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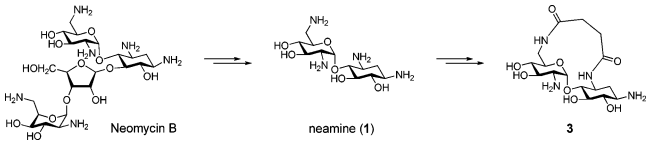
Design and Synthesis of a Structurally Constrained Aminoglycoside

Dale Kling, Dusan Hesek, Qicun Shi, and Shahriar Mobashery*

Department of Chemistry and Biochemistry, University of otre Dame, Notre Dame, Indiana 46556

mobashery@nd.edu

Received April 11, 2007



The synthesis of a constrained tricyclic aminoglycoside derivative is described. This constrained compound fixes the spatial orientation of two critical rings for the minimal motif for binding to biological macromolecules such as RNA and proteins. Methanolysis of neomycin B under acidic conditions produced the bicyclic neamine. Transient protection by the Cu^{2+} ion and regioselective introduction of protective groups led to intermediate **7**, which was used for a key annulation reaction that introduced the tricyclic nucleus into the structural framework. A final hydrogenolysis step to remove the protective groups produced the desired target molecule. The efficient eight-step synthesis was accomplished in 8% overall yield.

Aminoglycoside antibiotics have been in clinical use since the 1940s.[1−3] These antibiotics have been effective in light of their predictable pharmacokinetics and reliability in treatment of difficult bacterial infections. Although examples of resistance to these antibiotics have emerged over the years,[4−9] they continue to be used clinically to the present day.

These antibiotics bind to the 30S bacterial ribosomal subunit, interfering with the protein biosynthetic machinery, which results in a bactericidal outcome.[10,11] Whereas different mechanisms for resistance to these agents have emerged, the most common are acquisition of resistance enzymes.[9,12,13] These enzymes modify the structures of the antibiotics, whereby binding to the ribosomal site either is eliminated or becomes less effective.

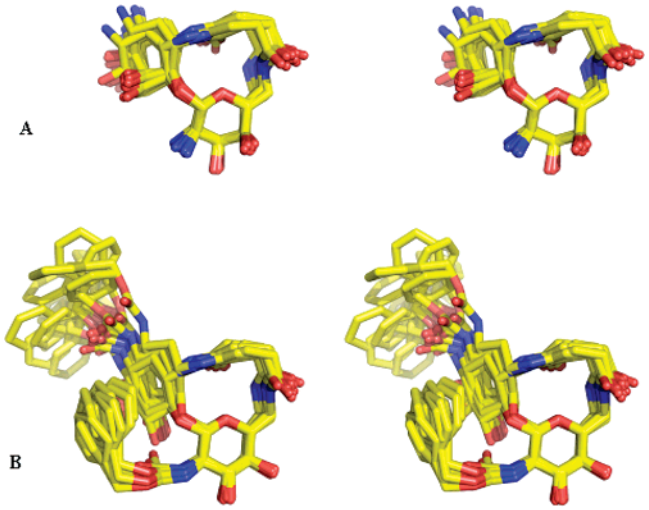


FIGURE 1. Stereoview of a capped stick representation for 15 superimposed conformers of (A) compound **3** and (B) compound **9**. Atoms are colored according to atom types (C, N, and O shown in yellow, blue, and red, respectively).

The typical aminoglycoside antibiotic may have up to five rings in its structure. Though each ring has its own preferred conformation, where these molecules exhibit considerable conformational flexibility is at the glycosidic bonds. Indeed, part of the effectiveness of aminoglycosides from a therapeutic standpoint comes from their ability to adopt multiple conformations. A manifestation of this structural flexibility is that aminoglycosides tend to lack high RNA target selectivity, binding not only to bacterial ribosomal RNA but also to biologically relevant ribozymes and the HIV-1 RNAs known as RRE and TAR.[14] In addition, bacterial resistance enzymes that target these compounds take advantage of their conformational adaptability, binding aminoglycosides in conformationally distinct manners.[15,16] There exist now opportunities for the generation of conformationally constrained aminoglycosides that could be used in studies of both RNA targets (ribosomal and otherwise) and resistance enzymes. These analogues might discriminate in binding to one or the other sites and thus prove to be valuable tools in studies of the functions of these biologically important targets.[17]

We describe herein the design and synthesis of a conformationally restricted aminoglycoside poised for mechanistic studies

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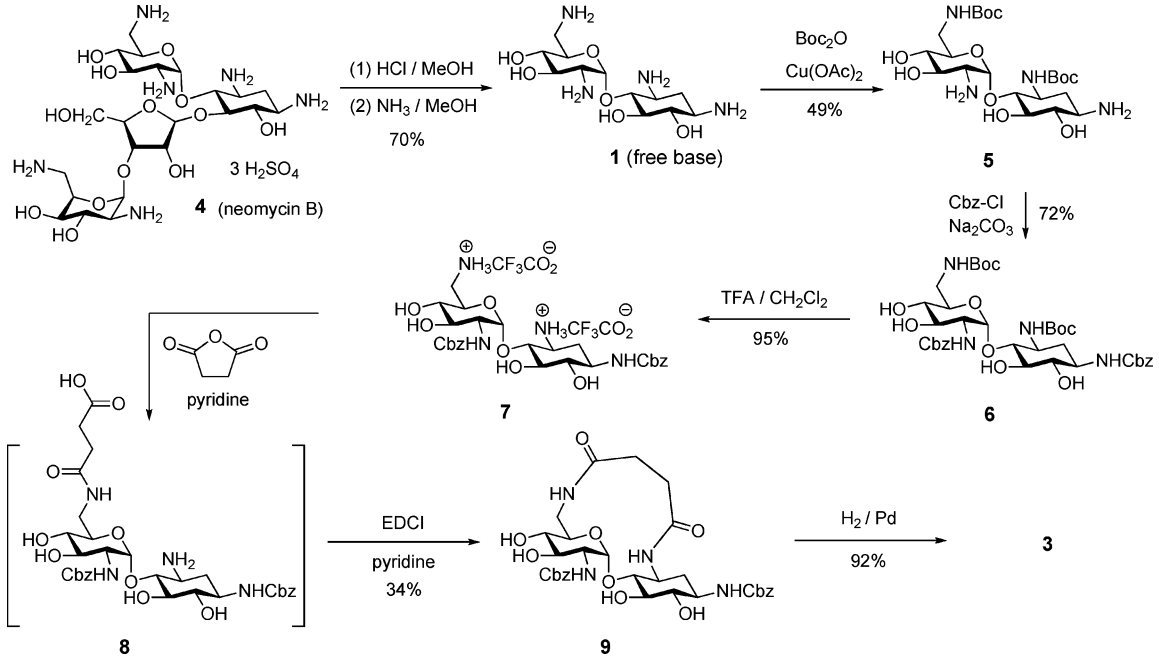
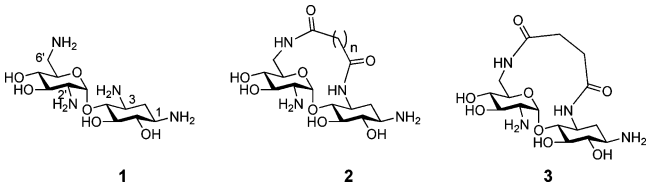
10.1021/jo0707636 CCC: $37.00
Published on Web 06/19/2007

SCHEME 1. Synthesis of a Structurally Constrained Aminoglycoside 3



with biological targets. The structural template is that of neamine (**1**). A series of computational analyses revealed that tethering



of the 6′-amine group to that at position 3 would be favorable in locking the conformations of the two rings of neamine roughly orthogonal to each other (Figure 1A), as will be described below.

Quantum mechanics methods were applied in computing the suitable number of methylenes within the tether while keeping the energy to a minimum (**2**, $n = 0-4$ were investigated). These analyses revealed that with both amide bonds in a trans configuration the minimal preferred number for methylenes is two. Molecular dynamics simulations were performed for the species with two methylene groups (**3**) to document the stability of the molecule. A 50 000-step energy minimization followed by 500 ps nonperiodic molecular dynamics ensued. We selected 15 conformations from the course of the simulation, which are overlapped in Figure 1A.

The synthesis of **3** (Scheme 1) started with methanolysis of commercially available neomycin B trisulfate (**4**) to afford neamine **1** (as an HCl salt), which was converted to the free base form in the presence of ammonia in methanol.[18−20] Protection of the 3-NH_2 and 6′-NH_2 groups in **1** by di-*tert*-butyldicarbonate was carried out in the presence of Cu^{2+}. This protection strategy, which relies upon preferential chelation of Cu^{2+} ions to the amines at positions 1 and 2′ of neamine, was investigated earlier by our group by detailed measurements of the paramagnetic contributions of the cupric ion to T1 relaxation times by H NMR spectroscopy.[21] This transient metal-ion protection strategy efficiently results in just two products: the desired di-Boc derivative **5** with protection at positions 3 and 6′ as the major component and a tri-Boc neamine species (Boc at positions 1, 3, and 6′), which was easily separated by column chromatography. Subjecting **5** to benzyl chloroformate and aqueous sodium carbonate gave **6** in good yield.

Deprotection of the Boc groups in **6** proceeded smoothly using 25% triflouroacetic acid in dichloromethane. Concentration of the reaction in vacuo and subsequent dilution with anhydrous ether led to precipitation of crude **7**, which was used in the next step without further purification. This material was dissolved in pyridine in high dilution before allowing it to react with 1 equiv of succinic anhydride. We presume that intermediate **8** should form preferentially over the corresponding 3-*N* regioisomer. Analysis by LC/MS at this point revealed a single new species with the correct mass, which was not isolated. Instead, EDCI was added directly to the reaction mixture, and stirring was continued for 2 days. This reaction lead to compound **9** and according to LC/MS analysis conversion was in excess of 95%. Removal of the two Cbz groups at positions 1 and 2′ over palladium under an atmosphere of hydrogen produced the desired **3** in 92% yield. The overall yield for the eight-step procedure was 8% (from neomycin trisulfate, **4**). A combination of homonuclear decoupling experiments, two-dimensional NMR, and IR spectroscopy were used to establish the connectivities of the linker element uniting the two rings.

Annulation of species **8** was key in the success of this synthesis. In designing this synthesis we were keenly aware that the spatial predisposition of the amines at positions 6′ and 3 would be critical in the success of the reaction. Computation revealed that this should be likely, and the product of the annulation, compound **9**, turned out to be stable. The results of

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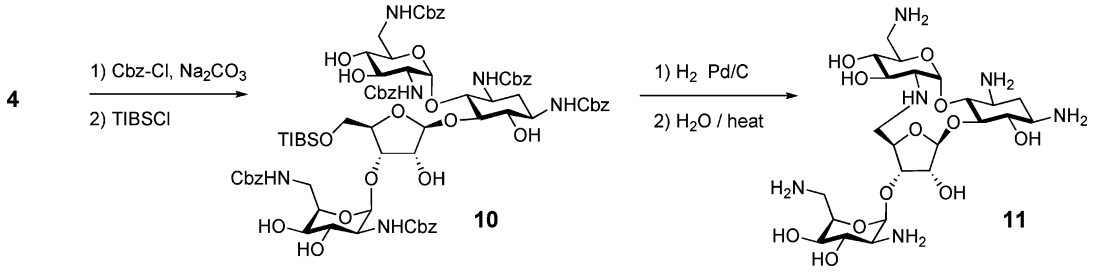
SCHEME 2. Synthesis of Constrained Aminoglycoside 11



the dynamics simulations of **9** are depicted in Figure 1B. These analyses revealed that the two Cbz groups segregate to one side of the molecule, leaving the 6′ and 3 amines available in close proximity for the annulation reaction to proceed.

We hasten to add that two other groups have also prepared a certain constrained aminoglycoside **11** previously.[15,17,22] This synthesis (Scheme 2) proceeded through protection of the four amino groups of the neomycin B (**4**) with benzyl chloroformate followed by protection of the primary alcohol function of the five-membered ring with TIBS chloride to give derivative **10**. Deprotection of the amines by hydrogenolysis was followed by heating the aqueous solution for 7 days to produce **11**.

Experimental Section

Free-base neamine **(1)**[18,19] and the di-Boc derivative **5**[21] were prepared by literature methods.

Compound 6. 3,6′-Di-*N*-(*tert*-butoxycarbonyl)neamine (**5**) (3.5 g, 6.7 mmol) was added to 300 mL of *p*-dioxane, and the mixture was stirred at room temperature for 15 min to dissolve most of the solid. Benzyl chloroformate (2.40 g, 14.1 mmol) and Na_2CO_3 (7.6 g, 20 mmol) were then added along with 100 mL of deionized water. The reaction proceeded for 3 h, after which time TLC ($CHCl_3$/MeOH/concentrated ammonia 4:1:0.1) revealed a single spot with $R_f = 0.35$. The solvent was removed in vacuo, and the remaining solid was washed with 150 mL portions of water (4×). Product **6** was obtained (3.85 g) in 72% yield. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.30 (overlapping multiplet, 10H, Cbz), 5.30 (s, 1H, H-1′), 5.10 (m, 4H, CH_2Ph), 3.76 (unresolved multiplet, 1H, H-5′), 3.40−3.64 (overlapping multiplets, 8H, H-1, H-2′, H-3, H-3′, H-4, H-5, H-6′), 3.24 (t, $J = 8.5$ Hz, 1H, H-4′), 3.20 (t, $J = 10$ Hz, 1H, H-6), 2.02 (m, 1H, H-2eq), 1.34 (s, 18H, OC(CH_3)$_3$), 1.25 (m, 1H. H-2ax). ^{13}C NMR (CD_3OD, 125 MHz) δ 157.9, 157.7, 157.2, 156.5 (C═O), 136.9, 128.1, 127.9, 127.6, 127.5 (C_6H_5), 98.8 (C-1′), 80.6, 79.3, 77.4, 75.2 (C-3′, C-4, C-4, C-6), 71.2 (C-5′) 70.9 (C-4′) 66.3 and 66.0 ($CH_2C_6H_5$), 56.0 (C-2′), 51.4 (C-1), 49.6 (C-3), 40.5 (C-6′), 35.5 (C-2), 27.5 and 27.4 (OC(CH_3)$_3$). LC/MS using a Pro C18 YMC reverse-phase column (Scan ES$^+$) provided a single peak at $R_t = 7.68$ min, corresponding to *m*/*z* 791 $[M + H]^+$. HRMS (FAB$^+$) for $C_{38}H_{54}N_4O_{14}Na$ $[M + Na+]^+$: calcd 813.3534, found 813.3535. Melting point: 245 °C.

Compound 7. To 1.47 g (1.9 mmol) of **6** was added 100 mL of 25% triflouroacetic acid in CH_2Cl_2. This solution was stirred for 1 h at room temperature under an atmosphere of nitrogen, after which time the solvent was evaporated. *p*-Dioxane (200 mL) was then added, stirred for 5 min, and decanted to help remove residual TFA. To the remaining golden brown residue was added 150 mL of diethyl ether, which resulted in precipitation of a white solid. This material corresponded to the desired **7**, which was filtered and dried under vacuum. ^{1}H NMR (D_2O, 500 MHz) δ 8.25, 8.10, and 7.56 (4H, NH′s), 6.90 (10H, Cbz), 5.18 (d, $J = 4$ Hz, 1-H, H-1′), 4.61 (m, 4H, Cbz CH_2Ph), 3.40 (s, 1H, H-5′), 3.28 (overlapping multiplet, 3H, H-3, H-3′, H-4 or H-5), 3.07−2.89 (overlapping muliplets, 7H, H-1, H-2′, H-3 H-4 or H-5, H-4′, H-6, H-6′), 1.98 (ddd, $\omega = 21$ Hz, assigned as a 1:2:1:1:2:1 six line m, $J_{2eq-2ax} = 13$ Hz, $J_{2eq-1} = J_{2eq-3} = 4$ Hz, 1H, H-2eq), 1.29 (ddd, $\omega = 37$, assigned as a 1:3:3:1 four line m, $J_{2ax-2eq} = 13$ Hz, $J_{2ax-1} = J_{2ax-3} = 12$ Hz, 1H, H-2ax). ^{13}C NMR (CD_3CN, 125 MHz) δ 162.6, 162.3, 158.4, and 157.7 (C═O), 138.1, 138.0, 129.6,129.0, 128.8, (C_6H_5), 118.4, 97.6 (C-1′), 77.8 (C-4), 75.8 (C-6), 73.6 (C-5), 71.7 (C-3′), 70.0 (C-4′), 67.7 (C-5′), 67.3 (C-2′), 56.4 (C-1), 52.0 (C-3), 51.2 (C-6′), 42.7 (C-2). LC/MS using a Pro C18 YMC reverse-phase column (Scan ES$^+$) provided a single peak at $R_t = 4.30$ min corresponding to *m*/*z* 591 $[M + H]^+$. HRMS (FAB$^+$) for $C_{28}H_{38}N_4O_{10}$ $[M + H]^+$: calcd 591.2666, found 591.2648. Melting point: 79−81 °C.

Derivative 9. To a flask containing 250 mg (0.3 mmol) of **7** was added 60 mL of pyridine. This mixture was stirred for 15 min at room temperature before addition of succinic anhydride (31 mg, 0.3 mmol). The reaction was aged for 12 h. Analysis by LC/MS (Scan ES+) revealed a single peak at $R_t = 4.5$ min representing *m*/*z* 691 $[M + H]^+$, which corresponds to intermediate **8**. No purification was performed at this point. To the crude product **8** was added EDCI (150 mg, 75 mmol, 2.5 equiv). After 16 h LCMS revealed a 50:50 mixture of starting material and the desired product **9**. The reaction was stirred for an additional 32 h (48 h total) to bring the reaction to completion. Pyridine was removed in vacuo to obtain a brown residue. Addition of 50 mL of water resulted in precipitation of a tan-colored solid, which was filtered. Product **9** was obtained (32 mg) in 34% yield. ^{1}H (DMSO, 500 MHz) δ 7.87, 7.64, 7.16, and 7.08 (4H, NH′s), 7.37 (m, 10H, C_6H_5), 5.68 (1H, H-1′), 5.04 (m, 4H, CH_2Ph), 3.77 (m, 1H, H-3), 3.57−3.13 (overlapping multiplets, 12H, H-2′ H-3′ H-4, H-4′, H-5, H-5′, H-6, H-6′ and 4 OH′s), 2.90 and 2.71 (m, 2H, H-1, H-6′), 2.34 (m, 4H, bridge CH_2's), 1.80 (1H, H-2eq), 1.25 (1H, H-2ax). ^{13}C (DMSO, 125 MHz) δ 172.4, 171.1, 156.8, and 156.2 (C═O), 147.7, 138.0, 137.7, 128.8, 128.0, 127.8, 127.6 (C_6H_5), 101.4 (C-1′), 85.6 (C-4), 76.6 (C-6), 74.6 and 73.9 (C-3′ and C-5), 72.8 (C-4′), 71.6 (C-5′), 65.6 and 65.5 (CH_2Ph), 57.3 (C-2′), 51.6 (C-1), 49.4 (C-3), 42.7 (C-6′), 35.2 and 33.6 (bridge CH_2 ‘s), 32.6 (C-2). LC/MS using a Pro C18 YMC reverse phase column (Scan ES$^+$) provided a single peak at $R_t = 4.93$ min corresponding to *m*/*z* 673 $[M + H]^+$. HRMS (FAB$^+$) for $C_{32}H_{40}N_4O_{12}$ $[M + H]^+$: calcd 673.2721, found 673.2722. Melting point: > 310 °C.

Compound 3. To a flask containing of **9** (82 mg 0.12 mmol) was added 30 mL of a DMF/MeOH/AcOH solution (5:4:1). The flask was placed under an atmosphere of nitrogen before addition of 50 mg of palladium (10%) on activated carbon. Evacuation was then carried out with hydrogen atmosphere replacements (3×). The mixture was stirred overnight at room temperature under an atmosphere of hydrogen. The mixture was filtered through celite, and the residue was washed with MeOH (10 mL, 3×) and water (10 mL). Upon evaporation of the solvent, compound **3** was isolated as a beige solid **(**44 mg, a 92%). ^{1}H (D_2O, 500 MHz) δ 5.1 (d, $J = 3.5$ Hz, 1H, H-1′), 3.75 (m, 1H, H-3), 3.61 (overlapping multiplets, 3H, H-4, H-5′, H-6′), 3.46 (m, 2H, H-6, H-3′ or H-5), 3.38 (t, $J = 10.5$ Hz, 1-H, H-3′ or H-5), 3.19 (overlapping multiplets, 2H, H-2′, H-4′), 3.09 (overlapping multiplets, 2H, H-1,

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H-6′), 2.43 (m, 4H, bridge CH_2's), 2.1 (ddd, $\omega = 21$ Hz, assigned as a 1:2:1:1:2:1 6 line m, $J_{2eq-2ax} = 13$ Hz, $J_{2eq-1} = J_{2eq-3} = 4$ Hz, 1H, H-2eq), 1.30 (ddd, $\omega = 38$ Hz, assigned as a 1:3:3:1 4 line m, $J_{2ax-2eq} = 13$ Hz, $J_{2ax-1} = J_{2ax-3} = 12.5$ Hz, 1H, H-2ax). ^{13}C (D_2O, 125 MHz) δ 174.8, 174.2 (C═O), 98.8 (C-1′), 84.4 (C-4), 75.4 (C-6), 72.8 and 72.4 (C-3′ and C-5), 71.5 (C-4′), 69.4 (C-5′), 55.0 (C-2′), 49.8 (C-1), 49.1 (C-3), 39.9 (C-6′), 33.4 and 31.8 (bridge CH_2 's), 30.3 (C-2). FT-IR (KBr pellet), 3395, 1654, 1560, 1412, 1050 cm^{-1}. LC/MS using a Pro C18 YMC reverse phase column (Scan ES^+) provided a single peak at $R_t = 0.85$ min corresponding to m/z 405 $[M + H]^+$. HRMS (FAB^+) for $C_{16}H_{28}N_4O_8$ $[M + H]^+$: calcd 405.1985, found 405.2003. Melting point: 170−172 °C.

Computational Methods. The structures of compounds **3** and **9** were prepared by Sybyl 7.0 (Tripos) (SYBYL 7.0, Tripos Inc., 1699 South Hanley Rd., St. Louis, MO, 63144), starting with the crystal coordinates for neamine.[23] The geometry of the structure was energetically optimized at the Hartree−Fock (HF) level with the 6-31G(d) basis set.[24] The structures were heated from 0 to 300 K in the gas phase using the Amber Molecular Dynamics Package programs (version 8).[25] The generated electrostatic potentials from the HF optimization were fit using the two-step RESP procedure.[26] The Antechamber program in the Amber package was used for assigning atom charge for the non-amino-acid molecules and documented in the format readable to Sybyl. Subsequently, Amber xleap was applied to generate a geometry documented in the PDB format, and all atom information was packed into a topological document. The structures were energy-minimized in 50 000 steps with the 30 steps steepest descent method followed by the conjugate gradient method for the remaining steps. The resultant structure was then used for molecular dynamic simulation. A total of 25 iterative simulation runs were carried out, each for 20 ps. The fluctuation of temperature about the preset value of 300 K was less than 0.6 K. The coordinate but not velocity parameters of the structures from each simulation were used as starting points in the next simulation. For each simulation run 10 000 snapshots were taken. In more than 200 000 conformations from the molecular dynamics sampling, 15 representative examples were selected for superimposition shown in Figure 1.

Supporting Information Available: NMR spectra for the synthetic molecules **3**, **5**, **6**, **7**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0707636

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