NaOH (3 N, 0.5 mL) and water (0.5 mL). The reaction mixture was heated at 100 °C for 1 h before it was cooled, neutralized with glacial acetic acid and concentrated. The crude mixture was desalted using a Sephadex column and the product-containing fractions were concentrated. A part of the solid residue (8.2 mg, 0.009 mmol) was dissolved in water (0.2 mL) and treated with **74** (2.4 µL, 0.014 mmol) and Et_3N (1 µL). The reaction mixture was stirred for 2 h and quenched with ammonium hydroxide (0.25 mL) followed by addition of PMe_3 (1 M in THF, 0.3 mL) and stirring at 60 °C for 3 h. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with acetic acid and lyophilized to afford **75** (4.5 mg, 42%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +82.2 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 7.98 (s, 1H), 5.67 (d, *J* = 3.9 Hz,

1H), 5.34 (d, *J* = 3.9 Hz, 1H), 5.14 (d, *J* = 2.9 Hz, 1H), 5.06 (d, *J* = 8.5 Hz, 1H), 4.46 – 4.39 (m, 1H), 4.02 (dd, *J* = 4.4, 3.2 Hz, 1H), 3.94 (t, *J* = 5.3 Hz, 1H), 3.90 (q, *J* = 5.7 Hz, 1H), 3.85 – 3.67 (m, 6H), 3.63 (dd, *J* = 12.5, 4.6 Hz, 1H), 3.56 (dd, *J* = 9.8, 3.8 Hz, 1H), 3.54 – 3.50 (m, 3H), 3.42 (dd, *J* = 14.6, 4.2 Hz, 1H), 3.32 (dd, *J* = 14.6, 6.2 Hz, 1H), 3.29 – 3.23 (m, 1H), 3.22 (dd, *J* = 8.5, 2.7 Hz, 1H), 3.16 (td, *J* = 11.6, 10.9, 4.3 Hz, 1H), 3.10 (t, *J* = 10.3 Hz, 1H), 2.63 (s, 3H), 2.30 – 2.16 (m, 2H), 1.94 – 1.83 (m, 1H), 1.68 – 1.55 (m, 1H); ^{13}C NMR (151 MHz, D_2O): δ 164.7, 110.0, 94.4, 94.0, 92.9, 85.1, 80.9, 76.4, 74.7, 72.5, 70.8, 70.2, 69.7, 69.7, 68.6, 65.9, 62.7, 60.3, 59.4, 52.0, 49.8, 48.5, 47.7, 40.0, 30.0, 28.9, 26.8; ESI-HRMS: m/z calcd. for $C_{27}H_{51}N_6O_{15}$ $[M+H]^+$ 699.3412; found, 699.3410.

5-*O*-β-[3-*O*-(2-Aminoethyl)-5-deoxy-5-formamido-D-ribofuranosyl]apramycin hexaacetate salt (76). A stirred solution of **75** (67 mg, 0.046 mmol) in dioxane (1.5 mL) was treated with 3 N NaOH (1.5 mL) and heated at 100 °C for 18 h. The reaction mixture was cooled to 0 °C and neutralized with glacial acetic acid before it was concentrated *in vacuo*. The crude mixture was passed through a silica gel column (eluent: 25% MeOH/DCM). The resulting solid (20 mg, 0.023 mmol) was dissolved in a MeOH:water mixture (1:1, 0.5 mL) and treated with **74** (30 µL, 0.17 mmol) and triethylamine (2 µL). The reaction mixture was stirred for 2 h and quenched with aqueous ammonium hydroxide (0.25 mL) and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5% to 15% ammonical MeOH in DCM). A portion of the resulting residue (20 mg, 0.022 mmol) was dissolved in dioxane:water (1:1, 0.6 mL) followed by the addition PMe_3 (1 M in THF, 0.3 mL), and stirred at 50 °C for 45 min. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (10%, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with acetic acid, and lyophilized to afford the hexaacetate salt of **76** in (13.9 mg, 57%) as a white solid; $[\alpha]_D^{25}$= +55.35 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 7.94 (s, 1H), 5.69 (d, *J* = 3.9 Hz, 1H), 5.30 (d, *J* = 4.0 Hz, 1H), 5.15 (d, *J* = 3.0 Hz, 1H), 5.03 (d, *J* = 8.6 Hz, 1H), 4.40 (t, *J* = 2.7 Hz, 1H), 4.16 (dd, *J* = 4.9, 3.0 Hz, 1H), 3.97 (q, *J* = 5.6 Hz, 1H), 3.88 (t, *J* = 9.6 Hz, 1H), 3.83 – 3.68 (m, 5H), 3.68 – 3.55 (m, 4H), 3.54 – 3.44 (m, 4H), 3.40 (dd, *J* = 14.5, 4.6 Hz, 1H), 3.33 (ddd, *J* = 14.3, 10.4, 4.3 Hz, 1H), 3.29 (dd, *J* = 14.5, 6.1 Hz, 1H), 3.20 (dd, *J* = 8.6, 2.8 Hz, 1H), 3.18 – 3.12 (m, 1H), 3.10 (t, *J* = 10.3 Hz, 1H), 3.05 – 2.98 (m, 2H), 2.59 (s, 3H), 2.28 (dt, *J* = 12.6, 4.3 Hz, 1H), 2.18 (dt, *J* = 11.2, 4.6 Hz, 1H), 1.88 (d, *J* = 11.8 Hz, 1H), 1.72 – 1.63 (m, 1H); ^{13}C NMR (151 MHz, D_2O): δ 164.8, 110.3, 94.4, 93.6, 92.8, 84.8, 79.3, 78.8, 74.8, 73.1, 72.3, 70.2, 69.7, 69.3, 68.2, 65.9, 65.8, 62.6, 60.2, 59.3, 52.0, 49.6, 48.5, 47.6, 40.1, 39.2, 30.0, 27.9, 26.7; ESI-HRMS: m/z calcd. for $C_{29}H_{56}N_7O_{15}$ $[M+H]^+$ 742.3834; found, 742.3861.

5-*O*-[(2,3-Di-*O*-acetyl-5-deoxy)-β-D-ribofuranosyl]-1,3,2′,4″-tetraazido-6,2″,3″,6″- tetra-*O*-benzoyl-1,3,2′,4″-tetradesamino-6′,7′-oxazolidinoapramycin (78). To a stirred solution of **77** (244 mg, 0.94 mmol) and **69** (480 mg, 0.44 mmol) in DCM (4.6 mL) at 0 °C with oven-dried 4Å molecular sieves was added $BF_3{\cdot}OEt_2$ (0.35 mL, 2.66 mmol). Additional $BF_3{\cdot}OEt_2$ (0.1 mL, 0.76 mmol) was added as progress slowed. After 1.5 h, the reaction was quenched with excess Et_3N and left to stir at 0 °C for 10 mins. The reaction mixture was then diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. The crude product was purified via silica gel chromatography (0.3-0.8% MeOH in DCM, R_f = 0.55 in 2% MeOH in DCM) to give **78** (154 mg, 27%) as a white solid. $[\alpha]_D^{23}$ = +108.6 (*c* = 1.0, EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 12.1, 7.9 Hz, 4H), 8.05 (d, *J* = 7.8 Hz, 2H), 8.00 (d, *J* = 7.8 Hz, 2H), 7.63 (dt, *J* = 12.7, 7.5 Hz, 2H), 7.55 (dt, *J* = 15.8, 7.3 Hz, 4H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.44 (q, *J* = 7.4 Hz, 4H), 6.03

(t, *J* = 10.1 Hz, 1H), 5.72 (d, *J* = 3.7 Hz, 1H), 5.69 (d, *J* = 3.5 Hz, 1H), 5.33 (d, *J* = 3.3 Hz, 1H), 5.23 (t, *J* = 9.9 Hz, 1H), 5.19 (dd, *J* = 10.6, 3.8 Hz, 1H), 4.93 (t, *J* = 4.0 Hz, 1H), 4.86 (t, *J* = 5.0 Hz, 1H), 4.80 (d, *J* = 5.7 Hz, 1H), 4.76 (dd, *J* = 7.0, 3.6 Hz, 1H), 4.72 – 4.62 (m, 2H), 4.15 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.14 – 4.09 (m, 1H), 4.03 (m, 2H), 3.91 (t, *J* = 10.1 Hz, 1H), 3.81 (t, *J* = 9.3 Hz, 1H), 3.76 (t, *J* = 6.3 Hz, 1H), 3.59 – 3.52 (m, 1H), 3.49 (td, *J* = 10.7, 4.2 Hz, 1H), 3.46 – 3.39 (m, 1H), 2.98 – 2.91 (m, 4H), 2.45 (dt, *J* = 13.2, 4.6 Hz, 1H), 1.80 (s, 3H), 1.73 (m, 2H), 1.66 (s, 3H), 1.36 (q, *J* = 12.0 Hz, 1H), 1.28 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.3, 169.2, 166.1, 165.9, 165.6, 164.8, 157.0, 133.7, 133.6, 133.6, 130.1, 130.0, 129.8, 129.7, 129.3, 129.0, 129.0, 128.8, 128.7, 128.6, 128.5, 128.5, 105.5, 100.6, 96.6, 95.6, 80.7, 77.4, 76.4, 75.5, 74.6, 74.4, 71.8, 71.3, 70.5, 69.9, 66.5, 65.9, 63.4, 60.9, 60.3, 59.3, 58.3, 55.5, 31.4, 30.3, 28.4, 20.3, 19.9, 19.4; ESI-HRMS: *m/z* calc for $C_{59}H_{59}N_{13}O_{21}Na$ $[M+Na]^+$ 1308.38407, found 1308.3816. Although not isolated pure, the minor α-anomer was identified in the crude reaction mixture by the following diagnostic signals: δ 5.86 (d, *J* = 10.1 Hz, 1H), 2.74 (s, 3H), 2.42 (dt, *J* = 13.1, 4.6 Hz, 1H).

5-*O*-[5-deoxy-β-D-ribofuranosyl]apramycin pentaacetate salt (79). To a stirred solution of **78** (51 mg, 0.040 mmol) in dioxane (1.5 mL) was added aqueous NaOH (1.5 mL, 3 N) and the reaction was heated to reflux. After 4 h, the temperature was reduced to 60 °C and PMe_3 solution (0.35 mL, 1.0 M in THF). After 2.5 h, the reaction was neutralized with AcOH and concentrated to dryness. The crude product was passed through a CM Sephadex C25 column, loading in 10% aqueous acetic acid and eluting with a gradient of 0.1-1.2% ammonium hydroxide in deionized water. The product-containing fractions were lyophilized in vacuo with glacial acetic acid to generate the peracetate salt of **79** (22 mg, 57% over 3 steps) $[\alpha]_D^{23}$ = +25.0 (*c* = 0.08, H_2O); ^{1}H NMR (900 MHz, D_2O) δ 5.79 (d, *J* = 4.1 Hz, 1H), 5.42 (d, *J* = 4.0 Hz, 1H), 5.19 (d, *J* = 2.2 Hz, 1H), 5.13 (d, *J* = 8.6 Hz, 1H), 4.50 (t, *J* = 2.9 Hz, 1H), 4.13 (dd, *J* = 4.9, 2.1 Hz, 1H), 3.98 (p, *J* = 6.5 Hz, 1H), 3.86 (ddd, *J* = 11.4, 9.4, 4.7 Hz, 4H), 3.82 (t, *J* = 9.7 Hz, 1H), 3.78 (dd, *J* = 12.4, 3.3 Hz, 1H), 3.76 (t, *J* = 9.1 Hz, 1H), 3.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 3.66 – 3.63 (m, 2H), 3.60 (dt, *J* = 12.8, 4.4 Hz, 1H), 3.56 (dd, *J* = 10.5, 9.1 Hz, 1H), 3.33 – 3.27 (m, 2H), 3.23 (ddd, *J* = 12.4, 10.4, 4.3 Hz, 1H), 3.16 (t, *J* = 10.3 Hz, 1H), 2.70 (s, 3H), 2.31 (m, 2H), 1.96 (q, *J* = 11.9 Hz, 1H,), 1.83 (s, 15H), 1.68 (q, *J* = 12.6 Hz, 1H), 1.28 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (226 MHz, D_2O) δ 181.1, 110.6, 94.5, 94.3, 93.0, 85.3, 78.6, 77.2, 75.1, 74.4, 72.8, 70.3, 69.9, 69.7, 68.8, 65.9, 62.8, 60.4, 59.5, 52.1, 49.9, 48.6, 47.7, 30.2, 29.3, 27.1, 23.0, 18.3; ESI-HRMS: *m/z* calc for $C_{26}H_{49}N_5O_{14}Na$ $[M+Na]^+$ 678.31682, found 678.3181.

Biological Testing

Cell-free translation inhibition assays. The S30 fraction of *Mycobacterium smegmatis* cell extracts was used for bacterial cell-free translation inhibition assays as described previously.[4] Inhibition of mammalian ribosomes has been assessed with a commercial Rabbit Reticulocyte Lysate System (Promega) as described previously.[4] Firefly luciferase mRNA was used as reporter to monitor translation activity. Luminescence was measured using a luminometer Flx800 (Bio-Tek Instruments).

Antibacterial inhibition assays. The minimal inhibitory concentrations (MIC) of synthesized compounds were determined by broth microdilution assays according to CLSI reference methodology M07[5] as described previously.[6] A summary of bacterial strains used in this study is provided in Table S1. Clinical bacterial isolates were obtained from the diagnostic laboratories of the Institute of Medical

Microbiology, University of Zurich. Whole genome sequencing of the bacterial isolates and bioinformatic annotation of resistance genes was done as described previously.[6]

References

[1] NMR spectra were complicated by the presence of multiple rotamers. These intermediates were characterized by mass spectrometry analysis.

[2] Kanazawa, T.; Sato, T., *Nippon Kagaku Zasshi* **1959,** *80*, 200-3.

[3] Raluy, E.; Pamies, O.; Dieguez, M., *Adv. Synth. Catal.* **2009,** *351*, 1648-1670.

[4] Matt, T.; Ng, C. L.; Lang, K.; Sha, S.-H.; Akbergenov, R.; Shcherbakov, D.; Meyer, M.; Duscha, S.; Xie, J.; Dubbaka, S. R.; Perez-Fernandez, D.; Vasella, A.; Ramakrishnan, V.; Schacht, J.; Böttger, E. C., *Proc. Natl. Acad. Sci., USA* **2012**, *109*, 10984-10989.

[5] Clinical Laboratory Standards Institute (2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Tenth Edition: Approved Standard M07-A10. CLSI, Wayne, PA, USA.

[6] Juhas, M.; Widlake. E.; Teo, J.; Huseby, D. L.; Tyrrell, J. M.; Polikanov, Y. S.; Ercan, O.; Petersson, A.; Cao, S.; Aboklaish, A.F.; Rominski, A.; Crich, D.; Böttger, E. C.; Walsh, T. R.; Hughes, D.; Hobbie, S. N., *J. Antimicrob. Chemother.* **2019**, *74*, 944-952.

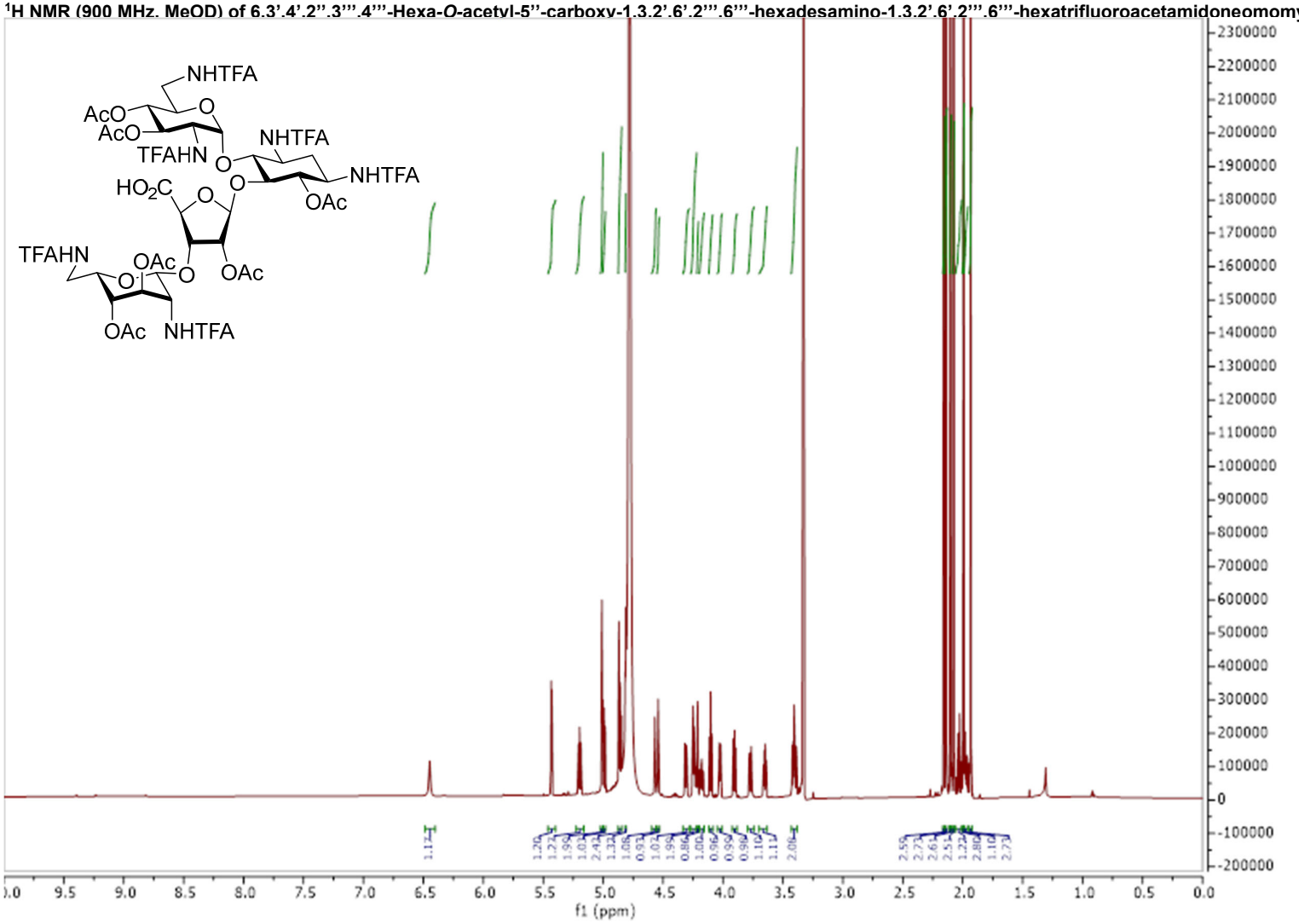
Author Contributions

ECB, AV, SNH and DC designed the project. JCKQ, GCS and AS prepared all new compounds. MG and KH carried our all microbiological assays. JCKQ, ECB, AV, SNH and DC analyzed the data and wrote the manuscript.

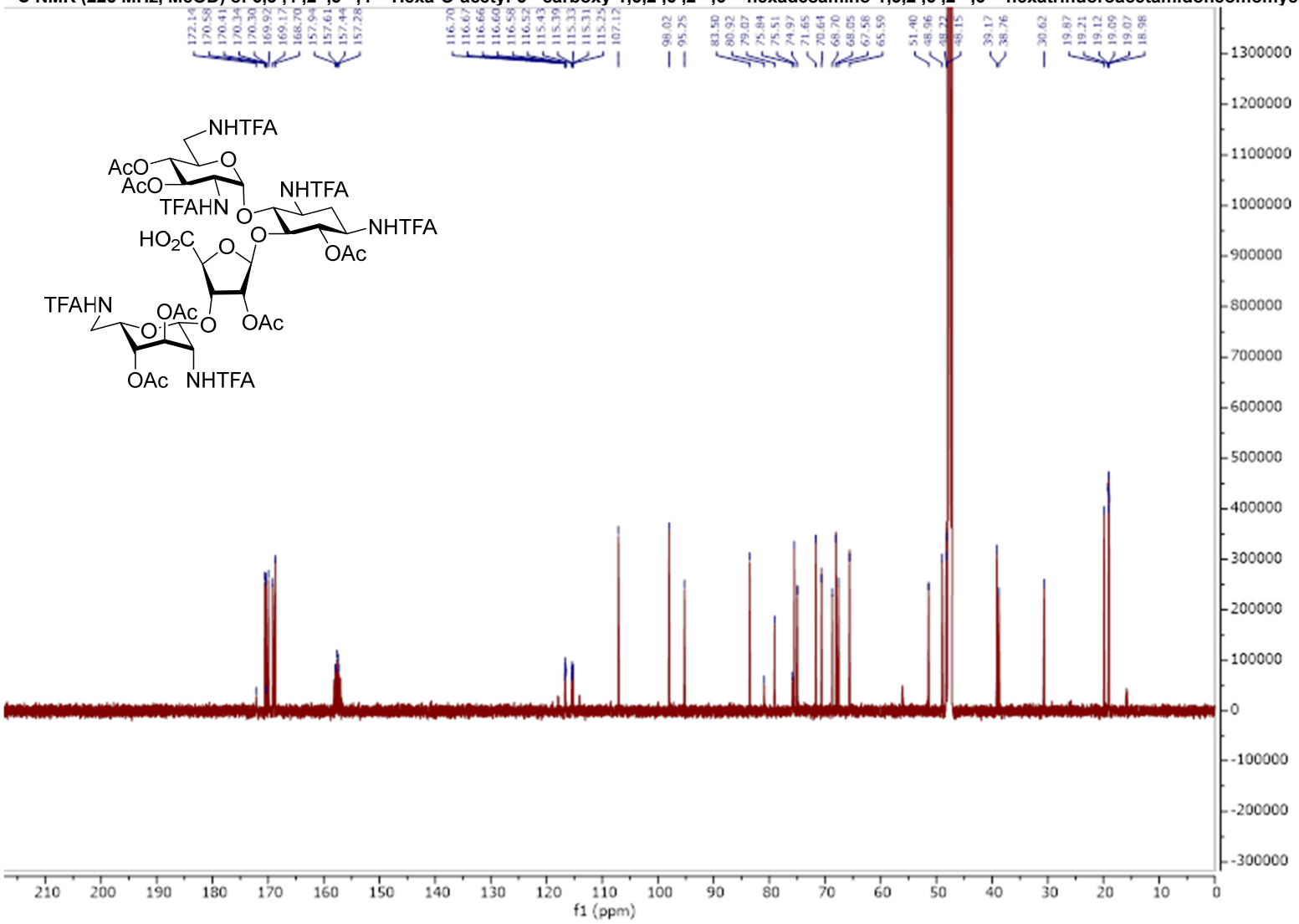
SUPPORTING INFORMATION

NMR Spectra

^{1}H NMR (900 MHz, MeOD) of 6.3',4',2'',3''',4'''-Hexa-*O*-acetyl-5''-carboxy-1,3,2',6',2''',6'''-hexadesamino-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomomycin (24).

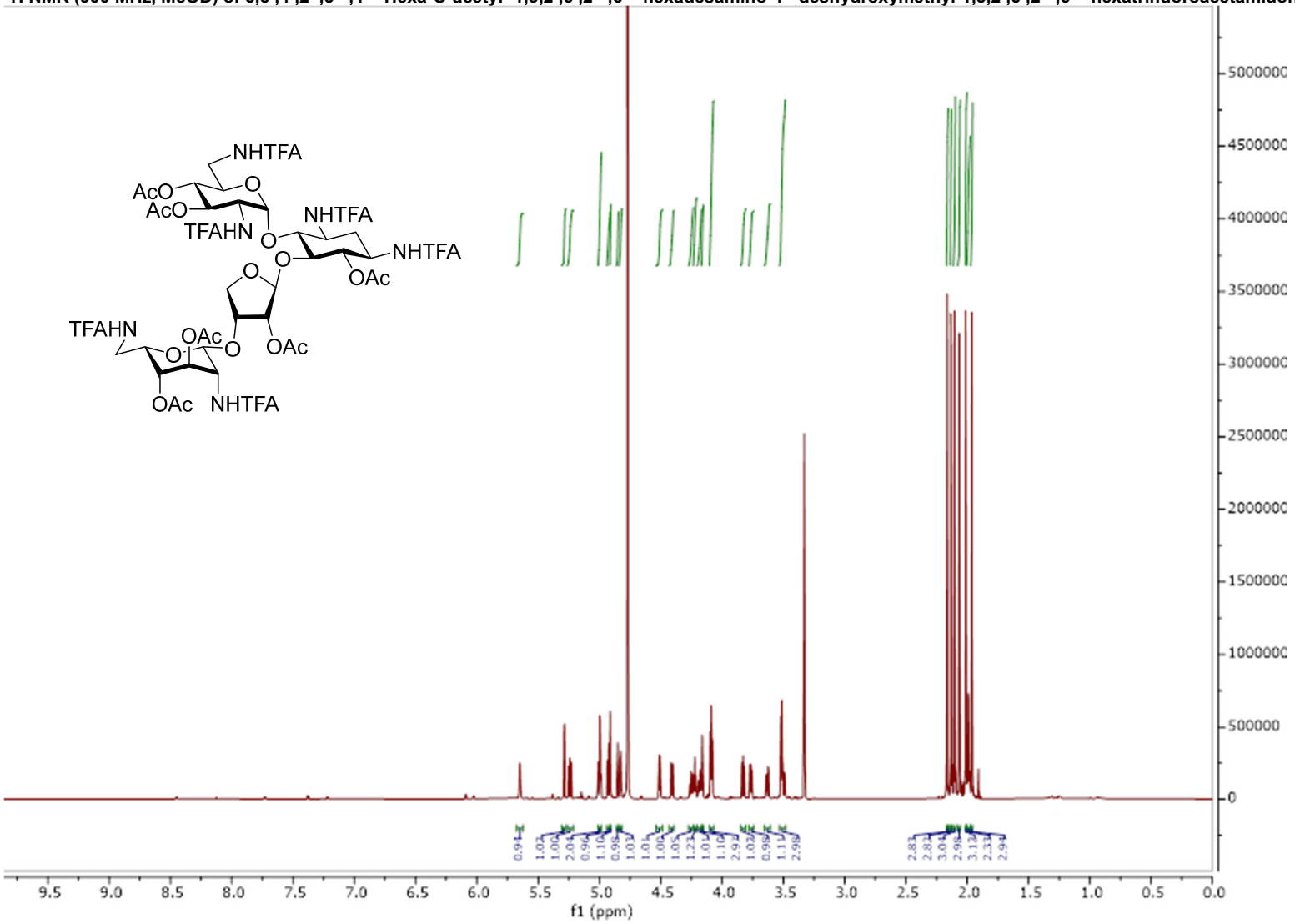


^{13}C NMR (225 MHz, MeOD) of 6,3',4',2'',3''',4'''-Hexa-*O*-acetyl-5''-carboxy-1,3,2',6',2''',6'''-hexadesamino-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomomycin (24).

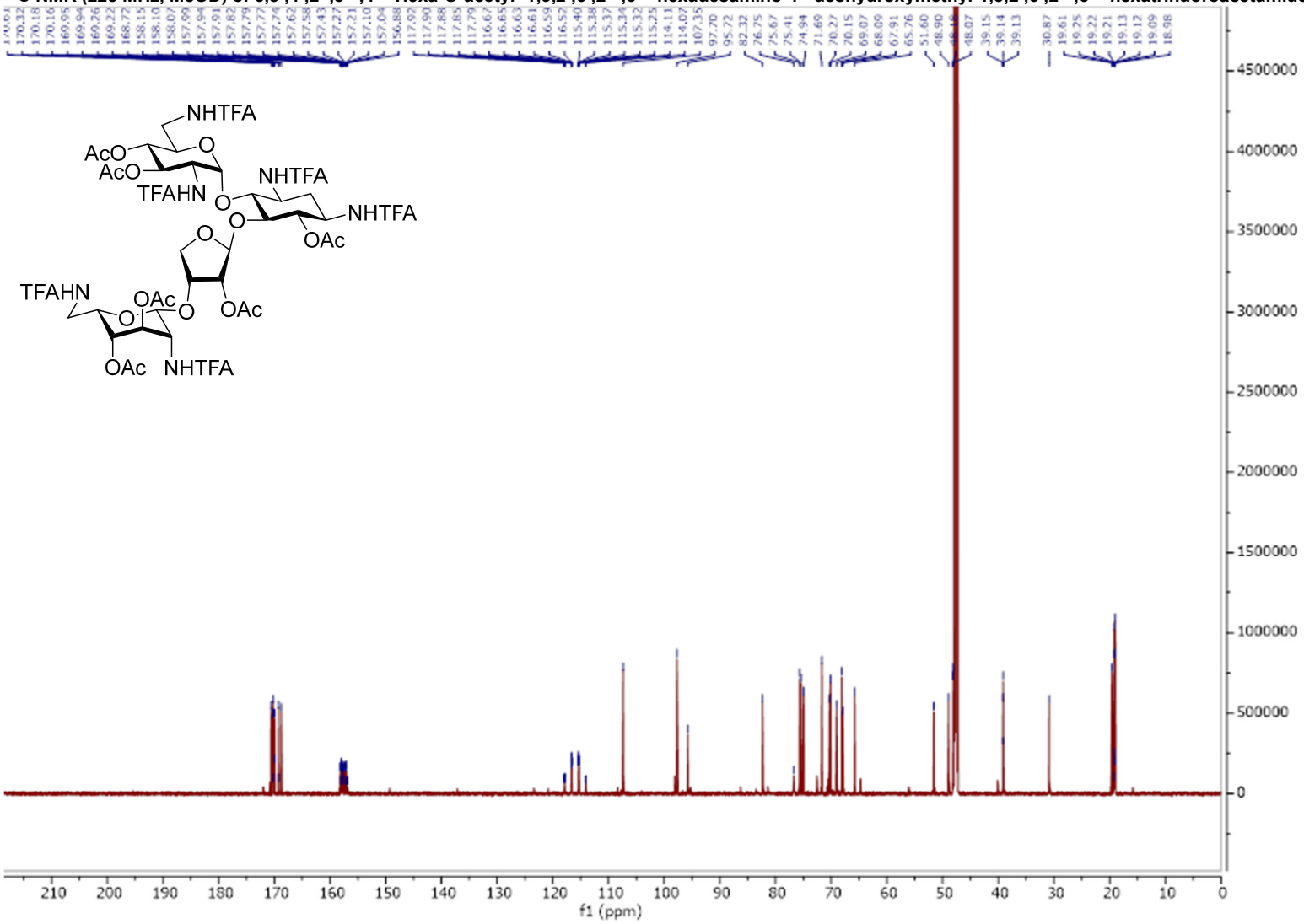


SUPPORTING INFORMATION

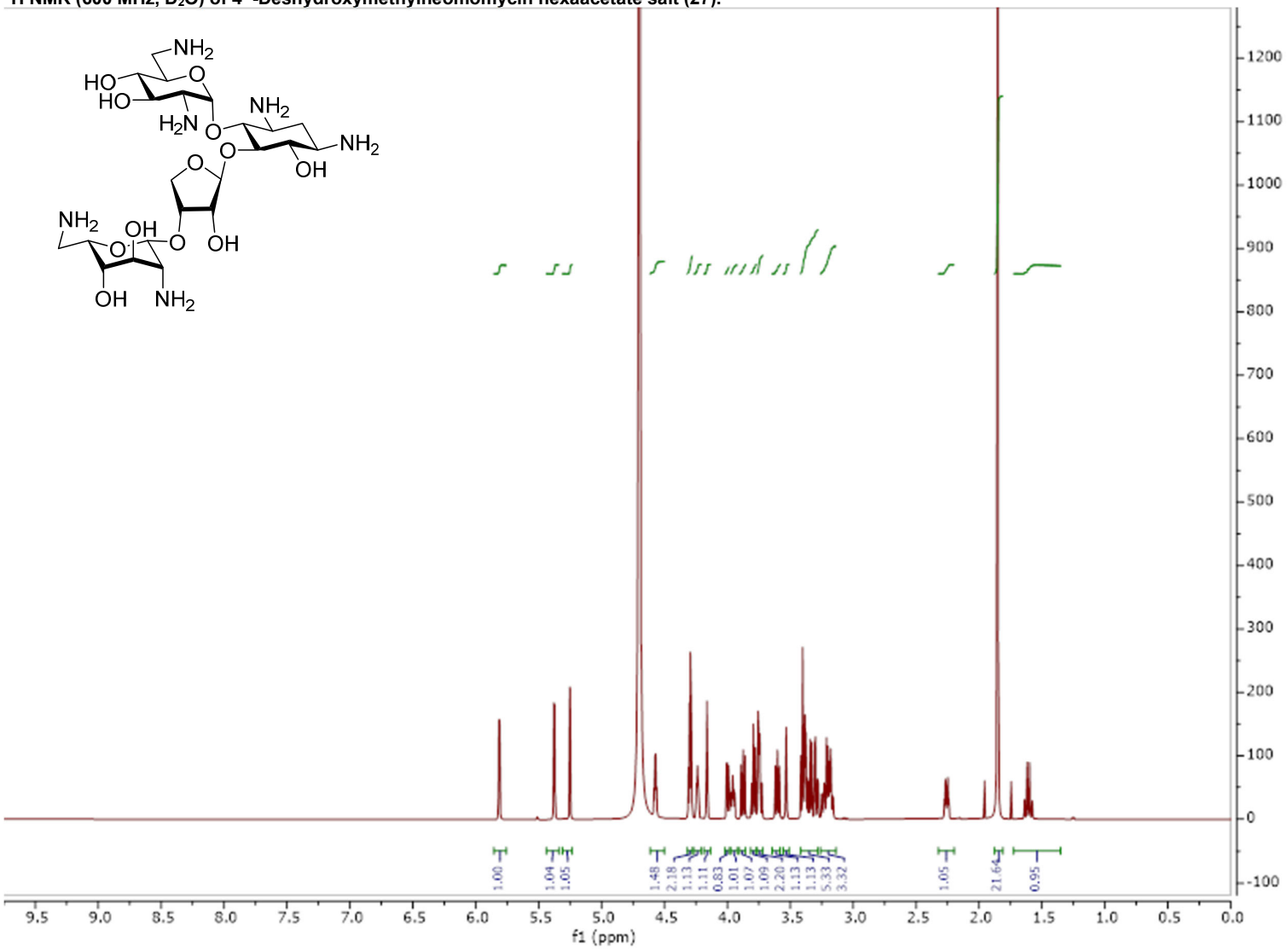
^{1}H NMR (900 MHz, MeOD) of 6,3',4',2'',3''',4'''-Hexa-*O*-acetyl -1,3,2',6',2''',6'''-hexadesamino-4''-deshydroxymethyl-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomycin (26).



^{13}C NMR (225 MHz, MeOD) of 6,3',4',2'',3''',4'''-Hexa-*O*-acetyl -1,3,2',6',2''',6'''-hexadesamino-4''-deshydroxymethyl-1,3,2',6',2''',6'''-hexatrifluoroacetamidoneomycin (26).

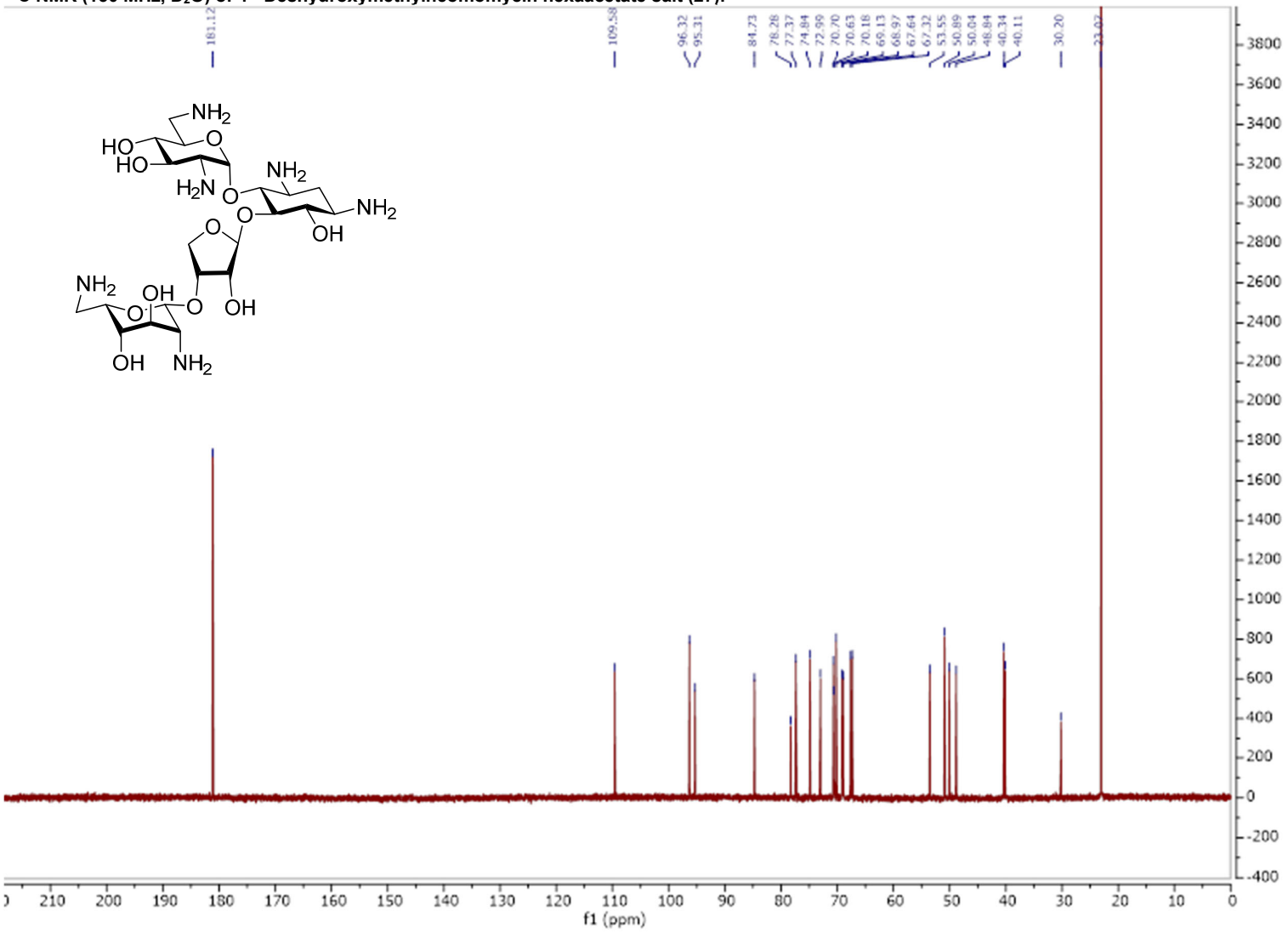


^{1}H NMR (600 MHz, D_2O) of 4''-Deshydroxymethylneomomycin hexaacetate salt (27).

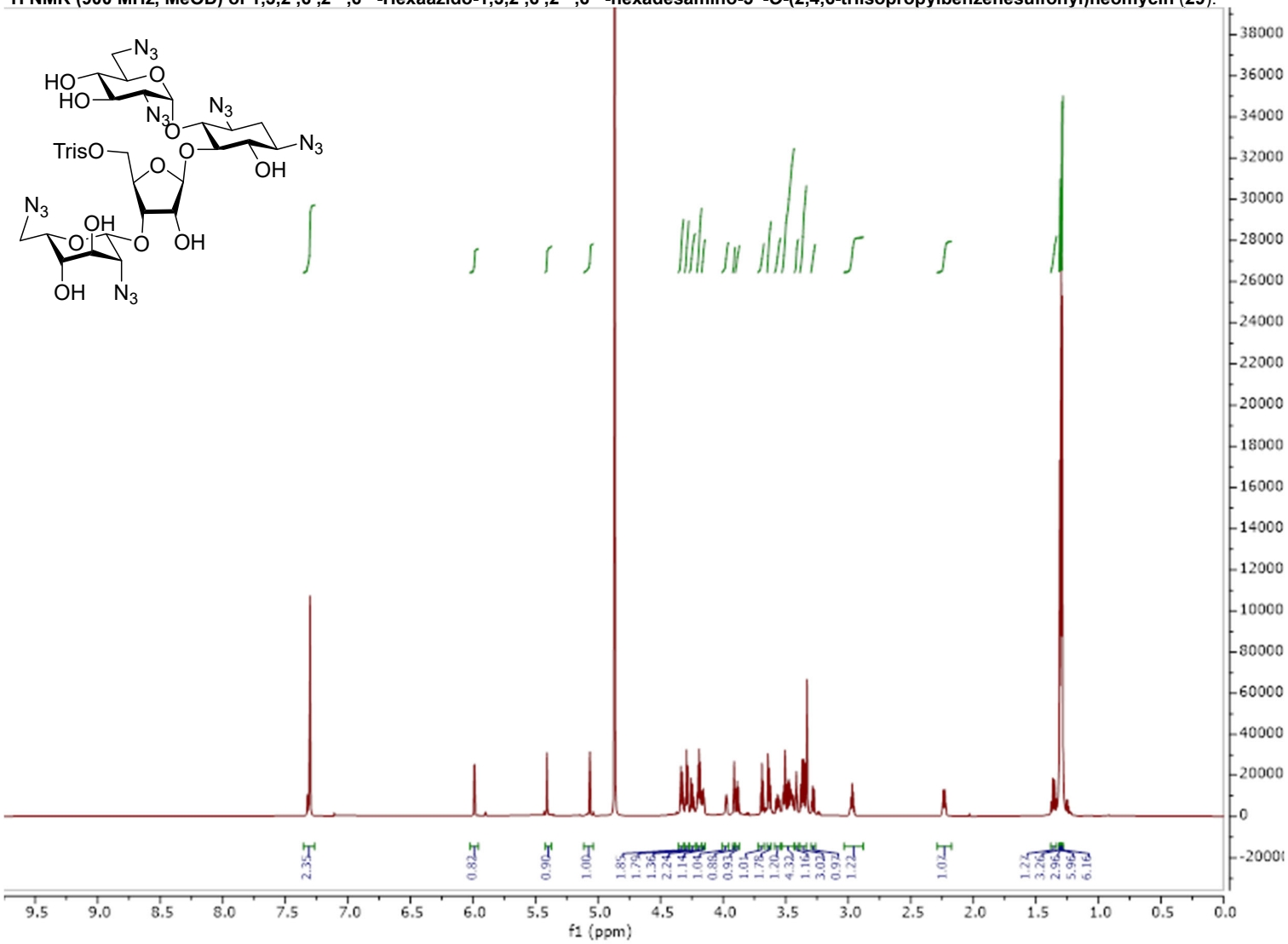


SUPPORTING INFORMATION

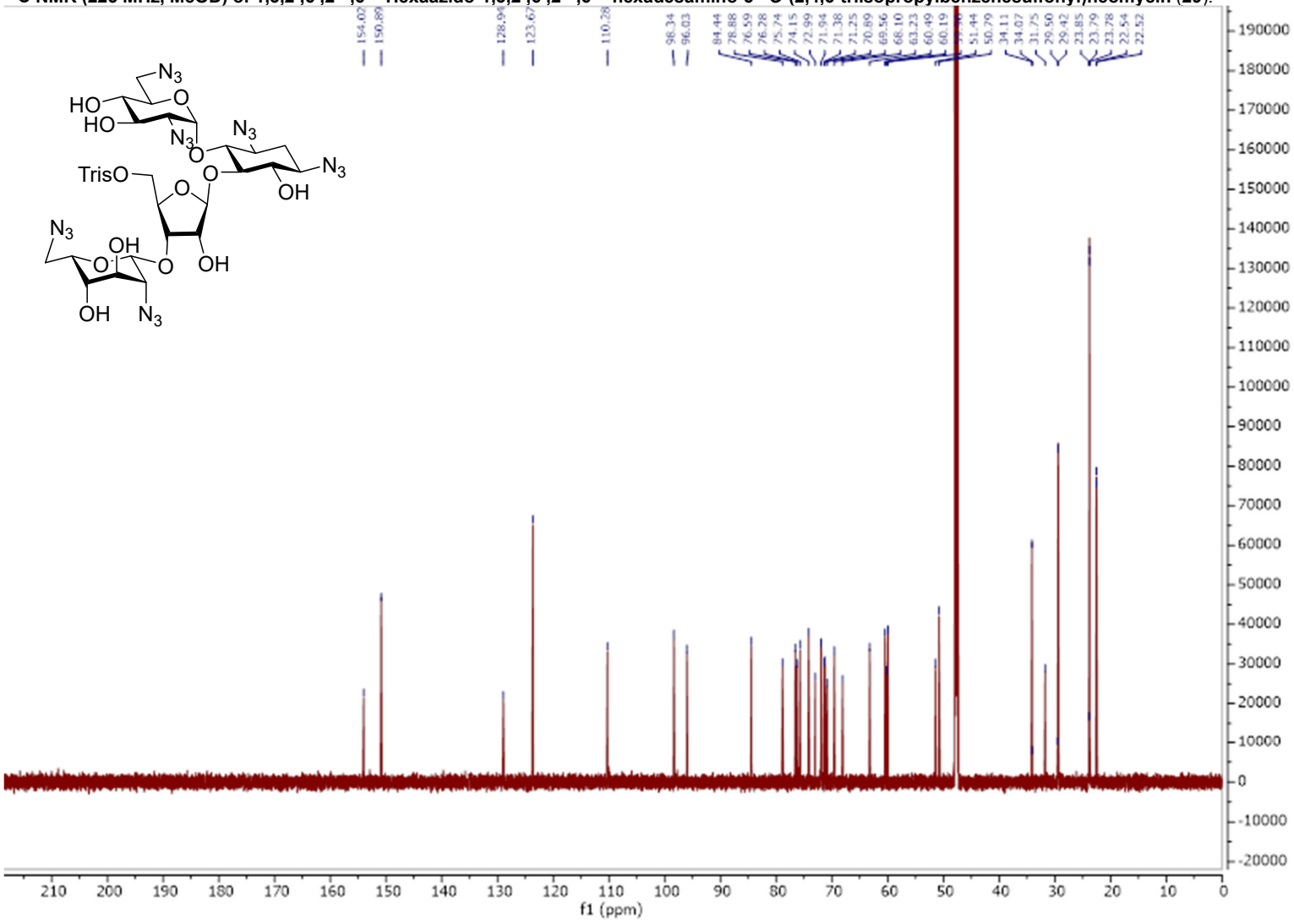
^{13}C NMR (150 MHz, D_2O) of 4''-Deshydroxymethylneomomycin hexaacetate salt (27).



^{1}H NMR (900 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-1,3,2',6',2''',6'''-hexadesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)neomycin (29).



^{13}C NMR (225 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-1,3,2',6',2''',6'''-hexadesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)neomycin (29).



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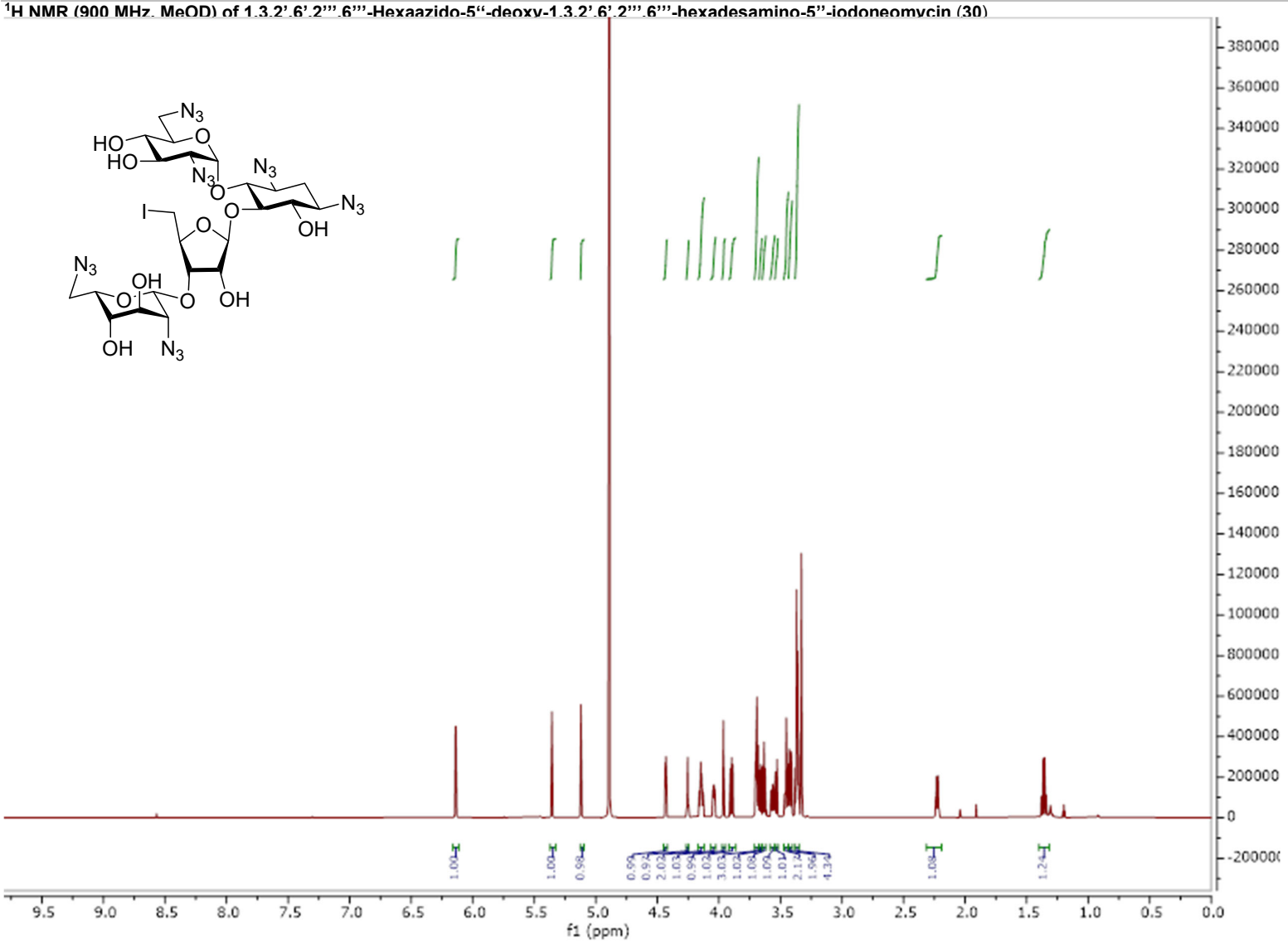
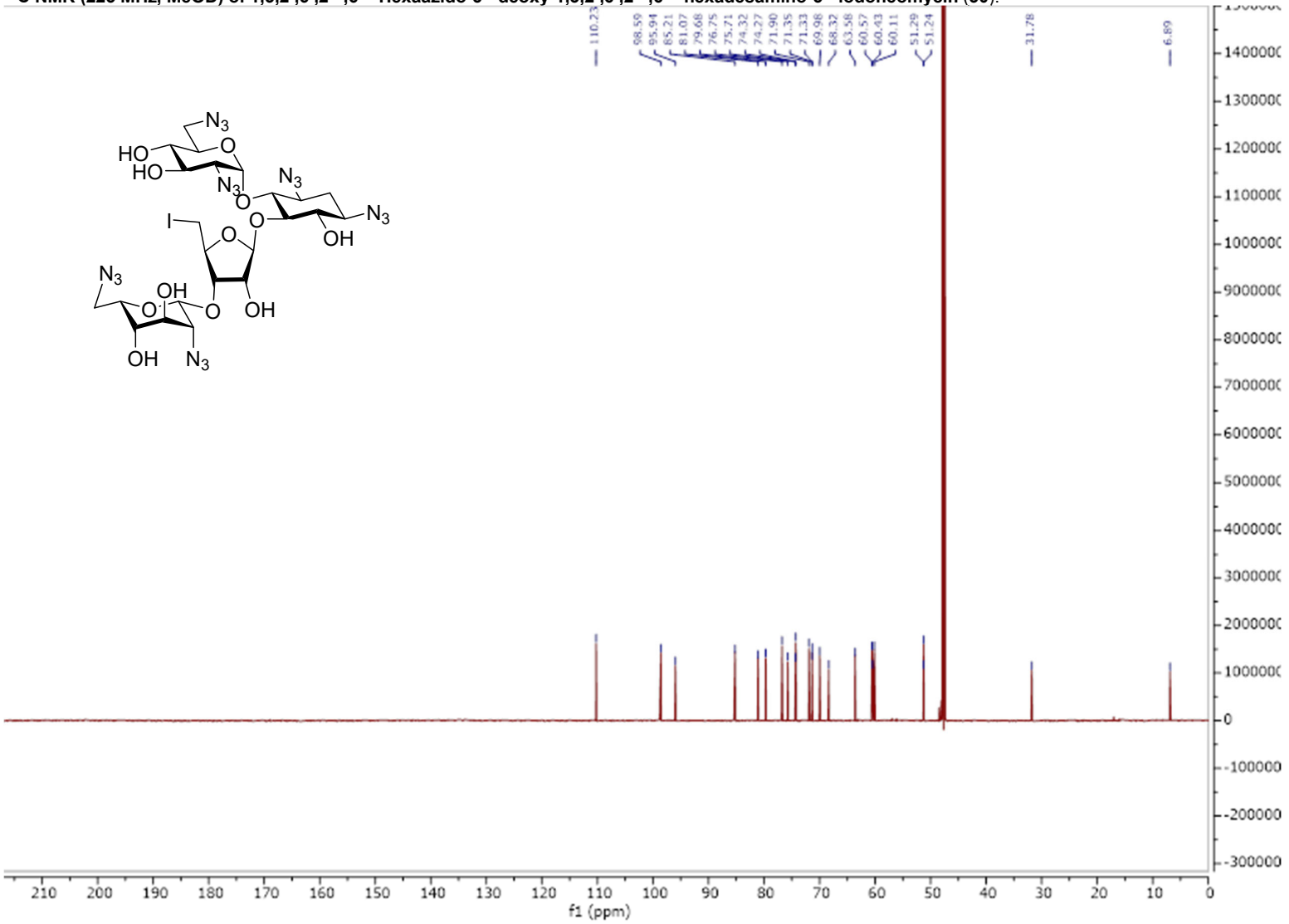
^{1}H NMR (900 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-iodoneomycin (30)



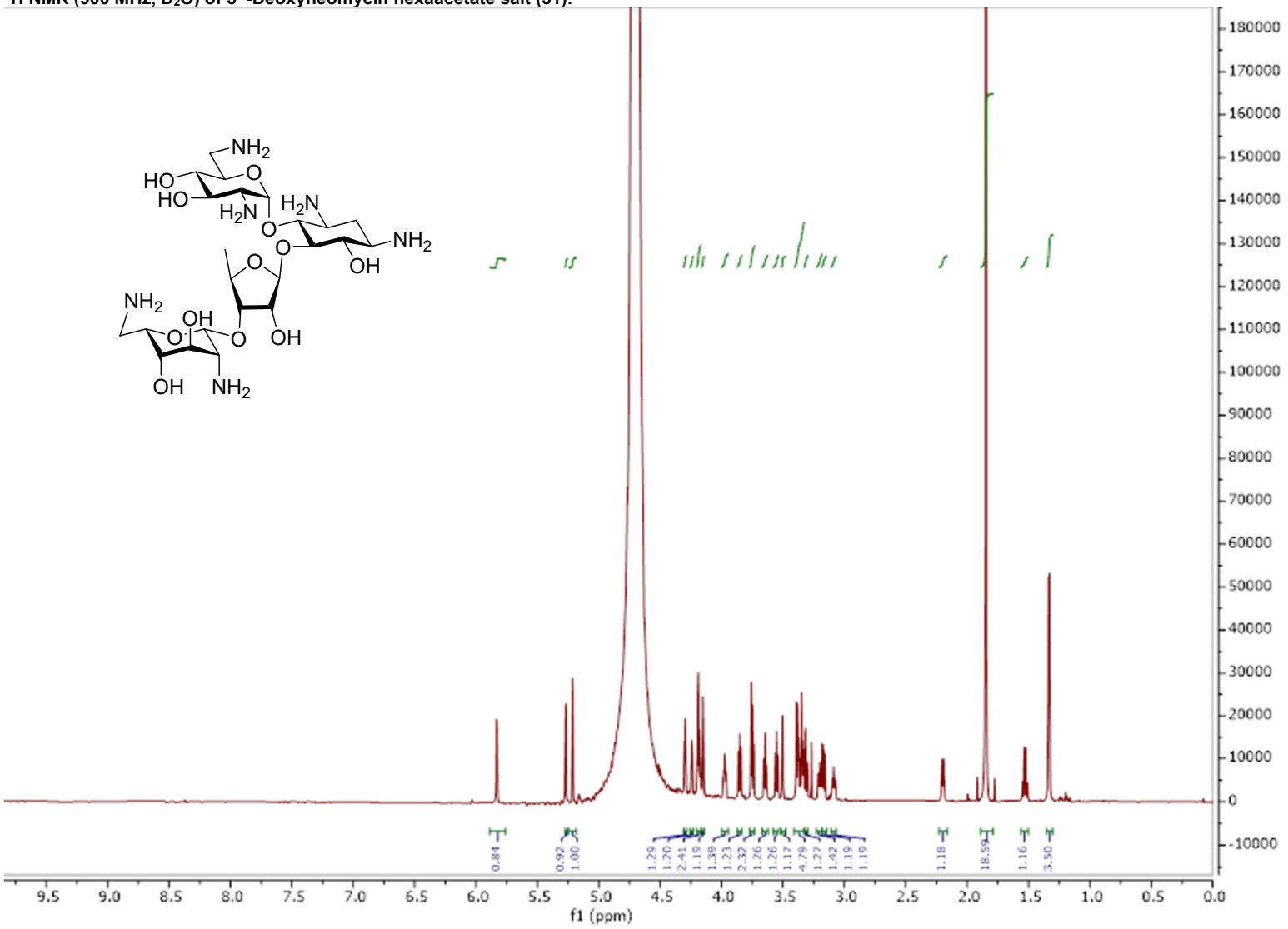
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-iodoneomycin (30).



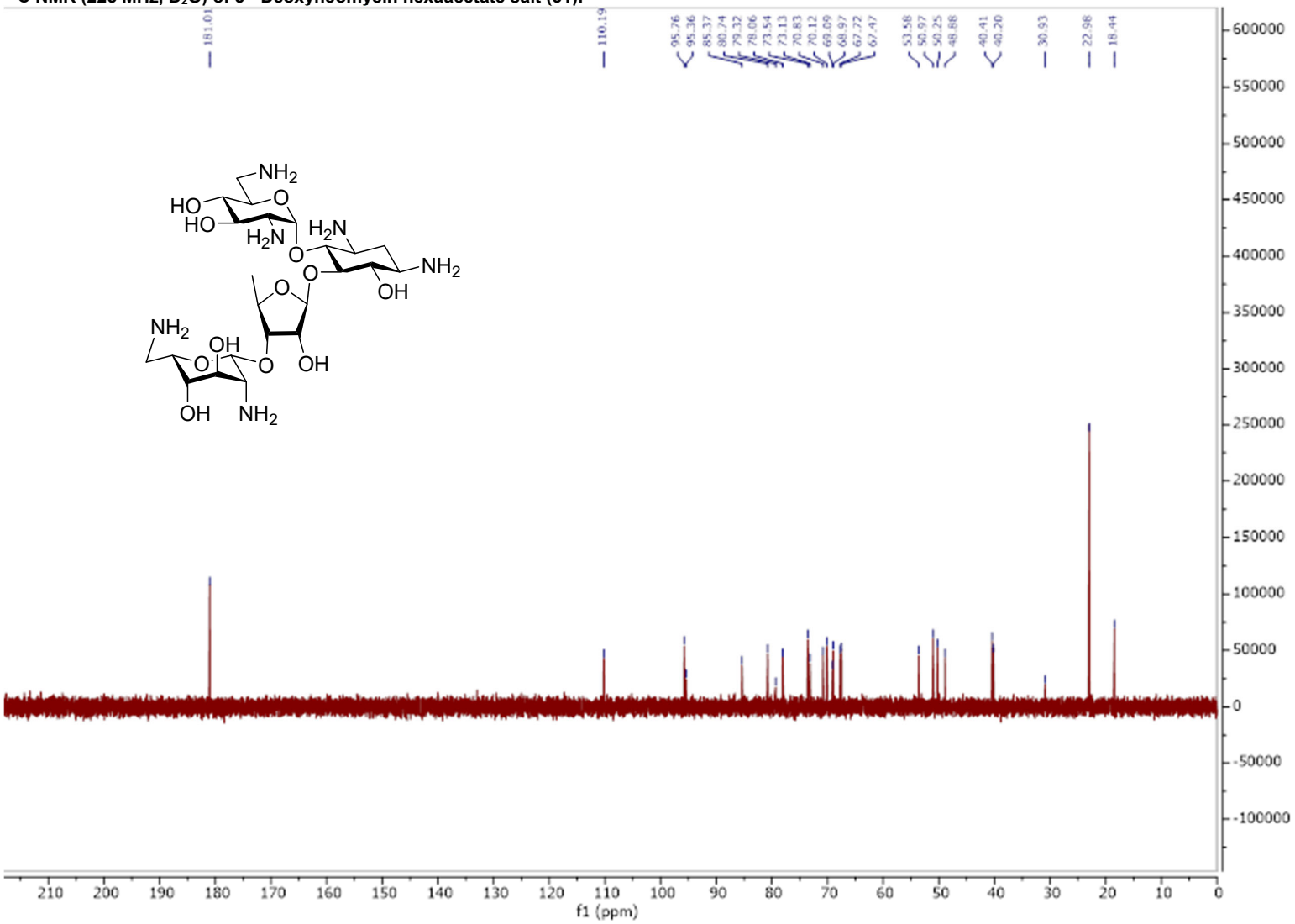
SUPPORTING INFORMATION

^{1}H NMR (900 MHz, D_2O) of 5''-Deoxyneomycin hexaacetate salt (31).

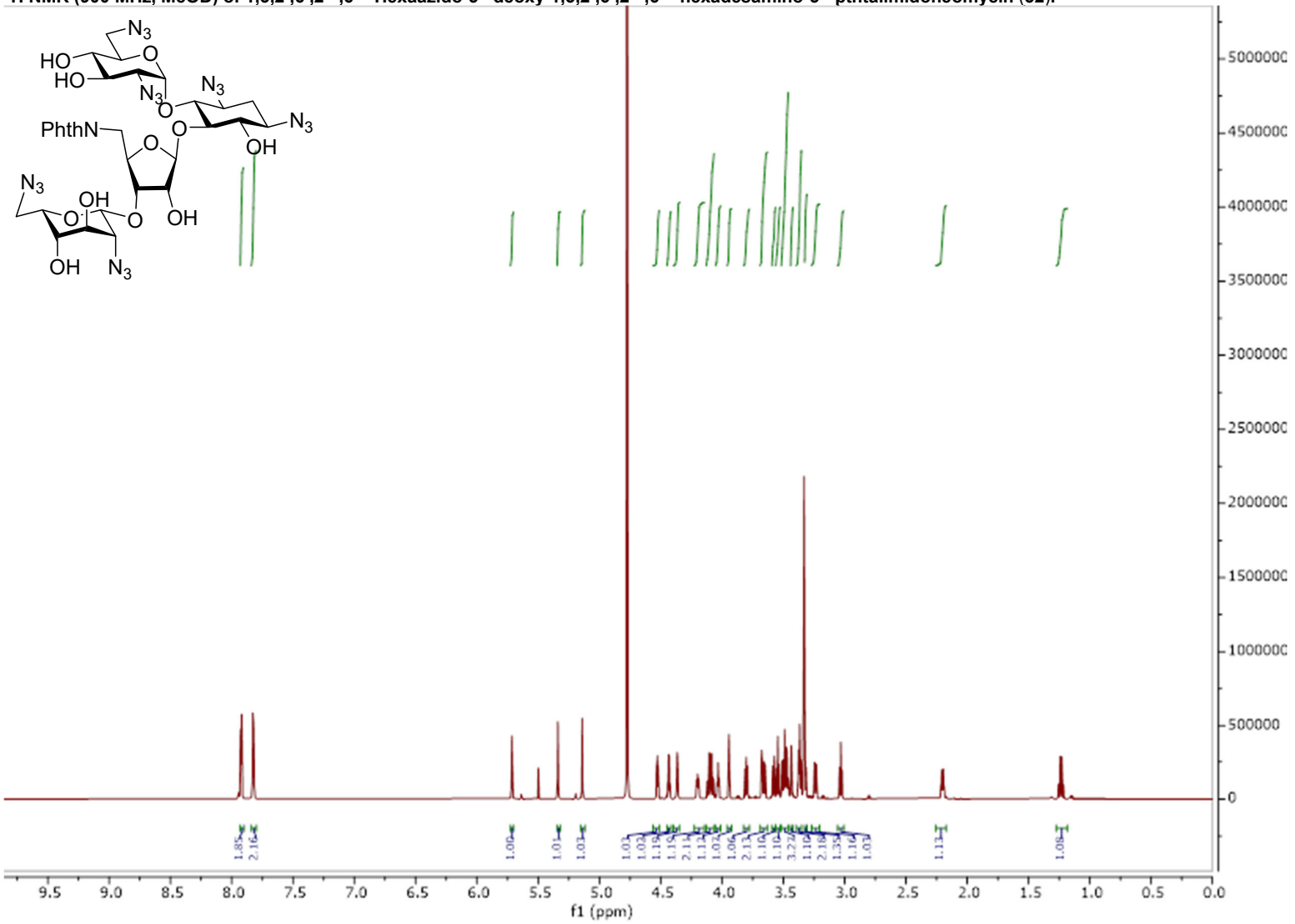


SUPPORTING INFORMATION

^{13}C NMR (225 MHz, D_2O) of 5''-Deoxyneomycin hexaacetate salt (31).

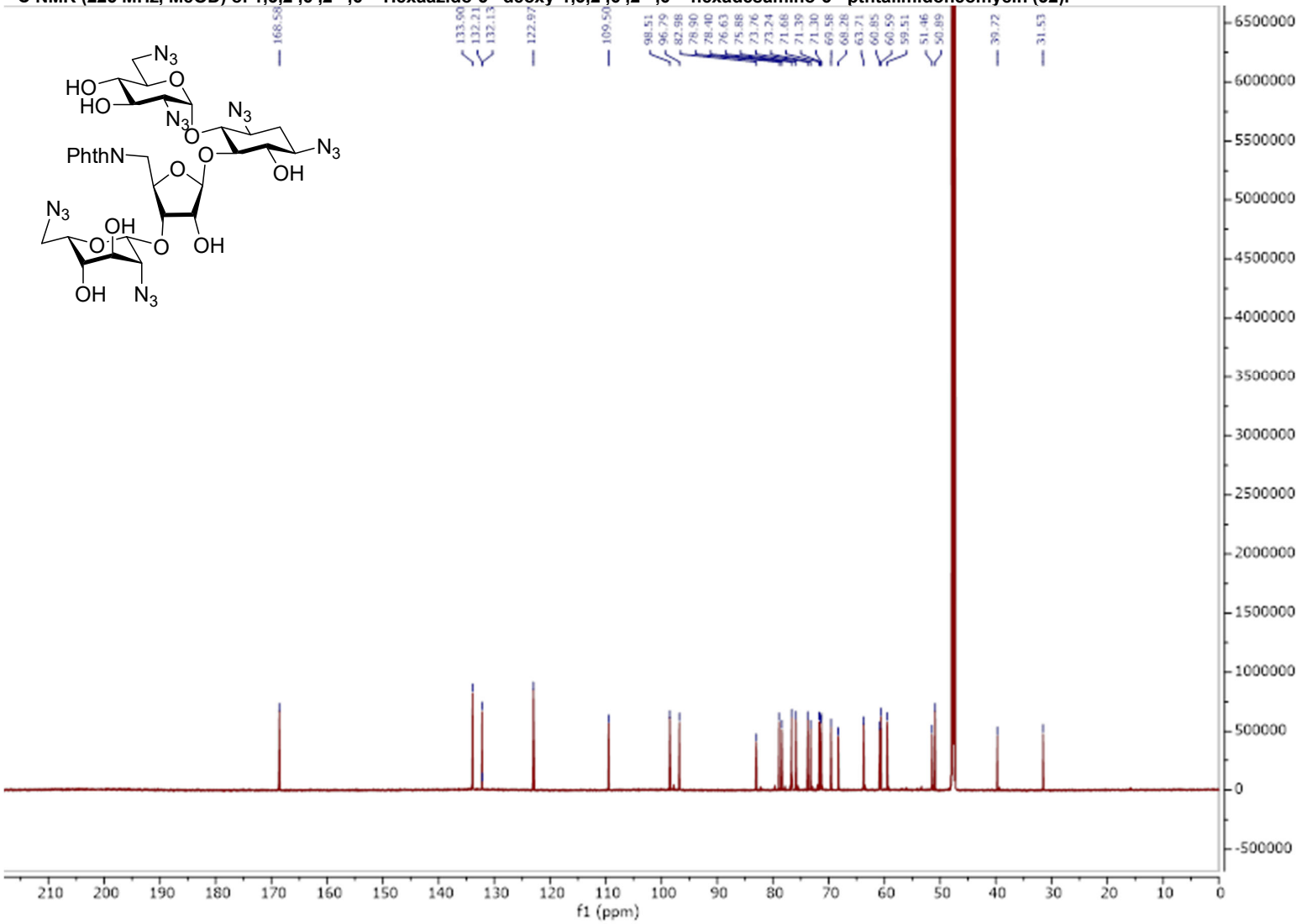


^{1}H NMR (900 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-pthtalimidoneomycin (32).



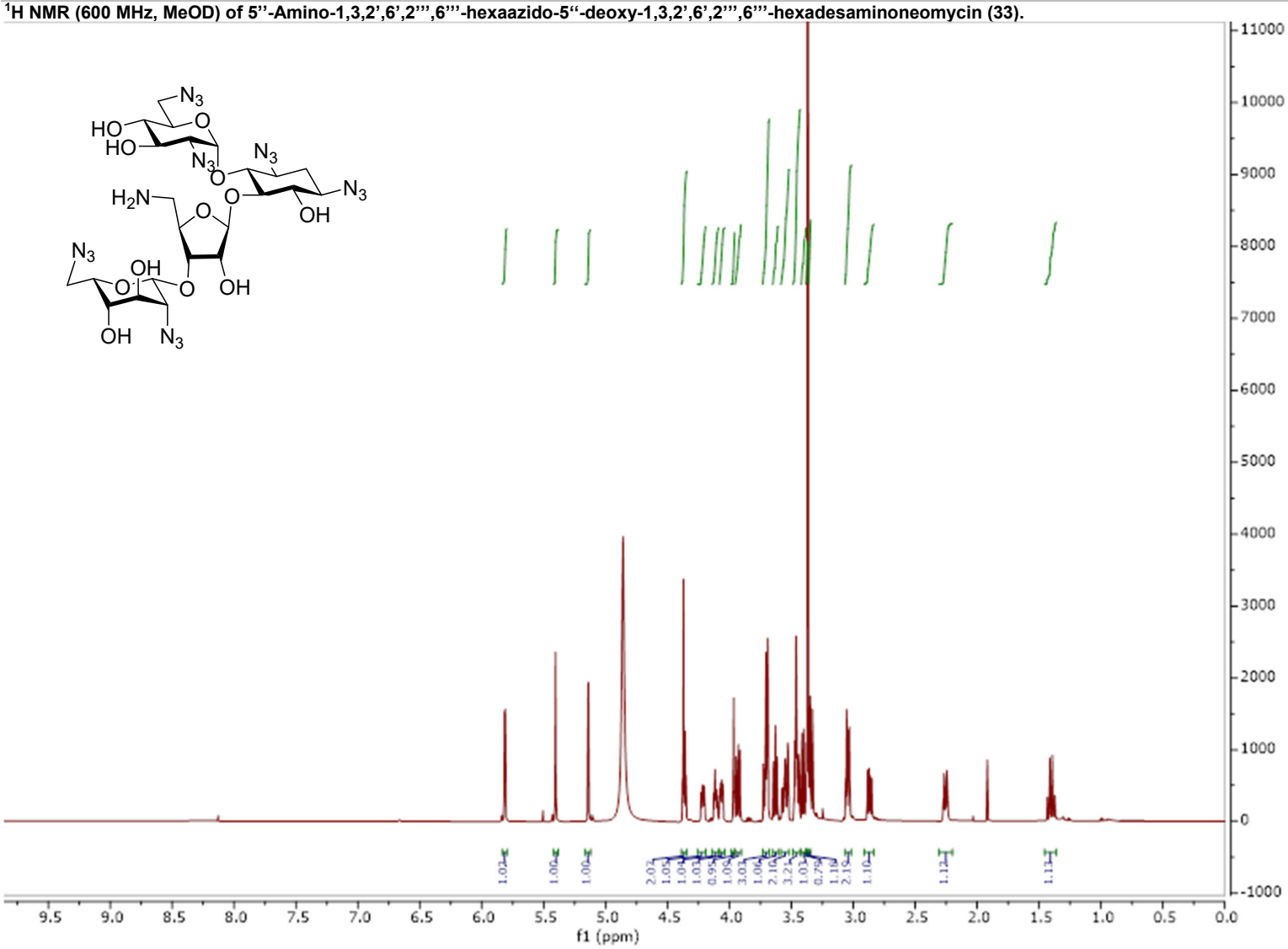
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 1,3,2',6',2''',6'''-Hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesamino-5''-pthtalimidoneomycin (32).

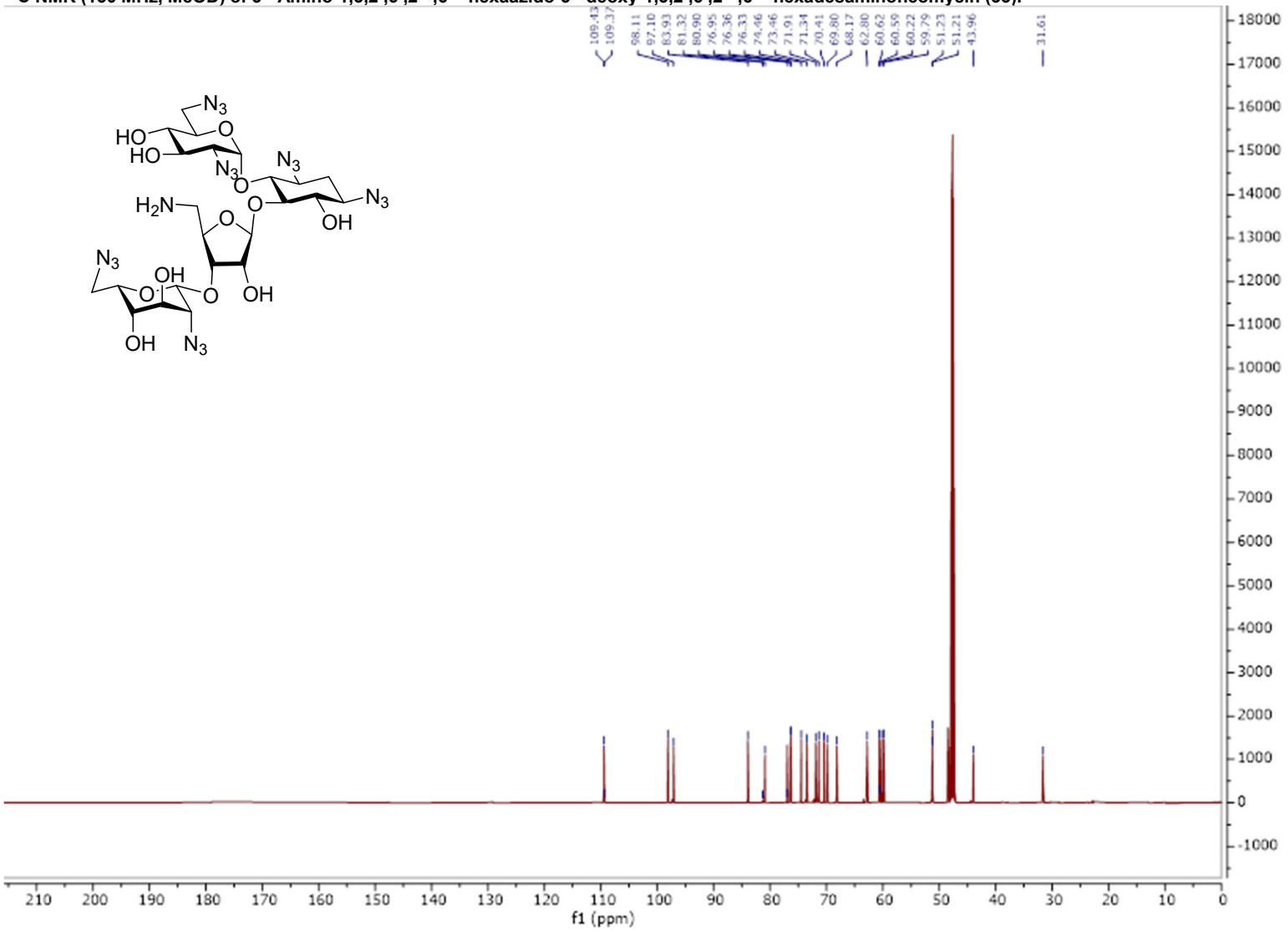


SUPPORTING INFORMATION

^{1}H NMR (600 MHz, MeOD) of 5''-Amino-1,3,2',6',2''',6'''-hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesaminoneomycin (33).

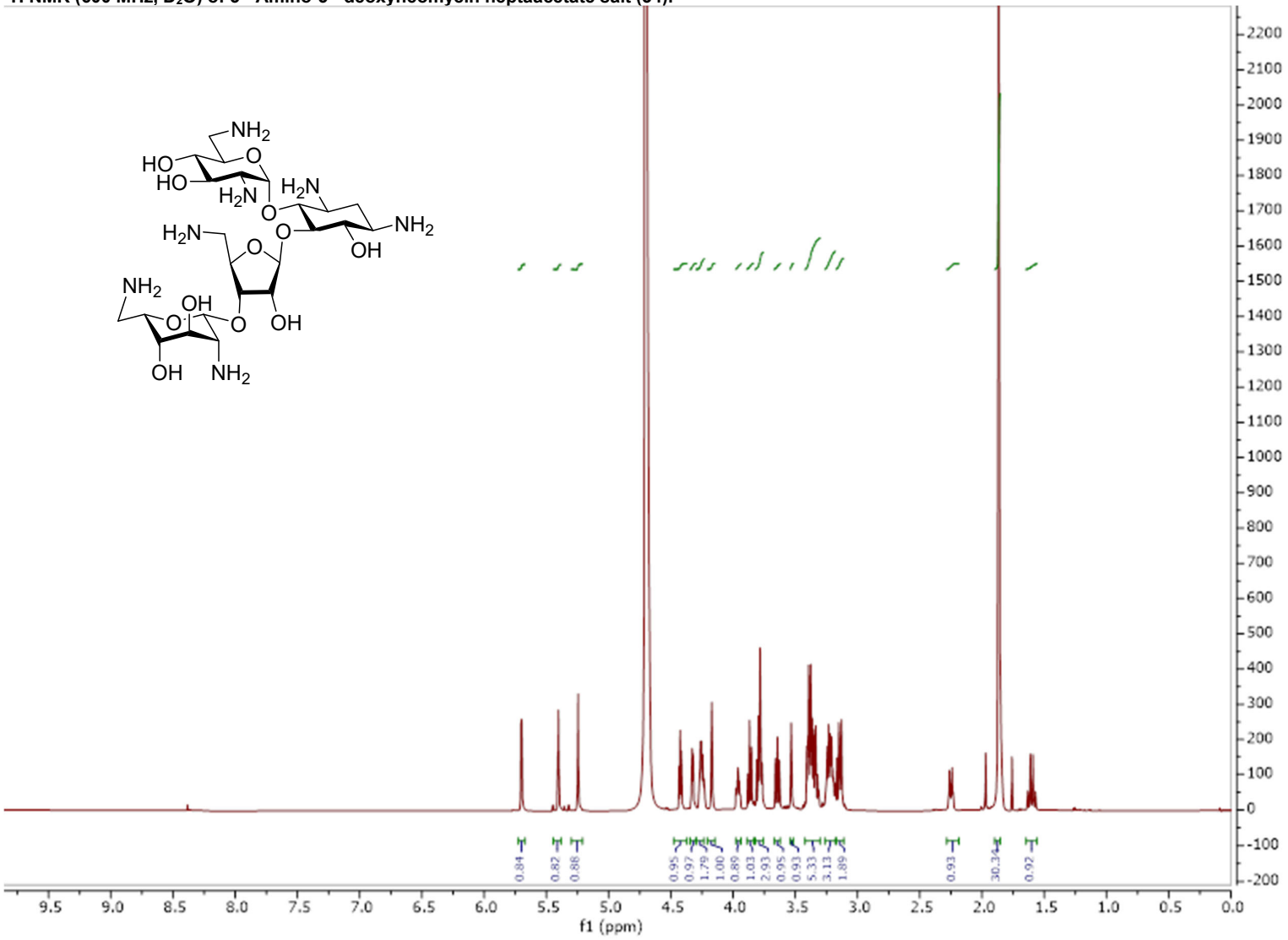


^{13}C NMR (150 MHz, MeOD) of 5''-Amino-1,3,2',6',2''',6'''-hexaazido-5''-deoxy-1,3,2',6',2''',6'''-hexadesaminoneomycin (33).



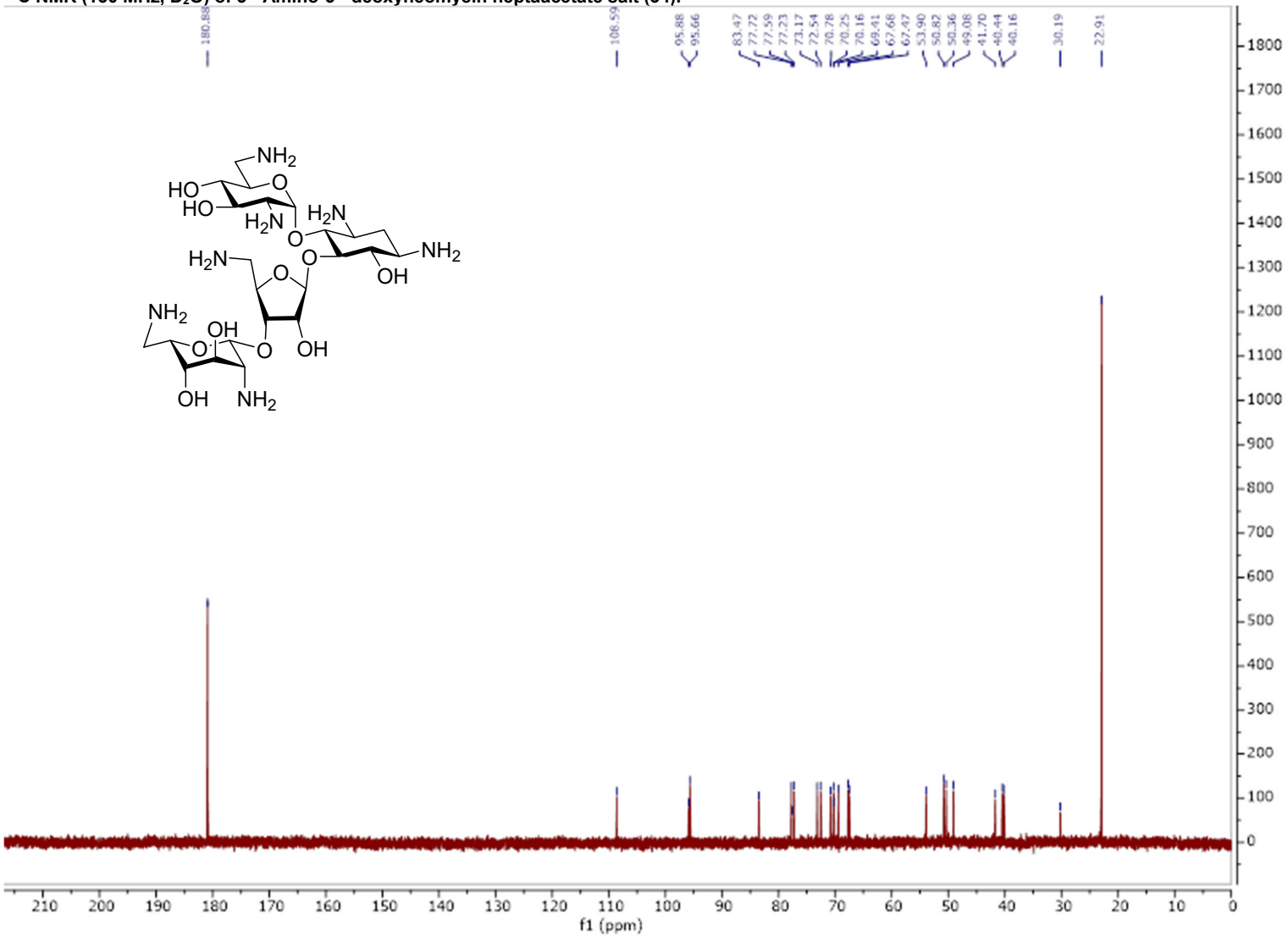
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 5''-Amino-5''-deoxyneomycin heptaacetate salt (34).

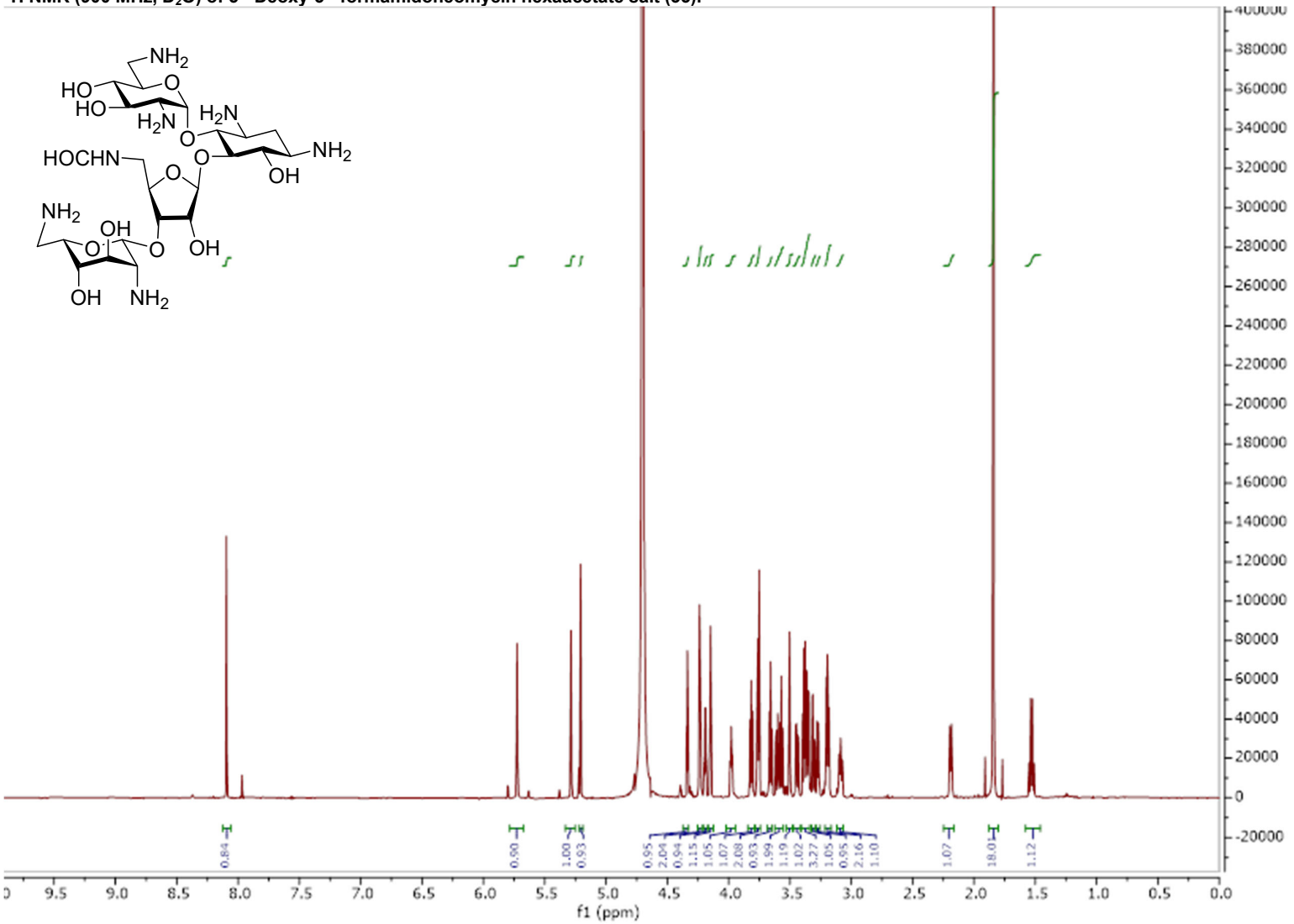


SUPPORTING INFORMATION

^{13}C NMR (150 MHz, D_2O) of 5''-Amino-5''-deoxyneomycin heptaacetate salt (34).

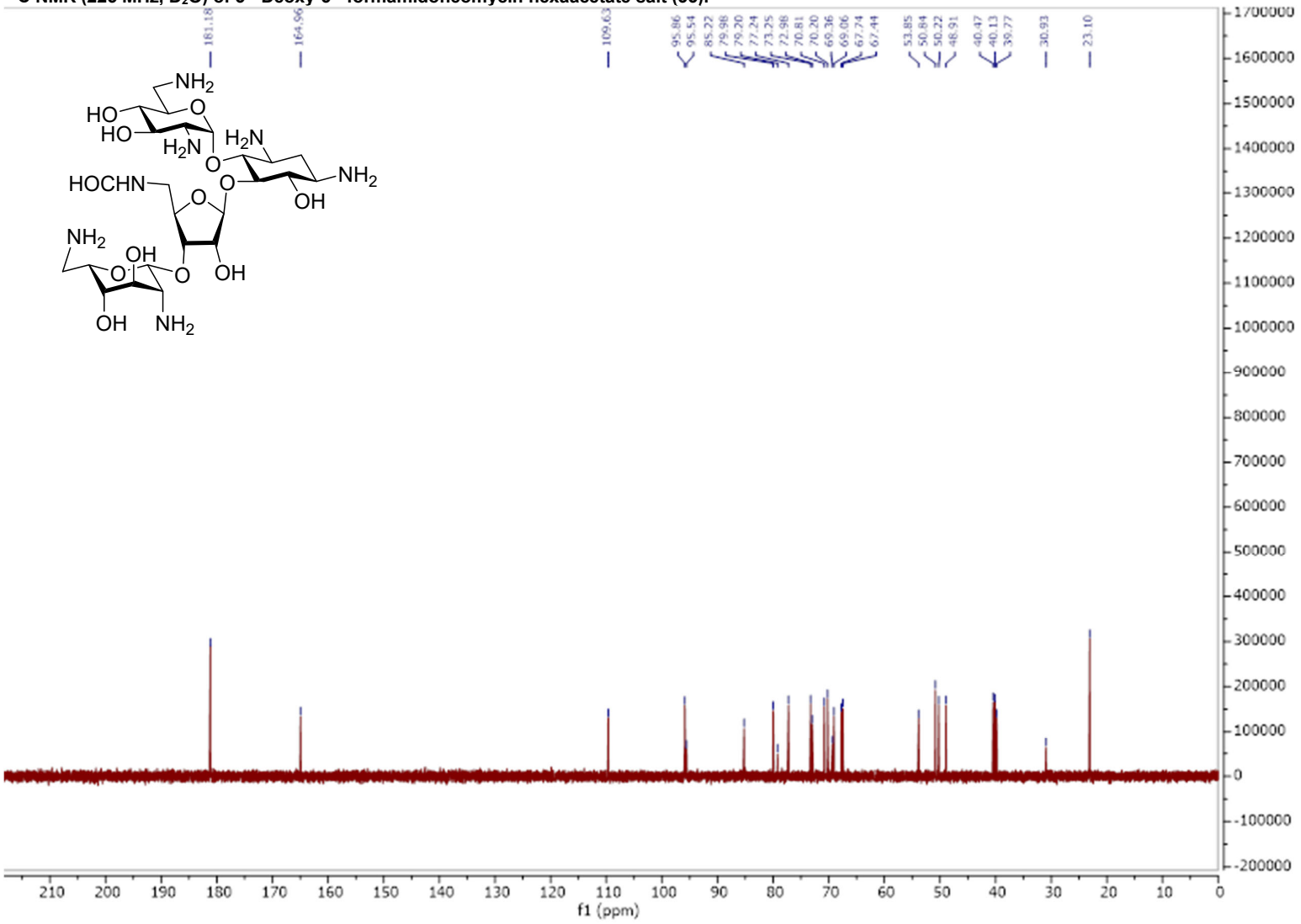


^{1}H NMR (900 MHz, D_2O) of 5''-Deoxy-5''-formamidoneomycin hexaacetate salt (35).



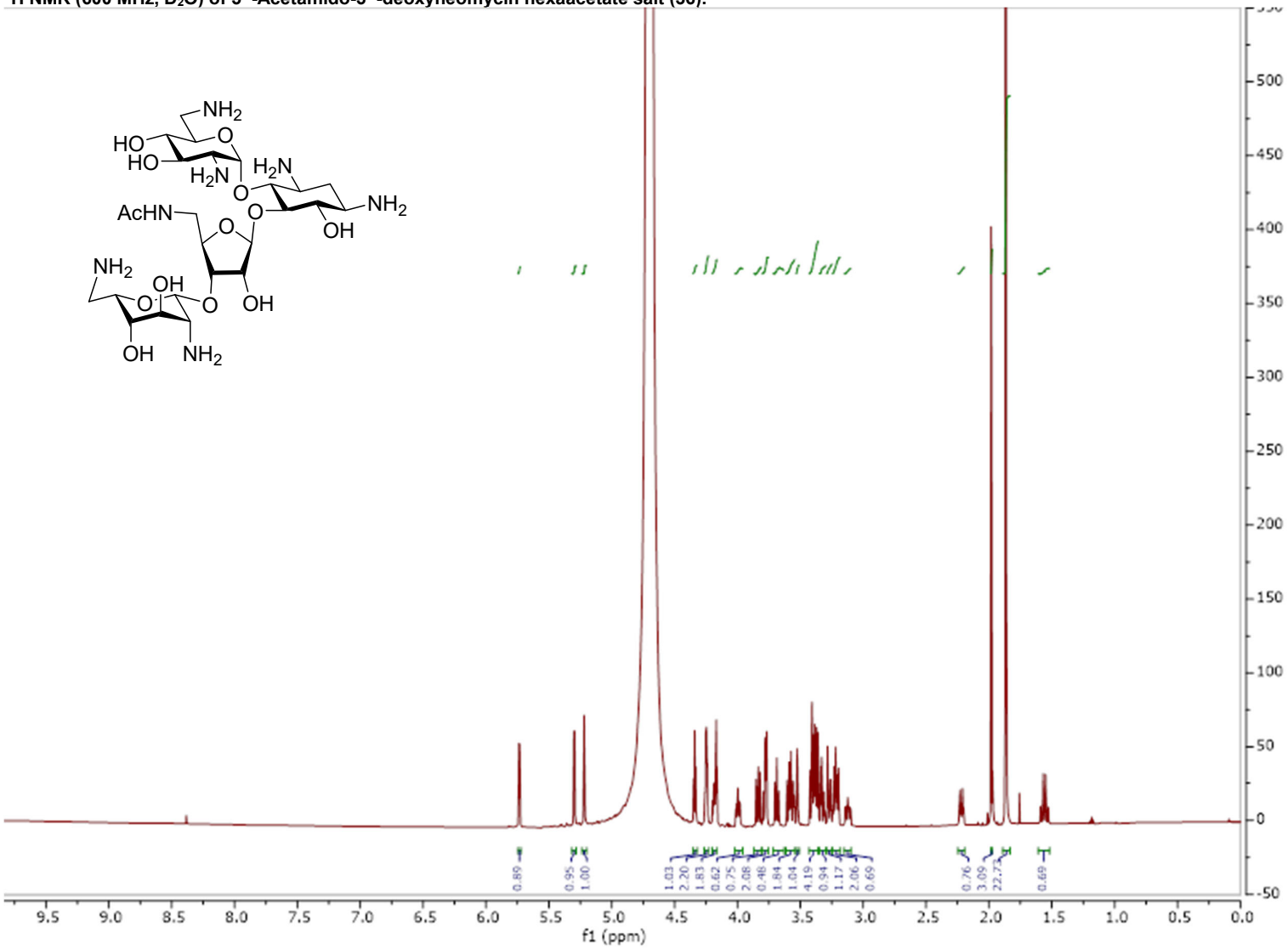
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, D_2O) of 5''-Deoxy-5''-formamidoneomycin hexaacetate salt (35).



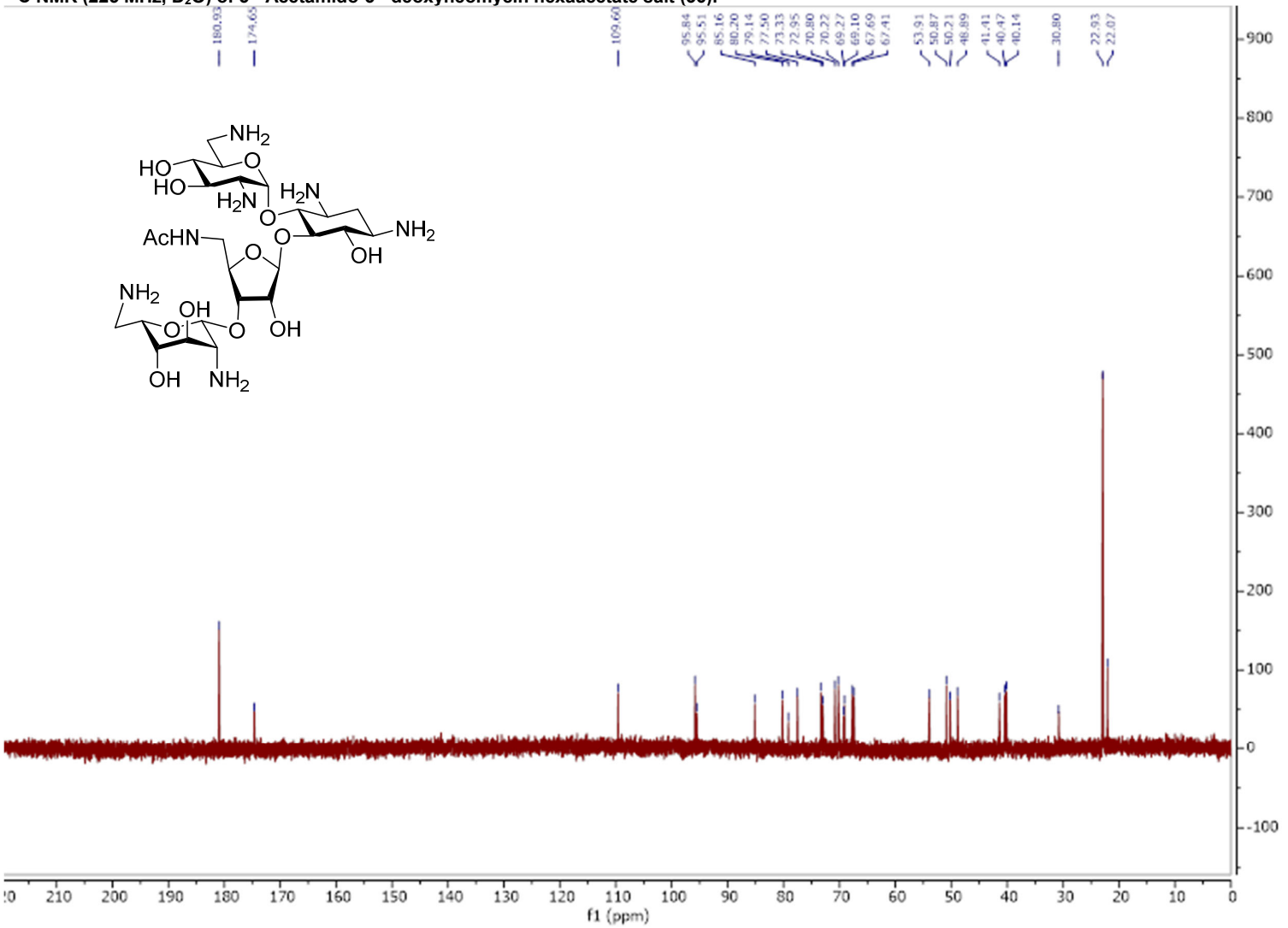
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 5''-Acetamido-5''-deoxyneomycin hexaacetate salt (36).



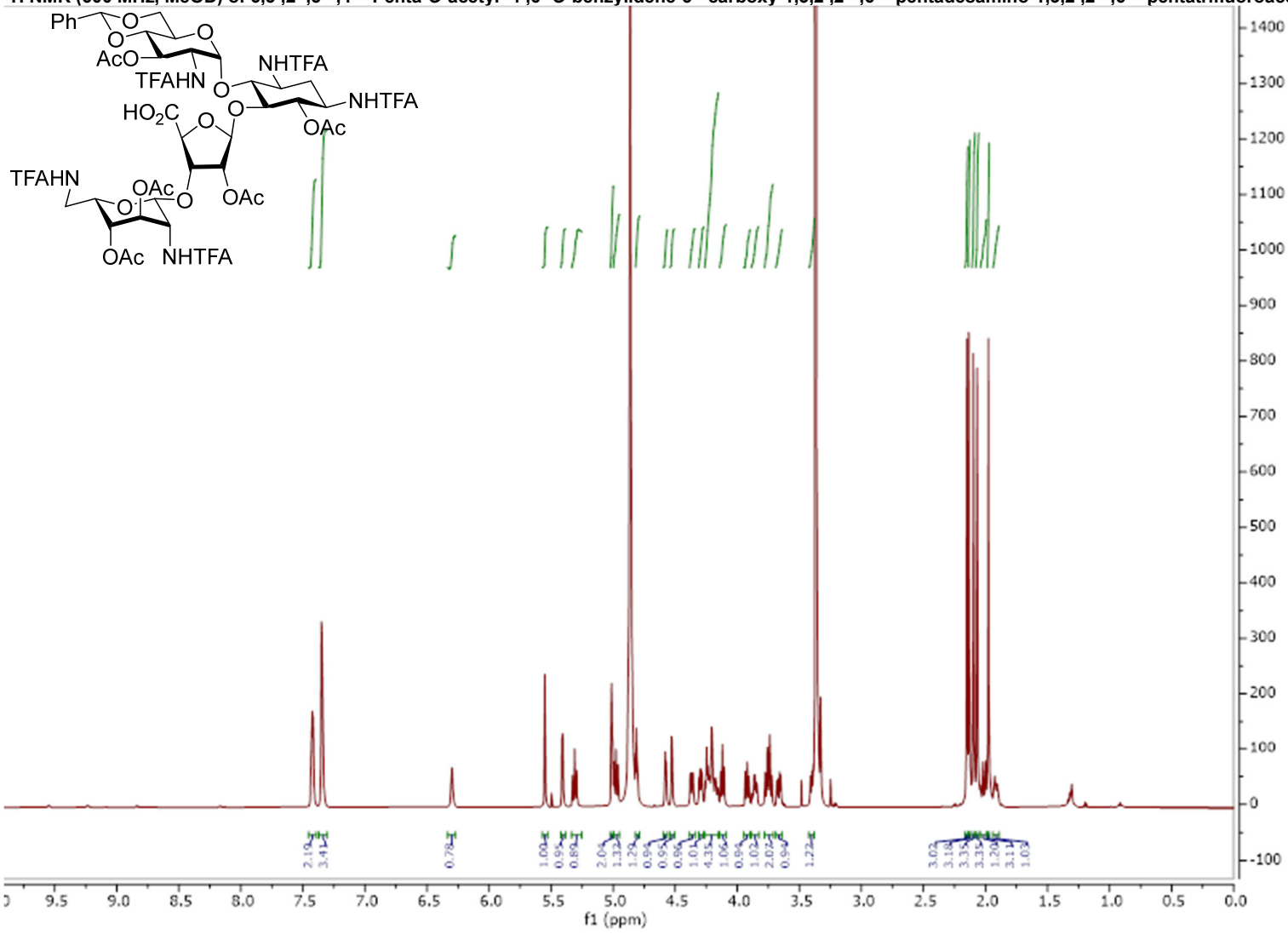
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, D_2O) of 5''-Acetamido-5''-deoxyneomycin hexaacetate salt (36).



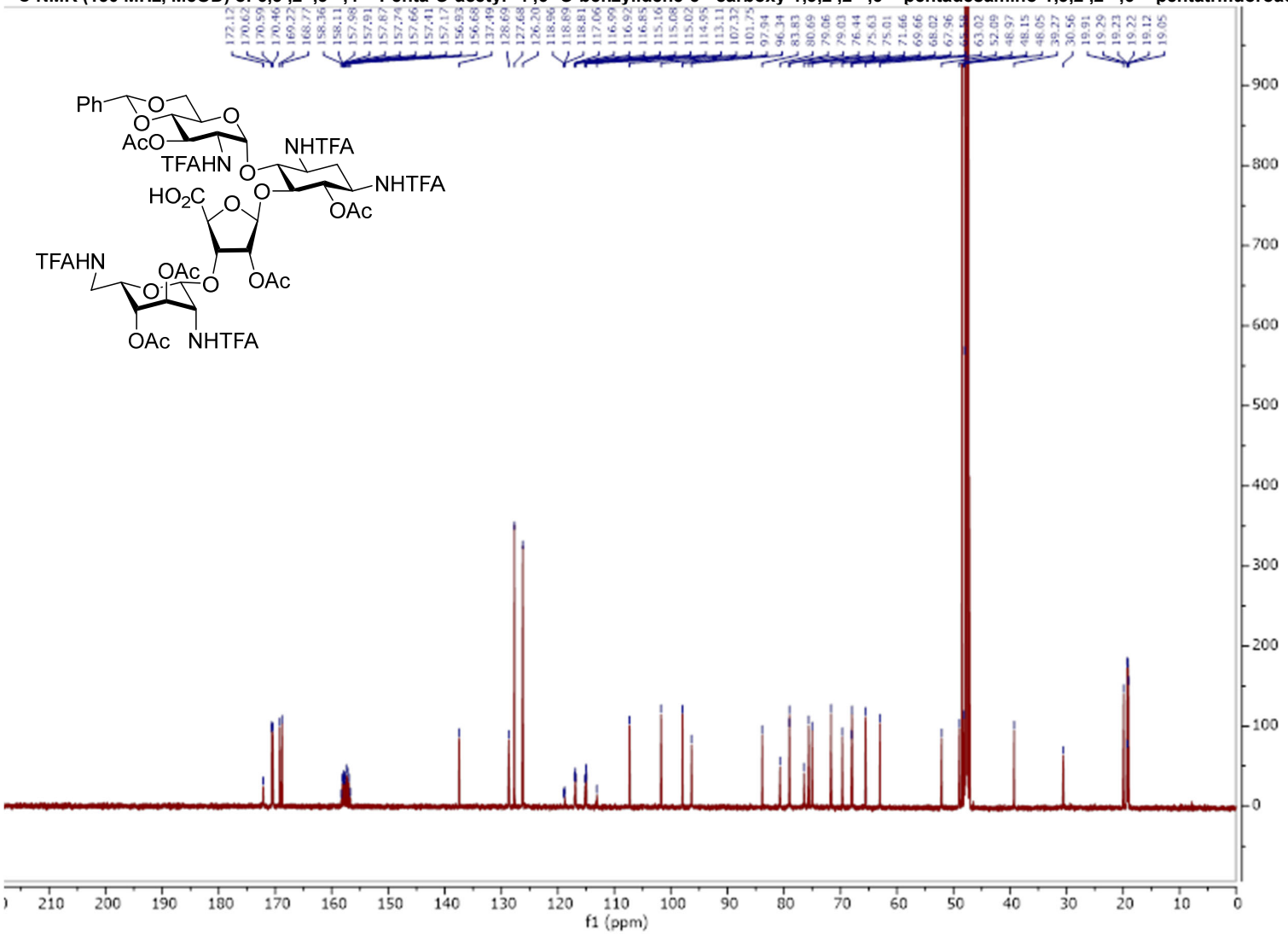
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, MeOD) of 6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene-5''-carboxy-1,3,2',2''',6'''-pentadesamino-1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (38).



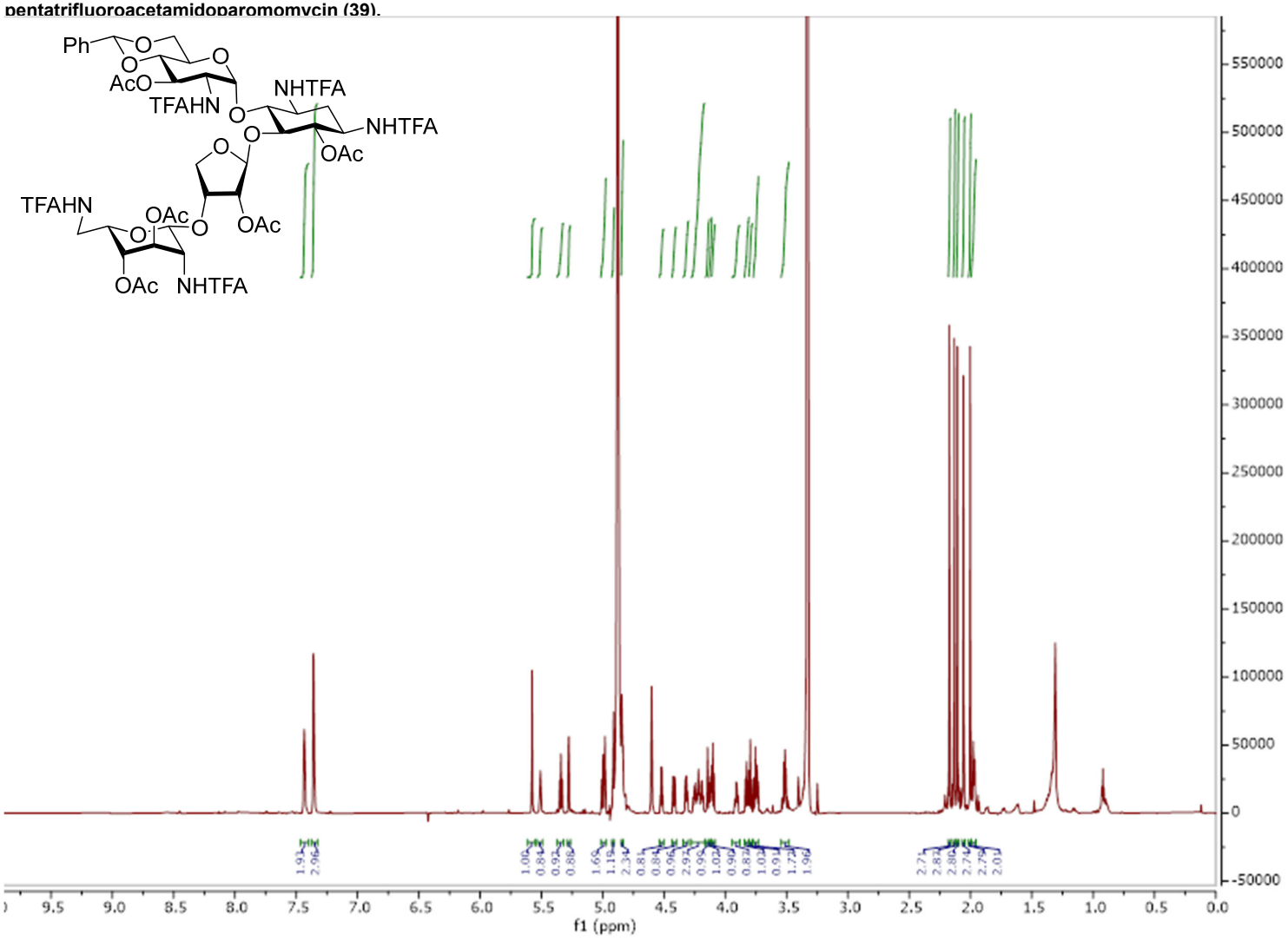
SUPPORTING INFORMATION

^{13}C NMR (150 MHz, MeOD) of 6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene-5''-carboxy-1,3,2',2''',6'''-pentadesamino-1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (38).



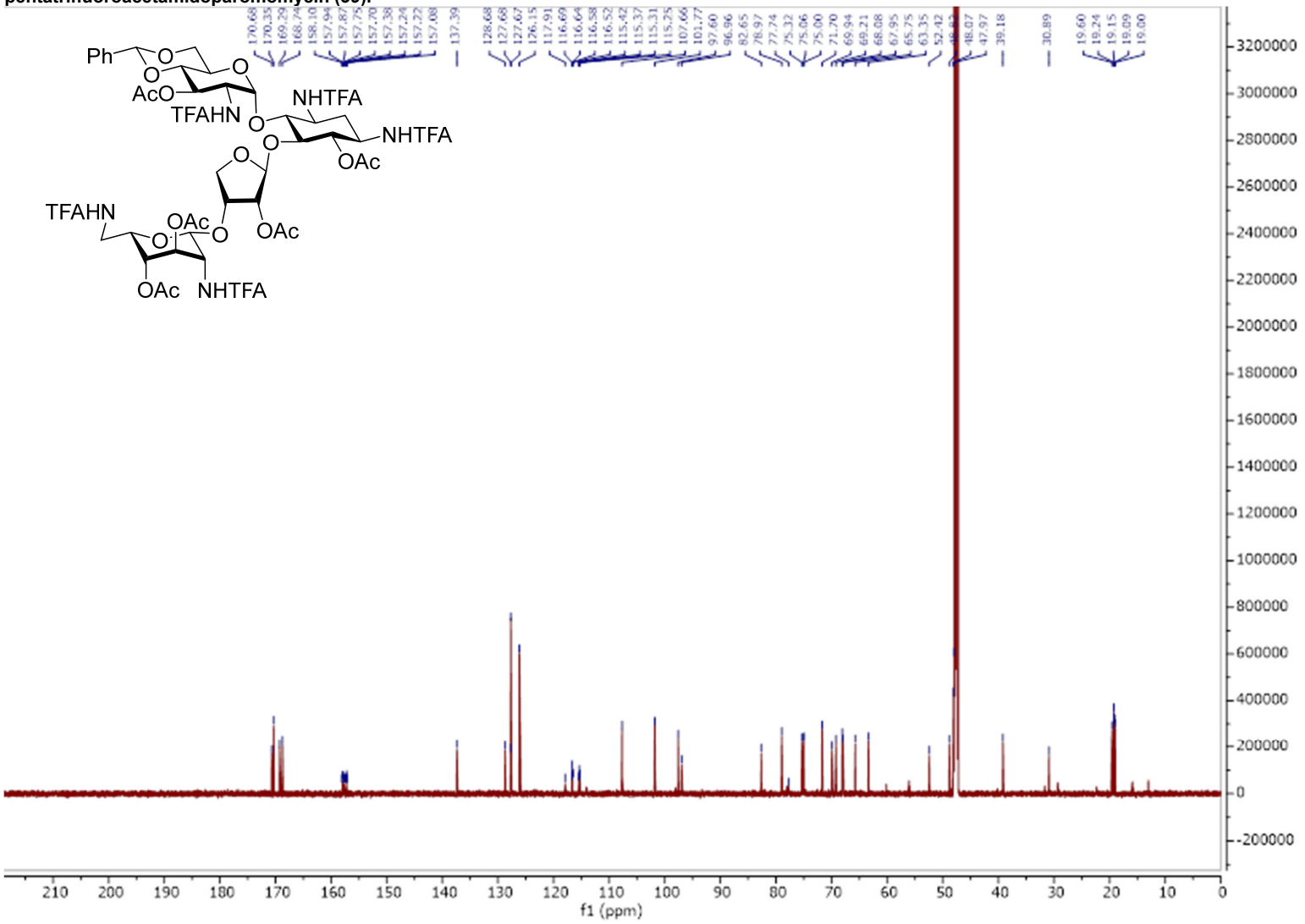
SUPPORTING INFORMATION

^{1}H NMR (900 MHz, MeOD) of 6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene -1,3,2',2''',6'''-pentadesamino-4''-deshydroxymethyl -1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (39).



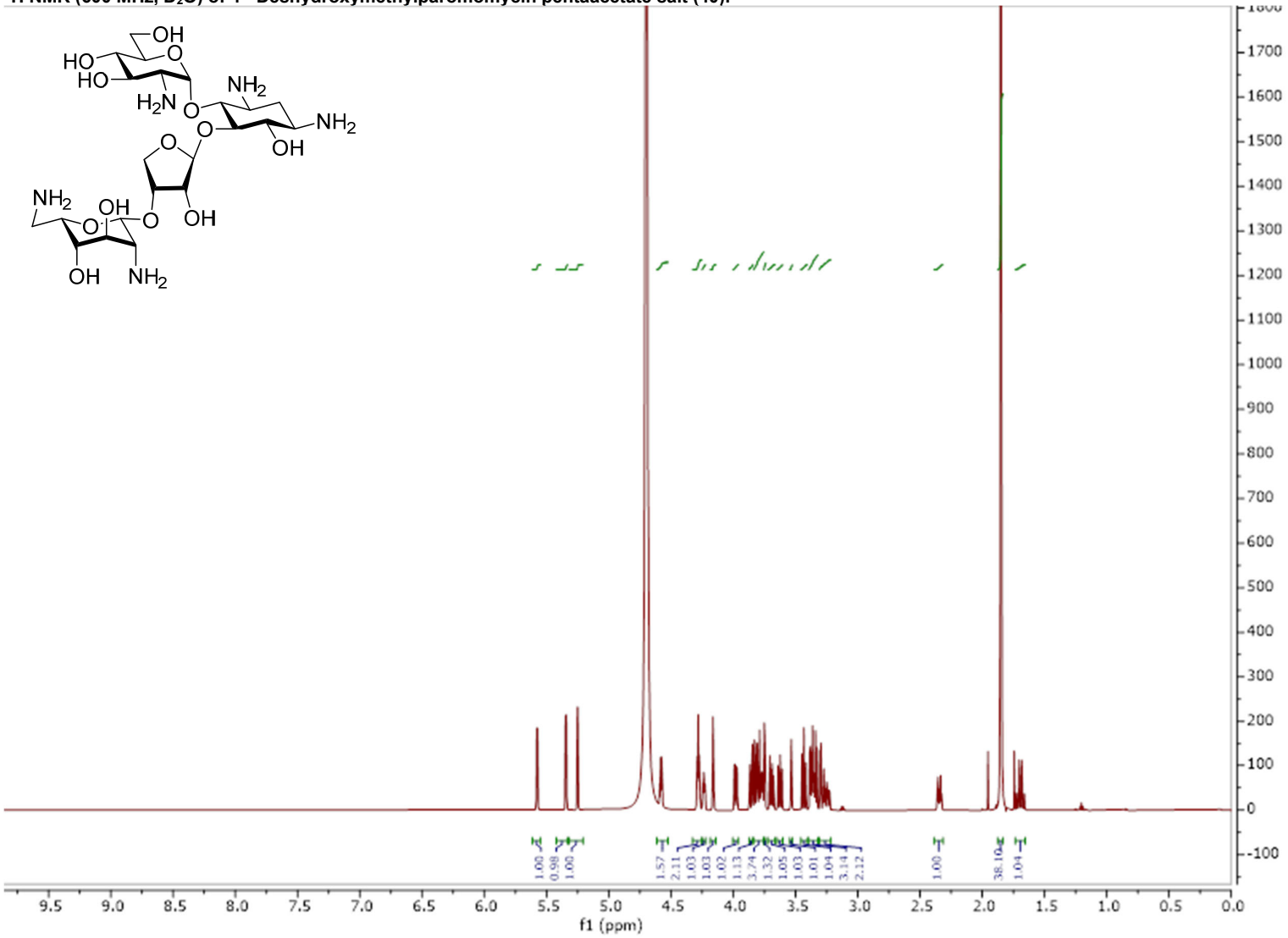
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 6,3',2'',3''',4'''-Penta-*O*-acetyl -4',6'-*O*-benzylidene -1,3,2',2''',6'''-pentadesamino-4''-deshydroxymethyl -1,3,2',2''',6'''-pentatrifluoroacetamidoparomomycin (39).



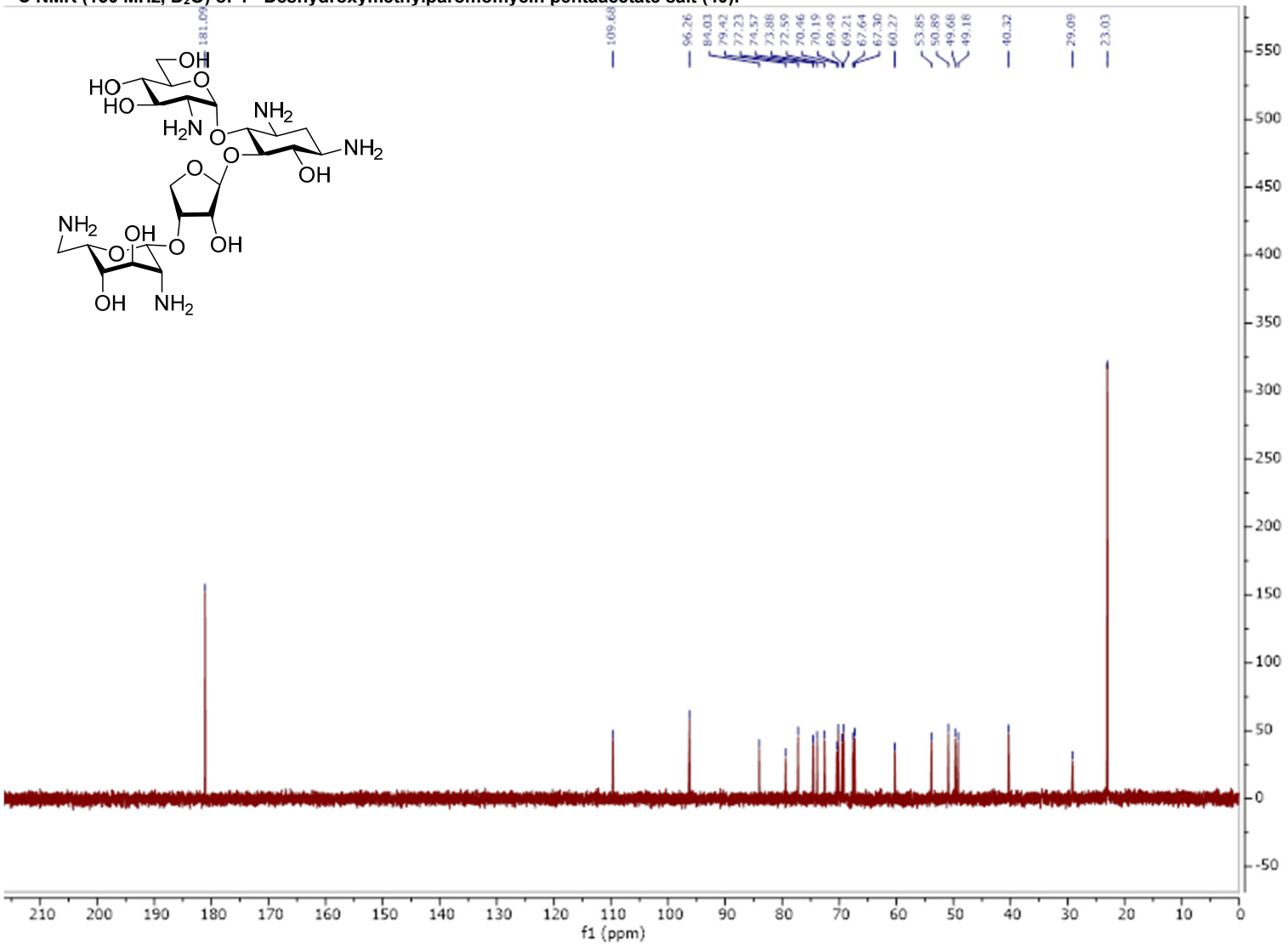
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 4''-Deshydroxymethylparomomycin pentaacetate salt (40).

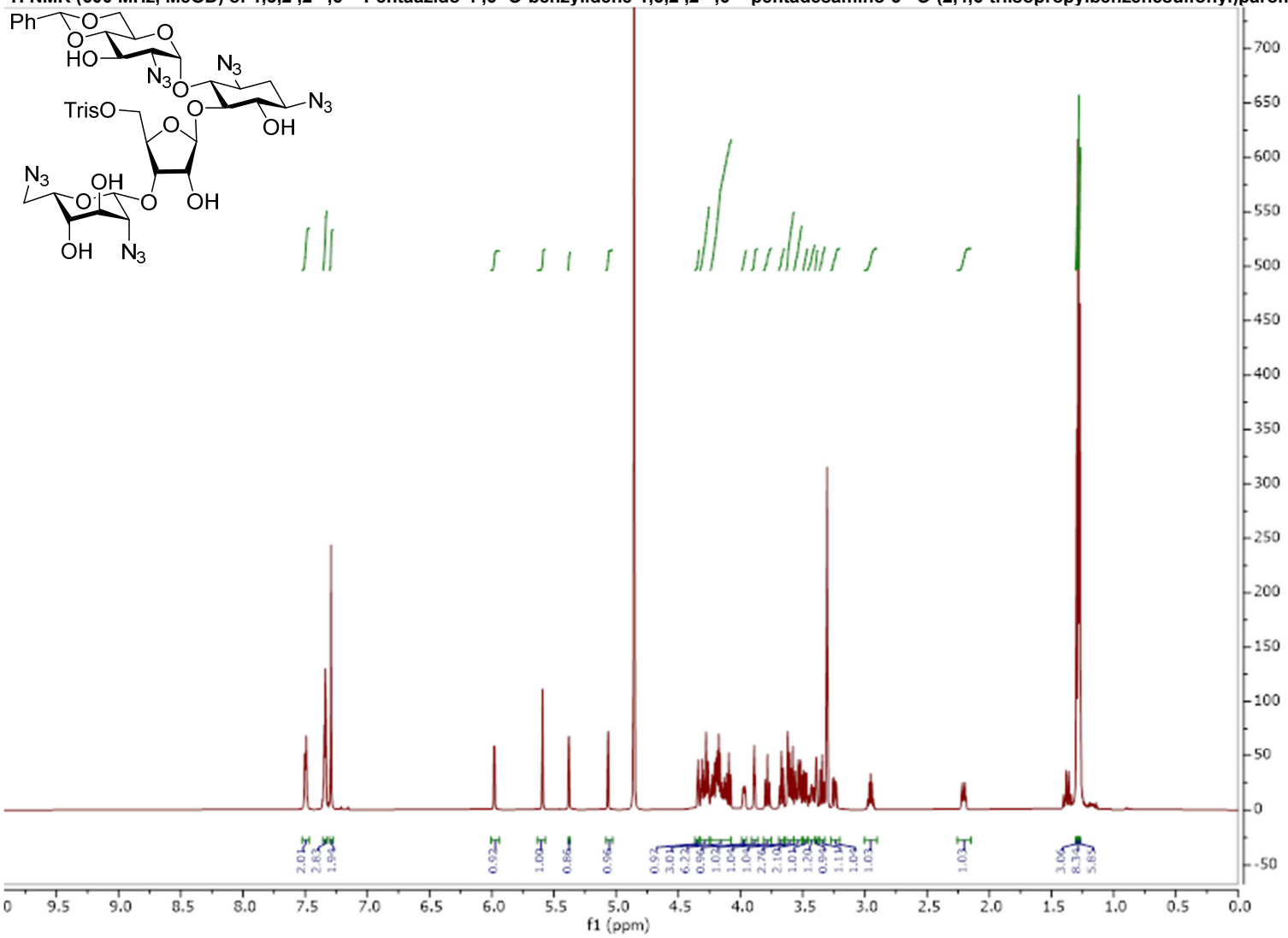


SUPPORTING INFORMATION

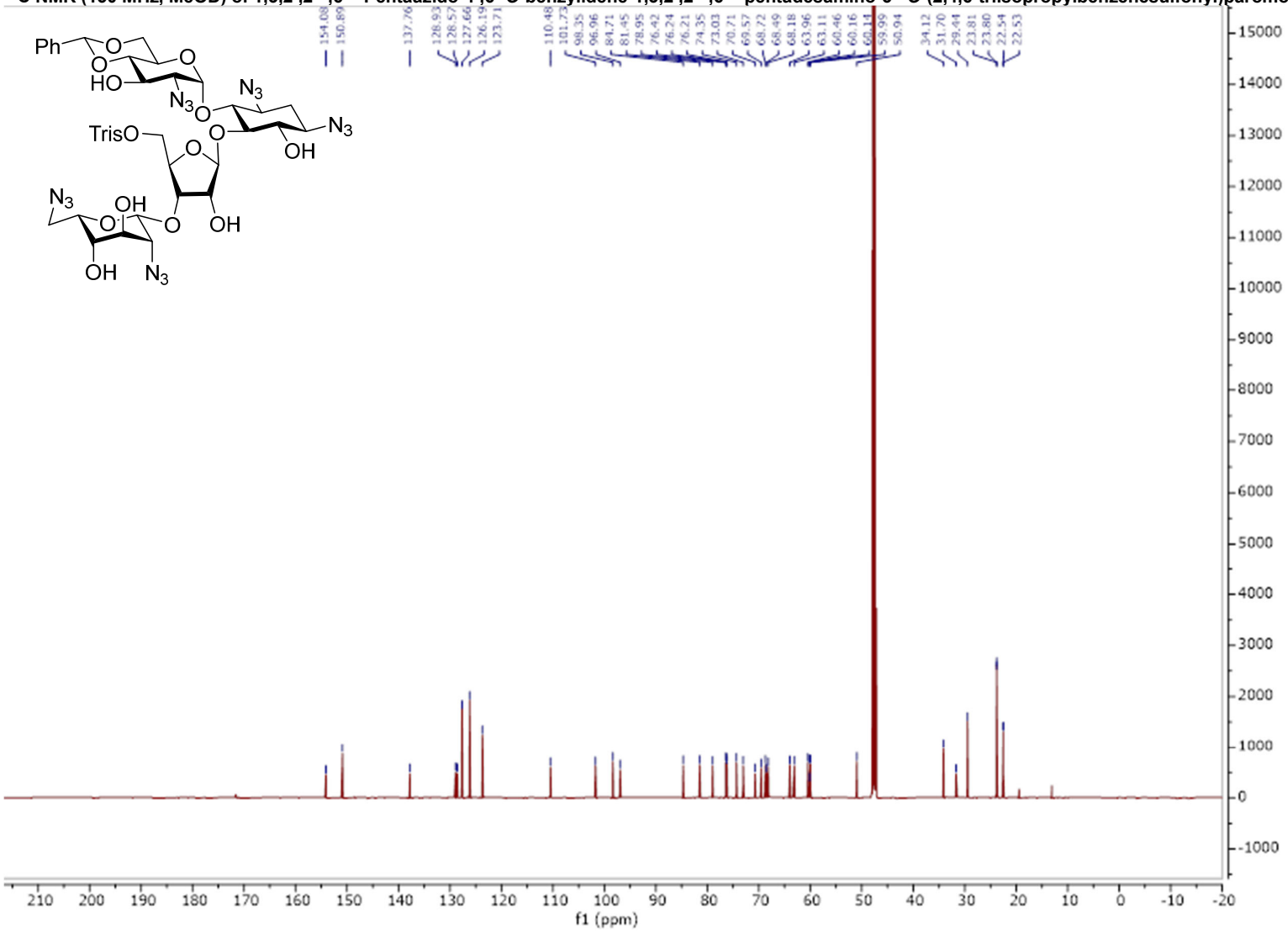
^{13}C NMR (150 MHz, D_2O) of 4''-Deshydroxymethylparomomycin pentaacetate salt (40).



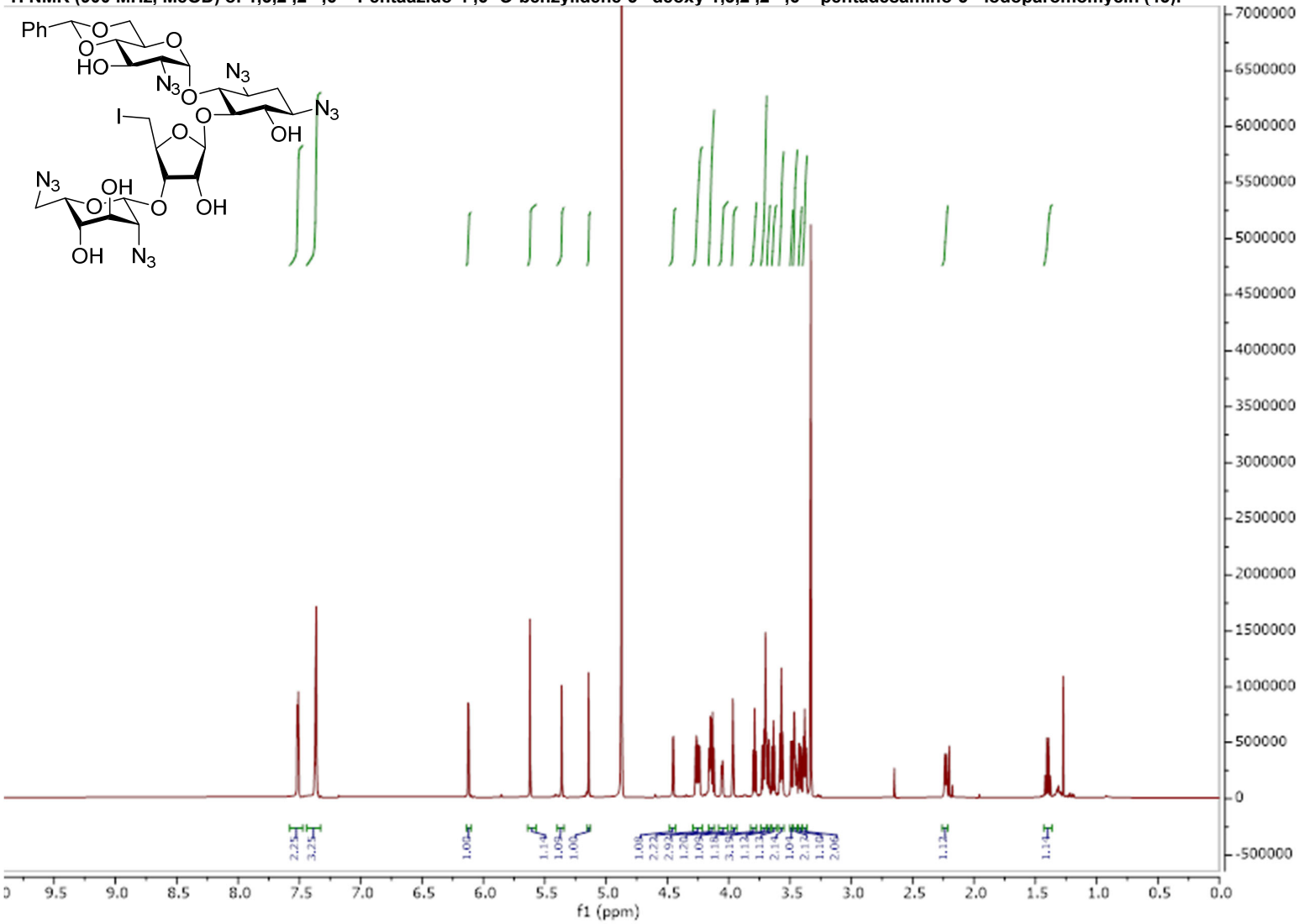
^{1}H NMR (600 MHz, MeOD) of 1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-1,3,2',2''',6'''-pentadesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)paromomycin (43).



^{13}C NMR (150 MHz, MeOD) of 1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-1,3,2',2''',6'''-pentadesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)paromomycin (43).

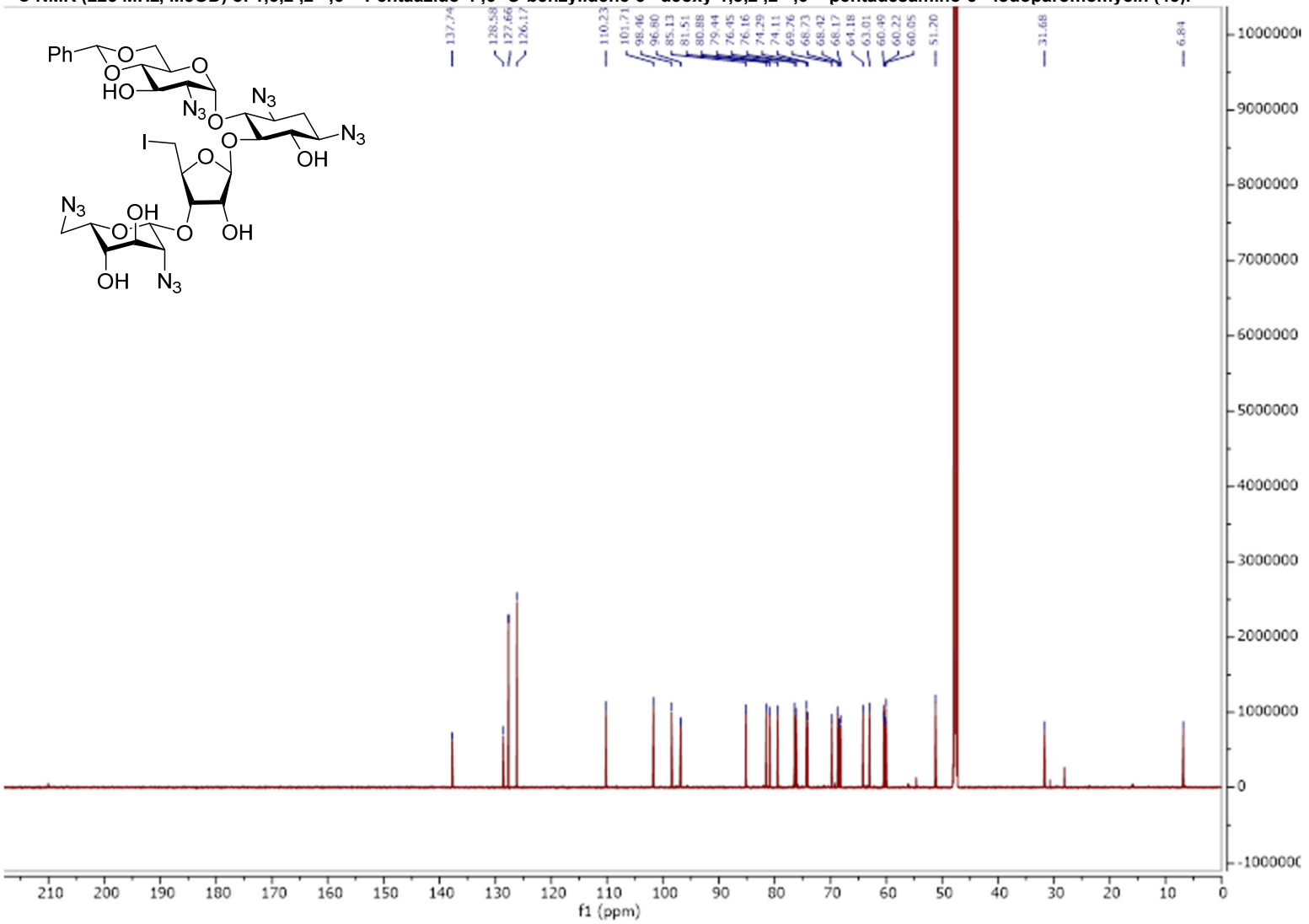


^{1}H NMR (900 MHz, MeOD) of 1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesamino-5''-iodoparomomycin (45).

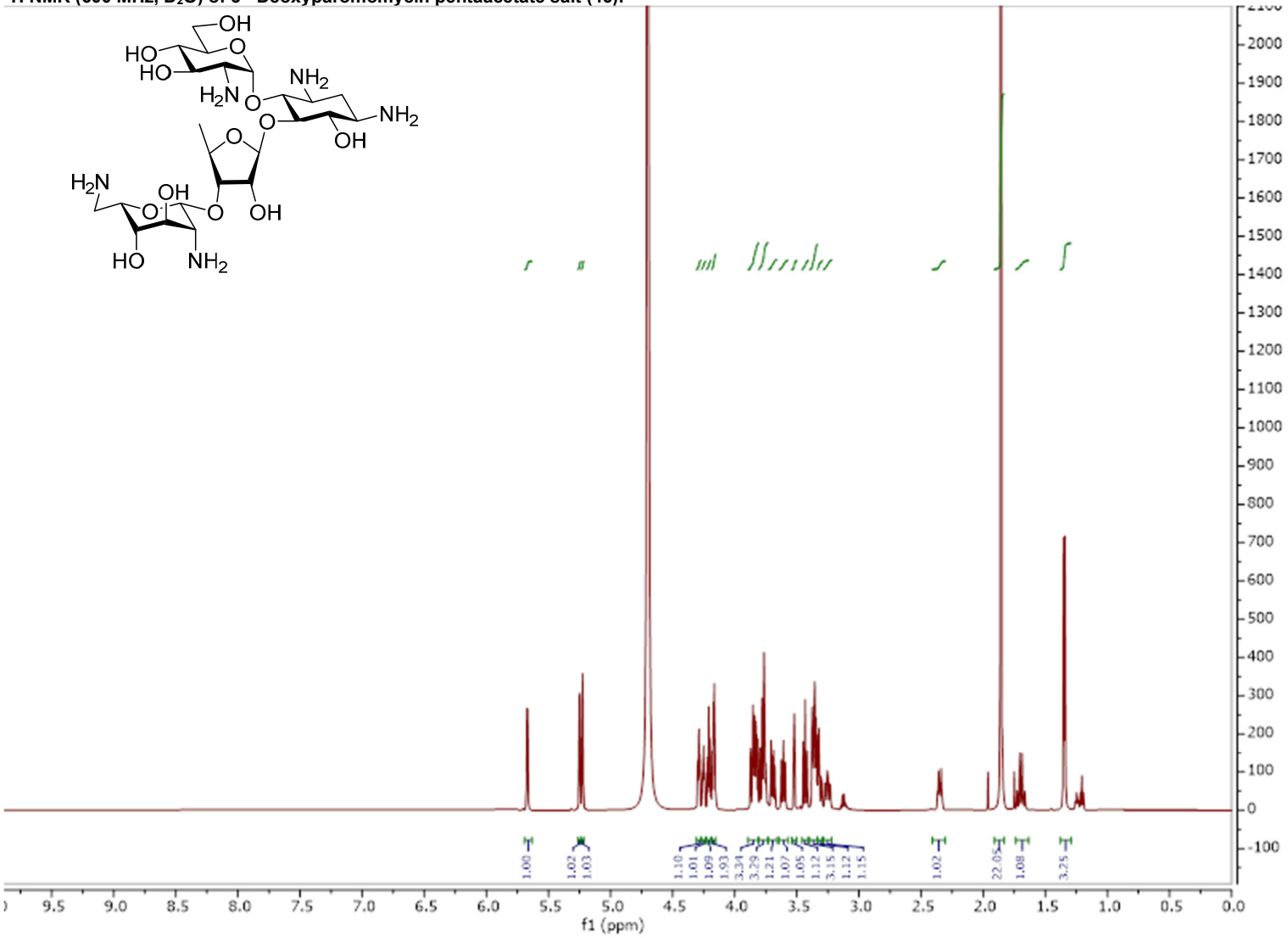


SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 1,3,2',2''',6'''-Pentaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesamino-5''-iodoparomomycin (45).

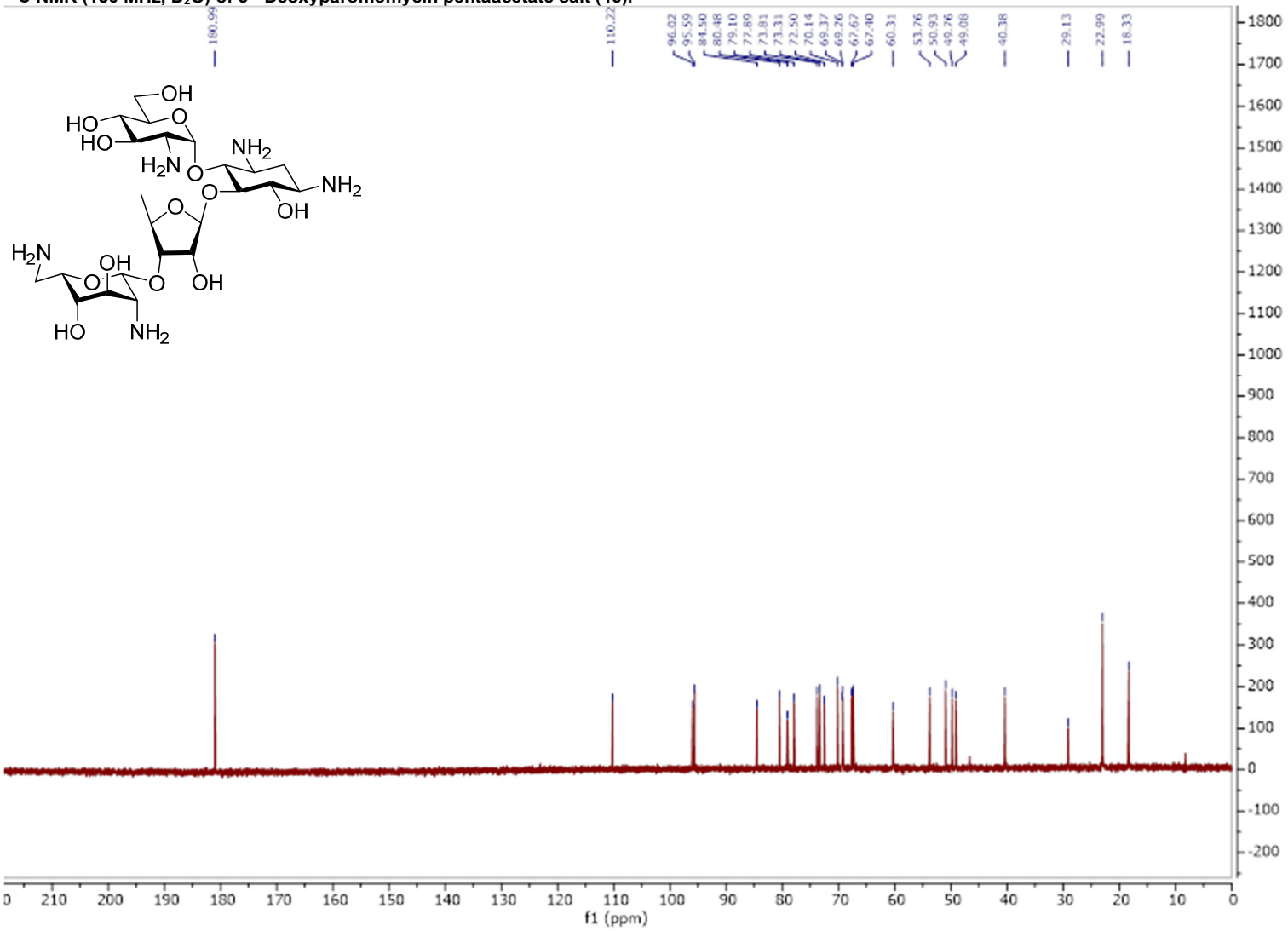


^{1}H NMR (600 MHz, D_2O) of 5''-Deoxyparomomycin pentaacetate salt (46).

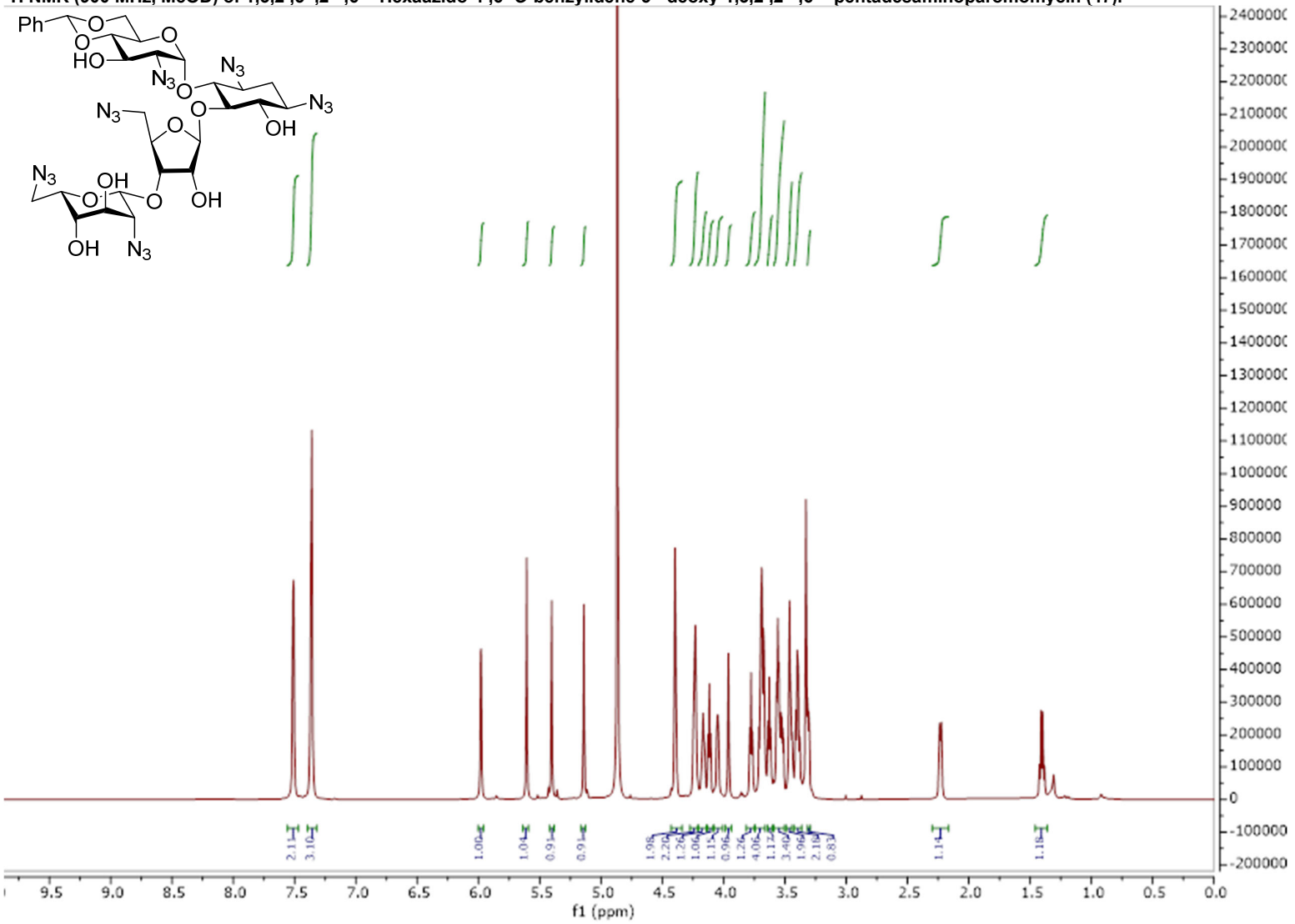


SUPPORTING INFORMATION

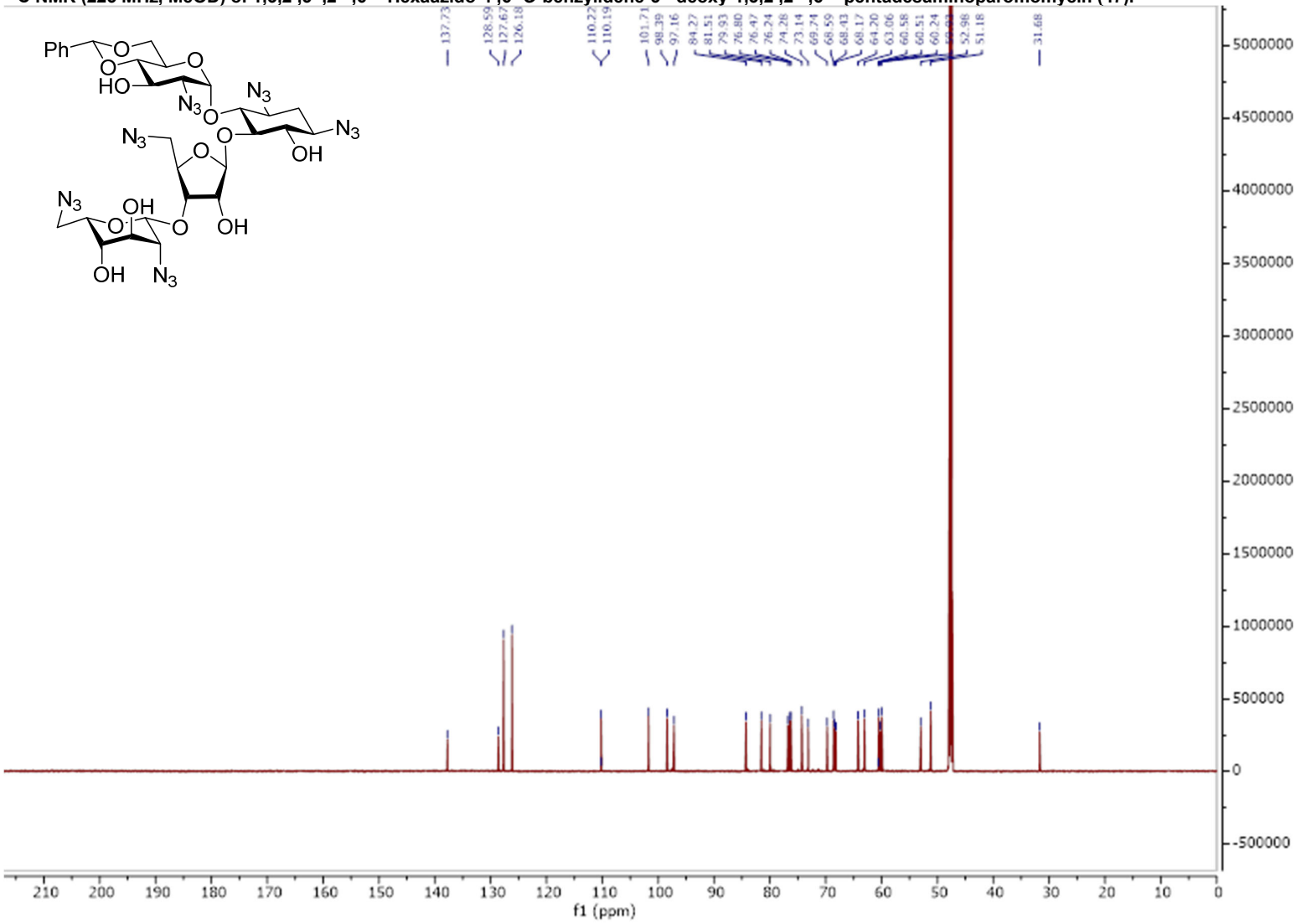
^{13}C NMR (150 MHz, D_2O) of 5''-Deoxyparomomycin pentaacetate salt (46).



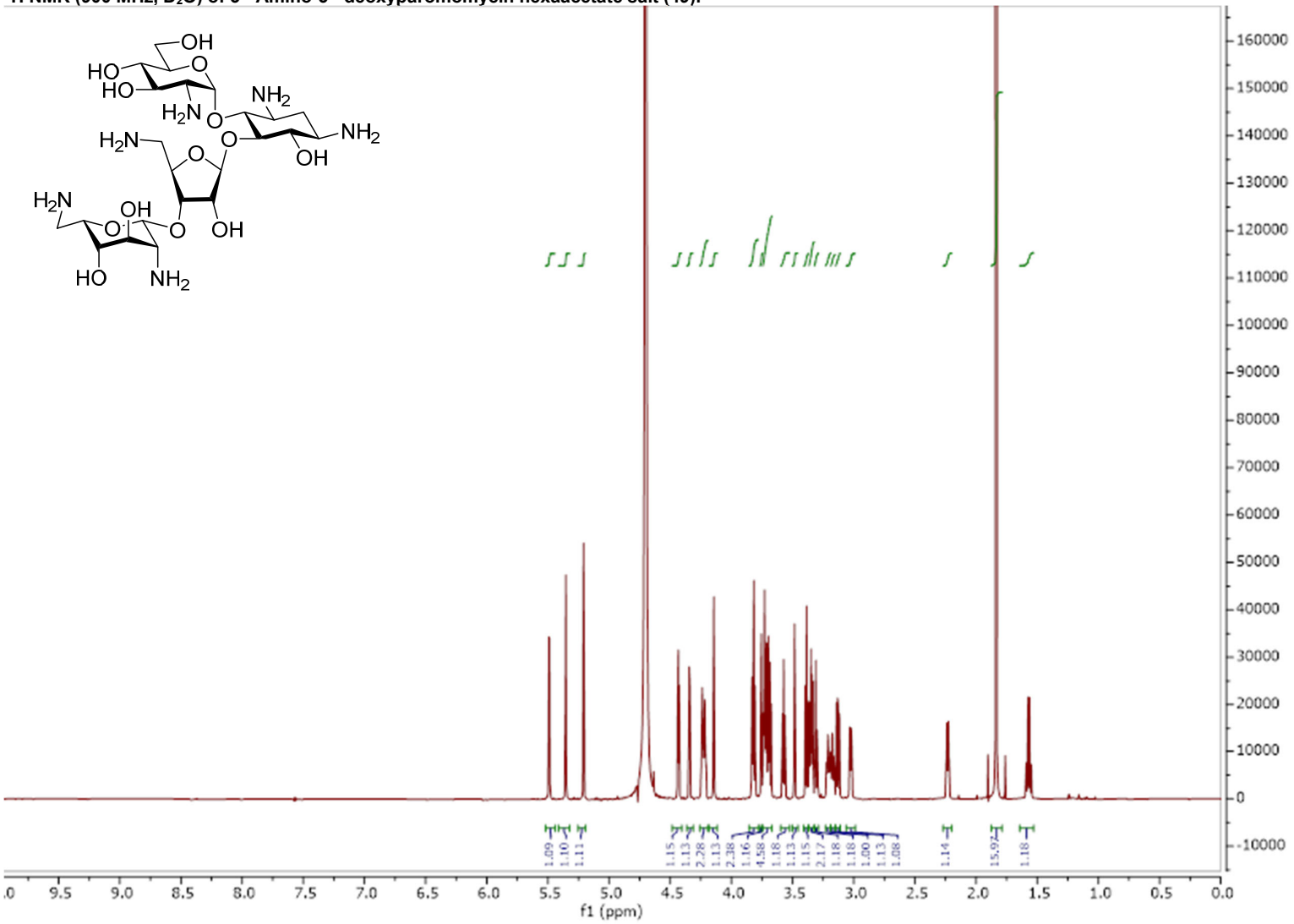
^{1}H NMR (900 MHz, MeOD) of 1,3,2',5'',2''',6'''-Hexaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesaminoparomomycin (47).



^{13}C NMR (225 MHz, MeOD) of 1,3,2',5'',2''',6'''-Hexaazido-4',6'-*O*-benzylidene-5''-deoxy-1,3,2',2''',6'''-pentadesaminoparomomycin (47).

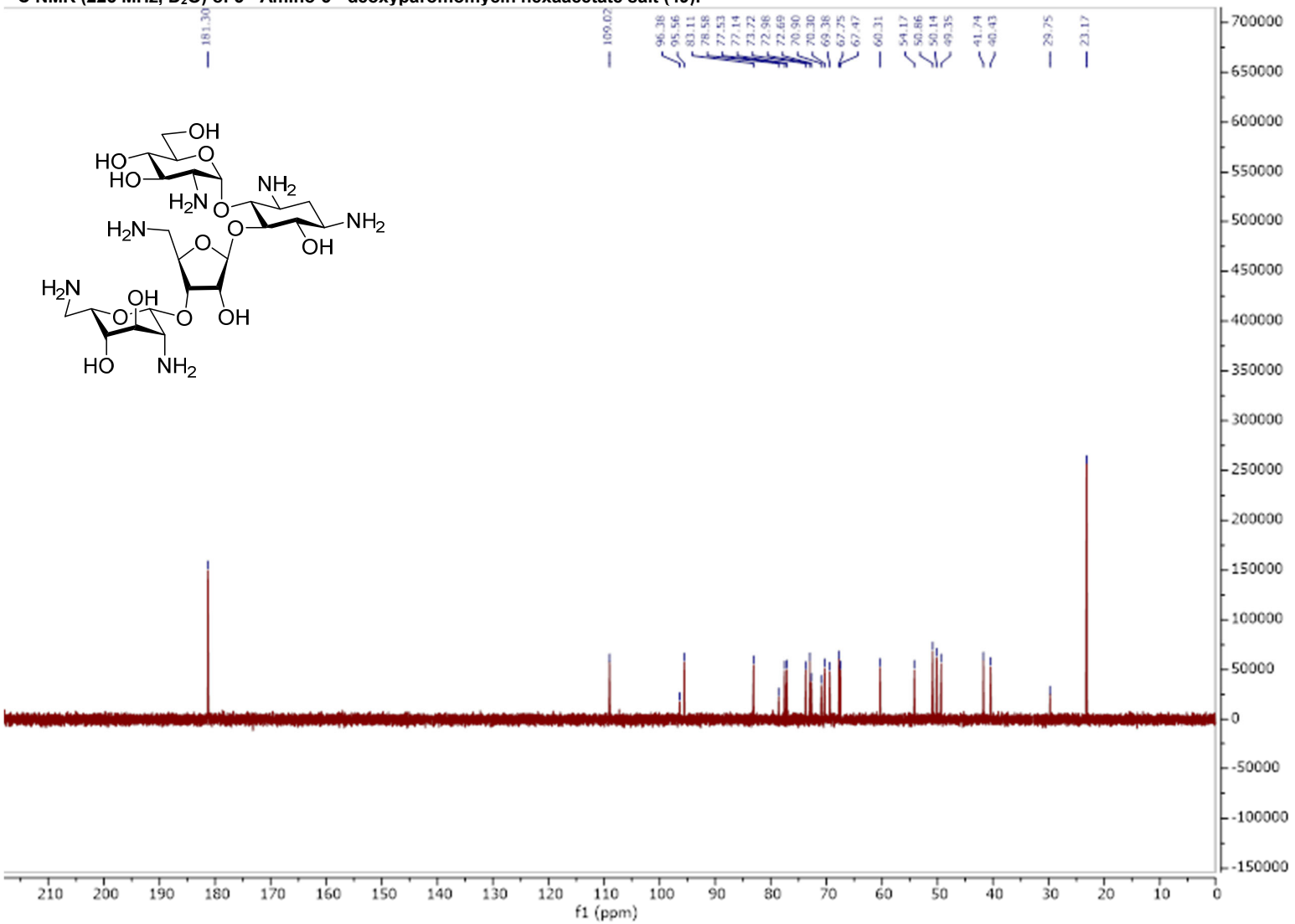


^{1}H NMR (900 MHz, D_2O) of 5''-Amino-5''-deoxyparomomycin hexaacetate salt (49).



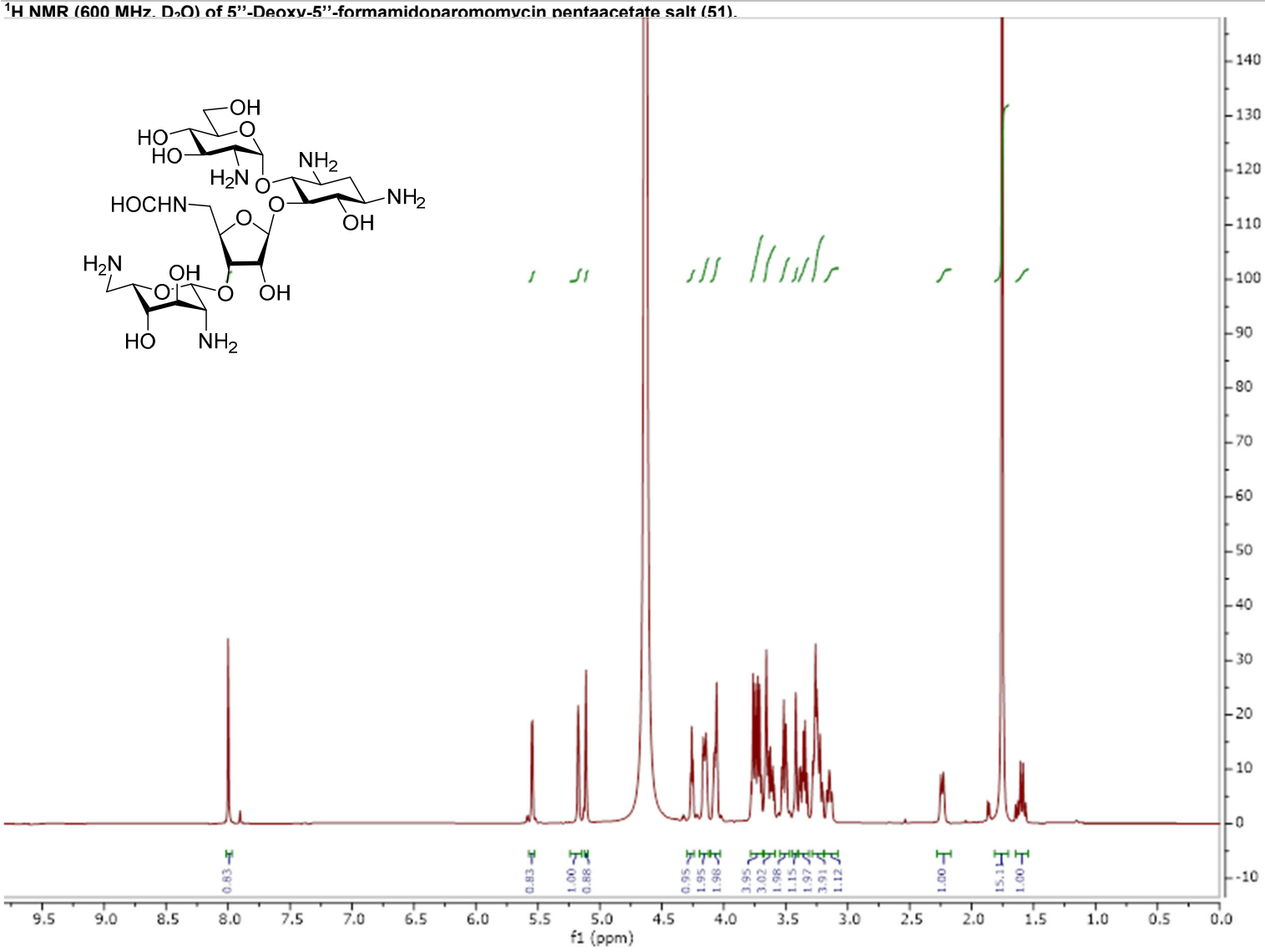
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, D_2O) of 5''-Amino-5''-deoxyparomomycin hexaacetate salt (49).

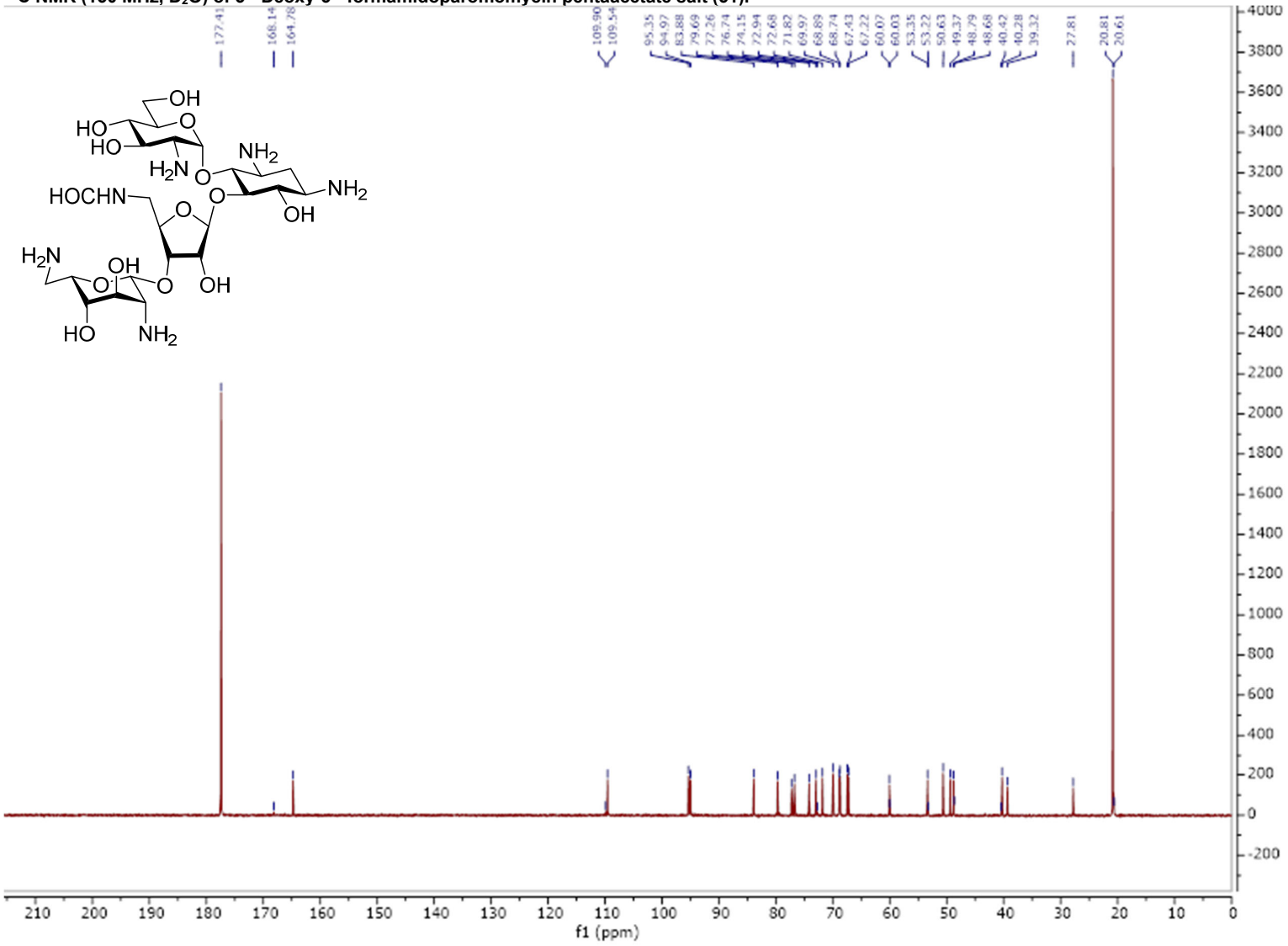


SUPPORTING INFORMATION

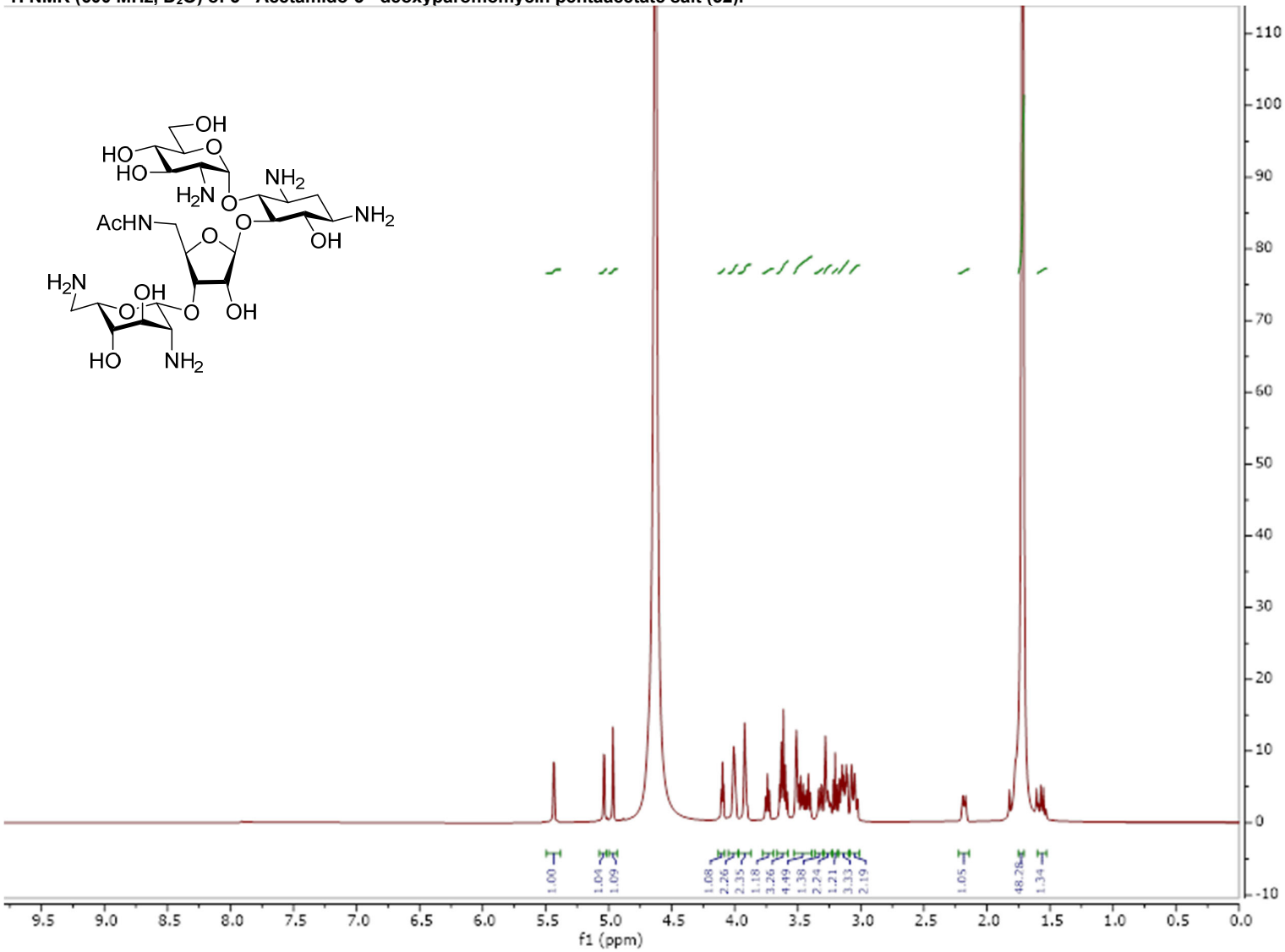
^{1}H NMR (600 MHz, D_2O) of 5''-Deoxy-5''-formamidoparomomycin pentaacetate salt (51).



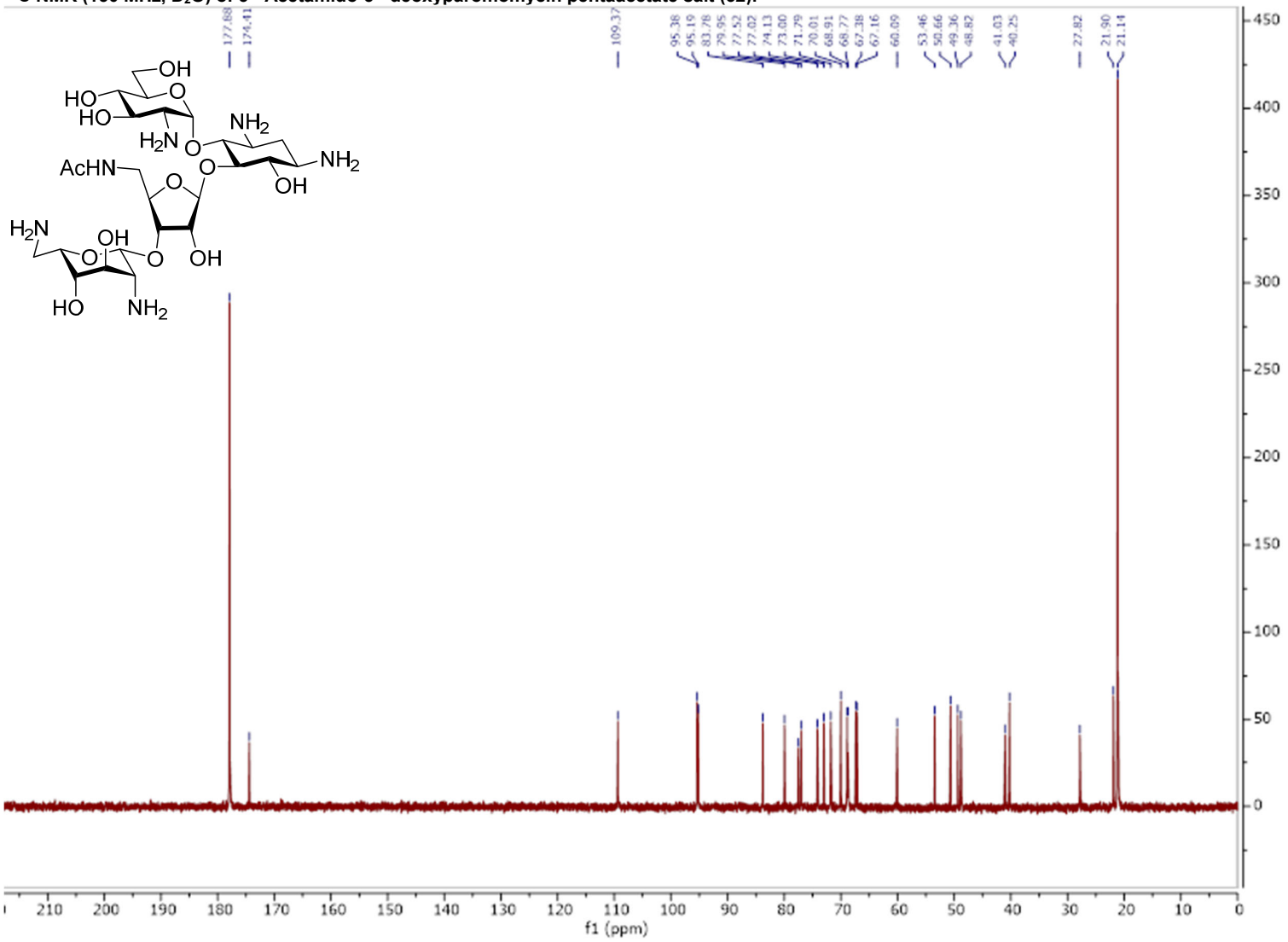
^{13}C NMR (150 MHz, D_2O) of 5''-Deoxy-5''-formamidoparomomycin pentaacetate salt (51).



^{1}H NMR (600 MHz, D_2O) of 5''-Acetamido-5''-deoxyparomomycin pentaacetate salt (52).

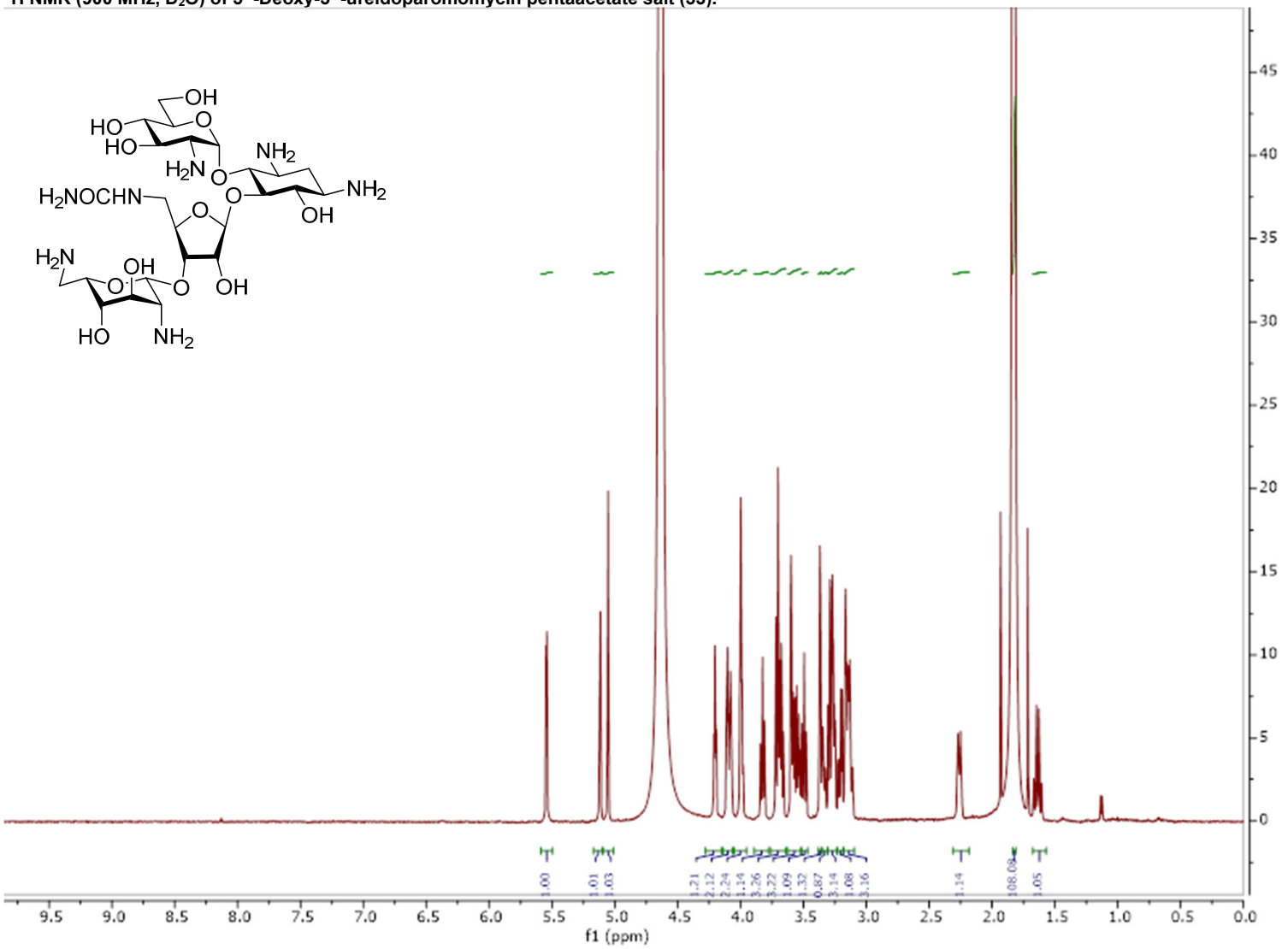


^{13}C NMR (150 MHz, D_2O) of 5''-Acetamido-5''-deoxyparomomycin pentaacetate salt (52).

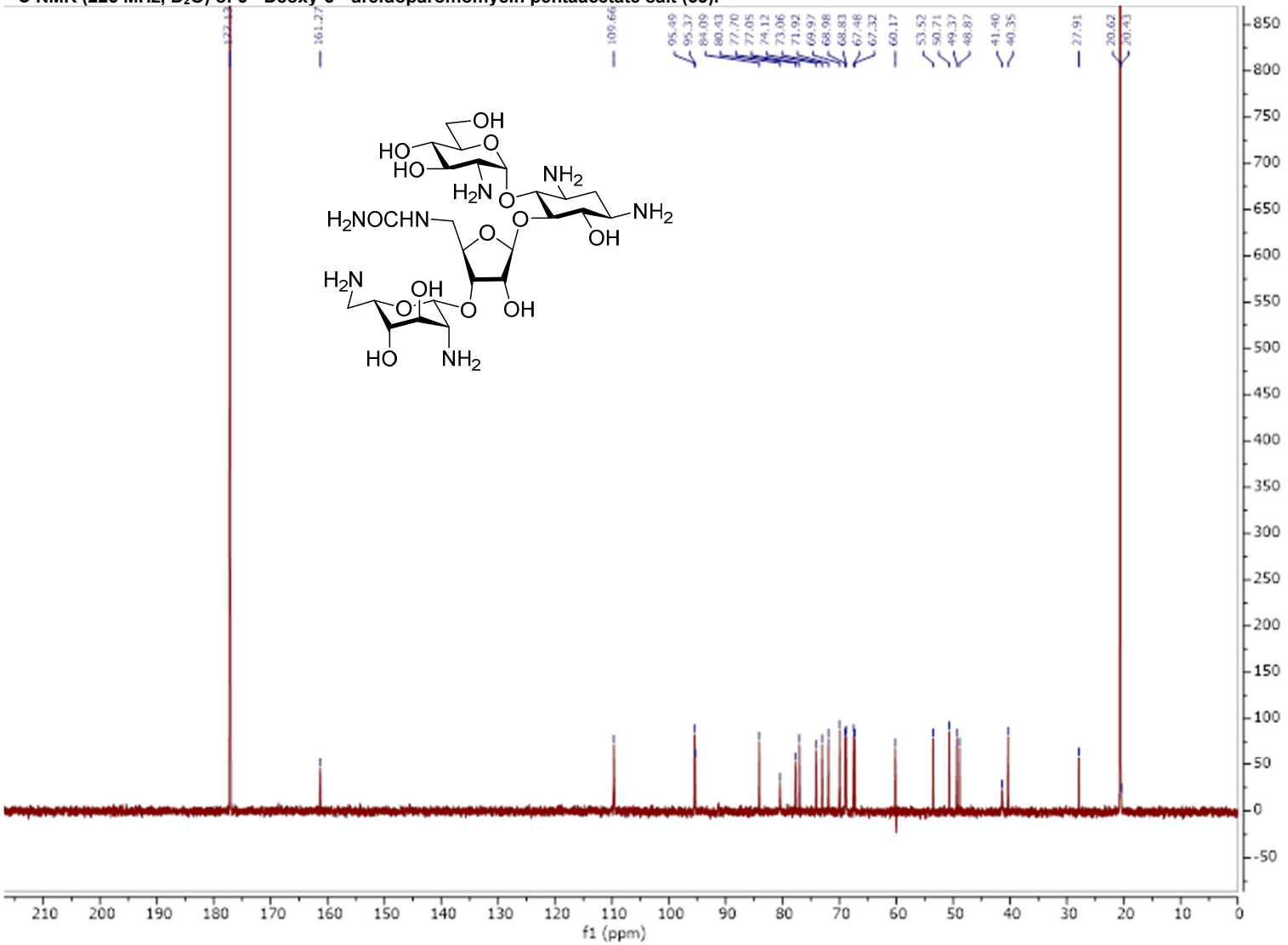


SUPPORTING INFORMATION

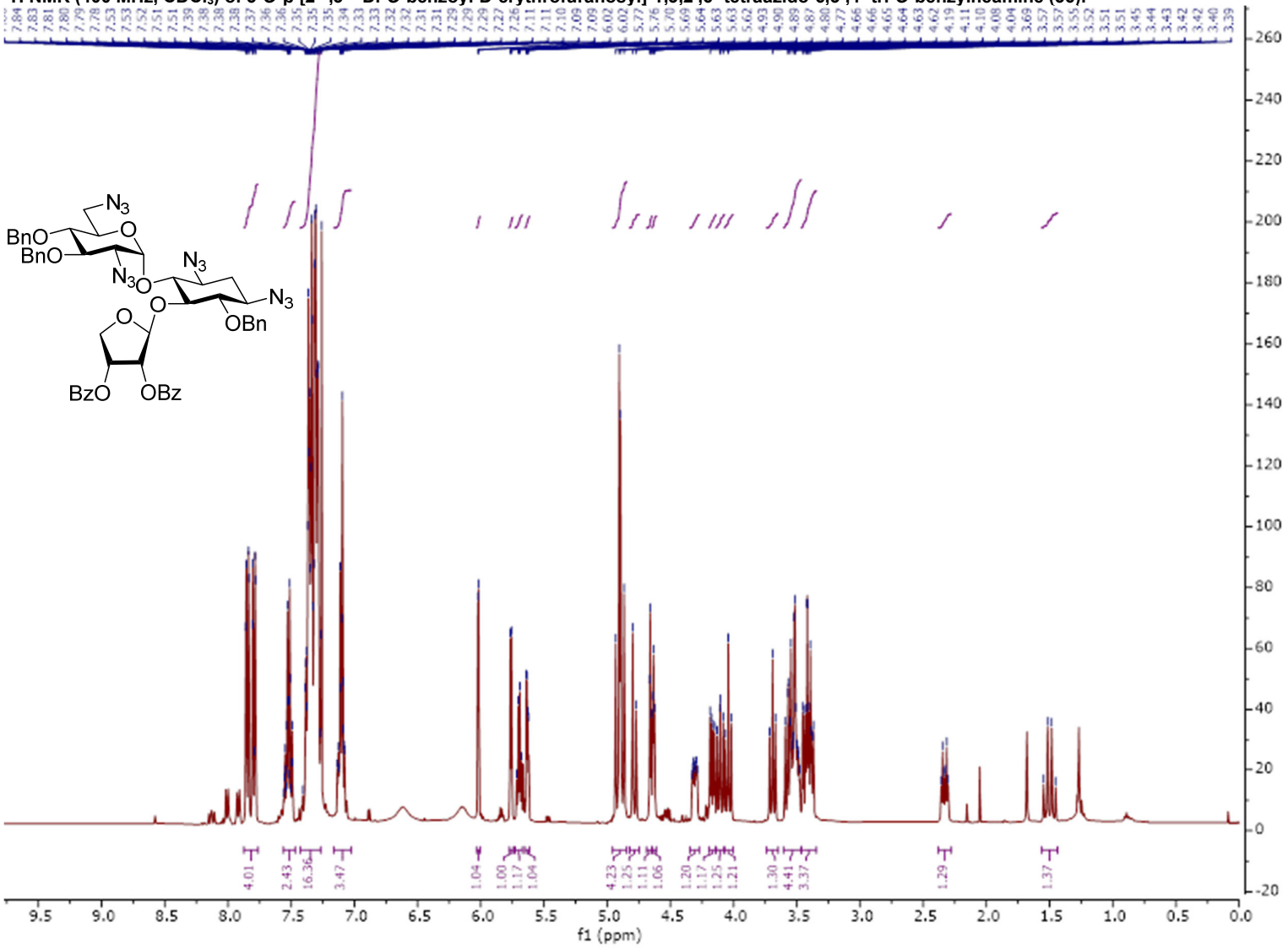
^{1}H NMR (900 MHz, D_2O) of 5''-Deoxy-5''-ureidoparomomycin pentaacetate salt (53).



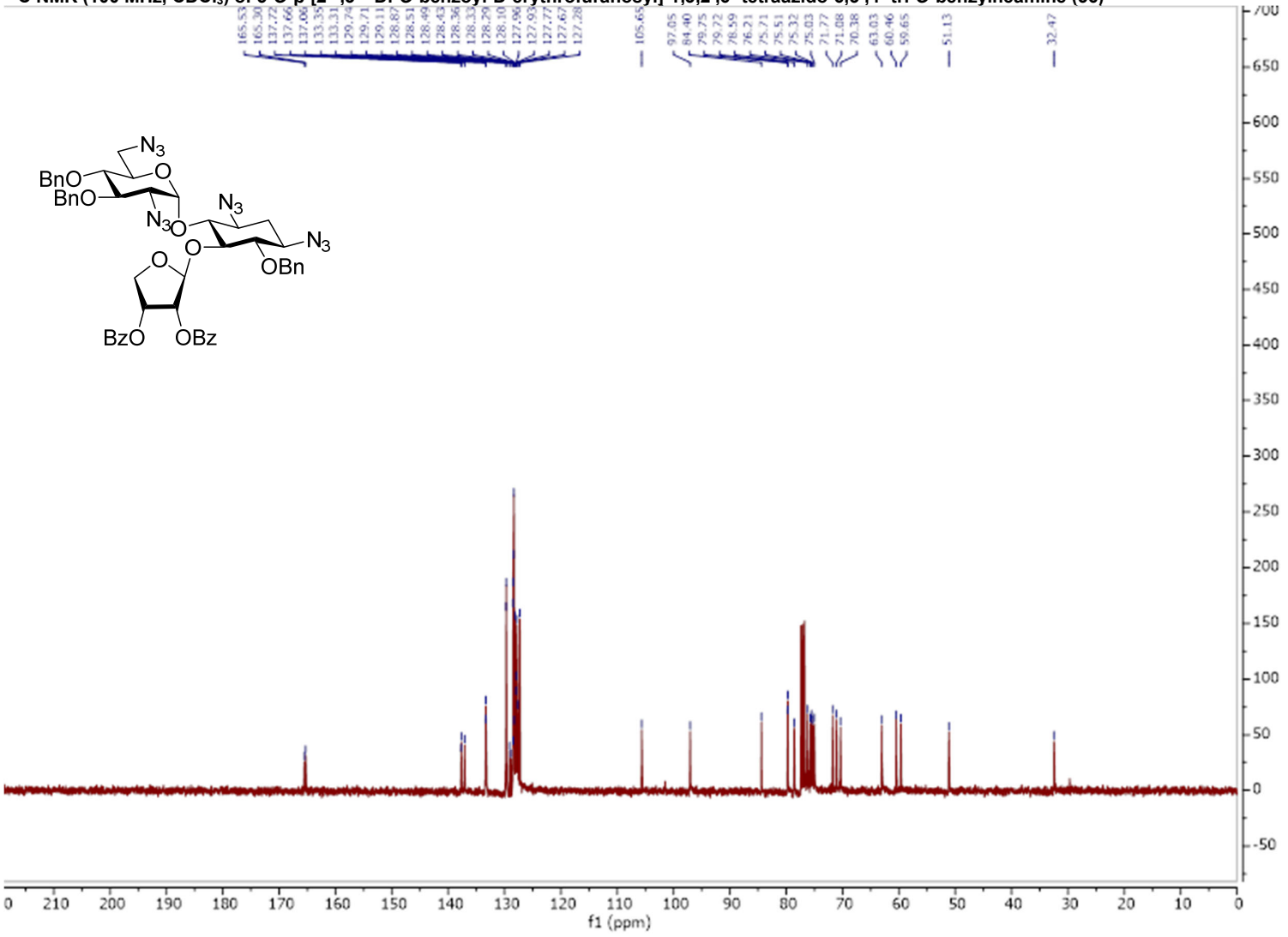
^{13}C NMR (225 MHz, D_2O) of 5''-Deoxy-5''-ureidoparomomycin pentaacetate salt (53).



^{1}H NMR (400 MHz, $CDCl_3$) of 5-*O*-β-[2‴,3‴-Di-*O*-benzoyl-D-erythrofuranosyl]-1,3,2′,6′-tetraazido-6,3′,4′-tri-*O*-benzylneamine (56).

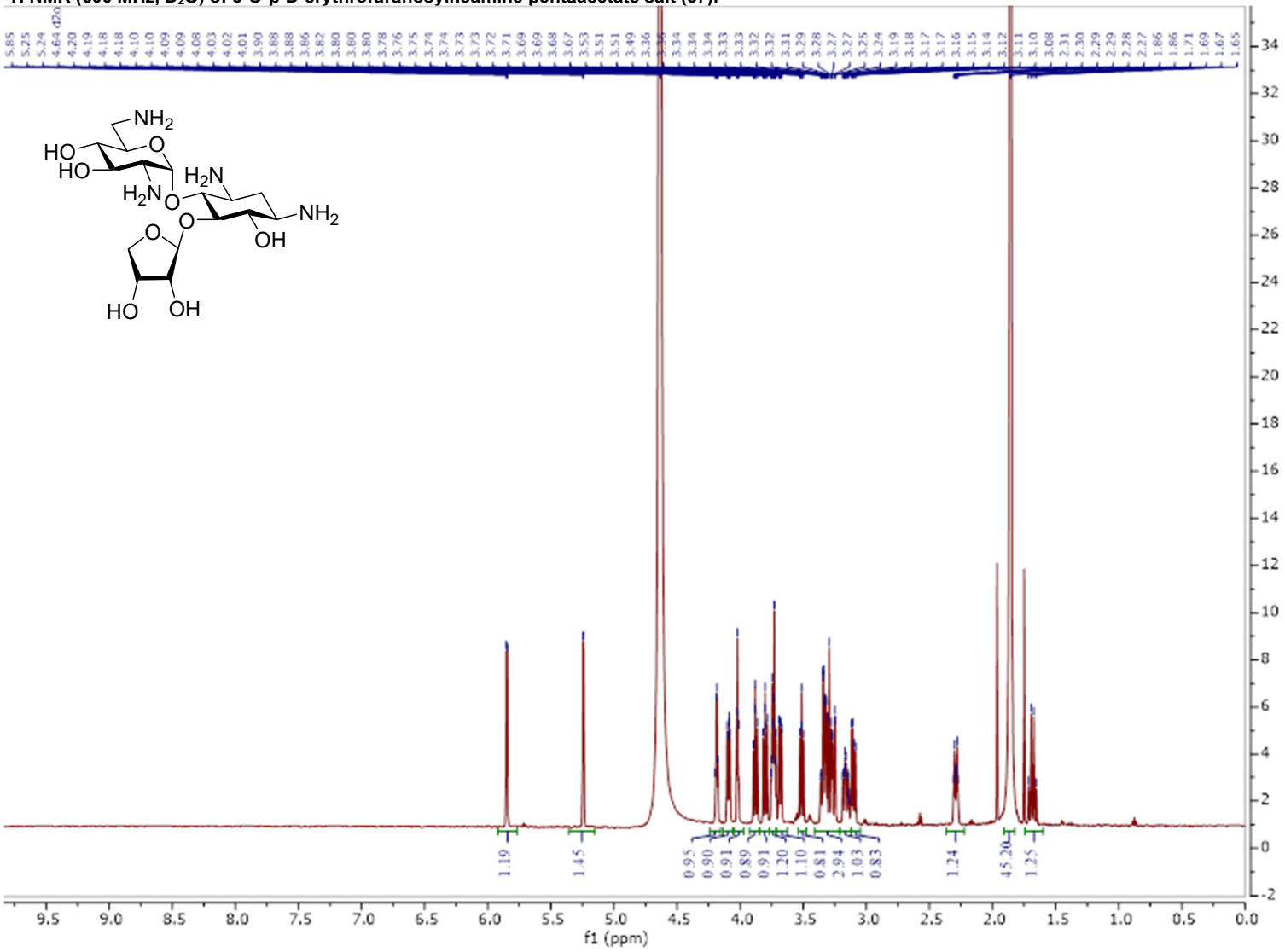


^{13}C NMR (100 MHz, $CDCl_3$) of 5-*O*-β-[2‴,3‴-Di-*O*-benzoyl-D-erythrofuranosyl]-1,3,2′,6′-tetraazido-6,3′,4′-tri-*O*-benzylneamine (56)



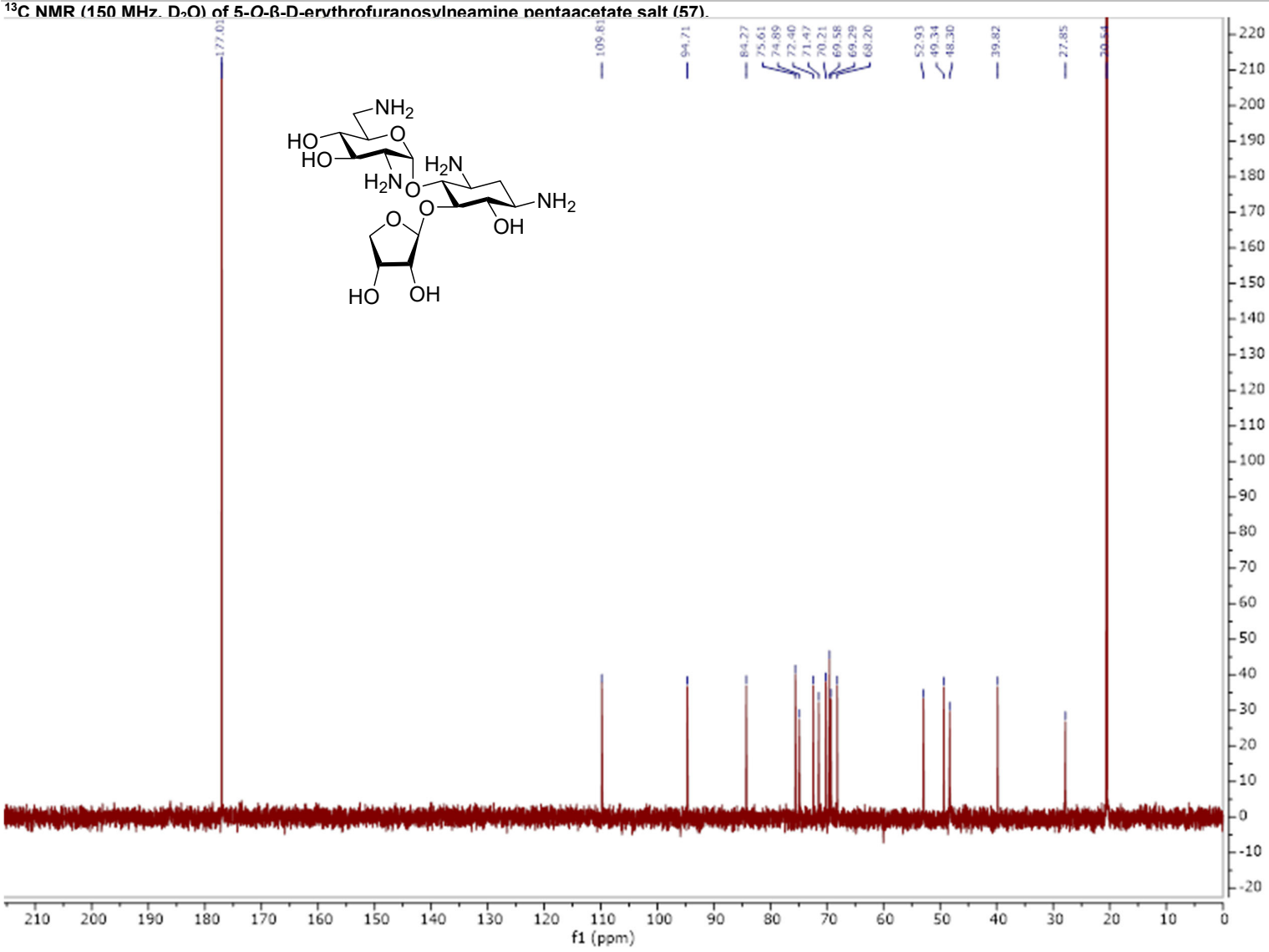
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 5-*O*-β-D-erythrofuranosylneamine pentaacetate salt (57).



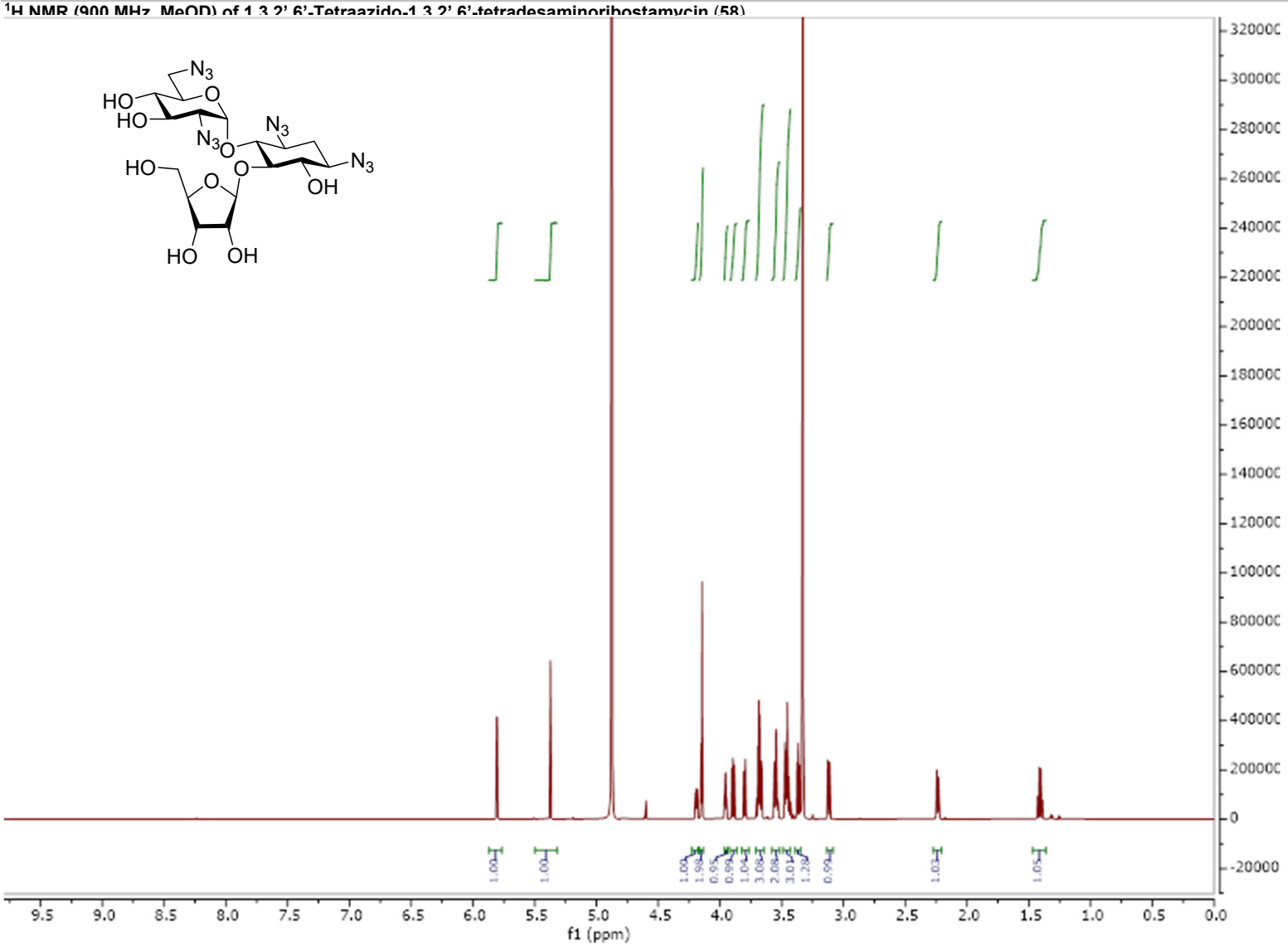
SUPPORTING INFORMATION

^{13}C NMR (150 MHz, D_2O) of 5-*O*-ß-D-erythrofuranosylneamine pentaacetate salt (57).

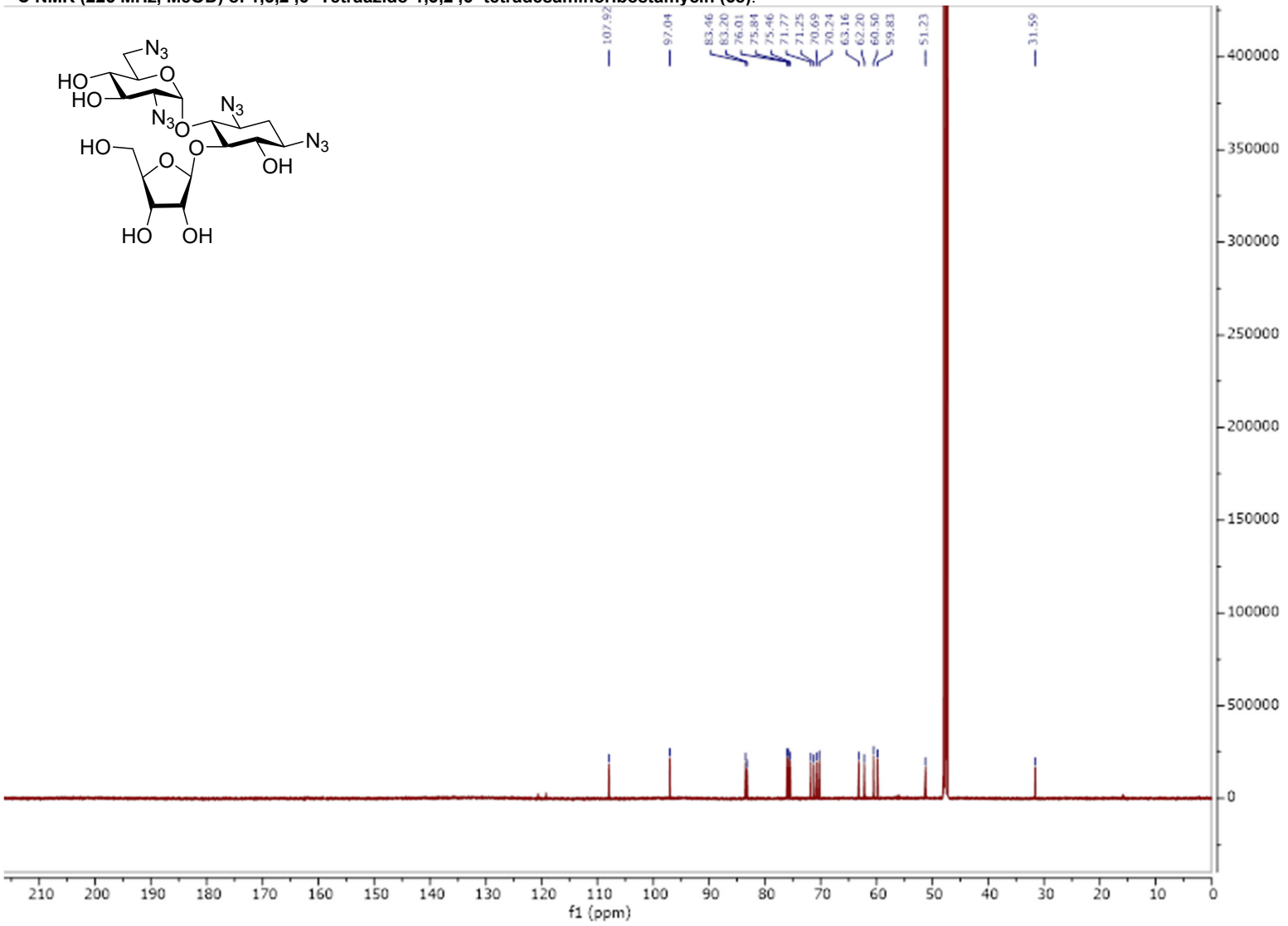


SUPPORTING INFORMATION

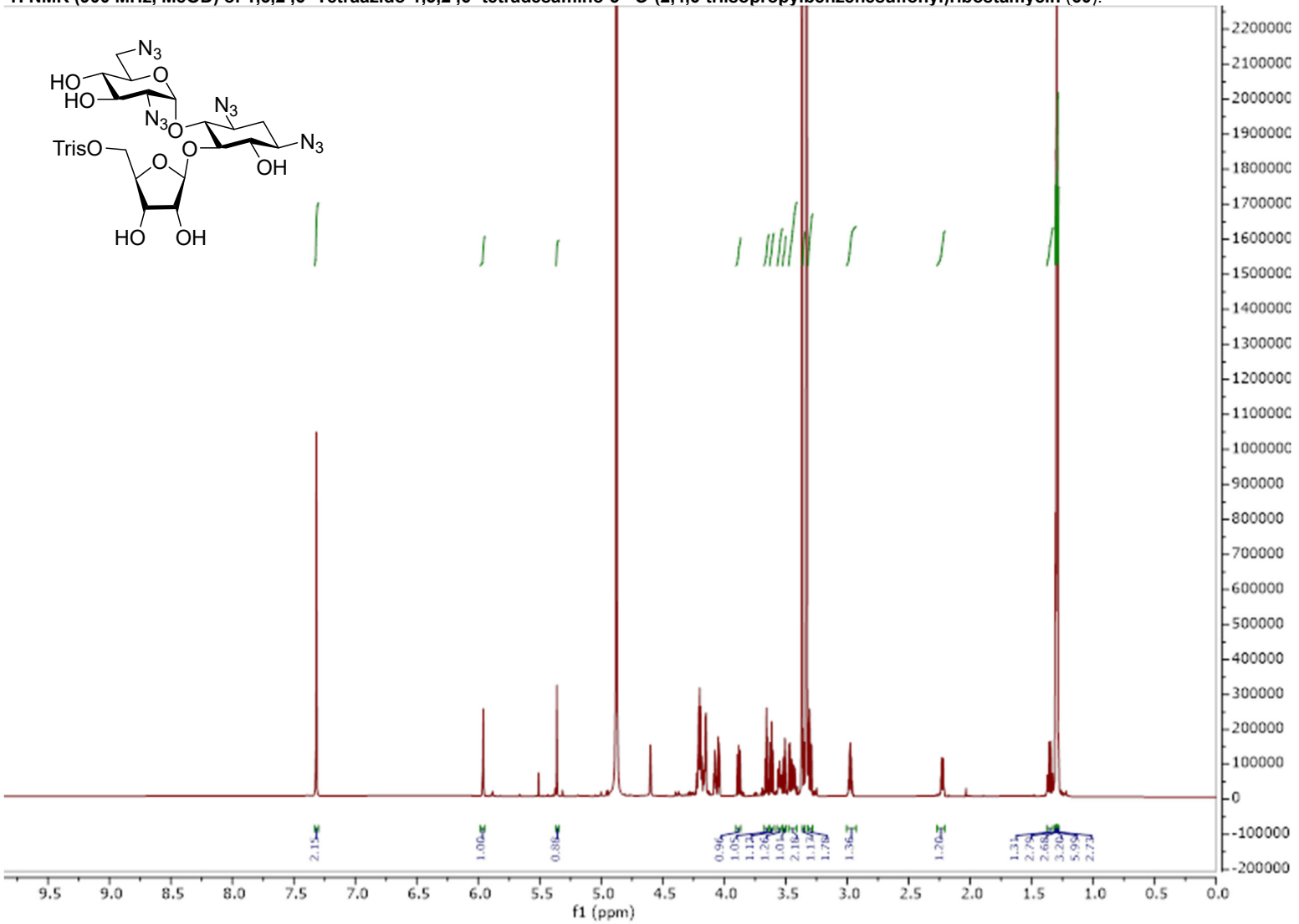
^{1}H NMR (900 MHz, MeOD) of 1,3,2',6'-Tetraazido-1,3,2',6'-tetradesaminoribostamycin (58)



^{13}C NMR (225 MHz, MeOD) of 1,3,2',6'-Tetraazido-1,3,2',6'-tetradesaminoribostamycin (58).

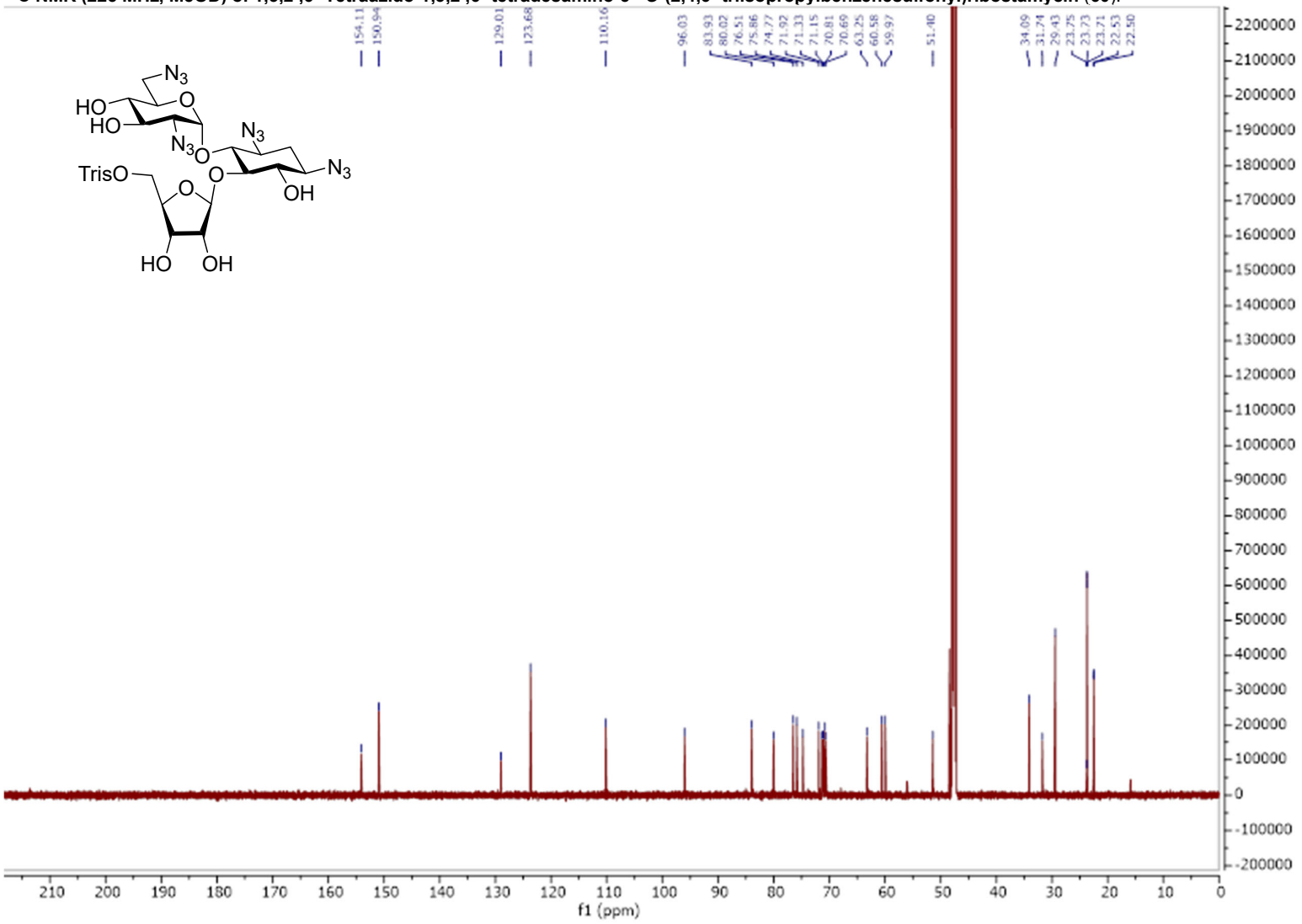


^{1}H NMR (900 MHz, MeOD) of 1,3,2',6'-Tetraazido-1,3,2',6'-tetradesamino-5''-*O*-(2,4,6-triisopropylbenzenesulfonyl)ribostamycin (60).

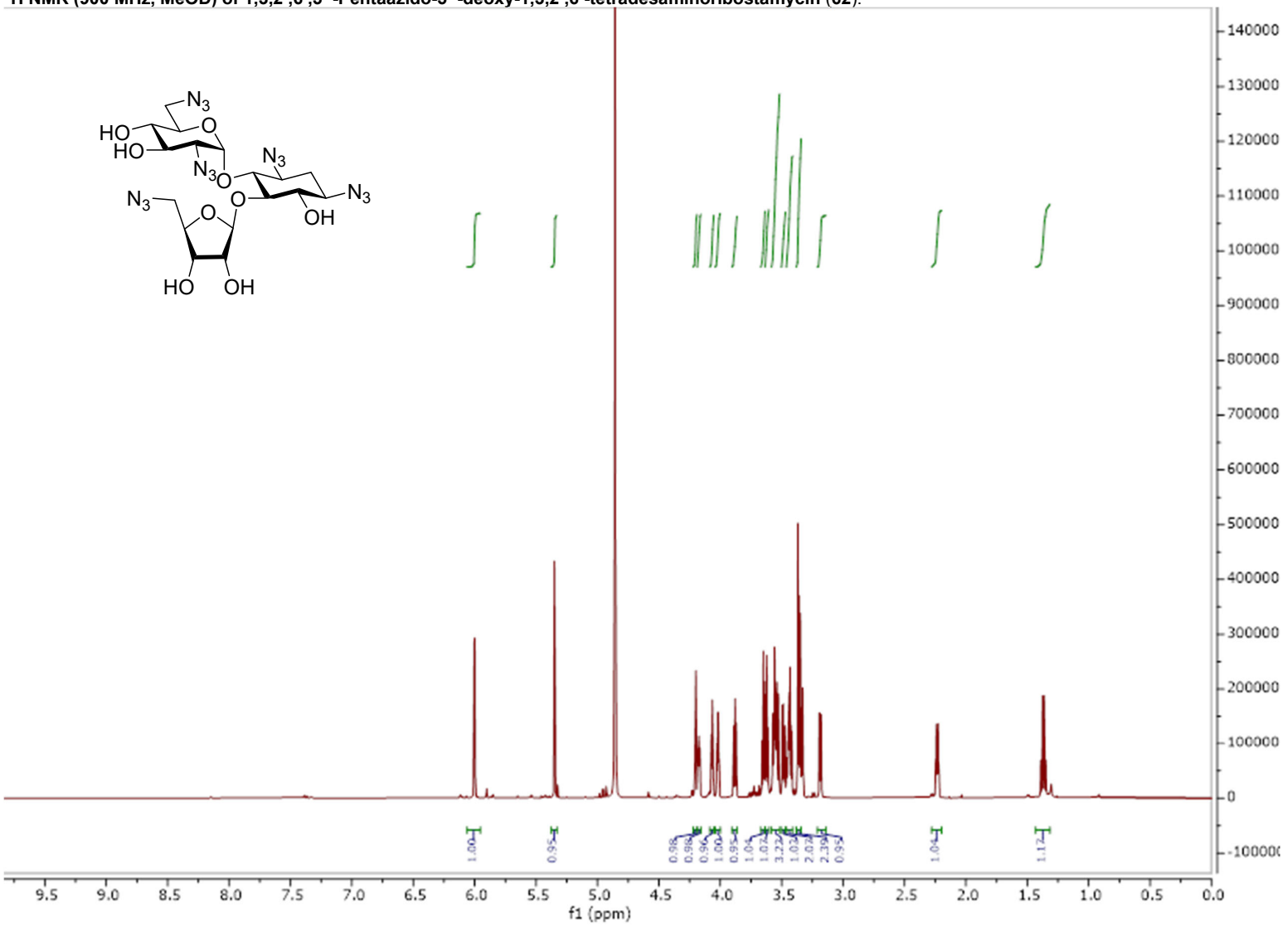


SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 1,3,2',6'-Tetraazido-1,3,2',6'-tetradesamino-5"-*O*-(2,4,6- triisopropylbenzenesulfonyl)ribostamycin (60).

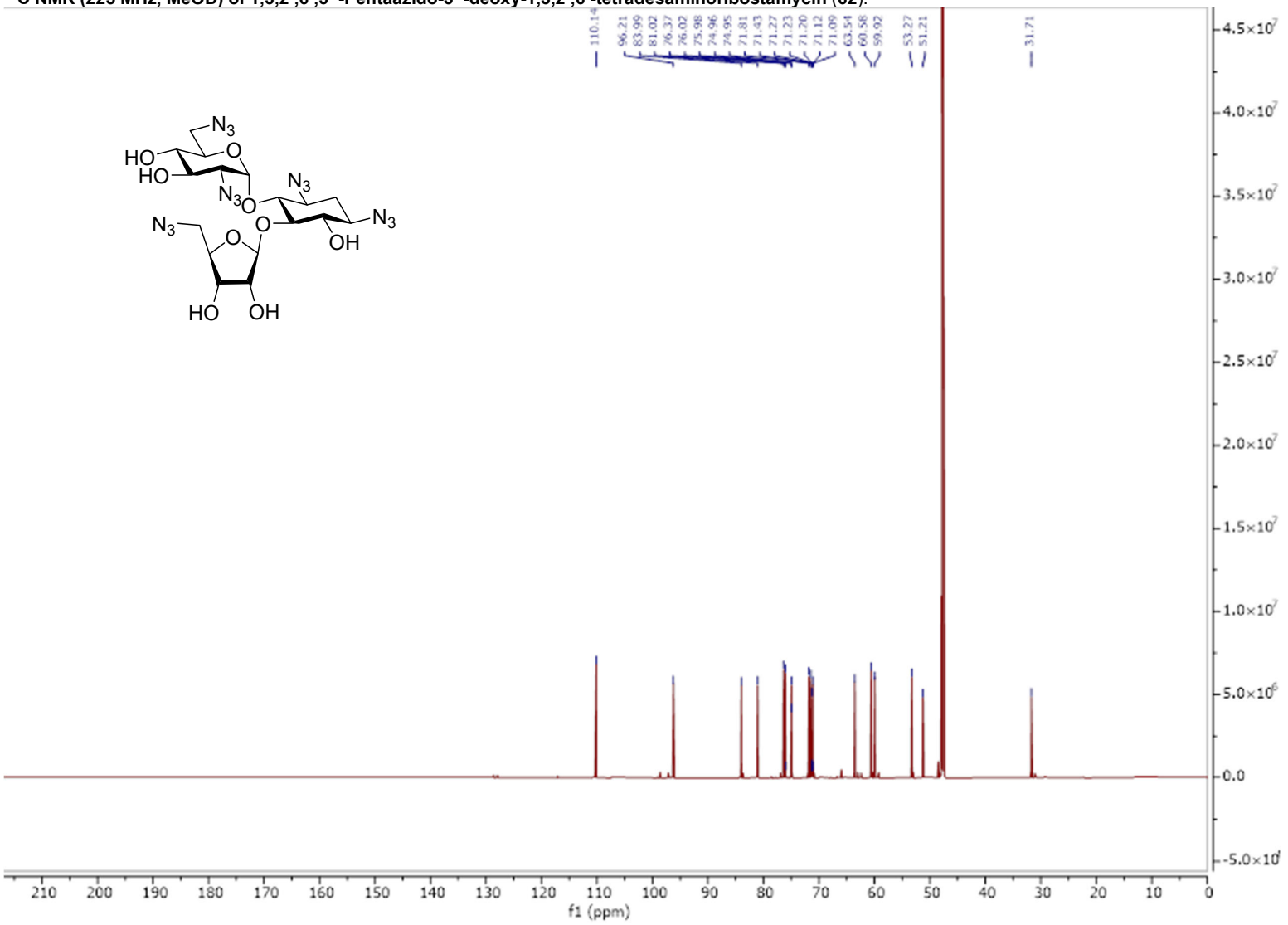


^{1}H NMR (900 MHz, MeOD) of 1,3,2',6',5''-Pentaazido-5''-deoxy-1,3,2',6'-tetradesaminoribostamycin (62).



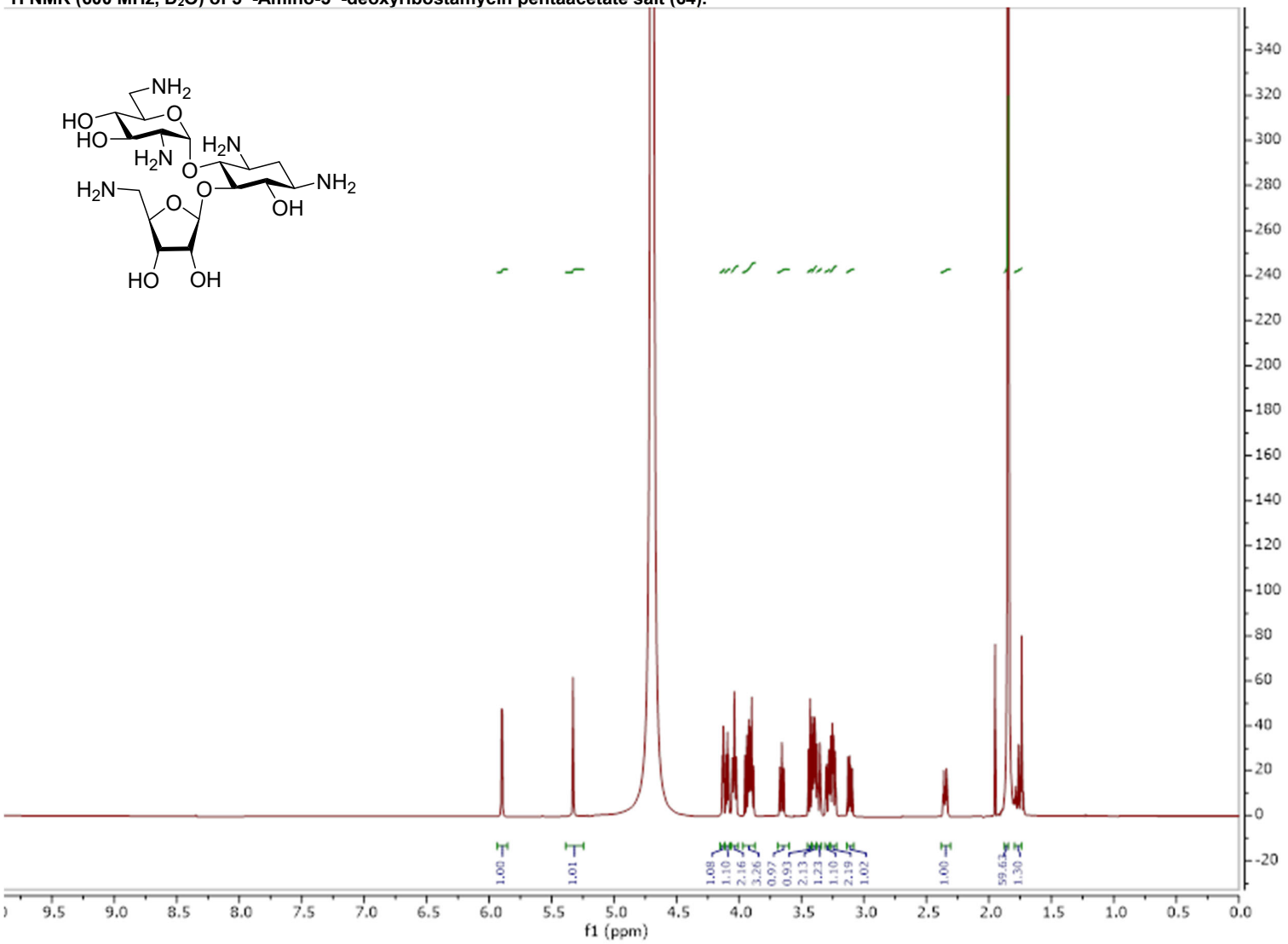
SUPPORTING INFORMATION

^{13}C NMR (225 MHz, MeOD) of 1,3,2',6',5''-Pentaazido-5''-deoxy-1,3,2',6'-tetradesaminoribostamycin (62).

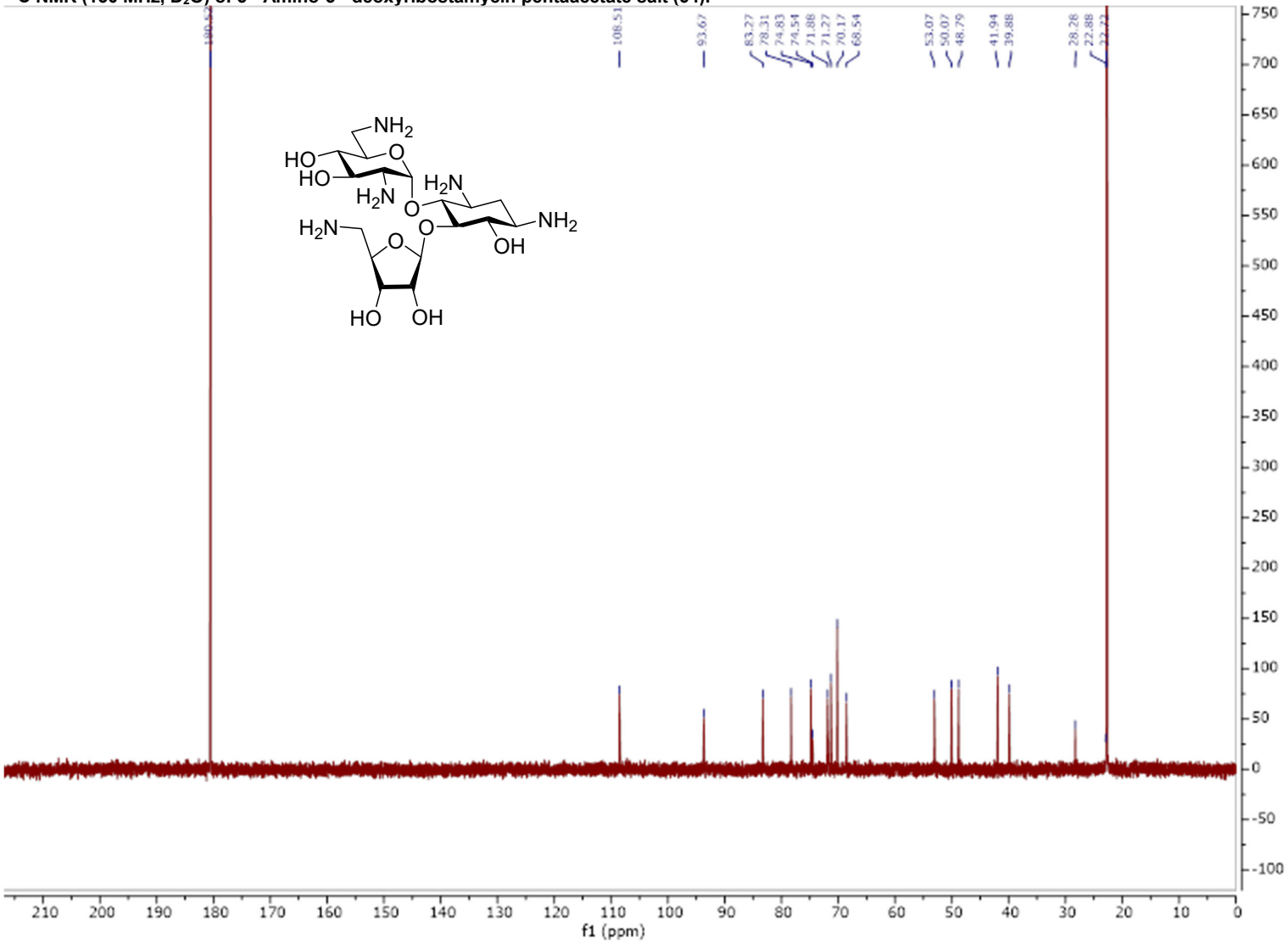


SUPPORTING INFORMATION

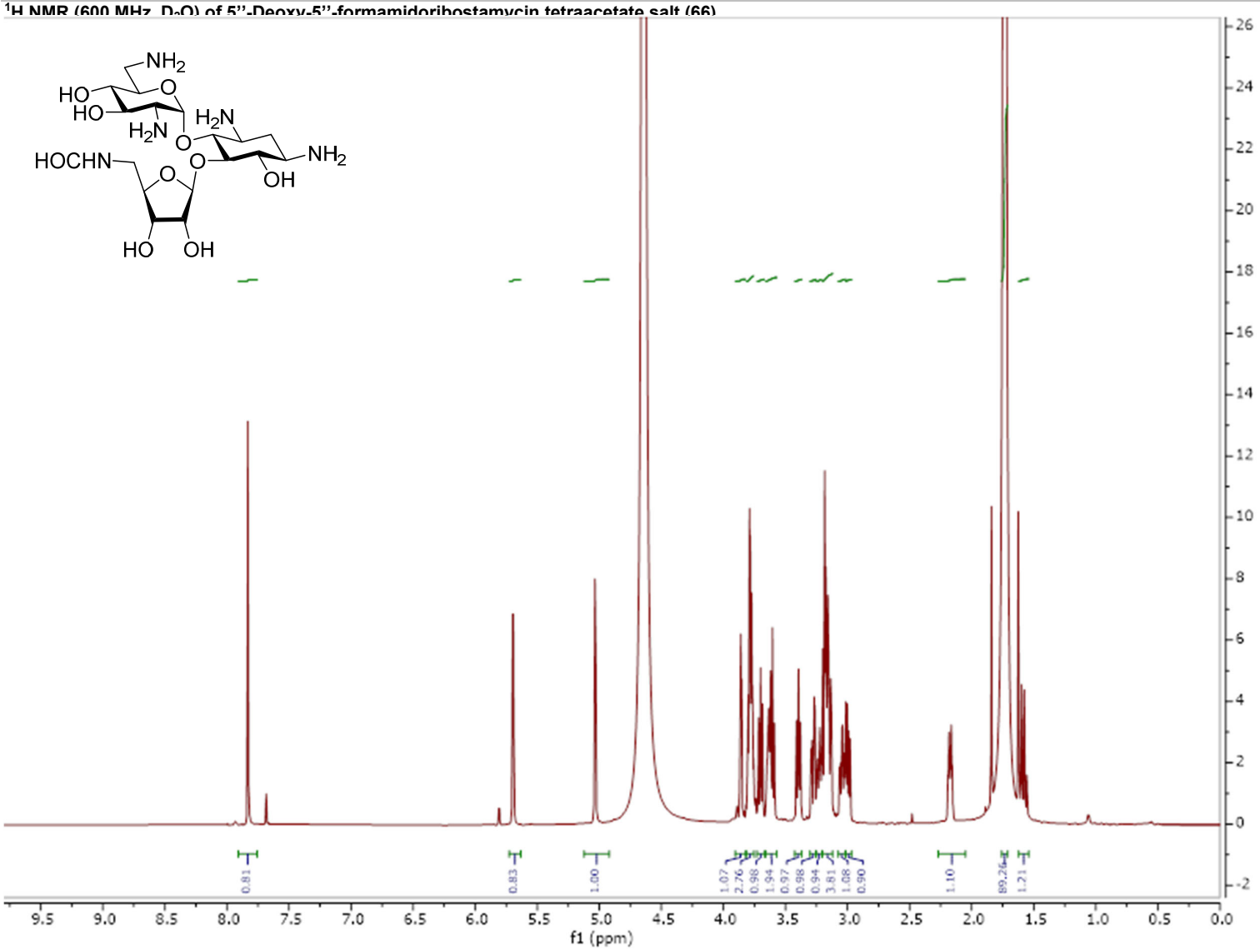
^{1}H NMR (600 MHz, D_2O) of 5''-Amino-5''-deoxyribostamycin pentaacetate salt (64).



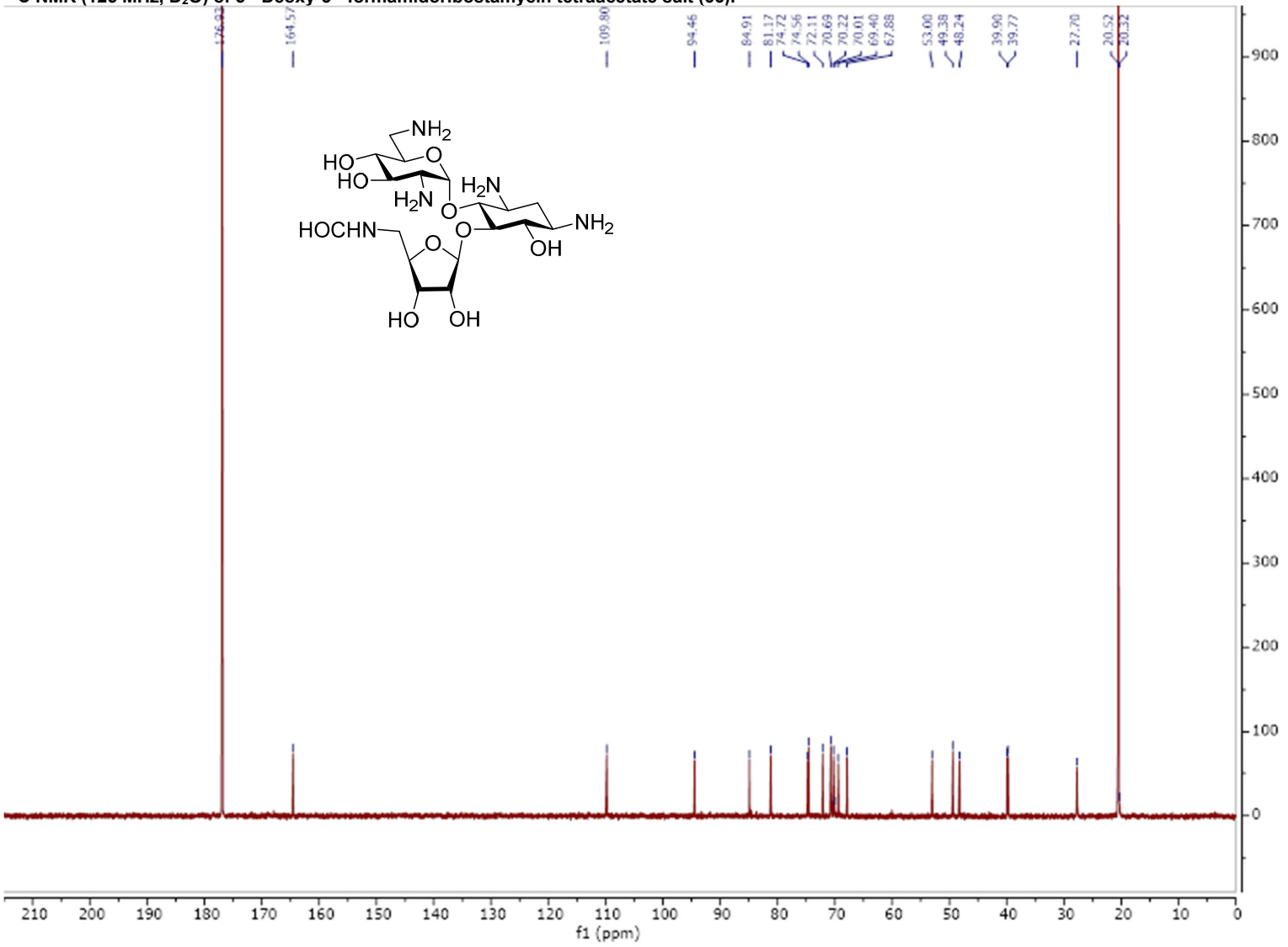
^{13}C NMR (150 MHz, D_2O) of 5''-Amino-5''-deoxyribostamycin pentaacetate salt (64).



^{1}H NMR (600 MHz, D_2O) of 5''-Deoxy-5''-formamidoribostamycin tetraacetate salt (66)

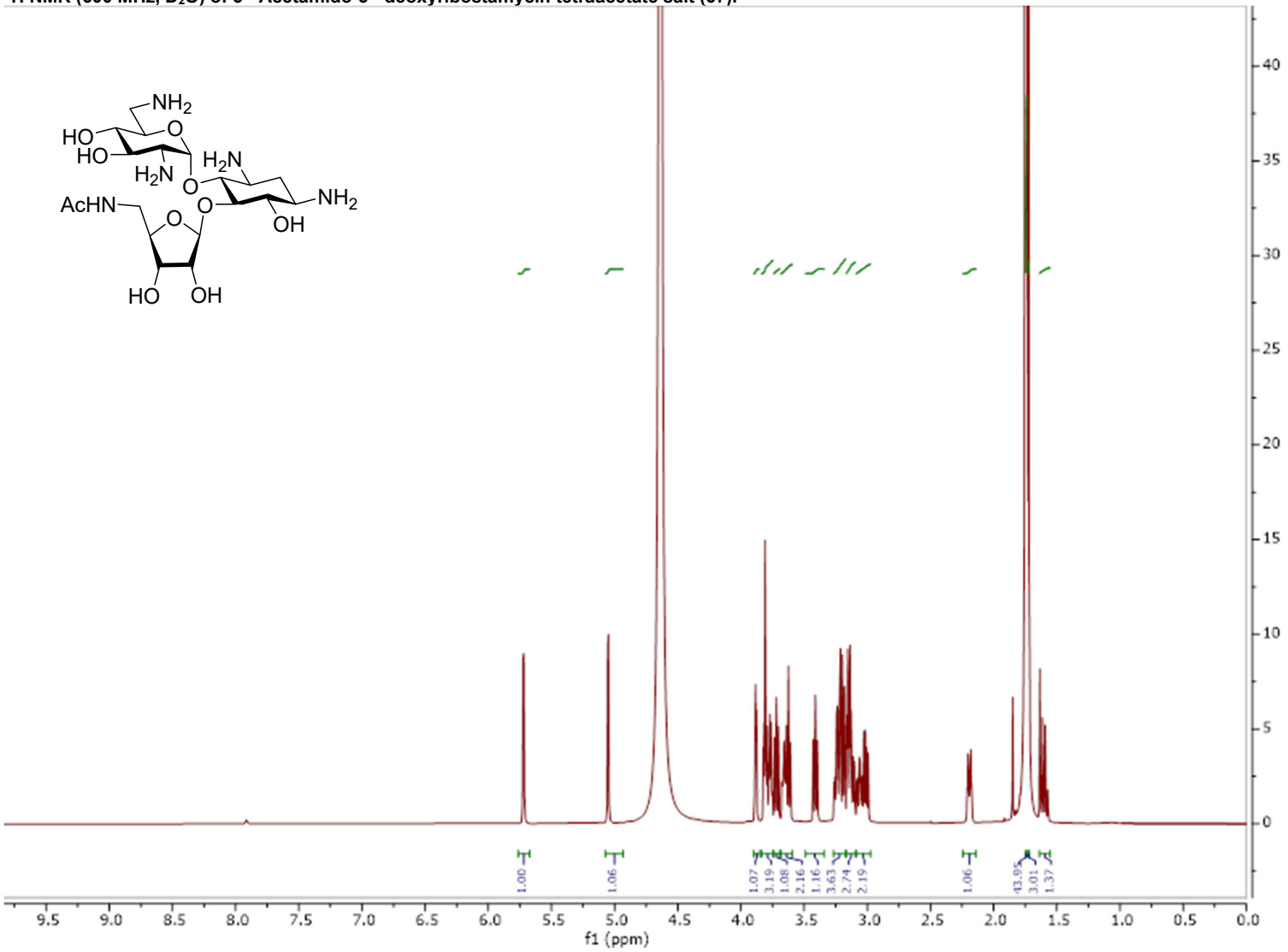


^{13}C NMR (125 MHz, D_2O) of 5''-Deoxy-5''-formamidoribostamycin tetraacetate salt (66).



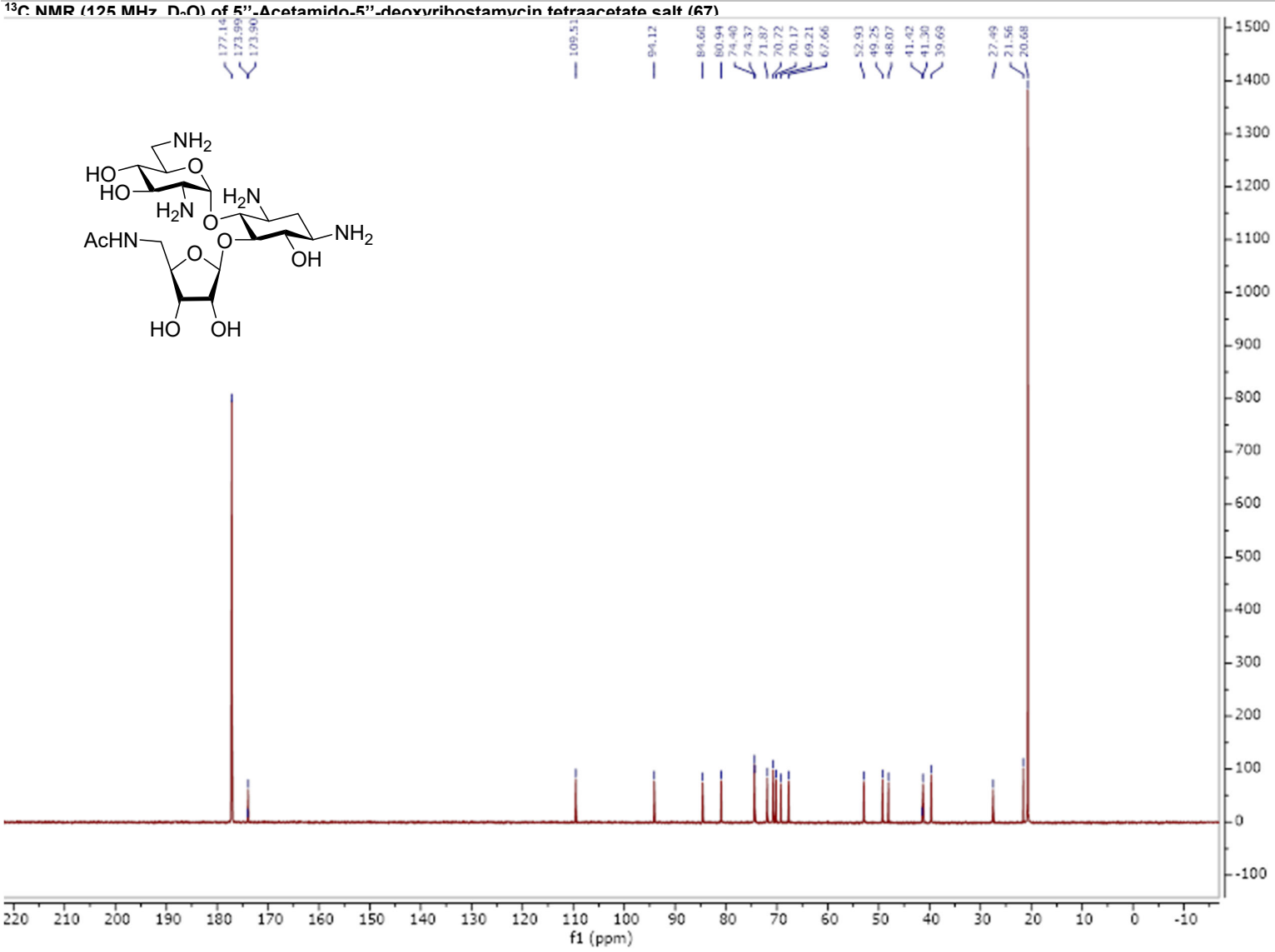
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 5''-Acetamido-5''-deoxyribostamycin tetraacetate salt (67).



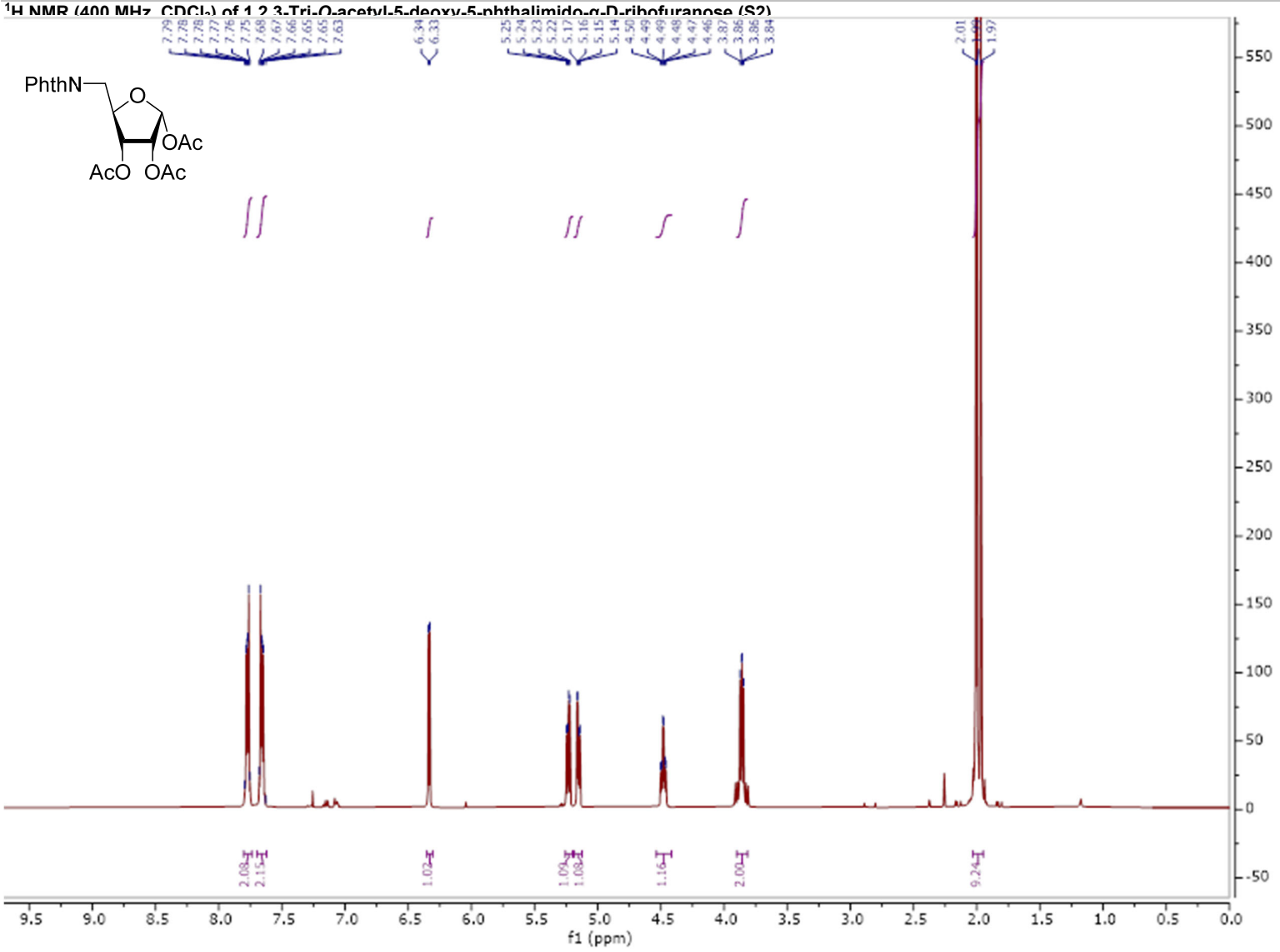
SUPPORTING INFORMATION

^{13}C NMR (125 MHz, D_2O) of 5''-Acetamido-5''-deoxyribostamycin tetraacetate salt (67)

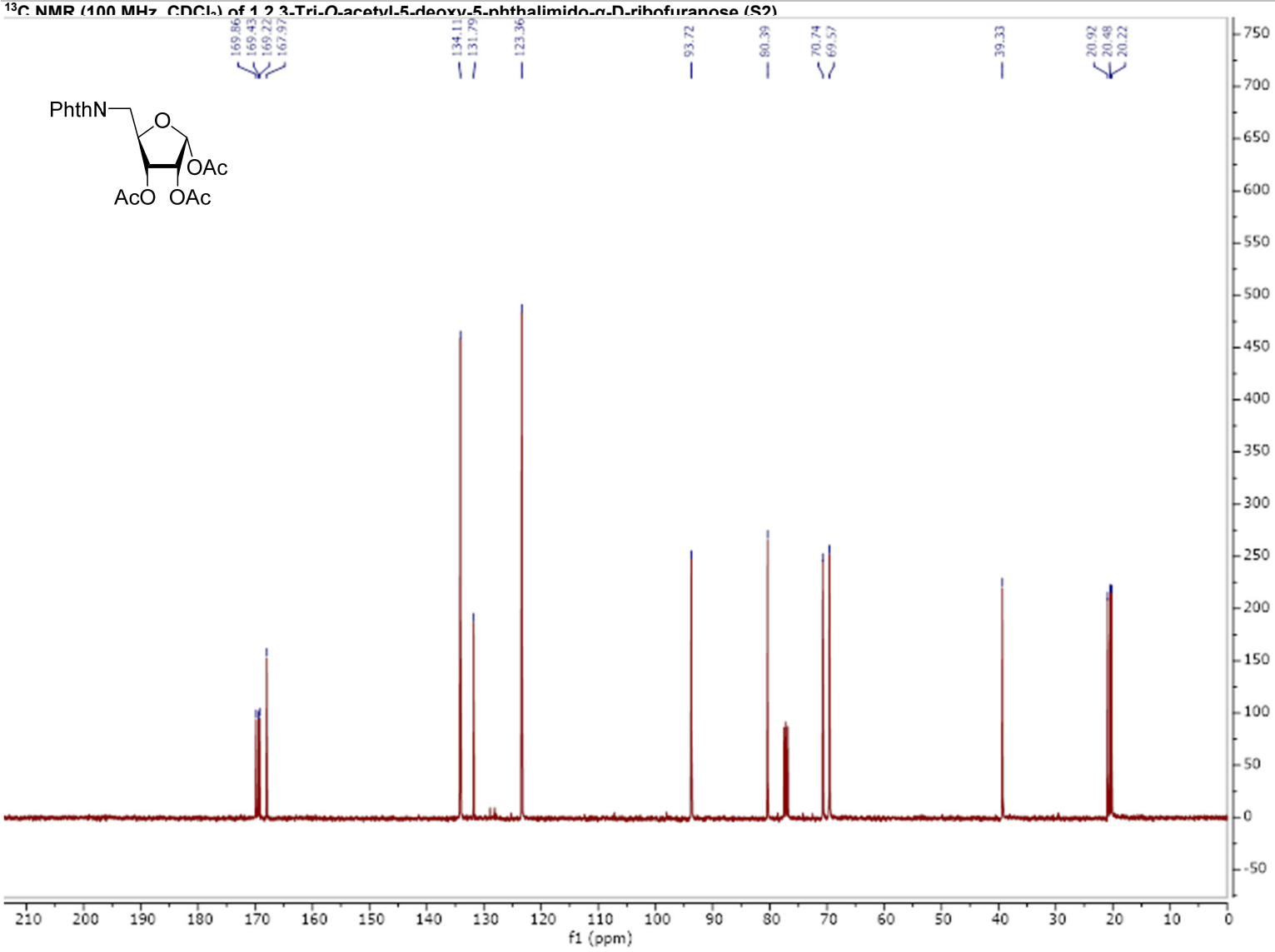


SUPPORTING INFORMATION

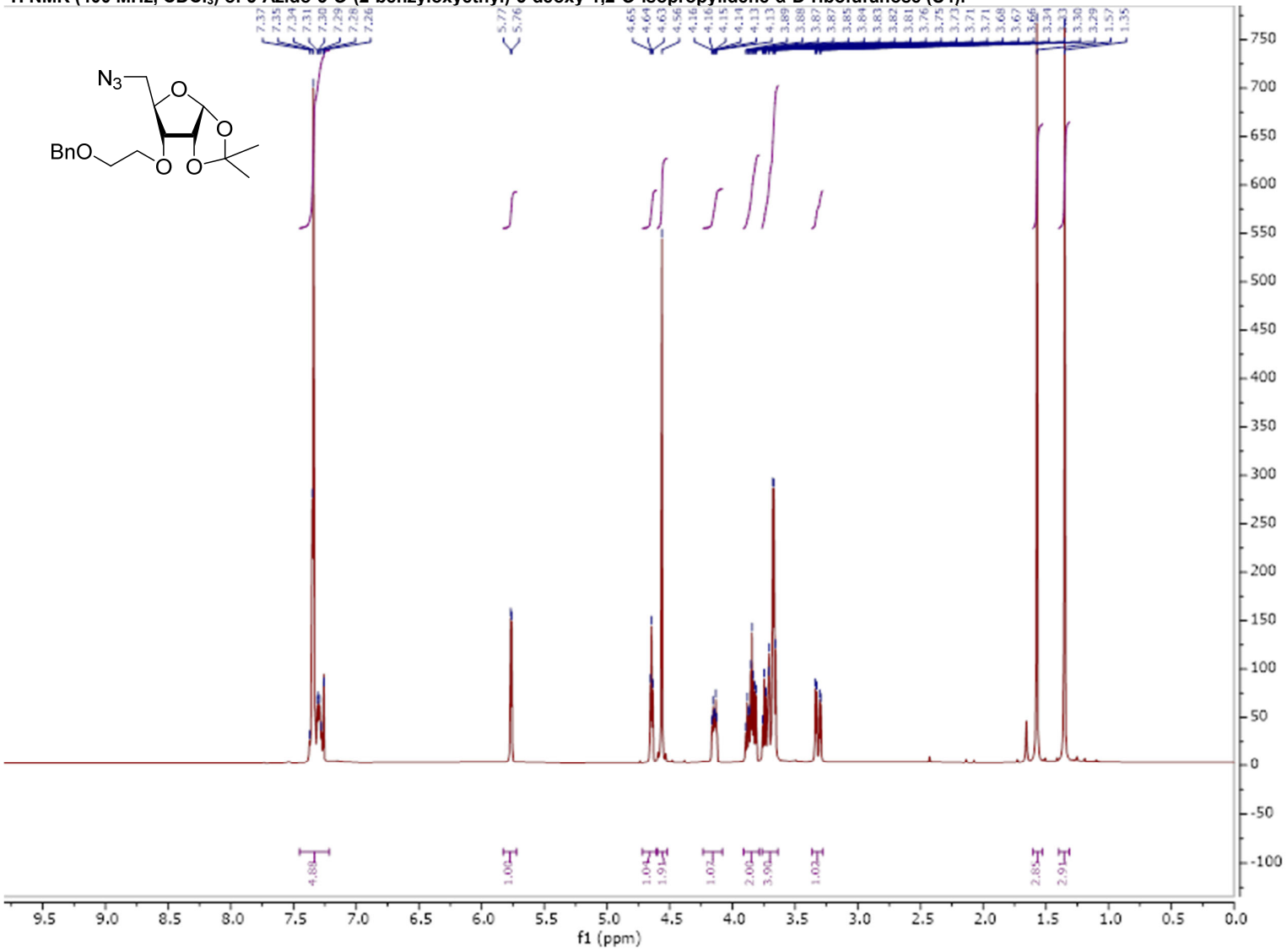
^{1}H NMR (400 MHz, $CDCl_3$) of 1,2,3-Tri-O-acetyl-5-deoxy-5-phthalimido-α-D-ribofuranose (S2)



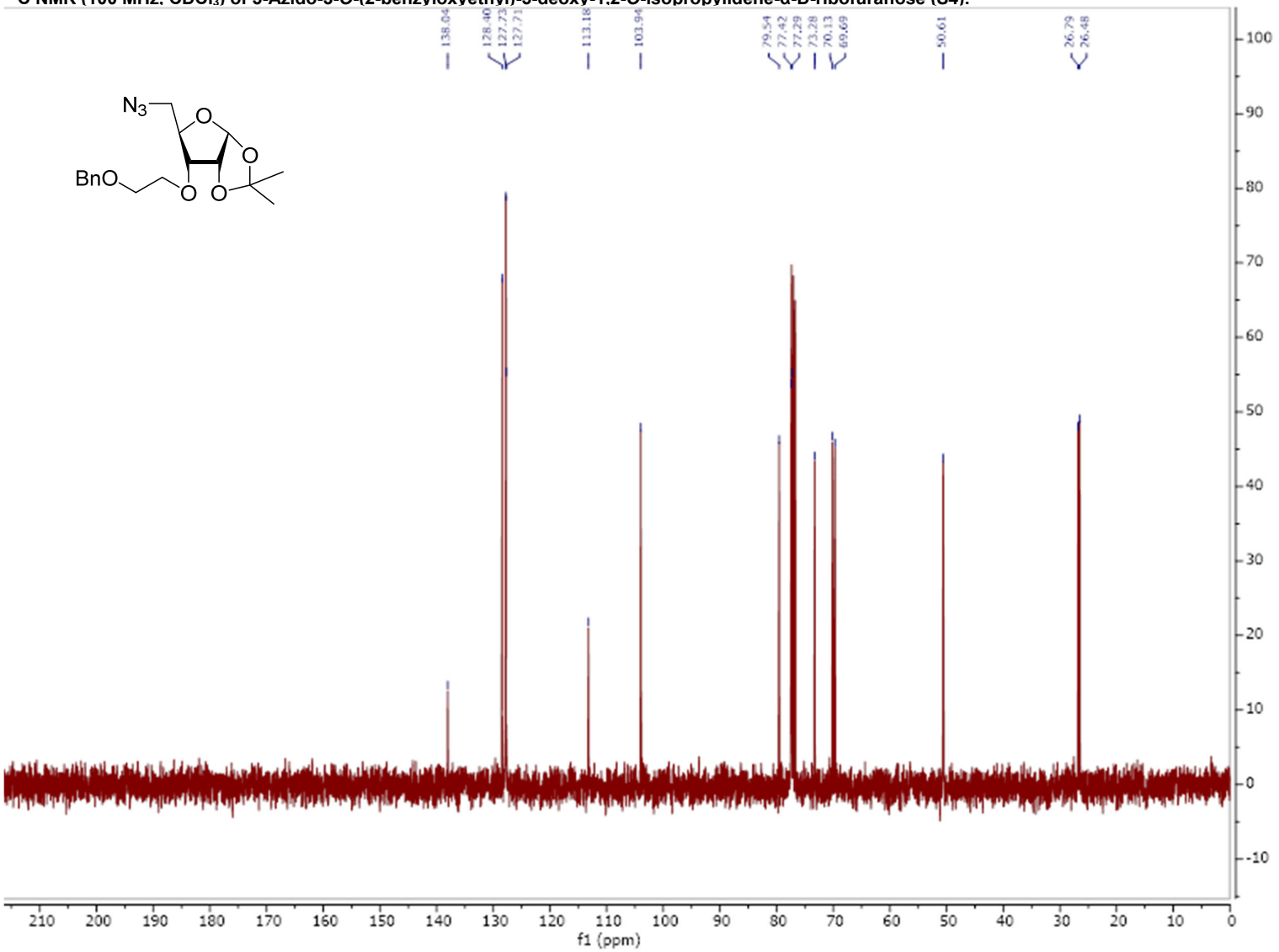
^{13}C NMR (100 MHz, CDCl$_3$) of 1,2,3-Tri-*O*-acetyl-5-deoxy-5-phthalimido-α-D-ribofuranose (S2)



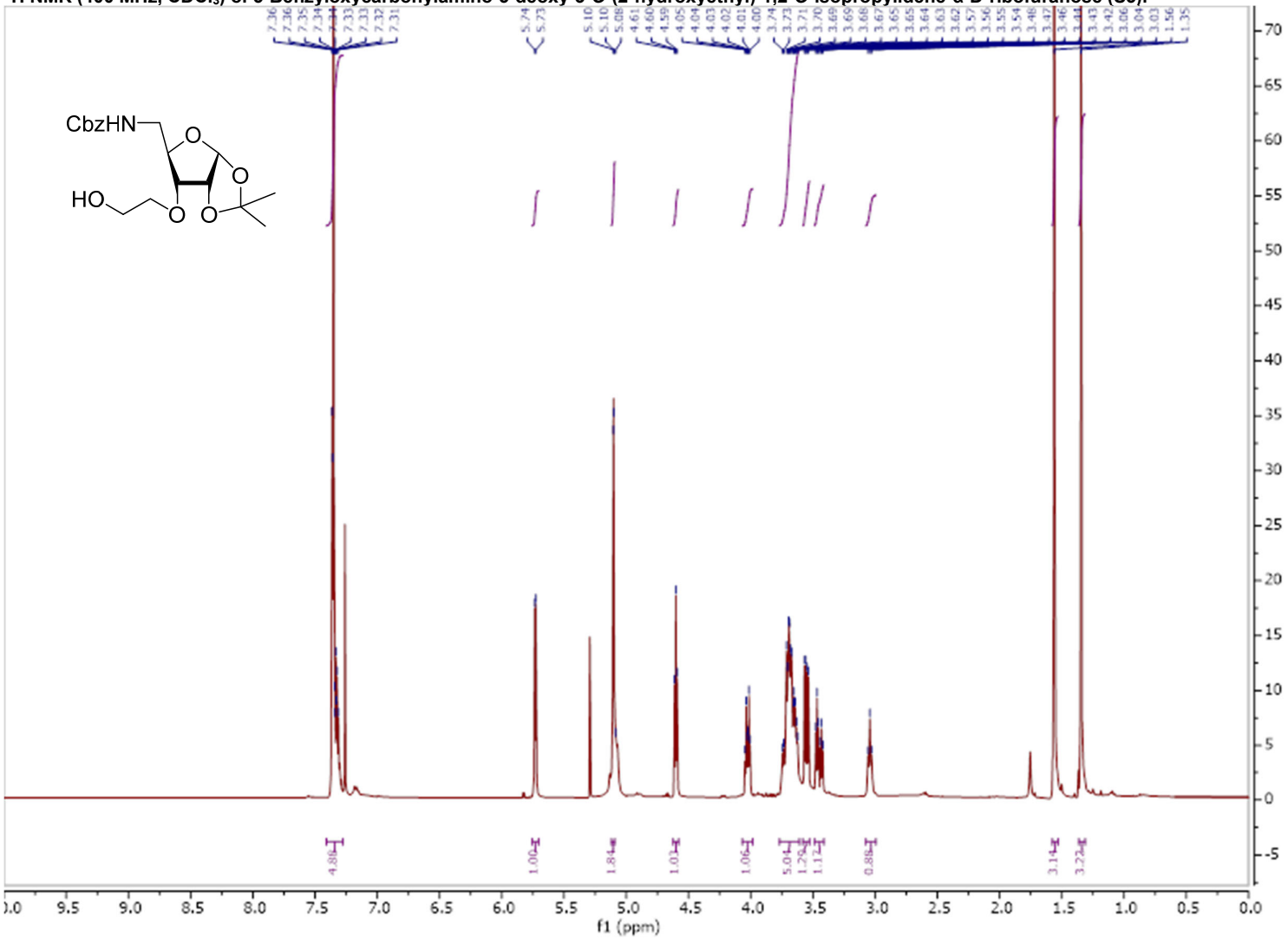
^{1}H NMR (400 MHz, $CDCl_3$) of 5-Azido-3-*O*-(2-benzyloxyethyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S4).



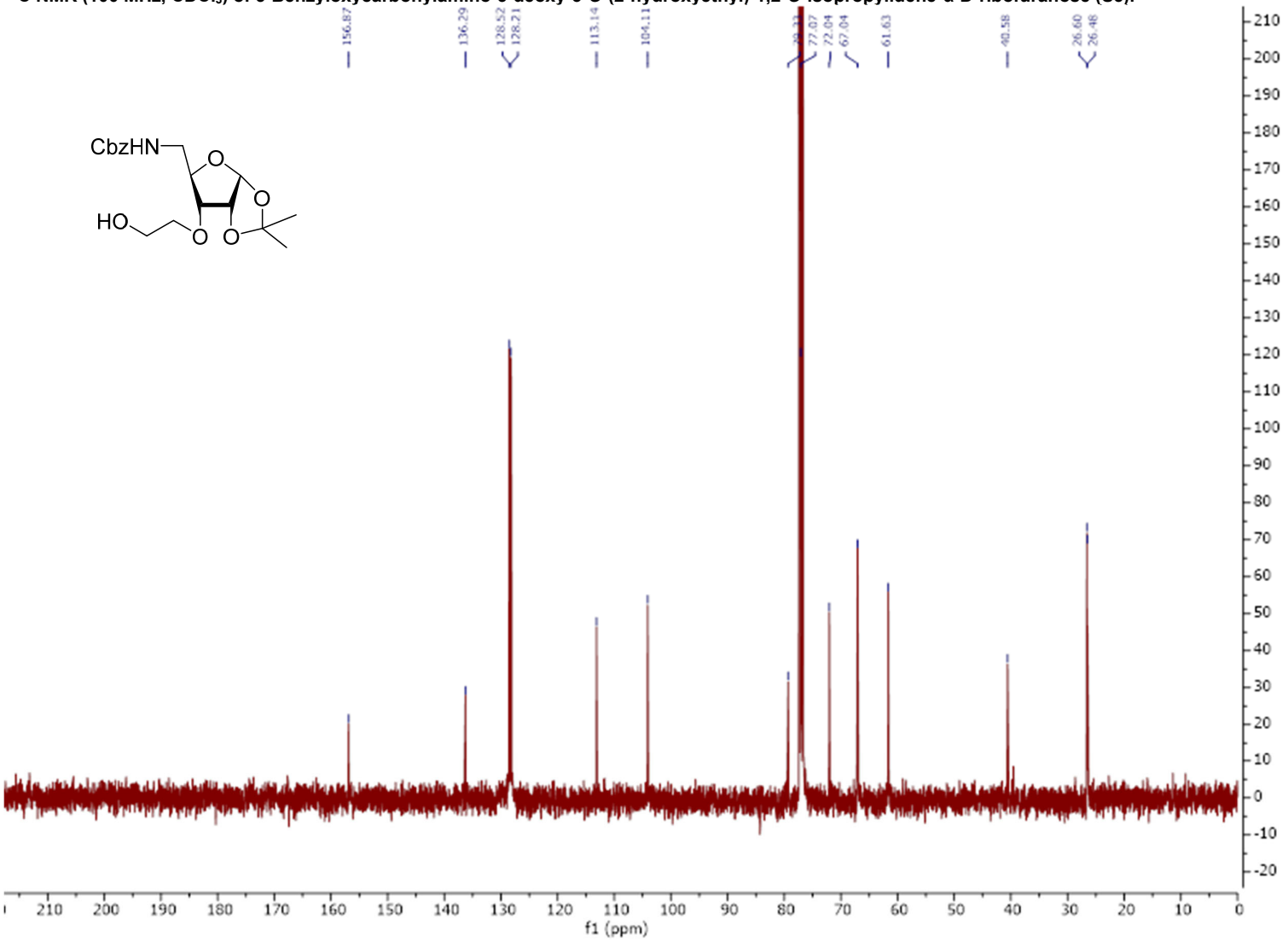
^{13}C NMR (100 MHz, $CDCl_3$) of 5-Azido-3-*O*-(2-benzyloxyethyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S4).



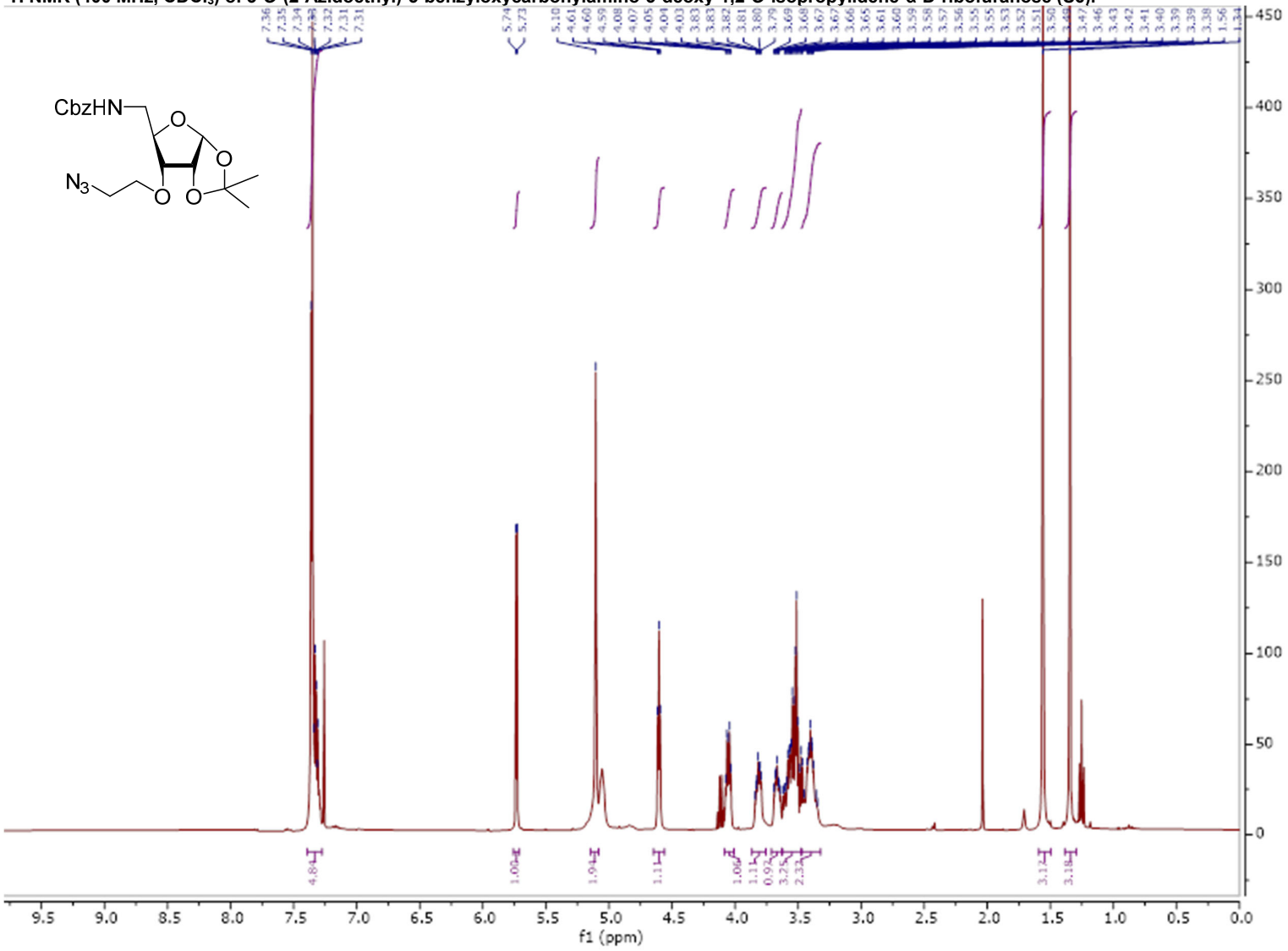
^{1}H NMR (400 MHz, $CDCl_3$) of 5-Benzyloxycarbonylamino-5-deoxy-3-*O*-(2-hydroxyethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (S5).



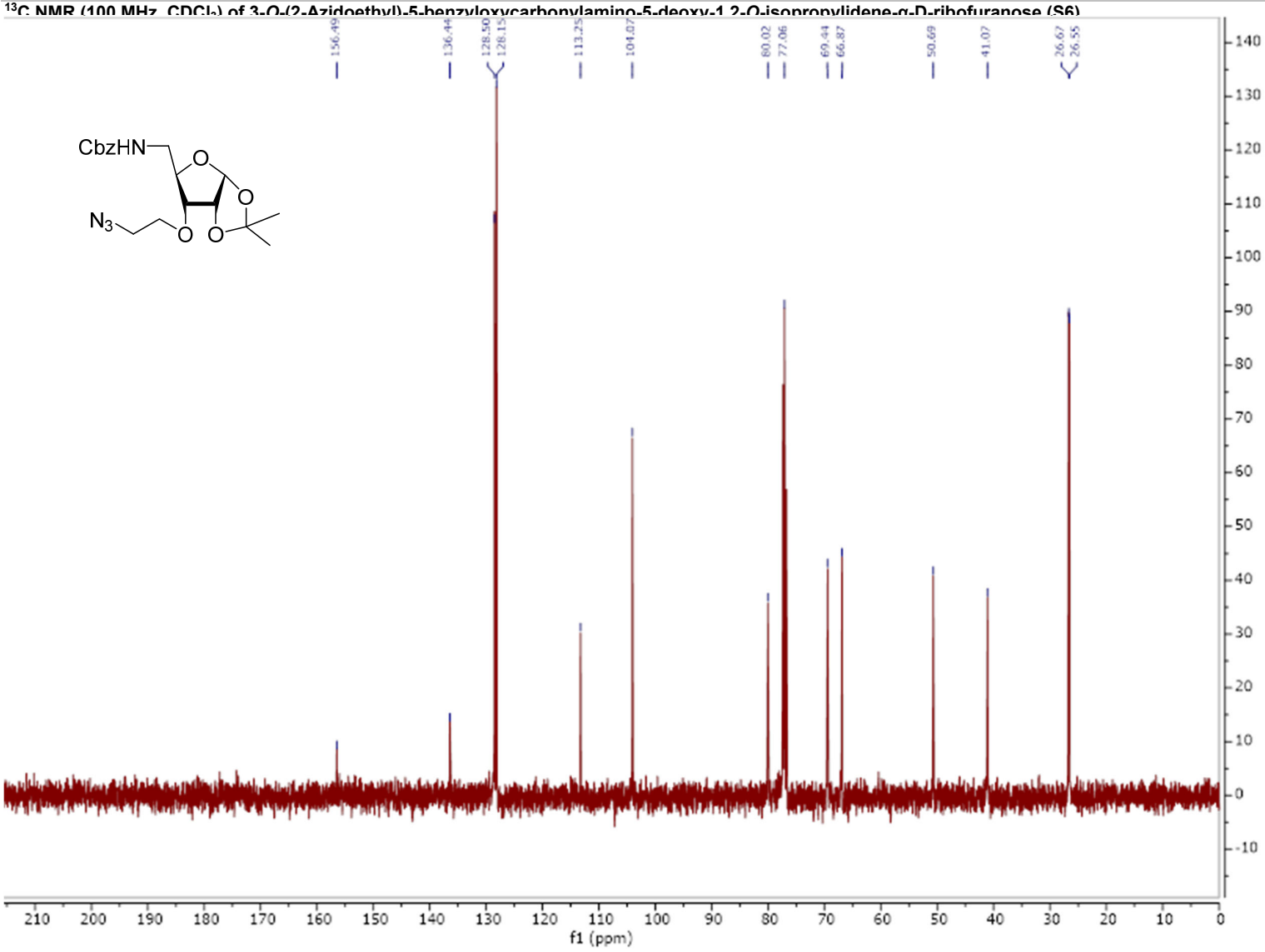
^{13}C NMR (100 MHz, $CDCl_3$) of 5-Benzyloxycarbonylamino-5-deoxy-3-*O*-(2-hydroxyethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (S5).



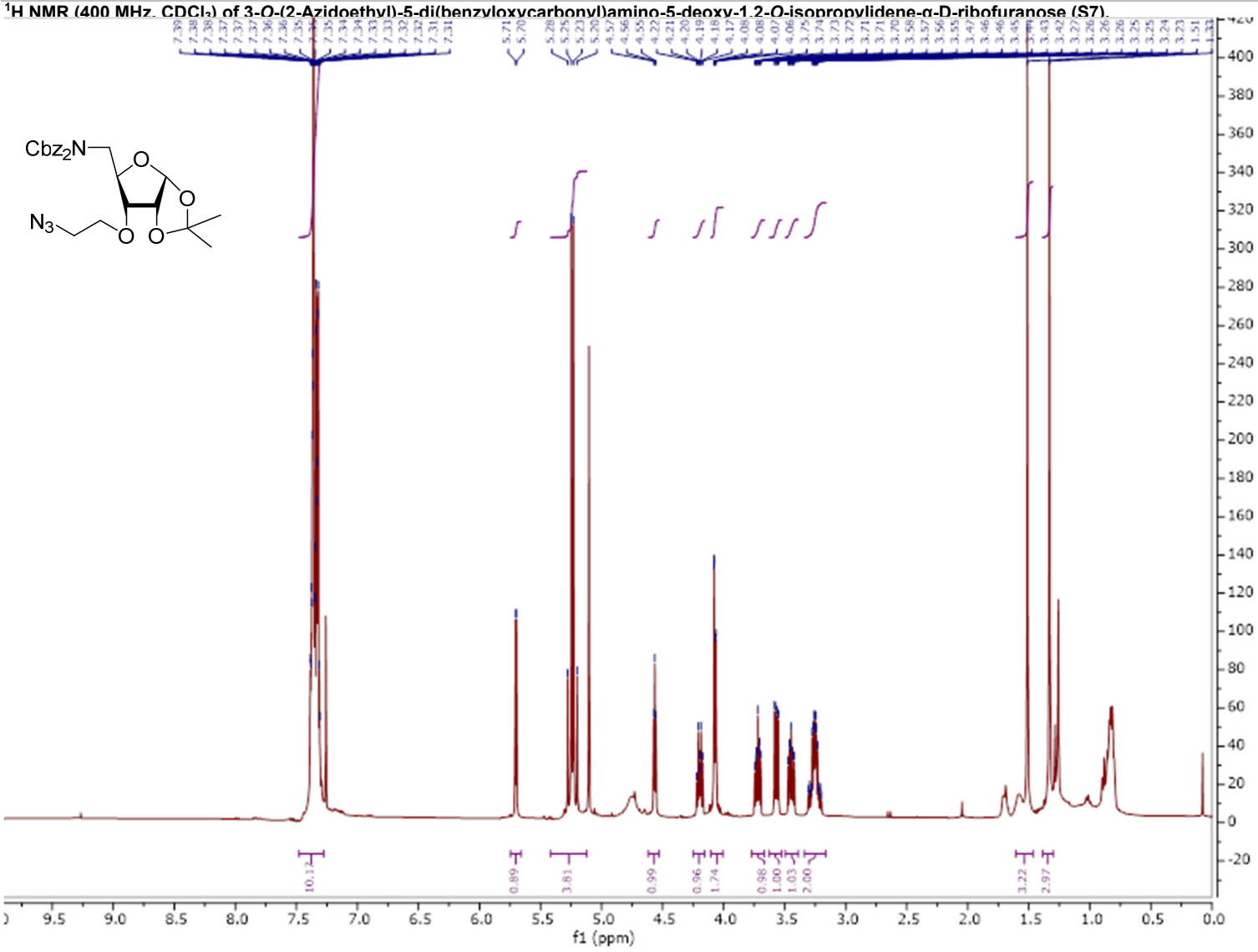
^{1}H NMR (400 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-benzyloxycarbonylamino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S6).



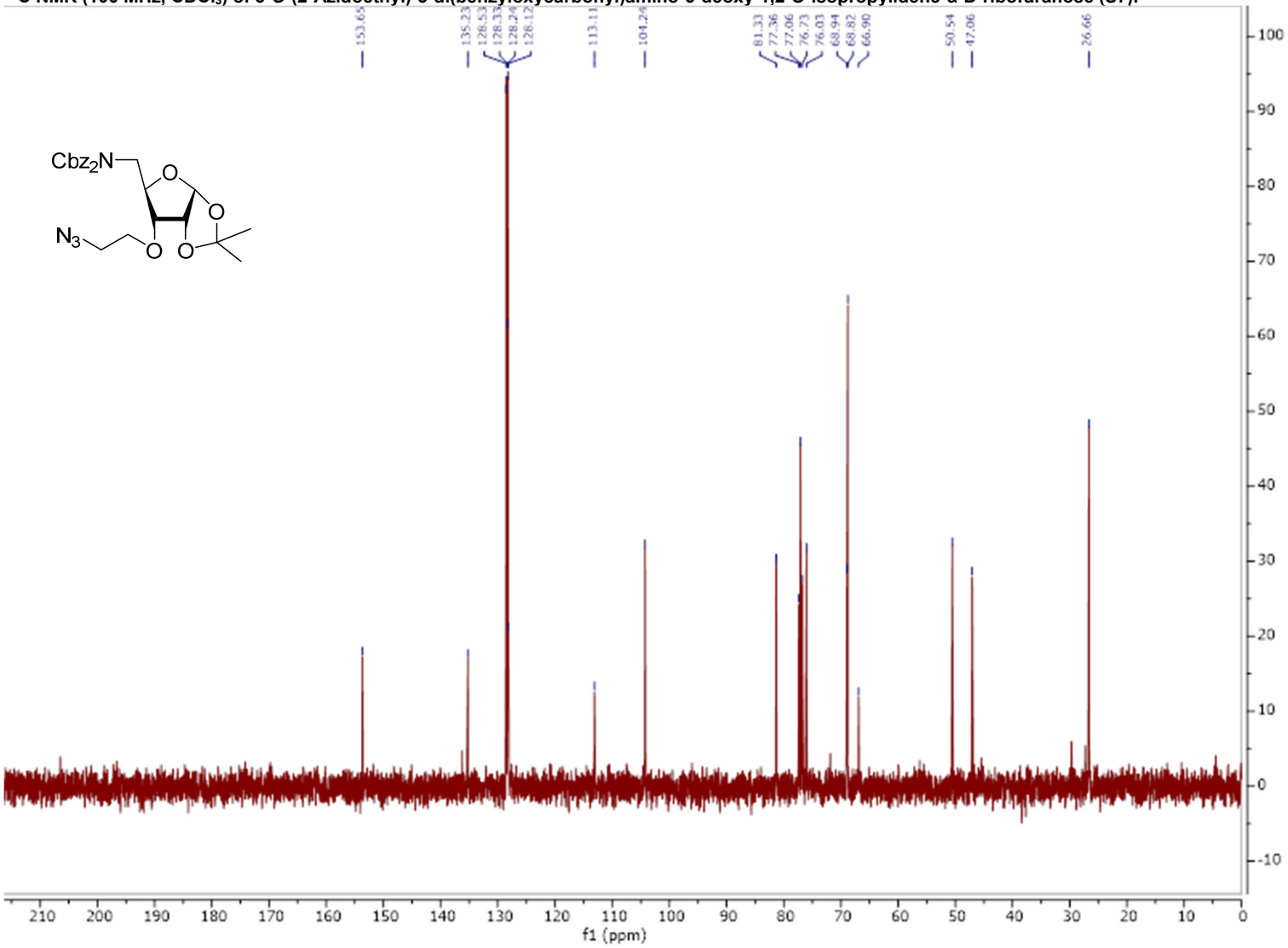
^{13}C NMR (100 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-benzyloxycarbonylamino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S6)



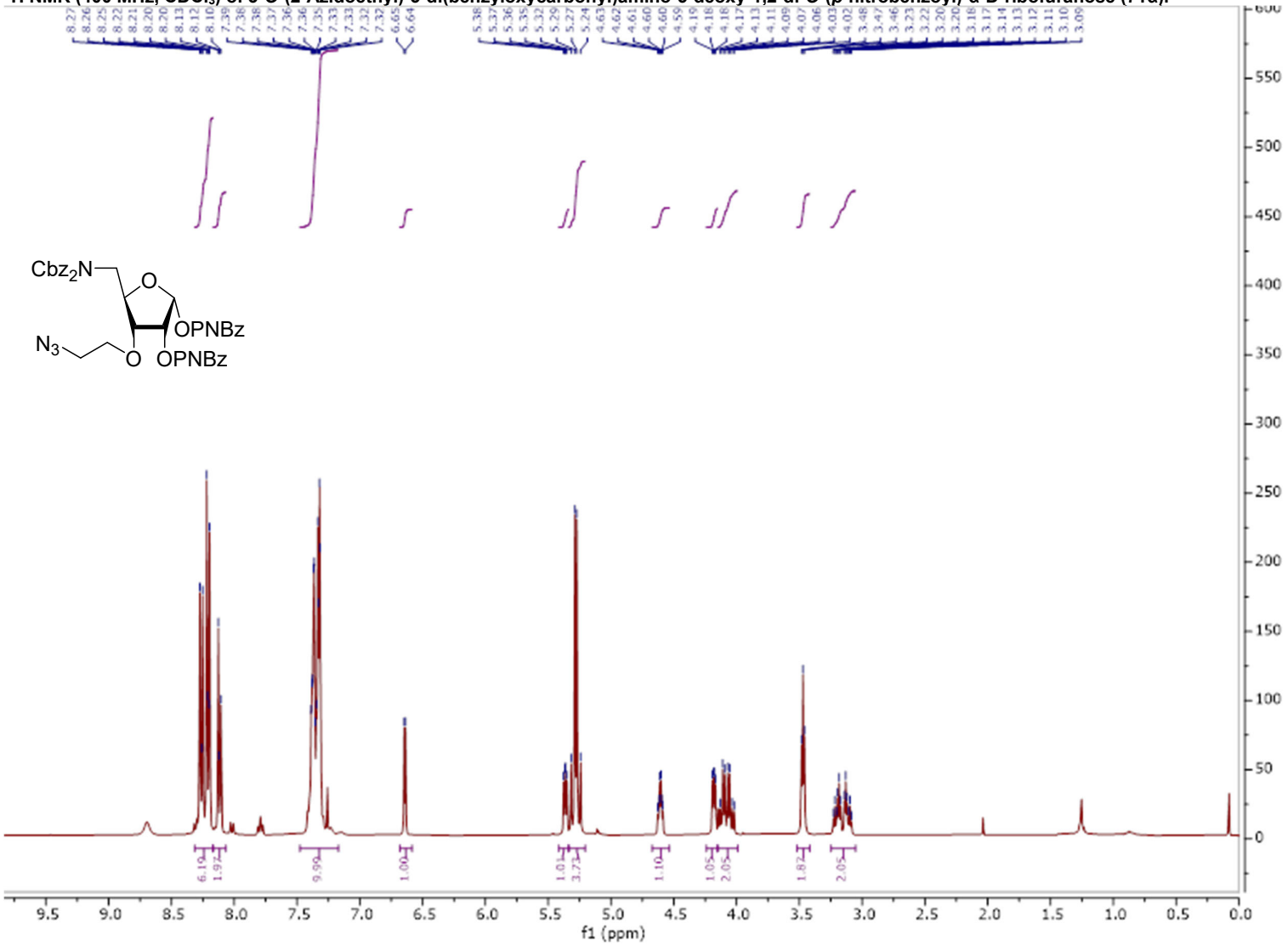
^{1}H NMR (400 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S7).



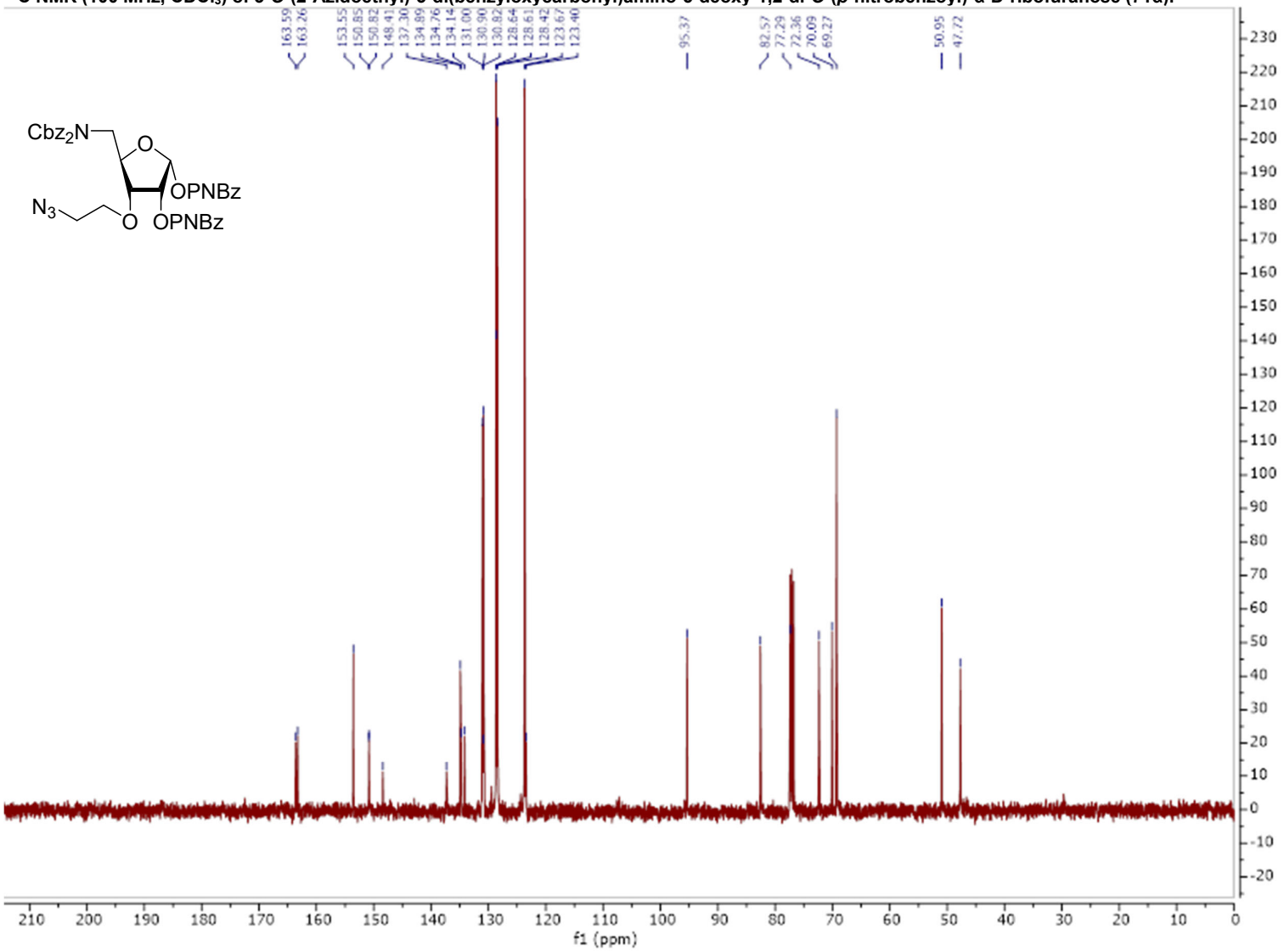
^{13}C NMR (100 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (S7).



^{1}H NMR (400 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-α-D-ribofuranose (71α).

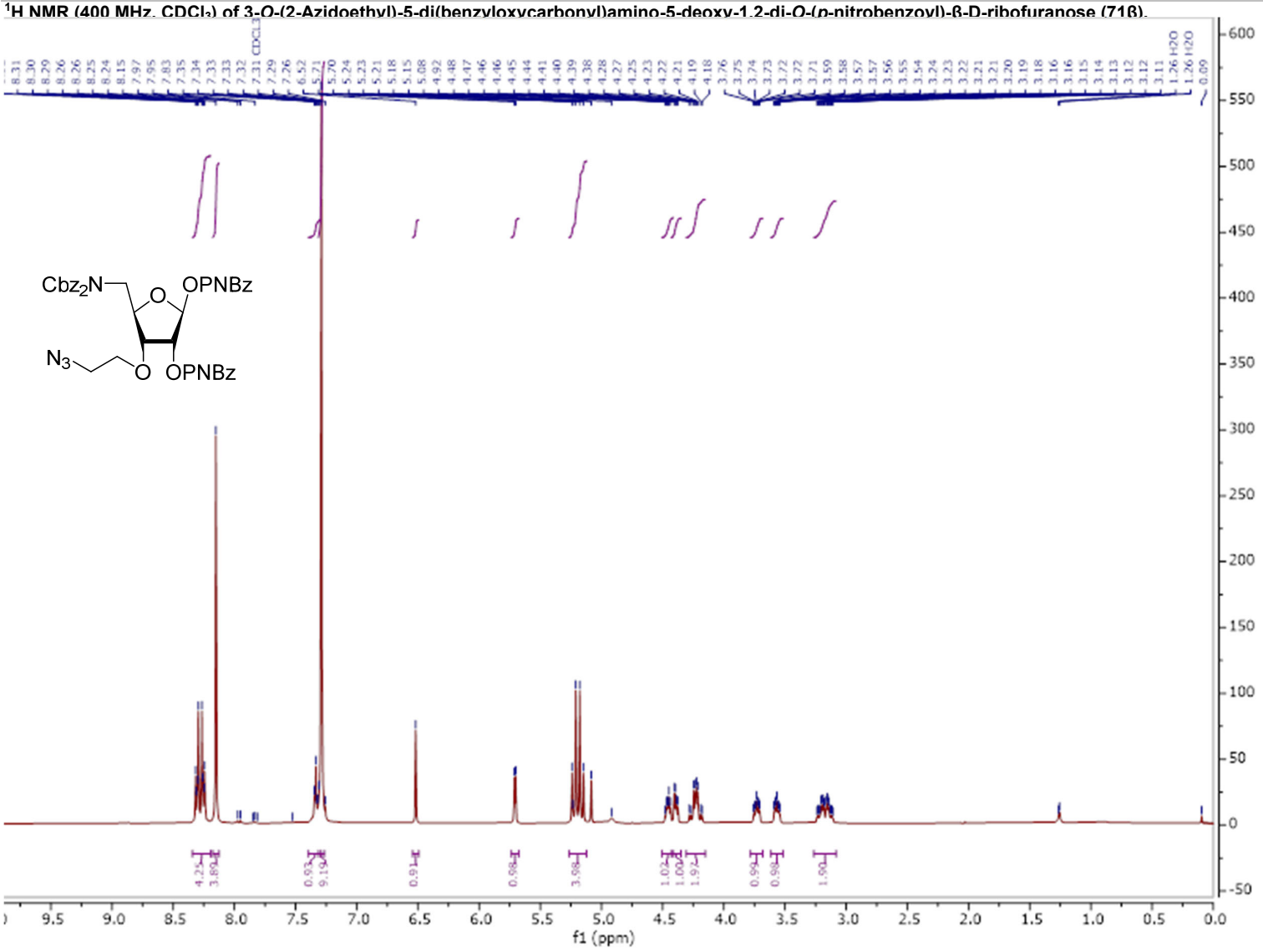


^{13}C NMR (100 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-α-D-ribofuranose (71α).

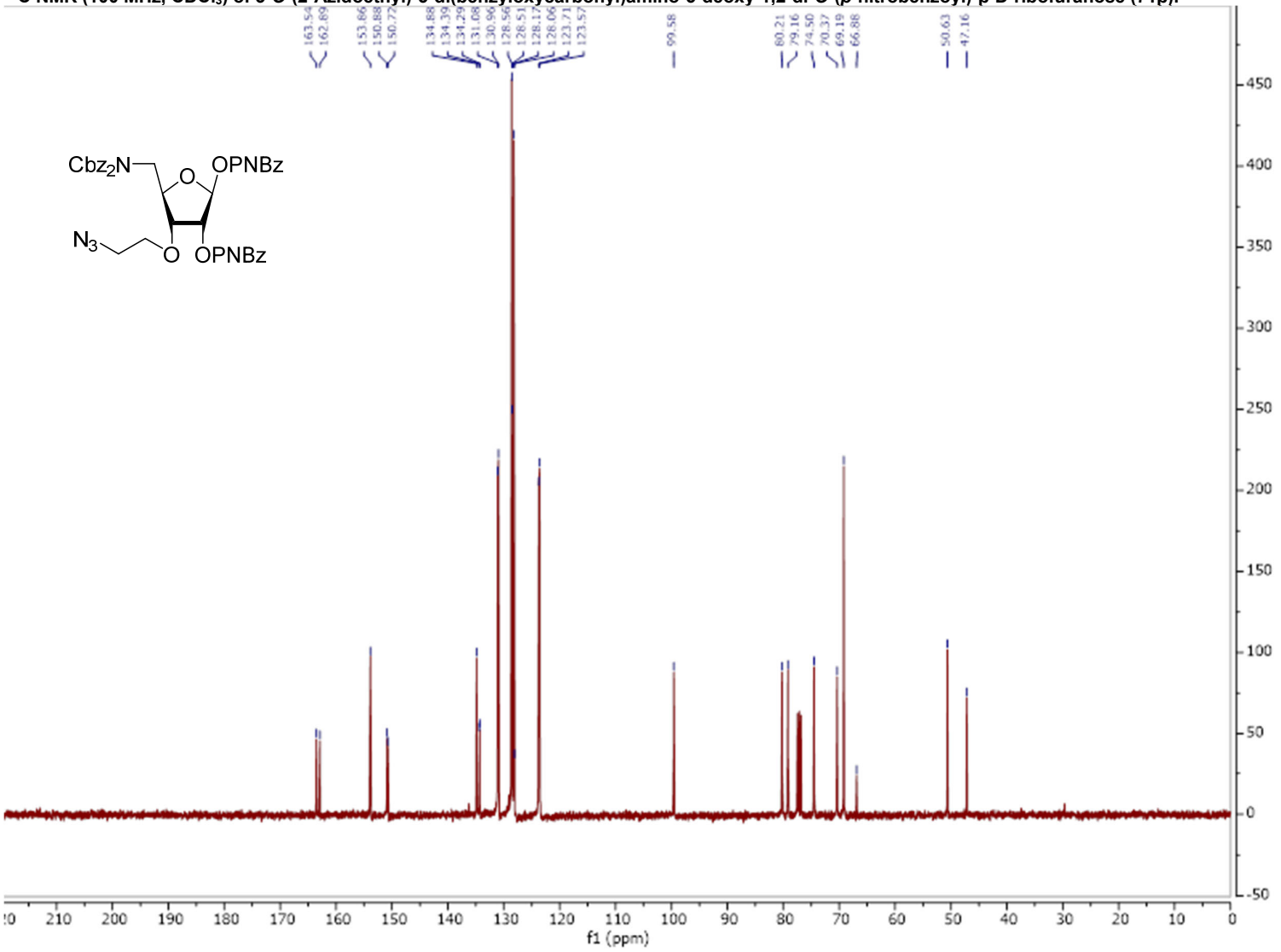


SUPPORTING INFORMATION

^{1}H NMR (400 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-ß-D-ribofuranose (71ß).

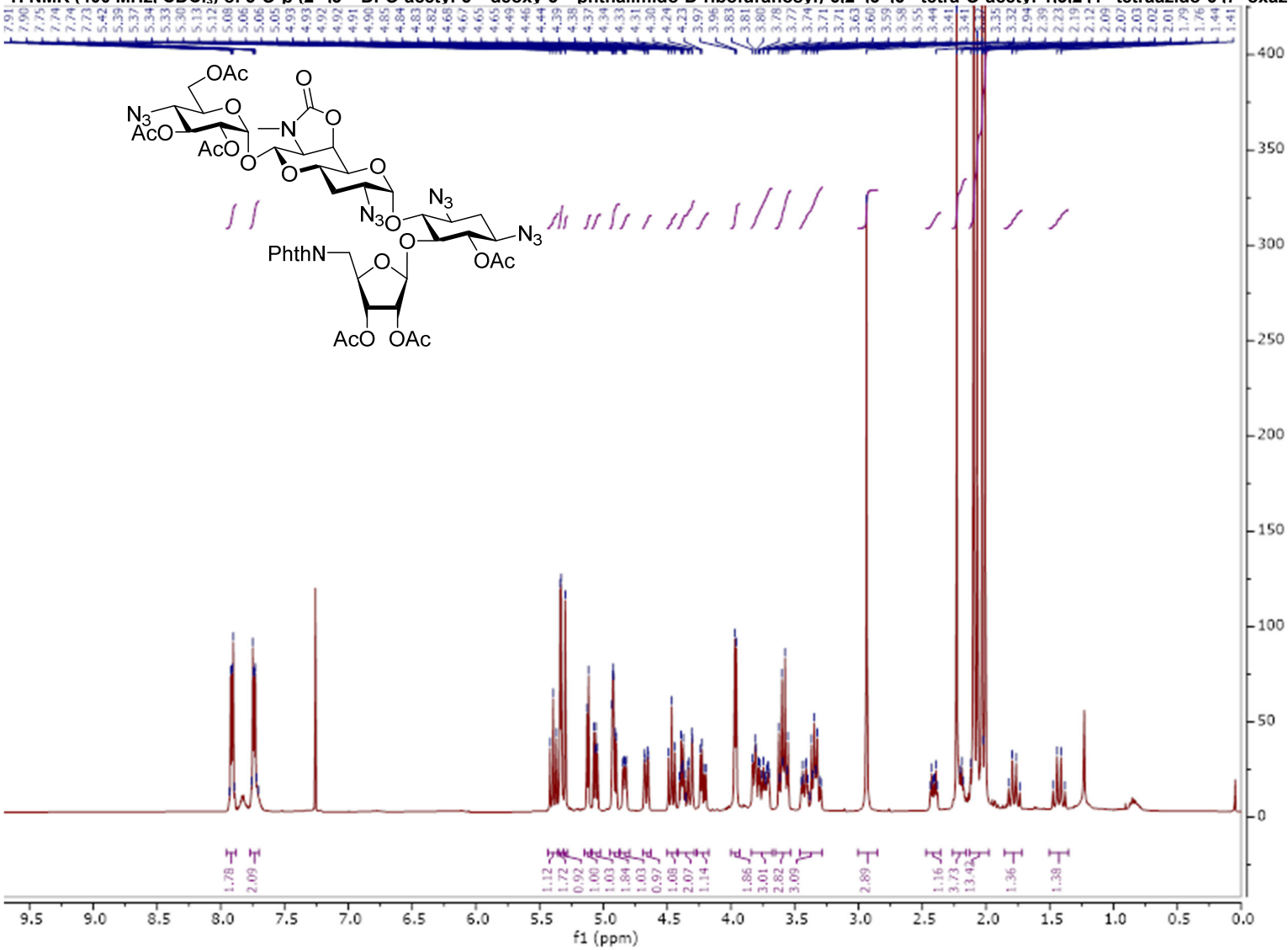


^{13}C NMR (100 MHz, $CDCl_3$) of 3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-β-D-ribofuranose (71β).

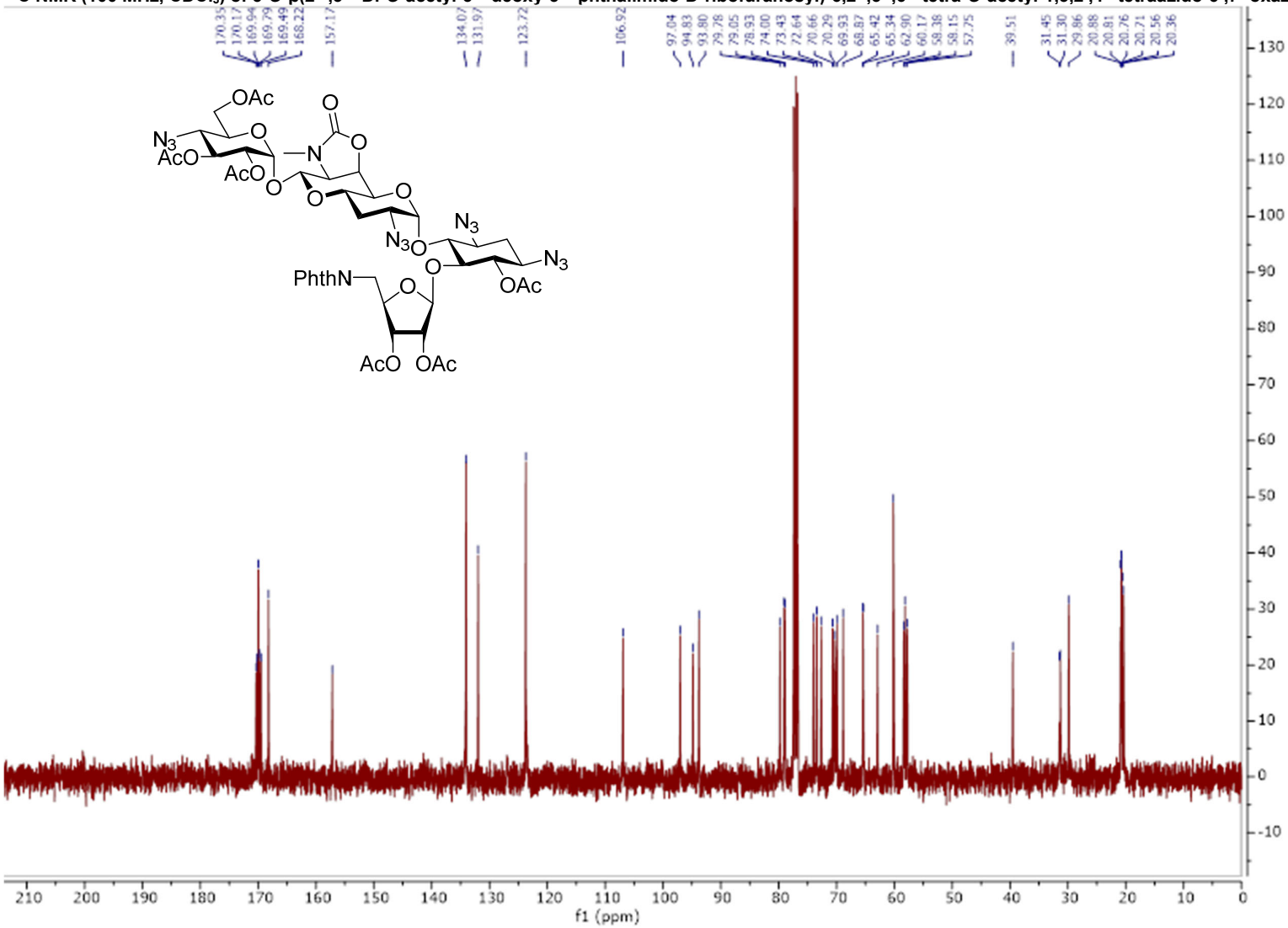


SUPPORTING INFORMATION

^{1}H NMR (400 MHz, $CDCl_3$) of 5-*O*-β-(2‴,3‴-Di-*O*-acetyl-5‴-deoxy-5‴-phthalimido-D-ribofuranosyl)-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (72).

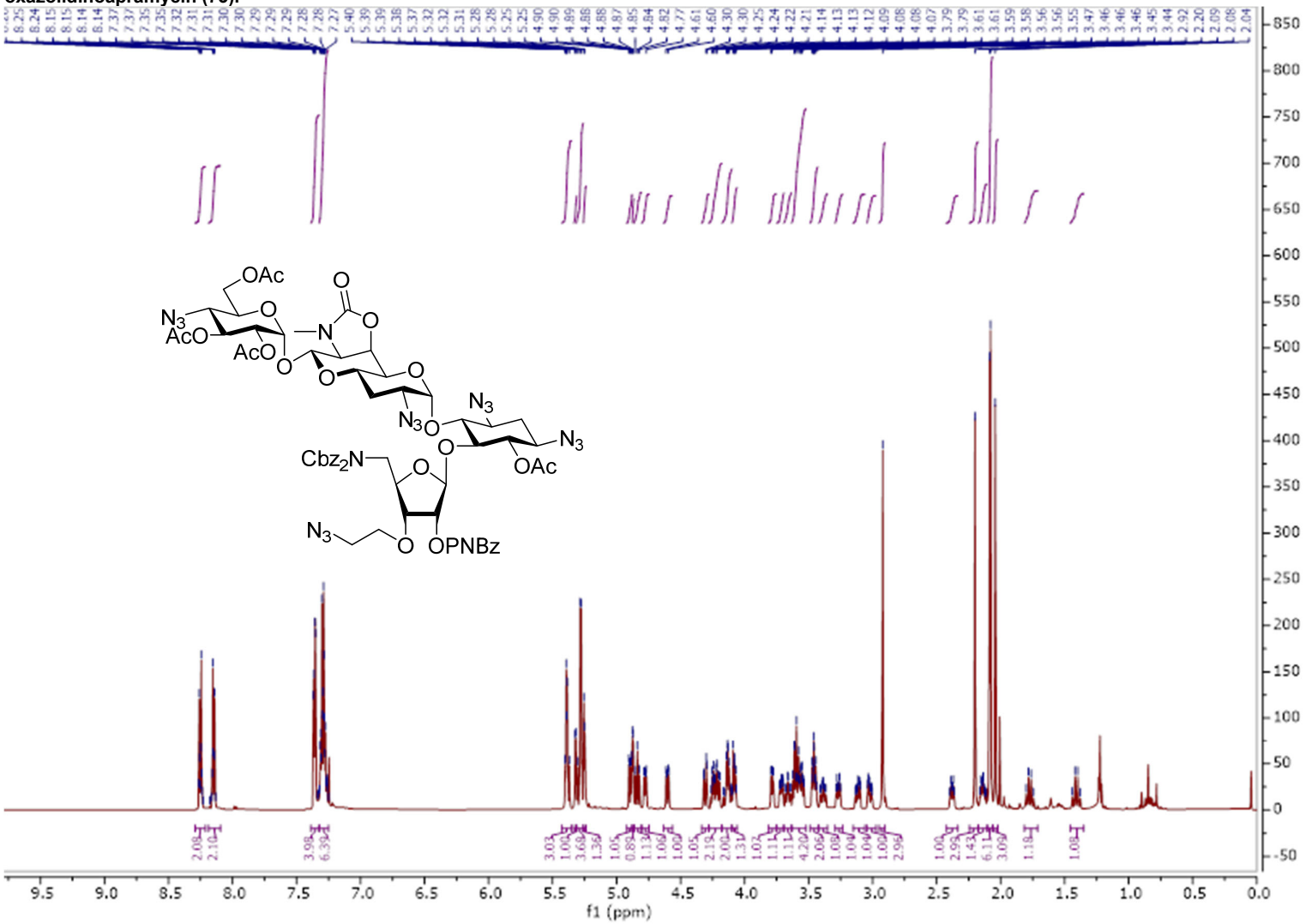


^{13}C NMR (100 MHz, $CDCl_3$) of 5-*O*-β(2‴,3‴-Di-*O*-acetyl-5‴-deoxy-5‴-phthalimido-D-ribofuranosyl)-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (72).



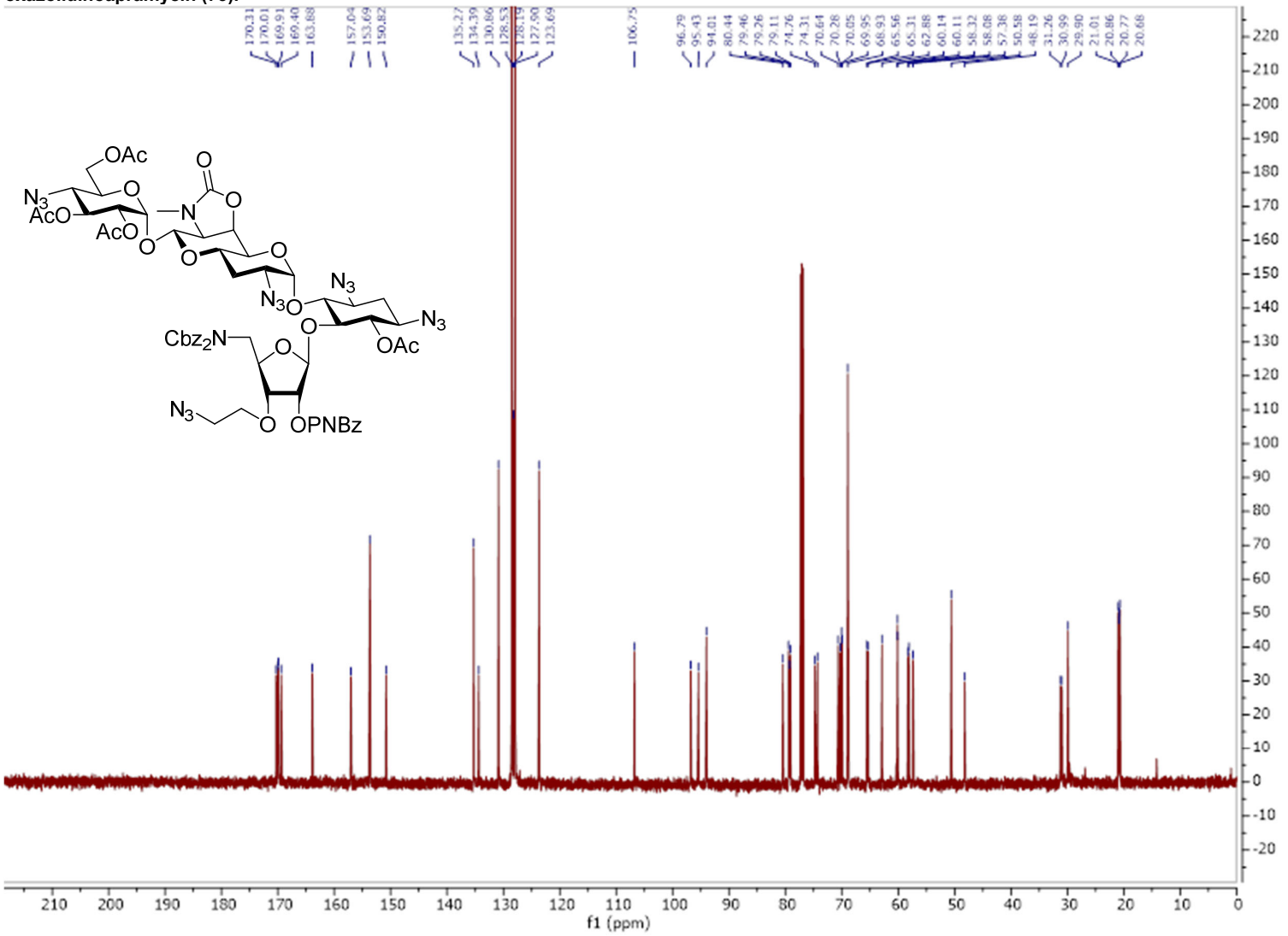
SUPPORTING INFORMATION

^{1}H NMR (600 MHz, $CDCl_3$) of 5-*O*-β-[3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-2-*O*-*p*-nitrobenzoyl-D-ribofuranose]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (73).



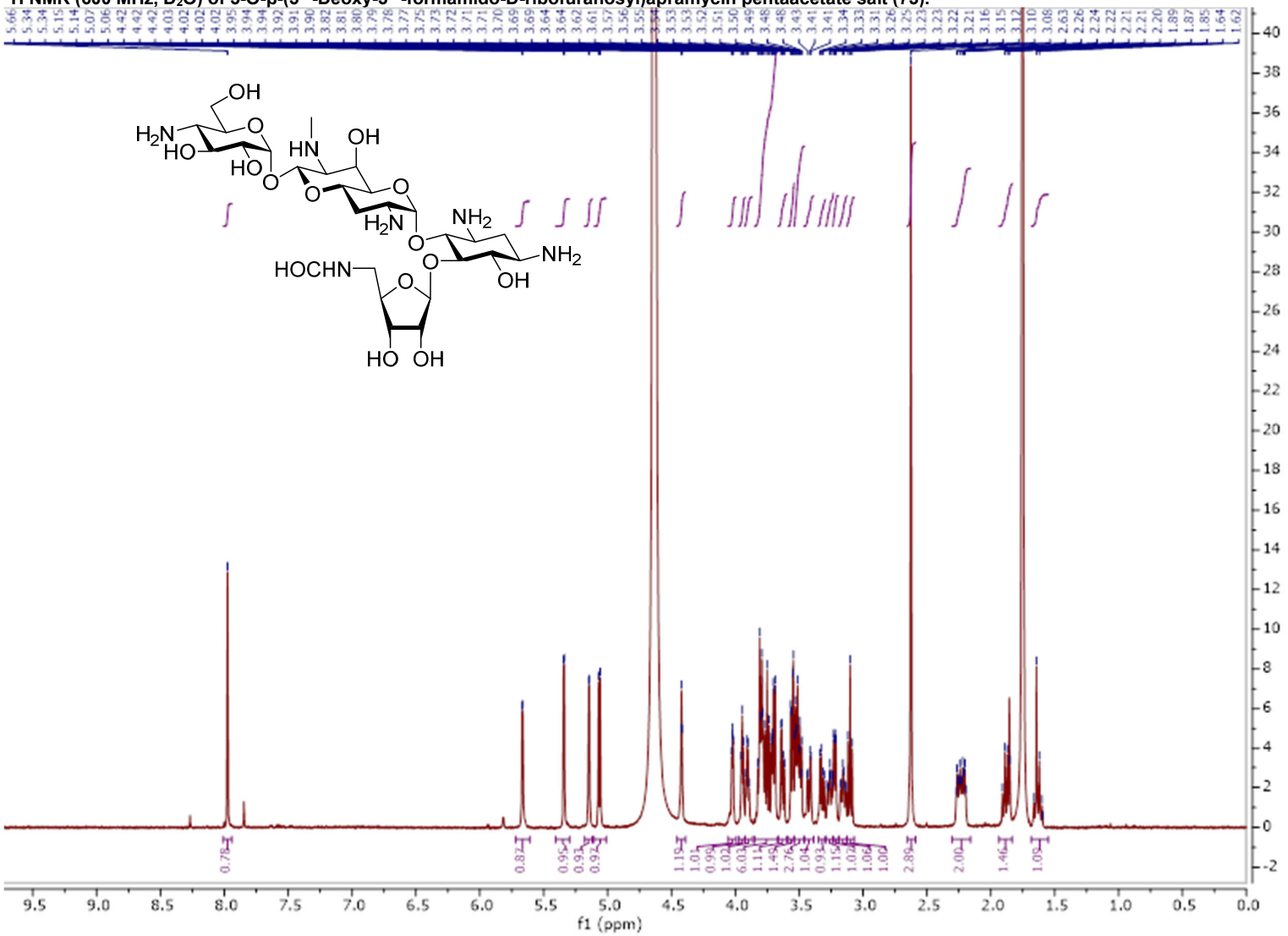
SUPPORTING INFORMATION

^{13}C NMR (125 MHz, $CDCl_3$) of 5-*O*-β-[3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-2-*O*-*p*-nitrobenzoyl-D-ribofuranose]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidinoapramycin (73).

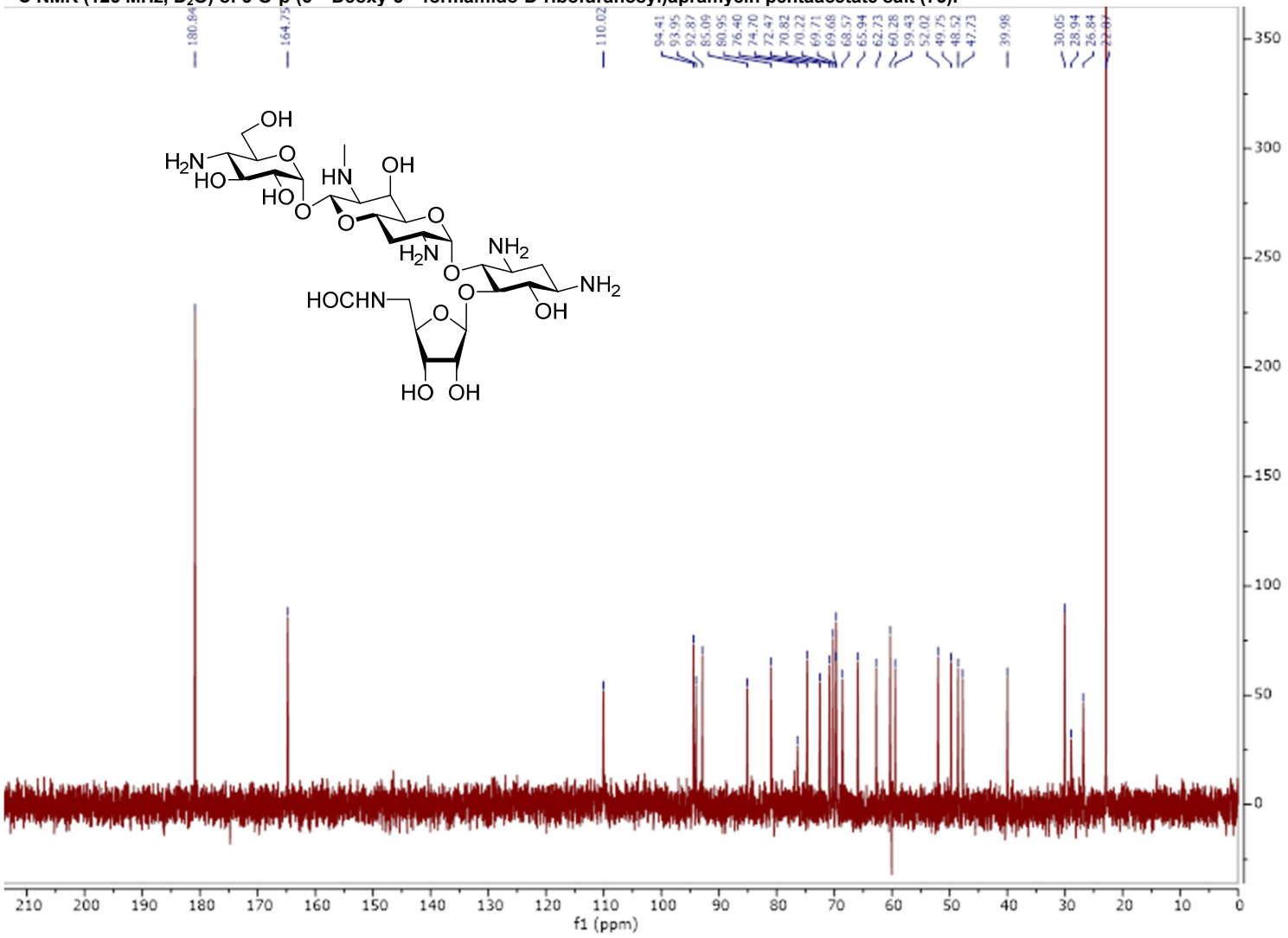


SUPPORTING INFORMATION

^{1}H NMR (600 MHz, D_2O) of 5-O-β-(5'''-Deoxy-5'''-formamido-D-ribofuranosyl)apramycin pentaacetate salt (75).

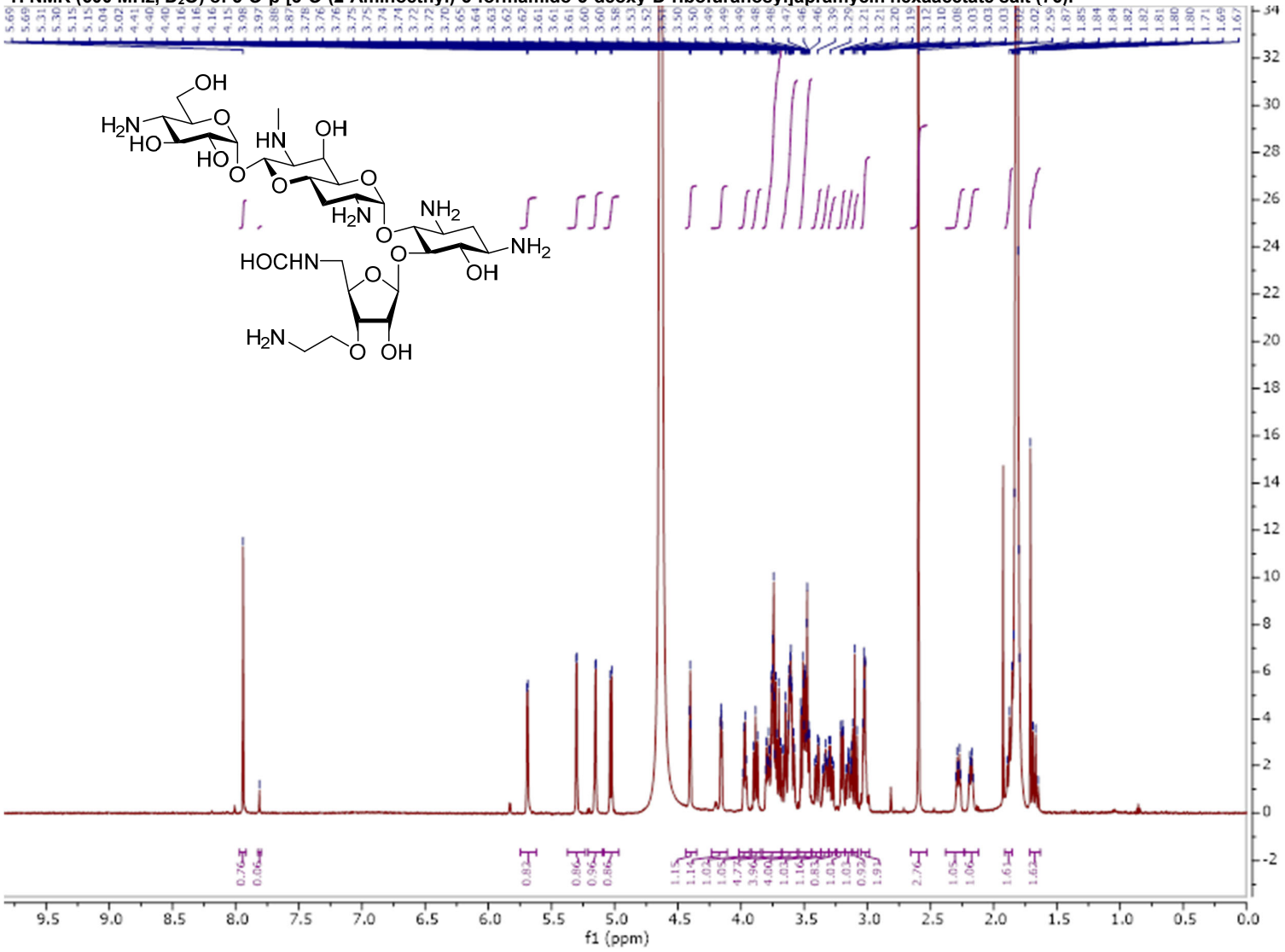


^{13}C NMR (125 MHz, D_2O) of 5-O-β-(5'''-Deoxy-5'''-formamido-D-ribofuranosyl)apramycin pentaacetate salt (75).

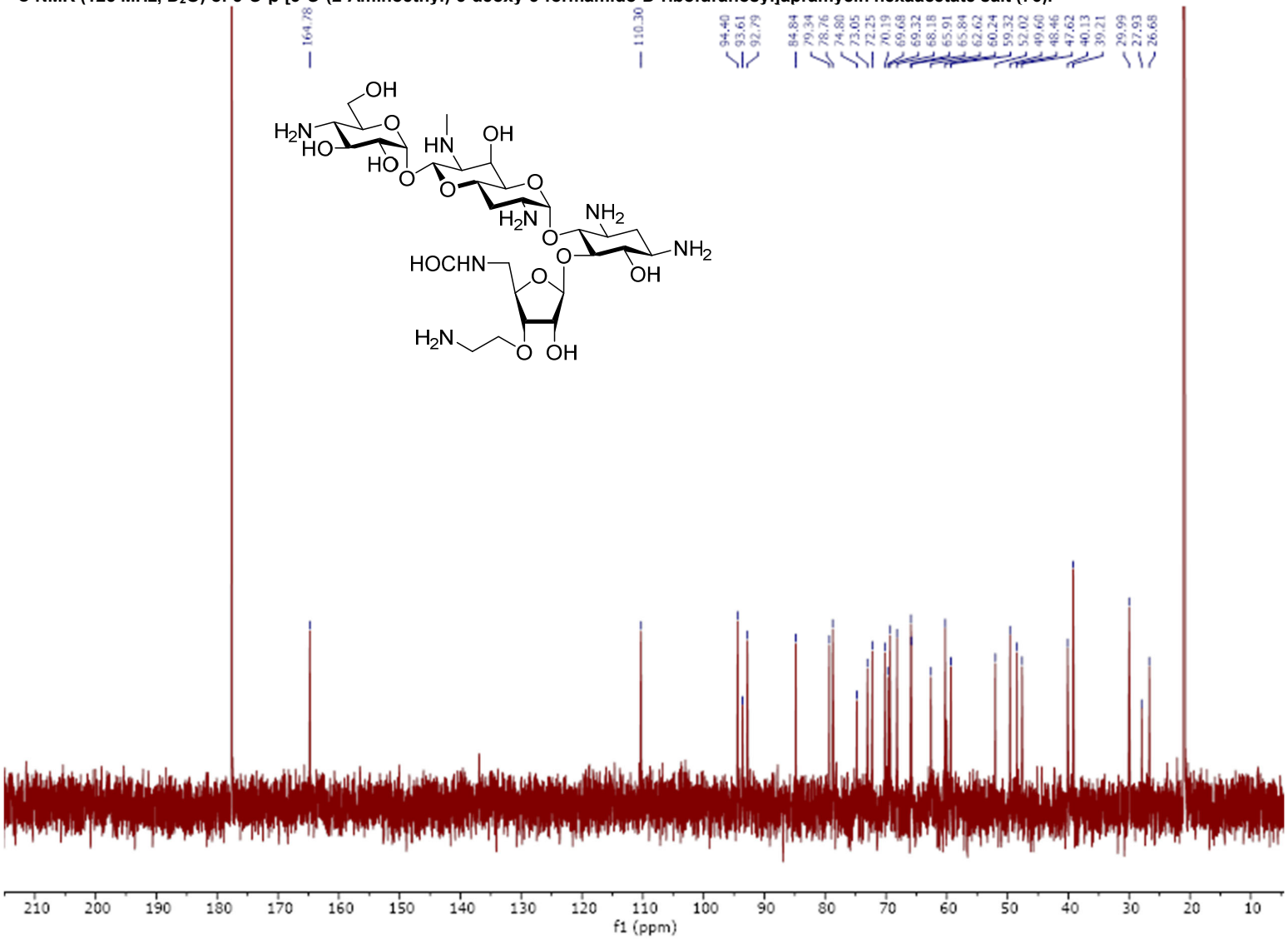


SUPPORTING INFORMATION

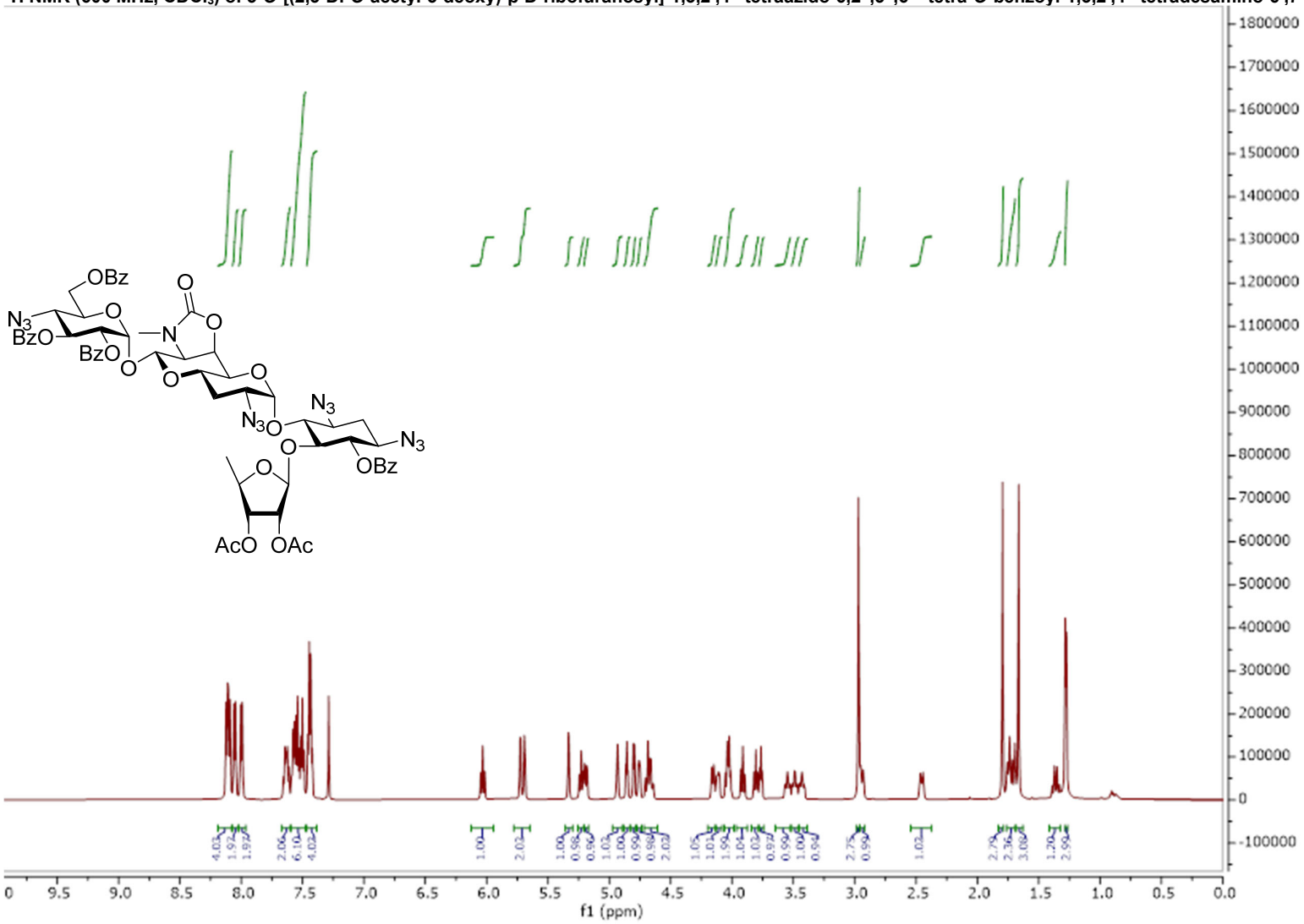
^{1}H NMR (600 MHz, D_2O) of 5-*O*-β-[3-*O*-(2-Aminoethyl)-5-formamido-5-deoxy-D-ribofuranosyl]apramycin hexaacetate salt (76).



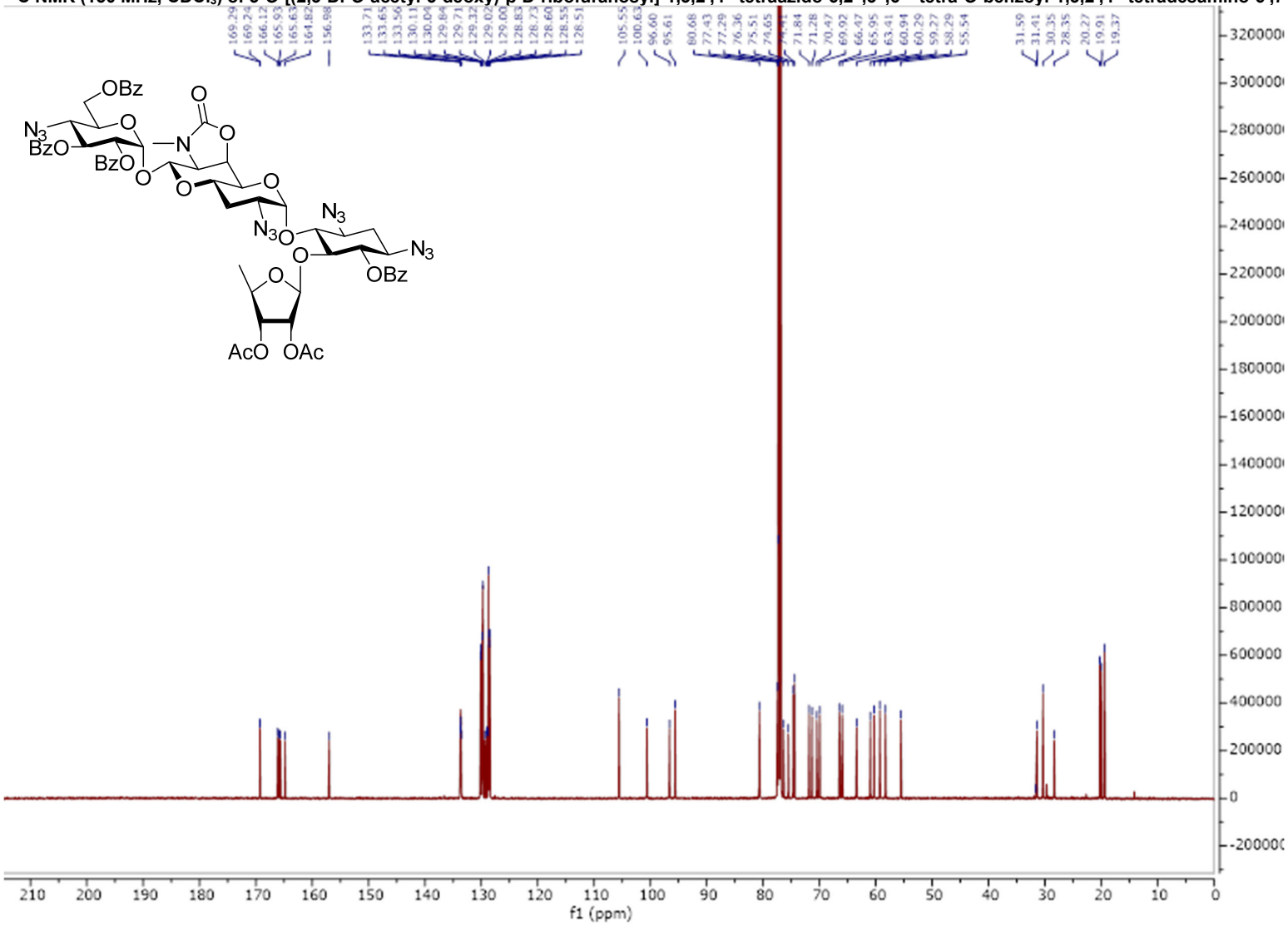
^{13}C NMR (125 MHz, D_2O) of 5-*O*-β-[3-*O*-(2-Aminoethyl)-5-deoxy-5-formamido-D-ribofuranosyl]apramycin hexaacetate salt (76).



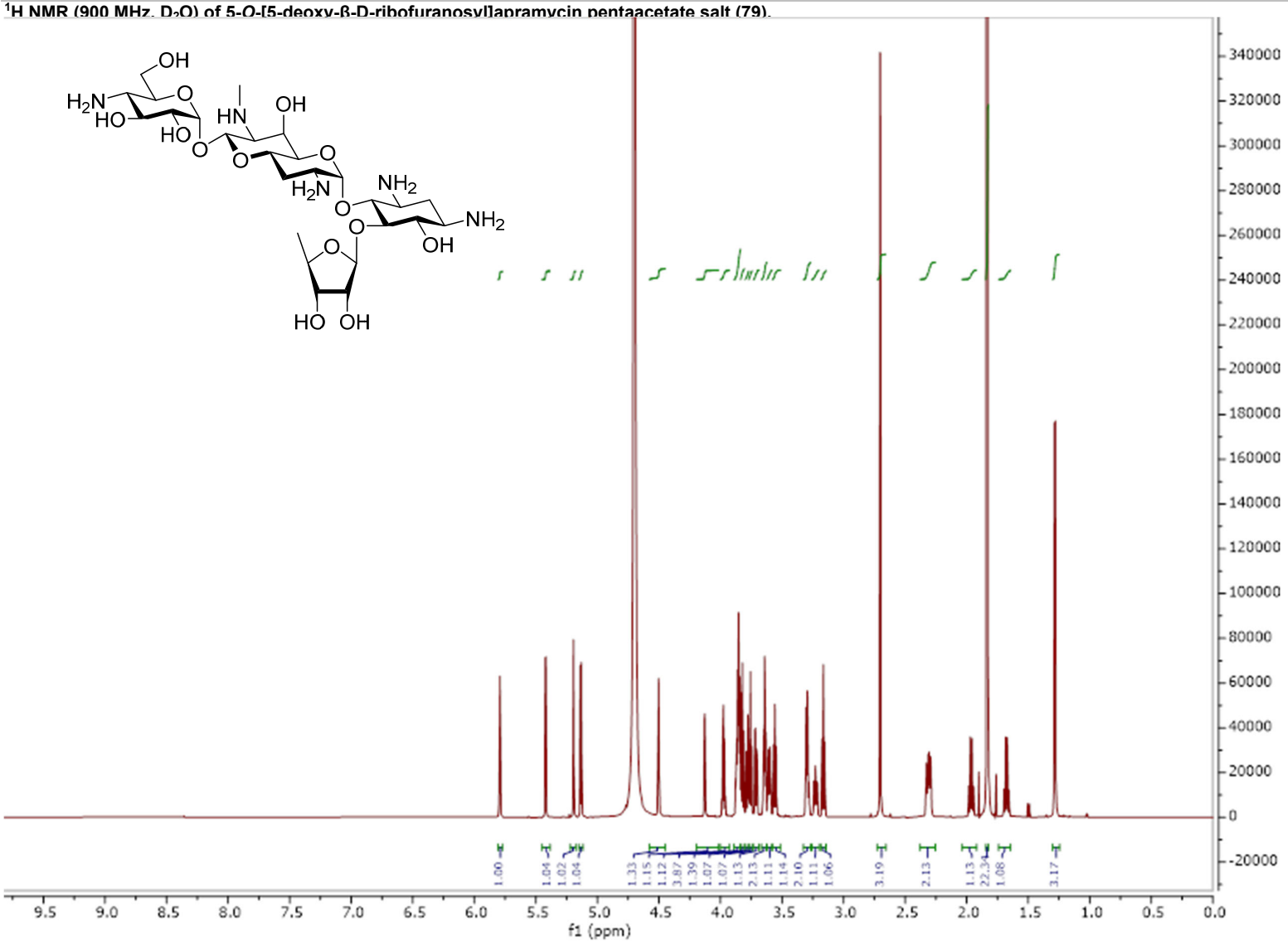
^{1}H NMR (600 MHz, $CDCl_3$) of 5-*O*-[(2,3-Di-*O*-acetyl-5-deoxy)-β-D-ribofuranosyl]-1,3,2′,4″-tetraazido-6,2″,3″,6″- tetra-*O*-benzoyl-1,3,2′,4″-tetradesamino-6′,7′-oxazolidinoapramycin (78).



^{13}C NMR (150 MHz, $CDCl_3$) of 5-*O*-[(2,3-Di-*O*-acetyl-5-deoxy)-β-D-ribofuranosyl]-1,3,2′,4″-tetraazido-6,2″,3″,6″- tetra-*O*-benzoyl-1,3,2′,4″-tetradesamino-6′,7′-oxazolidinoapramycin (78).



^{1}H NMR (900 MHz, D_2O) of 5-*O*-[5-deoxy-ß-D-ribofuranosyl]apramycin pentaacetate salt (79).



^{13}C NMR (225 MHz, D_2O) of 5-*O*-[5-deoxy-β-D-ribofuranosyl]apramycin pentaacetate salt (79).