<table>
<thead>
<tr>
<th>Compound</th>
<th>Modifications made</th>
</tr>
</thead>
</table>
| All compounds | • Added glycolysis reactions (SEED IDs rxn005226, rxn00558, rxn00545, rxn00786) when missing, to allow glucose uptake and catabolism  
• Added methionine biosynthesis reactions when missing (SEED IDs rxn00952, rxn00953, rxn01300, rxn01301, rxn01302, rxn01303, rxn00693, rxn01113, rxn05256, rxn05902, rxn09240), based on Kovaleva & Gelfand, 2006.  
• Removed metabolite 'Acyl_carrier_protein_c' from the pathway, as a synthesis reaction for this metabolite is often missing  
• Made KEGG pathway reactions irreversible to avoid flux block by looping  
• Added necessary reactions from KEGG pathway rxn00524 (RXB0809, RX0891, RX0892, RX0893, RX0894, RX0895, RX0896, RX0897, RX0899, RX0899, RX0899, RX0897, RX0890, RX0896, RX0890, RX0890, RX0890)  
• Made 5-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes  |
| Aureomycin | • Added KEGG pathway rxn00523 (R04060, R01919, R01919, R05456, R07442, R09198, R05459, R09194, R06641, R06642, R09187, R09188, R09195, R09198, R05463, R04642, R09192, R09196, R09199, R09200, R09193, R09190)  
• Added reaction for the conversion from 2-oxoglutaramate + H2O to 2-oxoglutarate + NH3 (KEGG R00269)  
• Added enterobactin biosynthesis reaction from 3.0 ATP, 3.0 L-serine and 3.0 2-3-dihydroxybenzoate to 3.0 enterobactin (based on Reimman et al., 2001)  
• Made enterobactin biosynthesis reactions irreversible to avoid flux block by looping  |
| Butirosin | • Added necessary reactions from KEGG pathway rxn00524 (RXB0809, RX0891, RX0890, RX0892, RX0893, RX0894, RX0895, RX0896, RX0897, RX0899, RX0899, RX0899, RX0897)  
• Made pyruvate secretable, as its buildup would otherwise block fluxes  |
| Cephalosporin | • Added KEGG pathway rxn00311 (R07402, R03063, R07400, R02170, R06363, R03062, R05228, R03064, R05230, R05229, R05301, R06361, R04147, R04872, R04870, R05303, R07401, R05302)  
• Added alpha-aminoadipate biosynthesis reactions (SEED IDs rxn00310, rxn02916, rxn01578, rxn06127, rxn01664, rxn02226) when missing (based on Madduri et al., 1991).  
• Made AMP secretable, as its buildup would otherwise block fluxes  |
| Clavulanic acid | • Added KEGG pathway rxn00331 (R05465, R04647, R05471, R05469, R05468, R05357, R05466, R05470)  
• Removed orphan metabolite 5-oxoproline  |
| Clorobiocin | • Added necessary reactions from KEGG pathway rxn00401 (R06749, R07474, R06747, R06775, R06774, R06773, R06776, R06766, R06750, R06753, R06752, R06751, R06759, R06758, R01728, R00734, R06757, R06764, R06765, R06748)  
• Added sugar unit biosynthesis steps and essential dTTP/dTDP-converting reactions when missing (SEED IDs rxn00527, which is a duplicate of a KEGG pathway rxn00401 reaction  
• Made 5-Adenosyl-L-homocysteine and dTDP secretable, as their buildup would otherwise block fluxes  |
| Cumaric acid | • Added KEGG pathway rxn00401 (R06768, R06767, R06777, R06749, R06746, R07474, R06750, R06753, R06752, R06751, R01728, R00734, R06757, R06764, R06765)  
• Added sugar unit biosynthesis steps and essential dTTP/dTDP-converting reactions when missing (SEED IDs rxn00527, which is a duplicate of a KEGG pathway rxn00401 reaction  
• Made S-Adenosyl-L-homocysteine and dTDP secretable, as their buildup would otherwise block fluxes  |
| Enterobactin | • Added KEGG pathway rxn01053 (R01505, R06602, R01717, R03037)  
• Added enterobactin biosynthesis reaction from 3.0 ATP, 3.0 L-serine and 3.0 2,3-dihydroxybenzoate to 3.0 ADP and 1.0 enterobactin/enterobactin (based on Reimman et al., 2001)  
• Added essential pyruvate-converting step (SEED ID rxn00499)  
• Made pyruvate secretable, as its buildup would otherwise block fluxes  |
| Erythromycin | • Added KEGG pathway rxn00522 (R06499, R06498, R06475, R06503, R06497, R06496, R0649, R06491, R06490, R06479, R06457, R06456, R06454, R06464, R06460, R05530, R02858, R02859, R06452, R06451, R06449, R06450, R06477, R06488, R06473, R06470, R06467, R05522, R06462, R06506, R05532, R06461, R06455, R05520, R05270, R00918, R06463, R05531, R05521)  
• Added polyketide sugar biosynthesis KEGG pathway rxn0523 (R06513, R02328, R06428, R06424, R02773, R08533, R06427, R06426, R06443, R06429, R06435, R06433)  
• Added reactions for methylenomalony-CoA biosynthesis, one from 1.0 succinyl-CoA and 1.0 methylenalCoA and one from 1.0 propanoyl-CoA and 1.0 CO2 to 1.0 methylenal-CoA (KEGG R05373), based on Gross et al., 2006.  
• Added reaction for propanoyl-CoA biosynthesis where missing (SEED ID rxn004794)  
• Added reactions for D-glucose-1P biosynthesis where missing (SEED IDs rxn02302, rxn00704)  
• Made S-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes  |
| Neomycin | • Added necessary reactions from KEGG pathway rxn00524 (RXB0809, RX0890, RX0892, RX0889, RX0895, RX0896, RX0897, RX0898, RX0899, RX0899, RX0890, RX0901, RX0903, RX0893, RX0890, RX0902, RX0904)  
• Made pyruvate secretable, as its buildup would otherwise block fluxes  |
| Novobiocin | • Added KEGG pathway rxn00401 (R06750, R06772, R06771, R06770, R06769, R06755, R06754, R06753, R06752, R06751, R06759, R06758, R01728, R00734, R06757, R06756)  
• Added conversion reactions to form 3-Methylpyrrole-2,4-dicarboxylic acid, CO2 and orthophosphate from L-
threonine and oxaloacetate (based on Siebenberg et al., 2011)

- Added sugar unit biosynthesis steps and essential dTTP/dTDP-converting reactions when missing (SEED IDs rxn00704, rxn01997, rxn02000, rxn02003, rxn01675, rxn01143)
- Made S-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes

Penicillin

- Added KEGG pathway m00311 (R07402, R03063, R07400, R02170, R06363, R03062, R05228, R03064, R05230, R05229, R05301, R06361, R04147, R04872, R04870, R05303, R07401, R05302)
- Made S-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes

Pyochelin

- Added KEGG pathway m00311 (R07402, R03063, R07400, R02170, R06363, R03062, R05228, R03064, R05301, R06361, R04147, R04872, R04870, R05303, R07401, R05302)
- Made S-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes

Streptomycin

- Added necessary reactions from KEGG pathway m00311 (R03384, R01187, R07334, R02777, R02225, R02228, R04222, R06514, R06513, R03238, R06369, R03477, R05516, R05515, R06365, R01183, R02781, R08843, R08844, R05547, R03496, R03502)
- Added reaction for the conversion from 2-oxoglutaramate + H₂O to 2-oxoglutarate + NH₃
- Removed orphan metabolites 'Amino_group_donor_c' and 'Activated_methyl_group_c'
- Added sugar unit biosynthesis steps and essential dTTP/dTDP-converting reactions when missing (SEED IDs rxn00704, rxn01997, rxn02000, rxn02003, rxn01675, rxn01143)
- Made AMP secretable, as its buildup would otherwise block fluxes

Tetracycline

- Added KEGG pathway m00311 (R04060, R09191, R09197, R05456, R00742, R09198, R05459, R09194, R06641, R06641, R06642, R06642, R01187, R09188, R09195, R09189, R05463, R05462, R09192, R09196, R09199, R09200, R09193, R09190)
- Made S-Adenosyl-L-homocysteine & NAD+ secretable, as their buildup would otherwise block fluxes

Tylosin

- Added KEGG pathway m00311 (R06499, R06498, R06475, R06503, R06497, R06496, R06491, R06490, R06479, R06457, R06456, R06454, R06464, R06460, R05530, R02858, R02859, R06452, R06451, R06449, R06440, R06450, R06477, R06488, R06473, R06470, R06407, R05522, R06462, R06506, R05532, R06461, R06455, R05520, R055270, R00919, R06448, R06463, R05531, R05521)
- Added polyketide sugar biosynthesis KEGG pathway m00311 (R06439, R06513, R02328, R06428, R06424, R06423, R02773, R08583, R06427, R06426, R06436, R06438, R06347, R06436, R06435, R06433)
- Added reactions for methylnalonal-CoA biosynthesis, one from 1.0 succinyl-CoA to 1.0 methylnalonal-CoA and one from 1.0 propanoyl-CoA to 1.0 methylnalonal-CoA (KEGG R05373), based on Gross et al., 2006.
- Added reactions for ethylmalonyl-CoA biosynthesis (based on KEGG R09291 and R03027)
- Added reaction for propanoyl-CoA biosynthesis where missing (SEED ID rxn04794)
- Added reactions for D-glucose-1P biosynthesis where missing (SEED IDs rxn02302, rxn00704)
- Made S-Adenosyl-L-homocysteine secretable, as its buildup would otherwise block fluxes

Table S1.

**Used methods for integration of KEGG pathways towards the biosynthesis of secondary metabolites.** To integrate the KEGG pathways for secondary metabolite biosynthesis in all 38 actinobacterial models, compound-specific Python scripts were written which used our in-house PyModelEditor to edit the models in such a way that they would allow simulation of compound biosynthesis. For the fifteen compounds chosen, different modifications had to be made to the models, as indicated in this table. The minimal medium used for flux balance analysis (FBA) consisted of H₂O (influx upper bound 10000), O₂ (10000), glucose (10), NH₃ (10), PO₄³⁻ (10), SO₄²⁻ (10), CO₂ (10), H⁺ (10), Cu²⁺ (10), Pb²⁺ (10), Zn²⁺ (10), Mn⁴⁺ (10), CrO₄²⁻ (10), Mg²⁺ (10), K⁺ (10), Co²⁺ (10), Ca²⁺ (10), Fe²⁺ (10), Fe³⁺ (10), Cl⁻ (10), Ni²⁺ (10), Na⁺ (10), Cd²⁺ (10). Also, low influx of octadecanoic acid (0.001) was allowed, which was necessary to ‘start up’ some essential biosynthesis reactions.