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Efficient synthesis of neomycin B related aminoglycosides

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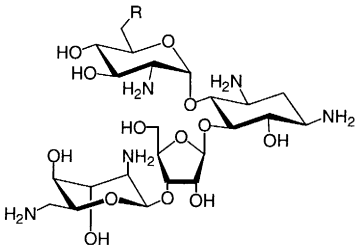
Abstract

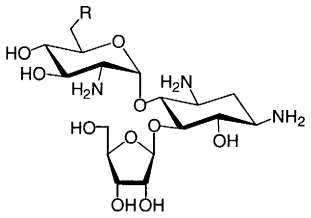
Aminoglycosides 6′-hydroxyl-ribostamycin and 5-(α-neobiosamine)-2-deoxystreptamine were chemically synthesized. These compounds will be used as standards to compare RNA binding affinity and specificity with neomycin B.

Keywords: aminoglycosides; neomycin B derivatives; synthesis.

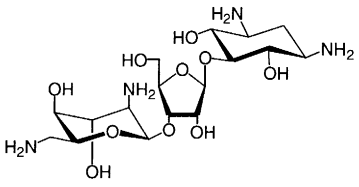
Aminoglycoside antibiotics are known to interact with a variety of RNA molecules, including ribosomal RNA and the hammerhead ribozyme.[1] These low-molecular weight antibiotics have also been found to block the binding of HIV-1 Rev protein to its viral RNA recognition site, thereby inhibiting the production of the virus.[2] Among these aminoglycosides, neomycin B is by far the most effective inhibitor.[3] But due to its toxicity, poor oral bioavailability, cell penetration and instability, neomycin B can not be used as an inhibitory drug directly.[4] Therefore, it is highly desirable to find compounds which are less toxic, more stable and more active than neomycin B.[5]



Neomycin B R = NH_2
Paromomycin R = OH



1 Ribostamycin R = NH_2
2 R = OH



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In order to determine which structural elements of neomycin B are necessary for its RNA binding affinity and specificity, ribostamycin (**1**),[6] 6′-hydroxyl-ribostamycin (**2**), and 5-(α- neobiosamine)-2-deoxystreptamine (**3**) are needed as standards to compare binding properties with neomycin B and paromomycin. Based on these results, we could design and synthesize neomycin B mimetics to seek

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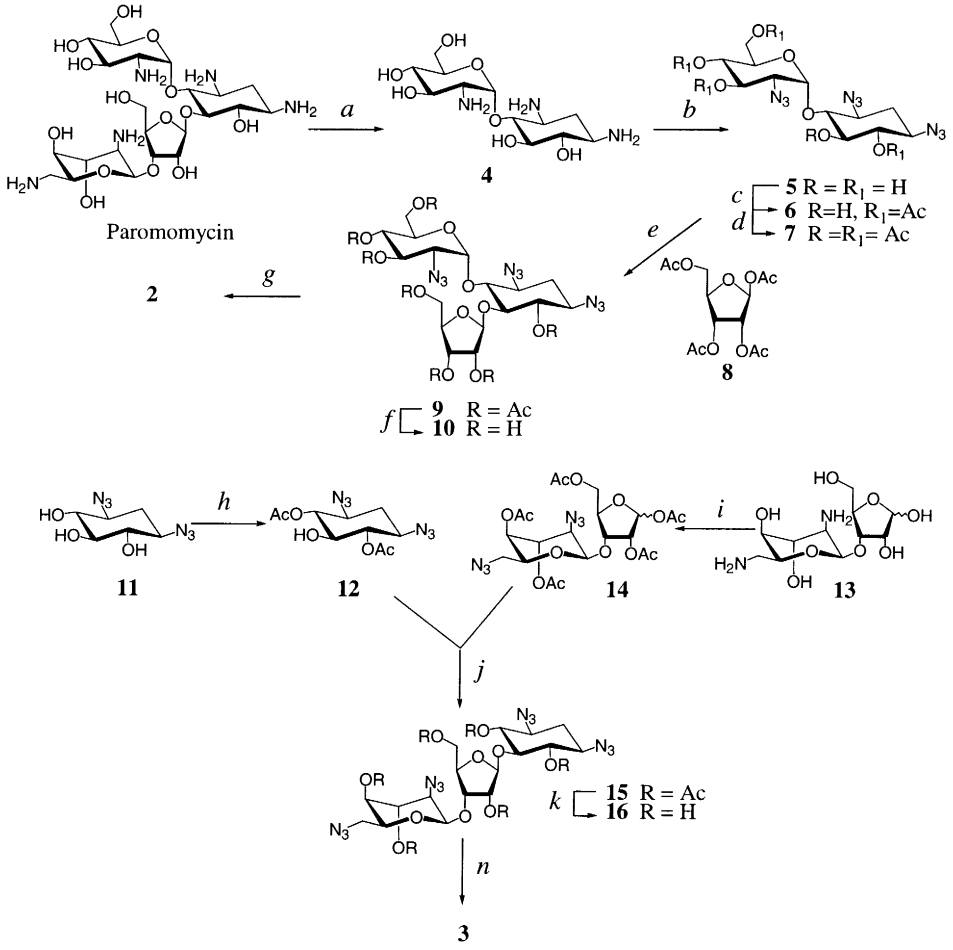
more efficient inhibitors of the Rev/RRE protein–RNA interaction. Compounds **2** and **3** have previously been obtained from degradation of paromomycin and neomycin B.[7,8] In this communication, we report the chemical synthesis of **2** and **3**, which not only afford the target compounds for screening, but also provide a synthetic methodology which will allow subsequent access to analogs of the parent structures.

The synthetic strategy is summarized in Scheme 1. Compound **2** was synthesized starting from paromamine **4**, which was obtained from mild hydrolysis of paromomycin.[9] Subsequent conversion of the amino groups of **4** into azides occurred with retention of stereochemistry and gave **5** in 65% yield.[10] Regioselective acetylation of **5** with 4 equiv. acetic anhydride in pyridine afforded compound **6** in 75% yield, in which all functional groups except the 5-hydroxyl group are protected. Acetylation of **6** gave the per-*O*-acetyl derivative **7**, in which the ^{1}H NMR signal of H-5 at 3.41 (1H, *t*, *J*=9.2 Hz) for **6** was shifted to 4.83 (1H, *t*, *J*=9.2 Hz) for **7**. Ribofuranosylation of **6** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**) was performed in CH_2Cl_2 in the presence of TMSOTf (1.2 equiv.) to give the β-linked coupled product



Scheme 1. Conditions and reagents: (a) 1N HCl/MeOH, 70°C, 10 h, 65%; (b) TfN_3 (6 equiv.), MeOH:H_2O (1:1), K_2CO_3, 24 h, 65%; (c) Ac_2O (4.2 equiv.), pyridine, −10°C, 4 h, 75%; (d) AC_2O/pyridine, 0°C, 1 h, 95%; (e) **8** (2 equiv.), MS, CH_2Cl_2, TMSOTf (2 equiv.), 10 h; (f) MeONa/MeOH, rt, 30 min, 56% for two steps; (g) Me_3P/THF (1N), H_2O, NaOH, rt, 10 h, 62%; (h) Ac_2O (2.3 equiv.), pyridine, −15°C, 4 h, 25%; (i) (1) TfN_3 (3 equiv.), H_2O/MeOH, K_2CO_3, rt, 30 h; (2) Ac_2O/pyridine, rt, 10 h, 52%; (j) 14 (1.5 equiv.), CH_2Cl_2, MS, TMSOTf (2 equiv.), rt, 10 h, 55%; (k) NaOMe/MeOH, rt, 30 min, 100%; (n) Me_3P/THF (1N), H_2O/NaOH, rt, 12 h, 73%

9. Deacetylation of **9** with sodium methoxide in methanol gave the pseudo-trisaccharide **10** in 56% yield over two steps. Reduction of azido groups in **10** by $Me_3P/THF/H_2O/NaOH$ yielded compound **2** in 62% yield.

Compound **3** was synthesized from the coupling reaction of 4,6-di-*O*-acetyl-1,3-diazido-2-deoxystreptamine (**12**) and 1,2,5,3′,4′-penta-*O*-acetyl-2′,6′-diazidoneobiosamine (**14**). Treatment of 1,3-diazido-2-deoxy-streptamine (**11**)[11] with 2 equiv. acetic anhydride in pyridine at −10°C gave the glycosyl acceptor **12** in 25% yield. NMR and mass spectral data of **12** confirmed that two acetyl groups were located at the 4 and 6 positions. The neobiosamine derivative **14**, in which all the functional groups are protected, was prepared from neobiosamine **13**.[12] Treatment of **13** with TfN_3, followed by acetylation with acetic anhydride in pyridine, gave the glycosyl donor **14** in 52% yield. Glycosylation of **12** with **14** in dichloromethane in the presence of TMSOTf gave the pseudo-trisaccharide **15** in 55% yield. Deacetylation of **15** with sodium methoxide in methanol gave the partially protected derivative **16**. Reduction of the azido groups of **16** by the reaction with $Me_3P/NaOH/THF/H_2O$ gave compound **3** in 73% yield for two steps.

Compounds **2** and **3** will be used as standards to compare RNA binding activities with ribostamycin (**1**), neomycin B and paromomycin, and then to determine the relationship between the structures of aminoglycosides and RNA binding activities.

Aminoglycosides provide several important lessons in the design of compounds that bind to RNA.[13] The synthesis of aminoglycosides reported here provides efficient access to some interesting aminoglycoside structures, and this work may be useful for synthesizing unnatural aminoglycoside derivatives.[14]

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14. Spectral data for selected compounds. Compound **6**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.42 (1H, t, *J*=9.2), 5.32 (1H, d, *J*=3.6, H-1′), 4.98 (1H, t, *J*=9.2), 4.89 (1H, t, *J*=9.2), 4.31 (1H, dt, *J*=2.0, 10.4), 4.23 (1H, dd, *J*=4.4, 12.4), 4.05 (1H, dd, *J*=2.4, 12.4), 3.61 (1H, t, *J*=9.2), 3.56 (1H, dd, *J*=4, 10.4), 3.48 (1H, ddd, *J*=4.8, 10.0, 12.4), 3.41 (1H, t, *J*=9.2), 3.30 (1H, ddd, *J*=4.8, 10.0, 12.4), 2.33 (1H, dt, *J*=4.4, 13.2, H-2α), 2.11 (3H, s), 2.03 (3H, s), 2.02 (3H, s), 1.99 (3H, s), 1.59 (1H, q, *J*=12.4, H-2β); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 99.1 (C-1′), 83.3, 75.2, 74.6, 71.3, 68.5, 68.4, 62.0, 61.8, 58.5, 58.1, 31.9 (C-2). Compound **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.36 (1H, t, *J*=9.2), 5.05 (2H, m), 4.96 (1H, t, *J*=9.2), 4.82 (1H, t, *J*=9.3), 4.36 (1H, d, *J*=10.2), 4.22 (1H, d, *J*=10.3), 4.04 (1H, d, *J*=10.3), 3.46 (2H, m), 3.36 (1H, m), 3.24 (1H, dt), 2.26 (1H, m, H-2α), 1.48 (1H, q, *J*=12.4, H-2β). Compound **9**: ^{1}H NMR (CD_3OD, 400 MHz) δ 6.01 (1H, d, *J*=3.9, H-1′), 5.46 (1H, t, *J*=7.6), 5.45 (1H, d, *J*=4.0, H-1′′), 5.22 (1H, t, *J*=3.0), 5.12 (1H, t, *J*=3.1), 5.02 (1H, t, *J*=7.8), 4.97 (1H, t, *J*=7.9), 4.46 (1H, dt), 4.40 (1H, dd), 4.27 (1H, m), 4.20 (2H, m), 3.99 (1H, t, *J*=7.8), 3.78 (1H, t, *J*=7.9), 3.65 (2H, m), 2.36 (1H, m, H-2α), 1.59 (1H, q, *J*=12.4, H-2β); ^{13}C NMR (CD_3OD, 400 MHz) δ 106.7 (C-1′), 96.2 (C-1′′), 81.6, 79.5, 76.2, 75.0,

74.4, 70.8, 70.5, 68.5, 68.4, 63.1, 62.0, 61.3, 59.8, 58.4, 31.4 (C-2). Compound **10**: ^{1}H NMR (CD_3OD, 400 MHz) δ 5.56 (1H, d, *J*=4.8, H-1′′), 5.36 (1H, s, H-1′), 4.16 (1H, dd), 4.08 (1H, d), 3.06 (1H, dd), 2.17 (1H, m, H-2α), 1.37 (1H, q, *J*=12.4, H-2β); ^{13}C NMR (CD_3OD, 400 MHz) δ 107.3 (C-1′), 96.7 (C-1′′), 83.9, 83.5, 75.8, 75.7, 75.0, 72.9, 70.9, 70.6, 70.2, 63.5, 61.9, 61.2, 60.5, 60.2, 31.0 (C-2). Compound **2**: ^{1}H NMR (D_2O, 400 MHz) δ 5.70 (1H, d, J<1, H-1′′), 5.25 (1H, s, H-1′), 4.20 (2H, s), 3.90 (2H, m), 2.40 (1H, d, *J*=12.4, H-2α), 1.75 (1H, q, *J*=12.4, H-2β); ^{13}C NMR (D_2O, 400 MHz) δ 110.4 (C-1′), 96.4 (C-1′′), 84.5, 82.8, 77.5, 75.3, 74.1, 72.5, 69.5, 69.1, 69.0, 60.9, 60.6, 54.0, 50.1, 49.1, 29.8 (C-2); ESMS: (*m/z*) 456 $[M+H]^+$, 478 $[M+Na]^+$. Compound **12**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.89 (2H, t, *J*=10.0, H-4 and H-6), 3.63 (2H, ddd, *J*=4.4, 10.0, 12.4, H-1 and H-3), 3.59 (1H, d, *J*=10.0, H-5), 2.25 (1H, dt, J=4.4, 12.4, H-2α), 1.51 (1H, q, *J*=12.4, H-2β), 2.15 (6H, s); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 172.4, 76.7, 75.1, 60.9, 32.0, 21.2; ESMS: (*m/z*) 298 $[M]^+$, 321 $[M+Na]^+$. Compound **14**: ^{13}C NMR ($CDCl_3$, 400 MHz) δ 98.5, 98.1, 97.2, 74.0, 69.4, 68.6, 68.6, 66.7, 66.2, 61.2, 57.1, 57.2. Compound **16**: ^{1}H NMR (CD_3OD, 400 MHz) δ 5.26 (1H, s, H-1′), 5.06 (1H, d, *J*=6, H-1′′), 4.25 (1H, s), 2.19 (1H, m, H-2α), 1.31 (1H, q, *J*=12.4, H-2β); ^{13}C NMR (CD_3OD, 400 MHz) δ 112.3 (C-1′′), 98.9 (C-1′), 84.9, 76.5, 74.9, 74.0, 70.4, 70.3, 68.8, 64.5, 61.5, 61.0, 60.6, 51.3, 32.1 (C-2); ESMS: (*m/z*) 581 $[M+Na]^+$. Compound **3**: ^{1}H NMR (D_2O, 400 MHz) δ 5.34 (1H, s, H-1′), 5.08 (1H, d, *J*=4.0, H-1′′), 4.26 (1H, d, *J*<1.0), 4.22 (1H, m), 4.15 (1H, d, *J*=3.2), 3.70 (2H, m), 3.87 (1H, d, J<1.0), 3.67 (1H, s), 2.48 (1H, dt, *J*=4, 12.8, H-2α), 1.72 (1H, q, *J*=12.8, H-2β); ^{13}C NMR (D_2O, 400 MHz) δ 101.6 (C-1′), 95.7 (C-1′′), 81.6, 75.1, 72.8, 71.1, 70.4, 69.4, 67.9, 67.7, 67.6, 63.9, 52.6, 51.4, 50.4, 50.1, 30.7 (C-2); ESMS: (*m/z*) 455 $[M+H]^+$, 477 $[M+Na]^+$.