Synthesis of Gentamicin Minor Components: Gentamicin B1 and Gentamicin X2

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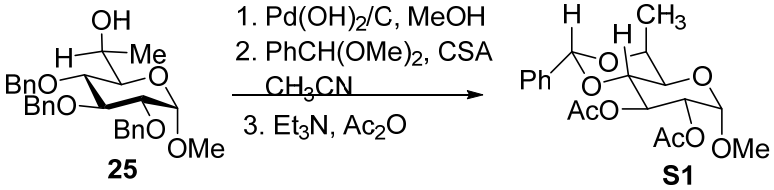
General Experimental

All experiments were carried out under a dry argon atmosphere unless otherwise specified. Compounds that only appear in the Supporting Information have numbers prefaced with S.. Heating of reaction mixtures was carried out on an aluminum heating block of appropriate size. Chromatographic purifications were carried over silica gel (230-400 mesh). Thin layer chromatography was performed with precoated glass backed plates (w/UV 254). TLC were visualized by UV irradiation (254 nm) and by charring with sulfuric acid in ethanol (20:80, v/v) or with ceric ammonium molybdate solution [$Ce(SO_4)_2$: 4 g, $(NH_4)_6Mo_7O_{24}$: 10 g, H_2SO_4: 40 mL, H_2O: 360 mL]. Optical rotations were measured at 589 nm and 21 ºC on a digital polarimeter with a path length of 10 cm. NMR spectra were recorded in $CDCl_3$ or D_2O using a 500, 600 or 900 MHz instrument, and assignments were made with the help of COSY, HMBC, and HSQC spectra. High-resolution (HRMS) mass spectra were recorded in the electrospray mode using a Orbitrap. Chemical shifts (δ) are recorded in ppm and multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad).

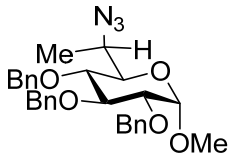
Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-α-L-glycero-D-gluco-heptopyranoside (28). To a stirred solution of **27**[1] (1.3 g, 2.79 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added Dess Martin periodinane (1.42 g, 3.35 mmol). The resulting mixture was brought to room temperature and stirred for 2 h then quenched with 20% aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the crude compound was used for the next step without further purification. The crude aldehyde was dissolved in THF (20 mL) and cooled to -78 °C. The solution was treated with methylmagnesium chloride solution (3 M in THF, 2.79 mL, 8.37 mmol), and the resulting solution was stirred with gradual warming to room temperature. Saturated NH_4Cl was added carefully, and the contents were extracted using EtOAc, dried (Na_2SO_4), and concentrated using a rotary evaporator. The crude product was purified using silica gel column chromatography (eluent: 30 – 40 % hexane/EtOAc) to give **28** (0.75 g, 56 % overall for the two steps) in the form of a colorless oil; $[\alpha]_D^{22}$ = +70.9 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 15H), 5.00 – 4.91 (m, 2H), 5.86 – 4.79 (m, 2H), 4.70 – 4.65 (m, 2H, $PhCH_2$), 4.60 (d, *J* = 3.6 Hz, 1H, H-1), 4.05 (q, *J* = 6.6 Hz, 1H, H-6), 4.00 (t, *J* = 9.3 Hz, 1H, H-3), 3.63 (dd, *J* = 10.0, 9.0 Hz, 1H, H-4), 3.50 (dd, *J* = 9.6, 3.6 Hz, 1H, H-2), 3.43 (dd, *J* = 9.9, 1.5 Hz, 1H, H-5), 3.35 (s, 3H, OMe), 1.25 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.9, 138.4, 138.3, 128.6, 128.3, 128.1, 128.0, 127.9, 127.7, 98.3, 82.3, 80.0, 77.8, 75.8, 75.2, 73.5, 72.9, 65.2, 55.1, 20.3. ESI-HRMS: m/z calcd. for $C_{29}H_{34}O_6Na$ $[M+Na]^+$ 501.2253; found, 501.2237.



Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-α-L-glycero-D-gluco-heptopyranoside (S1).[2] A suspension of compound **28** (60 mg, 0.13 mmol) and $Pd(OH)_2/C$ (10%, 15 mg) in methanol (1.0 mL) was subjected to hydrogenolysis under 45 psi of H_2 for 12 h. After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated to dryness. To the crude mixture in CH_3CN (0.8 mL) benzaldehyde dimethyl acetal (30 µL, 0.19 mmol) was added followed by camphor-1-sulfonic acid (CSA, 3 mg, 0.02 mmol). The reaction mixture was stirred at room temperature for 0.5 h. After

completion, the reaction was quenched with Et_3N (0.1 mL), the solvent was evaporated to dryness and the residue was re-dissolved in mixture Et_3N:Ac_2O (1:1, 0.5 mL) at 0 °C and stirred for 4 h at room temperature. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with CH_2Cl_2, washed (sat. aq. $NaHCO_3$), dried (Na_2SO_4), and concentrated. Purification of the crude compound by column chromatography (eluent: 20 – 30 % hexane/EtOAc) afforded **S1** (35 mg, 72 %) as a gum. $[\alpha]_D^{22}$ = +71.9 (*c* 0.53, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.39 (m, 2H), 7.38 – 7.30 (m, 3H), 5.79 (s, 1H, PhCH), 5.57 (t, *J* = 9.8 Hz, 1H, H-3), 4.92 (d, *J* = 3.7 Hz, 1H, H-1), 4.87 (dd, *J* = 10.0, 3.7 Hz, 1H, H-2), 4.49 (p, *J* = 6.7 Hz, 1H, H-6), 4.15 (dd, *J* = 10.3, 5.9 Hz, 1H, H-5), 3.91 (t, *J* = 9.9 Hz, 1H, H-4), 3.41 (s, 3H, OMe), 2.09 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 169.7, 137.4, 128.9, 128.2, 126.2, 97.5, 94.0, 72.7, 71.5, 70.5, 69.6, 64.4, 55.3, 20.8, 20.8, 11.3; ESI-HRMS: m/z calcd. for $C_{19}H_{24}O_8Na$ $[M+Na]^+$ 403.1363; found, 403.1360.

Methyl 6-azido-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α-D-glycero-D-gluco-heptopyranoside (29).

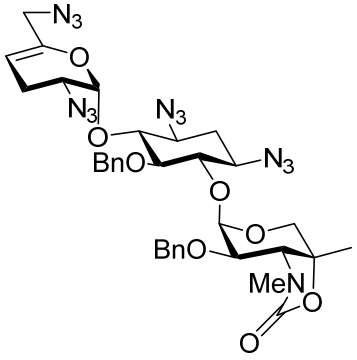


Alcohol **28** (0.60 g, 1.26 mmol) and PPh_3 (0.39 g 1.51 mmol) were dissolved in THF (10 mL), diisopropylethylamine (0.21 mL 20.8 mmol) was added and the mixture cooled to 10 ° C. Diisopropyl azodicarboxylate (DIAD, 0.3 mL, 1.51 mmol) was then added and the reaction mixture stirred for 10 min. Diphenylphosphoryl azide (DPPA, 0.39 mL 1.51 mmol) was then added at same temperature and the reaction mixture was warmed to room temperature and stirred for 2 h. It was quenched with satd. aq. NH_4Cl and extracted using EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (eluent: 10 – 15% hexane/EtOAc) to give **29** (0.52 g, 83%), in the form of a colorless oil. $[\alpha]_D^{22}$ = +16.3 (*c* 0.60, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.06 (m, 15H), 5.04 (d, *J* = 10.8 Hz, 1H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.87 – 4.78 (m, 2H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 3.6 Hz, 1H, H-1), 4.61 (d, *J* = 11.2 Hz, 1H), 4.02 (dd, *J* = 9.6, 8.7 Hz, 1H, H-3), 3.87 (dd, *J* = 10.2, 2.1 Hz, 1H, H-5), 3.58 (qd, *J* = 6.9, 2.1 Hz, 1H, H-6), 3.52 (dd, *J* = 9.6, 3.7 Hz, 1H, H-2), 3.45 (s, 3H), 3.34 (dd, *J* = 10.2, 8.7 Hz, 1H, H-4), 1.12 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.6, 138.0, 137.8, 128.5, 128.5, 128.5, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 127.7, 97.8, 82.3, 80.1, 77.7,

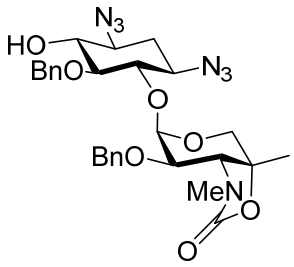
75.8, 74.6, 73.3, 72.0, 55.7, 55.3, 11.9; ESI-HRMS: m/z calcd. for $C_{29}H_{33}O_5N_3Na$ $[M+Na]^+$ 526.2312; found, 526.2310.

***p*-Tolyl 6-azido-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α,β-D-glycero-D-gluco-heptothiopyranoside (30)**. To a solution of **29** (200 mg, 0.40 mmol) and acetic anhydride (200 µL) in acetic acid (3 mL), H_2SO_4 (50 µL) was added at 0 °C. The reaction mixture was stirred for 1 h at r.t., and then quenched with water, diluted with CH_2Cl_2, washed (sat. aq. $NaCO_3$, and water), dried (Na_2SO_4), and concentrated. A quick filtration of the crude reaction mixture through a pad of silica gel (eluent: 20 – 30 % hexane/EtOAc) gave a mixture of the anomeric acetates. To this mixture in CH_2Cl_2 (3 mL), activated 4 Å powdered molecular sieves, and TolSH (99 mg, 0.8 mmol) were added, followed by slow addition of $BF_3{\cdot}OEt$ (60 µL, 0.48 mmol) at 0 ° C. The reaction mixture was stirred for 1 h at 0 °C, and an additional 12 h at room temperature, then was diluted with CH_2Cl_2, washed (sat. aq. $NaHCO_3$), dried (Na_2SO_4), and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5 – 10% hexane/EtOAc) to give an anomeric mixture of **30** (161 mg, 68%, α/β = 3/1) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.10 (m, 19H, both anomers), 5.58 (d, *J* = 5.4 Hz, 1H, α anomer), 5.07 – 4.63 (m, 6H, both anomers), 4.61 (d, *J* = 9.8 Hz, 1H, β isomer), 4.43 – 4.38 (m, 1H), 3.93 (dd, *J* = 9.6, 8.6 Hz, 1H, α anomer), 3.84 (dd, *J* = 9.6, 5.4 Hz, 1H, α anomer), 3.73 (t, *J* = 8.8 Hz, β isomer), 3.69 – 3.52 (m, 1H, α anomer, 2H, β isomer), 3.50 – 3.43 (m, 1H, 2H, β isomer), 3.41 (dd, *J* = 10.2, 8.6 Hz, 1H, α anomer), 2.37 (s,3H, β isomer), 2.36 (s, 3H, α anomer), 1.20 (d, *J* = 6.9 Hz, 1H, β isomer), 1.13 (d, *J* = 6.9 Hz, 3H, α anomer); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.9, 138.5, 138.2, 138.1, 138.0, 137.8, 137.6, 133.2, 132.6, 130.1, 130.1, 129.8, 129.8, 129.7, 128.6, 128.5, 128.5, 128.5, 128.5, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 126.1, 120.3, 120.2, 87.9, 87.5, 87.1, 82.6, 80.9, 80.6, 79.9, 77.6, 77.4, 75.8, 75.8, 75.4, 74.7, 74.6, 73.2, 72.3, 56.2, 56.0, 21.1, 12.4, 12.4; .ESI-HRMS: m/z calcd. for $C_{35}H_{37}O_4N_3Na$ $[M+Na]^+$ 618.2402; found, 618.2401.

1,3,2′,6′-Tetra(deamino)-5,2′′-di-*O*-benzyl-1,3,2′,6′-tetraazido-3′′-*N*,4′′-*O*-carbonyl-



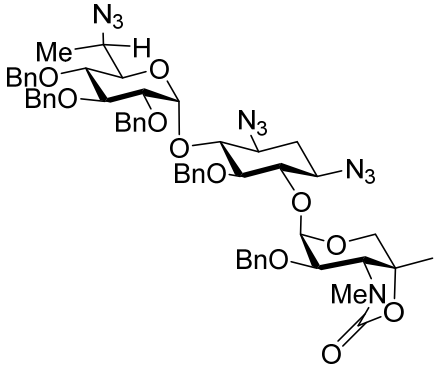
sisomicin (25). A stirred solution of 1,3,2′,6′-tetra-deamino-3′′-*N*-benzyloxycarbonyl-1,3,2′,6′-tetraazido-sisomicin **24**[3] (660 mg, 0.88 mmol) in DMF (8 mL) cooled to 0 °C was treated with NaH (105 mg, 60% in paraffin oil, 2.63 mmol) and stirred at same temperature for 20 min. After disappearance of the starting materials based on TLC, benzyl bromide (0.45 mL, 3.50 mmol) was added dropwise. The resulting reaction mixture was allowed to stir at room temperature for 2 h before the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl. The solvents were evaporated and the crude product was purified using silica gel column chromatography (eluent: 10 – 12% hexane/EtOAc) to give compound **25** (635 mg, 87%) as a yellow oil. $[\alpha]_D^{21}$ = +68.0 (*c* 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.23 (m, 10H), 5.67 (d, *J* = 2.5 Hz, 1H, H-1'), 5.16 (d, *J* = 3.4 Hz, 1H, H-1''), 5.11 (d, *J* = 10.8 Hz, 1H, $PhCH_2$), 4.93 (dd, *J* = 5.4, 2.4 Hz, 1H, H4'), 4.88 (d, *J* = 11.5 Hz, 1H, $PhCH_2$), 4.74 – 4.64 (m, 2H, $PhCH_2$), 3.97 (t, *J* = 3.5 Hz, 1H, H-2''), 3.82 – 3.63 (m, 5H, CH_2, H-5a'', H-4, H-5, CH_2, H-6'a, H-6'b), 3.50 (t, *J* = 9.4 Hz, 1H, H-6), 3.48 – 3.42 (m, 2H.H-1, H-3''), 3.40 – 3.34 (m, 2H, H-3, CH_2, H-5b''), 3.27 (ddd, *J* = 10.7, 6.1, 2.5 Hz, 1H, H-2), 2.79 (s, 3H, NCH_3), 2.50 – 2.39 (m, 1H, H-3a''), 2.28 – 2.19 (m, 2H, H-3b'', H-2a''), 1.41 (q, *J* = 12.8 Hz, 1H, H-2b''), 1.22 (s, 3H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.4, 145.7, 138.1, 137.4, 128.6, 128.3, 128.2, 128.2, 128.0, 127.5, 126.9, 126.9, 97.9, 96.6, 96.3, 82.8, 80.7, 78.4, 77.0, 75.1, 75.0, 72.3, 67.0, 63.2, 61.2, 60.3, 54.6, 52.2, 32.5, 29.9, 23.3, 21.2; m/z calcd. for $C_{34}H_{39}O_8N_{13}[M+Na]^+$ 780.2942; found, 780.2939.



5,2′-Di-*O*-benzyl-1,3-di(deamino)-1,3-diazido-3′-*N*,4′-*O*-carbonyl-garamine (26). Compound **25** (600 mg, 0.79 mmol) was dissolved in tetrahydrofuran (15 mL), and the pH of the solution adjusted to 1 by careful addition of concentrated sulfuric acid in tetrahydrofuran (2% solution of H_2SO_4 in THF, 1 mL was added). The solution was stirred at 40 ºC for 48 h, then was diluted with EtOAc, neutralized with aq. $NaHCO_3$, extracted using EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (eluent: 30 - 40%

hexane/EtOAc) to give **26** (390 mg, 85%), in the form of a colorless oil. $[\alpha]_D^{21}$ = +19.2 (*c* 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.23 (m, 10H), 5.20 (d, *J* = 3.5 Hz, 1H, H-1"), 5.00 (d, *J* = 11.4 Hz, 1H, $PhCH_2$), 4.91 (d, *J* = 11.6 Hz, 1H, $PhCH_2$), 4.66 (dd, *J* = 11.5, 3.0 Hz, 2H, $PhCH_2$), 3.98 (t, *J* = 3.7 Hz, 1H, H-2"), 3.95 (d, *J* = 12.5 Hz, 1H, CH_2, H-5a"), 3.68 (t, *J* = 9.6 Hz, 1H, H-6), 3.54 – 3.42 (m, 4H, CH_2, H-5b", H-3", H-4, H-1), 3.38 (ddd, *J* = 12.4, 9.7, 4.5 Hz, 1H, H-3), 3.25 (t, *J* = 9.3 Hz, 1H, H-5), 2.81 (s, 3H, NCH_3), 2.64 (d, *J* = 2.4 Hz, 1H, OH), 2.20 (dt, *J* = 13.3, 4.5 Hz, 1H, H-2a), 1.39 (q, *J* = 12.7 Hz, 1H, H-2b), 1.28 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.4, 138.1, 137.5, 128.6, 128.6, 128.2, 127.9, 127.8, 127.5, 96.1, 81.9, 80.3, 77.3, 76.0, 75.3, 74.8, 74.8, 72.7, 66.7, 63.0, 61.3, 59.6, 32.0, 30.0, 23.3. ESI-HRMS: m/z calcd. for $C_{28}H_{34}O_7N_7[M+H]^+$ 580.2520; found, 580.2510.

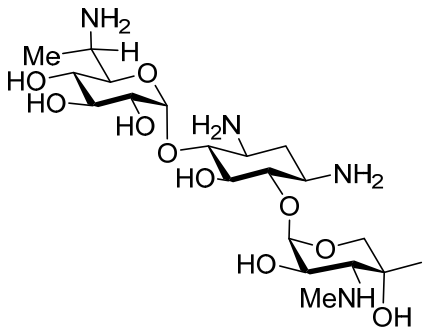
5,2′,3′,4′,2″-Penta-*O*-benzyl-1,3,6′-triazido-1,3,6′-tri(deamino)-3″-*N*,4″-*O*-carbonyl-



gentamicin B1 (31). A mixture of acceptor **26** (57 mg; 0.10 mmol), donor **30** (90 mg, 0.15 mmol), and freshly activated 4 Å acid-washed powdered molecular sieves in dichloromethane (2.0 mL) was stirred for 1 h at room temperature, then cooled to -30 °C, and treated with *N*-iodosuccinimide (41 mg; 0.18 mmol) and AgOTf (8 mg; 0.03 mmol). The reaction mixture was stirred at -30 °C for 1 h and then gradually warmed to room temperature and stirred for 6 h before it was quenched with triethylamine (0.2 mL). The mixture was diluted with dichloromethane, filtered through Celite®, washed with 20% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (eluent: 25 – 35 % hexane/EtOAc) to give **31** (75 mg, 76%), in the form of a colorless oil. $[\alpha]_D^{21}$ = +17.3 (*c* 0.53, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.18 (m, 20H), 7.18 – 7.11 (m, 2H), 7.04 – 6.96 (m, 3H), 5.65 (d, *J* = 3.8 Hz, 1H, H-1'), 5.18 (d, *J* = 3.6 Hz, 1H, H-1"), 4.94 – 4.84 (m, 4H, $PhCH_2$), 4.78 (dd, *J* = 11.2, 1.9 Hz, 2H, $PhCH_2$), 4.60 (d, *J* = 11.3 Hz, 2H, $PhCH_2$), 4.50 – 4.37 (m, 2H, $PhCH_2$), 4.36 (dd, *J* = 10.2, 1.8 Hz, 1H, H-5'), 4.04 (dd, *J* = 9.9, 8.8 Hz, 1H, H-3'), 3.92 (t, *J* = 3.8 Hz, 1H, H-2"), 3.77 (td, *J* = 9.6, 2.5 Hz, 2H, H-6, H-4), 3.64 – 3.52 (m, 3H, H-6', H-5, H-1), 3.52 – 3.44 (m, 2H, H-3,CH_2, H-5a"), 3.41 (d, *J* = 3.9 Hz, 1H, H-3"), 3.37 (dd, *J* = 9.9, 3.8 Hz, 1H, H-

2'), 3.33 (dd, *J* = 10.2, 8.8 Hz, 1H, H-4'), 3.09 (d, *J* = 12.8 Hz, 1H, CH_2, H-5b''), 2.79 (s, 3H, NCH_3), 2.35 (dt, *J* = 13.2, 4.4 Hz, 1H, H-2a), 1.59 (q, *J* = 12.8 Hz, 1H, H-2b), 1.12 (d, *J* = 7.0 Hz, 3H), 1.01 (s, 3H). 1.12 (d, *J* = 6.9 Hz, 3H, CH_3), 1.00 (s, 3H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 157.4, 138.4, 138.4, 137.9, 137.7, 137.5, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 126.9, 125.6, 96.6, 95.6, 82.8, 82.0, 79.7, 79.2, 78.0, 75.5, 74.8, 74.4, 73.7, 73.4, 72.9, 72.6, 66.2, 62.8, 61.1, 59.5, 56.8, 32.1, 30.0, 22.9, 12.9; ESI-HRMS: m/z calcd. for $C_{56}H_{62}O_{11}N_{10}Na$ $[M+Na]^+$ 1073.4497; found, 1073.4480.

Gentamicin B1 tetraaacetate salt (7). A suspension of compound **31** (23 mg, 21.9 μmol) and

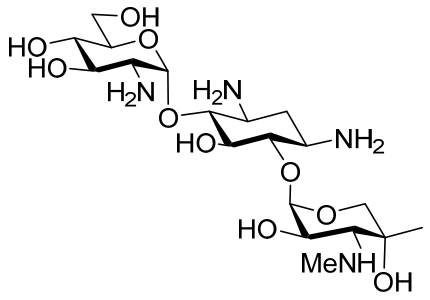


$Pd(OH)_2/C$ (10%, 50 mg) in 1,4-dioxane: H_2O (1:1, 0.8 mL) was subjected to hydrogenolysis under 40 psi of H_2 for 36 h. After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated to dryness. The crude residue was re-dissolved in saturated aqueous $Ba(OH)_2$ (0.5 mL) and heated to 60 °C for 12 h. After complete hydrolysis of the oxazolidinone, as indicated by mass spectrometry, the reaction mixture was neutralized to pH 7 by addition of dry ice and the precipitated solid was filtered off. The filter cake was washed with water and the filtrate was concentrated to dryness. The crude product was then taken up in 10% aqueous acetic acid and loaded on a prepacked Sephadex C-25 column and eluted with 0.1% to 1.5% ammonium hydroxide in deionized water. The product-containing fractions were combined, concentrated, acidified with glacial acetic acid, and lyophilized to give the pentaacetate salt of gentamicin B1 (**7**) as a white foam (6.1 mg, 56 %). $[\alpha]_D^{21}$ = +30 (*c* 0.1, H_2O); ^1H NMR (900 MHz, D_2O) δ 5.45 (d, *J* = 3.9 Hz, 1H, H-1'), 5.02 (d, *J* = 3.7 Hz, 1H, H-1''), 4.14 (dd, *J* = 10.8, 3.6 Hz, 1H, H-2''), 3.95 (dd, *J* = 10.2, 2.8 Hz, 1H, H-5'), 3.93 (d, *J* = 12.9 Hz, 1H, CH_2, H-5a''), 3.82 – 3.77 (m, 2H, H-5, H-6), 3.73 – 3.67 (m, 2H, H-6', H-3'), 3.66 – 3.63 (m, 1H, H-4), 3.61 – 3.54 (m, 2H, H-2', H-1), 3.46 (ddd, *J* = 12.9, 10.4, 4.2 Hz, 1H, H-3), 3.43 – 3.38 (m, 2H, H-3'', CH_2, H-5b''), 3.38 – 3.35 (m, 1H, H-4'), 2.83 (s, 3H), 2.46 (dt, *J* = 12.4, 4.3 Hz, 1H, H-2a), 1.83 (q, *J* = 12.5 Hz, 1H, H-2b), 1.26 (s, 3H, CH_3), 1.23 (d. *J* = 6.8 3H, CH_3); ^{13}C NMR (225 MHz, D_2O) δ 101.3, 94.9, 84.1, 78.2, 72.4, 71.7, 71.0, 70.6, 69.9, 69.6, 67.7,

66.3, 63.4, 49.8, 47.2, 47.0, 34.4, 27.5, 20.8, 10.9. ESI-HRMS: m/z calcd. for $C_{20}H_{41}O_{10}N_4[M+H]^+$ 497.2823; found, 497.2806.

5,3′,4′,6′,2′′-Penta-*O*-benzyl-1,3,2′-triazido-1,3,2′-tri(deamino)-3′′-*N*,4′′-*O*-carbonyl-gentamicin X2 (33). A mixture of thioglycoside **32**[4] (111 mg; 0.19 mmol), and freshly activated 4 Å acid-washed powdered molecular sieves were suspended in dichloromethane (1.6 mL). DMF (60 µL, 0.77 mmol) was added and the resulting mixture was stirred for 1 h at rt before it was cooled to 0 ºC and stirred for 0.25 h before NIS (43 mg, 0.19 mmol) and TMSOTf (35 µL, 0.19 mmol) were added. After stirring for an additional 0.5 h at 0 ºC a solution of glycosyl acceptor **23** (74 mg, 0.13 mmol) in dichloromethane (1.0 mL) was added and the reaction mixture was slowly allowed to warm to rt and stirred for 24 h before it was quenched with triethylamine (0.2 mL). The mixture was diluted with dichloromethane, filtered through Celite®, washed with 20% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (eluent: 20 – 30 % hexane/EtOAc) to give **33** (67 mg, 51% and recovered **26** (12 mg, 16%), in the form of a colorless oil. $[\alpha]_D^{21}$ = +18.3 (*c* 0.53, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.19 (m, 25H), 5.61 (d, *J* = 3.9 Hz, 1H, H-1'), 5.20 (d, *J* = 3.5 Hz, 1H, H-1''), 5.16 (d, *J* = 10.9 Hz, 1H, PhCH2), 4.94 – 4.83 (m, 3H, $PhCH_2$), 4.79 (d, *J* = 10.9 Hz, 2H, $PhCH_2$), 4.67 – 4.64 (m, 2H, $PhCH_2$), 4.56 – 4.48 (m, 2H, $PhCH_2$), 4.20 (dt, *J* = 10.1, 2.5 Hz, 1H, H-5''), 4.01 – 3.96 (m, 2H, H-2'', H-3'), 3.82 (dd, *J* = 10.8, 2.9 Hz, 1H, CH_2, H-6'a), 3.78 – 3.73 (m, 3H, H-5, CH_2, H-5''a, H-4'), 3.70 – 3.64 (m, 2H, H-4, CH_2, H-6'b), 3.52 (t, *J* = 9.3 Hz, 1H, H-6), 3.51 – 3.44 (m, 2H, H-1, H-3''), 3.41 (ddd, *J* = 12.5, 9.9, 4.6 Hz, 1H, H-3), 3.36 (d, *J* = 12.6 Hz, 1H, CH_2, H-6'b), 3.28 (dd, *J* = 10.4, 3.9 Hz, 1H, H-2''), 2.82 (s, 3H, NCH_3), 2.35 (dt, *J* = 13.1, 4.5 Hz, 1H, H-2a), 1.56 – 1.50 (m, 1H, H-2b), 1.20 (s, 3H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.4, 138.2, 138.1, 137.9, 137.8, 137.4, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.2, 126.4, 97.8, 96.1, 83.0, 80.5, 80.3, 78.1, 77.4, 77.1, 75.4, 75.0, 74.9, 74.9, 73.6, 72.2, 71.3, 68.0, 66.9, 63.2, 63.1, 61.0, 59.2, 31.8, 30.0, 23.2; ESI-HRMS: m/z calcd. for $C_{55}H_{60}O_{11}N_{10}Na$ $[M+Na]^+$1059.4335; found, 1059.4338.

Gentamicin X2 tetraaacetate salt (8). A suspension of compound **33** (23 mg, 21.9 μmol) and



$Pd(OH)_2/C$ (10%, 50 mg) in dioxane: H_2O (1:1, 0.8 mL) was subjected to hydrogenolysis under 40 psi of H_2 for 36 h. After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated to dryness. The crude residue was re-dissolved in a saturated aqueous solution of $Ba(OH)_2$ (0.5 mL) and heated to 60 °C for 12 h. After complete hydrolysis of the oxazolidinone, the reaction mixture was neutralized to pH 7 by addition of dry ice and the precipitated solid was filtered off. Th filter cake was washed with water and the filtrate was concentrated to dryness. The crude product was taken up in 10% aqueous acetic acid and loaded on a prepacked Sephadex C-25 column and eluted with 0.1% to 1.5% ammonium hydroxide in deionized water. The product-containing fractions were combined, concentrated, acidified with glacial acetic acid, and lyophilized to give the pentaacetate salt of gentamicin X2 (**8**) as a white foam (6.6 mg, 61 %). $[\alpha]_D^{21}$ = +64.2 (*c* 0.07, H_2O); 1H NMR (600 MHz, D_2O) δ 5.60 (d, *J* = 4.1 Hz, 1H, H-1'), 5.04 (d, *J* = 3.7 Hz, 1H, H-1"), 4.17 (dd, *J* = 10.9, 3.7 Hz, 1H, H-2"), 3.94 (d, *J* = 12.8 Hz, 1H, CH_2, H-5a"), 3.90 – 3.79 (m, 3H), 3.80 – 3.66 (m, 4H), 3.54 – 3.38 (m, 6H), 2.86 (s, 3H, NCH_3), 2.48 (dt, *J* = 12.7, 4.3 Hz, 1H, H-2a), 1.85 (q, *J* = 12.6 Hz, 1H, H-2b), 1.29 (s, 3H, CH_3).^{13}C NMR (151 MHz, D_2O) δ 101.2, 97.2, 83.5, 80.6, 73.8, 73.7, 69.8, 69.3, 69.0, 67.8, 66.2, 63.3, 60.4, 53.9, 49.5, 48.6, 34.5, 27.9, 20.9; ESI-HRMS: m/z calcd. for $C_{19}H_{39}O_{10}N_4[M+H]^+$ 483.2666; found, 483.2649.

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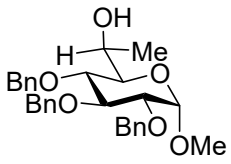
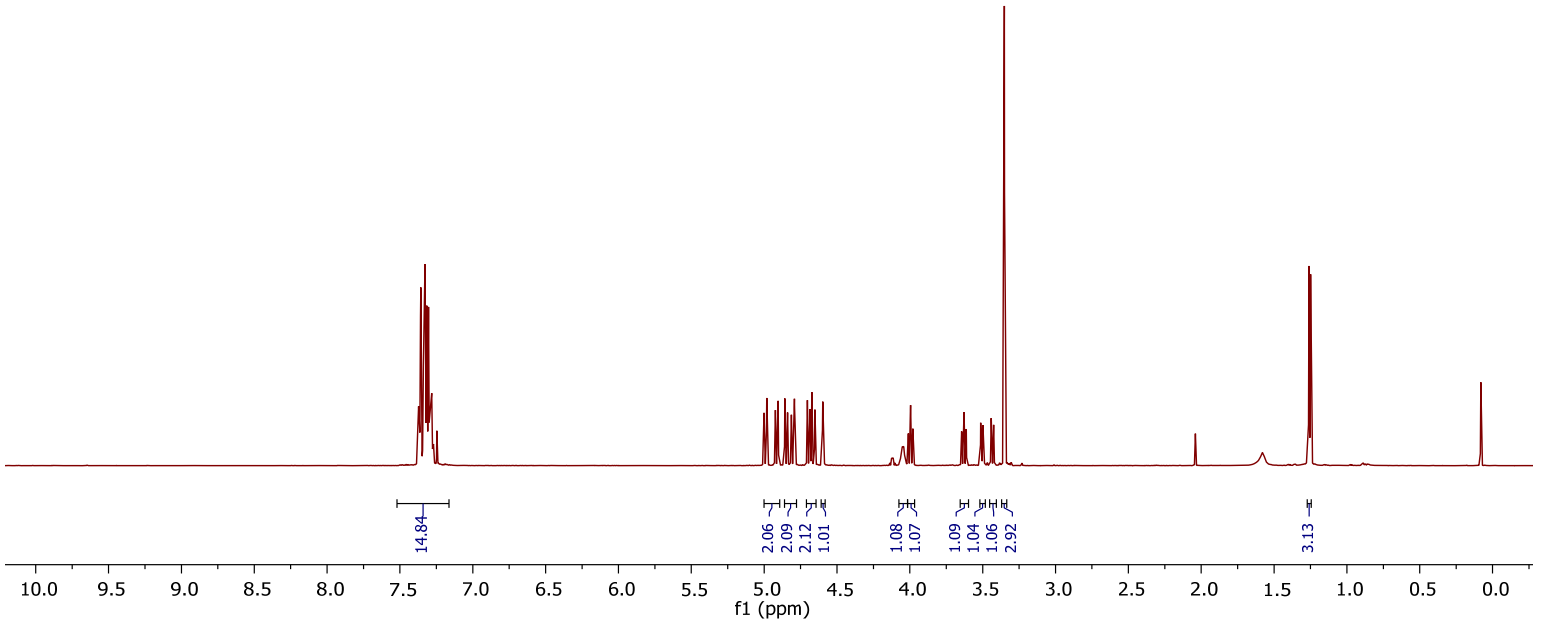
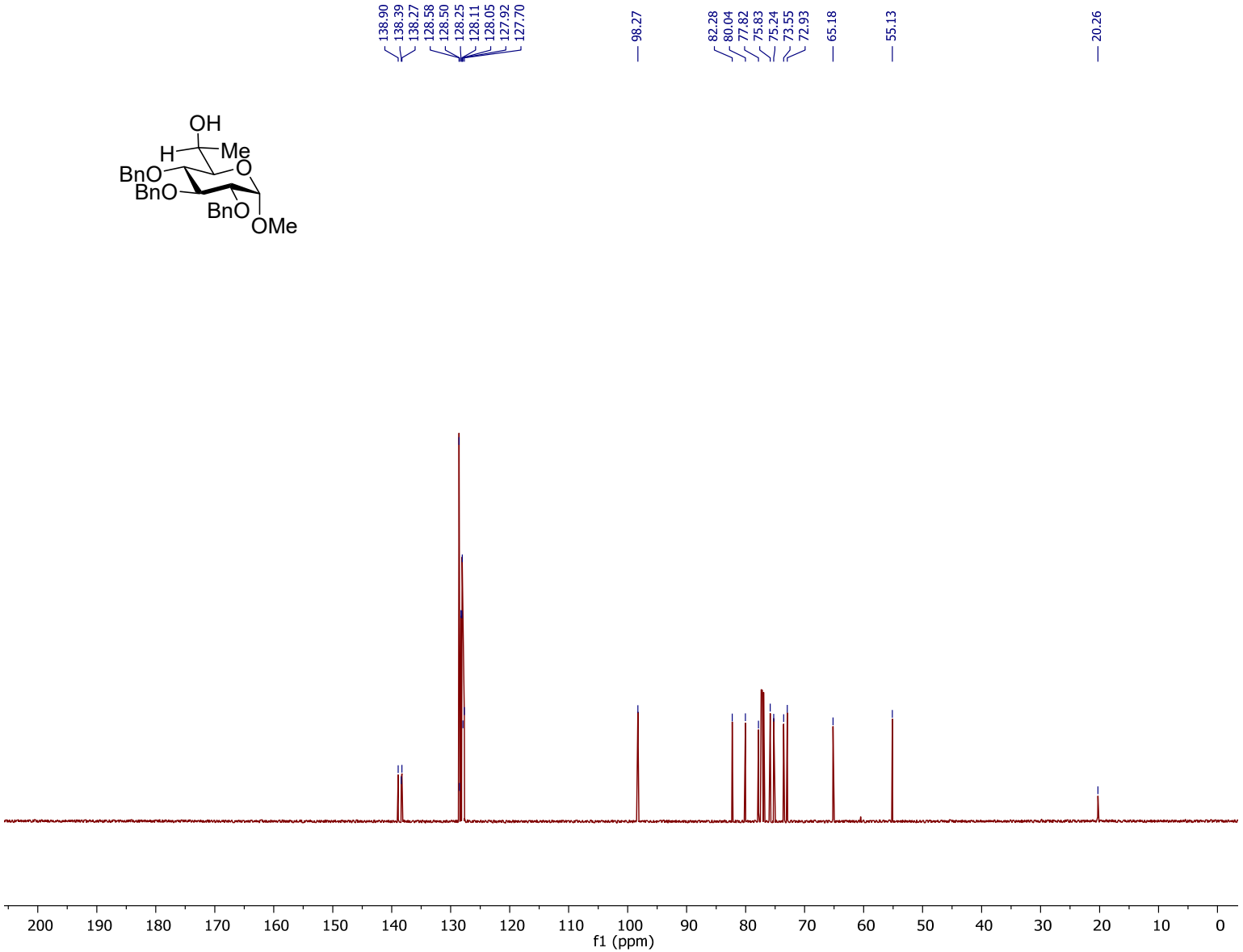
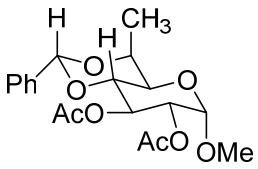


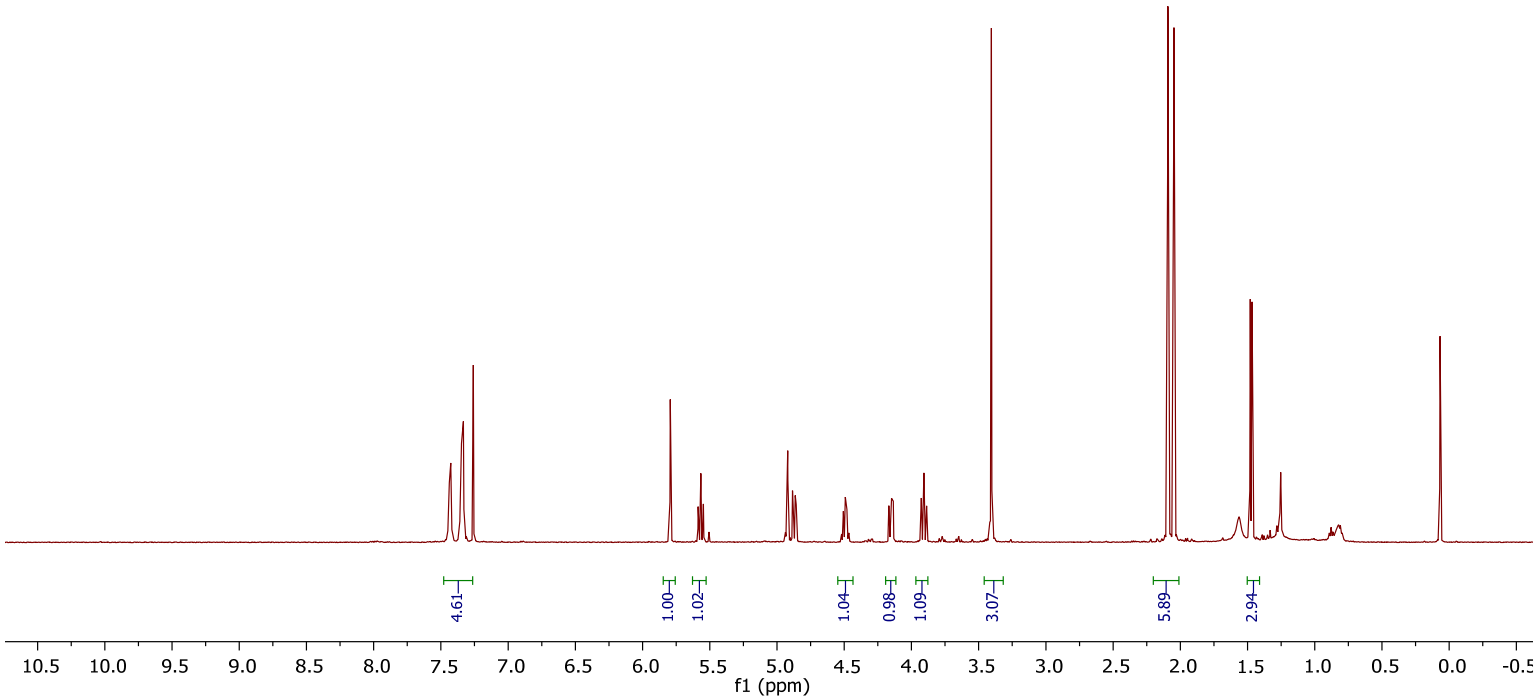


^{1}H NMR (600 MHz, $CDCl_3$) of Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-α-L-glycero-D-gluco-heptopyranoside **(28)**

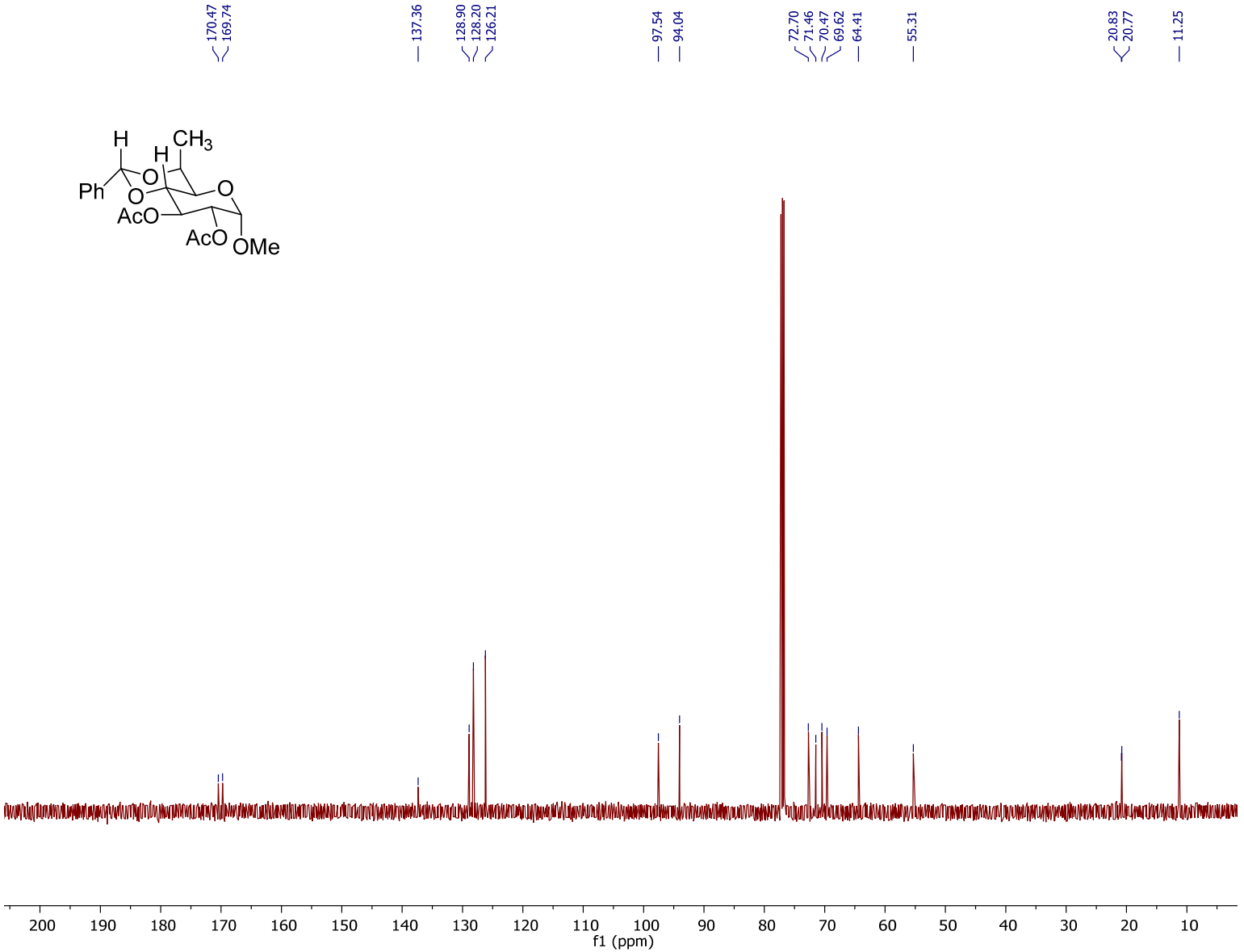


^{13}C NMR (150 MHz, $CDCl_3$) of Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-α-L-glycero-D-gluco-heptopyranoside **(28)**

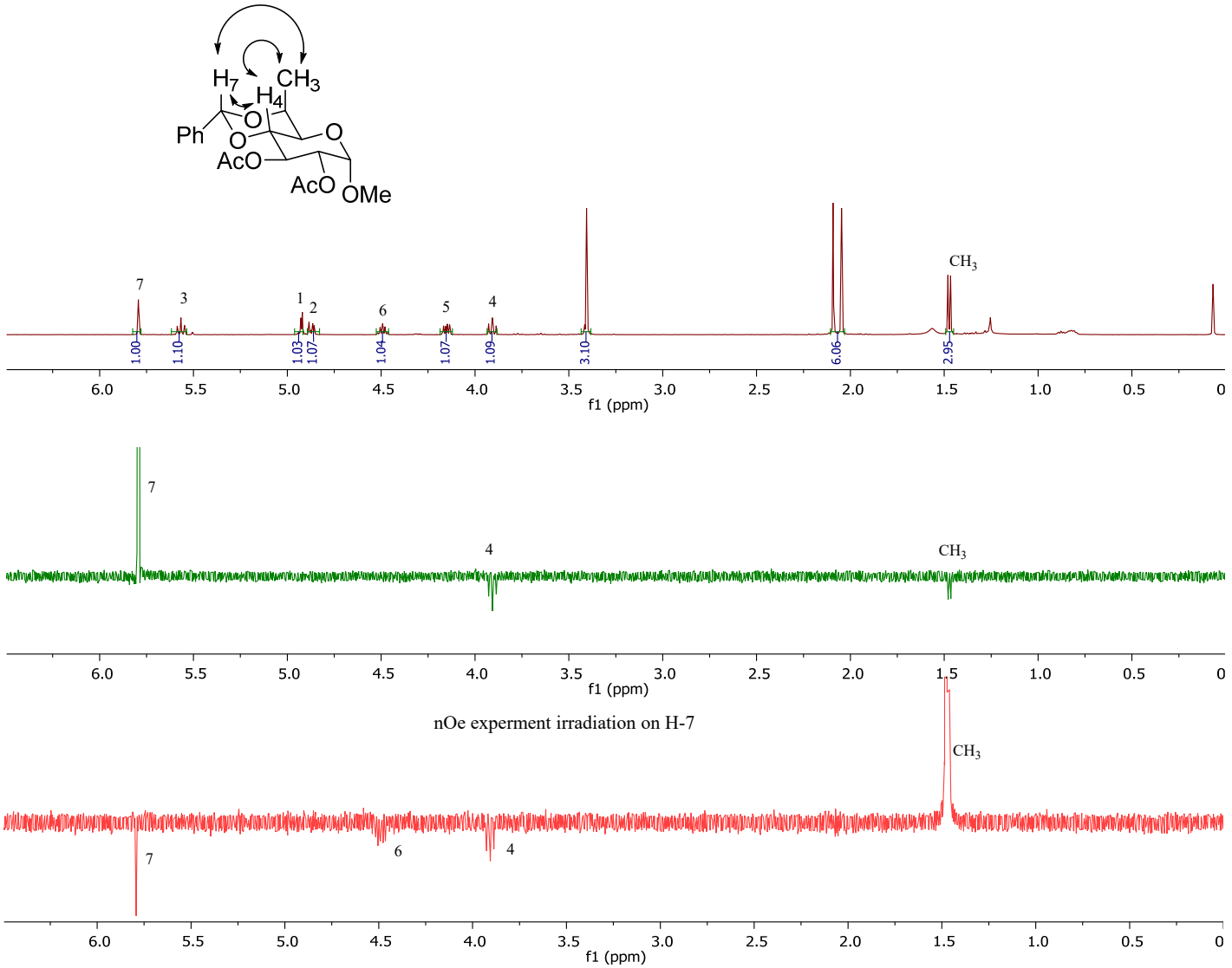




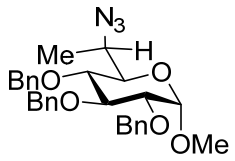
^{1}H NMR (500 MHz, $CDCl_3$) of Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-α-L-glycero-D-gluco-heptopyranoside **(S1)**

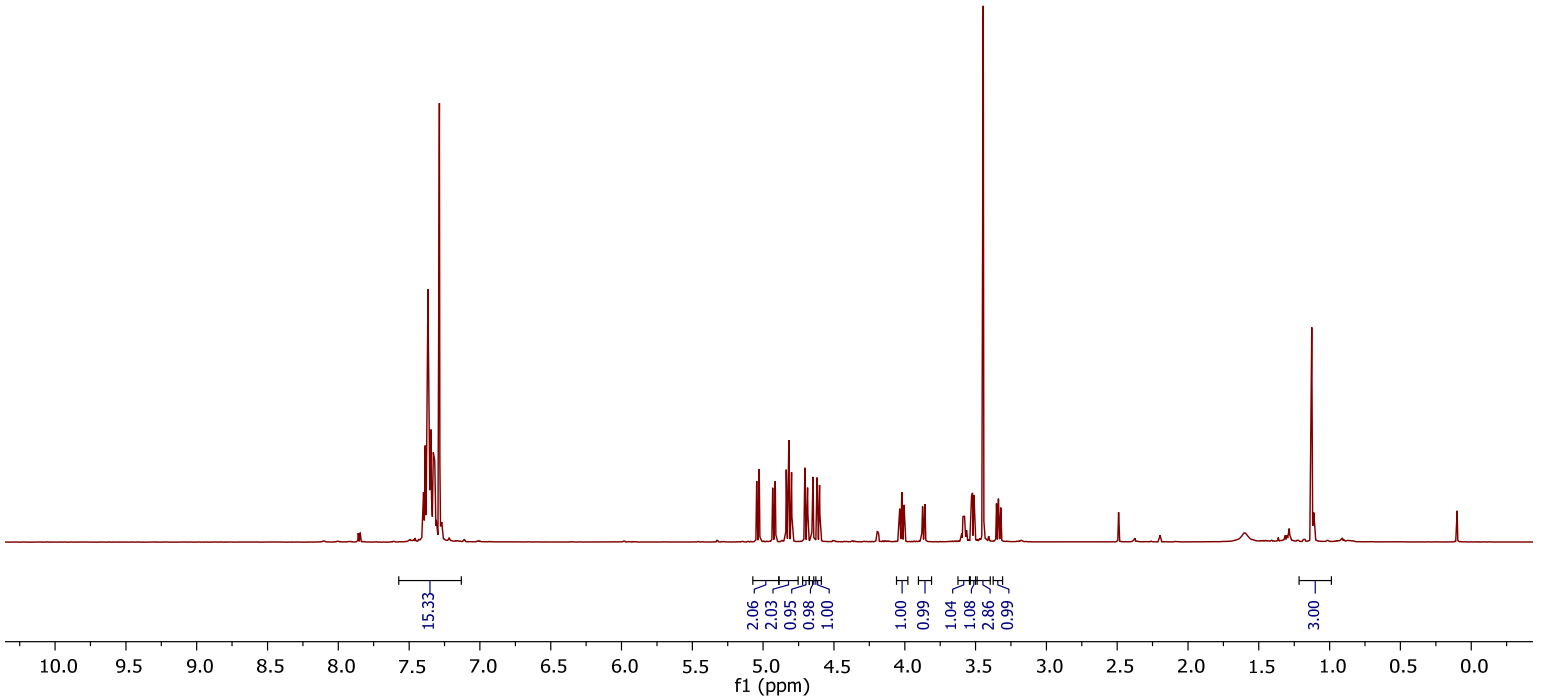


^{13}C NMR (125 MHz, $CDCl_3$) of Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-α-L-glycero-D-gluco-heptopyranoside **(S1).**

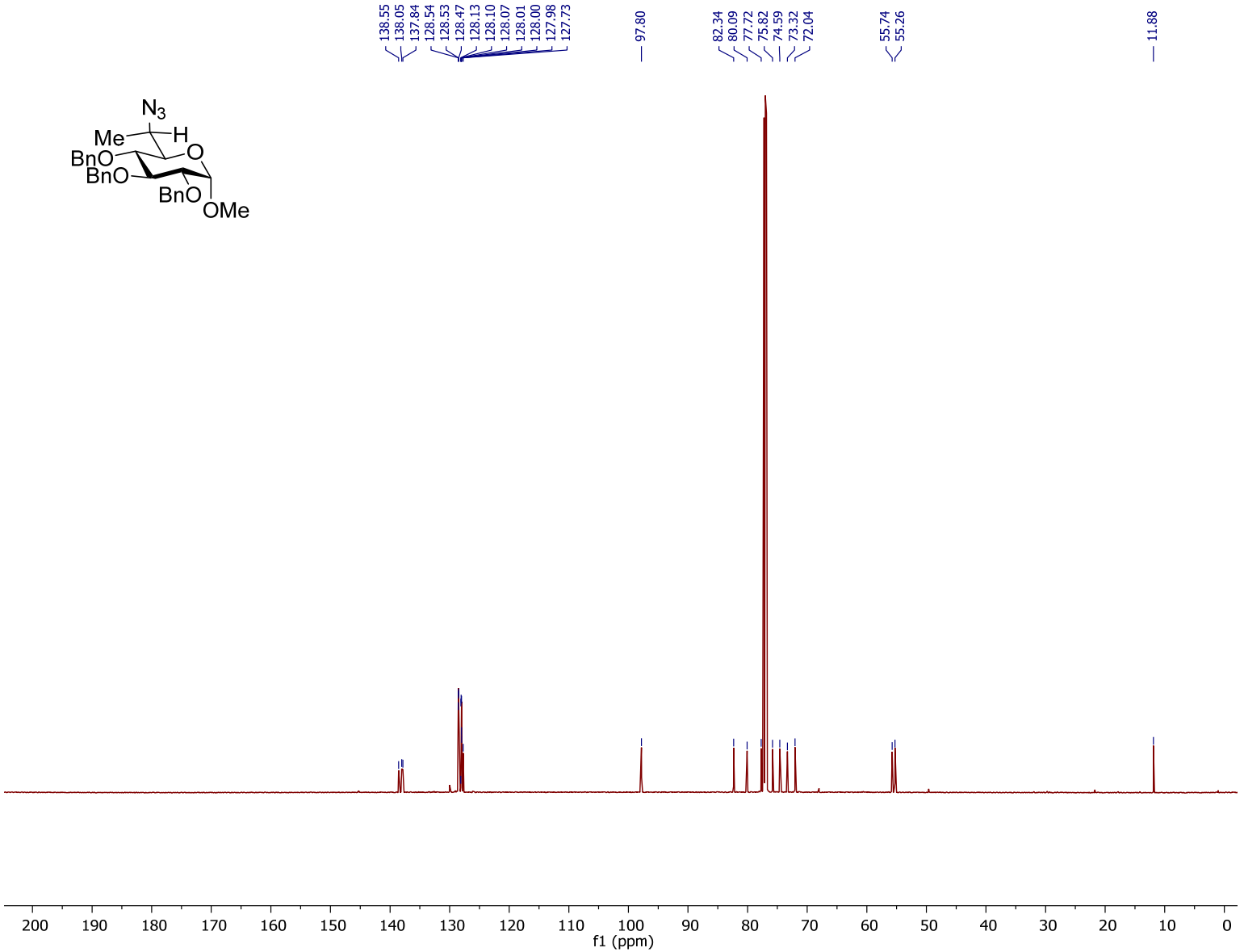


nOe Spectra (500 MHz, $CDCl_3$) of Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-α-L-glycero-D-gluco-heptopyranoside **(S1)**

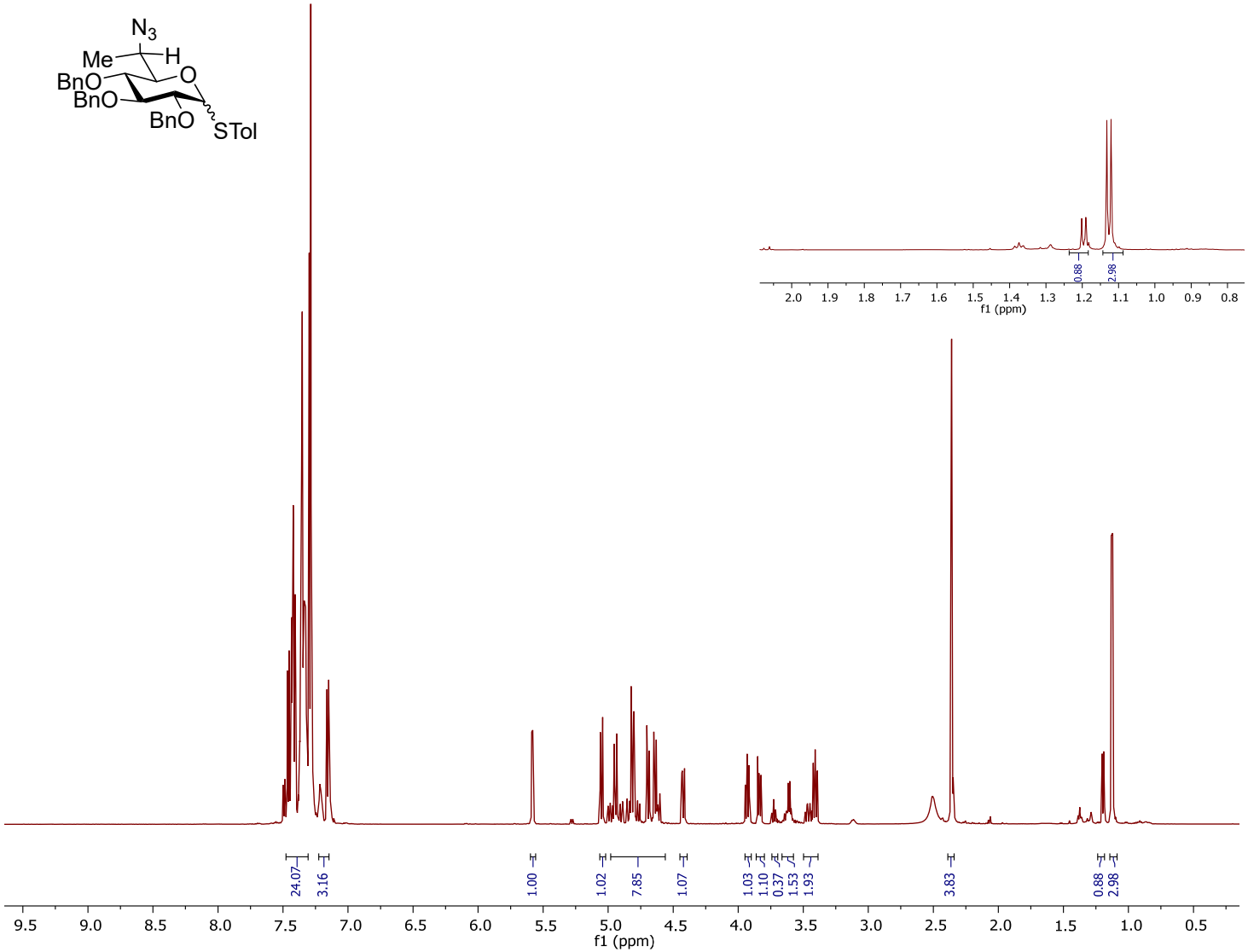




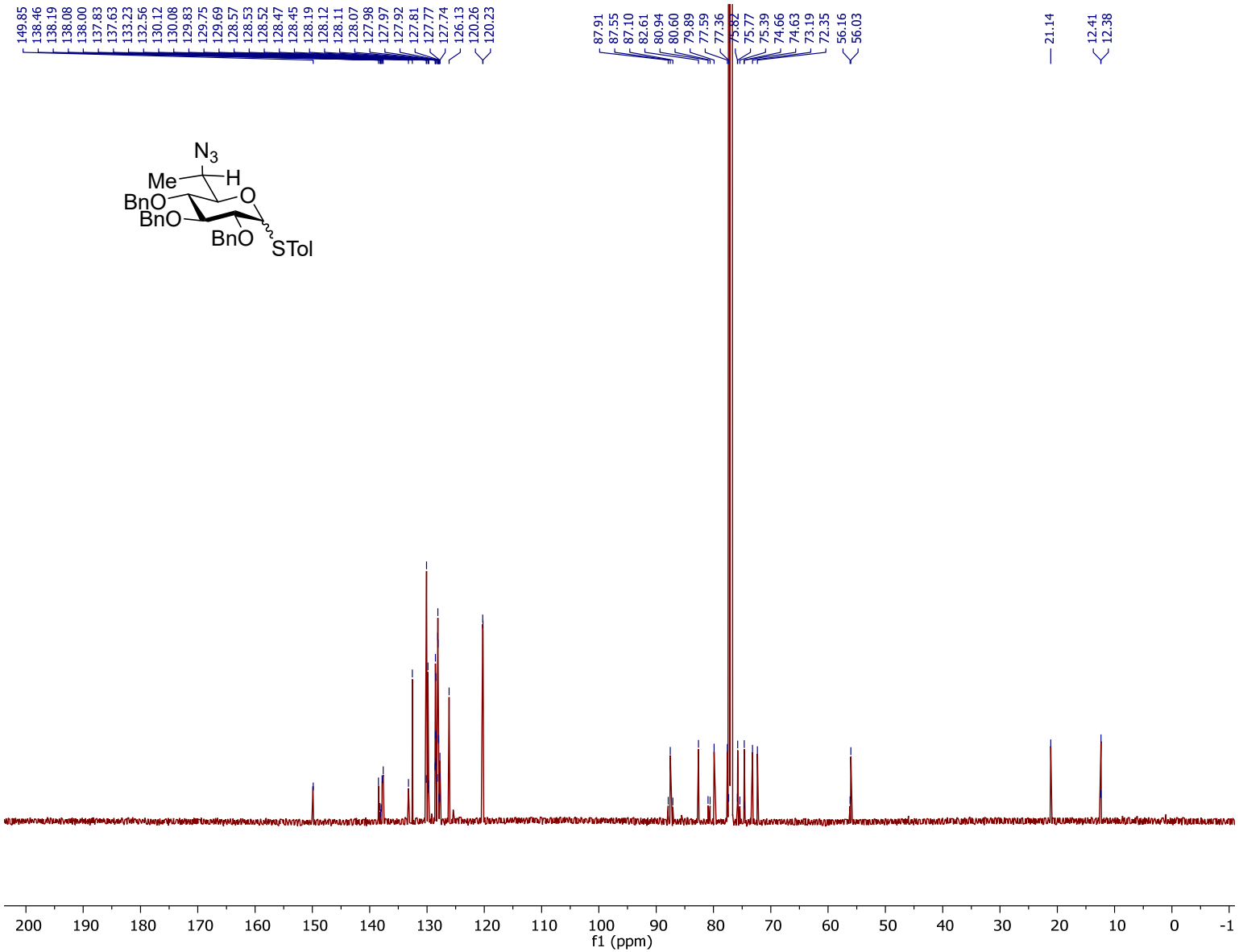
^{1}H NMR (600 MHz, $CDCl_3$) of Methyl 6-azido-2,3,4-tri-O-benzyl-6,7-dideoxy-α-D-glycero-D-gluco-heptopyranoside **(29).**



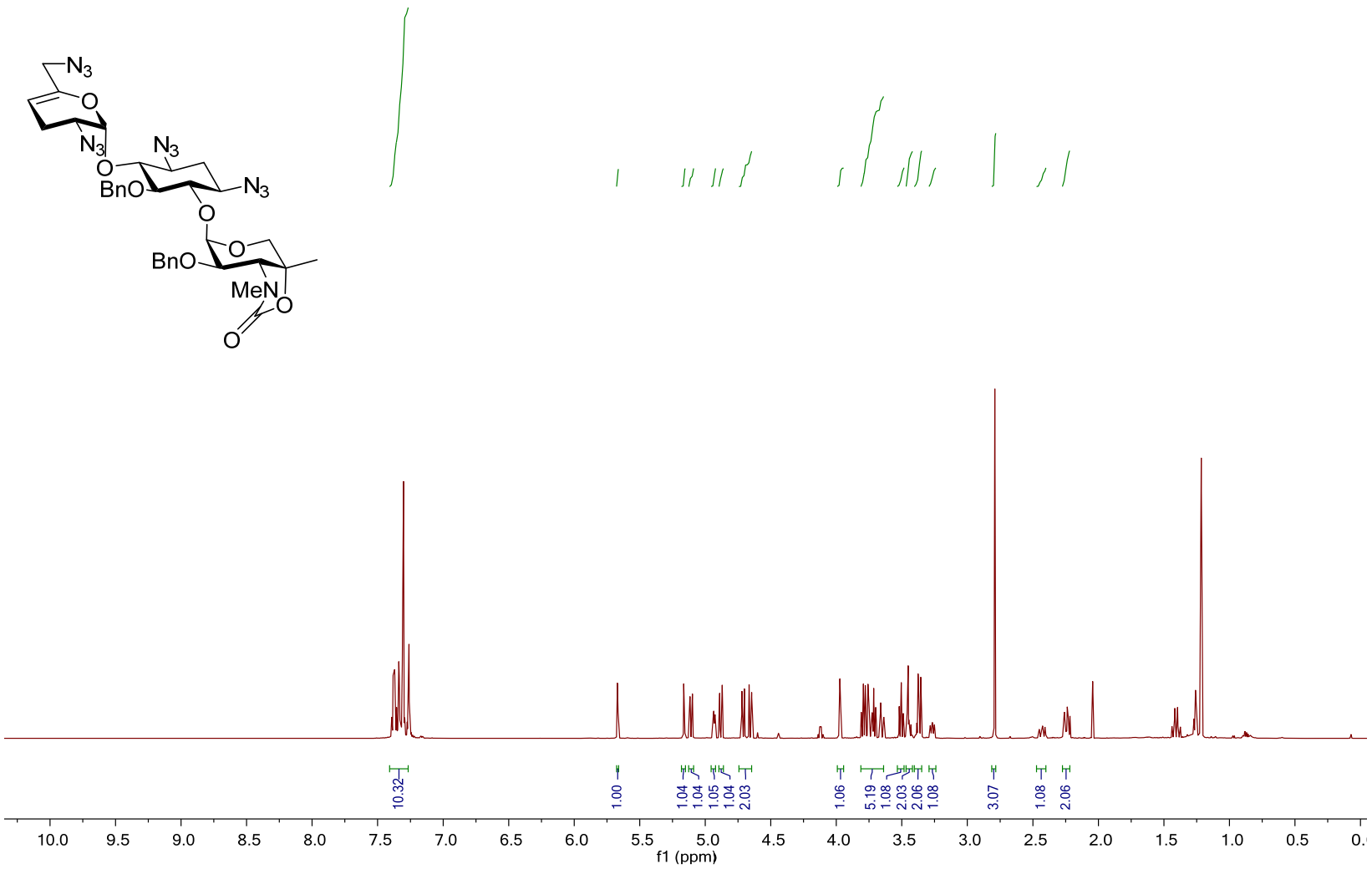
^{13}C NMR (150 MHz, $CDCl_3$) of Methyl 6-azido-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α-D-glycero-D-gluco-heptopyranoside **(29).**



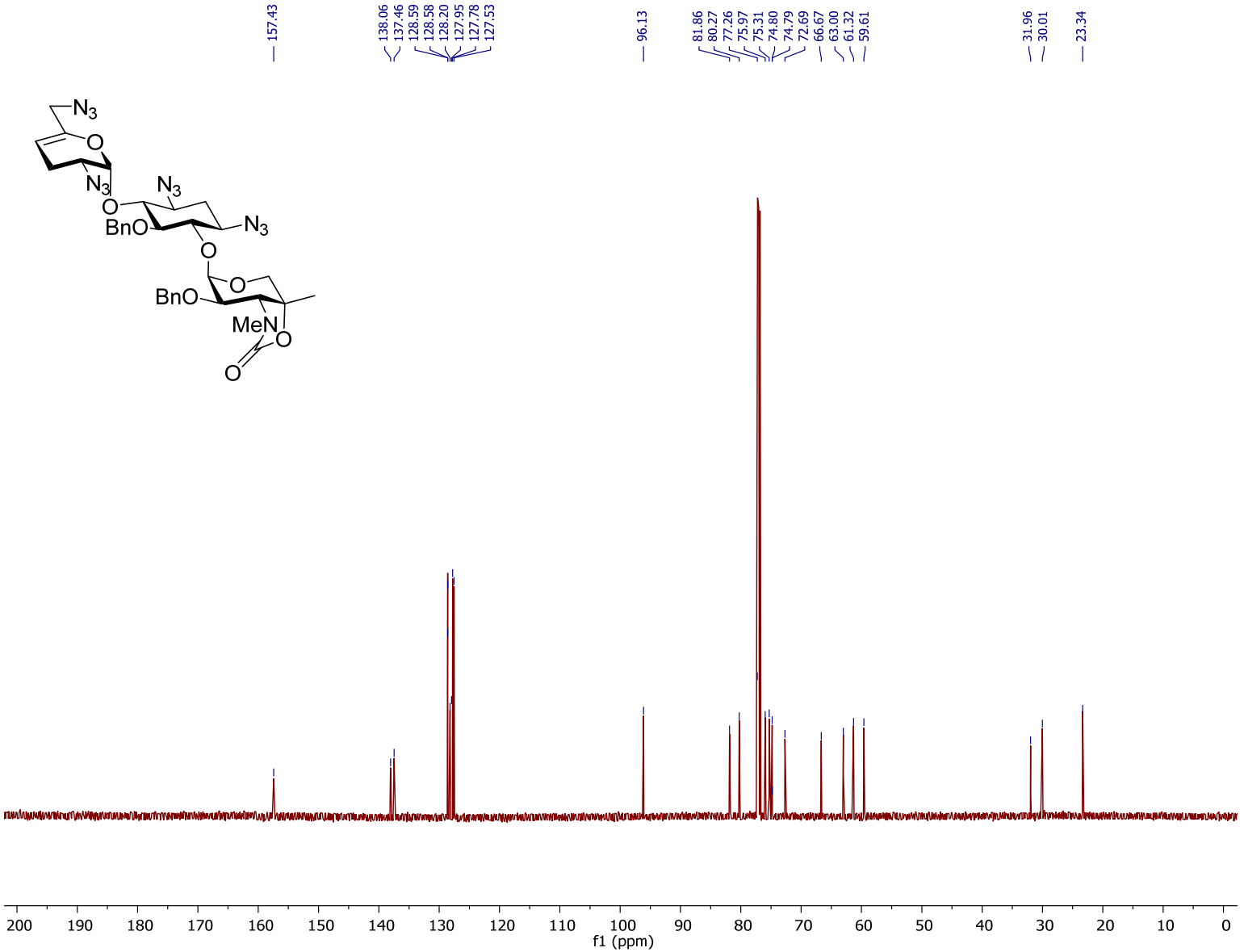
^{1}H NMR (600 MHz, $CDCl_3$) of *p*-Tolyl 6-azido-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α,β-D-glycero-D-gluco-heptothiopyranoside **(30).**



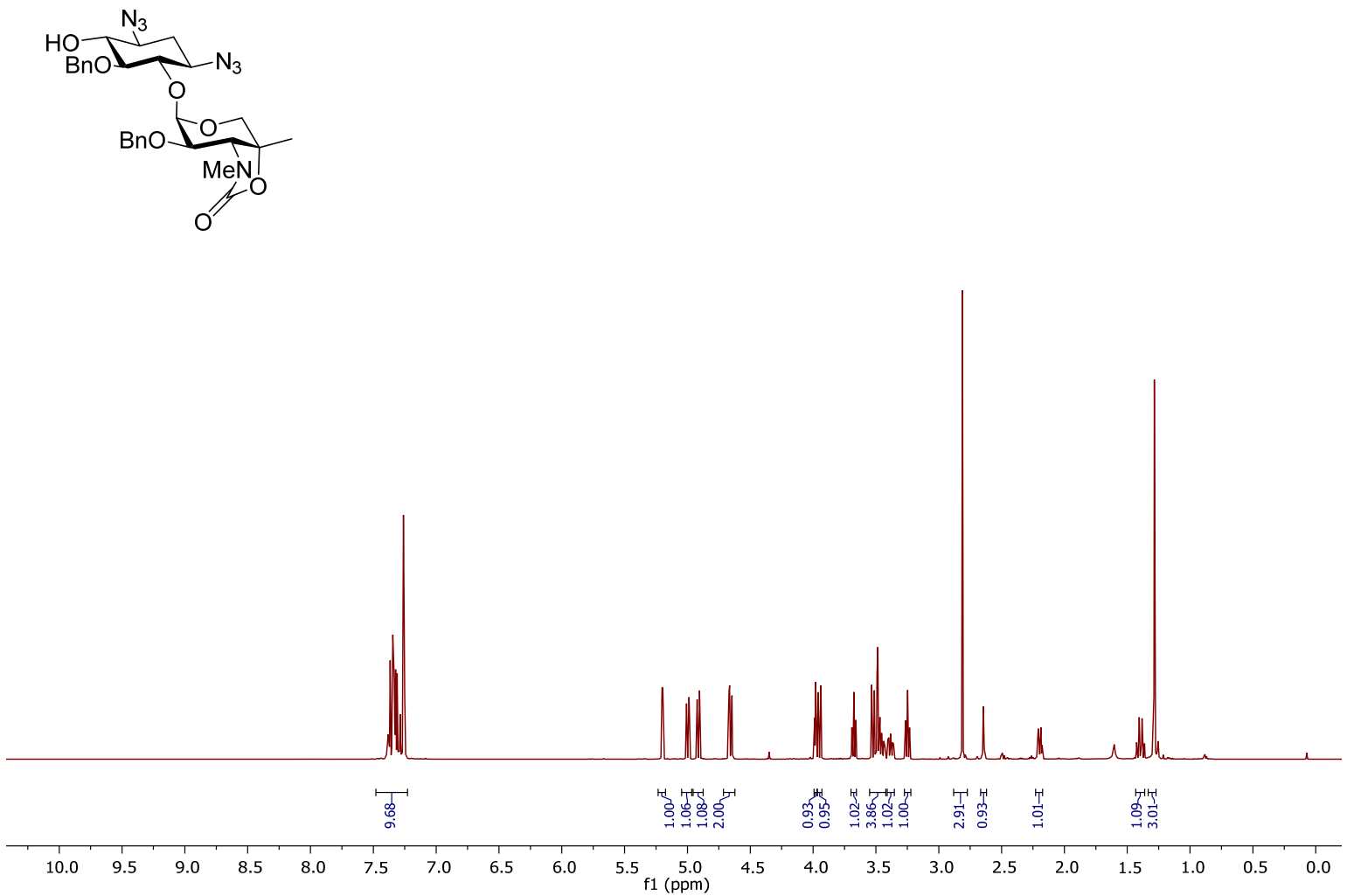
^{13}C NMR (150 MHz, $CDCl_3$) of *p*-Tolyl 6-azido-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α,β-D-glycero-D-gluco-heptothiopyranoside **(30).**



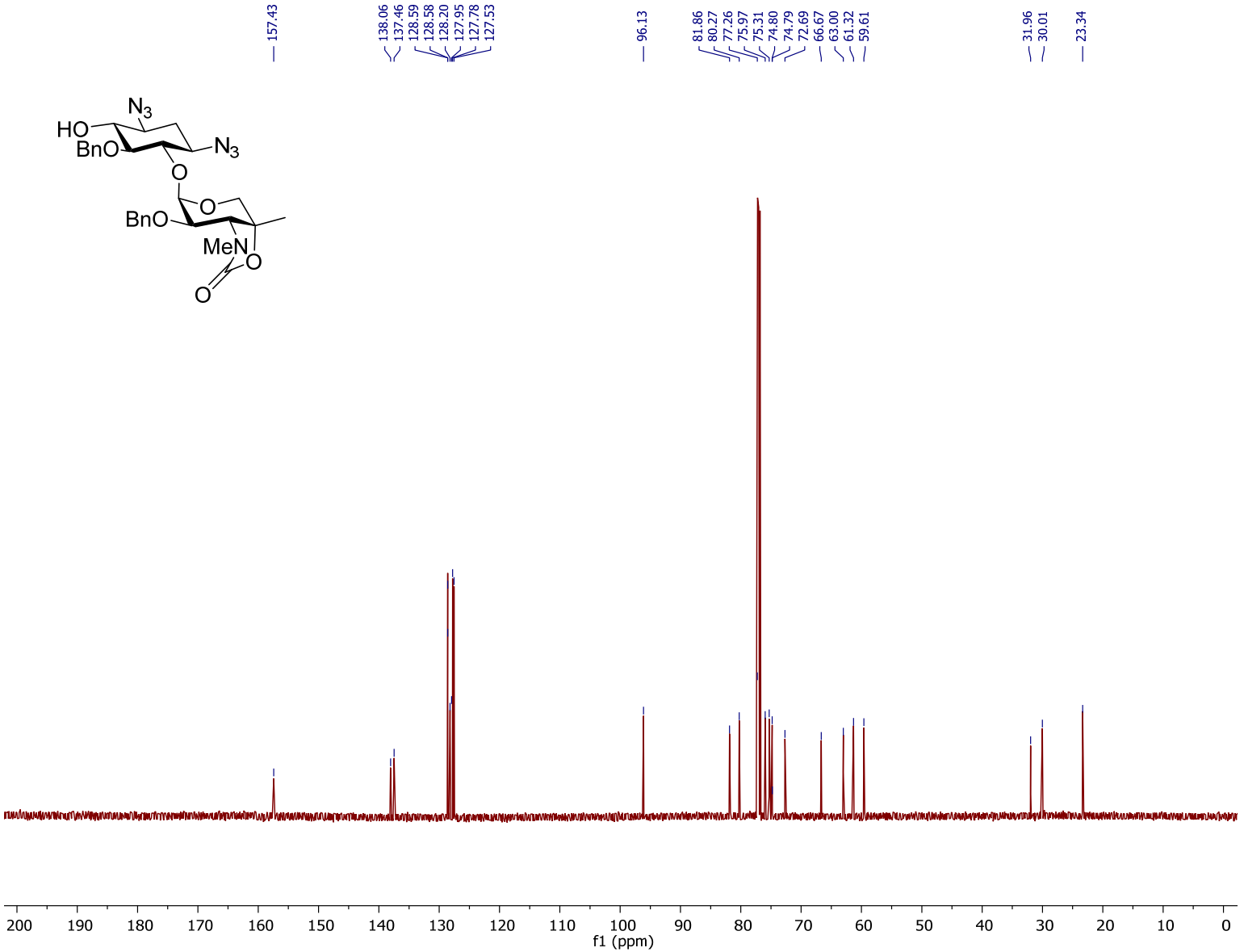
^{1}H NMR (600 MHz, $CDCl_3$) 1,3,2′,6′-Tetra(deamino)-5,2″-di-*O*-benzyl-1,3,2′,6′-tetraazido-3″-*N*,4″-*O*-carbonyl-sisomycin **(25)**.



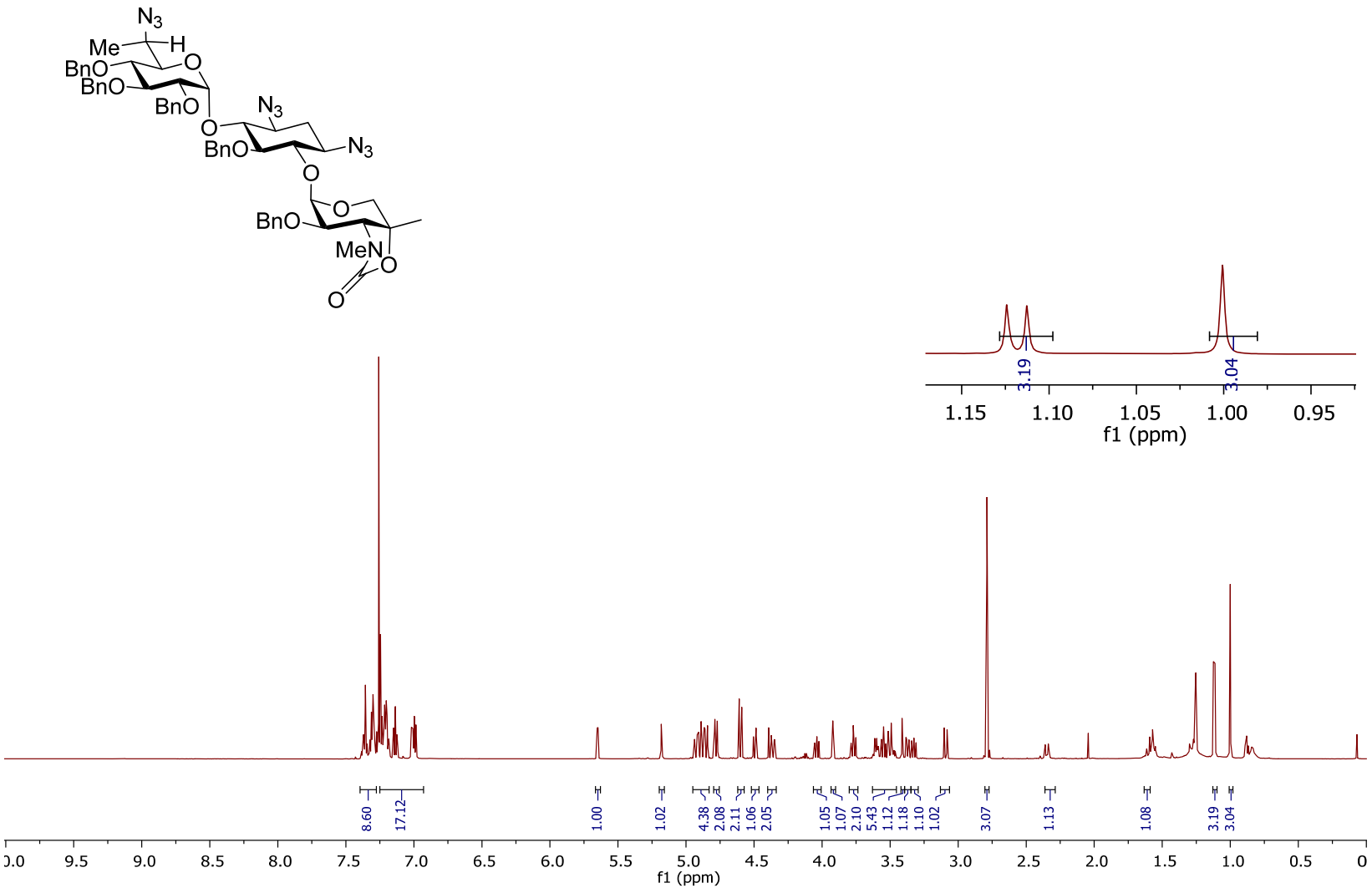
^{13}C NMR (150 MHz, $CDCl_3$) 1,3,2′,6′-Tetra(deamino)-5,2″-di-*O*-benzyl-1,3,2′,6′-tetraazido-3″-*N*,4″-*O*-carbonyl-sisomycin **(25)**.



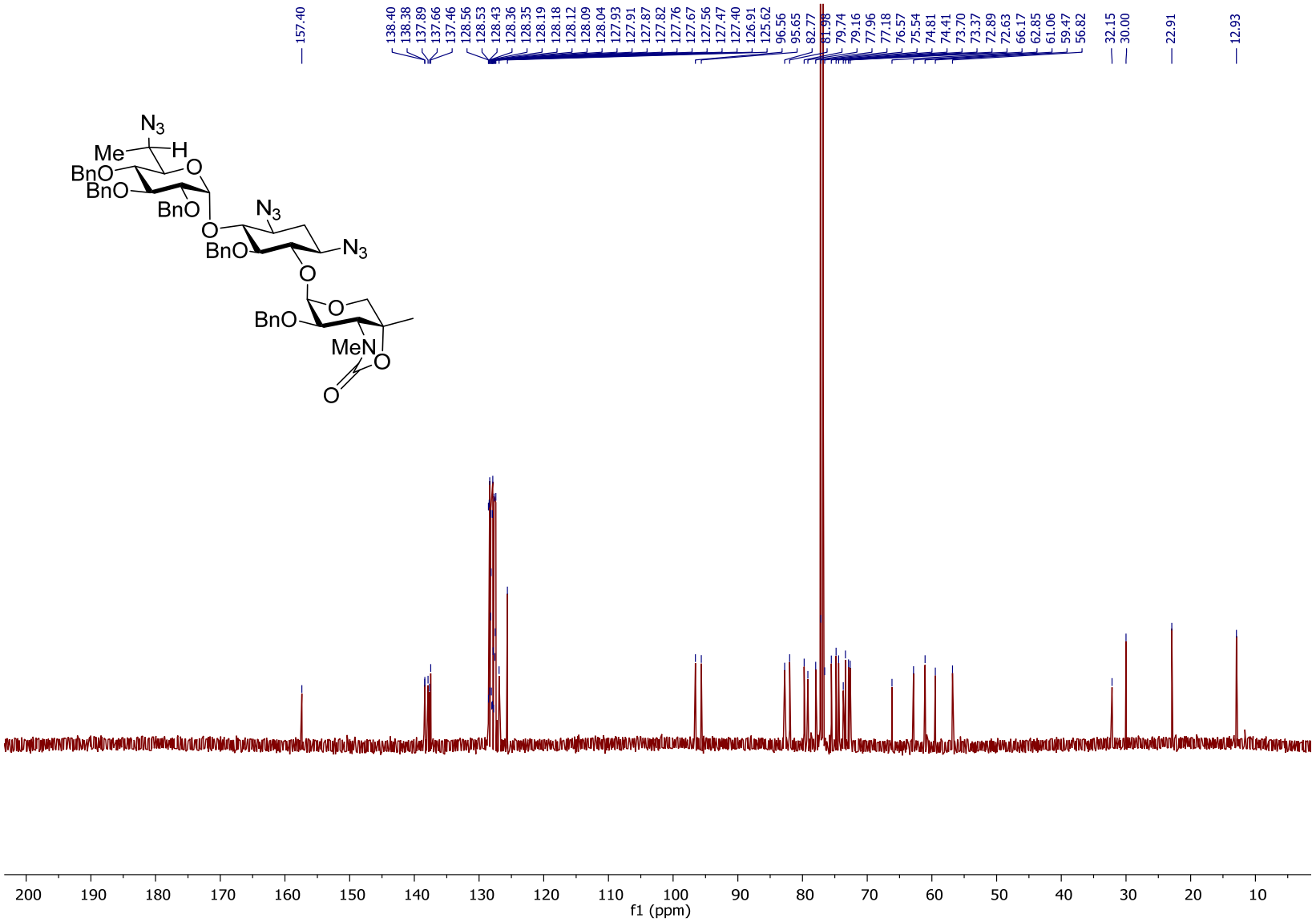
^{1}H NMR (600 MHz, $CDCl_3$) 5,2′-Di-*O*-benzyl-1,3-di(deamino)-1,3-diazido-3′-*N*,4′-*O*-carbonyl-garamine **(26)**.



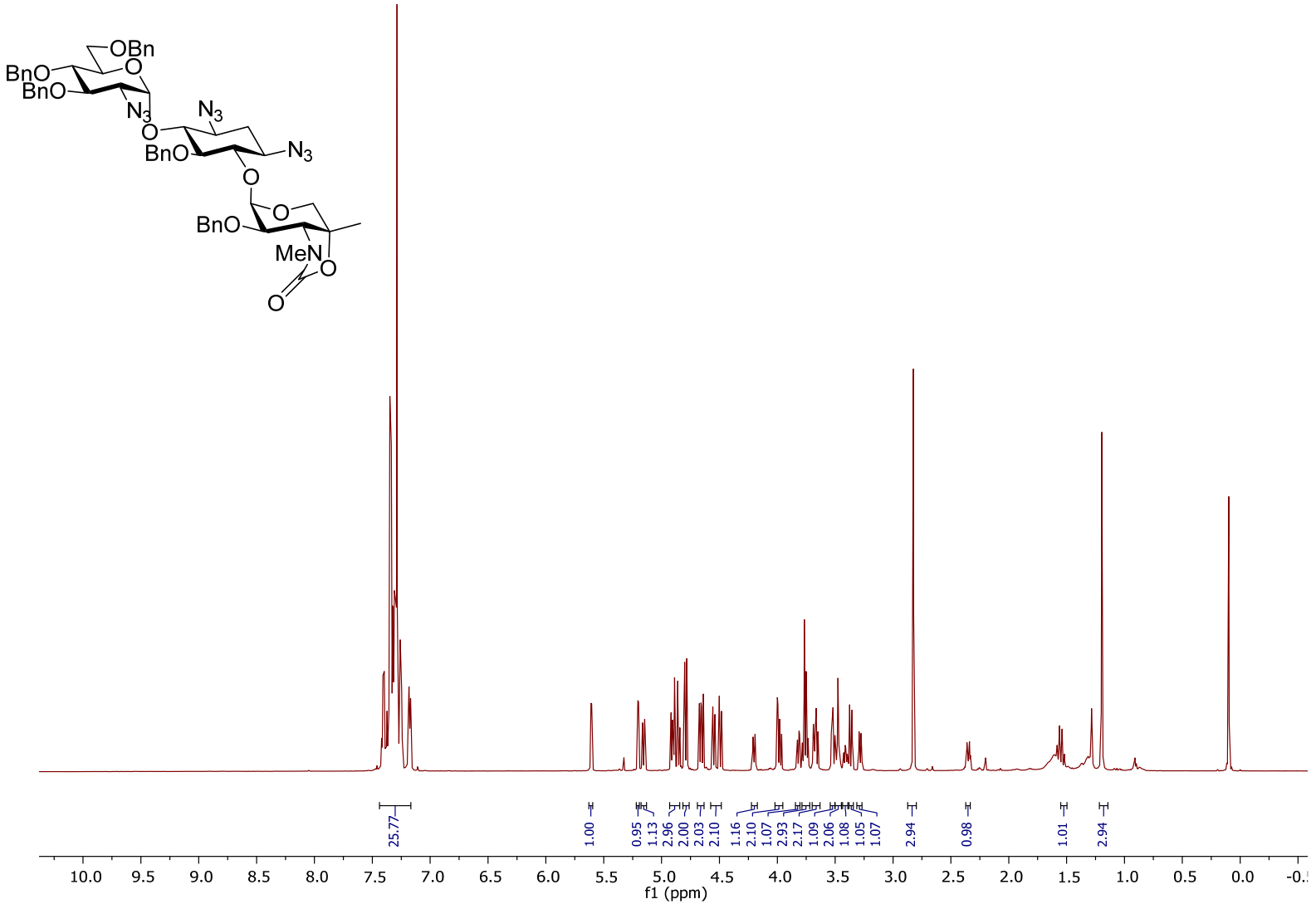
^{13}C NMR (150 MHz, $CDCl_3$) 5,2′-Di-*O*-benzyl-1,3-di(deamino)-1,3-diazido-3′-*N*,4′-*O*-carbonyl-garamine **(26)**.



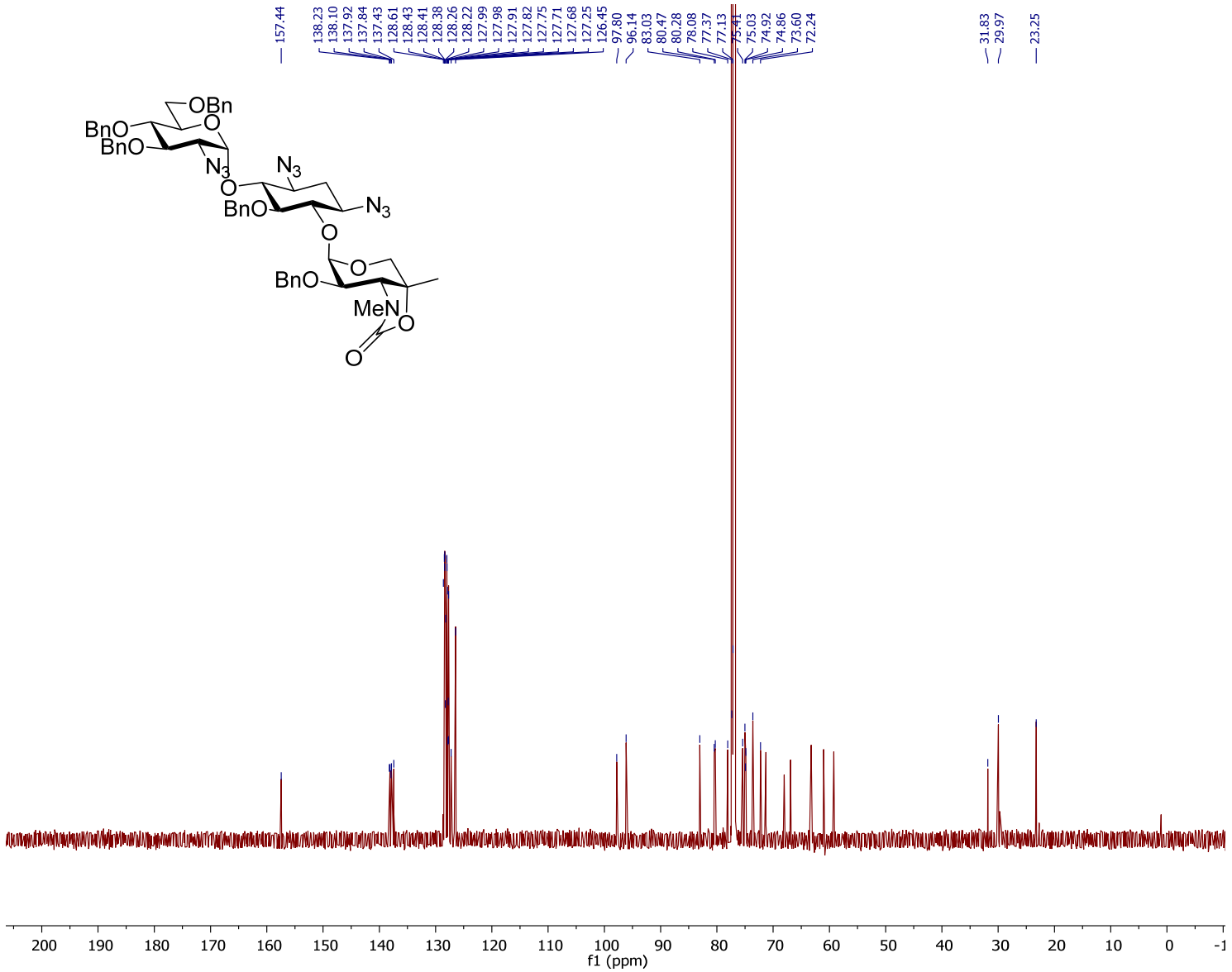
^{1}H NMR (600 MHz, $CDCl_3$) 5,2′,3′,4′,2″-Penta-*O*-benzyl-1,3,6′-triazido-1,3,6′-tri(deamino)-3″-*N*,4″-*O*-carbonyl-gentamicin B1 **(31)**.



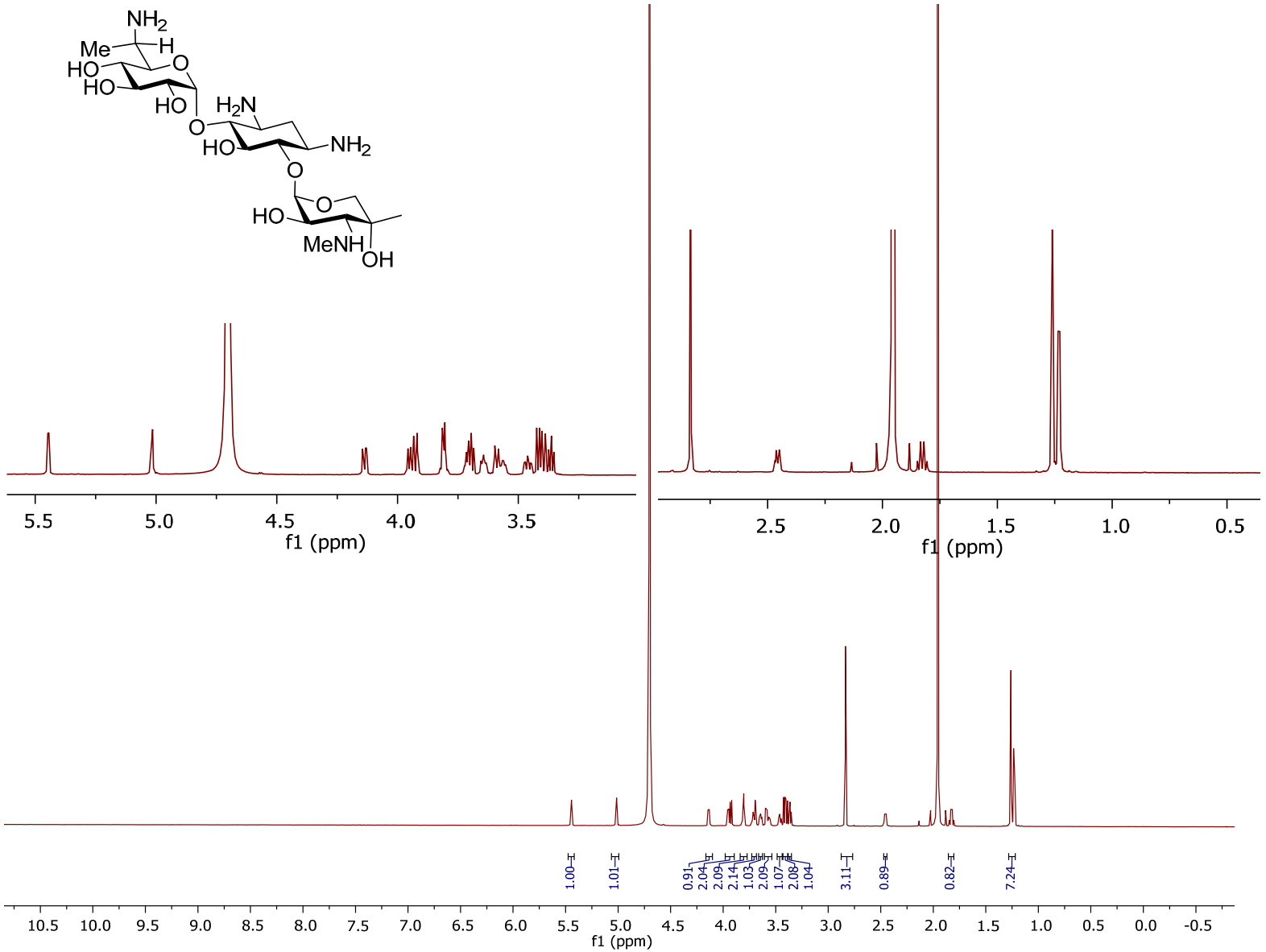
^{13}C NMR (150 MHz, $CDCl_3$) 5,2′,3′,4′,2″-Penta-*O*-benzyl-1,3,6′-triazido-1,3,6′-tri(deamino)-3″-*N*,4″-*O*-carbonyl-gentamicin B1 **(31)**.



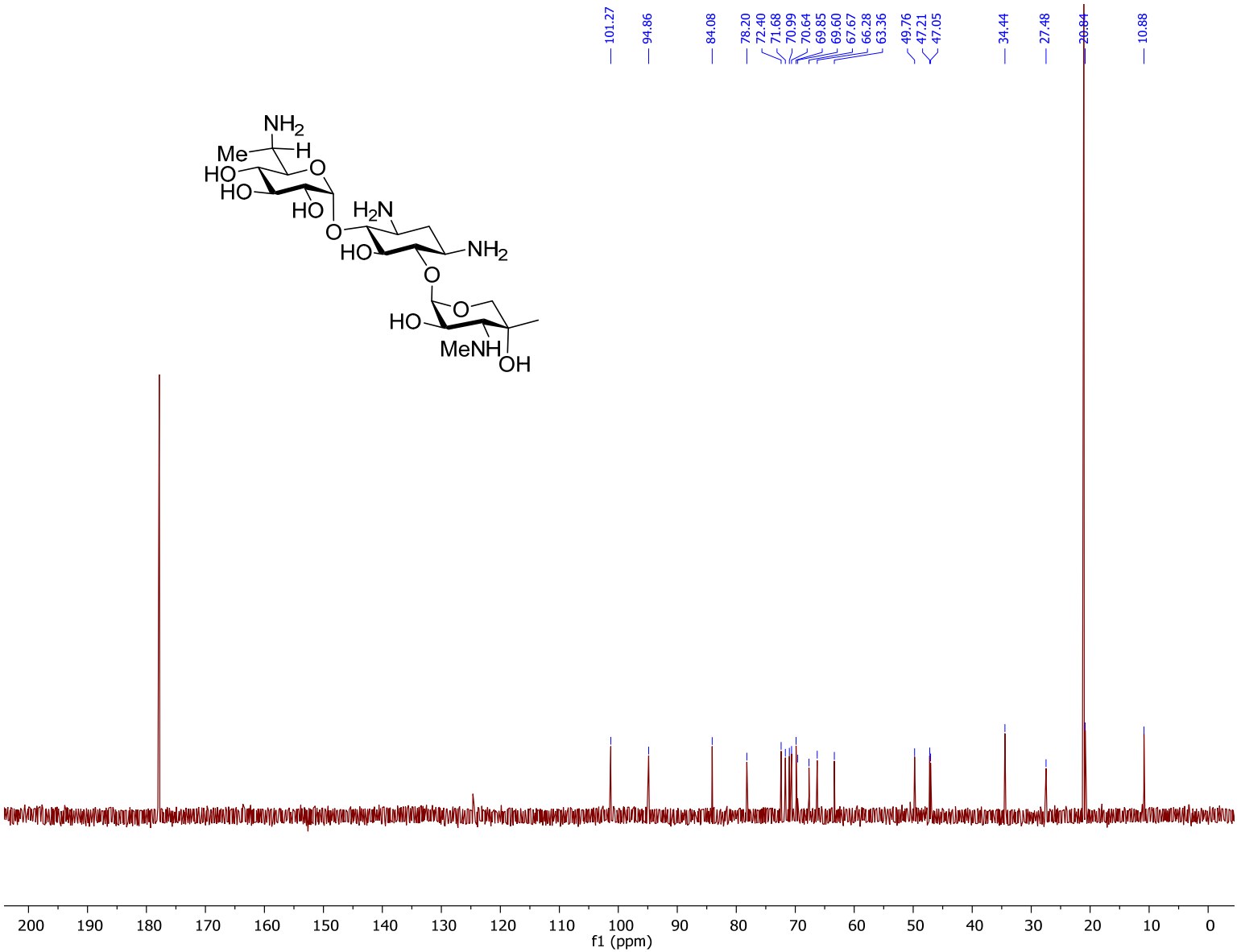
^{1}H NMR (600 MHz, $CDCl_3$) of 5,3′,4′,6′,2″-Penta-*O*-benzyl-1,3,2′-triazido-1,3,2′-tri(deamino)-3″-*N*,4″-*O*-carbonyl-gentamicin X2 **(33).**



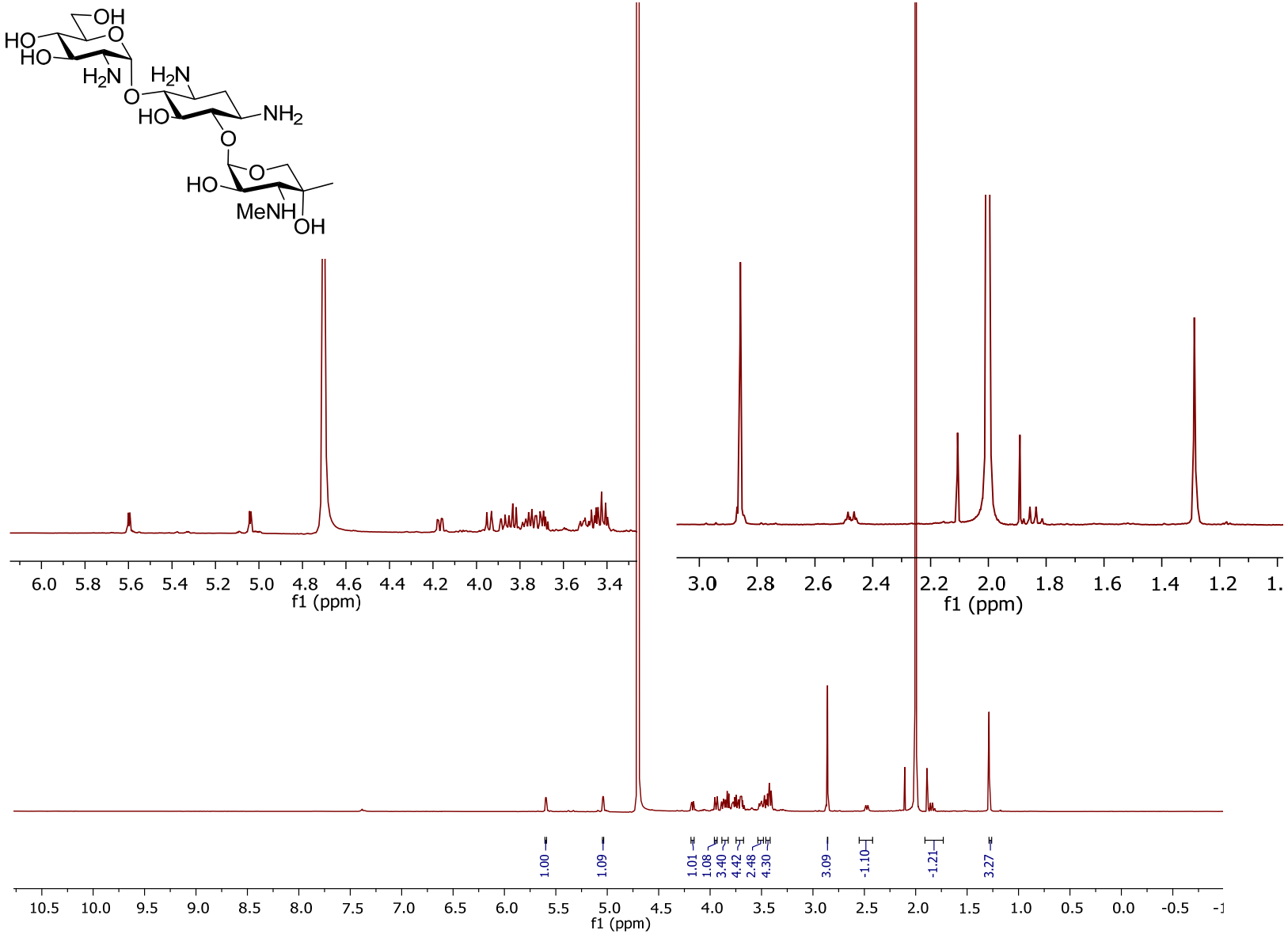
^{13}C NMR (150 MHz, $CDCl_3$) of 5,3′,4′,6′,2″-Penta-*O*-benzyl-1,3,2′-triazido-1,3,2′-tri(deamino)-3″-*N*,4″-*O*-carbonyl-gentamicin X2 **(33).**



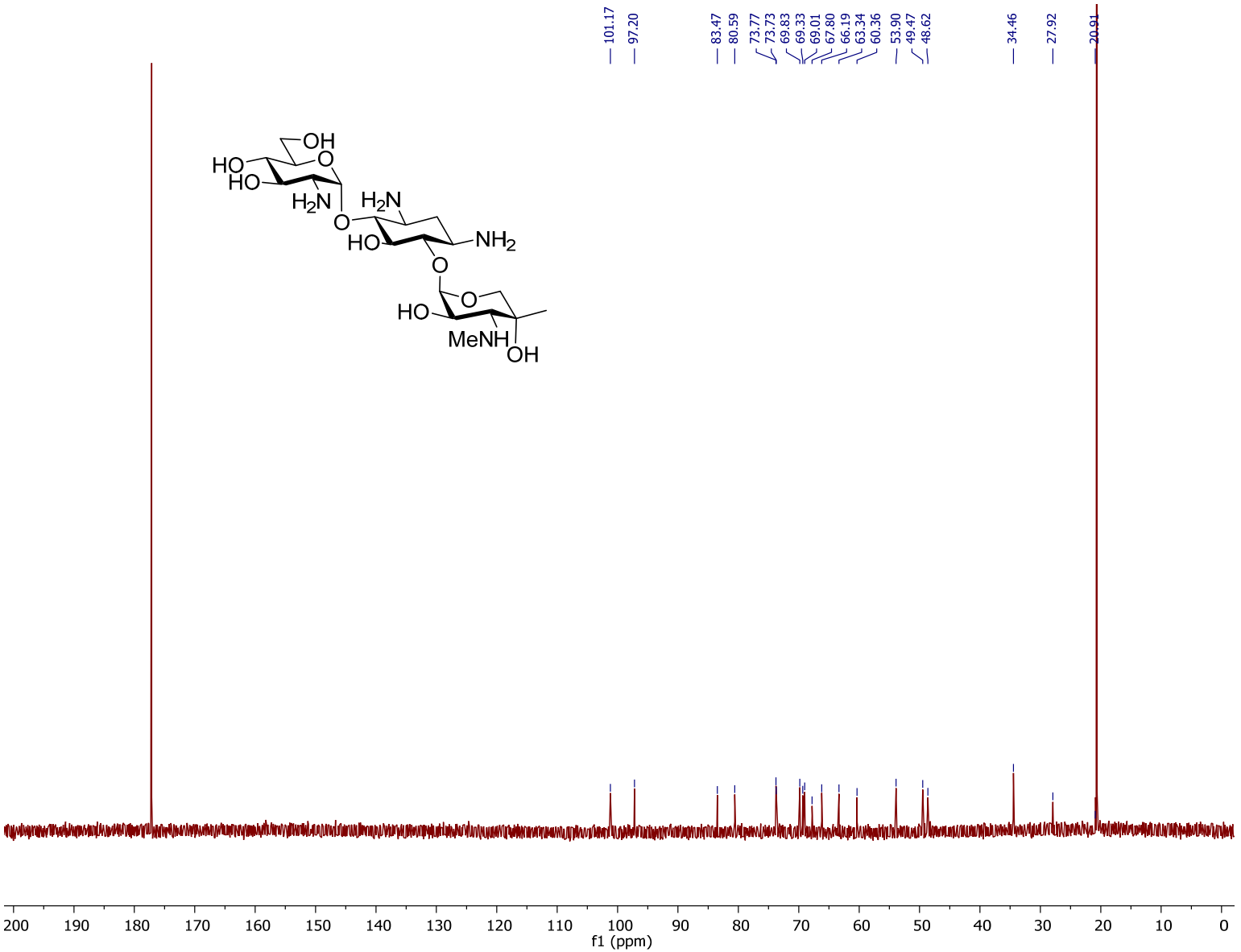
^{1}H NMR (900 MHz, D_2O) of Gentamicin B1 tetraaacetate salt **(7)**.



^{13}C NMR (225 MHz, D_2O) of Gentamicin B1 tetraaacetate salt **(7)**.



^{1}H NMR (600 MHz, D_2O) of Gentamicin X2 tetraaacetate salt **(8)**.



^{13}C NMR (150 MHz, D_2O) of Gentamicin X2 tetraaacetate salt **(8)**.