Disaccharide Mimetics of the Aminoglycoside Antibiotic Neamine

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A highly convergent approach has been employed for the facile synthesis of a library of 24 disaccharides that are α(1–3), β(1–3), α(1–4), or β(1–4) linked and contain 2–4 amino groups. Fourier-transformation ion cyclotron resonance mass spectrometry (FT-ICR MS) has been used to determine dissociation constant (K_d) values for the binding of the disaccharides to a prototypical fragment of 16S ribosomal RNA. Several derivatives bound with affinities similar to that of neamine. Structure–activity relationships have revealed the substitution pattern that is important for high-affinity binding. The compounds described here are unique lead compounds for the design of novel aminoglycoside antibiotics.

Introduction

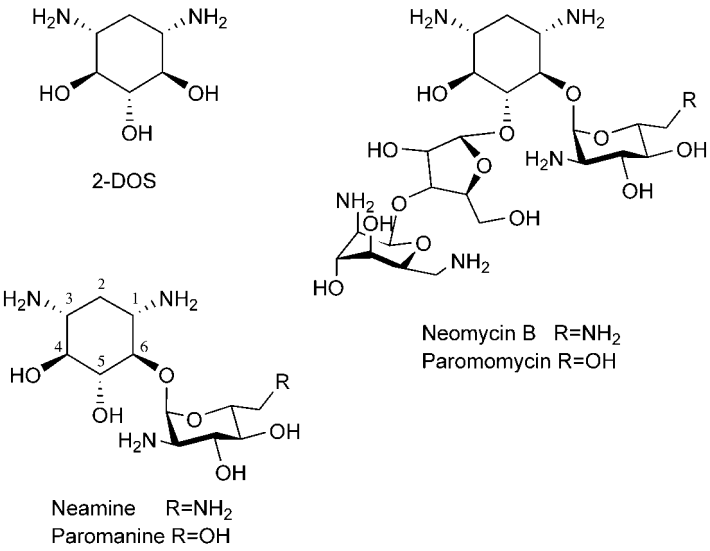
A unique property of RNA is that it is single stranded. It contains, however, short stretches of nucleotides capable of forming conventional base pairs with complementary sequences found elsewhere in the molecule. These interactions, along with additional "nonconventional" base pairs allow RNA to fold into a variety of three-dimensional structures determined by its sequence of nucleotides. Due to this well-defined shape, small molecules can target specific RNA domains and this may result in a modulation of biological activities.[1–6]

The paradigm of small-molecule–RNA interactions is provided by natural antibiotics, such as aminoglycosides, tetracyclines, and macrolides.[7] These compounds can bind to ribosomal RNA (rRNA), thereby resulting in interference or blockage of protein biosynthesis. The aminoglycoside antibiotics, for example, bind selectively in the major groove of the A-site of the 16S subunit of rRNA.[8] This binding interferes with two conformationally flexible adenine residues involved in the selection of cognate aminoacyl transfer RNA during translation. The resulting conformational changes increase the misincorporation of near-cognate amino acids or result in the termination of protein biosynthesis.

Analysis of the chemical structures of naturally occurring aminoglycosides reveals that they contain a 2-deoxystreptamine (2-DOS) or streptamine moiety (Scheme 1). This moiety is glycosylated with aminosugars at C-5 and C-6 to give the neomycin class or at C-4 and C-6 to give the kanamycin–gentamicin class. These compounds share a pseudoglycoside core, in which 2-DOS is glycosylated at the C-6 position with a 2-amino- or 2,6-diamino-substituted α-glucopyranosyl moiety, such as in paromamine and neamine. NMR spectroscopy[9–13] and X-ray crystallography[14–17] studies have indicated that this subunit is the minimal motif for selective binding to the A-site of 16S rRNA.

Although aminoglycosides are widely used in the treatment of various infections caused by gram-positive and -negative bacteria and mycobacteria, their use is associated with several drawbacks. Aminoglycosides are cytotoxic and may impair hearing and kidney function at high doses. Furthermore, strains of organisms resistant to aminoglycosides are emerging at an increasing rate; these resistant strains have enzymes capable of modifying these compounds by acetylation or phosphorylation, thus rendering them ineffective.[18,19]

Not surprisingly, these developments have triggered a search for novel compounds capable of binding to specific RNA structures, with the long-term goal of these studies being the development of antibiotics displaying minimal toxicity and



Scheme 1. *Chemical structures of aminoglycoside antibiotics. 2-DOS = 2-deoxystreptamine.*

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Supporting Information for this article is available on the WWW under http://www.chembiochem.org or from the author.

  DOI: 10.1002/cbic.200400105

inhibition of resistance development. The rational design of novel ligands for RNA remains in its infancy, compared to the well-tried routes to protein-based drug design. Prior approaches have been based on the chemical modification of natural aminoglycosides or part structures, such as neamine.[3,20–40] Due to the structural complexity of aminoglycosides, selective protection followed by chemical modification is very complex.

Here, we report a highly convergent synthesis of a wide range of disaccharides that contain several amino groups. It is shown that a number of these derivatives can bind to a prototypical segment of 16S rRNA with affinities similar to that of neamine. Structure–activity relationships have revealed the structural elements that are important for high-affinity binding.

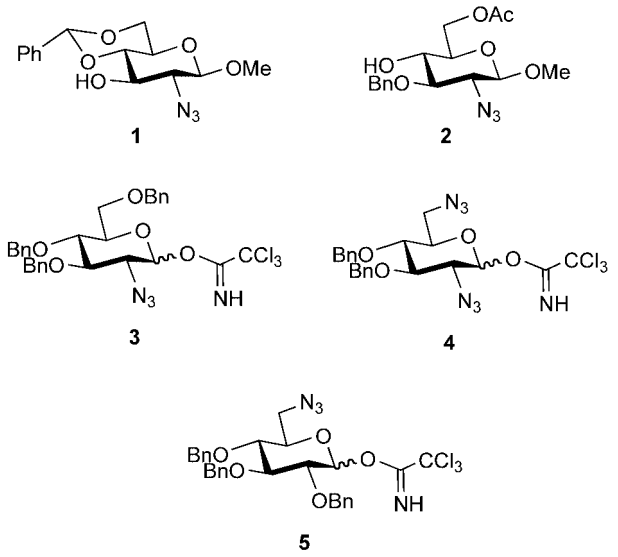
Results and Discussion

Synthesis of disaccharides

Neamine is the smallest structural motif of the neomycin-type aminoglycosides for selective binding to the A-site of the 16S rRNA. This pseudodisaccharide has been obtained by mild acid cleavage of the riboside glycosidic linkage of neomycin B.[24] Ingenious protecting group manipulations followed by chemical modifications have produced a limited number of derivatives, a few of which had noteworthy properties.

The possibility of discovering a synthetic disaccharide that would exhibit an affinity for rRNA similar to that of neamine was quite compelling to us. The rationale for this approach is that improved methods for oligosaccharide synthesis would allow a convenient preparation of a wide range of saccharides with a varying number of amino groups at different positions. If this were achieved, it would create a unique opportunity to perform detailed structure–activity relations. Furthermore, it was expected that these studies would provide new lead compounds that were readily available and more amenable for chemical modification than neamine.

A highly convergent building-block approach was employed for the convenient preparation of a library of disaccharides. Compounds **1** and **2**, which have free hydroxy groups at C-3 and C-4, respectively, were used as glycosyl acceptors (Scheme 2). As glycosyl donors, the trichloroacetimidates **3**–**5**, which have azido moieties at C-2, C-2 and C-6, and C-6, respectively, were employed. Coupling of each donor with each acceptor would give six disaccharides, with each as a mixture of anomers. Selective removal of the C-6′ protecting groups of the resulting disaccharides followed by conversion of the hydroxy groups into azido moieties would give an additional 12 disaccharides. Deprotection of the disaccharides with concomitant reduction of the azido groups to amines would give 24 disaccharides that are α(1–3), β(1–3), α(1–4), or β(1–4) linked and have 2–4 amino groups. It was expected that these disaccharides, which have different combinations of amines at C-2 and C-6, might be compounds that bind with high affinity to rRNA. As will be shown below, amino groups of disaccharides linked in α(1–3) or α(1–4) fashion overlay well with the amino groups of neamine and paromamine. NMR spectroscopy[9–13] and X-ray crystallography[14–17] studies have shown that the pseudodisaccharide moiety of aminoglycoside antibiotics is the minimal motif for selective binding to rRNA. It has also been shown that the 2,6-diamine-2,6-dideoxyglucopyranoside moiety of neamine makes many of the key interactions with RNA. Studies by Wong and co-workers have demonstrated that substitution patterns other than 2,6-diamines lead to loss of activity.[30] They argue that glyco-type 1,3-hydroxyamines can interact through multiple hydrogen bonds with phosphodiesters and the Hoogsteen face of guanidine.[35,38]

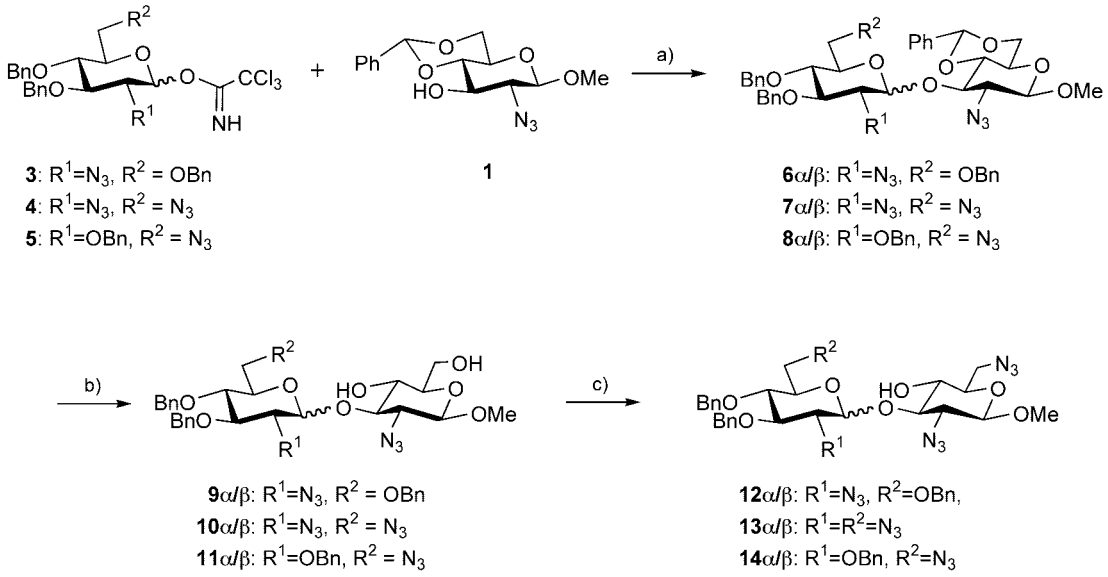


Scheme 2. *Glycosyl acceptors **1** and **2** and glycosyl donors **3**–**5** for preparation of the library of disaccharides.*

Glycosyl acceptors **1** and **2** and donors **3**–**5** were employed in the assembly of the library of disaccharides. First, each of the glycosyl donors was coupled with acceptor **1** to give (1–3)-linked disaccharides **6**–**8** (Scheme 3). When $BF_3{\cdot}OEt_2$ was employed as an activator in dichloromethane at −30 °C, the reactive glycosyl donors **3** and **5** gave mainly the disaccharides **6** and **8** as β anomers.[41] The 2,6-diazido donor **4** was not reactive under these conditions. Activation of the trichloroacetimidates **3**–**5** with the more powerful promoter TMSOTf gave, however, in each case, mixtures of anomers.[41] The anomers of disaccharides **6** and **7** could easily be separated by silica gel column chromatography, whereas acetylation, separation, and deacetylation were required to obtain the individual anomers of derivative **8**. The anomeric configuration for each compound was confirmed from $J_{1,2}$ coupling constant data.

Compounds **12**–**14**, which have an additional azido function at C-6′, could easily be prepared from **6**–**8**, respectively. Thus, removal of the benzylidene acetal moieties by using standard conditions gave **9**–**11**. Regioselective mesylation of these derivatives with mesyl chloride in pyridine followed by displacement of the mesyl groups with NaN_3 in DMF at 85 °C gave compounds **12**–**14**.

In a similar manner, individual anomers of the (1–4)-linked disaccharides **15**–**17** were obtained by coupling glycosyl acceptor **2** with glycosyl donors **3**–**5**, respectively, with TMSOTf



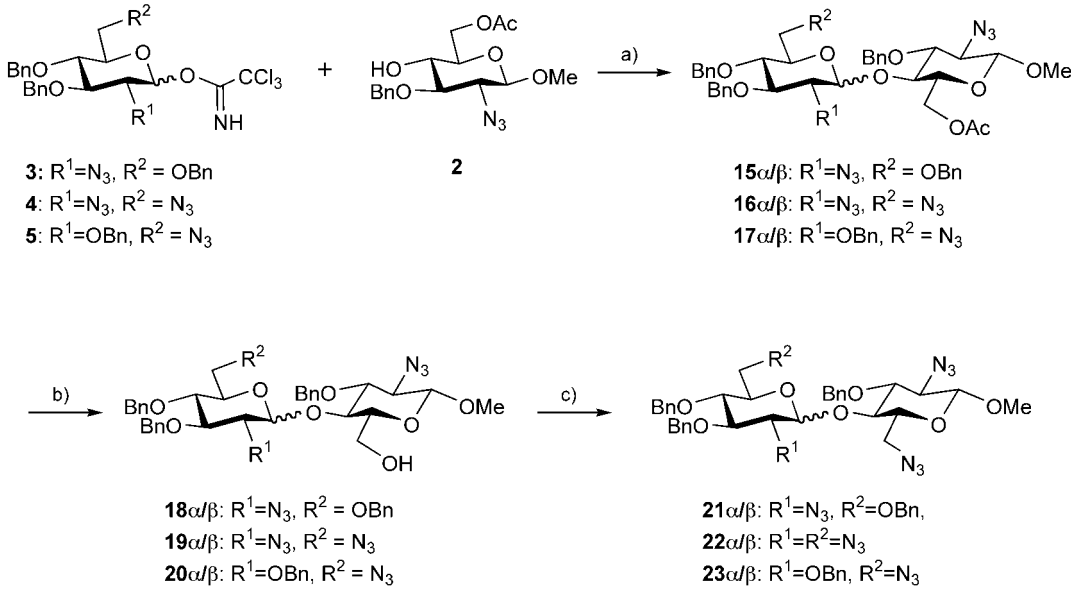
Scheme 3. *Reagents and conditions. a) TMSOTf, −55°C→−10°C; or BF_3·Et_2O, −35°C→−10°C); b) TFA, H_2O, CH_2Cl_2, RT; c) MsCl, Pyr, then NaN_3, DMF, 95°C. TMSOTf = trimethylsilyl trifluoromethanesulfonate, TFA = trifluoroacetic acid, Ms = mesyl = methanesulfonate, Pyr = pyridine, DMF = N,N-dimethylformamide.*

as the promoter (Scheme 4). These compounds could be converted into **21**–**23** by a three-step procedure involving removal of the acetyl ester followed by mesylation of the resulting hydroxy group and displacement of the mesylates with NaN_3 in DMF.

Finally, catalytic hydrogenation of the individual anomers of **9**–**14** and **18**–**23** over Pd/C, which was deactivated with pyridine, resulted in selective reduction of the azido groups to amines. The resulting amines were immediately subjected to a second catalytic hydrogenation, but in this case $Pd(OH)_2$[30] was used in a mixture of acetic acid and water to remove the benzyl ethers and give the individual anomers of target compounds **24**–**35** (see Table 1). Impure compounds were obtained when the reduction of the azido and benzyl ethers was performed as a one-step procedure.

Binding of disaccharides to rRNA

High-resolution mass spectrometry (HRMS) has considerable potential for monitoring complex formation between small molecules and RNA fragments.[42,43] The mild electrospray-ionization (ESI) process effectively transfers both free and complexed RNAs into the gas phase. The result is a representation of the relative distribution of bound and free RNA present in solution. This conveniently allows a determination of dissocia-

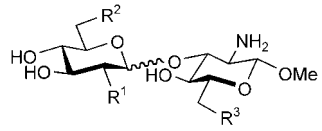
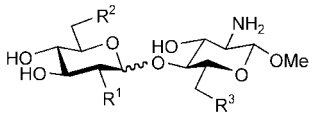


Scheme 4. *Reagents and conditions: a) TMSOTf, −55→−10°C; or BF_3·Et_2O, −35→−10°C; b) NaOMe, MeOH; c) MsCl, Pyr, then NaN_3, DMF, 95°C.*

tion constants by measuring ion abundances for the free and complexed RNAs as a function of ligand concentration. Fourier-transformation ion cyclotron resonance mass spectrometry (FT-ICR MS) coupled with this approach has successfully been used to determine dissociation constant (K_d) values for the binding of several aminoglycoside antibiotics to rRNA fragments.[44]

ESI–FT-ICR mass spectra were acquired under noncompetitive binding conditions from 0.5 μM mixtures of untagged 16S and mass-tagged 18S with 0.75, 2.5, 7.5, and 25 μM concentrations of disaccharides **24**–**35α/β**. The relative ion abundances were used to determine the dissociation constants. The K_d value for the binding of 16S rRNA to neamine was determined to be approximately 7 μM by using this method and provided a comparator for the compounds in this study. The dissociation constants of the synthetic compounds are listed in Table 1.

Table 1. *Binding studies with the 16S rRNA fragment. K_d values less than 50 μM are highlighted in bold.*

R^1	R^2	R^3	 (1–3)-linked	K_d [μM]	 (1–4)-linked	K_d [μM]
NH_2	OH	OH	**24α**	69	**30α**	166
NH_2	OH	OH	**24β**	121	**30β**	71
OH	NH_2	OH	**25α**	107	**31α**	n.d
OH	NH_2	OH	**25β**	97	**31β**	n.d
NH_2	NH_2	OH	**26α**	68	**32α**	**40**
NH_2	NH_2	OH	**26β**	119	**32β**	81
OH	NH_2	NH_2	**27α**	211	**33α**	187
OH	NH_2	NH_2	**27β**	108	**33β**	136
NH_2	OH	NH_2	**28α**	**49**	**34α**	**26**
NH_2	OH	NH_2	**28β**	81	**34β**	87
NH_2	NH_2	NH_2	**29α**	**37**	**35α**	**11**
NH_2	NH_2	NH_2	**29β**	85	**35β**	**39**
			neamine	7		

Similar values were obtained for the 16S and 18S rRNA fragment, a result indicating no selectivity for the bacterial rRNA over the eukaryotic rRNA fragment.

Most of the amino disaccharides bound to the 16S rRNA fragments with high micromolar affinities (Table 1). However, five compounds (**28α**, **29α**, **32α**, **34α**, **35α**, and **35β**) bound with K_d values of <50 μM. The best compound tested was the α(1–4)-linked derivative **35α**, which has four amino groups at C-2, C-2′, C-6, and C-6′. Importantly, this derivative has an affinity for the 16S rRNA similar to that of neamine, a result indicating that the amino disaccharides have the potential to mimic the interaction of neamine with RNA. A comparison of the data for the different compounds reveals that the α-linked compounds bind with higher affinities than analogous β-linked compounds. Furthermore, α(1–3)-linked compounds generate weaker complexes than similarly substituted α(1–4)-glycosides. Only compounds with three or four amino groups showed affinities better than 50 μM.

In an attempt to rationalize the binding date, structural features of the disaccharides were compared with those of neamine moieties of natural aminoglycoside antibiotics that complex to RNA fragments. Detailed three-dimensional information of aminoglycoside recognition by ribosomal RNA has come from recent NMR spectroscopy[9–13] and X-ray crystallography[14–17] studies. In each study, the common neamine moiety bound in such a way that adenine residues 1492 and 1493 were extruded from the helix and bulged out. This particular conformation of the aminoglycoside–A-site complex is stabilized by a precise set of specific interactions. In particular, the 2,6-diaminoglucoside moiety of neamine forms a pseudo base pair with two direct hydrogen bonds to the Watson–Crick sites of A1408 and its puckered ring makes C–H π interactions to G1491. In addition, the amino and hydroxy groups of the pseudosaccharide core make a large number of direct or water-bridged hydrogen bonds to phosphate oxygen atoms, N1 and N7 of several adenines, and N7 of several guanines. Additional saccharide moieties attached to neamine make additional hydrogen-bonding contacts that confer specific interactions.[45] There is evidence to support the idea that, in the bound state, the conformation of the neamine moiety is similar to the global minimal energy conformation in solution.[46]

Models of disaccharides **29α** and **35α** were built in which the Ψ and Φ torsional angles between the two sugar rings were identical to those proposed for the similar glycosidic linkage of neamine.[15] Next, the nonreducing 2,6-diamino-2,6-dideoxyglycosamine moieties of **29α** and **35α** were superimposed on the neamine moiety of paramomycin (Figure 1). In these overlays, the important amine at C-1 of the 2-deoxystreptamine moiety was mimicked by the amines on C-2 of the α(1–3)-linked disaccharide **29α** (Figure 1A) and on C-6 of the α(1–4)-linked disaccharide **35α** (Figure 1B). The amine on C-3 of the 2-deoxystreptamine moiety was not mimicked by these compounds, a fact that provides a possible explanation for the slight reduction in binding affinities. It is likely that the amines on C-6 of **29α** and C-2 of **35α** make indirect water-mediated interactions with RNA, as the removal of these functionalities leads to loss of affinity. In the case of the β anomers, no overlays with good root mean square deviation could be made with the 2-deoxystreptamine moiety. In this case, the reducing moiety of the disaccharides may make indirect interactions that contribute to the affinities.

Conclusion

Aminoglycoside antibiotics are a class of compounds that can bind to specific sites in prokaryotic ribosomal RNA and thereby disrupt protein biosynthesis. The emergence of bacterial resist-

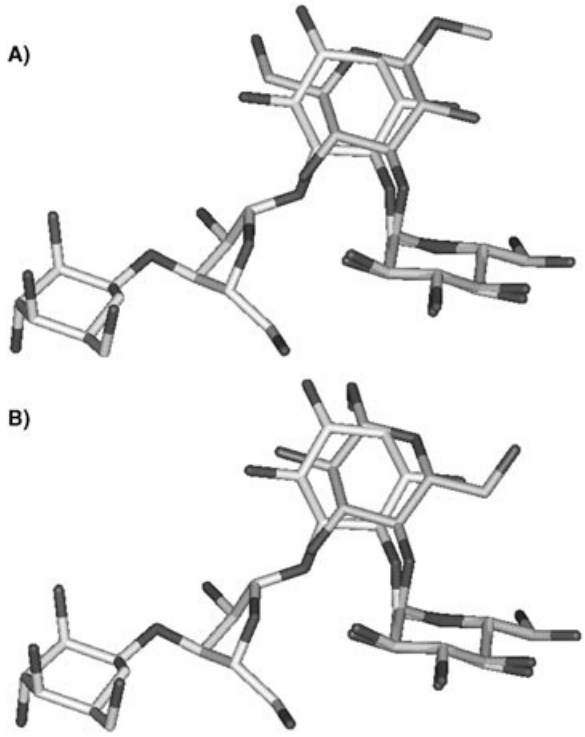


*Figure 1. Superimposition of compound A) **29α** and B) **35α** with paromomycin (grey). The conformation of paromomycin is the same as that observed in the crystal structure of paromomycin complexed with the ribosomal decoding site (PDB code 1J7T).[15] The ϕ and ψ dihedral angles in **29α** and **35α** were adjusted to the same values as those observed in the bound conformation of paromomycin. Atoms C-1′, C-2′, C-3′, C-4′, C-5′ and the ring oxygen atom in compounds **29α** and **35α** were superimposed with the corresponding atoms of the glucosamine ring of paromomycin. The superimposition was performed by using the InsightII 98 modeling package.*

ance in conjunction with cytotoxicity has triggered a search for analogues that retain antibiotic activity but have reduced toxicity and inhibit the development of resistance. Neamine is the smallest structural motif of the neomycin-type aminoglycosides for selective binding to the A-site of 16S rRNA. This unit is, however, most susceptible to modifications by enzymes of targeted pathogens. Here, we have outlined a novel strategy whereby amino disaccharides mimic the unique spatial arrangements of the functional groups of neamine that are required for the recognition of the RNA target. In a highly convergent fashion, 3 glycosyl donors and 2 glycosyl acceptors could be converted into 24 disaccharides that are α(1–3), β(1–3), α(1–4), or β(1–4) linked and have 2–4 amino groups. Fourier-transformation ion cyclotron resonance mass spectrometry (FT-ICR MS) was employed to determine K_d values for the binding of the disaccharides to rRNA fragments. Although most amino disaccharides bound to the 16S rRNA with high micromolar affinities, several compounds displayed more favorable dissociation constants. The best compound tested was the α(1–4)-linked derivative **35α**, with four amino groups at C-2, C-2′, C-6, and C-6′, which displayed an affinity similar to that of neamine. Superimposition of compound **35α** on the neamine moiety of paramomycin indicated that that three of the four amino groups have similar spatial orientations.

Several of the disaccharides described here are attractive lead compounds for further development as novel antibiotics. Neamine has only weak antibiotic activity at high concentrations. In this respect, it has been indicated that it can bind to 16S RNA in to different fashions. Attachment of other sugar residues is required for increasing the affinity and for proper positioning in the A-site of rRNA. Therefore, it is to be expected that only trisaccharides and larger structures will provide compounds with antibiotic activity. The preparation of these derivatives is under way and the results will be reported in due course.

Experimental Section

General procedures, syntheses, and characterization of all starting materials and intermediates are reported in the Supporting Information.

General procedure for the deprotection of disaccharides to form 24–30α/β and 32–35α/β: Pd/C (10%, 1–1.5 times the weight of the starting material) was added to a solution of the protected azido disaccharides (5–75 mg) in pyridine (2–5 mL) under Ar. After evacuation, the flask was placed under an atmosphere of H_2. The reaction was stirred overnight until TLC analysis (hexane/EtOAc (1:1), $CHCl_3/CH_3OH$ (90:10), and *i*PrOH/28% NH_4OH (95:5)) indicated the completion of the reaction. The mixture was filtered through a polytetrafluoroethene (PTFE) syringe filter (diameter 25 mm, pore size 0.2 μm), which was further washed with pyridine. The solvents were coevaporated with toluene. The residue was dried in vacuo for several hours. Matrix-assisted time-of-flight (MALDI) MS and NMR spectroscopy confirmed the reduction of the azido groups. $Pd(OH)_2$ (Degussa type, Aldrich,[30] 1–2 times the weight of the starting material) was added to the above-obtained material dissolved in a mixture of AcOH and H_2O (10:1, 2–5 mL) under Ar. The mixture was placed under an atmosphere of H_2 and stirred overnight. TLC (*i*PrOH/28% NH_4OH (95:5) and *i*PrOH/H_2O/28% NH_4OH (30:10:5 or 30:20:10)) indicated the presence of a single compound. The mixture was filtered through a PTFE syringe filter (as above) and further washed with AcOH. The solvents were coevaporated with toluene. The residue was dried in vacuo for several hours. A 1 M solution of HCl in Et_2O (1.1 equiv per NH_2 group) was added with a microsyringe to a solution of the recovered dried acetates of the amino disaccharides dissolved in a small amount of CH_3OH until a pH value of 2 was reached. The solution was then evaporated with an excess of CH_3OH and toluene and the products were dried in vacuo. The recovered materials were passed through a small amount of C-18 silica gel (0.35–0.5 g) and slowly eluted with water. The fractions containing the products were collected, filtered through a PTFE syringe filter (diameter 5 mm, pore size 0.2 μm), and freeze dried.

Methyl *O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (24α): Compound **9α** (25 mg, 0.037 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (3.5 mL, 10:1) in the presence of $Pd(OH)_2$ (30 mg). The product was transformed into its HCl salt to give **24α** (14.0 mg, 89%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.66$ (d, $J =$ 3.6 Hz, 1H; H-1′), 4.67 (d, $J = 8.7$ Hz, 1H; H-1), 4.02 (dd, $J = 9.4$ and 9.8 Hz, 1H; H-3), 3.95–3.90 (m, 3H; H-6a, H-3′, H-6′a), 3.83 (m, 1H; H-5′), 3.80–3.72 (m, 3H; H-4, H-6b, H-6′b), 3.59 (s, 3H; OCH_3), 3.57 (m, 1H; H-5), 3.49 (dd, 1H; $J = 9.5$ and 10.1 Hz, H-4′), 3.46 (dd, $J =$ 10.7 Hz, 1H; H-2′), 3.25 ppm (dd, $J = 10.1$ Hz, 1H; H-2); ^{13}C NMR

(D_2O, 75 MHz): $\delta = 99.5$ (C-1), 96.8 (C-1′), 80.0 (C-3), 75.7, 73.7, 69.6, 69.5, 69.1, 60.5 and 60.0 (C-6 and C-6′), 57.6 (OCH_3), 54.7 and 54.0 ppm (C-2 and C-2′).

Methyl *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (24β): Compound **9β** (40 mg, 0.059 mmol) was hydrogenated in pyridine (4 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (4.4 mL, 10:1) in the presence of $Pd(OH)_2$ (35 mg). The product was transformed into its HCl salt to give **24β** (24 mg, 95%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.09$ (d, $J =$ 8.6 Hz, 1H; H-1′), 4.65 (d, $J = 8.5$ Hz, 1H; H-1), 4.14 (t, $J = 9.8$ Hz, 1H; H-3), 3.97–3.90 (m, 2H; H-6a, H-6′a), 3.83–3.76 (m, 3H; H-4, H-6a, H-6′b), 3.72 (dd, $J = 8.9$ and 10.4 Hz, 1H; H-3′), 3.51–3.61 (m, 6H; including OCH_3), 3.28–3.22 ppm (m, 2H; H-2, H-2′); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.9$ (C-1), 96.2 (C-1′), 76.9 (C-3), 76.0, 72.0, 69.3, 67.2, 60.3 and 60.2 (C-6 and C-6′), 57.6 (OCH_3), 55.0 and 54.9 ppm (C-2 and C-2′).

Methyl *O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (25α): Compound **11α** (23 mg, 0.034 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (3.5 mL, 10:1) in the presence of $Pd(OH)_2$ (30 mg). The product was transformed into its HCl salt to give **25α** (14.0 mg, 96%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.48$ (d, $J =$ 4.9 Hz, 1H; H-1′), 4.70 (d, $J = 8.2$ Hz, 1H; H-1), 4.06–3.97 (m, 1H; H-3, H-5′), 3.94 (dd, $J = 3.0$ and 13.5 Hz, 1H; H-6a), 3.83 (t, $J = 9.8$ Hz, 1H; H-4), 3.81–3.75 (m, 2H; H-6b, H-3′), 3.65 (dd, $J = 9.1$ Hz, 1H; H-2′), 3.59 (s, 3H; OCH_3), 3.53 (m, 1H; H-5), 3.43 (dd, $J = 3.3$ and 13.3 Hz, 1H; H-6′a), 3.37 (t, $J = 9.4$ Hz, 1H; H-4′), 3.29 (dd, $J =$ 11.0 Hz, 1H; H-2), 3.19 ppm (dd, $J = 8.0$ Hz, 1H; H-6b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.6$ (C-1), 95.6 (C-1′), 77.6, 76.4, 72.2, 70.9, 70.8, 68.7, 68.1, 60.2, 57.7 (OCH_3), 53.5 (C-2), 40.4 ppm (C-6′).

Methyl *O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (25β): Compound **11β** (20 mg, 0.029 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (4.4 mL, 10:1) in the presence of $Pd(OH)_2$ (35 mg). The product was transformed into its HCl salt to give **25β** (14 mg, 97%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 4.70$ (m, 2H; H-1, H-1′), 3.98–3.92 (m, 2H; including H-3 (dd, $J = 8.9$ and 10.4 Hz), H-6a), 3.78 (dd, $J = 5.5$ and 12.5 Hz, 1H; H-6b), 3.72–3.63 (m, 2H; H-4, H-5′), 3.59 (s, 3H; OCH_3), 3.57–3.53 (m, 2H; including H-5, H-3′ (t, $J = 9.2$ Hz)), 3.47 (dd, $J = 2.8$ and 13.5 Hz, 1H; H-6′a), 3.42 (t, $J =$ 8.2 Hz, 1H; H-2′), 3.36 (t, 1H; H-4′), 3.28 (dd, $J = 8.5$ Hz, 1H; H-2), 3.17 ppm (dd, $J = 8.5$ Hz, 1H; H-6′b); ^{13}C NMR (D_2O, 75 MHz): $\delta =$ 103.6 and 99.6 (C-1 and C-1′), 82.2 (C-3), 76.0, 75.4, 73.7, 72.4, 71.0, 68.7, 60.4 (C-6), 57.7 (OCH_3), 55.4 (C-2), 40.6 ppm (C-6′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (26α): Compound **10α** (23 mg, 0.037 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (20 mg). The intermediate was further hydrogenated in AcOH and H_2O (2.6 mL, 10:1) in the presence of $Pd(OH)_2$ (18 mg). The product was transformed into its HCl salt to give **26α** (10.7 mg, 64%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.94$ (d, $J = 3.6$ Hz, 1H; H-1′), 4.64 (d, $J = 8.5$ Hz, 1H; H-1), 4.15 (dd, $J = 9.5$ and 9.7 Hz, 1H; H-3), 4.00–3.94 (m, 2H; H-3′, H-5′), 3.90 (dd, $J = 2.4$ and 12.5 Hz, 1H; H-6a), 3.81–3.74 (m, 2H; H-4, H-6b), 3.57 (s, 3H; OCH_3), 3.53 (m, 1H; H-5), 3.49–3.40 (m, 3H; H-2′, H-4′, H-6′a), 3.27 (dd, $J = 7.0$ and 13.6 Hz, 1H; H-6′b), 3.15 ppm (dd, $J = 9.5$ Hz, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.7$ (C-1), 95.3 (C-1′), 77.0 (C-3), 75.7, 70.7, 70.2, 69.1, 68.4, 59.9 (C-6), 57.6 (OCH_3), 54.4 and 53.5 (C-2 and C-2′), 40.1 ppm (C-6′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl)-(1–3)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (26β): Compound **10β** (12 mg, 0.019 mmol) was hydrogenated in pyridine (1.5 mL) in the presence of Pd/C (13 mg). The intermediate was further hydrogenated in AcOH and H_2O (2.2 mL, 10:1) in the presence of $Pd(OH)_2$ (17 mg). The product was transformed into its HCl salt to give **26β** (7 mg, 76%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.12$ (d, $J =$ 8.5 Hz, 1H; H-1′), 4.68 (d, $J = 8.6$ Hz, 1H; H-1), 4.18 (t, $J = 9.2$ Hz, 1H; H-3), 3.95 (dd, $J = 1.8$ and 12.2 Hz, 1H; H-6a), 3.82–3.72 (m, 3H; H-4, H-6b, H-3′, H-5′), 3.61–3.54 (m, 4H; H-5′, OCH_3), 3.53–3.45 (m, 2H; H-6′a, H-4′), 3.32–3.25 ppm (m, 3H; H-2, H-2′, H-6′b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.6$ (C-1), 96.4 (C-1′), 77.4 (C-3), 75,9, 72.8, 71.5, 70.9, 67.2, 60.1 (C-6), 57.6 (OCH_3), 55.1 and 54.9 (C-2 and C-2′), 40.1 ppm (C-6′).

Methyl *O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (27α): Compound **14α** (33 mg, 0.047 mmol) was hydrogenated in pyridine (4 mL) in the presence of Pd/C (25 mg). The recovered intermediate was further hydrogenated in AcOH and H_2O (4.4 mL, 10:1) in the presence of $Pd(OH)_2$ (39 mg). The product was transformed into its HCl salt to give **27α** (20.5 mg, 91%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.57$ (d, $J = 4.09$ Hz, 1H; H-1′), 4.76 (H-1), 4.06 (m, 1H; H-3), 3.98 (m, 1H; H-5′), 3.78–3.72 (m, 3H; H-4, H-5, H-3′), 3.63 (dd, $J = 9.8$ Hz, 1H; H-2′), 3.59 (s, 3H; OCH_3), 3.51 (dd, $J = 1.5$ and 13.0 Hz, 1H; C*H*NH_2), 3.43 (dd, $J = 3.4$ and 13.4, 1H; C*H*NH_2), 3.36 (t, $J = 8.8$ Hz, 1H; H-4), 3.33 (dd, $J = 10.6$ Hz, 1H; H-2), 3.22–3.10 ppm (m, 2H; 2×C*H*NH_2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.5$ (C-1), 95.9 (C-1′), 76.9 (C-3), 72.0 (×2), 70.7, 70.6, 70.0, 68.6, 57.7 (OCH_3), 53.4 (C-2), 40.2 and 40.2 ppm (C-6 and C-6′).

Methyl *O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (27β): Compound **14β** (21.4 mg, 0.30 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (4 mL, 10:1) in the presence of $Pd(OH)_2$ (34 mg). The product was transformed into its HCl salt to give **27β** (13.3 mg, 94%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 4.74$ (d, $J = 8.9$ Hz, H-1), 4.70 (d, $J = 8.0$ Hz, 1H; H-1′), 4.01 (dd, $J = 8.8$ and 10.5 Hz, 1H; H-3), 3.80 (m, 1H; H-5), 3.70 (m, 1H; H-5′), 3.64–3.59 (m, 4H; H-4, OCH_3), 3.57–3.52 (m, 2H; H-3′, H-6′a), 3.47 (dd, $J = 3.0$ and 13.7 Hz, 1H; H-6a), 3.43 (dd, $J = 9.4$ Hz, 1H; H-2′), 3.37 (dd, $J =$ 9.1 and 9.5 Hz, 1H; H-4′), 3.31 (dd, 1H; H-2), 3.24–3.20 ppm (m 2H; H-6b, H-6′b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 103.7$ and 99.6 (C-1 and C-1′), 81.7(C-3), 75.4, 73.7, 72.4, 71.9, 70.9, 70.5, 57.7 (OCH_3), 55.2 (C-2), 40.5 and 40.4 ppm (C-6 and C-6′).

Methyl *O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (28α): Compound **12α** (12.6 mg, 0.018 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (20 mg). The recovered intermediate was further hydrogenated in AcOH and H_2O (2.0 mL, 10:1) in the presence of $Pd(OH)_2$ (25 mg). The product was transformed into its HCl salt to give **28α** (7.6 mg, 91%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.64$ (d, $J = 3.8$ Hz,1H; H-1′), 4.70 (d, $J = 8.5$ Hz, 1H; H-1), 4.03 (dd, $J = 8.9$ and 10.0 Hz, 1H; H-3), 3.93 (dd, $J = 2.1$ and 12.2 Hz, 1H; H-6′a), 3.90 (dd, $J = 9.5$ and 10.7 Hz, 1H; H-3′), 3.83–3.75 (m, 2H; H-5, H-5′), 3.73 (dd, $J = 7.0$ Hz, 1H; H-6′b), 3.69 (t, 1H; H-4), 3.59 (s, 3H; OCH_3), 3.53 (dd, $J = 3.0$ and 13.5 Hz, 1H; H-6a), 3.47 (t, 1H; H-4′), 3.44 (dd, 1H; H-2′), 3.33 (dd, 1H; H-2), 3.17 ppm (dd, $J = 9.2$ Hz, 1H; H-6b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.5$ (C-1), 96.8 (C-1′), 79.3

(C-3), 73.8, 71.8, 71.2, 69.5, 69.0, 60.5 (C-6′), 57.7 (OCH_3), 54.5 and 54.0 (C-2 and C-2′), 40.2 ppm (C-6).

Methyl *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (28β): Compound **12β** (71 mg, 0.101 mmol) was hydrogenated in pyridine (5 mL) in the presence of Pd/C (30 mg). The recovered intermediate was further hydrogenated in CH_3CO_2H and H_2O (4.4 mL, 10:1) in the presence of $Pd(OH)_2$ (40 mg). The product was transformed into its HCl salt to give **28β** (25.8 mg, 56%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.08$ (d, $J = 8.8$ Hz, 1H; H-1′), 4.71 (d, $J = 8.2$ Hz, 1H; H-1), 4.17 (dd, $J = 9.0$ and 10.2 Hz, 1H; H-3), 3.93 (dd, $J = 1.8$ and 12.2 Hz, 1H; H-6′a), 3.81–3.76 (m, 2H; H-5, H-6′b), 3.75–3.70 (m, 2H; H-3′, H-4), 3.61–3.56 (m, 4H; H-5′, OCH_3), 3.55–3.51 (m, 2H; H-4′, H-6a), 3.33 (dd, 1H; H-2), 3.25 (dd, $J = 10.4$ Hz, 1H; H-2′), 3.21 ppm (dd, $J = 8.8$ and 13.5 Hz, 1H; H-6b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.5$ (C-1), 96.1(C-1′), 76.8, 76.3 (C-3), 71.9, 71.8, 69.2, 68.9, 60.1 (C-6′), 57.7 (OCH_3), 54.9 and 54.5 (C-2 and C-2′), 40.2 ppm (C-6).

Methyl *O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (29α): Compound **13α** (20 mg, 0.029 mmol) was hydrogenated in pyridine (3.5 mL) in the presence of Pd/C (25 mg). The intermediate was further hydrogenated in AcOH and H_2O (3.5 mL, 10:1) in the presence of $Pd(OH)_2$ (33 mg). The product was transformed into its HCl salt to give **29α** (13.5 mg, 87%): ^{1}H NMR (D_2O, 500 MHz): $\delta =$ 5.92 (d, $J = 3.5$ Hz, 1H; H-1′), 3.75 (H-1), 4.13 (dd, $J = 9.5$ and 9.8 Hz, 1H; H-3), 4.02–3.97 (m, 2H; H-3′, H-5′), 3.79 (m, 1H; H-5), 3.74 (t, 1H; H-4), 3.59 (s, 3H; OCH_3), 3.52–3.42 (m, 3H; H-2′, 2×C*H*NH_2), 3.28 (dd, $J = 6.8$ and 13.5 Hz, 1H; C*H*NH_2), 3.24 (t, $J = 9.0$ Hz, 1H; H-2), 3.17 ppm (dd, $J = 9.1$ and 13.1 Hz, 1H; C*H*NH_2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.1$ (C-1), 95.0 (C-1′), 75.6 (C-3), 71.8, 71.6, 70.6, 69.1, 68.1, 57.6 (OCH_3), 53.9 and 53.3 (C-2 and C-2′), 40.0 and 39.9 ppm (C-6 and C-6′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl)-(1–3)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (29β): Compound **13β** (18 mg, 0.028 mmol) was hydrogenated in pyridine (2.5 mL) in the presence of Pd/C (18 mg) as above. The intermediate was further hydrogenated in CH_3CO_2H and H_2O (4.4 mL, 10:1) in the presence of $Pd(OH)_2$ (19 mg). The reduction was incomplete due to unexpected inactivation of the catalyst. The recovered crude acetate was then chromatographed on Iatro beads (0.350 mg, applied in *i*PrOH and eluted with *i*PrOH/H_2O/28% NH_4OH (40:10:5)). The recovered amino disaccharide was then transformed into its HCl salt. Due to loss of material, only 5 mg (36%) of hydrochloride salt **29β** were recovered: ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.11$ (d, $J = 8.5$ Hz, 1H; H-1′), 4.68 (d, $J = 8.2$ Hz, 1H; H-1), 4.15 (t, $J = 9.5$ Hz, 1H; H-3), 3.80–3.70 (m, 4H; H-4, H-5, H-3′, H-5′), 3.60 (s, 3H; OCH_3), 3.55 (dd, $J = 2.4$ and 13.4 Hz, 1H; C*H*NH_2), 3.49 (dd, $J = 2.7$ and 13.7 Hz, 1H; C*H*NH_2), 3.46 (t, $J = 9.5$ Hz, 1H; H-4′), 3.34–3.23 (m, 3H; H-2, H-2′, C*H*NH_2), 3.20 ppm (dd, $J = 9.5$ and 13.7 Hz, 1H; C*H*NH_2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.7$ (C-1), 96.7 (C-1′), 76.9 (C-3), 72.9, 72.1, 71.7, 71.0, 69.3, 57.7 (OCH_3), 55.2 and 54.8 (C-2 and C-2′), 40–4 and 40.2 ppm (C-6 and C-6′).

Methyl *O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1–4)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (30α): Compound **18α** (18 mg, 0.023 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (14 mg) as above. The intermediate was further hydrogenated in CH_3CO_2H and H_2O (3.3 mL, 10:1) in the presence of $Pd(OH)_2$ (15 mg). The product was transformed into its HCl salt to give **30α** (10 mg, 89%): ^{1}H NMR (D_2O, 800 MHz): $\delta = 5.66$ (d, $J = 3.7$ Hz, 1H; H-1′), 4.63 (d, $J = 8.4$ Hz, 1H; H-1), 3.96–3.92 (m, 2H; H-3, C*H*OH), 3.88–3.78 (m, 5H; H-4, H-3′, 3×C*H*OH), 3.72 (m, 1H; H-5 or H-5′), 3.65 (m, 1H; H-5 or H-5′), 3.58 (s, 3H; OCH_3), 3.57 (t, $J =$ 9.6 Hz, 1H; H-4′), 3.37 (dd, $J = 10.8$ Hz, 1H; H-2′), 3.37 ppm (dd, $J =$ 10.7, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.8$ (C-1), 96.2 (C-1′), 75.0, 74.7, 73.3, 72.6, 69.4, 69.2, 60.3 and 60.1 (C-6 and C-6′), 57.6 (OCH_3), 56.1 and 54.2 ppm (C-2 and C-2′).

Methyl *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1–4)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (30β): Compound **18β** (21 mg, 0.027 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (26 mg). The intermediate was further hydrogenated in AcOH and H_2O (2.2 mL, 10:1) in the presence of $Pd(OH)_2$ (18 mg). The product was transformed into its HCl salt to give **30β** (9.5 mg, 80%): ^{1}H NMR (D_2O, 800 MHz): $\delta = 4.79$ (d, $J =$ 8.3 Hz, 1H; H-1′), 4.65 (d, $J = 8.2$ Hz, 1H; H-1), 3.95–3.90 (m, 2H; H-6a, H-6′a), 3.87–3.82 (m, 2H; H-3, H-4), 3.78–3.74 (m, 2H; H-6b, H-6′b), 3.67 (m, 1H; H-5), 3.65 (m, 1H; H-3′), 3.58 (s, 3H; OCH_3), 3.53 (m, 1H; H-5′), 3.47 (m, 1H; H-4′), 3.07 (dd, $J = 10.6$ Hz, 1H; H-2′), 3.04 ppm (dd, $J = 10.0$ Hz, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): $\delta =$ 99.7 and 97.9 (C-1 and C-1′), 76.9, 76.5, 74.6, 71.9, 70.4, 69.7, 60.4 and 60.2 (C-6 and C-6′), 57.6 (OCH_3), 55.9 and 55.7 ppm (C-2 and C-2′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-(1–4)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (32α): Compound **19α** (13 mg, 0.023 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (14 mg) as above. The intermediate was further hydrogenated in AcOH and H_2O (2.2 mL, 10:1) in the presence of $Pd(OH)_2$ (14 mg). The product was transformed into its HCl salt as to give **32α** (7 mg, 82%): ^{1}H NMR (D_2O, 800 MHz): $\delta = 5.80$ (d, $J = 3.8$ Hz, 1H; H-1′), 4.65 (d, $J = 8.5$ Hz, 1H; H-1), 4.10–3.92 (m, 3H; H-3, H-4, H-6a), 3.89 (m, 1H; H-5′), 3.85 (dd, $J = 9.1$ and 10.5 Hz, 1H; H-3′), 3.82 (dd, $J = 3.1$ and 12.7 Hz, 1H; H-6b), 3.62 (m, 1H; H-5), 3.57 (s, 3H; OCH_3), 3.48–3.44 (m, 2H; H-4, H-6′a), 3.43 (dd, 1H; H-2′), 3.21 (dd, $J = 8.5$ and 13.5 Hz, 1H; H-6′b), 3.07 ppm (dd, $J = 9.4$ Hz, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.9$ (C-1), 95.7 (C-1′), 74.4, 73.3, 72.9, 71.0, 69.4, 69.1, 59.8 (C-6), 57.7 (OCH_3), 56.3 and 53.9 (C-2 and C-2′), 40.3 ppm (C-6′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl)-(1–4)-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (32β): Compound **19β** (8.0 mg, 0.011 mmol) was hydrogenated in pyridine (1.5 mL) in the presence of Pd/C (11 mg). The intermediate was further hydrogenated in AcOH and H_2O (1.65 mL, 10:1) in the presence of $Pd(OH)_2$ (12 mg). The product was transformed into its HCl salt to give **32β** (4.6 mg, 90%): ^{1}H NMR (D_2O, 600 MHz): $\delta = 4.87$ (d, $J = 8.3$ Hz, 1H; H-1′), 3.96–3.90 (m, 2H; H-4, H-6a), 3.95 (dd, $J = 9.4$ and 10.4 Hz, 1H; H-3), 3.77–3.68 (m, 3H; H-6b, H-3′, H-5′), 3.66 (m, 1H; H-5), 3.57 (s, 3H; OCH_3), 3.50 (dd, $J = 2.4$ and 13.5 Hz, 1H; H-6′a), 3.44 (t, $J = 9.4$ Hz, 1H; H-4′), 3.21 (dd, $J = 9.1$ Hz, 1H; H-6′b), 3.16 (dd, $J = 10.2$ Hz, 1H; H-2′), 3.07 ppm (dd, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): $\delta = 99.9$ and 97.4 (C-1 and C-1′), 75.2, 74.6, 72.4, 71.4, 71.3, 70.3, 59.9 (C-6), 57.6 (OCH_3), 55.8 and 55.6 (C-2 and C-2′), 40.2 ppm (C-6′).

Methyl *O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (33α): Compound **23α** (10 mg, 0.0126 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (14 mg). The intermediate was further hydrogenated in AcOH and H_2O (2.5 mL, 10:1) in the presence of $Pd(OH)_2$ (20 mg). The product was transformed into its HCl salt to give **33α** (5.8 mg, 93%): ^{1}H NMR (D_2O, 500 MHz): $\delta = 5.50$ (d, $J =$ 3.7 Hz, 1H; H-1′), 4.72 (d, $J = 8.6$ Hz, 1H; H-2), 4.04 (dd, $J = 8.1$ and 10.7 Hz, 1H; H-3), 3.91 (m, 1H; H-5), 3.83 (m, 1H; H-5′), 3.73 (dd, $J = 9.5$ Hz, 1H; H-4), 3.69 (dd, $J = 8.5$ and 10.1 Hz, 1H; H-3′), 3.63

(dd, 1H; H-2′), 3.60 (s, 3H; OCH_3), 3.51 (dd, J=3.0 and 13.0 Hz, 1H; H-6a), 3.44–3.36 (m, 2H; H-4′, H-6′a), 3.28 (dd, J=10.0 Hz, 1H; H-6b), 3.23 (dd, J=7.0 and 13.4 Hz, 1H; H-6b′), 3.14 ppm (dd, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): δ=100.1 (C-1), 99.2 (C-1′), 78.4, 72.4, 72.0, 71.4, 71.0, 70.7, 69.1, 58.0 (OCH_3), 56.0 (C-2), 40.9 and 40.6 ppm (C-6 and C-6′).

Methyl *O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (33β): Compound **23β** (8.0 mg, 0.010 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (18 mg). The intermediate was further hydrogenated in AcOH and H_2O (1.6 mL, 10:1) in the presence of $Pd(OH)_2$ (15 mg). The product was transformed into its HCl salt to give **33β** (4.7 mg, 90%): ^{1}H NMR (D_2O, 500 MHz): δ=4.72 (d, J=8.2 Hz, 1H; H-1), 4.53 (d, J=7.6 Hz, 1H; H-1′), 3.90–3.83 (m, 2H; including H-3 (dd, J=8.8 and 9.1 Hz)), 3.73–3.63 (m, 3H), 3.61 (s, 3H; OCH_3), 3.53 (t, J=9.1 Hz, H-4), 3.47 (dd, J=2.7 and 13.7 Hz, 1H; C*H*NH_2), 3.37–3.32 (m, 2H), 3.24 (dd, 1H; J=9.8 and 13.1 Hz, C*H*NH_2), 3.20 (dd, J=9.3 and 13.7 Hz, 1H; C*H*NH_2), 3.13 ppm (dd, J=10.7 Hz, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): δ=103.0 (C-1′), 99.9 (C-1), 80.1, 75.2, 73.2, 72.0, 71.4, 70.9, 70.3, 57.7 (OCH_3), 55.4 (C-2), 40.4 and 40.0 ppm (C-6 and C-6′).

Methyl *O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (34α): Compound **21α** (20 mg, 0.025 mmol) was hydrogenated in pyridine (2.5 mL) in the presence of Pd/C (20 mg). The intermediate was further hydrogenated in AcOH and H_2O (3.3 mL, 10:1) in the presence of $Pd(OH)_2$ (15 mg). The recovered product was transformed into its HCl salt as to give **34α** (9.9 mg, 85%): ^{1}H NMR (D_2O, 800 MHz): δ=5.61 (d, J=3.7 Hz, 1H; H-1′), 4.69 (d, J=8.3 Hz, 1H; H-1), 3.97 (dd, J=8.8 and 10.6 Hz, 1H; H-3), 3.92–3.87 (m, 2H; H-5, H-6′a), 3.82 (dd, J=9.2 and 10.8 Hz, 1H; H-3′), 3.80–3.75 (m, 2H; H-4, H-6′b), 3.67 (m, 1H; H-5′), 3.59 (s, 3H; OCH_3), 3.56 (dd, J=2.9 and 13.6 Hz, 1H; H-6a), 3.50 (t, 1H; H-4′), 3.40 (dd, 1H; H-2′), 3.24 (dd, J=9.7 Hz, 1H; H-6b), 3.15 ppm (dd, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): δ=99.7 (C-1), 96.9 (C-1′), 78.2, 73.7, 72.0, 70.0, 69.3, 69.2, 60.5 (C-6′), 57.7 (OCH_3), 55.8 and 54.1 (C-2 and C-2′), 40.7 ppm (C-6).

Methyl *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (34β): Compound **21β** (79 mg, 0.099 mmol) was hydrogenated in pyridine (4.0 mL) in the presence of Pd/C (26 mg). The intermediate was further hydrogenated in AcOH and H_2O (5.5 mL, 10:1) in the presence of $Pd(OH)_2$ (55 mg). The product was transformed into its HCl salt to give **34β** (33.5 mg, 72%): ^{1}H NMR (D_2O, 500 MHz): δ=3.98–3.93 (m, 3H; H-3, H-5, H-6′a), 3.87 (t, J=5.7 and 12.5 Hz, 1H; H-6′b), 3.70 (dd, J=8.8 and 10.7 Hz, 1H; H-3′), 3.60 (s, 3H; OCH_3), 3.58–3.52 (m, 2H; H-6a, H-5′), 3.49 (t, J=9.5 Hz, 1H; H-4′), 3.27 (dd, J=9.5 and 13.0 Hz, 1H; H-6b), 3.19–3.13 ppm (m, 2H; H-2, H-2′); ^{13}C NMR (D_2O, 75 MHz): δ=99.5 and 97.0 (C-1 and C-1′), 77.6, 76.4, 71.6, 70.7, 69.5, 69.4, 60.2 (C-6), 57.6 (OCH_3), 40.1 ppm (C-6).

Methyl *O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (35α): Compound **22α** (14.5 mg, 0.020 mmol) was hydrogenated in pyridine (2.0 mL) in the presence of Pd/C (14 mg). The intermediate was further hydrogenated in CH_3CO_2H and H_2O (1.6 mL, 10:1) in the presence of $Pd(OH)_2$ (18 mg). The product was transformed into its HCl salt to give **35α** (9.0 mg, 91%): ^{1}H NMR (D_2O, 800 MHz): δ=5.58 (d, J=3.5 Hz, 1H; H-1′), 4.70 (d, J=8.6 Hz, 1H; H-1), 4.04 (dd, J=8.8 and 10.5 Hz, 1H; H-3), 3.92 (m, 1H; H-5), 3.86 (dd, J=9.1 and 10.7 Hz, 1H; H-3′), 3.84–3.80 (m, 2H; H-4, H-5′), 3.60 (s, 3H; OCH_3), 3.52–3.43 (m, 3H; H-4′, H-6a, H-6′a), 3.33–3.28 (m, 2H; H-6b, H-6′b), 3.16 ppm (dd, 1H; H-2); ^{13}C NMR (D_2O, 75 MHz): δ=99.9 (C-1), 96.0 (C-1′), 76.8, 72.2, 70.6, 70.4, 69.5, 68.7, 57.8 (OCH_3), 56.0 and 53.8 (C-2 and C-2′), 40.7 and 40.2 ppm (C-6 and C-6′).

Methyl *O*-(2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl)-(1–4)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride (35β): Compound **22β** (4.5 mg, 0.099 mmol) was hydrogenated in pyridine (1.2 mL) in the presence of Pd/C (12 mg). The intermediate was further hydrogenated in AcOH and H_2O (1.65 mL, 10:1) in the presence of $Pd(OH)_2$ (9 mg). The recovered product was transformed into its HCl salt to give **35β** (2.4 mg, 82%): ^{1}H NMR (D_2O, 500 MHz): δ=4.81 (d, J=8.2 Hz, 1H; H-1 or H-1′), 4.70 (d, J=8.5 Hz, 1H; H-1 or H-1′), 3.93 (m, 1H; H-5), 3.89–3.83 (m, 2H), 3.73 (m, 1H; H-5′), 3.64 (t, J=9.6 Hz, 1H), 3.60 (s, 3H; OCH_3), 3.53–3.47 (m, 2H; H-6a, H-6′a), 3.44 (t, J=9.3 Hz, 1H), 3.27 (dd, J=9.8 and 13.9 Hz, 1H; C*H*NH_2), 3.23 (dd, J=8.5 and 13.5 Hz, 1H; C*H*NH_2), 3.16–3.09 ppm (m, 2H; H-2, H-2′); ^{13}C NMR (D_2O, 75 MHz): δ=100.25 and 99.99 (C-1 and C-1′), 77.69, 71.5, 71.8, 71.2, 71.0, 70.1, 57.7 (OCH_3), 55.8, 55.4, 40.2 ppm (C-6 and C-6′).

Acknowledgements

The authors would like to thank Dr. Kristin A. Sannes-Lowery for determination of the K_d values.

Keywords: aminoglycosides · antibiotics · glycosylations · RNA · substituent effects

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Received: April 14, 2004

