## Supporting Information

for

## Synthesis of Saccharocin from Apramycin and Evaluation of its Ribosomal Selectivity

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General Experimental. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. All organic extracts were dried over sodium sulfate and concentrated under vacuum. Chromatographic purifications were carried out over silica gel or CM Sephadex C-25 as stated. Analytical thin-layer silica gel chromatography was performed with pre-coated glass-backed plates and visualized by UV irradiation ( 254 nm ) or by staining with $20 \% \mathrm{H}_{2} \mathrm{SO}_{4}$ in EtOH , or ceric ammonium molybdate solution. Specific rotations were obtained using a digital polarimeter in the solvent specified. High resolution mass spectra were recorded with an electrospray source coupled to a time-offlight mass analyzer (Waters). ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$ and 2D NMR spectra were recorded on $600 \mathrm{MHz}, 500$ MHz and 400 MHz instruments. Ammonical methanol was prepared from ammonium hydroxide solution ( $28 \%$ in water) and methanol in a 1:9 ratio.

## $7^{\prime}-N$-Acetyl-5,6,6',2', $3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2', $4^{\prime \prime}$-tetradeamino-1,3,2', $\mathbf{4}^{\prime \prime}$-tetraazidoapra-

 mycin (5) and $7^{\prime}, 4^{\prime \prime}$-Di- $N$-acetyl-5,6,6', $2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6): Trifluoromethanesulfonyl azide was prepared fresh for each reaction as described here: Sodium azide ( $5.0 \mathrm{~g}, 76.9 \mathrm{mmol}$ ) was dissolved in water ( 16.0 mL ) and an equal volume of dichloromethane was added while stirring at room temperature. The resulting suspension was cooled to $0{ }^{\circ} \mathrm{C}$ and $\mathrm{Tf}_{2} \mathrm{O}(5 \mathrm{~mL}, 29.8 \mathrm{mmol})$ was added dropwise over $8-10 \mathrm{~min}$ with vigorous stirring. The reaction mixture was stirred at $0{ }^{\circ} \mathrm{C}$ for 3 h before saturated aqueous $\mathrm{NaHCO}_{3}(18.0 \mathrm{~mL})$ was added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with dichloromethane ( 8.0 mL ). The organic layers were combined (triflyl azide solution) and immediately used for next step.In a 250 mL round bottom flask, apramycin sulphate (1) ( $2.0 \mathrm{~g}, 2.54 \mathrm{mmol}$ ), $\mathrm{NaHCO}_{3}(4.8 \mathrm{~g}$, $57.2 \mathrm{mmol})$ and $\mathrm{CuSO}_{4} \cdot 5 \mathrm{H}_{2} \mathrm{O}(60 \mathrm{mg}, 0.4 \mathrm{mmol})$ were dissolved in water $(32.0 \mathrm{~mL})$ and cooled to $0{ }^{\circ} \mathrm{C}$. The above triflyl azide solution was added slowly to the reaction mixture over 15 min , followed by dropwise addition of $\mathrm{MeOH}(35.0 \mathrm{~mL})$ over 10 min . The reaction mixture was stirred for 8 h below $10{ }^{\circ} \mathrm{C}$ before $n$-butylamine ( $0.5 \mathrm{~mL}, 5.1 \mathrm{mmol}$ ) was added to quench the excess $\mathrm{TfN}_{3}$. The solvents were evaporated under vacuum and the blue-colored residue was
taken up in 1:1 methanol:dichloromethane and the residual solid was filtered off through a sintered funnel and washed with same solvent mixture. The filtrate was evaporated, dried, redissolved in pyridine ( 25 mL ) and treated with acetic anhydride $(5 \mathrm{~mL})$ in presence of 4 dimethylaminopyrydine $(25 \mathrm{mg})$ at $50^{\circ} \mathrm{C}$ for 20 h . After completion of the reaction, the solvents were evaporated and the crude product was subjected to column chromatographic purification over silica gel (eluent: 50\% Ethyl acetate:Hexane to 100\% Ethyl acetate to 1\% Methanol in Ethyl acetate) to give 5 ( $619 \mathrm{mg}, 26 \%$ ) and 6 ( $895 \mathrm{mg}, 37 \%$ ).

Compound 5: $[\alpha]^{21}{ }_{\mathrm{D}}=+112.2\left(c=1.0\right.$, chloroform). ${ }^{1} \mathrm{H}$ NMR ( $500 \mathrm{MHz}, \mathrm{DMF}-d_{7}$, at $\left.100{ }^{\circ} \mathrm{C}\right) \delta$ 5.89 - 5.72 (m, 1H, H-6'), 5.66 (d, $\left.J=3.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}\right), 5.62-5.55\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-8^{\prime}\right), 5.50(\mathrm{t}, J=$ $\left.9.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime \prime}\right), 5.34(\mathrm{t}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5), 5.22(\mathrm{t}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6), 5.19(\mathrm{~d}, J=3.5$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime}$ ), 4.96 (dd, J = 9.8, 3.7 Hz, 1H, H-2'), 4.70 (br s, 1H, H-7'), $4.58-4.42$ (m, 2H, H$6^{\prime \prime}$ ), 4.25 (br d, J = $9.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5^{\prime}$ ), $4.19-3.88$ (m, 6H, H-1, H-4', H-5', H-4, H-4', H-3), 3.64 - 3.53 (m, 1H, H-2'), 3.22 (br s, 3H, N-CH3), 2.65 (dt, $J=12.8,4.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{a}$ ), $2.47-2.34$ ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{a}^{\prime}$ ), $2.10\left(\mathrm{q}, J=12.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{~b}^{\prime}\right), 2.02(\mathrm{q}, J=12.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{~b}) .{ }^{13} \mathrm{C}$ NMR ( 126 $\mathrm{MHz}, \mathrm{DMF}-\mathrm{d} 7$, at $100^{\circ} \mathrm{C}$ ) $\delta 171.0,170.2,169.9,169.6,169.4,98.5,96.8,94.6,78.5,74.5,74.3$, $70.7,70.1,69.5,69.1,67.7,63.3,60.7,59.3,58.2,56.6,32.9,31.3,28.3,21.2,20.3,20.2,20.0$, 19.96, 19.94. ESIHRMS calculated for $\mathrm{C}_{35} \mathrm{H}_{47} \mathrm{~N}_{13} \mathrm{O}_{18}[\mathrm{M}+\mathrm{Na}]^{+}, 960.3060$; found, 960.3082 .

Compound 6: $[\alpha]^{21}{ }_{\mathrm{D}}=+82.1\left(c=1.6\right.$, chloroform). ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMF- $d_{7}$, at $\left.100{ }^{\circ} \mathrm{C}\right) \delta$ 7.87 (br d, $J=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{NH}$ ), $5.86-5.74$ ( $\left.\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-6^{\prime}\right), 5.67$ (d, $\left.J=5.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}\right), 5.55$ (d, $\left.J=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-8^{\prime}\right), 5.48\left(\mathrm{t}, J=10.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime \prime}\right), 5.33$ (t, $\left.J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5\right), 5.23-$ 5.18 (m, 2H, H-6, H-1'), 4.93 (m, 1H, H-2"), $4.76-4.64$ (m, 1H, H-7'), $4.54-4.20$ (m, 4H, H$6^{\prime \prime}$, H-5', H-4'), 4.17 - 3.92 (m, 5H, H-1, H-5', H-4', H-4, H-3), 3.60 (br d, J = $12.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ $2^{\prime}$ ), $3.22\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{N}-\mathrm{CH}_{3}\right), 2.65(\mathrm{dt}, J=12.8,4.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{a}), 2.39\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{a}^{\prime}\right), 2.30-1.90$ (m, 26H, $8 \times \mathrm{COCH}_{3}, \mathrm{H}-3 \mathrm{~b}^{\prime}, \mathrm{H}-2 \mathrm{~b}$ ). ${ }^{13} \mathrm{C}$ NMR ( 125 MHz , DMF- $d_{7}$, at $100^{\circ} \mathrm{C}$ ) $\delta 171.4,170.3$, $170.2,170.1,170.0,169.7,169.5,98.4,97.1,95.0,78.4,74.5,74.3,71.4,70.7,69.9,69.6,69.0$, 67.7, 63.6, 59.3, 58.2, 56.6, 50.6, 32.7, 31.3, 28.3, 22.2, 21.2, 20.3, 20.2, 20.12, 20.10, 20.05, 19.98, 19.95. ESIHRMS calculated for $\mathrm{C}_{37} \mathrm{H}_{51} \mathrm{~N}_{11} \mathrm{O}_{19}[\mathrm{M}+\mathrm{Na}]^{+}, 976.3260$; found, 976.3245 .

1,3,2',-Trideamino-1,3,2'-triazido-4'-trifluoroacetamidoapramycin (7): Sodium azide (28.0 $\mathrm{g}, 0.43 \mathrm{~mol})$ was dissolved in water $(80.0 \mathrm{~mL})$ and an equal volume of dichloromethane ( 80.0 mL ) was added while stirring at room temperature. The resulting suspension was cooled to $0^{\circ} \mathrm{C}$
and $\mathrm{Tf}_{2} \mathrm{O}(50.0 \mathrm{~g}, 0.18 \mathrm{~mol})$ was added dropwise over 40 min with vigorous stirring. The mixture was stirred at $0^{\circ} \mathrm{C}$ for 3 h before sat. $\mathrm{NaHCO}_{3}(90.0 \mathrm{~mL})$ was added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (20.0 mL ). The organic layers were combined and immediately used for next step.

In a 500 mL round bottom flask, apramycin sulphate (1) ( $10.0 \mathrm{~g}, 15.7 \mathrm{~mol}$ ), $\mathrm{NaHCO}_{3}(24.0 \mathrm{~g}$, $286.0 \mathrm{mmol})$ and $\mathrm{CuSO}_{4} \cdot 5 \mathrm{H}_{2} \mathrm{O}(0.5 \mathrm{~g}, 2.4 \mathrm{~mol})$ were dissolved in $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ and cooled to 0 ${ }^{\circ} \mathrm{C}$. the above triflyl azide solution was added slowly to the reaction mixture at $0{ }^{\circ} \mathrm{C}$, over 30 min , followed by dropwise addition of $\mathrm{MeOH}(170 \mathrm{~mL})$ over 30 min . The reaction mixture was held at $0^{\circ} \mathrm{C}$ for 6 h and monitored by LCMS before $n$-butylamine ( $2.0 \mathrm{~g}, 27.3 \mathrm{mmol}$ ) was added to quench the excess $\mathrm{TfN}_{3}$. The solvents were evaporated and the residue was purified by column chromatography over silica gel (eluent: gradient of $4 \%$ to $8 \%$ to $12 \%$ to $14 \%$ and $20 \%$ to $45 \%$ of ammonical methanol in dichloromethane) gave first 3 ( $4.27 \mathrm{~g}, 52 \%$ ) as a white foamy solid and then a blue-colored solid mass comprised mainly of compound $4(2.40 \mathrm{~g})$.

Compound $4(250 \mathrm{mg})$ obtained in the previous step, was suspended in trifluoroacetic anhydride $(3.4 \mathrm{~mL}, 24.3 \mathrm{mmol})$ and potassium acetate $(600 \mathrm{mg}, 6.1 \mathrm{mmol})$ at $0^{\circ} \mathrm{C}$. After 5 min . the ice bath was removed, and reaction mixture was stirred at room temperature for 10 min before it was heated to $55^{\circ} \mathrm{C}$ for 4 h . Once LCMS and TLC showed complete trifluoroacetyl protection of all the free amino- and hydroxyl groups, the reaction mixture was cooled to room temperature, evaporated under vacuum, and co-evaporated with toluene to remove the excess trifluoroacetic anhydride. The crude product was dissolved in ethyl acetate and washed with saturated aq. $\mathrm{NaHCO}_{3}$ solution. The organic layer was separated, washed again with saturated aq. $\mathrm{NaHCO}_{3}$ solution followed by brine. The organic layer was evaporated, dried, and taken up in 2 M acetic acid in ethanol and heated to $100^{\circ} \mathrm{C}$ for 2.5 h until all the trifluoroacetyl esters and the $7^{\prime}-\mathrm{N}$ trifluoroacetamide were cleaved off. The solvents were evaporated, and the crude product was purified by column chromatography on flash silica gel by eluting with $10 \%$ ammonical methanol in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ to $25 \%$ ammonical methanol in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ to obtain 7 as an off-white solid ( $84 \mathrm{mg}, 29 \%$ from 1). $[\alpha]^{21}{ }_{\mathrm{D}}=+135.3\left(c=2.0\right.$, methanol). ${ }^{1} \mathrm{H} \operatorname{NMR}\left(600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}\right) \delta 5.60(\mathrm{~d}, \mathrm{~J}=3.5 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{H}-1^{\prime}$ ), 5.34 (d, $J=3.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}$ ), 4.97 (d, $J=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-8^{\prime}$ ), 4.29 (br s, 1H, H-6'), 3.94 (t, $\left.J=9.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime \prime}\right), 3.88-3.81$ (m, 3H, H-5', H-5', H-4'), 3.74 (t, J = $9.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ $4^{\prime \prime}$ ), 3.61 (dd, $\left.J=12.3,2.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{a}^{\prime \prime}\right), 3.54-3.45$ (m, 5H, H-2", H-4, H-6b", H-5, H-3),
$3.41(\mathrm{td}, J=12.0,4.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1), 3.28-3.21\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-6, \mathrm{H}-2^{\prime}\right), 2.75(\mathrm{dd}, J=8.3,2.8 \mathrm{~Hz}$, $\left.1 \mathrm{H}, \mathrm{H}-7{ }^{\prime}\right), 2.51\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{N}-\mathrm{CH}_{3}\right), 2.24(\mathrm{dt}, J=12.0,4.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{a}), 2.19(\mathrm{dt}, J=11.0,4.2 \mathrm{~Hz}$, $\left.1 \mathrm{H}, \mathrm{H}-3 \mathrm{a}^{\prime}\right), 2.03\left(\mathrm{q}, J=11.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{~b}^{\prime}\right), 1.41(\mathrm{q}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{~b}) .{ }^{13} \mathrm{C}$ NMR ( 151 $\left.\mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}\right) \delta 158.3,158.0,157.8,157.6,121.1,119.0,118.9,116.99,115.1,97.7,94.5,94.3$, $79.3,76.5,76.5,71.8,70.8,70.4,69.4,66.5,64.3,62.2,61.2,60.3,59.8,56.4,52.5,48.0,47.9$, 47.8, 47.6, 47.5, 47.3, 47.2, 31.8, 31.3, 27.9. $\mathrm{C}_{23} \mathrm{H}_{35} \mathrm{~N}_{11} \mathrm{~F}_{3} \mathrm{O}_{12}[\mathrm{M}+\mathrm{H}]^{+}, 714.2419$; found, 714.2442 .

1,3,2'-Trideamino-1,3,2'-triazidoapramycin (4): A solution of 7 ( $20 \mathrm{mg}, 28.0 \mu \mathrm{~mol}$ ) in methanol ( 0.8 mL ) was treated with sodium methoxide ( $8 \mathrm{mg} ; 148.1 \mu \mathrm{~mol}$ ) for 12 h , at room temperature. After complete consumption of the starting material, the base was neutralized with acidic resin Amberlyst 15. The resin was filtered off, washed with methanol, and the solvents were evaporated and the residue was absorbed on silica gel and purified by column chromatography on silica gel (eluent: $10 \%$ to $25 \%$ ammonical methanol in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ ) to give the title compound 4 in $55 \%$ yield $(9.6 \mathrm{mg}) .[\alpha]^{21}{ }_{\mathrm{D}}=+120.0\left(c=0.33\right.$, methanol). ${ }^{1} \mathrm{H}$ NMR ( 600 $\left.\mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}\right) \delta 5.66\left(\mathrm{~d}, J=3.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime}\right), 5.35\left(\mathrm{~d}, J=3.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}\right), 5.05(\mathrm{~d}, J=8.3$ Hz, 1H, H-8'), 4.34 (t, J = 2.6 Hz, 1H, H-6'), $3.95-3.82$ (m, 2H, H-5', H-4'), $3.80-3.74$ (m, 1H, H-6a"), $3.75-3.62$ (m, 3H, H-3", H-6b", H-5"), $3.57-3.47$ (m, 4H, H-5, H-4, H-3, H-2"), 3.42 ( $\mathrm{td}, J=12.0,4.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1$ ), $3.28-3.18\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-6, \mathrm{H}-2^{\prime}\right), 2.96(\mathrm{dd}, J=8.3,2.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ $7^{\prime \prime}$ ), 2.84 (app. t, $J=9.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4^{\prime \prime}$ ), 2.64 (S, 3H, N-CH3), $2.26-2.19$ (m, 2H, H-2a, H-3a'), $2.06\left(\mathrm{q}, J=12.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{~b}^{\prime}\right), 1.41(\mathrm{q}, J=12.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{~b}) .{ }^{13} \mathrm{C}$ NMR ( $151 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) $\delta 98.9,95.9,95.2,80.5,78.0,77.9,73.1,72.8,72.4,71.6,68.0,65.4,62.9,62.8,61.7,61.1,57.7$, 54.7, 33.2, 32.1, 29.1. ESIHRMS calculated for $\mathrm{C}_{21} \mathrm{H}_{35} \mathrm{~N}_{11} \mathrm{O}_{11}[\mathrm{M}+\mathrm{H}]^{+}, 618.2596$; found, 618.2585 .

## $7^{\prime}-N$-Acetyl-5,6,6', $2^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}-h e p t a-O-a c e t y l-1,3,2^{\prime}$-trideamino-1,3,2'-triazidosaccharocin

(9): A solution of $\mathbf{6}(140 \mathrm{mg}, 0.16 \mathrm{mmol})$ in anhydrous $\mathrm{CH}_{2} \mathrm{Cl}_{2}(5 \mathrm{~mL})$ and anhydrous pyridine $(118 \mu \mathrm{~L}, 116 \mathrm{mmol})$ was cooled to $-10{ }^{\circ} \mathrm{C}$. After stirring for 10 min , nitrosyl tetrafluoroborate $(85.7 \mathrm{mg}, 0.78 \mathrm{mmol})$ was added in one portion to the mixture and the mixture was stirred at -10 ${ }^{\circ} \mathrm{C}$ for 6 h with monitoring by LRMS. After 6 h , another 5 equiv ( 85.7 mg ) of nitrosyl tetrafluoroborate was added and reaction mixture was warmed to $10^{\circ} \mathrm{C}$ and stirred overnight. The reaction mixture was diluted with cold $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ and washed with cold 1 N HCl , followed by
cold saturated aqueous $\mathrm{NaHCO}_{3}$ and cold brine. The organic layer was dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and then concentrated keeping the bath temp below $10^{\circ} \mathrm{C}$ to obtain the $4^{\prime \prime}-N$-nitroso derivative $\mathbf{8}$. The crude nitroso-derivative 8 was dissolved $\mathrm{CH}_{2} \mathrm{Cl}_{2}(1.5 \mathrm{~mL})$, and cooled to $-10{ }^{\circ} \mathrm{C}$. In a separate flask, $\mathrm{NaOCH}_{2} \mathrm{CF}_{3}$ and 18-crown-6 were dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ ( 1.5 mL ), and cooled in an ice bath. This cooled solution was transferred to the solution of nitroso-derivative quickly and stirred for about 3-4 min until LCMS showed complete consumption of the starting material. Then 20 equiv of acetic acid $(168 \mu \mathrm{~L})$ were added to the reaction flask quickly. After stirring for 5 min , the reaction mixture was quenched with saturated aqueous $\mathrm{NaHCO}_{3}$ solution. The organic layer was separated, washed with brine, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$ and concentrated. The crude product was purified by column chromatography on flash silica gel (eluent: $30 \%$ ethyl acetate in hexane to $60 \%$ ethyl acetate in hexane) to obtain 9 as a white solid ( $83 \mathrm{mg}, 59 \%$ ). $35 \%$ ). $[\alpha]^{21}{ }_{\mathrm{D}}=+39.5$ $\left(c=0.57\right.$, chloroform). ${ }^{1} \mathrm{H}$ NMR ( 500 MHz, DMF- $d_{7}$, at $100^{\circ} \mathrm{C}$ ) $\delta 5.86-5.76\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-6^{\prime}\right), 5.69$ (d, $J=3.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}$ ), 5.61 (br d, $\left.J=8.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-8^{\prime}\right), 5.52\left(\mathrm{t}, J=9.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime \prime}\right), 5.35$ $(\mathrm{t}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-5), 5.23(\mathrm{t}, J=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6), 5.21-5.17$ (m, 2H, H-1', H-4), 5.00 (dd, J $\left.=10.4,3.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2^{\prime \prime}\right), 4.73$ (br s, 1H, H-7'), $4.44-4.36$ (m, 1H, H-6a"), $4.35-4.24$ (m, 3H, H-6b", H-5', H-5'), 4.19 - 4.03 (m, 3H, H-1, H-4', H-4), 4.02 - 3.96 (m, 1H, H-3), 3.61 (br d, J $=12.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2$ '), $3.24\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{N}-\mathrm{CH}_{3}\right), 2.65(\mathrm{dt}, J=12.8,4.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{a}), 2.46-2.35(\mathrm{~m}$, $\left.1 \mathrm{H}, \mathrm{H}-3 \mathrm{a}^{\prime}\right), 2.27-2.14\left(\mathrm{~m}, 24 \mathrm{H}, \mathrm{COCH}_{3}\right), 2.16-2.09\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{~b}^{\prime}\right), 2.08(\mathrm{q}, J=12.8,1 \mathrm{H}, \mathrm{H}-$ 2b). ${ }^{13} \mathrm{C}$ NMR ( $125 \mathrm{MHz}, \mathrm{DMF}_{7} \mathrm{~d}_{7}$, at $100^{\circ} \mathrm{C}$ ) $\delta 171.0,170.1,169.8,169.6,169.5,169.5,169.3$, $98.5,94.7,78.4,74.5,74.3,70.7,70.6,69.8,69.4,69.1,67.7,62.7,59.3,58.2,56.6,35.1,35.1$, $34.97,34.89,34.80,34.6,34.5,34.3,34.1,32.9,31.3,30.27,30.1,29.9,29.8,29.6,29.4,29.3$, $28.3,21.2,20.3,20.2,20.0,19.93,19.89,19.86$. ESIHRMS calculated for $\mathrm{C}_{37} \mathrm{H}_{50} \mathrm{~N}_{10} \mathrm{O}_{20}$ $[\mathrm{M}+\mathrm{Na}]^{+}, 977.3101$; found, 977.3146 .

Saccharocin 2: To a solution of $9(60 \mathrm{mg}, 62.8 \mu \mathrm{~mol})$ in dioxane $(1.5 \mathrm{~mL})$, a saturated aqueous solution of barium hydroxide ( 1.5 mL ) was added and the reaction mixture was heated to $60^{\circ} \mathrm{C}$ for 8 h . After hydrolysis of all acetates, the reaction mixture was neutralized by addition of dry ice. The precipitated barium carbonate was filtered off and the filtrate was concentrated, redissolved in a minimum amount of $10 \%$ ammoniacal methanol in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(0.25 \mathrm{~mL})$ and eluted through a pad of flash silica gel using $25 \%$ to $30 \%$ ammoniacal methanol in $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ as eluent. The fractions containing saponified material were concentrated, dried under vacuum and subjected to hydrogenation over $\operatorname{Pd}(\mathrm{OH})_{2} / \mathrm{C}(30 \mathrm{mg})$ in water using a balloon to maintain 1 atm
of $\mathrm{H}_{2}$. After completion ( 5 h ), the catalyst was filtered off on Celite ${ }^{\circledR}$, and washed. The filtrate was concentrated and the residue was taken up in $10 \%$ aqueous acetic acid and loaded on a Sephadex column (CM Sephadex C-25) from which it was flushed initially with D.I. water and then $0.1 \%-1 \% \mathrm{NH}_{4} \mathrm{OH}$ in D.I. water. The product containing fractions were combined, acidified with glacial acetic acid, and lyophilized to give saccharocin 2 as a white solid ( $19.2 \mathrm{mg}, 40 \%$ ). $[\alpha]^{21}{ }_{\mathrm{D}}=+126.7(c=0.8$, Water $) .{ }^{1} \mathrm{H}$ NMR $\left(600 \mathrm{MHz}, \mathrm{D}_{2} \mathrm{O}\right) \delta 5.53\left(\mathrm{~d}, J=3.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime}\right), 5.22$ (d, $J=4.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime \prime}$ ), 5.02 (d, $\left.J=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-8^{\prime}\right), 4.37$ (br s, $1 \mathrm{H}, \mathrm{H}-6^{\prime}$ ), 3.74 (td, $J=11.2$, $\left.3.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4^{\prime}\right), 3.71$ (t, $\left.J=9.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 3.65$ (dd, $\left.J=12.0,1.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{a}^{\prime \prime}\right), 3.61-$ 3.50 (m, 3H, H-5', H6b"' H-3'), $3.47-3.42$ (m, 4H, H-2', H-5, H-2', H-5'), 3.37 (t, J = 9.8 Hz , $1 \mathrm{H}, \mathrm{H}-6), 3.34-3.30(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3), 3.25$ (t, $\left.J=9.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4^{\prime \prime}\right), 3.18$ (dd, $J=8.5,2.5 \mathrm{~Hz}, 1 \mathrm{H}$, H-7'), 3.11 (td, $J=12.0,4.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1$ ), $2.60\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{N}-\mathrm{CH}_{3}\right), 2.30(\mathrm{dt}, J=8.4,4.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ 2a), $2.22-2.14\left(\mathrm{~m}, 1 \mathrm{H}, 3 \mathrm{a}^{\prime}\right), 1.87-1.81\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3 \mathrm{~b}^{\prime}\right), 1.67$ (q, $\left.J=12.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2 \mathrm{~b}\right) .{ }^{13} \mathrm{C}$ NMR (151 MHz, $\left.\mathrm{D}_{2} \mathrm{O}\right) \delta 180.1,96.7,95.8,94.0,79.4,76.3,74.3,73.7,73.5,71.4,70.9,70.4$, $67.2,64.1,61.7,60.6,50.9,49.6,49.1,31.4,29.6,28.0,22.9$. ESIHRMS calculated for $\mathrm{C}_{21} \mathrm{H}_{40} \mathrm{~N}_{4} \mathrm{O}_{12}[\mathrm{M}+\mathrm{H}]^{+}, 541.2721$; found, 541.2731.

Bacterial strains. Clinical isolates of E. coli and S. aureus were obtained from the Diagnostic Department, Institute of Medical Microbiology, University of Zurich. MIC values were determined by broth microdilution assays as described. ${ }^{1}$

Recombinant microorganisms. The construction of these strains derived from single rRNA allelic M. smegmatis $\Delta \mathrm{rrnB}$, has been described previously. ${ }^{2-3}$

Cell-free translation assays. S-30 extracts and purified ribosomes were used for cell-free translation assays as described previously. ${ }^{1}$ Firefly luciferase mRNA was used as reporter to monitor translation activity. Luminescence was measured using a luminometer Flx800 (Bio-Tek Instruments).

Cochlear Explants. Drugs were screened for toxicity to hair cells in cochlear explants from CBA/J mice on postnatal day 2 to $3 .{ }^{4}$ The dissected tissue was placed on a collagen-coated incubation dish in 1 mL of serum-free Basal Medium Eagle plus serum-free supplement (Invitrogen), $1 \% \mathrm{BSA}, 2 \mathrm{mM}$ glutamine, and $5 \mathrm{mg} / \mathrm{mL}$ glucose. The explants were incubated $\left(37^{\circ} \mathrm{C}, 5 \% \mathrm{CO}_{2}\right)$ for 4 h , and an additional 1 mL of culture medium was added to submerse the
explants. After 24 h the medium was exchanged for new medium containing the drugs, and the explants were incubated for an additional 24 or 72 h . Cultures were fixed overnight in $4 \%$ (vol/vol) paraformaldehyde at $4{ }^{\circ} \mathrm{C}$, and then permeabilized for 30 min with $3 \%$ (vol/vol) Triton X-100 in PBS, and washed three times with PBS. Following incubation at room temperature for 40 min with rhodamine-phalloidin (Molecular Probes), hair cell presence was determined by light microscopy of the phalloidin-stained stereociliary bundles and circumferential F-actin rings on the cuticular plate.

## References

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${ }^{1} \mathrm{H}$ NMR (500 MHz, DMF- $\left.\boldsymbol{d}_{7}, 100{ }^{\circ} \mathrm{C}\right) \quad 7^{\prime}-N-A c e t y l-5,6,6^{\prime}, 2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2',4"-tetradeamino-1,3,2',4'-tetraazidoapra-mycin (5):

${ }^{13}$ C NMR ( 125 MHz, DMF- $d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2', $4^{\prime \prime}$-tetradeamino-1,3,2', $4^{\prime \prime}$ -tetraazidoapra-mycin (5):


COSY (500 MHz, DMF- $d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N-A c e t y l-5,6,6^{\prime}, 2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2',4'-tetradeamino-1,3,2',4'-tetraazidoapra-mycin (5):


HSQC (500 MHz, DMF- $\boldsymbol{d}_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N-A c e t y l-5,6,6^{\prime}, 2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2',4'-tetradeamino-1,3,2',4'-tetraazidoapra-mycin (5):


HMBC ( $500 \mathrm{MHz}, \mathrm{DMF}-d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}$ - $N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2',4'-tetradeamino-1,3,2', $\mathbf{4}^{\prime \prime}$ -tetraazidoapra-mycin (5):

${ }^{1}$ H NMR ( $500 \mathrm{MHz}, \mathrm{DMF}-\mathrm{d}_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}, 4^{\prime \prime}$-Di-N-acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6):

${ }^{13}$ C NMR ( 125 MHz, DMF- $d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}, 4^{\prime \prime}$-Di-N-acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6):


COSY (500 MHz, DMF- $d_{7}, 100{ }^{\circ}$ C) Spectrum of $7^{\prime}, 4^{\prime \prime}-$ Di-N-acetyl-5,6,6', $2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6):


HSQC (500 MHz, DMF- $d_{7}, 100{ }^{\circ}$ C) Spectrum of $7^{\prime}, 4^{\prime \prime}-$ Di-N-acetyl-5,6,6', $2^{\prime \prime}, 3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6):


HMBC (500 MHz, DMF- $d_{7}, 100{ }^{\circ}$ C) Spectrum of $7^{\prime}, 4^{\prime \prime}$-Di-N-acetyl-5,6,6',2', $3^{\prime \prime}, 6^{\prime \prime}$-hexa-O-acetyl-1,3,2'-trideamino-1,3,2'triazidoapramycin (6):

${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-trideamino-1,3,2'-triazido-4"-trifluoroacetamidoapramycin (7):

${ }^{13} \mathrm{C}$ NMR ( $151 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of $1,3,2^{\prime}$,-trideamino-1,3,2'-triazido-4'-trifluoroacetamidoapramycin (7):


COSY ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-trideamino-1,3,2'-triazido-4'-trifluoroacetamidoapramycin (7):


HSQC ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-trideamino-1,3,2'-triazido-4'-trifluoroacetamidoapramycin (7):


HMBC ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-trideamino-1,3,2'-triazido-4'-trifluoroacetamidoapramycin (7):

${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of $1,3,2^{\prime}$,-Trideamino-1,3,2'-triazidoapramycin (4):

${ }^{13} \mathrm{C}$ NMR ( $151 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of $1,3,2^{\prime}$,-Trideamino-1,3,2'-triazidoapramycin (4):


COSY ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-Trideamino-1,3,2'-triazidoapramycin (4):


HSQC ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-Trideamino-1,3,2'-triazidoapramycin (4):


HMBC ( $600 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) Spectrum of 1,3,2',-Trideamino-1,3,2'-triazidoapramycin (4):

${ }^{1}$ H NMR ( 500 MHz, DMF- $\boldsymbol{d}_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}$-hepta-O-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):


VT ${ }^{1}$ H NMR ( 500 MHz, DMF- $\boldsymbol{d}_{7}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}$-hepta-O-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):

VAS-476-39-100C-1H
The triplet for $\mathrm{H}-4^{\prime \prime}$ at 5.21 at $60^{\circ} \mathrm{C}\left(5.24\right.$ at $\left.40^{\circ} \mathrm{C}\right)$ shifted towards right side with rising temperature
and eventually merged with $\mathrm{H}-1^{\prime}$ proton at $100^{\circ} \mathrm{C}$. Its coupling constant at $60^{\circ} \mathrm{C}$ was found to be
9.95 Hz


VAS-476-39-60C-1H


VAS-476-39-40C-1H


${ }^{13}$ C NMR ( 125 MHz, DMF- $d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}$-hepta-O-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):


COSY (500 MHz, DMF- $d_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6', $\mathbf{2}^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}-h e p t a-O$-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):


HSQC (500 MHz, DMF- $\boldsymbol{d}_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6,6',2', $3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}-h e p t a-O$-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):


HMBC (500 MHz, DMF- $\boldsymbol{d}_{7}, 100{ }^{\circ} \mathrm{C}$ ) Spectrum of $7^{\prime}-N$-Acetyl-5,6, $6^{\prime}, 2^{\prime \prime}, 3^{\prime \prime}, 4^{\prime \prime}, 6^{\prime \prime}$-hepta-O-acetyl-1,3,2'-trideamino-1,3,2'triazidosaccharocin (9):

${ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{D}_{2} \mathrm{O}$ ) Spectrum of Saccharocin (2):

${ }^{13} \mathrm{C}$ NMR (151 MHz, $\left.\mathrm{D}_{2} \mathrm{O}\right)$ Spectrum of Saccharocin (2):


COSY ( $600 \mathrm{MHz}, \mathrm{D}_{2} \mathrm{O}$ ) Spectrum of Saccharocin (2):


HSQC ( $600 \mathrm{MHz}, \mathrm{D}_{2} \mathrm{O}$ ) Spectrum of Saccharocin (2):


## HMBC ( $600 \mathrm{MHz}, \mathrm{D}_{2} \mathrm{O}$ ) Spectrum of Saccharocin (2):



