Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supplementary Information for

Synthesis, Antiribosomal and Antibacterial Activity of 4'-*O*-Glycopyranosyl Paromomycin Aminoglycoside Antibiotics

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4′-*O*-(2′′′′,3′′′′,4′′′′,6′′′′-Tetra-*O*-benzyl-α-D-glucopyranosyl)-1,3,2′, 2′′′,6′′-pentaazido-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (16α) and 4′-*O*-(2′′′′,3′′′′,4′′′′,6′′′′-Tetra-*O*-benzyl-β-D-glucopyranosyl)-1,3,2′, 2′′′,6′′-pentaazido-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (16β). Compounds **16α** (221 mg), **16β** (69 mg) were prepared from **11**[1] (500 mg) by the general glycosylation procedure, which was conducted at -60 °C for 1 h and then at -50 °C for 1 h before quenching with saturated aqueous $NaHCO_3$. The pure anomers were isolated in the form of white gums by with gradient chromatography over silica gel (toluene:EtOAc 98:2 to 95:5) followed by preparative HPLC over silica gel (Agilent dynamax 250*21.4 mm SI, Hexane: EtOAc 90:10, 21.5 ml/min) in a α:β ratio of 3.2: 1 and a combined yield of 42%.

16α: $[\alpha]_D^{RT}$ +71.7 (*c* 4.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 7.50-7.00 (m, 55H, aromatic), 6.11 (d, *J* = 4.03 Hz, 1 H: H1′), 5.64 (d, *J* = 5.5 Hz, 1 H: H1′′), 5.44 (d, *J* = 2.94 Hz, 1 H: H1′′′′), 4.95 (m, 22 H: PhC*H*$_2$), 4.56 (s, 1 H, H1′′′), 4.30 (s, 1 H: H4′′), 4.29 (s, 1 H: H3′′), 4.22 (d, *J* = 9.9 Hz, 1 H: H5′), 4.12 (t, *J* = 9.35 Hz, 1 H: H3′), 4.00-3.90 (m, 5 H: H2′′, H3′′′′, H6′, H4′, H5), 3.83 (d, *J* = 10.64, 1 H: H5′′′′), 3.81-3.75 (m, 4 H: H6′, H5′′, H5′′′, H3′′′), 3.69 (t, *J* = 9.17 Hz, 1 H: H4), 3.66-3.60 (m, 2 H: H6′′′, H4′′′′), 3.55 - 3.60 (m, 2 H: H5′′, H6′′′′), 3.40 - 3.51 (m, 4 H: H2′′′′, H6′′′′, H3, H1), 3.36 (s, 1 H: H2′′′), 3.25 (t, *J* = 9.35 Hz, 1 H: H6), 3.16 (s, 1 H: H4′′′), 3.11 (dd, *J* = 3.9 Hz, 9.8 Hz, 1 H: H2′), 2.92 (dd, *J* = 4.2 Hz, 12.8 Hz, 1 H: H6′′), 2.24 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.31-1.41 (m, 1 H: $H2_{ax}$). ^{13}C NMR (600 MHz, $CDCl_3$) δ 106.27 (C1′′), 98.57 (C1′′′), 97.47 (C1′′′′), 95.99 (C1′), 83.95 (C6), 82.17 (C2′′), 81.91 (C4′′), 81.89 (C3′′′′), 81.86 (C5), 79.80 (C3′), 79.59 (C2′′′′), 77.61 (C4′′′′), 75.53 (C3′′), 75.44 (C4), 74.26 (C4′), 73.67 (C5′′), 71.49 (C5′), 71.10 (C5′′′′), 70.88 (C3′′′), 70.15 (C6′), 69.34 (C5′′), 68.41(C6′′′′), 62.78 (C2′), 60.38 (C1), 59.90 (C3), 57.33 (C2′′′), 51.03 (C6′′), 32.38 (C2); Ph*C*H$_2$ (11C: 75.31, 75.00, 74.90, 73.39, 73.23, 73.14, 73.07, 72.91, 72.85, 72.40, 71.74). HRESIMS calcd for $C_{106}H_{111}N_{15}O_{19}Na$ $[M+Na]^+$, 1920.8078; found, 1920.8175.

16β: $[\alpha]_D^{RT}$ +60.3 (*c* 0.80, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70-7.00 (m, 55H, aromatic), 6.09 (d, *J* = 4.03 Hz, 1 H: H1′), 5.62 (d, *J* = 5.50 Hz, 1 H: H1′′), 5.13-4.22 (m, 22 H: PhC*H*$_2$), 4.82 (s, 1 H: H1′′′), 4.38 (m, 1H: H1′′′′), 4.24 (s, 1 H: H4′′), 4.20 (s, 1 H, H3′′), 4.13 (m, *J* = 9.9 Hz, 1 H: H5′), 3.97-4.12 (m, 2 H: H3′, H4′), 3.81-3.96 (m, 3 H: H6′, H2′′, H5), 3.59-3.73 (m, 5

H: H6‴″, H5″, H3‴, H6′, H5‴), 3.65 (t, *J* = 11.2 Hz, 1H: H4), 3.61 (t, *J* = 9.35 Hz, 1 H: H4‴″), 3.49-3.56 (m, 4 H: H6‴, H5″, H3‴″, H6‴″), 3.34-3.46 (m, 3 H: H3, H2‴″, H1), 3.30 (m, 1 H: H5‴″), 3.34 (s, 1 H: H2″),) 3.22 (t, *J* = 9.35 Hz, 1 H: H6), 3.12 (m, 1 H: H2′), 3.10 (s, 1 H: H4‴), 2.86-2.92 (dd, *J* = 4.4 Hz, 13.2 Hz, 1 H: H6‴), 2.18 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.27-1.34 (m, 1 H: $H2_{ax}$). ^{13}C NMR (600 MHz, $CDCl_3$) δ 105.99 (C1′), 102.87 (C1‴), 98.66 (C1‴″), 95.84 (C1′), 84.71 (C3‴″), 84.05 (C6), 82.61 (C2‴″), 82.23 (C4″), 81.97 (C2″), 81.70 (C5), 77.99 (C4‴″),77.50 (C3′), 77.21 (C4′), 75.59 (C3‴), 75.17 (C5‴″), 74.72 (C4), 74.09 (C5‴), 73.08 (C3‴), 71.71 (C5′), 69.86 (C6‴″), 69.00 (C5″), 67.96 (C6′), 62.62 (C2′), 60.34 (C1), 60.19 (C3), 57.30 (C2‴), 50.89 (C6‴), 32.64 (C2); Ph*C*H_2 (11C: 75.55, 75.10, 75.01, 74.90, 74,83, 73.23, 73.21, 72.90, 72.35, 71.41, 70.99). HRESIMS calcd for $C_{106}H_{111}N_{15}O_{19}Na$ $[M+Na]^+$, 1920.8078; found, 1920.8156.

4′-*O*-(4‴″,6‴″-*O*-Benzylidene-2‴″,3‴″-*O*-benzyl-α-D-mannopyranosyl)-1,3,2′,2‴,6‴-pentaazido-6,3′,6′,2″,5‴,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (17α) and 4′-*O*-(4‴″,6‴″-*O*-Benzylidene-2‴″,3‴″-*O*-benzyl-β-D-mannopyranosyl)-1,3,2′,2‴,6‴-pentaazido-6,3′,6′,2″,5‴,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeaminoparomomycin (17β). Compounds **17α** (76 mg), **17β** (272 mg) were prepared from **11**[1] (520 mg) by the general glycosylation procedure. The glycosylation reaction was conducted at -60 °C for 2 h and then gradually increased to room temperature before quenching by saturated aqueous $NaHCO_3$. The pure anomers were isolated in the form of white gums by gradient chromatography over silica gel (toluene:EtOAc 98:2 to 94:6) followed by preparative reverse phase-HPLC (Varian dynamax 250*21.4 mm C_{18}, acetonitrile:H_2O 50:50 to 100:0, 21.5 ml/min) in a α:β ratio of 1: 3.6 with a total yield of 51%.

17α: $[\alpha]_D^{RT}$ -27.3 (*c* 0.21, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.50-7.00 (m, 50H, aromatic), 6.15 (d, *J* = 3.7 Hz, 1 H: H1'), 5.63 (d, *J* = 5.9 Hz, 1 H: H1″), 5.61 (s, 1 H: PhC*H*), 5.23 (s, 1H: H1‴″), 4.30-4.94 (m, 16 H: PhCH_2), 4.86 (s, 1 H: H1‴), 4.21-4.27 (m, 4 H: H4″, H4‴″, H3″, PhCH_2), 4.08-4.11 (m, 2 H: H6‴″, PhCH_2), 4.05-4.10 (d, *J* = 9.9 Hz, 1 H: H5′), 3.97 (t, *J* = 9.2 Hz, 1 H: H3′), 3.90-3.94 (m, 3 H: H2″, H3‴″, H5), 3.84-3.87 (m, 1 H: H5‴″), 3.78 (t, *J* = 9.9 Hz, 1 H: H6‴″), 3.74-3.80 (m, 6 H: H5″, H6′, H6′, H2‴″, H5‴, H3‴), 3.66-3.70 (m, 2 H: H4′, H4), 3.62 (dd, *J* = 8.4 Hz, 12.9 Hz, 1 H: H6‴), 3.55 (dd, *J* = 3.3 Hz, 10.3 Hz, 1 H: H5″), 3.39-3.50 (m,

2 H: H3, H1), 3.33 (s, 1 H: H2‴), 3.22 (t, *J* = 9.5 Hz, 1 H: H6), 3.11 (s, 1 H: H4‴), 2.93 (dd, *J* = 3.7 Hz, 10.3 Hz, 1 H: H2′), 2.90 (d, *J* = 4.0 Hz, 12.8 Hz, 1 H: H6‴), 2.20-2.24 (dt, *J* = 4.8 Hz, 13.2 Hz, 1 H: $H2_{eq}$), 1.34 (m, 1 H: $H2_{ax}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 106.24 (C1″), 101.40 (Ph*C*H), 101.29 (C1⁗), 98.64 (C1‴), 95.84 (C1′), 84.14 (C6), 82.28(C5), 82.05 (C4″), 81.96 (C2″), 79.94 (C3′), 78.99 (C4⁗), 77.96 (C2⁗),77.76 (C4′), 76.27 (C3⁗), 75.51 (C3″), 75.08 (C4), 74.31 (C5⁗), 72.89 (C3‴), 71.48 (C4‴), 70.61 (C5′), 70.16 (C5″), 69.25 (C6′), 68.68 (C6⁗), 65.32 (C5⁗), 62.86 (C2′), 60.45 (C1), 60.27 (C3), 57.29 (C2‴), 51.07 (C6‴), 32.12 (C2); Ph*C*H_2 (9C: 74.88, 74.82, 74.11, 73.93, 73.36, 73.25, 73.16, 72.40, 71.73). HRESIMS calcd for $C_{99}H_{103}N_{15}O_{19}Na$ $[M+Na]^+$ 1828.7452; found, 1828.7511.

17β: $[\alpha]_D^{RT}$ +63.0 (*c* 0.33, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70-7.00 (m, 50H, aromatic), 6.20 (d, *J* = 4.0 Hz, 1 H: H1′), 5.70 (d, *J* = 5.9 Hz, 1 H: H1″), 5.54 (s, 1 H: PhC*H*), 4.39-5.19 (m, 16 H: PhCH_2), 4.95 (s, 1 H: H1‴), 4.45 (m, 1H: H1⁗), 4.32-4.35 (m, 2 H: H4″, PhCH_2), 4.27-4.29 (d, *J* = 12.1 Hz, PhCH_2), 4.26 (br s, 1 H, H3″), 4.17-4.20 (m, *J* = 9.9 Hz, 1 H: H5′), 4.03 - 4.14 (m, 3 H: H4⁗, H6⁗, H3′), 3.94-4.03 (m, 3 H: H4′, H2″, H5), 3.71 - 3.81 (m, 6 H: H5⁗, H2⁗, H5″, H3⁗, H4, H5⁗), 3.67-3.70 (d, *J* = 11.3 Hz, 1H: H6′), 3.58-3.63 (m, 2 H: H5″, H6⁗), 3.50-3.55 (dt, *J* = 3.8 Hz, 10.3 Hz, 1 H: H3), 3.42-3.49 (m, 2 H: H6⁗, H1), 3.39 (dd, *J* = 0.7 Hz, 9.9 Hz, 1 H: H3⁗), 3.36 (br s, 1 H: H2‴), 3.29-3.34 (t, *J* = 9.2 Hz, 1 H: H6), 3.24-3.28 (dd, *J* = 4.0 Hz, 10.3 Hz, 1 H: H2′), 3.15 (br s, 1 H: H4‴), 3.07-3.11 (dt, *J* = 4.8 Hz, 9.6 Hz, 1 H: H5⁗), 2.92-2.95 (dd, *J* = 4.4 Hz, 13.2 Hz, 1 H: H6‴), 2.26 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.41 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 106.17 (C1″), 101.71 (C1⁗), 101.33 (Ph*C*H), 98.71 (C1‴), 95.81 (C1′), 84.14 (C6), 82.35 (C4″), 82.10 (C5), 81.86 (C2″), 78.53 (C3′), 78.15 (C4⁗), 77.92 (C3⁗),77.49 (C4′), 76.70 (C2⁗), 75.63 (C3′), 74.99 (C4), 73.74 (C5⁗), 73.48 (C3‴), 71.46 (C4″), 70.66 (C5′), 70.03 (C5″), 68.61 (C6⁗), 68.49 (6′), 67.38 (C5⁗), 62.60 (C2′), 60.39 (C1), 60.31 (C3), 57.34 (C2⁗), 51.00 (C6‴), 32.72 (C2); Ph*C*H_2 (9C: 75.10, 75.05, 74.98, 74.21, 73.30, 72.94, 72.42, 72.25, 71.76). HRESIMS calcd for $C_{99}H_{103}N_{15}O_{19}Na$ $[M+Na]^+$ 1828.7452; found, 1828.7444.

4′-*O*-(2⁗,3⁗,4⁗,6⁗-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,2′, 2‴,6″-pentaazido-6,3′,6′,2″,5‴,3⁗,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (18α) and 4′-*O*-(2⁗,3⁗,4⁗,6⁗-Tetra-*O*-benzyl-β-D-galactopyranosyl)-1,3,2′, 2‴,6″-pentaazido-

6,3′,6′,2″,5″,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (18β). Compounds **18α** (358 mg), **18β** (56 mg) were prepared from **11**[1] (500 mg) by the general glycosylation procedure, which was conducted at -72 °C for 2 h before quenching by saturated aqueous $NaHCO_3$. The pure anomers were isolated as white gums by gradient chromatography over silica gel (toluene:EtOAc 98:2 to 94:6). The two anomers were obtained as white gums with a α:β ratio of 6.4: 1 with a total yield of 60%.

18α: $[\alpha]_D^{RT}$ +82.97 (*c* 6.33, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50-7.00 (m, 55H, aromatic), 6.12 (d, *J* = 4.03 Hz, 1 H: H1′), 5.66 (d, *J* = 5.50 Hz, 1 H: H1″), 5.54 (d, *J* = 3.67 Hz, 1 H: H1‴), 4.91-4.34 (m, 21 H: PhCH_2), 4.88-4.91 (br s, 1 H, H1‴), 4.31-4.30 (m, 3 H: H4″, H3″, PhCH_2), 4.22-4.27 (dd, *J* = 3.3 Hz, 9.9 Hz, 1 H: H5′), 4.11 (t, *J* = 10.27 Hz, 1 H: H3′), 4.02-4.04 (dd, *J* = 3.45 Hz, 10.30 Hz, 1 H: H2‴), 4.01-3.98 (m, 2 H: H5‴, H2″), 3.96-3.98 (br s, 1 H: H4‴), 3.85-3.96 (m, 4 H: H3‴, H5, H6′, H4′), 3.76-3.84 (m, 4 H: H6′, H5″, H3‴, H5‴), 3.73 (t, *J* = 9.35 Hz, 1 H: H4), 3.65 (dd, *J* = 8.44 Hz, 12.84Hz, 1 H: H6‴), 3.57-3.62 (dd, *J* = 3.11Hz, 10.27 Hz, 1 H: H5″), 3.50-3.55 (m, 2 H: H6‴),) 3.41-3.50 (m, 2 H: H3, H1), 3.36-3.41 (br s, 1 H: H2‴), 3.27 (t, *J* = 9.35 Hz, 1 H: H6), 3.14-3.18 (s, 1 H: H4″), 3.09 (dd, *J* = 3.67 Hz, 10.27 Hz, 1 H: H2′), 2.95 (dd, *J* = 4.2 Hz, 13.02 Hz, 1 H: H6‴), 2.24 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.33-1.39 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120-144 (55C: aromatic), 106.27 (C1″), 98.63 (C1‴), 98.22 (C1‴), 95.91 (C1′), 84.01 (C6), 82.24 (C2″), 81.96 (C4″), 81.94 (C3″), 79.94 (C3′), 78.87 (C5), 75.97 (C2‴),75.53 (C3″), 75.28 (C4), 75.09 (C4′), 75.07 (C4‴), 74.28 (C5‴), 72.98 (C3‴), 71.57 (C4‴), 70.82 (C5′), 70.16 (C5″), 70.14 (C5‴), 69.73 (C6′), 69.08 (C6‴), 62.92 (C2′), 60.42 (C1), 60.00 (C3), 57.38 (C2‴), 51.05 (C6‴), 32.46 (C2); Ph*C*H_2 (11C: 74.94, 74.72, 73.80, 73.38, 73.30, 73.28, 73.19, 72.93, 72.89, 72.43, 71.79). HRESIMS calcd for $C_{106}H_{111}N_{15}O_{19}Na$ $[M+Na]^+$, 1920.8078; found, 1920.8009.

18β: $[\alpha]_D^{RT}$ +42.37 (*c* 3.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50-7.00 (m, 55H, aromatic), 6.12 (d, *J* = 3.7 Hz, 1 H: H1′), 5.65 (d, *J* = 5.9 Hz, 1 H: H1″), 5.14-4.23 (m, 22 H: PhCH_2), 4.81 (s, 1 H: H1‴), 4.38 (d, *J* = 7.0 Hz, 1H: H1‴), 4.20-4.27 (m, 3 H: H4″, H5′, H3″), 4.03-4.07 (t, *J* = 9.2 Hz, 1 H: H3′), 3.99-4.02 (t, *J* = 9.2 Hz, 1 H: H4′), 3.90 - 3.98 (m, 4 H: H6′, H2″, H5, H5‴), 3.82 (dd, *J* = 9.9, 7.7 Hz, 1 H: H2‴), 3.66 - 3.79 (m, 5 H: H4, H5″, H3‴, H6′, H5‴), 3.52-3.58 (m, 3 H: H6‴, H5″, H6‴), 3.42-3.50 (m, 2 H: H3, H1), 3.40 (dd, *J* = 3.0 Hz, 9.9 Hz,

1 H: H3‴), 3.33-3.37 (m, 2 H: H4‴, H6‴), 3.32 (br s, 1 H: H2″), 3.24-3.27 (t, *J* = 9.5 Hz, 1 H: H6), 3.18-3.23 (dd, *J* = 4.8 Hz, 12.8 Hz, 1H: H2′), 3.10-3.15 (br s, 1H: H4″), 2.93 (dd, *J* = 4.8 Hz, 12.8 Hz, 1 H: H6″), 2.24 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.30-1.40 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120-144 (55C: aromatic), 106.04 (C1′), 103.08 (C1‴), 98.68 (C1‴), 95.78 (C1′), 84.06 (C6), 82.29 (C5), 82.26 (C3‴), 82.00 (C4″), 81.76 (C2″), 79.95 (C2‴), 78.09 (C3′), 76.65 (C4′), 75.63 (C3″), 74.75 (C4), 74.10 (C5‴), 73.81 (C5‴), 73.14 (C4‴), 72.99 (C3‴), 71.07 (C5′), 69.92 (C5″), 68.13 (C6‴), 68.06 (C6′), 62.74 (C2′), 60.20 (C1), 60.03 (C3), 57.34 (C2‴), 50.91 (C6‴), 32.65 (C2), Ph*C*H_2 (11C: 75.35, 75.27, 75.01, 74.80, 73.37, 73.26, 73.24, 72.91, 72.69, 72.39, 71.76). HRESIMS calcd for $C_{106}H_{111}N_{15}O_{19}Na$ $[M+Na]^+$, 1920.8078; found, 1920.8101.

4′-*O*-(2‴,3‴,6‴-Tri-*O*-benzyl-4‴-azido-4‴-deoxy-α-D-glucopyranosyl)-1,3,2′,2‴,6″-pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (19α) and **4′-*O*-(2‴,3‴,6‴-Tri-*O*-benzyl-4‴-azido-4‴-deoxy-β-D-glucopyranosyl)-1,3,2′,2‴,6″-pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (19β)**. Compounds **19α** (272 mg), **19β** (61 mg) were prepared from **11**[1] (500 mg) by the general glycosylation procedure conducted at -60 °C for 1 h and then -50 °C for 1 h before quenching by saturated aqueous $NaHCO_3$. The two anomers were isolated as white gums by gradient chromatography over silica gel (toluene:EtOAc 98:2 to 94:6) followed by to preparative reverse phase HPLC ((Varian dynamax 250*21.4 mm C_{18}, acetonitrile:H_2O 70:30 to 100:0, 21.5 ml/min)) in a α:β ratio of 4.5: 1 with a total yield of 50%.

19α: $[\alpha]_D^{RT}$ +93.21 (*c* 3.73, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.50-7.00 (m, 50H, aromatic), 6.10 (d, *J* = 4.0 Hz, 1 H: H1′), 5.61 (d, *J* = 5.5 Hz, 1 H: H1″), 5.41 (d, *J* = 3.7 Hz, 1 H: H1‴), 4.94-4.30 (m, 19 H: PhCH_2), 4.85 (s, 1 H, H1‴), 4.24-4.29 (m, 3 H: H4″, H3″, PhCH_2), 4.14-4.20 (d, *J* = 9.9 Hz, 1 H: H5′), 4.07-4.13 (t, *J* = 9.2 Hz, 1 H: H3'), 3.94-3.96 (t, *J* = 5.1 Hz, 1 H: H2″), 3.90-3.93 (m, 3 H: H4′, H5, H6′), 3.82 (t, *J* = 9.4 Hz, 1 H: H3‴), 3.74-3.78 (m, 3 H: H5″, H3‴, H5‴), 3.73 (t, *J* = 8.4 Hz, 1 H: H6′), 3.67 (t, *J* = 9.5 Hz, 1 H: H4), 3.59-3.63 (m, 3 H: H6‴, H4‴, H5‴), 3.55 (dd, *J* = 3.3 Hz, 10.3 Hz, 1 H: H5″), 3.39-3.49 (M, 5 H: H3, H6‴, H2‴, H6‴, H1), 3.32-3.36 (br s, 1 H: H2‴), 3.24 (t, *J* = 9.4 Hz, 1 H: H6), 3.11-3.15 (br s, 1 H: H4″), 3.08 (dd, *J* = 10.1, 3.9 Hz, 1 H: H2′), 2.91 (dd, *J* = 13.0, 4.2 Hz, 1 H: H6″), 2.24 (dt, *J* = 4.5 Hz,

8.6 Hz, 1 H: $H2_{eq}$), 1.30-1.39 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120-144 (55C: aromatic), 106.30 (C1″), 98.57 (C1‴), 97.26 (C1⁗), 95.96 (C1′), 81.89 (C5), 79.76 (C3⁗), 79.69 (C3′), 79.42 (C2⁗), 77.21 (C6), 76.99 (C2″), 76.78 (C4″), 75.53 (C3″), 75.36 (C4), 74.25 (C4′), 72.39 (C3‴), 71.52 (C4‴), 70.84 (C5′), 70.19 (C4⁗), 70.14 (C5″), 69.20 (C6′), 68.51 (C6⁗), 62.66 (C2′), 61.60 (C4⁗), 60.38 (C1), 60.03 (C3), 57.32 (C2‴), 51.04 (C6‴), 32.45 (C2); Ph*C*H_2 (10C: 75.44, 75.01, 74.61, 73.39, 73.21, 73.17, 73.11, 72.93, 72.75, 71.75). HRESIMS calcd for $C_{99}H_{104}N_{18}O_{18}Na$ $[M+Na]^+$, 1855.7674; found, 1855.7692.

19β: $[\alpha]_D^{RT}$ +78.7 (*c* 1.87, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.50-7.00 (m, 50H, aromatic), 6.12 (d, *J* = 4.0 Hz, 1 H: H1′), 5.63 (d, *J* = 5.9 Hz, 1 H: H1″), 5.09-4.29 (m, 21 H: PhCH_2), 4.80 (s, 1 H: H1‴), 4.32 (d, *J* = 7.7 Hz, 1H: H1⁗), 4.24-4.26 (m, 2 H: H4″, PhCH_2), 4.20 (m, H3″), 4.18 (d, *J* = 9.5 Hz, *J* 1 H: H5′), 4.01 (m, *2* H: H3′, H4′), 3.88-3.96 (m, 3 H: H6′, H2″, H5), 3.68-3.76 (m, 4 H: H5″, H3⁗, H5‴, H6′), 3.65-3.67 (m, 2 H: H4, H6⁗), 3.60 (t, *J* = 9.9 Hz, 1 H: H4⁗), 3.52-3.59 (m, 2 H: H6‴, H5″), 3.48 (dd, *J* = 4.4 Hz, 11.0 Hz, 1 H: H6⁗), 3.39-3.43 (m, 2 H: H1, H3), 3.37 (t, *J* = 8.4 Hz, 1 H: H2⁗), 3.31 (br s, 1 H: H2‴), 3.27 (t, *J* = 9.2 Hz, 1 H: H3⁗), 3.24 (t, *J* = 9.5 Hz, 1 H: H6), 3.13 (dd, *J* = 4.0 Hz, 9.9 Hz), 3.11 (br s, 1 H: H4‴), 3.02 (ddd, *J* = 1.5 Hz, 4.0 Hz, 6.2 Hz, 1 H: H5⁗), 2.91 (dd, *J* = 4.4 Hz, 12.8 Hz, 1 H: H6‴), 2.24 (dt, *J* = 4.5 Hz, 8.6 Hz, 1 H: $H2_{eq}$), 1.29-1.36 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 120-144 (50C: aromatic), 106.04 (C1″), 102.71 (C1‴), 98.66 (C1⁗), 95.89 (C1′), 84.05 (C6), 82.77 (C3⁗), 82.26 (C2″, C2⁗), 81.99 (C4″), 81.73 (C5), 77.79 (C3′), 77.02 (C4′), 76.77 (C3"), 74.82 (C4), 74.11 (C5""), 74.07 (C5'"), 72.91 (C3'"), 71.43 (C4'"), 70.90 (C5'), 69.89 (C5"), 69.16 (C6""), 67.88 (C6'), 62.64 (C2'), 62.08 (C4""), 60.35 (C1), 60.19 (C3), 57.31 (C2'"), 50.92 (C6'"), 32.64 (C2), Ph*C*H_2 (10C: 76.77, , 75.19, 75.03, 74.87, 73.31, 73.24, 73.21, 72.91, 72.37, 71.73). HRESIMS calcd for $C_{99}H_{104}N_{18}O_{18}Na$ $[M+Na]^+$, 1855.7674; found, 1855.7615.

4′-*O*-(2⁗,3⁗,4⁗,6⁗-Tetra-*O*-benzyl-α-D-glucopyranosyl)-6,3′,6′,2″,5″,3‴,4‴-hepta-*O*-benzyl-1,3,2′,2‴,6‴-pentadeamino paromomycin (20α). Compound **20α** (35 mg, 64%) was obtained as a white gum by the Staudinger reaction of **16α** (60 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ +27.50 (*c* 2.00, MeOH); HRESIMS calcd for $C_{106}H_{122}N_5O_{19}$ $[M+H]^+$, 1768.8734; found, 1768.8747. This compound was taken forward to the next step without further characterization.

4′-*O*-(2′′′′,3′′′′,4′′′′,6′′′′-Tetra-*O*-benzyl-β-D-glucopyranosyl)-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (20β). Compound **20β** (17 mg, 55%) was obtained as a white gum by Staudinger reaction of **16β** (33 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ -2.1 (*c* 0.52, MeOH); HRESIMS calcd for $C_{106}H_{122}N_5O_{19}$ $[M+H]^+$, 1768.8734; found, 1768.8700. This compound was taken forward to the next step without further characterization.

4′-*O*-(4′′′′,6′′′′-*O*-Benzylidene-2′′′′,3′′′′-*O*-benzyl-α-D-mannopyranosyl)-6, 3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (21α). Compound **21α** (17 mg, 91%) was obtained in the form of a white gum from by Staudinger reaction **17α** (20 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99) . $[\alpha]_D^{RT}$ +10.2 (*c* 0.93, MeOH); HRESIMS calcd for $C_{99}H_{114}N_5O_{19}[M+H]^+$, 1676.8108; found, 1676.8175. This compound was taken forward to the next step without further characterization.

4′-*O*-(4′′′′,6′′′′-*O*-Benzylidene-2′′′′,3′′′′-*O*-benzyl-β-D-mannopyranosyl)-6, 3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (21β). Compound **21β** (68 mg, 66%) was obtained in the form of a white gum by Staudinger reaction of 110 mg **17β** (110 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ -5.5 (*c* 1.75, MeOH); HRESIMS calcd for $C_{99}H_{114}N_5O_{19}$ $[M+H]^+$, 1676.8108; found, 1676.8066. This compound was taken forward to the next step without further characterization.

4′-*O*-(2′′′′,3′′′′,4′′′′,6′′′′-Tetra-*O*-benzyl-α-D-galactopyranosyl)-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (22α). Compound **22α** (83 mg, 81%) was obtained as a white gum by Staudinger reaction of 110 mg **18α** (110 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ +42.7 (*c* 6.67, MeOH); HRESIMS calcd for $C_{106}H_{122}N_5O_{19}$ $[M+H]^+$, 1768.8734; found, 1768.8707. This compound was taken forward to the next step without further characterization.

4′-*O*-(2′′′′,3′′′′,4′′′′,6′′′′-Tetra-*O*-benzyl-β-D-galactopyranosyl)-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeaminoparomomycin (22β). Compound **22β** (24 mg, 78%) was obtained as a white gum by Staudinger reaction of **18β** (33 mg) after silica gel chromatography

(NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ -5.2 (*c* 0.56, MeOH); HRESIMS calcd for $C_{106}H_{122}N_5O_{19}$ $[M+H]^+$, 1768.8734; found, 1768.8732. This compound was taken forward to the next step without further characterization.

4′-*O*-(2′′′′,3′′′′,6′′′′-Tri-*O*-benzyl-4′′′′-amino-4′′′′-deoxy-α-D-glucopyranosyl)-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-*O*-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (23α). Compound **23α** (23 mg, 64%) was obtained as a white gum by Staudinger reaction of 40 mg **19α** (40 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ +23.0 (*c* 0.80, MeOH); HRESIMS calcd for $C_{99}H_{117}N_6O_{18}$ $[M+H]^+$, 1677.8424; found, 1677.8425. This compound was taken forward to the next step without further characterization.

4′-*O*-(2′′′′,3′′′′,6′′′′-Tri-*O*-benzyl-4′′′′-amino-4′′′′-deoxy-β-D-glucopyranosyl)-6,3′,6′,2′′,5′′,3′′′,4′′′-hepta-O-benzyl-1,3,2′,2′′′,6′′′-pentadeamino paromomycin (23β). Compound **23β** (18 mg, 48%) was obtained as a white gum by Staudinger reaction of **19β** (28 mg) after silica gel chromatography (NH_4OH: MeOH 0: 100 to 1:99). $[\alpha]_D^{RT}$ +5.9 (*c* 1.20, $CHCl_3$); HRESIMS calcd for $C_{99}H_{117}N_6O_{18}$ $[M+H]^+$, 1677.8424; found, 1677.8474. This compound was taken forward to the next step without further characterization.

4'-*O*-α-D-Glucopyranosyl paramomycin (24α). Compound **24α** (29 mg, 39%) was obtained as a white solid by hydrogenolysis of **20α** (120 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +73.2 (*c* 0.50, H_2O); ^1H NMR (600 MHz, $CDCl_3$) δ 5.59 (d, *J* = 4.0 Hz, 1 H: H1'), 5.21 (d, *J* = 3.0 Hz, 1H: H1′′′′), 5.18 (br s, 1 H: H1′′), 5.11 (s, 1 H: H1′′′), 4.34 (t, *J* = 5.1 Hz, 1 H: H3′′), 4.20 (s, 1 H: H2′′), 4.12 (s, 1 H: H5′′′), 4.00-4.05 (m, 3 H: H3′′′, H3′, H4′′), 3.79 (t, *J* = 8.8 Hz, 1 H: H5′), 3.63-3.76 (m, 7 H: H5′′, H4, H5, H6′′′′, H6′, H6′, H4′′′), 3.55-3.60 (m, 3 H: H5′′, H6′′′′, H4′), 3.46-3.51 (m, 3 H: H3′′′′, H5′′′′, H6), 3.41-3.44 (dd, *J* = 3.7 Hz, 9.9 Hz, 1 H: H2′′′′), 3.40 (s, 1 H: H2′′′), 3.28 (dd, *J* = 4.0 Hz, 9.9 Hz, 1 H: H2′), 3.22-3.26 (m, 3 H: H4′′′′, H6′′′, H3), 3.16 (dd, *J* = 4.0 Hz, 14.0 Hz, 1 H: H2′), 3.22-3.27 (m, 3 H: H3, H4′′′′, H6′′′), 3.16-3.19 (dd, *J* = 4.0 Hz, 14.0 Hz, 1 H: H6′′′), 3.13 (dt, *J* = 3.6 Hz, 10.1 Hz, 1 H: H1), 2.23 (m, 1 H: $H2_{eq}$), 1.60 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 109.98 (C1′′), 99.44 (C1′′′′), 95.26 (C1′, C1′′′), 84.64 (C5), 81.07

(C4″), 79.22 (C4), 77.79 (C4′), 76.39 (C5‴), 74.98 (C3″), 73.28 (C2″), 72.86 (C3‴), 73.63 (C6), 72.01 (C5′), 70.38 (C2‴), 70.15 (C5‴), 67.99 (C3′), 67.62 (C3‴), 67.19 (C4‴), 66.52 (C4‴), 60.37 (C6′), 59.93 (C6‴, C5″), 53.29 (C2′), 50.74 (C2‴), 49.78 (C1), 48.70 (C3), 40.29 (C6‴), 28.85 (C2), 23.05 (*C*H$_3$). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3594.

4'-*O*-β-D-Glucopyranosyl paramomycin (24β). Compound **24β** (9 mg, 87%) was obtained in the form of a white solid by hydrogenolysis of **20β** (17 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +30.0 (*c* 0.45, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.59 (d, *J* = 3.7 Hz, 1 H: H1'), 5.19 (d, *J* = 2.9 Hz, 1 H: H1″), 5.09 (s, 1H: H1‴), 4.34 (t, *J* = 6.6 Hz, 1 H: H3″), 4.31 (d, *J* = 8.1 Hz, 1 H: H1⁗), 4.19 (br s, 1 H: H2″), 4.12 (t, *J* = 4.4 Hz, 1 H: H5‴), 4.03 (t, *J* = 3.3 Hz, 1 H: H3‴), 4.01(m, 1 H: H4″), 3.82 (t, *J* = 10.6 Hz, 1 H: H3′), 3.77 (m, 1 H: H5⁗), 3.72-3.74 (m, 2 H: H6⁗, H5″), 3.68 (dd, *J* = 4.1 Hz, 12.4 Hz, 1 H: H6⁗), 3.62-3.65 (m, 2 H: H5, H4‴), 3.59 (dd, *J* = 4.8 Hz, 12.5 Hz, 1 H: H5″), 3.52-3.57 (m, 4 H: H6′, H4′, H6′, H6), 3.43 (t, *J* = 10.3 Hz, 1 H: H4), 3.37 (s, 1 H: H2‴), 3.31 (t, *J* = 9.1 Hz, 1 H: H3⁗), 3.27 (dd, *J* = 2.4 Hz, 5.8 Hz, 1 H: H5′), 3.16-3.26 (m, 4 H: H6‴, H4⁗, H2′, H6″), 3.11 (t, *J* = 9.2 Hz, 1 H: H2⁗), 2.99-3.07 (m, 2 H: H1, H3), 2.10 (dt, *J* = 4.0 Hz, 12.5 Hz, 1 H: $H2_{eq}$), 1.44 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 109.80 (C1″), 102.44 (C1⁗), 95.80 (C1′), 95.44 (C1‴), 84.67 (C5), 81.03 (C4″), 79.94 (C6), 77.73 (C4′), 75.90 (C5′), 75.38 (C3⁗), 74.99 (C3″), 73.25 (C2⁗), 73.08 (C2″), 73.04 (C4), 72.14 (C5⁗), 70.19 (C5‴), 69.33 (C4⁗), 68.21 (C3′), 67.71 (C3‴), 67.23 (C4‴), 60.46 (C5″), 59.96 (C6′), 59.93 (C6⁗), 53.84 (C2′), 50.82 (C2‴), 50.12 (C1), 48.93 (C3), 40.30 (C6‴), 30.55 (C2), 23.12 (*C*H$_3$). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3537.

4'-*O*-α-D-Mannopyranosyl paramomycin (25α). Compound **25α** (4 mg, 33%) was obtained in the form of a white solid by hydrogenolysis of **21α** (17 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +55.0 (*c* 0.18, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.59 (d, *J* = 4.1 Hz, 1 H: H1′), 5.21 (br s, 1 H: H1″), 5.12 (s, 1 H: H1‴), 5.09 (m, 1H: H1⁗), 4.37 (t, *J* = 5.5 Hz, 1 H: H3″), 4.23 (s, 1 H: H2″), 4.15 (s, 1 H: H5‴), 4.06 (s, 1 H: H3‴), 4.04 (s, 1 H: H4″), 3.92 (t, *J* = 8.8 Hz, 1 H: H3′), 3.88 (s, 1 H: H2⁗), 3.63-3.79 (m, 7 H: H6⁗, H5″, H5′, H6′, H5, H6′, H4‴, H4), 3.54-3.63 (m, 4 H: H3⁗, H6⁗, H5″, H4′), 3.43-3.52 (m, 3 H: H5⁗, H4⁗, H6), 3.41 (s, 1 H: H2‴), 3.17-3.28 (m, 3 H: H6‴, H6‴, H2′), 3.09-3.15 (m, 2 H: H1, H3), 2.17 (m, 1 H: $H2_{eq}$), 1.50 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 109.98 (C1″), 101.49 (C1⁗), 95.64 (C1′), 95.27 (C1‴), 84.63 (C5),

81.01 (C4″), 79.02 (C4), 75.54 (C4′), 74.93 (C3″), 73.82 (C5‴″), 73.27 (C6), 72.79 (C2″), 72.48 (C5′), 70.19 (C3‴″), 70.16 (C5‴), 69.98 (C2‴″), 69.41 (C3′), 67.62 (C3‴), 67.20 (C4‴), 66.36 (C4‴″), 60.80 (C6‴″), 60.03 (C6′), 59.94 (C5″), 53.84 (C2′), 50.78 (C2‴), 50.00 (C1), 48.80 (C3), 40.29 (C6‴), 30.02 (C2), 22.95 (*C*H_3). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3600.

4'-*O*-β-D-Mannopyranosyl paramomycin (25β). Compound **25β** (11 mg, 33%) was obtained in the form of a white solid by hydrogenolysis of **21β** (68 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +31.9 (*c* 0.54, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.57 (d, *J* = 4.0 Hz, 1 H: H1′), 5.20 (br s, 1 H: H1″), 5.11 (s, 1 H: H1‴), 4.57 (s, 1H: H1‴″), 4.35 (t, *J* = 5.5 Hz, 1 H: H3″), 4.20 (s, 1 H: H2″), 4.12 (s, 1 H: H5‴), 4.04 (s, 1 H: H3‴), 4.02 (s, 1 H: H4″), 3.87 (m, 2 H: H3′, H2‴″), 3.72-3.78 (m, 3 H: H6‴″, H5′, H5″), 3.57-3.70 (m, 7 H: H6′, H6′, H5, H4‴, H4, H5″, H4′), 3.52-3.55 (dd, *J* = 6.6 Hz, 12.1 Hz, 1 H: H6‴″), 3.43-3.47 (m, 2 H: H3‴″, H6), 3.39 (s, 1 H: H2‴), 3.37 (t, *J* = 9.9 Hz, 1 H: H4‴″), 3.16-3.28 (m, 4 H: H6‴, H5‴″, H6‴, H2′), 3.07-3.11 (m, 2 H: H1, H3), 2.15 (m, 1 H: $H2_{eq}$), 1.49 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 109.87 (C1″), 99.96 (C1‴″), 95.61 (C1′), 95.35 (C1‴), 84.64 (C5), 81.07 (C4″), 79.22 (C4), 77.79 (C4′), 76.39 (C5‴″), 74.98 (C3″), 73.28 (C2″), 72.86 (C3‴″), 73.63 (C6), 72.01 (C5′), 70.38 (C2‴″), 70.15 (C5‴), 67.99 (C3′), 67.62 (C3‴), 67.19 (C4‴), 66.52 (C4‴″), 60.84 (C6‴″), 59.91 (C5″), 59.56 (C6′), 53.61 (C2′), 50.76 (C2‴), 50.01 (C1), 48.83 (C3), 40.29 (C6‴), 30.01 (C2), 23.00 (*C*H_3). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3561.

4'-*O*-α-D-Galactopyranosyl paramomycin (26α). Compound **26α** (36 mg, 62%) was obtained as a white solid by hydrogenolysis of **22α** (100 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +9.2 (*c* 0.45, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.59 (s, 1 H: H1′), 5.20 (s, 1H: H1‴″), 5.16 (s, 1 H: H1″), 5.09 (s, 1 H: H1‴), 4.32 (br s, 1 H: H3″), 4.19 (s, 1 H: H2″), 4.10 (s, 1 H: H5‴), 3.98-4.05 (m, 3 H: H3‴, H3′, H4″), 3.80 (t, *J* = 10.3 Hz, 1 H: H4), 3.73-3.79 (m, 3 H: H5′, H3‴″, H5‴″), 3.65-3.72 (m, 5 H: H5″, H6‴″, H5, H2‴″, H6‴″), 3.60-3.64 (m, 2 H: H4‴, H4‴″), 3.51-3.59 (m, 4 H: H6′, H6′, H5″, H4′), 3.48 (t, *J* = 9.5 Hz, 1 H: H6), 3.38 (s, 1 H: H2‴″), 3.29-3.34 (m, 2 H: H3, H2′), 3.22 (m, 1 H: H6‴″), 3.11-3.18 (m, 2 H: H6‴, H1), 2.24-2.28 (m, 1 H: $H2_{eq}$), 1.63 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 110.01 (C1″), 99.66 (C1‴″), 95.30 (C1‴″), 95.11 (C1′), 84.19 (C5), 81.22 (C4″), 77.43 (C4), 75.38 (C4′), 75.13 (C3″), 73.31 (C2″), 73.20 (C5′), 72.21

(C6), 71.89 (C5′′′′), 70.16 (C5′′), 69.13 (C4′′′′), 69.06 (C3′′′′), 68.83 (C3′), 68.22 (C2′′′′), 67.56 (C3′′), 67.19 (C4′′), 61.15 (C6′), 60.03 (C5′′), 59.97 (C6′′′′), 53.17 (C2′), 50.78 (C2′′), 49.69 (C1), 48.68 (C3), 40.32 (C6′′), 28.27 (C2), 22.59 (*C*H_3). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3577.

4'-*O*-β-D-Galactopyranosyl paramomycin (26β). Compound **26β** (5.5 mg, 39%) was obtained in the form of a white solid by hydrogenolysis of **22β** (24 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +38.7 (*c* 0.37, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.65 (s, 1 H: H1′), 5.23 (s, 1H: H1′′), 5.15 (s, 1 H: H1′′′), 4.38 (s, 1 H: H3′′), 4.31 (s, 1 H: H1′′′′), 4.23 (s, 1 H: H2′′), 4.17 (s, 1 H: H5′′′), 4.05-4.10 (m, 2 H: H3′′′, H4′′), 3.95 (t, *J* = 10.3 Hz, 1 H: H3′), 3.86 (t, *J* = 9.5 Hz, 1 H: H4), 3.69-3.84 (m, 6 H: H6′′′′, H5′′′′, H4′′′′, H5′′, H5, H6′′′′), 3.68 (s, 1 H: H4′′), 3.56-3.67 (m, 4 H: H5′′, H6′, H5, H4′, H6′), 3.50-3.57 (m, 3 H: H5′, H3′′′′, H6), 3.45 (s, 1 H: H2′′′), 3.32-3.40 (m, 3 H: H2′′′′, H3, H2′), 3.27-3.30 (br s, 1 H: H6′′′), 3.18-3.25 (m, 2 H: H1, H6′′′), 2.34 (m, 1 H: $H2_{eq}$), 1.68 (m, 1 H: $H2_{ax}$).^{13}C NMR (151 MHz, $CDCl_3$) δ 109.97 (C1′′), 102.82 (C1′′′′), 95.31 (C1′′′), 95.18 (C1′), 84.24 (C5), 81.21 (C4′′), 77.27 (C4), 77.19 (C4′), 75.39 (C5′), 75.06 (C3′′), 73.33 (C2′′), 72.94 (C5′′′′), 72.39 (C6), 72.22 (C3′′′′), 68.22 (C2′′′′), 70.11 (C5′′′), 68.41 (C4′′′′), 67.58 (C3′), 67.55 (C3′′′), 67.19 (C4′′′), 61.06 (C6′), 60.03 (C5′′), 59.39 (C6′′′′), 53.22 (C2′), 50.73 (C2′′′), 49.63 (C1), 48.66 (C3), 40.31 (C6′′′), 28.19 (C2), 21.83 (*C*H_3). HRESIMS calcd for $C_{29}H_{56}N_5O_{19}$ $[M+H]^+$, 778.3570; found, 778.3588.

4'-*O*-(4′′′′-Amino-4′′′′-deoxy-α-D-glucopyranosyl) paramomycin (27α). Compound **27α** (20 mg, 64%) was obtained as a white solid by hydrogenolysis of **23α** (54 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +53.8 (*c* 0.40, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.63 (s, 1 H: H1′), 5.32 (s, 1H: H1′′′′), 5.19 (s, 1 H: H1′′), 5.11 (s, 1 H: H1′′′), 4.34 (t, *J* = 5.5 Hz, 1 H: H3′′), 4.21 (s, 1 H: H2′′), 4.12 (br s, 1 H: H5′′′), 4.01-4.07 (m, 3 H: H3′′′, H3′, H4′′), 3.79-3.84 (m, 2 H: H5′′′′, H4), 3.68-3.77 (m, 5 H: H5′′, H4, H6′′′′, H6′, H3′′′′), 3.57-3.66 (m, 6 H: H5′′, H6′, H6′′′′, H4′, H4′′′, H5), 3.49-3.54 (m, 2 H: H2′′′′, H6), 3.41 (s, 1 H: H2′′′), 3.30-3.39 (m, 2 H: H3, H2′), 3.24 (dd, *J* = 6.6 Hz, 13.9 Hz, 1 H: H6′′′), 3.15-3.20 (m, 2 H: H1, H6′′′), 3.07 (t, *J* = 10.6 Hz, 1 H: H4′′′′), 3.13 (dt, *J* = 3.6 Hz, 10.1 Hz, 1 H: H1), 2.29 (m, 1 H: $H2_{eq}$), 1.66 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 110.03 (C1′′), 99.66 (C1′′′′), 95.29 (C1′′′), 95.25 (C1′), 84.24 (C5), 81.20 (C4′′), 77.18 (C4), 75.15 (C3′′), 75.13 (C4′), 73.31 (C2′′), 72.68 (C5′), 72.20 (C2′′′′), 71.22

(C6), 70.10 (C5″), 69.03 (C5‴), 68.93 (C3‴), 68.81 (C3′), 67.53 (C3″), 67.18 (C4″), 60.24 (C6′), 59.94 (C5″), 59.89 (C6‴), 53.31 (C2′), 52.08 (C4‴), 50.73 (C2″), 49.63 (C1), 48.61 (C3), 40.30 (C6″), 28.15 (C2), 21.74 (*C*H_3). HRESIMS calcd for $C_{29}H_{57}N_6O_{18}$ $[M+H]^+$, 777.3729; found, 777.3718.

4'-*O*-(4‴-Amino-4‴-deoxy-β-D-glucopyranosyl) paramomycin (27β). Compound **27β** (5 mg, 29%) was obtained as a white solid by hydrogenolysis of **23β** (22 mg) after Sephadex chromatography. $[\alpha]_D^{RT}$ +66.86 (*c* 0.17, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.66 (s, 1 H: H1′), 5.25 (s, 1 H: H1″), 5.16 (s, 1H: H1‴), 4.42-4.47 (m, 2 H: H1‴′, H3″), 4.24 (s, 1 H: H2″), 4.17 (s, 1 H: H5‴), 4.07-4.10 (m, 2 H: H3‴, H4″), 3.94 (br s, 1 H: H3′), 3.88 (br s, 1 H: H4), 3.73-3.84 (m, 7 H: H5′, H5, H5″, H6′, H6‴′, H6‴″, H6′), 3.69 (s, 1 H: H4‴), 3.59-3.66 (m, 3 H: H5‴′, H4′, H5″), 3.54-3.58 (m, 2 H: H3‴′, H6), 3.46 (s, 1 H: H2‴), 3.40 (br s, 1 H: H3) 3.35 (br s, 1H: H2′), 3.15-3.31 (m, 4 H: H6‴, H2‴′, H6‴′, H1), 3.12 (t, *J* = 10.6 Hz, 1 H: H4‴′), 3.13 (dt, *J* = 3.6 Hz, 10.1 Hz, 1 H: H1), 2.33 (m, 1 H: $H2_{eq}$), 1.70 (m, 1 H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 110.01 (C1″), 102.45 (C1‴′), 95.42 (C1‴), 95.35 (C1′), 84.27 (C5), 81.20 (C4″), 77.50 (C4), 77.36 (C4′), 75.26 (C3″), 73.44 (C2‴′), 73.42 (C2″), 72.98 (C5′), 72.28 (C5‴′), 72.22 (C3‴′), 71.74 (C6), 70.19 (C5‴), 67.59 (C3′), 67.56 (C3‴), 67.28 (C4‴), 60.32 (C6′), 60.03 (C5″), 59.37 (C6‴′), 53.38 (C2′), 52.18 (C4‴′), 50.84 (C2‴), 49.70 (C1), 48.75 (C3), 40.40 (C6‴), 28.22 (C2), 21.47 (*C*H_3). HRESIMS calcd for $C_{29}H_{57}N_6O_{18}$ $[M+H]^+$, 777.3729; found, 777.3704.

Phenylthiomethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**29**)**.** To a stirred solution of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranosyl trichloroacetimidate **28**[2] (628 mg, 1.06 mmol, α:β = 4:1) and phenythiomethanol[3] (163 mg, 1.17 mmol) in dichloromethane/diethyl ether (1:1, *v*/*v*; 7 mL) was added TMSOTf (21 μL, 26 μmol) at −78 °C under argon atmosphere. After 2 h of stirring at −78 °C, triethylamine (20 μL) was added and the reaction mixture was warmed to room temperature. The solution was diluted with dichloromethane (10 ml), washed with water (1 × 10 mL), sat. $NaHCO_3$ (1 × 10 ml), and brine (1 × 10 mL), then dried over $MgSO_4$. After concentration of the filtrate under reduced pressure, the residue was purified by Flash chromatography (eluent: 0.5% triethylamine in hexane:ethyl acetate from 9:1 to 6:1) to afford **29** (460 mg, 76%). $[\alpha]_D^{23}$ +77.4 (c 1.25, CH_2Cl_2). ^{1}H NMR (500

MHz, $CDCl_3$) δ 7.58 – 7.48 (m, 4H), 7.44 – 7.36 (m, 5H), 7.35 – 7.18 (m, 11H), 5.58 (s, 1H: PhC*H*O_2), 5.44 (d, *J* = 3.9 Hz, 1H: H1), 5.15 (dd, *J* = 59.5, 12.2 Hz, 2H: PhSC*H*$_2$O-C1), 4.90 (dd, *J* = 39.5, 11.4 Hz, 2H: PhC*H*$_2$O-C2), 4.71 (dd, *J* = 32.6, 11.4 Hz, 2H: PhC*H*$_2$O-C3), 4.27 (dd, *J* = 10.2, 4.8 Hz, 1H: H6a), 4.08 (t, *J* = 9.3 Hz, 1H: H3), 3.87 (td, *J* = 10.0, 4.8 Hz, 1H: H5), 3.77 – 3.63 (m, 3H: H6b, H2, H4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.74, 137.92, 137.35, 135.44, 130.06, 129.03, 128.94, 128.31, 128.28, 128.23, 128.05, 127.97, 127.94, 127.87, 127.71, 127.56, 126.90, 126.03, 101.29 (Ph*C*HO_2), 93.86 (C1), 81.99 (C4), 78.79 (C3), 78.55 (C2), 75.26 (Ph*C*H$_2$O-C2), 73.24 (Ph*C*H$_2$O-C3), 71.56 (PhS*C*H$_2$O-C1), 68.89 (C6), 63.17 (C5). HRESIMS *m*/*z* calcd for $C_{34}H_{34}O_6SNa$ $[M+Na]^+$ 593.1974, found 593.1973.

4′-*O*-(2,3-Di-*O*-benzyl-4,6-benzylidene-α-D-glucopyranosyloxymethyl)-1,3,2′,2‴,6‴-pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-*O*-benzyl-1,3,2′,,2‴,6‴-pentadeamino-paromomycin (30). To a solution of **11**[1] (111 mg, 194 μmol) and **29** (77.8 mg, 56.5 μmol) in dry dichloromethane (1 mL) were added *N*-iodosuccinimide (44.2 mg, 196 μmol) and trifluoromethanesulfonic acid (3.4 μL, 38 μmol) at −30 °C under argon atmosphere. After 2 h of stirring at −30 °C, sat. $NaHCO_3$ (2.5 mL) was added and warmed to room temperature. The reaction mixture was diluted with dichloromethane (4 mL) and the organic layer was separated, which was washed with sat. $Na_2S_2O_3$ (1 × 5 mL) and brine (1 × 5 mL), then dried over $MgSO_4$. After concentration of the filtrate under reduced pressure, the residue was purified by flash chromatography (eluent: toluene:ethyl acetate = 25:1) to afford **30** (34.6 mg, 33%). $[\alpha]_D^{23}$ +66.7 (c 1.14, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 – 7.11 (m, 50H), 6.17 (d, *J* = 3.7 Hz, 1H: H1′,), 5.67 (d, *J* = 5.8 Hz, 1H: H1″), 5.56 (s, 1H: PhC*H*O_2), 5.14 (d, *J* = 3.7 Hz, 1H: H1‴), 5.03 (d, *J* = 5.8 Hz, 1H: OC*H*$_2$O), 4.98 (d, *J* = 10.7 Hz, 1H: PhC*H*$_2$-C6), 4.95 – 4.81 (m, 4H: PhC*H*$_2$-C3⁗, H1‴, PhC*H*$_2$-C3′, OC*H*$_2$O), 4.81 – 4.74 (m, 3H: PhC*H*$_2$-C3⁗, PhC*H*$_2$-C2⁗, PhC*H*$_2$-C3′), 4.74 – 4.67 (m, 2H: PhC*H*$_2$-C2⁗, PhC*H*$_2$-C6), 4.67 – 4.50 (m, 5H: PhC*H*$_2$-C4‴, PhC*H*$_2$-C2″, PhC*H*$_2$-C6′, PhC*H*$_2$-C5″), 4.50 – 4.40 (m, 3H: PhC*H*$_2$-C2″, PhC*H*$_2$-C5″, PhC*H*$_2$-C3‴), 4.37 – 4.23 (m, 4H: PhC*H*$_2$-C3‴, H4″, PhC*H*$_2$-C4‴, H3″), 4.23 – 4.15 (m, 2H: H6a⁗, H5′), 4.10 – 4.00 (m, 2H: H3′, H3⁗), 4.00 – 3.88 (m, 3H: H2″, H5, H6a′), 3.88 – 3.75 (m, 5H: H6b′, H5⁗, H5a″, H3‴, H5‴), 3.75 – 3.66 (m, 2H: H4, H6b⁗), 3.66 – 3.52 (m, 5H: H4⁗, H6a‴, H5b″, H2⁗, H4′), 3.51 – 3.38 (m, 2H: H3, H1), 3.36 (t, *J* = 2.5 Hz, 1H: H2‴), 3.23 (t, *J* = 9.4 Hz, 1H: H6), 3.14 (d, *J* =

2.6 Hz, 1H: H4‴), 3.03 (dd, *J* = 10.3, 3.7 Hz, 1H: H2′), 2.92 (dd, *J* = 13.0, 4.2 Hz, 1H: H6b‴), 2.21 (dt, *J* = 13.3, 4.6 Hz, 1H: $H2_{eq}$), 1.31 (q, *J* = 12.7 Hz, 1H: $H2_{ax}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.66, 138.64, 138.23, 138.06, 138.00, 137.96, 137.59, 137.41, 137.05, 136.99, 128.92, 128.69, 128.55, 128.51, 128.46, 128.42, 128.39, 128.38, 128.36, 128.33, 128.29, 128.25, 128.21, 128.18, 127.98, 127.95, 127.90, 127.84, 127.78, 127.72, 127.57, 127.53, 127.51, 127.48, 127.45, 126.07, 115.90, 106.07 (C1″), 101.30 (Ph*C*HO_2), 98.69 (C1‴), 95.85 (C1′), 95.35 (C1⁗), 93.65 (O*C*H_2O), 84.14 (C6), 82.34 (C2″), 82.15 (C4⁗), 82.01 (C4″), 81.89 (C5), 79.90 (C3′), 78.79 (C2⁗), 78.38 (C3⁗), 77.51 (C4′), 75.59 (C3″), 75.20 (Ph*C*H_2-C3′), 75.11 (Ph*C*H_2-C3⁗), 75.06 (Ph*C*H_2-C6), 74.68 (C4), 74.24 (C5″), 73.35 (Ph*C*H_2-C2⁗), 73.30 (Ph*C*H_2-C2″), 73.26 (Ph*C*H_2-C5″), 72.95 (C3‴, Ph*C*H_2-C6′), 72.40 (Ph*C*H_2-C3‴), 71.76 (Ph*C*H_2-C4‴), 71.50 (C4‴), 70.81 (C5′), 70.07 (C5″), 69.09 (C6′), 68.93 (C6⁗), 63.03 (C2′), 62.98 (C5⁗), 60.40 (C1), 60.21 (C3), 57.32 (C2‴), 51.02 (C6‴), 32.59 (C2). HRESIMS *m/z* calcd for $C_{100}H_{105}N_{15}O_{20}Na$ $[M+Na]^+$ 1858.7558, found 1858.7540.

4'-*O*-(α-D-Glucopyranosyloxymethyl) paromomycin (32). To a stirred solution of **30** (34.6 mg, 18.8 μmol) in THF (2.2 mL) and 0.1 M NaOH (0.4 mL) was added a solution of 1 M trimethylphosphine in THF (107 μL, 107 μmol) at room temperature. After 6 h of stirring at 50 °C, the reaction mixture was concentrated under reduced pressure and the residue was purified by Flash chromatography (eluent: $CHCl_3$:2-propanol:25% ammonia = 5:3:0.2). The resulting product (34.2 mg) was dissolved in a solution of methanol (0.5 mL), water (1 mL), and acetic acid (0.15 mL), and 20% $Pd(OH)_2$/C (80.5 mg) was added. After 19 h of stirring under H_2 atmosphere (48 psi) at room temperature, the reaction mixture was filtered through Celite, neutralized with Amberlite IRA-400 (OH^- form), and concentrated under reduced pressure. The curde product was purified through a Sephadex C-25 ion exchange column (stepwise elution using water, 0.125% ammonia, and 0.375% ammonia) to afford **32** (4.1 mg, 27%), which was followed by titration with acetic acid in water and lyophilized *in vacuo* to give the corresponding pentaacetate salt (4.4 mg, 21%). $[\alpha]_D^{23}$+78.2 (c 0.29, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.59 (d, *J* = 3.9 Hz, 1H: H1′), 5.20 (d, *J* = 2.4 Hz, 1H: H1″), 5.12 (d, *J* = 1.8 Hz, 1H: H1‴), 5.02 (d, *J* = 3.8 Hz, 1H: H1⁗), 4.90 (dd, *J* = 10.9, 6.9 Hz, 2H: O*C*H_2O), 4.36 (dd, *J* = 6.9, 4.9 Hz, 1H: H3″), 4.21 (dd, *J* = 5.0, 2.4 Hz, 1H: H2″), 4.14 (ddd, *J* = 6.4, 4.0, 1.5 Hz, 1H: H5‴), 4.06 (t, *J* = 3.1 Hz,

1H: H3‴), 4.03 (ddd, *J* = 7.2, 4.5, 3.0 Hz, 1H: H4″), 3.91 (dd, *J* = 10.4, 8.7 Hz, 1H: H3′), 3.77 – 3.46 (m, 14H: H5a″, H5′, H4, H5, H6′, H4‴, H6⁗, H5b″, H4′, H5⁗, H3⁗, H6), 3.44 (dd, *J* = 9.9, 3.8 Hz, 1H: H2⁗), 3.41 (dt, *J* = 3.0, 1.3 Hz, 1H: H2‴), 3.30 – 3.16 (m, 5H: H2′, H6a‴, H4⁗, H6b‴, H3), 3.13 (ddd, *J* = 12.7, 10.5, 4.3 Hz, 1H: H1), 2.22 (dt, *J* = 12.8, 4.4 Hz, 1H: $H2_{eq}$), 1.75 (s, 15H: C*H*$_3$COOH), 1.56 (q, *J* = 12.6 Hz, 1H: $H2_{ax}$). ^{13}C NMR (151 MHz, D_2O) δ 180.98 (CH_3*C*OOH), 109.96 (C1″), 95.61 (C1′), 95.39 (C1⁗), 95.35 (C1‴), 92.31 (O*C*H$_2$O), 84.49 (C5), 81.17 (C4″), 78.64 (C4), 75.76 (C4′), 75.10 (C3″), 73.33 (C2″), 72.81 (C5⁗), 72.74 (C5′), 72.64 (C6), 72.33 (C3⁗), 70.81 (C2⁗), 70.19 (C5‴), 69.35 (C4⁗), 68.96 (C3′), 67.62 (C3‴), 67.23 (C4‴), 60.41 (C6⁗), 60.03 (C5″), 59.95 (C6′), 53.62 (C2′), 50.81 (C2‴), 49.91 (C1), 48.82 (C3), 40.35 (C6‴), 29.38 (C2), 23.05 (*C*H$_3$COOH). HRESIMS *m*/*z* calcd for $C_{30}H_{58}N_5O_{20}$ $[M+H]^+$ 808.3675, found 808.3674.

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