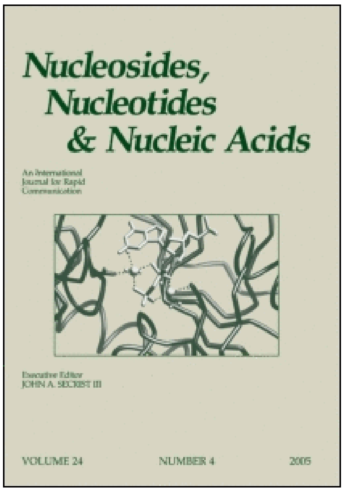

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Synthesis of Biomimetic Analogs of Neomycin B: Potential Inhibitors of the HIV RNA Rev Response Element

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SYNTHESIS OF BIOMIMETIC ANALOGS OF NEOMYCIN B: POTENTIAL INHIBITORS OF THE HIV RNA REV RESPONSE ELEMENT [+]

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Dedicated to the memory of Gertrude B. Elion

ABSTRACT: The aminoglycosidic antibiotic, neomycin B, is an inhibitor of the binding of Rev to RRE. This paper reports on the synthesis of analogs of neomycin B as potential anti-HIV compounds designed to function as inhibitors of Rev/ RRE binding.

Introduction

The viral regulatory protein, Rev, plays an important role in the expression of HIV genes. Its mechanism of action in the nucleus appears to be through recognition and binding to a highly structured RNA sequence, the Rev response element, RRE, located within the coding sequence of the *env* gene (Figure 1).[1] Binding to the protein is tight (Kd ~1 nM) and apparently involves an arginine-rich domain.[2,3] The aminoglycosidic antibiotic, neomycin B (Figure 1), is an inhibitor of the binding of Rev to RRE with an IC_{50} value in the low μM range.[4] This compound represents an excellent lead molecule for the design of novel aminoglycoside inhibitors of the binding of Rev to RRE. However, the ototoxicity of neomycin B is a major drawback to its therapeutic usefulness.[5] There is interest therefore in the discovery of analogs of neomycin B with comparable or greater activity and lower toxicity.[6-9]

Biological evidence indicates that the underlying mechanism of aminoglycoside toxicity is electrostatic binding of polycationic aminoglycosides to anionic phospho-

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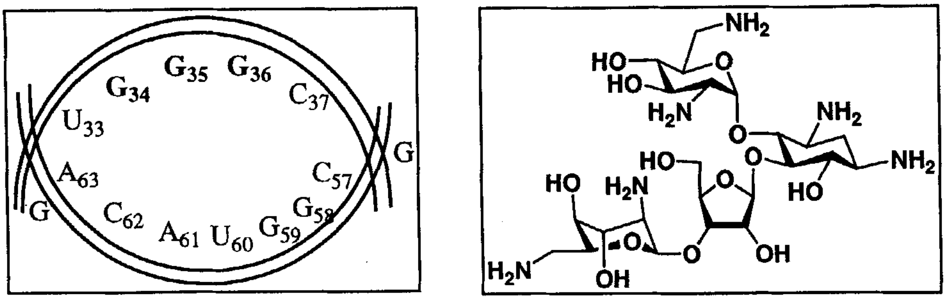


Rev Response Element (RRE) Neomycin B

Figure 1. Rev binding sequence of HIV mRNA, RRE (left), and the structure of neomycin B, an inhibitor (right). Neomycin B binding sequence is identified in bold in the structure of RRE.

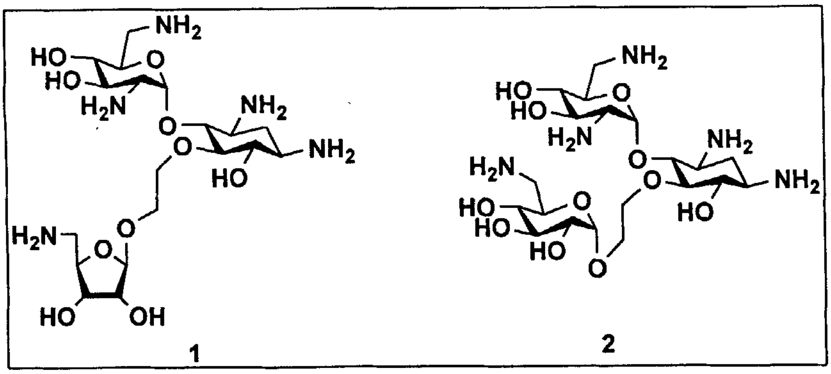
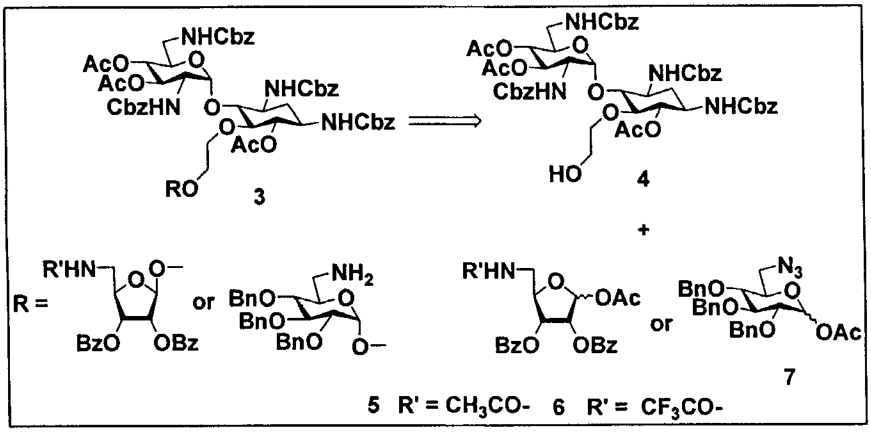


Figure 2. The structures of target compounds **1** and **2**

lipids.[5] However, polycationic function is essential for RNA binding.[10] Interestingly, the neamine part of neomycin B is found in a number of aminoglycosides that have RNA binding activity,[2,3] but in contrast to neomycin B, neamine does not have significant inhibitory activity against Rev binding to RRE (IC_{50} >100 μM). For these and other reasons outlined above, we designed structures **1** and **2** that were intermediate in complexity and aminoglycosidic composition between neomycin B and neamine, with the expectation of the discovery of the smallest aminoglycoside structure with inhibitory activity against HIV through inhibition of Rev/ RRE binding. This paper describes the synthesis and antiviral studies of **1** and **2**.

Results and Discussion

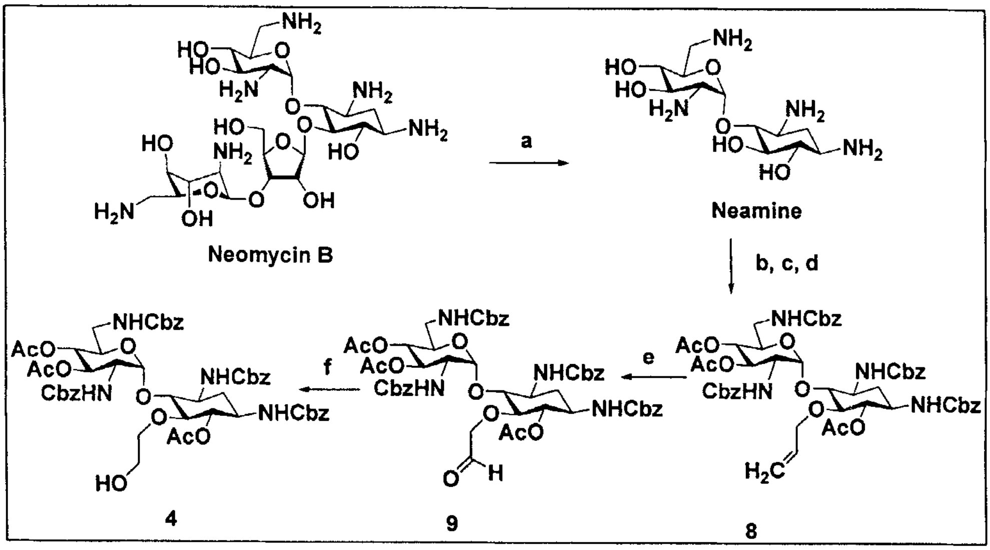
A retrosynthetic analysis is shown in Scheme 1. We planned that the target compound would be synthesized by glycosidation of a glycosyl acceptor **4** with an appropriate donor (**5**, **6** or **7**). The glycosyl acceptor would retain the key elements of neomycin B for activity and would be synthesized from the readily available natural product.



Scheme 1. Retrosynthetic analysis of targeted analogs of neomycin B

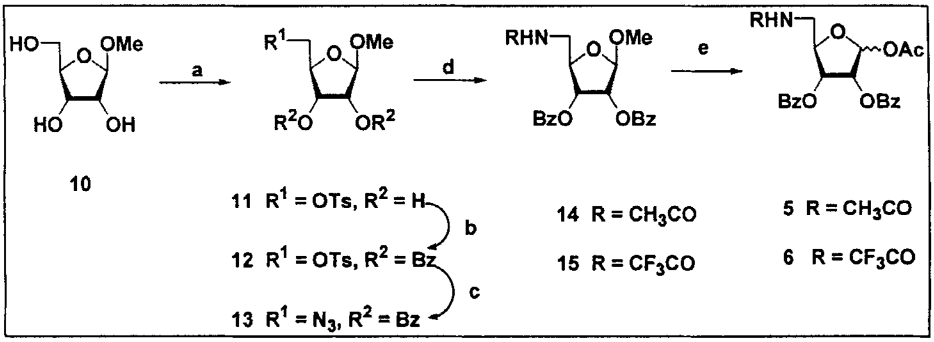
Synthesis of the glycosyl acceptor **4** is shown in Scheme 2. Aldehyde **9**,[6] was prepared from neomycin B in several steps. Treatment of commercially available neomycin B with methanolic-HCl gave crystalline neamine hydrochloride in about 70 % yield. The amino groups were protected (Cbz) (74%) and the hydroxyl groups were then selectively acetylated (37%). O-Allylation converted the resulting compound to intermediate **8** (46%). Oxidative cleavage of the O-allyl functionality in **8** gave the aldehyde **9** (71%), which was reduced chemoselectively with sodium triacetoxyborohydride[11] to afford **4** in 90% yield.

The next stage in the synthesis was the preparation of the ribose type glycosyl donors **5** and **6** (Scheme 3). The primary hydroxyl group of **10** was tosylated regioselectively followed by benzoylation to afford **12** (68% for two steps). Treatment of compound **12** with NaN$_3$/LiCl in DMF at 100 °C afforded **13**[12] in 70% yield. The azido



Reagents and conditions: a) HCl/MeOH; b) CbzCl, satd. aq. Na_2CO_3, acetone, toluene; c) Ac_2O, DMF, pyridine; d) allyl iodide, LiHMDS, DMSO; e) OsO_4, NMO, $NaIO_4$, dioxane, H_2O; f) $NaBH(OAc)_3$, benzene, reflux

Scheme 2. Synthesis of glycosyl acceptor 4



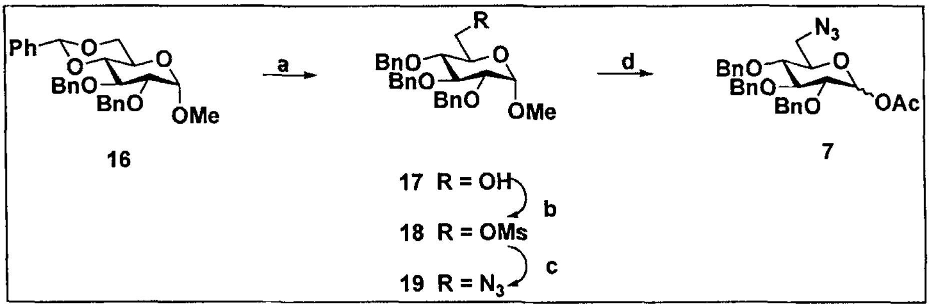
Reagents and conditions: a) TsCl, pyridine; b) BzCl, pyridine; c) NaN_3, LiCl, DMF, 100 °C; d) (i) H_2, Pd/C, (ii) Ac_2O, pyridine or $(CF_3CO)_2O$, pyridine; e) AcOH, Ac_2O, conc. H_2SO_4, 0 °C

Scheme 3. Synthesis of glycosyl donors 5 and 6

group of **13** was converted into the amino group by catalytic hydrogenation and the product was acetylated to afford **14** and **15** (75% and 51%). Glycosyl donors **5** and **6** were obtained by acetolysis of **14** and **15** (96% and 60%).

The other glycosyl donor **7** was synthesized as depicted in Scheme 4. 4,6-O-Benzylidene acetal **16** was regioselectively converted to 4-O-benzyl ether **17** (72%).[8]

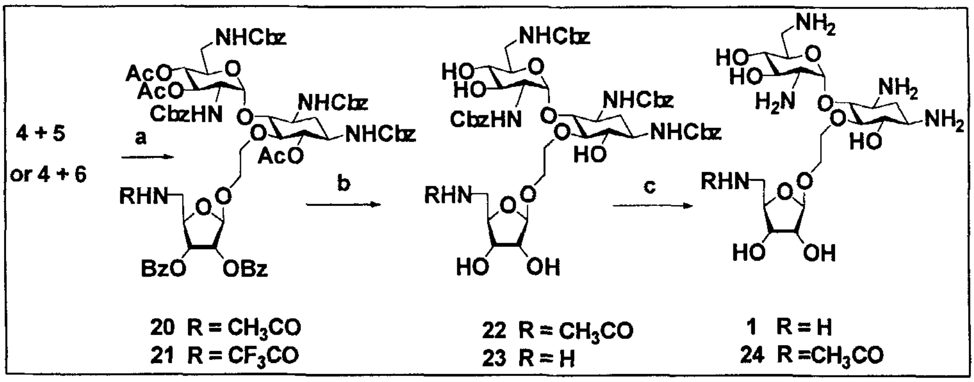
The azido sugar **19** was prepared from **17** by conversion to its mesylate **18** (90%) and treatment of the latter with lithium azide (75% yield). Glycosyl donor **7** was obtained in quantitative yield by acetolysis of **19**.



Reagents and conditions : a) BH_3-THF, Bu_2BOTf, 0 °C; b) MsCl, pyridine; c) NaN_3, LiCl, DMF, 100 °C; d) AcOH, Ac_2O, conc. H_2SO_4, 0 °C

Scheme 4. Synthesis of glycosyl donor 7

The reaction of **4** with **5** and **6** provided the desired β-linked pseudotrisaccharide **20** (87%) and **21** (49%) as the only anomer in each case.[13] Compound **20** was then treated with sodium methoxide to give **22** (Scheme 5). However, the amide group was not

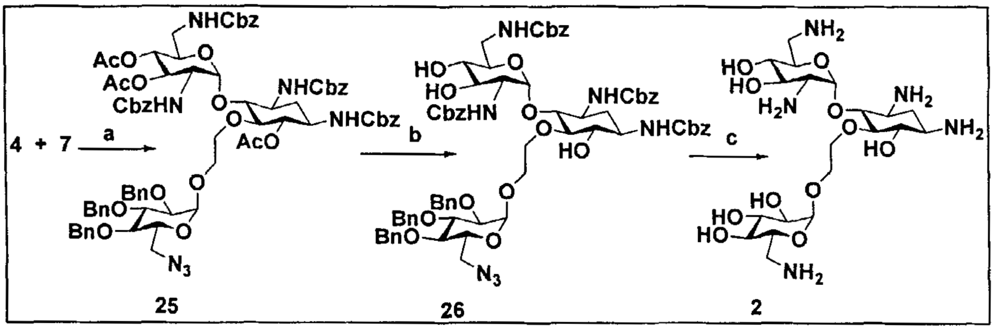


Reagents and conditions : a) TMSOTf, 4A Molecular Sieves; b) NaOMe, MeOH; c) Pd-C, H_2, H_2O, AcOH, 1,4-dioxane

Scheme 5. Coupling reactions to target compound 1

deprotected under these conditions and crude **22** was converted to **24** (i.e., the acylated analog of **1**) by catalytic hydrogenation (78% for two steps). On the other hand, deprotection of **21** afforded **23** smoothly under the same conditions. Finally, the desired compound **1** was obtained by hydrogenation of **23** (87% overall yield for two final steps).

Target compound **2** was synthesized as described for **1** (Scheme 6). The reaction of **4** and **7** afforded the α-linked pseudotrisaccharide **25** as the major product (37%) along with minor amounts of the β-anomer. The desired compound **2** was obtained by stepwise deprotection of **25** (78%).



Reagents and conditions : a) TMSOTf, 4A Molecular Sieves, CH_2Cl_2; b) NaOMe, MeOH; c) Pd-C, H_2, H_2O, AcOH, 1,4-dioxane

Scheme 6. Coupling reactions to target compound **2**

The structures of the target compounds and intermediates were established by ^{1}H and ^{13}C NMR spectra and HRMS data. The anti-HIV studies of target molecules **1** and **2** and selected precursors did not exhibit significant antiviral activity in HIV-1 infected CEM-SS cell lines. Further studies on these and other biomimetic analogs of neomycin B are in progress.

Experimental

NMR spectra were recorded on a Brüker Model AC-300 spectrometer. Chemical shifts (δ, ppm) are relative to TMS. High resolution mass spectra were determined on a VG ZAB-HF instrument. Preparative layer chromatography used plates prepared with E. Merck PF_{254} silica gel. Flash chromatography was carried out on columns packed with 240-400 mesh silica gel.

5-*O*-Hydroxyethyl-4,3',4'-tri-*O*-acetyl-1,3,2',6'-tetra-*N*-benzyloxycarbonylneamine (4). To a solution of **9**[6] (145 mg, 0.14 mmol) in benzene (4 mL) was added $NaBH(OAc)_3$ (240 mg, 1.12 mmol), and the mixture was stirred under reflux for 3 h. It was cooled to room temperature, diluted with ethyl acetate (30 mL) and washed with water (20 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (1:1 hexane : ethyl acetate) to afford **4** (130 mg, 90%) as a white foam. ^{1}H-NMR ($CDCl_3$) 7.39-7.26 (m, 20 H), 5.97 (br s, 1 H), 5.81 (br s, 1 H), 5.56 (br s, 1 H), 5.27-4.69 (m, 16 H), 4.06-3.93 (m, 2 H), 3.69-3.37 (m, 10 H), 3.08 (br s, 1 H), 2.07 (br m, 1 H), 1.94-1.82 (m, 9 H), 1.24 (br m, 1 H). FAB HRMS Calcd for $C_{52}H_{61}N_4O_{18}$ 1029.3981 $(M + H)^+$. Found 1029.4008.

Methyl 5-azido-2,3-di-*O*-benzoyl-5-deoxy-β-D-ribofuranoside (13). To a solution of **10** (1312 mg, 8.0 mmol) in pyridine (100 mL) was added a solution of tosyl chloride (2292 mg, 12.0 mmol) in chloroform (20 mL) at 0 °C, and the mixture was stirred at room temperature for 5 h. The reaction was quenched with ice and the solvent was then removed under reduced pressure. The residue was partitioned between chloroform (200 mL) and water (100 mL), and the oraganic layer was washed with water (100 mL x 3) and brine (200 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (1:1 hexane : ethyl acetate) to afford **11** (1945 mg, 76%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 7.81 (d, 2 H, *J* = 8.3 Hz), 7.36 (d, 2 H, *J* = 8.3 Hz), 4.81 (s, 1 H), 4.30-3.99 (m, 6 H), 3.26 (s, 3 H), 2.88 (br s, 2 H, OH), 2.45 (s, 3 H). ^{13}C-NMR ($CDCl_3$) 129.9, 128.0, 108.1, 8-.3, 74.8, 72.0, 70.3, 55.1, 21.6.

To a solution of **11** (820 mg, 2.6 mmol) in pyridine (20 mL) was added benzoyl chloride (724 µL, 6.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 5 h. The reaction was quenched with ice and the solvent was then removed under reduced pressure. The residue was partitioned between ethyl acetate (100 mL) and water (100 mL), and the organic layer was washed with water (100 mL x 3) and brine (200 mL) and dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (4: 1 hexane : ethyl acetate) to afford **12** (1234 mg, 90%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 8.14-7.30 (m, 14 H), 5.58-5.52 (m, 2 H), 5.09 (s, 1 H), 4.55 (ddd, 1 H, *J* = 3.7, 6.2 and 6.8 Hz), 4.40 (dd, 1 H, *J* = 3.7 and 10.6 Hz), 4.21 (dd, 1 H, *J* = 6.8 and 10.6 Hz), 3.35 (s, 3 H), 2.40 (s, 3 H). ^{13}C-NMR ($CDCl_3$) 165.2, 165.1, 144.9, 133.7, 133.5, 133.4, 132.7, 130.2, 129.9, 129.8, 129.7, 129.1, 128.7, 128.5, 128.4, 128.3, 128.0, 106.4, 78.8, 75.1, 72.3, 70.3, 55.2, 21.6.

To a solution of **12** (100 mg, 0.2 mmol) in DMF (10 mL) was added sodium azide (40 mg, 0.6 mmol) and lithium chloride (26 mg, 0.6 mmol), and the mixture was stirred at

100 °C for 18 h. It was diluted with ethyl acetate (50 mL) and washed with water (50 mL x 3) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (7: 1 hexane : ethyl acetate) to afford **13**[12] (55 mg, 70%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 8.04-7.30 (m, 10 H), 5.65-5.61 (m, 2 H), 5.16 (s, 1 H), 4.52 (m, 1 H), 3.65 (dd, 1 H, J = 3.5 and 13.2 Hz), 3.53 (dd, 1 H, J = 6.9 and 13.2 Hz), 3.52 (s, 3 H). ^{13}C-NMR ($CDCl_3$) 165.4, 165.2, 133.5, 133.4, 129.8, 129.7, 129.2, 128.8, 128.5, 128.4, 106.7, 80.6, 75.4, 72.8, 55.8, 53.9. FAB HRMS Calcd for $C_{20}H_{19}N_3O_6$ 420.1172 $(M + Na)^+$. Found 420.1183.

1-*O*-Acetyl-5-acetylamino-2,3-di-*O*-benzoyl-5-deoxy-α,β-D-ribofuranose (5). To a solution of **13** (309 mg, 0.78 mmol) in methanol (20 mL) was added 10% Pd-C (30 mg), and the mixture was stirred under 30 psi of H_2 for 1 h. The Pd-C was removed by filtration with celite. The filtrate was evaporated and the residue was then dissolved in pyridine (5 mL), to which acetic anhydride (110 μL, 1.17 mmol) was added at room temperature. The mixture was stirred for 6 h. The reaction was quenched with ice and the solvent was then removed under reduced pressure. The residue was partitioned between ethyl acetate (50 mL) and water (50 mL), and the organic layer was washed with water (30 mL x 3) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified with column chromatography (1: 4 hexane: ethyl acetate) to afford **14** (227 mg, 75%) as a light-yellow syrup. ^{1}H-NMR ($CDCl_3$) 8.01 (d, 2 H, J = 5.1 Hz), 7.89 (d, 2 H, J = 5.1 Hz), 7.61-7.30 (m, 6 H), 6.08 (br s, 1 H, NH), 5.61-5.54 (m, 2 H), 5.10 (s, 1 H), 4.51 (m, 1 H), 3.71 (ddd, 1 H, J = 5.0, 5.1 and 14.3 Hz), 3.61-3.50 (m, 4 H), 2.01 (s, 3 H).

To a solution of **14** (186 mg, 0.39 mmol) in acetic acid (7 mL) and acetic anhydride (1.5 mL) was added conc.H_2SO_4 (0.5 mL) at 0 °C, and the mixture was stirred at room temperature for 1 h. It was poured into sat. $NaHCO_3$ (30 mL), then extracted with chloroform (20 mL x 3). The organic layer was washed with satd. $NaHCO_3$ (30 mL), water (20 mL) and brine (20 mL) and dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (1:2 hexane : ethyl acetate) to afford **5** (189 mg, 96%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 8.05-7.30 (m, 16 H), 6.63 (d, 0.6 H, J = 4.4 Hz), 6.37 (s, 1 H), 6.06 (m, 0.6 H, NH), 5.97 (m, 1 H, NH), 5.71 (d, 1 H, J = 4.9 Hz), 5.59-5.51 (m, 1.6 H), 5.45 (dd, 0.6 H, J = 4.4 and 6.8 Hz), 4.59-4.52 (m, 1.6 H), 3.85 (m, 1 H), 3.76-3.58 (m, 1.2 H), 3.47 (m, 1 H), 2.17 (s, 3 H), 2.11 (s, 1.8H), 2.05 (s, 1.8 H), 2.00 (s, 3 H). FAB HRMS Calcd for $C_{23}H_{23}NO_8$ 464.1321 $(M + Na)^+$. Found 464.1321.

Methyl-5-deoxy-5-trifluoroacetylamino-2,3-di-*O*-benzoyl-β-D-ribofuranoside (15). Compound **13** (179 mg, 0.45 mmol) was converted to **15** using the procedure described for **14**. Purification by column chromatography (5:1 hexane: ethyl acetate) afforded **15** (112 mg, 51 %) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 8.17-7.31 (m, 10 H), 7.18 (br s, 1 H, NH), 5.63 (d, 1 H, *J* = 4.9 Hz), 5.57 (dd, 1 H, *J* = 4.9 and 6.9 Hz), 5.12 (s, 1 H), 4.56 (dt, 1 H, *J* = 4.5 and 6.9 Hz), 3.84-3.70 (m, 2 H), 3.51 (s, 3 H). FAB HRMS Calcd for $C_{22}H_{20}F_3NO_7$ 474.1352 $(M + Li)^+$. Found 474.1362.

1-*O*-Acetyl-5-deoxy-5-trifluoroacetylamino-2,3-di-*O*-benzoyl-α,β-D-ribofuranose (6). Compound **15** (181 mg, 0.39 mmol) was converted to **6** using the procedure described for **5**. Purification by column chromatography (3:1 hexane: ethyl acetate) afforded **6** (116 mg, 60%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 8.05-7.29 (m, 10 H), 7.05 (br m, 1 H, NH), 5.73 (d, 1 H, *J* = 4.9 Hz), 5.57 (dd, 1 H, *J* = 4.9 and 7.6 Hz), 5.48 (m, 1 H), 4.58 (m, 1 H), 3.93-3.65 (m, 2 H), 2.16 (s, 3 H). FAB HRMS Calcd for $C_{23}H_{20}F_3NO_8$ 502.1301 $(M + Li)^+$. Found 502.1296.

Methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glycopyranoside (19). A solution of 1M BH_3 in THF (22 mL, 22.0 mmol) was added to **16** (2.000 g, 4.4 mmol) at 0 °C, and a solution of 1M Bu_2BOTf in ether (4.4 mL, 4.4 mmol) was then added slowly to the clear solution. The resulting mixture was stirred at 0 °C for 1 h. Triethylamine (3 mL) was then added to the mixture followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction mixture was evaporated with methanol three times and the residue was purified by column chromatography (3:1 hexane : ethyl acetate) to afford **17** (1.500 g, 72%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) 7.41-7.26 (m, 15 H), 5.01-4.78 (m, 4 H), 4.68-4.62 (m, 2 H), 4.56 (d, 1 H, *J* = 3.5 Hz), 4.01 (t, 1 H, *J* = 9.2 Hz), 3.78-3.62 (m, 3 H), 3.55-3.41 (m, 3 H), 3.36 (s, 3H). ^{13}C-NMR ($CDCl_3$) 55.2, 61.8, 70.6, 73.4, 75.0, 75.7, 77.3, 79.9, 81.9, 98.1, 127.6, 127.9, 128.0, 128.1, 128.4, 138.1, 138.7.

To a solution of **17** (1.000 g, 2.2 mmol) in pyridine (10 mL) was added MsCl (225 μL, 2.9 mmol) at 0°C, and the mixture was stirred at room temperature for 3 h. The reaction was quenched with ice and diluted with ethyl acetate (50 mL). The mixture was washed with H_2O (50 mL x 3) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (3:1 hexane : ethyl acetate) to afford **18** (1.074 g, 90%) as a light yellow solid. ^{1}H-NMR ($CDCl_3$) 7.40-4.27 (m, 15 H), 5.02-4.77 (m, 4 H), 4.67-4.58 (m, 3 H), 4.36-4.35 (m, 2 H), 4.01 (t, 1 H, *J* = 9.3 Hz), 3.84 (m, 1 H), 3.54-3.48 (m, 2 H), 3.37 (s, 3 H), 2.97 (s, 3 H). ^{13}C-NMR ($CDCl_3$) 138.5, 137.9, 137.7, 128.5, 128.4, 128.0, 127.9, 127.7, 98.2, 81.8, 79.7, 76.9, 75.7, 75.1, 73.5, 68.6, 68.4, 55.5, 37.5.

A suspension of **18** (1.073 g, 1.98 mmol), NaN_3 (387 mg, 5.94 mmol) and LiCl (252 mg, 5.94 mmol) in DMF (10 mL) was stirred at 100 °C for 18 h. The mixture was diluted with ethyl acetate (75 mL) and washed with H_2O (50 mL x 3) and brine (50 mL), dried, and evaporated. The residue was purified by column chromatography (5:1 hexane : ethyl acetate) to afford **19** (806 mg, 75%) as light yellow syrup. ^{1}H-NMR ($CDCl_3$): 7.33-7.27 (m, 15 H), 5.00 (d, 1 H, *J* = 10.9 Hz), 4.90 (d, 1 H, *J* = 11.0 Hz), 4.81 (d, 1 H, *J* = 10.9 Hz), 4.79 (d, 1 H, *J* = 12.1 Hz), 4.66 (d, 1 H, *J* = 12.1 Hz), 4.61 (d, 1 H, *J* = 3.6 Hz), 4.57 (d, 1 H, *J* = 11.0 Hz), 3.98 (d, 1 H, *J* = 9.2 Hz), 3.78 (m, 1 H), 3.54 (dd, 1 H, *J* = 3.5 and 9.6 Hz), 3.47-3.41 (m, 2 H), 3.41 (s, 3 H), 3.32 (dd, 1 H, *J* = 5.6 and 13.0 Hz). ^{13}C-NMR ($CDCl_3$) : 138.6, 138.0, 137.9, 128.5, 128.4, 128.1, 127.9, 127.7, 98.0, 81.8, 79.9, 78.3, 75.7, 75.1, 73.4, 69.9, 55.3, 51.3. FAB HRMS Calcd for $C_{28}H_{31}N_3O_5$ 496.2424 $(M + Li)^+$. Found 496.2408.

1-*O*-Acetyl-6-azido-6-deoxy-2,3,4-tri-*O*-benzyl-α,β-D-glycopyranoside (7). To a solution of **19** (50 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) was added a solution of $AcOH/Ac_2O/c.H_2SO_4$(1: 3: 0.1) at 0 °C and the mixture was stirred at 0 °C for 30 min. The mixture was poured into ice water and extracted with $CHCl_3$. The combined organic layer was washed with satd. Na_2CO_3 and brine, then dried, and evaporated. The residue was purified with column chromatography (5:1 hexane : ethyl acetate) to afford 7 (68 mg, 100%) as a colorless syrup. (α : β = 85 : 15). α–anomer: ^{1}H-NMR ($CDCl_3$) 7.38-7.27 (m, 15 H), 6.34 (d, 1 H, J = 3.5 Hz), 4.99-4.58 (m, 6 H), 3.958 (t, 1 H, *J* = 9.3 Hz), 3.88 (m, 1 H), 3.68 (dd, 1 H, *J* = 3.5 and 9.5 Hz), 3.61-3.50 (m, 2 H), 3.36 (dd, 1 H, *J* = 4.5 and 13.3 Hz), 2.15 (s, 3 H). FAB HRMS Calcd for $C_{29}H_{31}N_3O_6$ 540.2111 $(M + Na)^+$. Found 540.2120.

5-*O*-[Ethyl-2-(5-deoxy-5-acetylamino-β-D-ribofuranosidyl)]neamine tetrahydrochloride (24). To a suspension of **4** (314 mg, 0.31 mmol), **5** (148 mg, 0.34 mmol), and 4A molecular sieves (50 mg) in CH_2Cl_2 (6 mL) was added TMSOTf (136 μL, 0.68 mmol) at room temperature and the mixture was stirred for 2h. It was diluted with ethyl acetate (50 mL) and washed with satd. Na_2CO_3 (50 mL), H_2O (50 mL) and brine (50 mL). The organic layer was dried and evaporated. The residue was purified by column chromatography (1:1 $CHCl_3$: ethyl acetate) to afford crude **20** (387 mg) as white foam. To a solution of **20** (215 mg, 0.15 mmol) in methanol (5 mL) was added sodium methoxide (50 mg) at room temperature and the mixture was stirred for 3h. The solvent was removed to give crude **22**, which was used for next reaction without futher purification. To a suspension of **22** in H_2O (5 mL), 1,4-dioxane (5 mL) and acetic acid (2.5 mL) was added 10% Pd-C (300 mg) and the mixture was stirred under 30 psi of H_2 for 4h. The Pd-C was removed (Celite) and the filtrate was concentrated. A solution of the residue in 5mM NH_4OH was applied to a column of CM-Sephadex C-25 (NH_4^+ form) and, after washing with 5mM of NH_4OH (20 mL), elution was continued with 1.0 mM (20 mL), 10 mM (20 mL), 50 mM (20 mL), 100 mM (20 mL), 200 mM (20 mL) and 400 mM (20 mL) of NH_4OH to give **24** (79 mg, 78%) as a light yellow solid which was converted into the hydrochloride salt by Amberlite A-21 resin (Cl^- form). ^{1}H-NMR (D_2O) : 5.74 (d, 1 H, *J* = 3.7 Hz), 4.85 (s, 1 H), 4.07-3.07 (m, 22 H), 2.30 (m, 1 H), 1.83 (s, 3 H), 1.72 (m, 1 H). FAB-HRMS Calcd. for $C_{21}H_{41}N_5O_{11}.4HCl$ (MH^+-4HCl) 540.2881. Found 540.2884.

4,3′,4′-Tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-benzyloxycarbonyl-5-*O*-[ethyl-2-(5-deoxy-5-trifluoroacetylamino-2,3-di-*O*-benzoyl-β-D-ribofuranosidyl)]neamine (21). To a suspension of **4** (274 mg, 0.27 mmol), **6** (116 mg, 0.27 mmol), and 4A molecular sieves (50 mg) in CH_2Cl_2 (2 mL) was added TMSOTf (162 μL, 0.81 mmol) at room temperature and the reaction was stirred for 4h. The mixture was diluted with ethyl acetate (50 mL) and washed with satd. Na_2CO_3 (50 mL), H_2O (50 mL) and brine (50 mL). The organic layer was dried and evaporated. The residue was purified by column chromatography (1:1 hexane : ethyl acetate) to afford crude **21** (184 mg, 49%) as a white foam. ^{1}H-NMR ($CDCl_3$) 9.52 (br m, 1 H, NH), 7.95-7.15 (m, 30 H), 6.39 (br d, 1 H, *J* = 12.0 Hz), 6.22 (br m, 1 H), 6.10 (br m, 1 H), 5.52 (d, 1 H, *J* = 3.0 Hz), 5.42 (br m, 1 H), 5.21-4.59 (br m, 16 H), 4.21 (br m, 1 H), 4.10 (br m, 1 H), 3.97-3.28 (br m, 11 H), 2.86 (br m, 1 H), 2.03-1.72 (m, 9 H), 1.33 (br m, 1 H). FAB-HRMS Calcd. for $C_{73}H_{76}N_5O_{24}$ $(M+Na)^+$ 1486.4730. Found 1486.4780.

5-*O*-[Ethyl-2-(5-amino-5-deoxy-β-D-ribofuranosidyl)]neamine pentahydrochloride (1). To a solution of **21** (84 mg, 0.06 mmol) in methanol (2 mL) was added sodium methoxide (20 mg) at room temperature and the mixture was stirred for 3h. The solvent was removed to give crude **23**, which was used for the next reaction without futher purification. To a suspension of **23** in H_2O (2 mL), 1,4-dioxane (2 mL) and acetic acid (1 mL) was added 10% Pd-C (100 mg) and the mixture was stirred under 30 psi of H_2 for 4h. The Pd-C was removed (Celite) and the filtrate was concentrated. A solution of the residue in 5mM NH_4OH was applied to a column of CM-Sephadex C-25 (NH_4^+ form) and after washing with 5mM of NH_4OH (20 mL), elution was continued with 1.0 mM (20 mL), 10 mM (20 mL), 50 mM (20 mL), 100 mM (20 mL), 200 mM (20 mL) and 400 mM (20 mL) of NH_4OH to give **1** (26 mg, 87%) as a light yellow solid which was converted into its hydrochloride salt by Amberlite A-21 resin (Cl^- form). ^{1}H-NMR (D_2O) 5.82 (d, 1 H, J = 4.0 Hz), 4.67 (m, 1 H), 4.14 -3.14 (m, 20 H), 2.38 (m, 1 H), 1.80 (m, 1 H). FAB-HRMS Calcd. for $C_{19}H_{39}N_5O_{10}.5HCl$ 498.2775 (MH^+-5HCl). Found 498.2774.

5-*O*-[Ethyl-2-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glycopyranosidyl)]-4,3′,4′-tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-benzyloxycarbonylneamine (25). To a suspension of **4** (113 mg, 0.11 mmol), **7** (57 mg, 0.11 mmol), and 4A molecular (5 mg) in CH_2Cl_2 (2 mL) was added TMSOTf (44 μL, 0.22 mmol) at room temperature and the mixture was stirred for 4h. The mixture was diluted with ethyl acetate (20 mL) and washed with satd. Na_2CO_3 (10 mL), H_2O (10 mL) and brine (10 mL). The organic layer was dried and evaporated. The residue was purified by column chromatography (1:1 hexane : ethyl acetate) to afford crude **25** (61 mg, 37%) as a white foam. ^{1}H-NMR ($CDCl_3$)7.35-7.27 (m, 35 H), 5.60-4.51 (br m, 25 H), 4.07-3.21 (br m, 15 H), 2.83 (br m, 1 H), 2.05 (br m, 1 H), 1.95 (s, 3 H), 1.80 (s, 3 H), 1.78 (s, 3 H), 0.95 (br m, 1 H). FAB-HRMS Calcd. for $C_{79}H_{87}N_7O_{22}$ $(M+Na)^+$ 1508.5802. Found 1508.5793.

5-*O*-[Ethyl-2-(6-amino-6-deoxy-α-D-glycopyranosidyl)]neamine pentahydrochloride (2). Compound **22** (159 mg, 0.11 mmol) was converted to **2** (45 mg, 78%) as described for **1**. Compound **2** was converted into its hydrochloride salt by Amberlite A-21 resin (Cl^- form). ^{1}H-NMR (D_2O) 5.88 (d, 1 H, $J = 3.8$ Hz), 4.83 (d, 1 H, $J = 2.7$ Hz), 4.10 (dd, m, 1 H), 4.03-3.15 (m, 19 H), 2.99 (m, 1 H), 2.34 (m, 1 H), 1.79 (m, 1 H). FAB-HRMS Calcd. for $C_{20}H_{41}N_5O_{11}.5HCl$ 528.2881(MH^+-5HCl). Found 528.2886.

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