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Supporting Information

Selectively Guanidinylated Aminoglycosides as Antibiotics

Richard J. Fair,[a] Mary E. Hensler,[b] Wdee Thienphrapa,[b] Quang N. Dam,[b] Victor Nizet,*[b] and Yitzhak Tor*[a]

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Supporting Information

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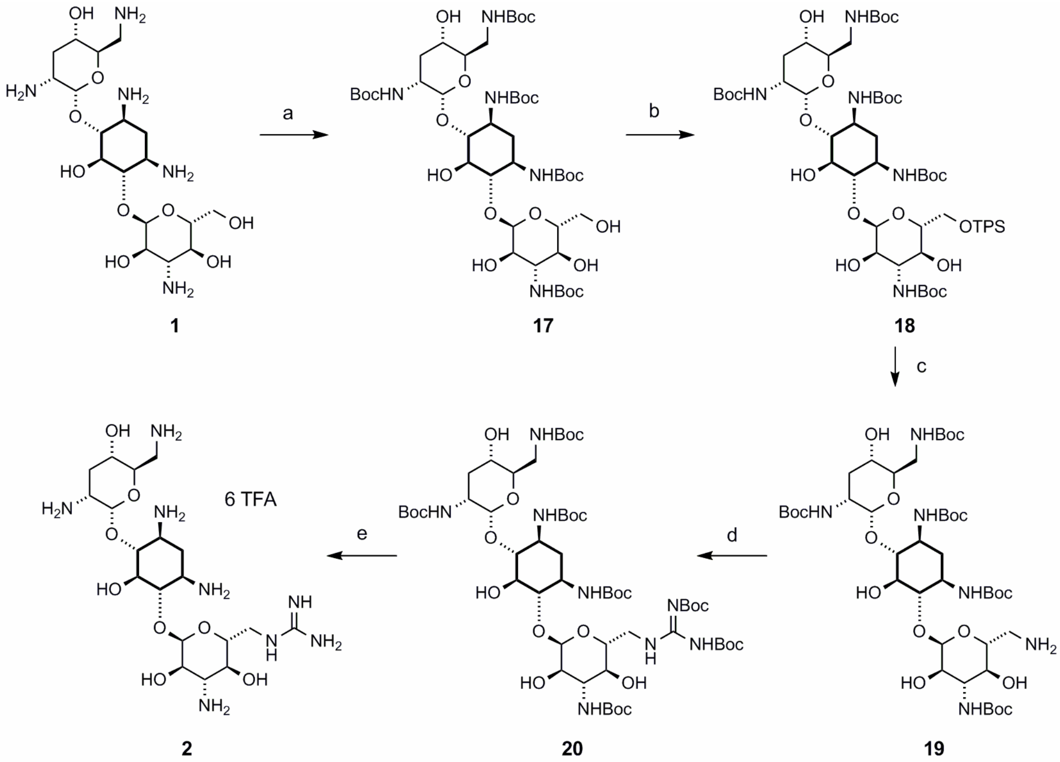
- ACN = acetonitrile
- DCM = dichloromethane
- DMF = dimethylformamide
- rt = room temperature
- TEA = triethylamine
- TIPS = triisopropylsilane

S.1 – Synthesis

General Procedures

Primary Alcohol to Guanidinium Conversions



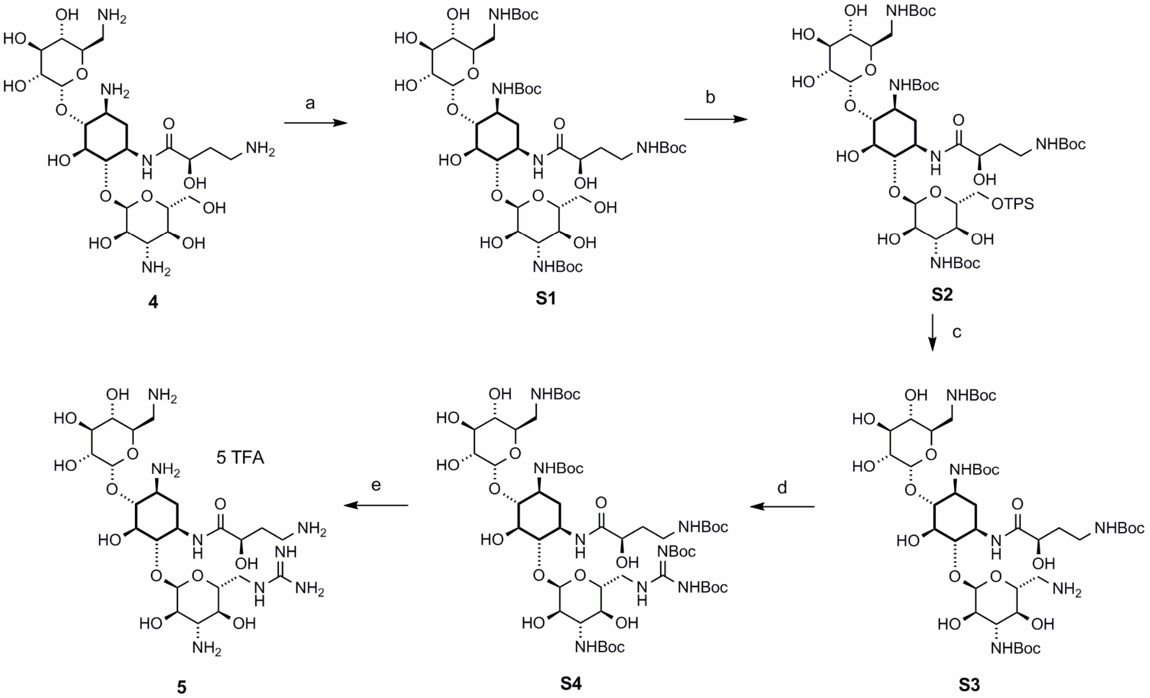
Scheme S1: 6"-Deoxy-6"-guanidinotobramycin synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NH_3, MeOH, (d) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (e) TFA, TIPS, DCM.

6''-Deoxy-6''-amino-(Boc)$_5$tobramycin (19). Synthesis and characterization of precursors 17 and 18 previously reported.[S1] Anhydrous methanol (18 mL) was added to 6''-deoxy-6''-triisopropylbenzylsulfonyl-(Boc)$_4$tobramycin (18) (411 mg, 0.33 mmol) in a pressure tube. The yellow solution was cooled to 0 °C and anhydrous ammonia was bubbled into the solution for 10 mins. The vessel was capped and heated to 80 °C for 2.5 days. The vessel was cooled to 0 °C and opened. After 5 mins DOWEX® Monosphere® 550A ion exchange resin ($^{-}$OH form) was added. The reaction was stirred for 12 hours at rt and filtered. The solvent was removed under reduced pressure and the resulting solid was dissolved in DCM and washed with a 2 M sodium bicarbonate solution. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Product: White solid (184 mg, 0.19 mmol, 57% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.11 (s, 2H), 4.21 (dd, J_1 = 5.6 Hz, J_2 = 2 Hz, 1H), 4.20 – 4.11 (m, 1H), 4.01 – 3.89 (m, 2H), 3.71 – 3.33 (m, 7H), 3.19 – 3.02 (m, 2H), 2.95 (t, J = 7.2 Hz, 1H), 2.80 (q, J = 7.2 Hz, 1H), 2.76 – 2.68 (m, 1H), 2.18 – 1.91 (m, 3H), 1.64 (q, J = 12.4 Hz, 1H), 1.56 – 1.10 (m, 43H), 0.96 – 0.90 (m, 2H); HR-ESI-MS calculated for $C_{43}H_{79}N_6O_{18}$ $[M+H]^+$ 967.5445, found 967.5450

6''-Deoxy-6''-guanidino-(Boc)$_7$tobramycin (20). DCM (3.3 mL), methanol (0.1 mL), and TEA (37 μL, 0.26 mmol) were added to 6''-deoxy-6''-amino-(Boc)$_4$tobramycin (19) (169 mg, 0.18 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (685 mg, 1.75 mmol) was added. The light yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 7% methanol in DCM). Product: White solid (169 mg, 0.14 mmol, 85% yield). ^{1}H-

NMR (400 MHz, CD_3OD): δ 5.13 (s, 1H), 5.09 (s, 1H), 4.16 – 4.11 (m, 1H), 3.91 – 3.86 (m, 1H), 3.79 – 3.19 (m, 14H), 2.17 – 1.95 (m, 3H), 1.57 – 1.51 (m, 13H), 1.51 – 1.41 (m, 42H); HR-ESI-MS calculated for $C_{49}H_{89}N_8O_{20}$ $[M+H]^+$ 1109.6188, found 1109.6186

6''-Deoxy-6''-guanidinotobramycin· 6 TFA (2). DCM (3.75 mL) and TIPS (0.2 mL) were added to 6''-deoxy-6''-guanidino-$(Boc)_6$kanamycin A (20) (150 mg, 0.12 mmol). TFA (3.75 mL) was added. The yellow solution was stirred for 2.5 hours. Toluene (8 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 0.1% ACN in water (0.1% TFA) over 13 min) eluted after 10.2 min, then lyophilized. Product: White powder (117 mg, 0.098 mmol, 79% yield). ^{1}H-NMR (400 MHz, D_2O): δ 5.74 (d, J = 3.2 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 4.05 – 3.90 (m, 4H), 3.83 – 3.66 (m, 4H), 3.64 – 3.47 (m, 6H), 3.41 (dd, J_1 = 14 Hz, J_2 = 3.6 Hz, 1H), 3.27 (q, J = 7 Hz, 1H), 2.54 (dt, J_1 = 12.8 Hz, J_2 = 4.2 Hz, 1H), 2.29 (dt, J_1 = 12.4 Hz, J_2 = 4.4 Hz, 1H), 2.03 (q, J = 11.6 Hz, 1H), 1.92 (q, J = 12.8 Hz, 1H); ^{13}C-NMR (100 MHz, D_2O): δ 163.63 (J = 35 Hz), 158.48, 116.93 (J = 290 Hz), 101.42, 94.74, 84.25, 78.17, 74.73, 71.84, 71.10, 68.61, 66.41, 64.91, 55.29, 50.49, 48.94, 48.32, 41.86, 40.32, 29.82, 28.35; HR-ESI-MS calculated for $C_{19}H_{41}N_8O_8$ $[M+H]^+$ 509.3042, found 509.3041



Scheme S2: 6''-Deoxy-6''-guanidinoamikacin synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NH_3, MeOH, (d) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (e) TFA, TIPS, DCM.

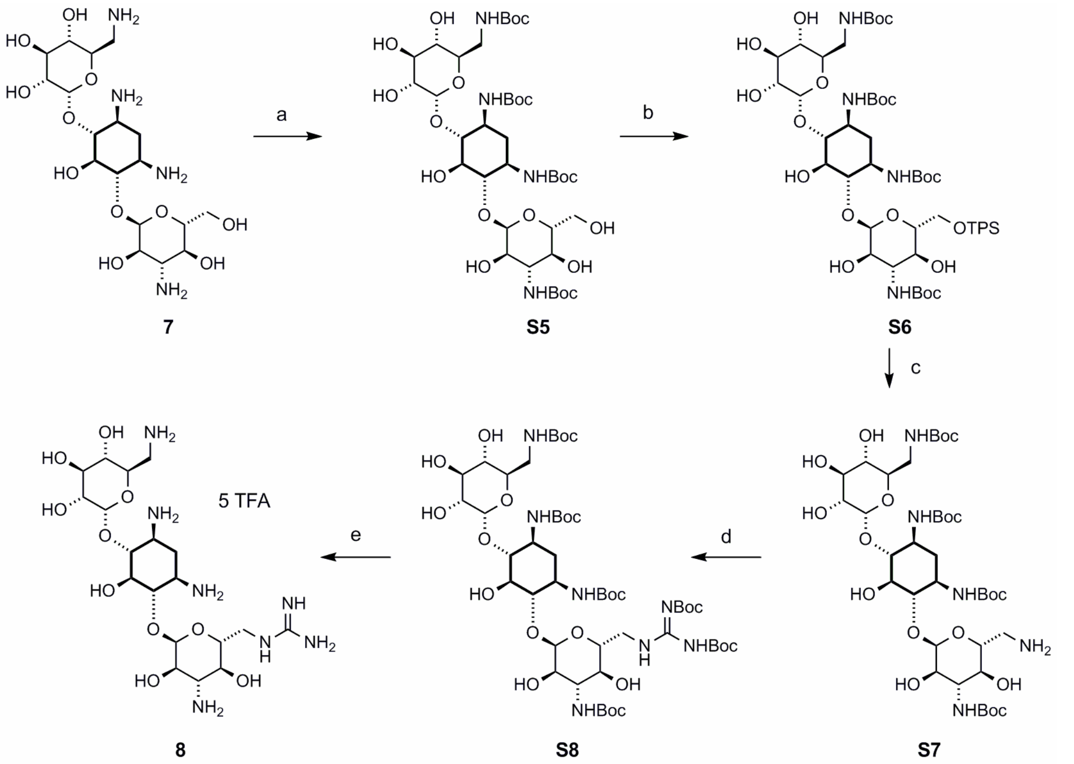
$(Boc)_4$Amikacin (S1). Water (2.1 mL), DMF (2.5 mL), and TEA (1.3 mL, 12.81 mmol) were added to amikacin sulfate (5) (500 mg, 0.85 mmol). The reaction was heated to 55 °C and di-tert-butyl dicarbonate (1.12 g, 5.12 mmol) dissolved in DMF (8 mL) was added slowly. The pale yellow solution was stirred for 6 hours. The solvent was removed under reduced pressure and the resulting solid was suspended in warm water. The solid was filtered and washed thoroughly with water. The product was dissolved in ACN and the solvent was removed under reduced pressure. Product: White solid (816 mg, 0.83 mmol, 97% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.12 (s, 1H), 5.04 (d, J = 4.4 Hz, 1H), 4.06 – 4.00 (m, 1H), 3.98 (dd, J_1 = 8.8 Hz, J_2 = 3.6 Hz, 1H), 3.80 – 3.59 (m, 7H), 3.50 – 3.17 (m, 13H), 2.16 – 2.09 (m, 1H), 1.98 – 1.90 (m, 1H), 1.80 – 1.71 (m, 1H), 1.50 – 1.33 (m, 36H), 1.31 (t, J = 7.2 Hz, 1H); HR-ESI-MS calculated for $C_{42}H_{75}N_5O_{21}Na$ $[M+Na]^+$ 1008.4847, found 1008.4833.

6’’-Deoxy-6’’-triisopropylbenzylsulfonyl-(Boc)$_4$amikacin (S2). Anhydrous pyridine (11.3 mL) was added to (Boc)$_4$amikacin (S1) (816 mg, 0.83 mmol). Triisopropylbenzylsulfonyl chloride (3.41 g, 11.27 mmol) was added. The orange solution was stirred for 2 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 5% methanol in DCM). Product: White solid (530 mg, 0.42 mmol, 51% yield). ^{1}H-NMR (300 MHz, CD$_3$OD): δ 7.27 (s, 2H), 5.49 (s, 2H), 4.40 – 4.28 (m, 3H), 4.19 – 4.08 (m, 4H), 4.00 – 3.82 (m, 3H), 3.76 – 3.31 (m, 8H), 3.28 – 3.16 (m, 4H), 2.99 – 2.86 (m, 3H), 2.08 – 1.90 (m, 3H), 1.52 – 1.38 (m, 30H), 1.38 – 1.13 (m, 22H), 0.98 – 0.86 (m, 3H); HR-ESI-MS calculated for $C_{57}H_{97}N_5O_{23}SNa$ [M+Na]$^+$ 1274.6187, found 1274.6190

6’’-Deoxy-6’’-amino-(Boc)$_4$amikacin (S3). Anhydrous methanol (7.5 mL) was added to 6’’-deoxy-6’’-triisopropylbenzylsulfonyl-(Boc)$_4$amikacin (S2) (480 mg, 0.39 mmol) in a pressure tube. The yellow solution was cooled to 0 °C and anhydrous ammonia was bubbled into the solution for 10 mins. The vessel was capped and heated to 80 °C for 2 days. The vessel was cooled to 0 °C and opened. After 5 mins DOWEX® Monosphere® 550A ion exchange resin ($^-$OH form) was added. The reaction was stirred for 12 hours at rt and filtered. The solvent was removed under reduced pressure and the resulting solid was dissolved in DCM and washed with a 2 M sodium bicarbonate solution. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Product: Tan solid (360 mg, 0.37 mmol, 93% yield). ^{1}H-NMR (400 MHz, CD$_3$OD): δ 5.12 (s, 1H), 5.08 (s, 1H), 4.53 – 4.44 (m, 2H), 4.00 – 3.84 (m, 3H), 3.72 – 3.59 (m, 5H), 3.51 – 3.46 (m, 2H), 3.30 – 3.08 (m, 6H), 2.88 – 2.81 (m, 2H), 2.14 (t, J = 7.6 Hz, 1H), 2.08 – 1.89 (m, 2H), 1.80 – 1.68 (m, 1H), 1.62 – 1.40 (m, 15H), 1.36 – 1.19 (m, 18H), 0.94 – 0.84 (m, 3H); HR-ESI-MS calculated for $C_{42}H_{76}N_6O_{20}Na$ [M+Na]$^+$ 1007.5007, found 1007.5002

6’’-Deoxy-6’’-guanidino-(Boc)$_6$amikacin (S4). DCM (2 mL), methanol (0.2 mL), and TEA (65 μL, 0.47 mmol) were added to 6’’-deoxy-6’’-amino-(Boc)$_4$amikacin (S3) (150 mg, 0.151 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (616 mg, 1.58 mmol) was added. The yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 7% methanol in DCM). Product: Tan solid (144 mg, 0.117 mmol, 78% yield). ^{1}H-NMR (400 MHz, CD$_3$OD): δ 5.12 (s, 1H), 5.01 (s, 1H), 4.60 (s, 1H), 4.35 – 4.28 (m, 1H), 4.20 – 4.08 (m, 3H), 4.06 – 3.95 (m, 2H), 3.90 – 3.42 (m, 8H), 3.20 – 3.12 (m, 3H), 2.95 (t, J = 7.2 Hz, 2H), 2.10 – 1.91 (m, 3H), 1.78 – 1.69 (m, 1H), 1.55- 1.50 (m, 6H), 1.48 – 1.38 (s, 27H), 1.32 – 1.23 (m, 21H); HR-ESI-MS calculated for $C_{53}H_{94}N_8O_{24}Na$ [M+Na]$^+$ 1249.6273, found 1249.6259

6’’-Deoxy-6’’-guanidinoamikacin· 5 TFA (5). DCM (0.9 mL) and TIPS (40 μL) were added to 6’’-deoxy-6’’-guanidino-(Boc)$_6$amikacin (S4) (43 mg, 0.033 mmol). TFA (0.9 mL) was added. The yellow solution was stirred for 2.5 hours. Toluene (2 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 6% ACN in water (0.1% TFA) over 12 min) eluted after 5.8 min, then lyophilized. Product: White powder (34 mg, 0.028 mmol, 85% yield). ^{1}H-NMR (500 MHz, D$_2$O): δ 5.47 (d, J = 3.8 Hz, 1H), 5.11 (d, J = 3.8 Hz, 1H), 4.24 – 4.20 (m, 2H), 4.07 – 4.03 (m, 1H), 3.98 – 3.95 (m, 1H), 3.84 – 3.68 (m, 5H), 3.63 (dd, J_1 = 10 Hz, J_2 = 4 Hz, 1H), 3.55 – 3.42 (m, 4H), 3.39 – 3.31 (m, 3H), 3.17 (q, J = 7 Hz, 1H), 3.11 (t, J = 7.5 Hz, 2H), 2.18 – 2.09 (m, 2H), 1.93 – 1.86 (m, 1H), 1.74 (q, J = 12.6 Hz, 1H); ^{13}C-NMR (125 MHz, D$_2$O): δ 176.15, 163.63 (J = 34 Hz), 158.36, 116.96 (J = 290 Hz), 98.60, 97.82, 73.61, 72.72, 71.48, 71.20, 71.03, 70.10, 69.36, 68.57, 66.98, 55.78, 49.46, 48.69, 41.94, 40.81, 37.51, 31.44, 30.81; HR-ESI-MS calculated for $C_{23}H_{47}N_8O_{12}$ [M+H]$^+$ 627.3308, found 627.3306



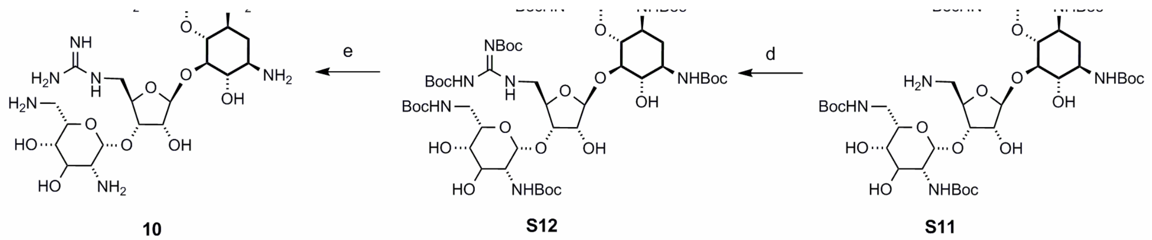
Scheme S3: 6''-Deoxy-6''-guanidinotkanamycin A synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NH_3, MeOH, (d) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (e) TFA, TIPS, DCM.

6''-Deoxy-6''-amino-(Boc)$_4$kanamycin A (S7). Synthesis and characterization of precursors S5 and S6 were previously reported.[S1] Anhydrous methanol (10 mL) was added to 6''-deoxy-6''-triisopropylbenzylsulfonyl-(Boc)$_4$kanamycin A (S6) (325 mg, 0.28 mmol) in a pressure tube. The yellow solution was cooled to 0 °C and anhydrous ammonia was bubbled into the solution for 10 mins. The vessel was capped and heated to 80 °C for 2 days. The vessel was cooled to 0 °C and opened. After 5 mins DOWEX® Monosphere® 550A ion exchange resin ($^{-}$OH form) was added. The reaction was stirred for 12 hours at rt and filtered. The solvent was removed under reduced pressure and the resulting solid was dissolved in DCM and washed with a 2 M sodium bicarbonate solution. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Product: White solid (227 mg, 0.26 mmol, 91% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.12 (s, 1H), 5.08 (s, 1H), 4.20 – 3.80 (m, 3H), 3.71 – 3.40 (m, 7H), 3.23 – 3.07 (m, 5H), 3.01 – 2.94 (m, 1H), 2.62 (q, J = 7 Hz, 1H), 2.16 (dt, J_1 = 18.4 Hz, J_2 = 8.4 Hz, 1H), 2.08 – 1.98 (m, 1H), 1.63 – 1.40 (m, 21H), 1.38 – 1.17 (m, 12H), 0.94 – 0.85 (m, 3H); HR-ESI-MS calculated for $C_{38}H_{69}N_6O_{18}Na$ $[M+Na]^+$ 906.4530, found 906.4527

6''-Deoxy-6''-guanidino-(Boc)$_6$kanamycin A (S8). DCM (3 mL), methanol (0.6 mL), and TEA (65 µL, 0.45 mmol) were added to 6''-deoxy-6''-amino-(Boc)$_4$kanamycin A (S7) (200 mg, 0.23 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (885 mg, 2.26 mmol) was added. The light yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 7% methanol in DCM). Product: White solid (202 mg, 0.18 mmol, 79% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.12 (s, 1H), 5.06 (s, 1H), 3.88 – 3.82 (m, 1H), 3.77 – 3.65 (m, 3H), 3.63 – 3.30 (m, 12H), 3.13 (q, J = 6.6 Hz, 1H), 2.07 – 2.00 (m, 1H), 1.60 – 1.38 (m, 16H), 1.30 – 1.14 (m, 39H); HR-ESI-MS calculated for $C_{49}H_{88}N_7O_{22}$ $[M+H]^+$ 1126.5977, found 1126.5973

6''-Deoxy-6''-guanidinokanamycin A· 5 TFA (8). DCM (1.5 mL) and TIPS (0.1 mL) were added to 6''-deoxy-6''-guanidino-(Boc)$_6$kanamycin A (S8) (67 mg, 0.060 mmol). TFA (1.5 mL) was added. The yellow solution was stirred for 2.5 hours. Toluene (3 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 7%

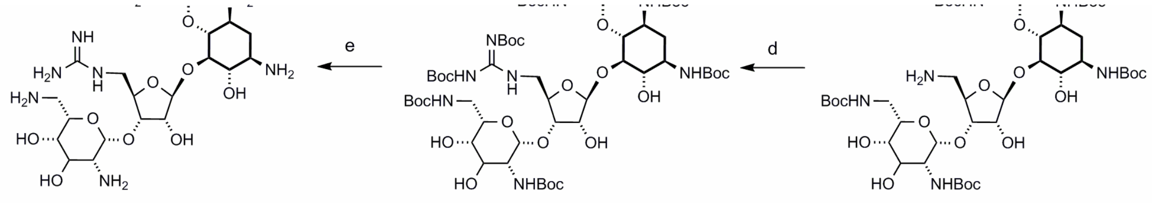
ACN in water (0.1% TFA) over 15 min) eluted after 6 min, then lyophilized. Product: White powder (56 mg, 0.051 mmol, 82% yield). ^{1}H-NMR (500 MHz, D_2O): δ 5.53 (d, J = 4 Hz, 1H), 5.05 (d, J = 4.2 Hz, 1H), 4.01 (dt, J_1 = 9.8 Hz, J_2 = 3.4 Hz, 1H), 3.97 – 3.94 (m, 1H), 3.90 (dd, J_1 = 10.9 Hz, J_2 = 3.8 Hz, 1H), 3.86 – 3.81 (m, 2H), 3.75 – 3.68 (m, 2H), 3.64 (dd, J_1 = 10 Hz, J_2 = 3.7 Hz, 1H), 3.58 – 3.45 (m, 5H), 3.40 – 3.34 (m, 2H), 3.20 (q, J = 6.8 Hz, 1H), 2.50 (dt, J_1 = 12.6 Hz, J_2 = 4 Hz, 1H), 1.87 (q, J = 12.6 Hz, 1H); ^{13}C-NMR (125 MHz, D_2O): δ 163.62 (J = 36 Hz), 158.37, 116.96 (J = 290 Hz), 101.42, 98.64, 84.25, 80.02, 73.95, 72.54, 71.78, 71.47, 71.17, 69.38, 68.70, 66.85, 55.41, 50.63, 48.63, 42.12, 40.78, 28.25; HR-ESI-MS calculated for $C_{19}H_{40}N_7O_{10}$ $[M+H]^+$ 526.2831, found 526.2826



Scheme S4: 5''-Deoxy-5''-guanidinoneomycin synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NH_3, MeOH, (d) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (e) TFA, TIPS, DCM.

5''-Deoxy-5''-guanidino-(Boc)$_8$neomycin (S12). Synthesis and characterization of precursors S9 – S11 were previously reported.[S2-S4] DCM (2.5 mL), methanol (0.1 mL), and TEA (40 µL, 0.28 mmol) were added to 5''-deoxy-5''-amino-(Boc)$_6$neomycin (S11) (226 mg, 0.19 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (728 mg, 1.86 mmol) was added. The yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 7% methanol in DCM). Product: White solid (218 mg, 0.149 mmol, 80% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.50 (s, 1H), 5.29 (s, 1H), 5.15 (s, 1H), 4.39 – 4.11 (m, 3H), 4.05 – 3.97 (m, 2H), 3.90 – 3.72 (m, 5H), 3.65 – 3.43 (m, 8H), 3.40 – 3.26 (m, 4H), 3.23 – 3.16 (m, 2H), 2.00 – 1.94 (m, 1H), 1.55 (s, 9H), 1.50 – 1.30 (m, 64H); HR-ESI-MS calculated for $C_{64}H_{114}N_9O_{28}$ $[M+H]^+$ 1456.7768, found 1456.7771

5''-Deoxy-5''-guanidinoneomycin· 7 TFA (10). DCM (1.26 mL) and TIPS (65 µL) were added to 5''-deoxy-5''-guanidino-(Boc)$_8$neomycin (S12) (73 mg, 0.05 mmol). TFA (1.26 mL) was added. The yellow solution was stirred for 3 hours. Toluene (3 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (5 – 8% ACN in water (0.1% TFA) over 10 min) eluted after 6 min, then lyophilized. Product: White powder (70 mg, 0.05 mmol, 96% yield). ^{1}H-NMR (500 MHz, D_2O): δ 6.08 (d, J = 4 Hz, 1H), 5.42 (d, J = 2.9 Hz, 1H), 5.30 (d, J = 1.7 Hz, 1H), 4.46 (t, J = 5.3 Hz, 1H), 4.41 (t, J = 3.8 Hz, 1H), 4.34 – 4.30 (m, 2H), 4.23 (t, J = 3.1 Hz, 1H), 4.12 (t, J = 9.5 Hz, 1H), 4.03 (t, J = 9.6 Hz, 1H), 3.97 – 3.92 (m, 2H), 3.84 (t, J = 1.6 Hz, 1H), 3.69 (t, J = 9.7 Hz, 1H), 3.62 – 3.54 (m, 3H), 3.49 – 3.27 (m, 9H), 2.49 (dt, J_1 = 12.6 Hz, J_2 = 4.2 Hz, 1H), 1.89 (q, J = 12.7 Hz, 1H); ^{13}C-NMR (125 MHz, D_2O): δ 163.63 (J = 35 Hz), 157.91, 117.00 (J = 290 Hz), 111.15, 96.17, 94.62, 85.88, 79.63, 77.89, 75.39, 73.86, 72.96, 71.28, 70.76, 70.56, 68.70, 68.24, 68.11, 53.91, 51.46, 50.28, 49.20, 44.63, 41.13, 40.87, 28.53; HR-ESI-MS calculated for $C_{24}H_{50}N_9O_{12}$ $[M+H]^+$ 656.3573, found 656.3571



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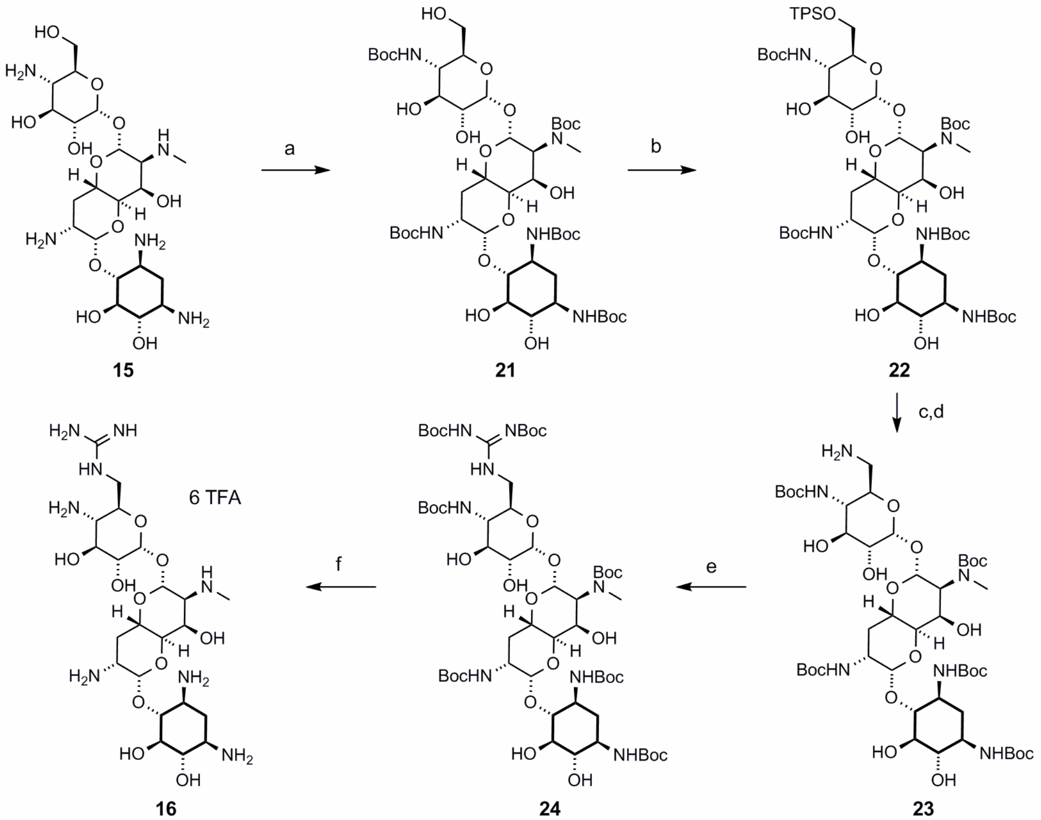
Scheme S5: 5'',6'-Dideoxy-5'',6'-diguanidinoparomomycin synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NH_3, MeOH, (d) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (e) TFA, TIPS, DCM.

5'',6'-Dideoxy-5'',6'-di(triisopropylbenzylsulfonyl)-(Boc)$_5$paromomycin (S14). Synthesis and characterization of precursor S13 was previously reported.[S5] Anhydrous pyridine (22 mL) was added to (Boc)$_5$paromomycin (S13) (1.63 g, 1.46 mmol). Triisopropylbenzylsulfonyl chloride (8.84 g, 29.2 mmol) was added. The orange solution was stirred for 2 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 4% methanol in DCM). Product: White solid (1.36 g, 0.83 mmol, 57% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 7.31 (s, 2H), 7.30 (s, 2H), 5.43 (s, 1H), 5.17 – 5.13 (m, 2H), 4.40 – 4.08 (m, 11H), 4.01 – 3.95 (m, 1H), 3.87 (s, 1H), 3.82 – 3.72 (m, 2H), 3.63 – 3.20 (m, 11H), 3.15 (t, J = 6.8 Hz, 1H), 2.98 – 2.93 (m, 3H), 1.98 – 1.91 (m, 1H), 1.50 – 1.35 (m, 42H), 1.34 – 1.19 (m, 40H); HR-ESI-MS calculated for $C_{78}H_{129}N_5O_{28}S_2Na$ $[M+Na]^+$ 1670.8158, found 1670.8154

5'',6'-Dideoxy-5'',6'-diamino-(Boc)$_5$paromomycin (S15). Anhydrous methanol (15.8 mL) was added to 5'',6'-dideoxy-5'',6'-di(triisopropylbenzylsulfonyl)-(Boc)$_5$paromomycin (S14) (1.31 g, 0.83 mmol) in a pressure tube. The pale yellow solution was cooled to 0 °C and anhydrous ammonia was bubbled into the solution for 10 mins. The vessel was capped and heated to 80 °C for 2 days. The vessel was cooled to 0 °C and opened. After 5 mins DOWEX® Monosphere® 550A ion exchange resin ($^-$OH form) was added. The reaction was stirred for 12 hours at rt and filtered. The solvent was removed under reduced pressure and the resulting solid was dissolved in DCM and washed with a 2 M sodium bicarbonate solution. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Product: Light yellow solid (811 mg, 0.73 mmol, 88% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.48 (s, 1H), 5.22 – 5.11 (m, 2H), 4.26 – 4.08 (m, 5H), 3.98 – 3.86 (m, 4H), 3.76 (s, 2H), 3.70 – 3.65 (m, 1H), 3.60 – 3.45 (m, 6H), 3.17 – 2.95 (m, 4H), 2.86 – 2.79 (m, 1H), 2.67 (q, J = 6.8 Hz, 1H), 1.98 – 1.87 (m, 1H), 1.59 – 1.35 (m, 39H), 1.35 – 1.21 (m, 6H), 0.95 – 0.88 (m, 1H); HR-ESI-MS calculated for $C_{48}H_{88}N_7O_{22}$ $[M+H]^+$ 1114.5977, found 1114.5970

5'',6'-Dideoxy-5'',6'-diguanidino-(Boc)$_9$paromomycin (S16). DCM (9.2 mL), methanol (0.5 mL), and TEA (0.29 mL, 2.05 mmol) were added to 5'',6'-dideoxy-5'',6'-diamino-(Boc)$_5$paromomycin (S15) (761 mg, 0.68 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (1.80 g, 4.78 mmol) was added. The yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 7% methanol in DCM). Product: White solid (770 mg, 0.482 mmol, 71% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.47 (s, 1H), 5.18 – 5.14 (m, 2H), 4.39 – 4.05 (m, 6H), 4.03 – 3.97 (m, 2H), 3.91 – 3.22 (m, 16H), 2.06 – 1.96 (m, 1H), 1.61 – 1.51 (m, 13H), 1.51 – 1.36 (m, 60H), 1.31 – 1.23 (m, 9H); HR-ESI-MS calculated for $C_{70}H_{124}N_{11}O_{30}$ $[M+H]^+$ 1598.8510, found 1598.8505

5'',6'-Dideoxy-5'',6'-diguanidinoparomomycin· 7 TFA (14). DCM (3 mL) and TIPS (0.15 mL) were added to 5'',6'-dideoxy-5'',6'-diguanidino-(Boc)$_9$paromomycin (S16) (183 mg, 0.12 mmol). TFA (3 mL) was added. The yellow solution was stirred for 3 hours. Toluene (6 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 0.1% ACN in water (0.1% TFA) over 10 min) eluted after 8.5 min, then lyophilized. Product: White powder (149 mg, 0.10 mmol, 82% yield). ^{1}H-NMR (300 MHz, D_2O): δ 5.95 (d, J = 3.9 Hz, 1H), 5.38 (d, J = 3 Hz, 1H), 5.27 (s, 1H), 4.42 (t, J = 5.4 Hz, 1H), 4.37 – 4.35 (m, 1H), 4.32 – 4.24 (m, 2H), 4.19 (t, J = 3 Hz, 1H), 4.09 – 3.87 (m, 3H), 3.84 – 3.76 (m, 2H), 3.67 (t, J = 9.6 Hz, 1H), 3.60 – 3.47 (m, 5H), 3.45 – 3.25 (m, 6H), 2.46 (dt, J_1 = 12.6 Hz, J_2 = 4 Hz, 1H), 1.85 (q, J = 12.6 Hz, 1H); ^{13}C-NMR (125 MHz, D_2O): δ 163.60 (J = 37 Hz), 158.30, 157.95, 116.99 (J = 290 Hz), 110.82, 96.19, 94.99, 85.54, 79.58, 77.76, 75.75, 73.84, 72.91, 72.83, 70.77, 70.09, 69.14, 68.23, 68.09, 54.11, 51.48, 50.29, 49.28, 44.45, 42.14, 41.12, 28.52; HR-ESI-MS calculated for $C_{25}H_{52}N_{11}O_{12}$ $[M+H]^+$ 698.3791, found 698.3785



Scheme S6: 6''-Deoxy-6''-guanidinoapramycin synthesis. (a) Boc_2O, TEA, H_2O, DMF. (b) TPSCl, Pyridine. (c) NaN_3, DMF. (d) Pd/C, H_2, MeOH (e) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (f) TFA, TIPS, DCM.

(Boc)$_5$Apramycin (21). Water (1.9 mL), DMF (2.1 mL), and TEA (1.2 mL, 11.76 mmol) were added to apramycin sulfate (15) (500 mg, 0.78 mmol). The reaction was heated to 55 °C and di-tert-butyl dicarbonate (1.03 g, 4.70 mmol) dissolved in DMF (7.5 mL) was added slowly. The yellow solution was stirred for 8 hours. The solvent was removed under reduced pressure and the resulting solid was suspended in warm water. The solid was filtered and washed thoroughly with water. The product was dissolved in ACN and the solvent was removed under reduced pressure. Product: White solid (749 mg, 0.72 mmol, 92% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.52 (s, 1H), 5.32 – 5.30 (m, 2H), 4.18 (s, 1H), 4.01 (d, J = 7.2 Hz, 1H), 3.81 (t, J = 7.6 Hz, 1H), 3.69 – 3.34 (m, 10H), 3.14 (t, J = 9 Hz, 2H), 3.05 (s, 3H), 2.06 – 2.01 (m, 1H), 1.98 – 1.92 (m, 1H), 1.71 (q, J = 11.6 Hz, 1H), 1.52 – 1.28 (m, 46H); HR-ESI-MS calculated for $C_{46}H_{81}N_5O_{21}Na$ $[M+Na]^+$ 1062.5316, found 1062.5319.

6''-Deoxy-6''-triisopropylbenzylsulfonyl-(Boc)$_5$apramycin (22). Anhydrous pyridine (4.6 mL) was added to (Boc)$_5$apramycin (21) (420 mg, 0.40 mmol). Triisopropylbenzylsulfonyl chloride (1.22 g, 4.03 mmol) was added. The orange solution was stirred for 36 hours. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 5% methanol in DCM). Product: White solid (160 mg, 0.12 mmol, 31% yield). ^{1}H-NMR (500 MHz, CD_3OD): δ 7.29 (s, 2H), 5.54 (s, 1H), 5.29 (d, J = 9 Hz, 1H), 5.26 (d, J = 3.5 Hz, 1H), 4.20 – 4.12 (m, 3H), 4.03 (d, J = 7 Hz, 1H), 3.93 (d, J = 8 Hz, 1H), 3.82 (q, J = 9.5 Hz, 1H), 3.72 – 3.68 (m, 1H), 3.62 (q, J = 9.5 Hz, 1H), 3.53 – 3.36 (m, 4H), 3.17 (q, J = 10.5 Hz, 1H), 3.09 – 3.04 (m, 2H), 3.00 (s, 3H), 2.99 – 2.93 (m, 3H), 2.08 – 2.03 (m, 1H), 2.02 – 1.95 (m, 1H), 1.72 (q, J = 12 Hz, 1H), 1.54 – 1.50 (m, 8H), 1.50 – 1.44 (m, 29H), 1.40 – 1.37 (s, 9H), 1.28 – 1.26 (m, 18H); HR-ESI-MS calculated for $C_{61}H_{103}N_5O_{23}SNa$ $[M+Na]^+$ 1328.6657, found 1328.6649

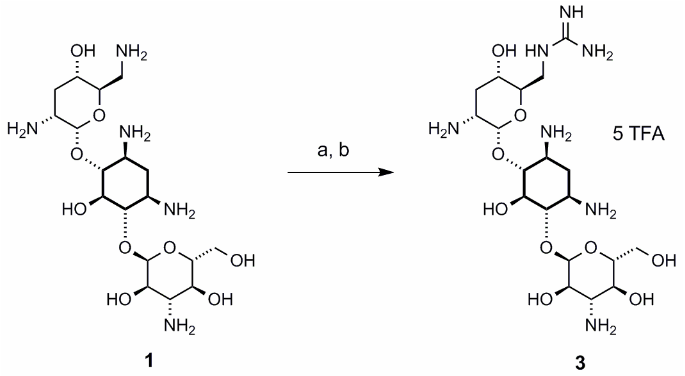
6''-Deoxy-6''-amino-(Boc)$_5$apramycin (23). DMF (2.5 mL) was added to 6''-deoxy-6''-triisopropylbenzylsulfonyl-(Boc)$_5$apramycin (22) (160 mg, 0.12 mmol). Sodium azide (64 mg, 0.98 mmol) was added. The yellow solution was heated to 55 °C and stirred for 2 days. The solvent was removed under reduced pressure and the resulting solid was dissolved in DCM and washed with water. The organic layers were dried with sodium sulfate and the solvent was removed under reduced pressure. Anhydrous methanol (1.1 mL) and acetic acid (10 µL) were added to the resulting white solid. The solution was degassed by bubbling through argon. Pd/C (10%, 14 mg, 0.013 mmol) was added and the reaction was stirred under atmospheric H_2 overnight. The solution was filtered through celite and the solvent was removed under reduced pressure. The product was isolated by flash chromatography (10% methanol, 1% TEA in DCM). Product: White solid (102 mg, 0.098 mmol, 80% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.47 (s, 1H), 5.30 (s, 1H), 5.27 (s, 1H), 4.24 – 4.14 (m, 3H), 4.02 – 3.94 (m, 2H), 3.86 – 3.31 (m, 6H), 3.17 – 3.09 (m, 2H), 3.03 (s, 3H), 2.94 (q, J = 6.6 Hz, 1H), 2.80 – 2.63 (m, 1H), 2.08 – 1.89 (m, 3H), 1.52 – 1.04 (m, 44H), 0.97 – 0.89 (m, 2H); HR-ESI-MS calculated for $C_{46}H_{83}N_6O_{20}$ $[M+H]^+$ 1039.5657, found 1039.5658

6''-Deoxy-6''-guanidino-(Boc)$_7$apramycin (24). DCM (0.7 mL), methanol (0.14 mL), and TEA (15 µL, 0.11 mmol) were added to 6''-deoxy-6''-amino-(Boc)$_5$apramycin (23) (55 mg, 0.05 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (207 mg, 0.53 mmol) was added. The light yellow solution was stirred for 3 days. The solvent was removed under reduced pressure. The product was isolated by flash chromatography (0 – 6% methanol in DCM). Product: White solid (50 mg, 0.04 mmol, 74% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ 5.39 (s, 1H), 5.28 (s, 1H), 5.13 (s, 1H), 4.22 – 4.15 (m, 2H), 4.02 – 3.96 (m, 2H), 3.86 – 3.80 (m, 2H), 3.70 – 3.35 (m, 6H), 3.22 – 3.14 (m, 3H), 2.97 (s, 3H), 2.08 – 1.95 (m, 3H), 1.74 – 1.68 (m, 1H), 1.55 (s, 6H), 1.49 – 1.39 (m, 36H), 1.32 – 1.26 (m, 21H); HR-ESI-MS calculated for $C_{57}H_{101}N_8O_{24}$ $[M+H]^+$ 1281.6923, found 1281.6925

6''-Deoxy-6''-guanidinoapramycin· 6 TFA (16). DCM (1 mL) and TIPS (50 µL) were added to 6''-deoxy-6''-guanidino-(Boc)$_6$kanamycin A (24) (50 mg, 0.039 mmol). TFA (1 mL) was added. The yellow solution was stirred for 2 hours. Toluene (2 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 0.1% ACN in water (0.1% TFA) over 12 min) eluted after 9.6 min, then lyophilized. Product: White powder (38 mg, 0.030 mmol, 78% yield). ^{1}H-NMR (500 MHz, D_2O): δ 5.76 (d, J = 4 Hz, 1H), 5.59 (d, J = 3.5 Hz, 1H), 5.25 (d, J = 8.5 Hz, 1H), 4.61 (t, J = 2 Hz, 1H), 4.17 (dq, J_1 = 9.5 Hz, J_2 = 3 Hz, 1H), 3.99 – 3.92 (m, 3H), 3.80 (dd, J_1 = 9.5 Hz, J_2 = 2.3 Hz, 1H), 3.76 (dd, J_1 = 9.5 Hz, J_2 = 4 Hz, 1H), 3.71 – 3.67 (m, 3H), 3.60 – 3.54 (m, 3H), 3.42 (dd, J_1 = 8.5 Hz, J_2 = 3 Hz, 1H), 3.37 – 3.30 (m, 2H), 2.83 (s, 3H), 2.53 (dt, J_1 = 12.5 Hz, J_2 = 4 Hz, 1H), 2.40 (dt, J_1 = 11.5 Hz, J_2 = 4 Hz, 1H), 2.07 (q, J = 11.5 Hz, 1H), 1.89 (q, J = 12.5 Hz, 1H); ^{13}C-NMR (125 MHz, D_2O): δ 163.66 (J = 35 Hz), 158.58, 116.99 (J = 290 Hz), 96.23, 94.88, 93.60, 78.83, 75.77, 73.20, 70.63, 70.38, 69.45, 68.71, 66.83, 63.41, 60.15, 57.99, 52.50, 50.32, 49.09, 48.60, 30.73, 28.98, 27.44; HR-ESI-MS calculated for $C_{22}H_{45}N_8O_{10}$ $[M+H]^+$ 581.3253, found 581.3250

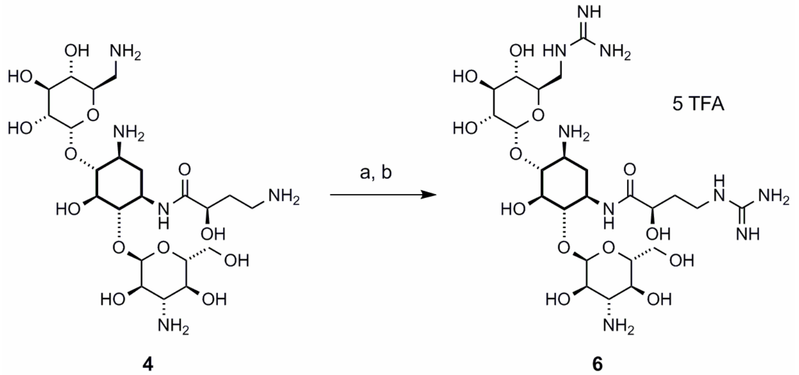
Amine to Guanidinium Conversions

6'-guanidinoneamine (12) was previously synthesized.[S6]



Scheme S7: 6'-Guanidinotobramycin synthesis. (a) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (b) TFA, TIPS, DCM.

6'-Guanidinotobramycin· 5 TFA (3). Water (2.6 mL), methanol (3.4 mL), and TEA (38 μL, 0.29 mmol) were added to tobramycin · 5 TFA (1) (60 mg, 0.058 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (16 mg, 0.041 mmol) was added. The light yellow solution was stirred for 5 days. The solvent was removed under reduced pressure. DCM (1.5 mL) and TIPS (80 μL) were added to the remaining solid. TFA (1.5 mL) was added. The pale yellow solution was stirred for 2 hours. Toluene (3 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 0.1% ACN in water (0.1% TFA) over 14 min) eluted after 10.2 min, then lyophilized. Product: White powder (14 mg, 0.013 mmol, 22% yield). ^{1}H-NMR (400 MHz, D_2O): δ 5.64 (d, J = 4 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 3.97 – 3.88 (m, 3H), 3.87 – 3.82 (m, 3H), 3.79 – 3.70 (m, 4H), 3.68 – 3.53 (m 5H), 3.47 (t, J = 10.6 Hz, 1H), 2.55 (dt, J_1 = 12.8 Hz, J_2 = 4.2 Hz, 1H), 2.29 (dt, J_1 = 12 Hz, J_2 = 4.2 Hz, 1H), 2.02 – 1.88 (m, 2H); ^{13}C-NMR (100 MHz, D_2O): δ 163.63 (J = 35 Hz), 158.31, 116.95 (J = 290 Hz), 101.32, 95.54, 84.44, 78.88, 74.62, 73.57, 73.42, 68.60, 65.88, 63.90, 60.39, 55.45, 50.15, 48.96, 48.52, 41.83, 30.09, 28.40; HR-ESI-MS calculated for $C_{19}H_{40}N_7O_9$ $[M+H]^+$ 510.2882, found 510.2878



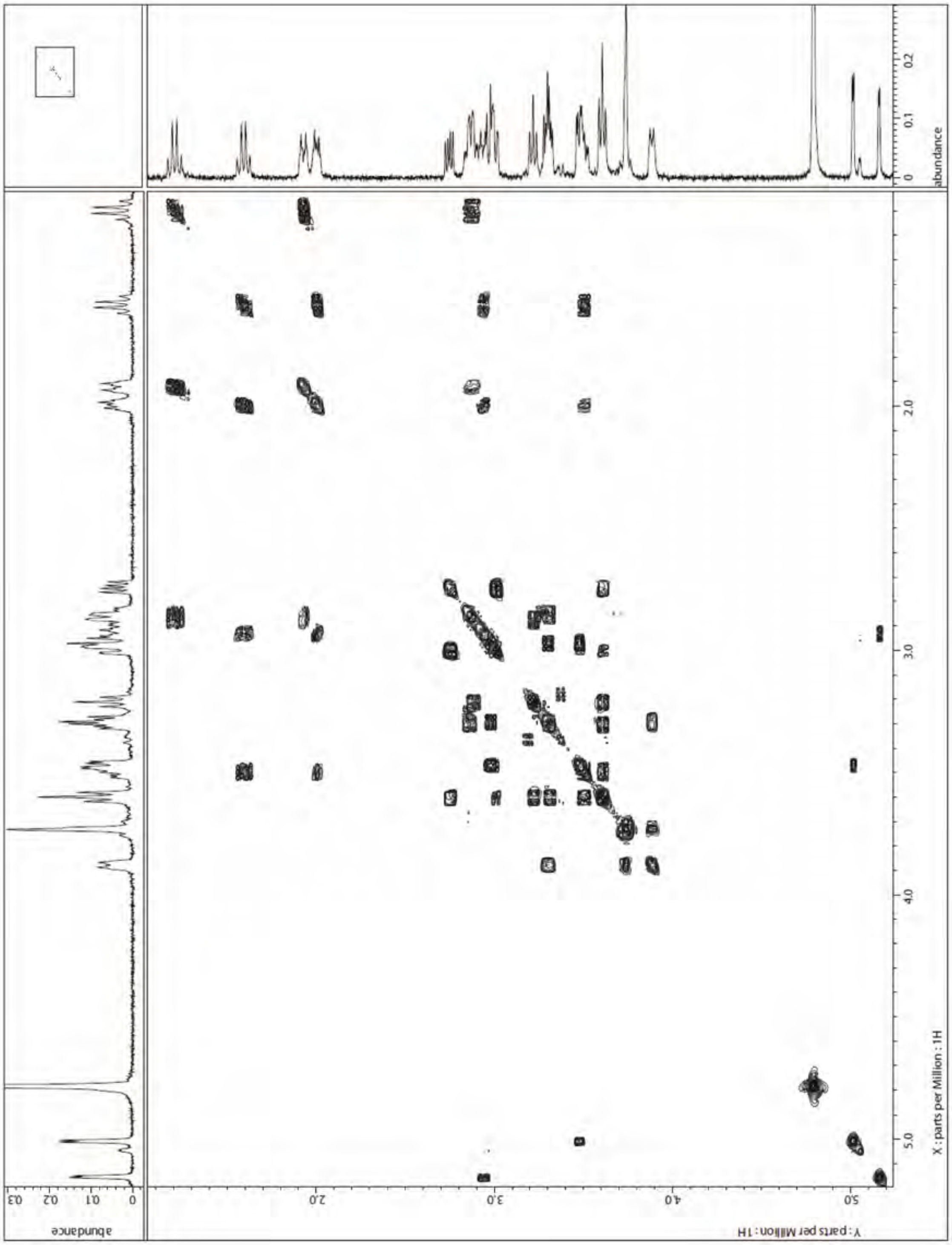
Scheme S8: 6',γ-Diguanidinoamikacin synthesis. (a) 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine, TEA, DCM, MeOH. (b) TFA, TIPS, DCM.

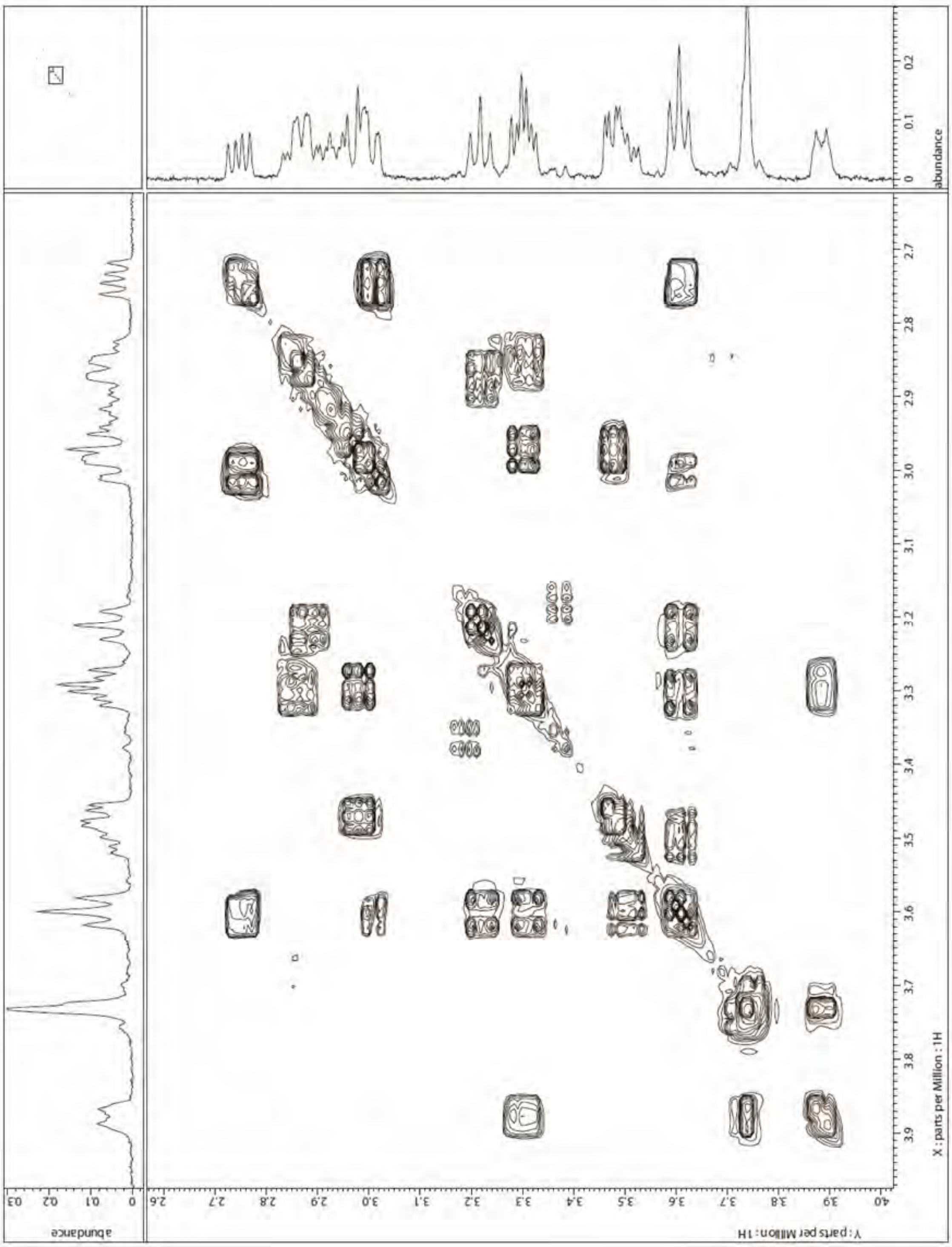
6', γ-Diguanidinoamikacin· 4 TFA (6). Water (15.5 mL), methanol (20.1 mL), and TEA (0.23 mL, 1.72 mmol) were added to amikacin sulfate (4) (300 mg, 0.34 mmol). 1,3-Di-boc-2-(trifluoromethylsulfonyl)guanidine (245 mg, 0.62 mmol) was added. The light yellow solution was stirred for 5 days. The solvent was removed under reduced pressure. DCM (8.6 mL) and TIPS (0.5 mL) were added to the remaining solid. TFA (8.6 mL) was added. The pale yellow solution was stirred for 2 hours. Toluene (17 mL) was added and the solvent was removed under reduced pressure. The remaining white solid was dissolved in water and purified by reverse phase HPLC (0 – 0.1% ACN in water (0.1% TFA) over 13 min) eluted after 8.2 min, then lyophilized. Product: White powder (48 mg, 0.042 mmol, 12% yield). ^{1}H-NMR (300 MHz, D_2O): δ 5.48 (d, J = 3.6 Hz, 1H), 5.15 (d, J = 3.6 Hz, 1H), 4.17 (dd, J_1 = 9.6 Hz, J_2 = 3.3 Hz, 1H), 4.11 – 4.05 (m, 2H), 3.93 – 3.60 (m, 10H), 3.53 – 3.47 (m, 3H), 3.42 – 3.31 (m, 4H), 2.20 (dt, J_1 = 12.6 Hz, J_2 = 4 Hz, 1H), 2.13 – 2.03 (m, 1H), 1.87 – 1.71 (m, 2H); ^{13}C-NMR (75 MHz, D_2O): δ 177.35, 163.60 (J = 38 Hz), 158.33, 157.46, 116.94 (J = 290 Hz), 98.01, 97.80, 80.54, 79.89, 73.62, 72.84, 71.94, 71.56, 70.27, 69.58, 68.65, 65.96, 65.55, 60.10, 55.86, 49.35, 48.84, 42.40, 38.38, 33.03, 30.85; HR-ESI-MS calculated for $C_{24}H_{48}N_9O_{13}$ $[M+H]^+$ 670.3366, found 670.3365

S.2 – 2-D NMR

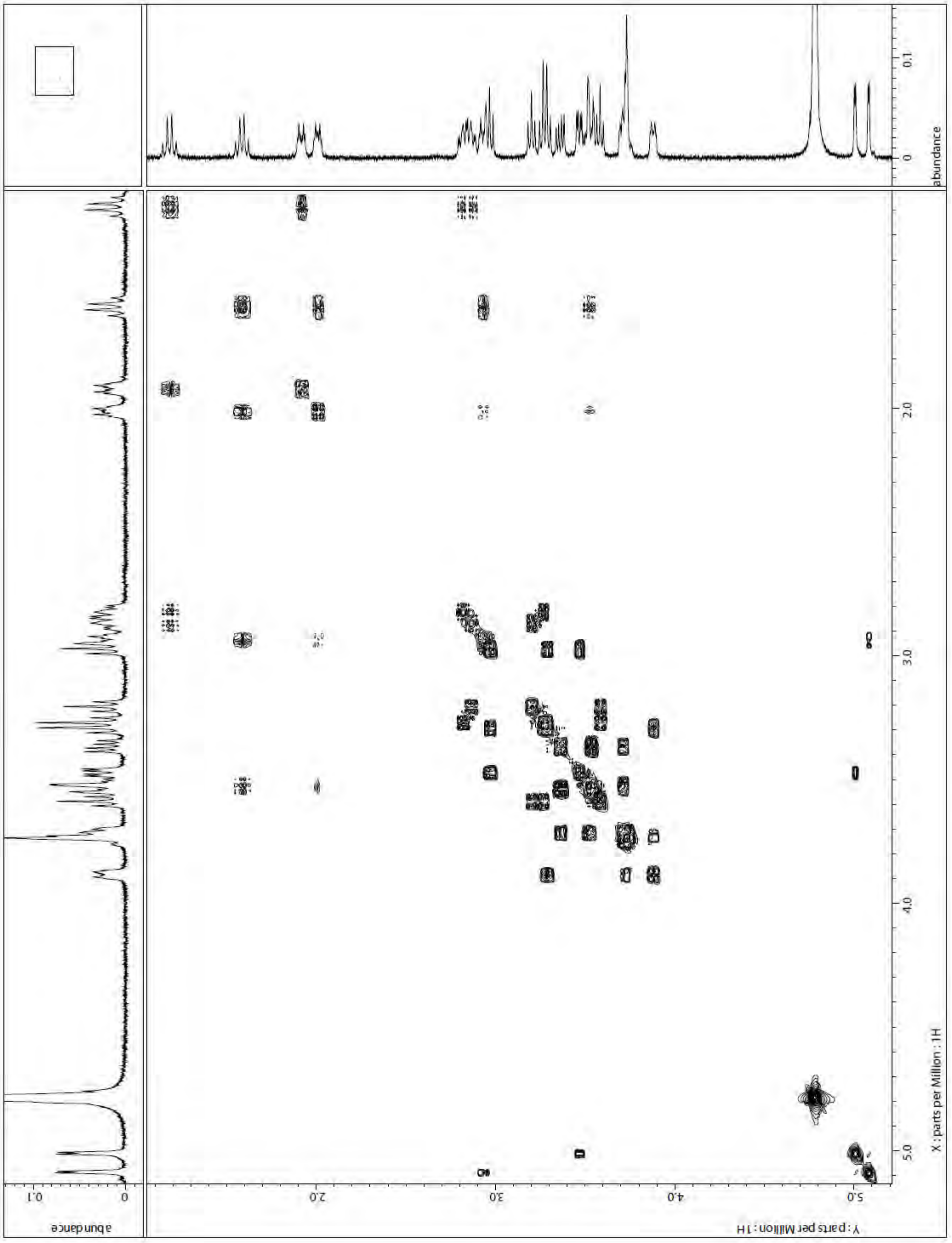
Tobramycin (1), 6’-guanidinotobramycin (3), amikacin (4), and 6’,γ-diguanidinoamikacin (6) were fully assigned by COSY and chemical shifts were compared to verify that only amines at primary sites were converted to guanidinium groups. It should be noted that all 2-D NMR spectra were performed on desalted aminoglycosides, unlike the 1-D ^{1}H NMR spectra, which were taken on TFA salts.

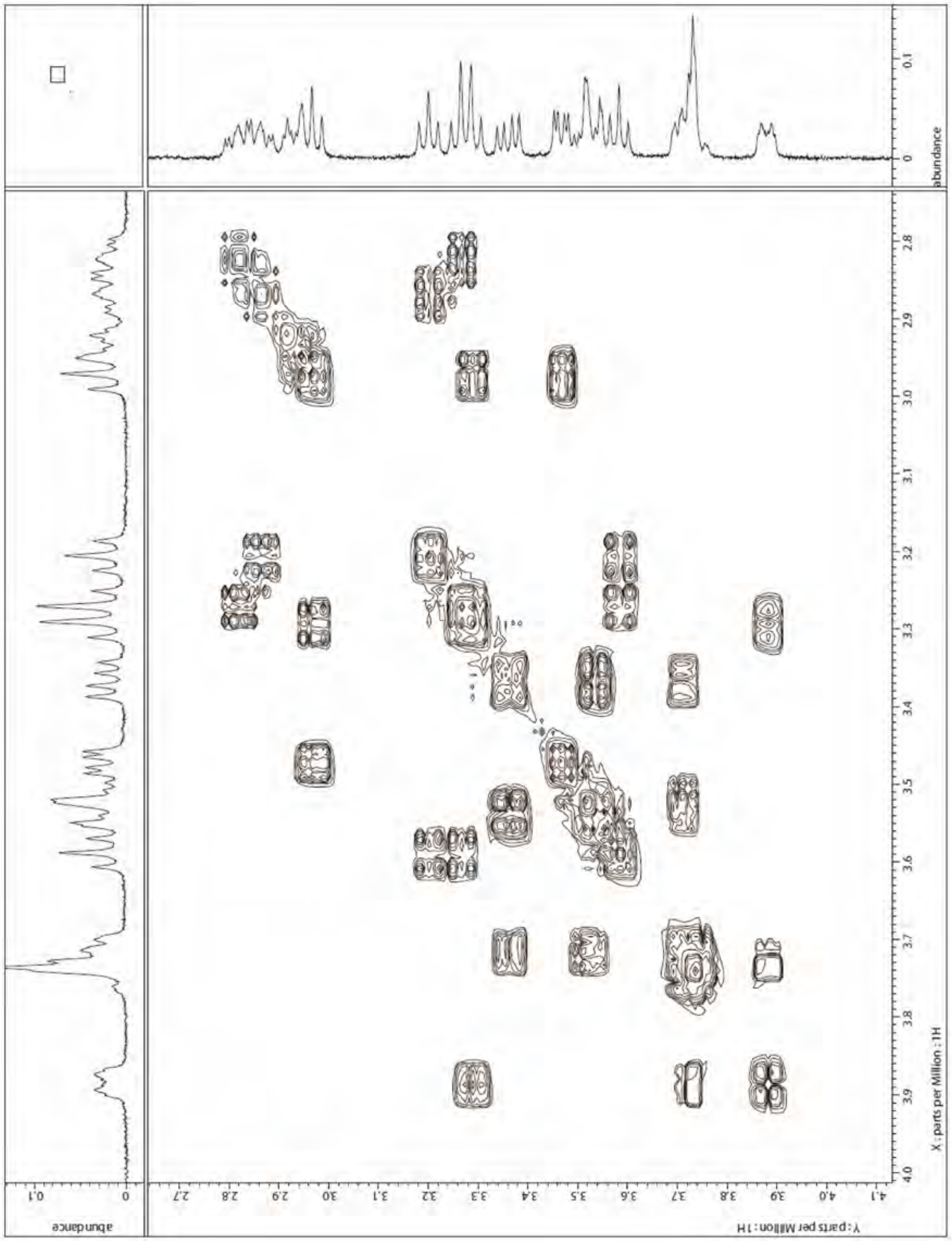
Tobramycin (1):




X : parts per Million : 1H
Y : parts per Million : 1H
abundance
abundance
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
2.6
4.0
0
0.1
0.2
0.3

6’-Guanidinotobramycin (3):

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3.9
3.8
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3.5
3.4
3.3
3.2
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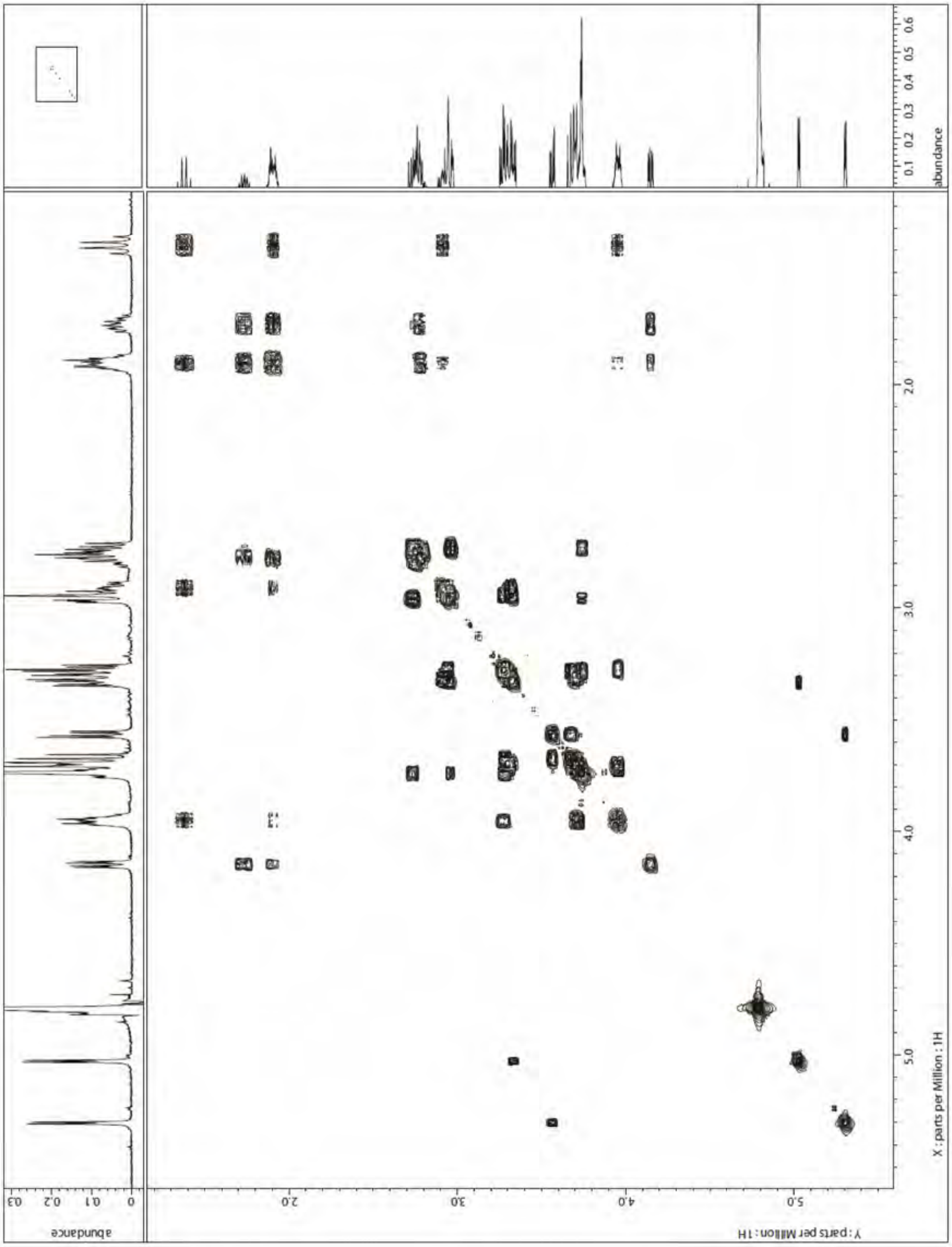
3.0
2.9
2.8
Y : parts per Million : 1H
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
abundance
0
0.1
abundance
0
0.1

Table S1. ^{1}H NMR Chemical Shift Comparison

Proton(s)	Tobramycin (1)	6'-Guanidinotobramycin (3)	Δ ppm
1'	5.14	5.09	-0.05
2'	2.92	2.94	0.02
3'	1.57	1.59	0.02
	1.98	2.01	0.03
4'	3.48	3.54	0.06
5'	3.58	3.72	0.14
6'	2.73	3.37	0.64
	2.99	3.55	0.56
1	2.84	2.85	0.01
2	1.39	1.20	-0.19
	1.90	1.92	0.02
3	2.86	2.87	0.01
4	3.19	3.22	0.03
5	3.59	3.59	0.00
6	3.28	3.27	-0.01
1"	5.00	5.01	0.01
2"	3.45	3.48	0.03
3"	2.96	2.97	0.01
4"	3.28	3.29	0.01
5"	3.87	3.89	0.01
6" (2H)	3.72	3.74	0.02

Amikacin (4):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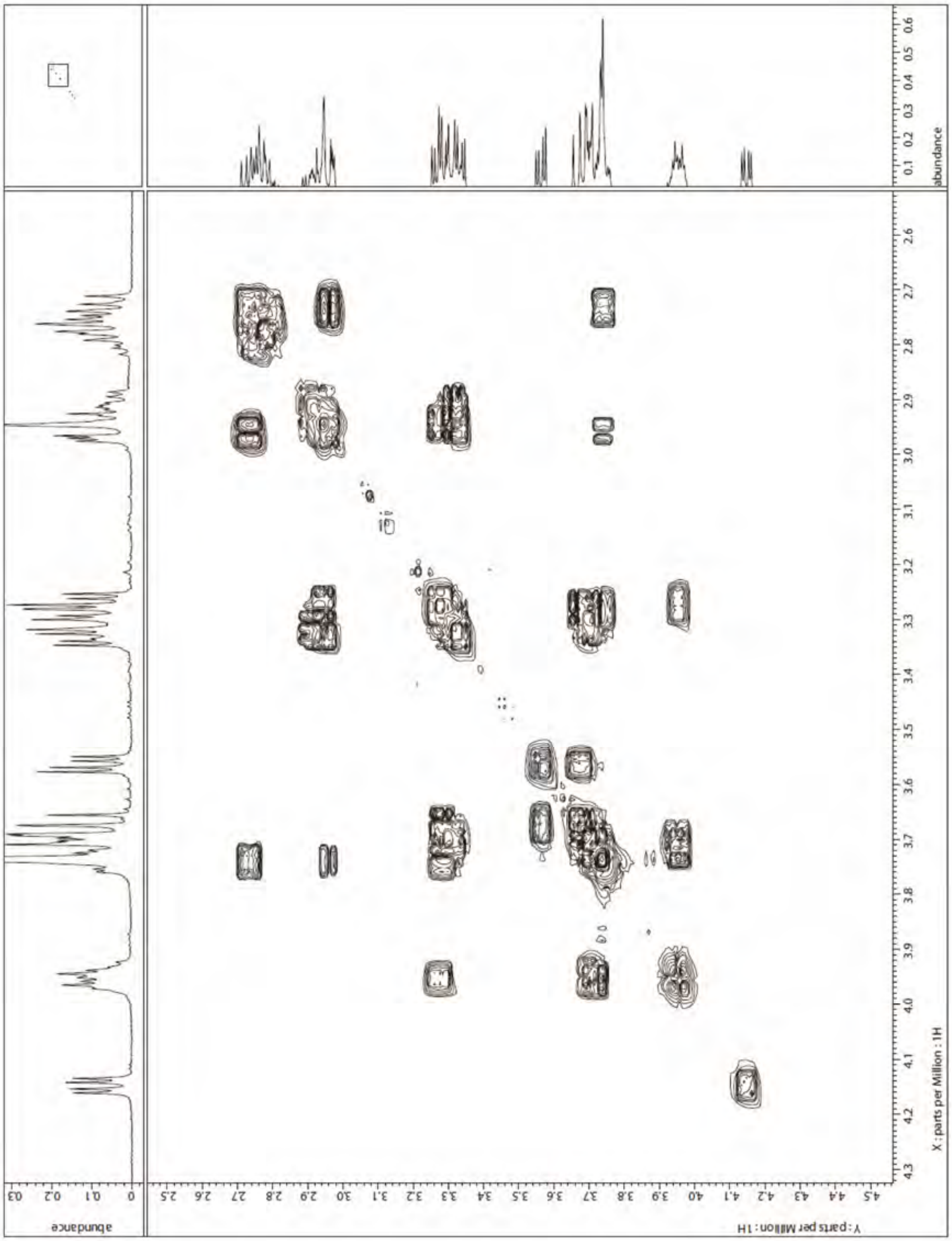
X : parts per Million : 1H
2.0
3.0
4.0
5.0
abundance
0 0.1 0.2 0.3
Y : parts per Million : 1H
2.0
3.0
4.0
5.0


abundance
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X : parts per Million : 1H
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Y : parts per Million : 1H
2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1 4.2 4.3 4.4 4.5
abundance
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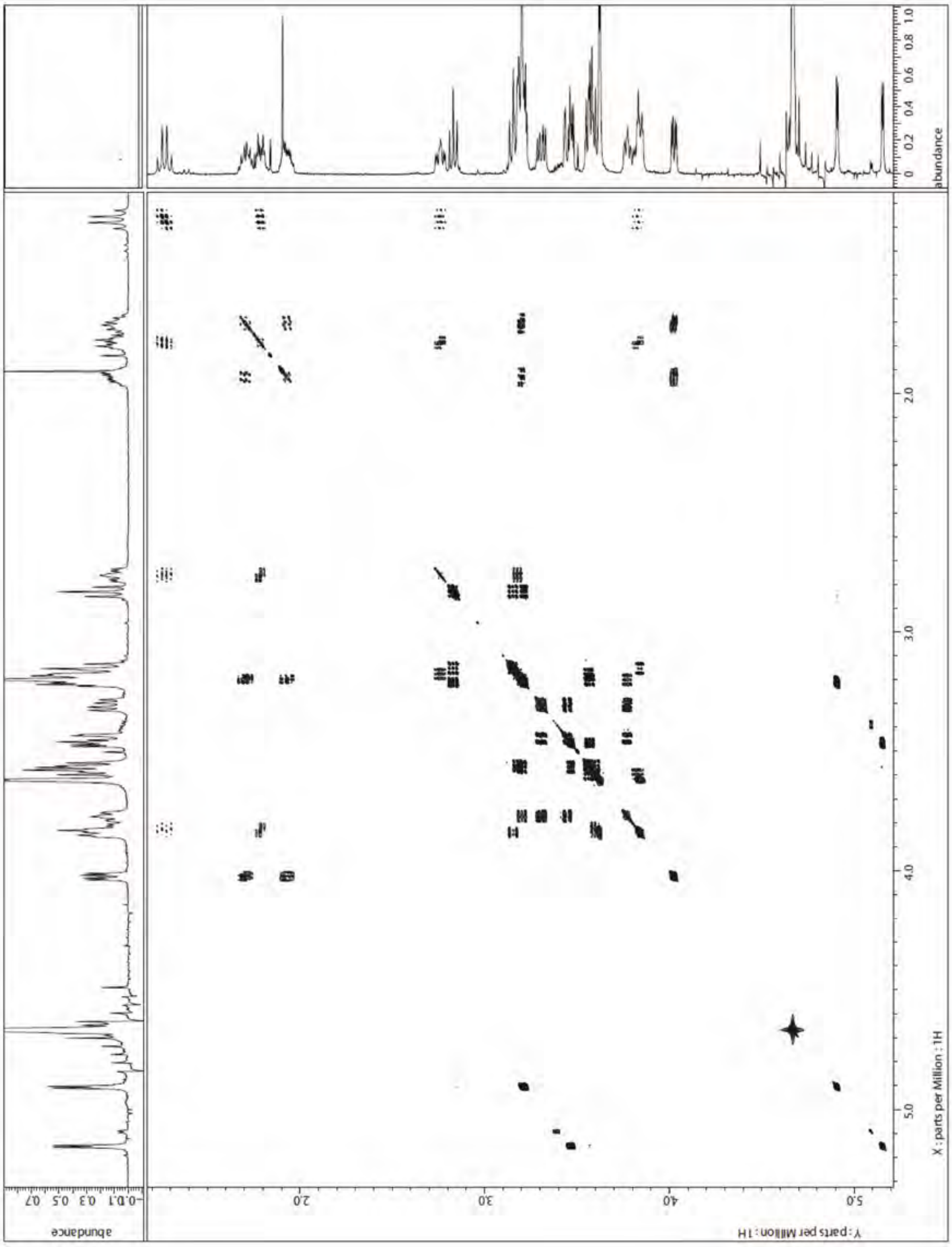
6’,γ-Diguanidinoamikacin (6):



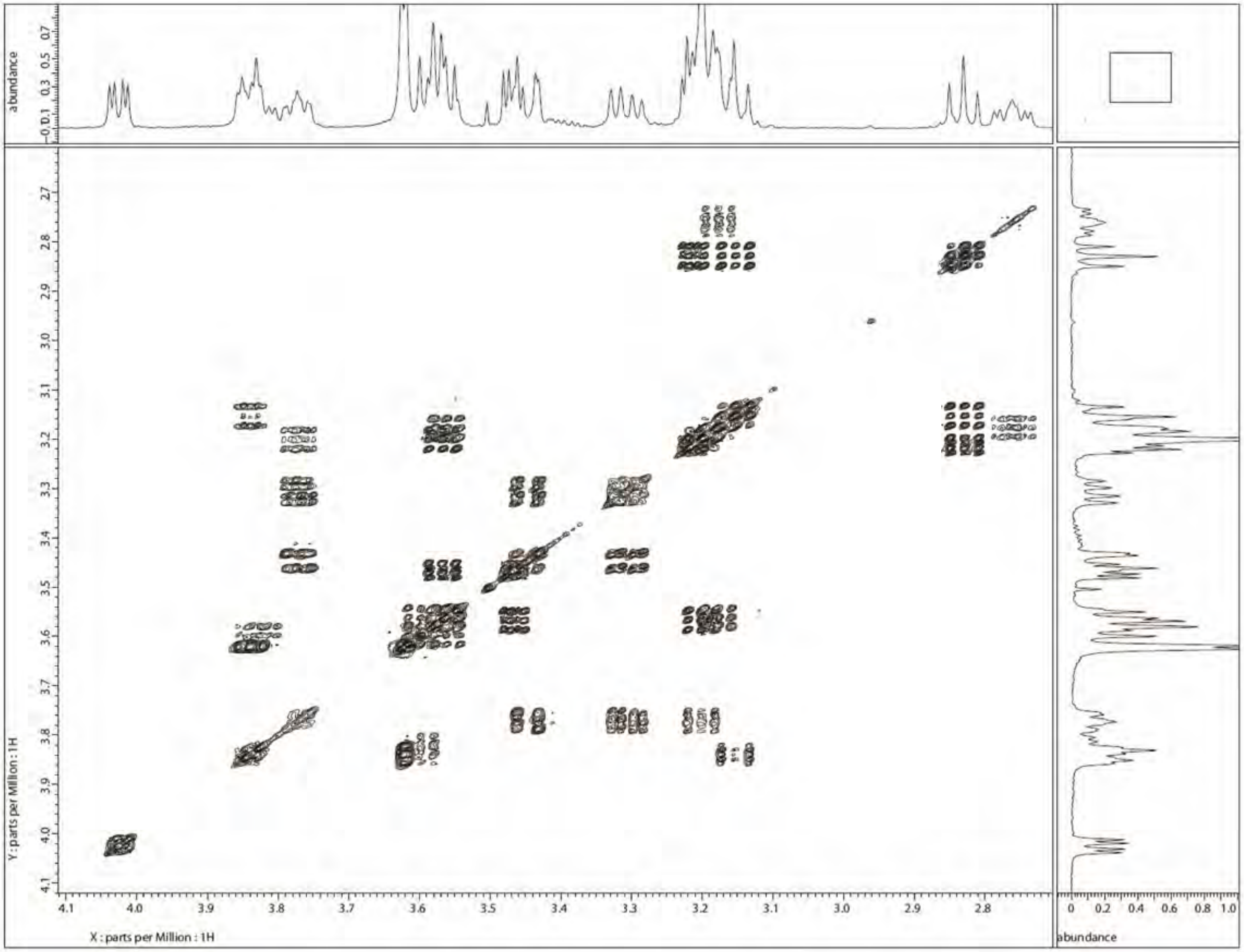

abundance
-0.0 0.1 0.3 0.5 0.7
2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1
Y : parts per Million : 1H
4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8
X : parts per Million : 1H
0 0.2 0.4 0.6 0.8 1.0
abundance

Table S2. ^{1}H NMR Chemical Shift Comparison

Proton(s)	Amikacin (4)	6',γ-Diguanidinoamikacin (6)	Δ ppm
1'	5.30	5.28	-0.02
2'	3.57	3.60	0.03
3'	3.67	3.70	0.03
4'	3.29	3.33	0.04
5'	3.74	3.91	0.17
6'	2.74	3.43	0.69
	2.96	3.57	0.61
1	3.96	3.96	0.00
2	1.37	1.39	0.02
	1.90	1.92	0.02
3	2.91	2.88	-0.03
4	3.31	3.31	0.00
5	3.71	3.70	-0.01
6	3.72	3.74	0.02
1"	5.03	5.03	0.00
2"	3.34	3.34	0.00
3"	2.93	2.95	0.02
4"	3.28	3.28	0.00
5"	3.97	3.97	0.00
6" (2H)	3.72	3.75	0.03
α	4.14	4.15	0.01
β	1.73	1.83	0.10
	1.90	2.07	0.17
γ (2H)	2.78	3.33	0.55

S.3 – A-Site Binding Assay

Aminoglycoside Titrations

All titrations were performed with working solutions of 1 μM Dy-547 labeled A-site in 20 mM cacodylate buffer (pH = 7.0, 100 mM NaCl, 0.5 mM EDTA). The solutions were heated to 75 °C for 5 min, cooled to room temperature over 2 h, cooled to 0 °C for 30 min, then allowed to warm back to room temperature. Kanamycin-courmarin or neomycin-coumarin was added, to give a working concentration of 0.53 μM, just prior to aminoglycoside titrations. Steady state fluorescence experiments were carried out at ambient temperature (20 °C). Excitation and emission slit widths were 9 nm for kanamycin-coumarin experiments and 7 nm for neomycin-coumarin. The system was excited at 400 nm and changes in Dy-547 emission were monitored at 561 nm. Errors were generated from three sets of measurements. IC_{50} values were calculated using OriginPro 8.5 software by fitting a dose response curve (eq 1) to the fractional fluorescence saturation (F_s) plotted against the log of antibiotic (A) concentration.

$$F_s = F_0 + (F_\infty[A]^n)/([IC_{50}]^n + [A]^n) \qquad (1)$$

F_s is the fluorescence intensity at each titration point. F_0 and F_∞ are the fluorescence intensity in the absence of aminoglycoside or at saturation, respectively, and n is the Hill coefficient or degree of cooperativity associated with binding.

Binding Curves

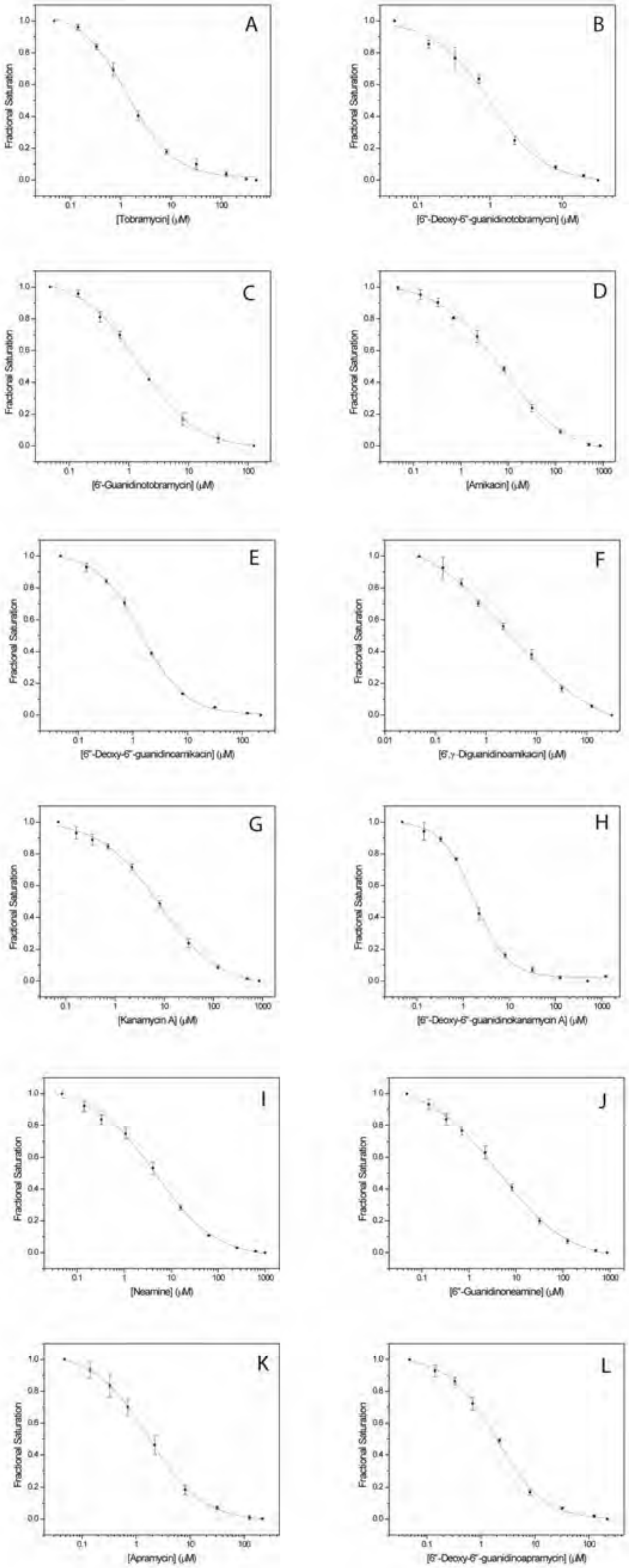

A
Fractional Saturation
[Tobramycin] (μM)
B
Fractional Saturation
[6"-Deoxy-6"-guanidinotobramycin] (μM)
C
Fractional Saturation
[6'-Guanidinotobramycin] (μM)
D
Fractional Saturation
[Amikacin] (μM)
E
Fractional Saturation
[6"-Deoxy-6"-guanidinoamikacin] (μM)
F
Fractional Saturation
[6',γ-Diguanidinoamikacin] (μM)
G
Fractional Saturation
[Kanamycin A] (μM)
H
Fractional Saturation
[6"-Deoxy-6"-guanidinokanamycin A] (μM)
I
Fractional Saturation
[Neamine] (μM)
J
Fractional Saturation
[6"-Guanidinoneamine] (μM)
K
Fractional Saturation
[Apramycin] (μM)
L
Fractional Saturation
[6"-Deoxy-6"-guanidinoapramycin] (μM)
0.0
0.2
0.4
0.6
0.8
1.0
0.01
0.1
1
10
100
1000

Figure S1: Kanamycin-Coumarin displacement curves. A = Tobramycin (1), B = 6''-Deoxy-6''-guanidinotobramycin (2), C = 6'-Guanidinotobramycin (3), D = Amikacin (4), E = 6''-Deoxy-6''-guanidinoamikacin (5), F = 6',γ-Diguanidinoamikacin (6),
G = Kanamycin A (7), H = 6''-Deoxy-6''-guanidinokanamycin A (8), I = Neamine (11),
J = 6'-guanidinoneamine (12), K = Apramycin (15),
L = 6''-Deoxy-6''-guanidinoapramycin (16)

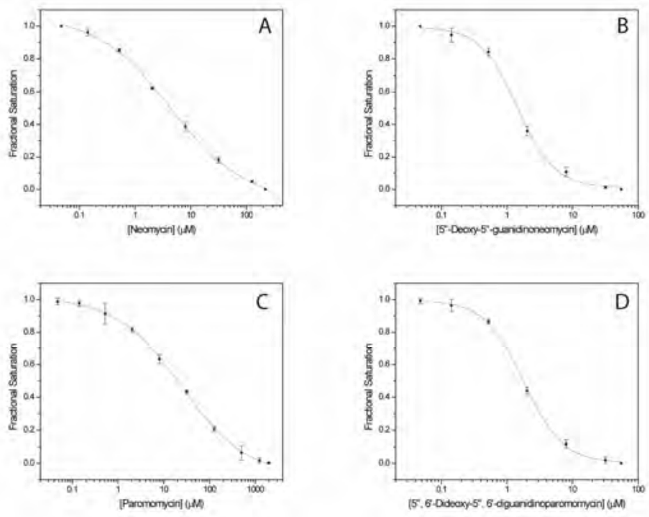


Figure S2: Neomycin-Coumarin displacement curves. A = Neomycin (9), B = 5''-Deoxy-5''-guanidinoneomycin (10), C = Paromomycin (13),
D = 5'',6'-Dideoxy-5'',6'-diguanidinoparomomycin (14)

S.4 – Parent Aminoglycoside Crystal Structures

All crystal structure representations were made using PyMOL Molecular Graphics Systems, Version 1.4.1, Schrödinger, LLC. All structures were adapted from PDB files: Tobramycin (1LC4), amikacin (2GSQ), kanamycin A (2E5I), neomycin (2ET4), neamine (2ET8), paromomycin (1J7T), apramycin (1YRJ).

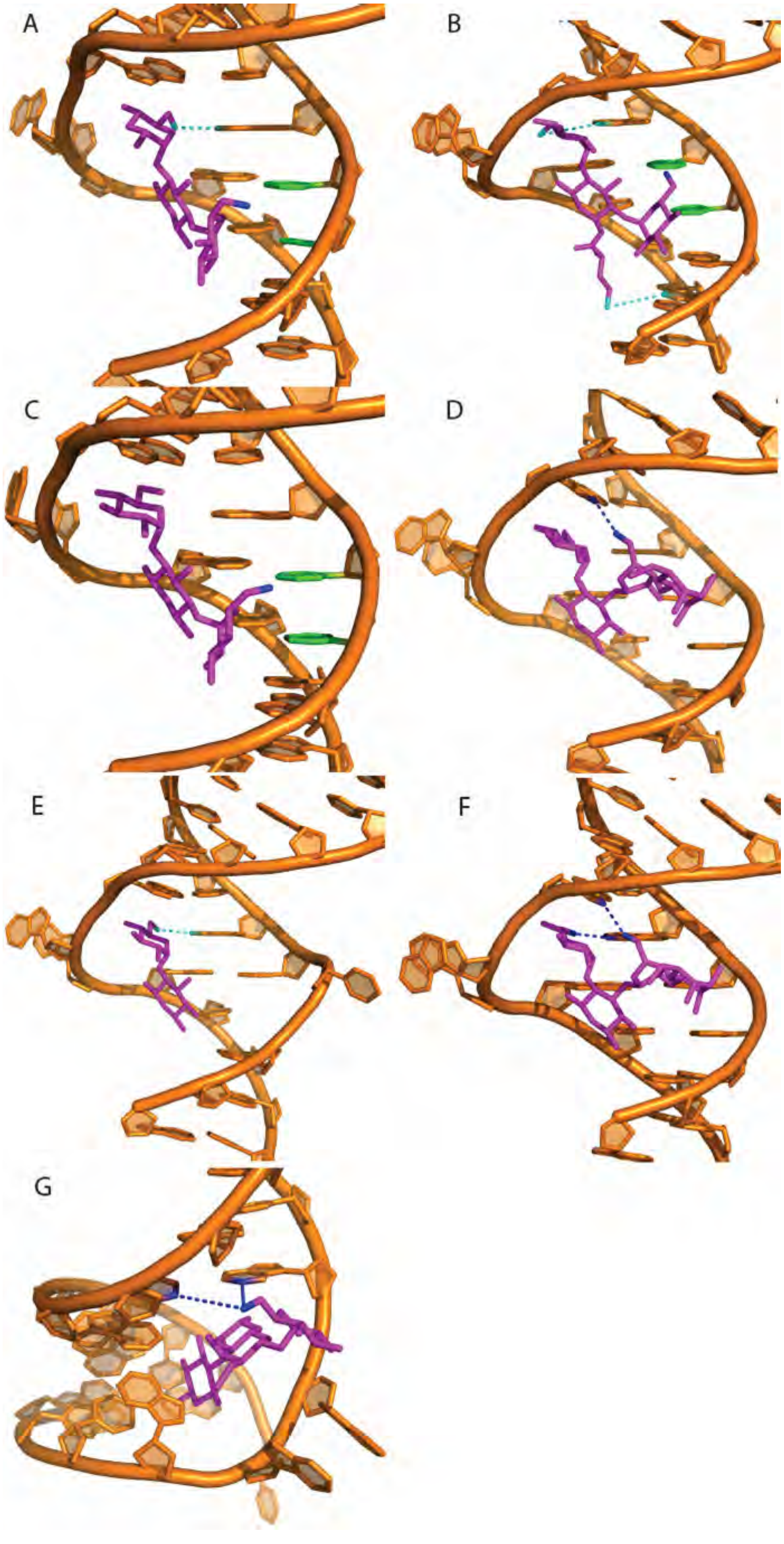

A
B
C
D
E
F
G

Figure S3: Parent aminoglycoside crystal structures. Orange = A-site RNA, Magenta = Aminoglycoside, Dark blue = Primary alcohols modification sites with hydrogen bonds, Light blue = Aminomethyl modification sites with hydrogen bonds, Green = Possible new contants for kanamycin class 6'' alcohol modifications. A = Tobramycin (1), B = Amikacin (4), C = Kanamycin A (7), D = Neomycin (9), E = Neamine (11), F = Paromomycin (13), G = Apramycin (15).

S.5 - References

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