Trisaccharide mimetics of the aminoglycoside antibiotic neomycin†

Yu Rao,[a] Andre Venot,[a] Eric E. Swayze,[b] Richard H. Griffey[b] and Geert-Jan Boons*[a]

Received 14th December 2005, Accepted 25th January 2006
First published as an Advance Article on the web 22nd February 2006
DOI: 10.1039/b517725a

A highly convergent approach for the chemical synthesis of eight structurally related trisaccharides that contain 3 to 5 amino groups has been described. Fourier-transformation ion cyclotron resonance mass spectrometry (FT-ICR MS) has been employed to determine the dissociation constants (K_d) for the binding of the trisaccharides to a prototypical fragment of 16S ribosomal RNA. A compound that contained a 4,6-dideoxy-4-amino-β-D-glucopyranoside moiety at C-3 displayed binding in the low micromolar range. It was found that small structural changes of the saccharides resulted in large differences in affinity. The described structure–activity relationship is expected to be valuable for the development of novel antibiotics that target rRNA.

Introduction

Aminoglycoside antibiotics such as neomycin (Fig. 1) have been used for more than fifty years as an effective treatment for gram-positive and gram-negative bacterial infections. These antibiotics exert their antibacterial activity by binding to the A-site of the 16S ribosomal RNA of bacteria.[1–4] During translation, the binding interferes with two conformationally flexible adenine residues involved in the selection of cognate aminoacyl-tRNA. The resulting conformational changes either increase the misincorporation of near cognate amino acids, or terminate protein biosynthesis.

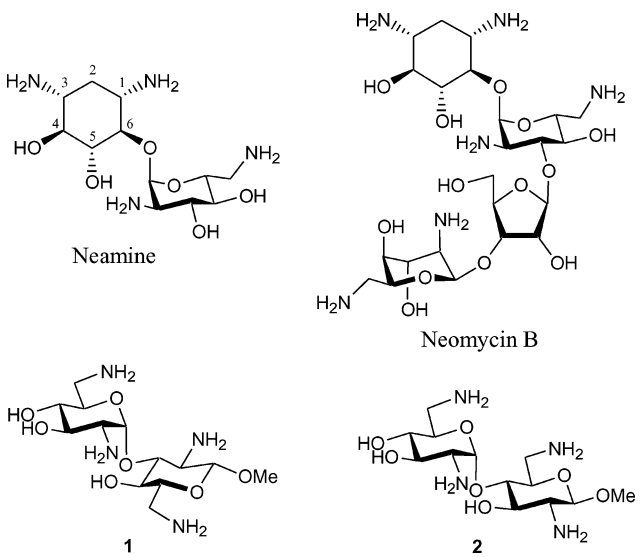


Fig. 1 Chemical structures of aminoglycoside antibiotics.

Although neomycin is widely employed for the treatment of serious infections, there are several problems associated with its use. For example, resistant bacterial strains are emerging due to overuse. The strains contain enzymes that can modify antibiotics by acetylation or phosphorylation, thus rendering the antibiotics ineffective.[5,6] An additional drawback is that aminoglycoside antibiotics display cytotoxicity and at high doses may impair hearing and kidney function.

All naturally occurring aminoglycoside antibiotics contain a 2-deoxystreptamine (2-DOS) or streptamine moiety, which is glycosylated with aminosugars at C-4 and C-5 to give the neomycin class or at C-6 to give the kanamycin–gentamicin class. Furthermore, the neomycin class shares a pseudoglycoside commonly known as neamine, in which 2-DOS is glycosylated at the C-4 position with an amino-substituted glucopyranoside moiety (Fig. 1). NMR[7–11] and X-ray[12–15] crystallographic studies have indicated that this subunit is the minimal motif for selective binding to the A-site of the 16S rRNA. This unit is, however, most susceptible to modifications by resistance-inducing enzymes of targeted pathogens.

The emergence of aminoglycoside-resistant pathogens has triggered a search for novel compounds capable of binding to specific RNA structures. Prior approaches have been based on the chemical modification of natural aminoglycosides or part structures such as neamine.[16–39] Our group has chosen a different approach by designing and synthesizing a series of amino-containing disaccharides which mimic the unique spatial arrangements of the functional groups of neamine that are required for the recognition of the RNA target.[40] Fourier-transformation ion cyclotron resonance mass spectrometry (FT-ICR MS) was used to determine K_d values for the binding of the disaccharides with rRNA fragments. The compound tested that yielded the best results was the α(1–3)-linked disaccharide **1** (Fig. 1), which displayed an affinity similar to that of neamine. Superimposition of compound **1** on the neamine moiety of paramomycin indicated that three of the four amino groups have similar spatial orientations. Overlay studies also showed that 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 **1** and C-6 of the α(1–4)-linked disaccharide **2**.

Neamine demonstrates exclusively weak antibiotic activity at high concentrations and requires the attachment of other sugar residues for increasing the affinity and proper positioning at the A-site of rRNA. Therefore, it is to be expected that oligosaccharides

[a]*Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA. E-mail: gjboons@ccrc.uga.edu*
[b]*Ibis Therapeutics, 2292 Faraday. Av., Carlsbad, CA 02008, USA*

† Electronic supplementary information available: ^{1}H NMR spectra for compounds **7–18** and **20–31**. See DOI: 10.1039/b517725a

that are more complex than **1** and **2** may bind with higher affinity to RNA. In order to test this hypothesis, we report here the design and synthesis of eight trisaccharides which are derived from compounds **1** and **2** but contain an additional β-ribofuranoside or 4,6-dideoxy-4-amino-β-D-glucopyranoside moiety. The binding of these compounds with a prototypical 16S ribosomal RNA fragment has been studied by FT-ICR mass spectrometry.

Results and discussion

Design and synthesis of trisaccharides

Neomycin contains a β-linked riboside at C-5 of the neamine core (Fig. 1). It has been shown that a replacement of the riboside by a 4,6-dideoxy-4-amino-β-D-glucopyranoside results in a compound with a potency similar to that of neomycin and ribostamycin.[41] Therefore, it was expected that the attachment of a β(1–3) or β(1–4) linked riboside or 4,6-dideoxy-4-aminoglucopyranoside to disaccharides **1** and **2** may result in compounds that have an increased affinity for rRNA. To this end, trisaccharides **12**, **13**, **16**, **17**, **23**, **24**, **30** and **31** (Schemes 1 and 2) were prepared.

It was envisaged that the targeted trisaccharides could be prepared by a highly convergent approach employing the glycosyl acceptor **3** and glycosyl donors **4–6** (Fig. 2).[42–44] The β(1–4) linked ribosides **12** and **13** and 4,6-dideoxy-4-aminoglucopyranosides **16** and **17** could be prepared by coupling **3** with **4** to give disaccharide **7**, which after removal of the benzylidene acetal and modification of C-6 could be glycosylated at the C-4 hydroxyl with glycosyl donors **5** or **6**. The β(1–3) linked ribosides **23** and **24** or 4,6-dideoxy-4-aminoglucopyranosides **30** and **31** could be prepared by first coupling **3** with **5** or **6** to give disaccharides **18** and **25**, respectively, which after removal of the benzylidene acetal and modification of C-6 could be glycosylated at the C-4 hydroxyl with glycosyl donor **4**.

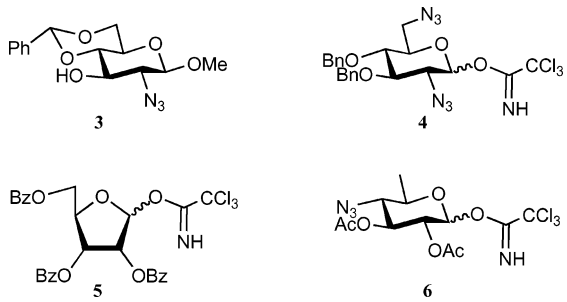
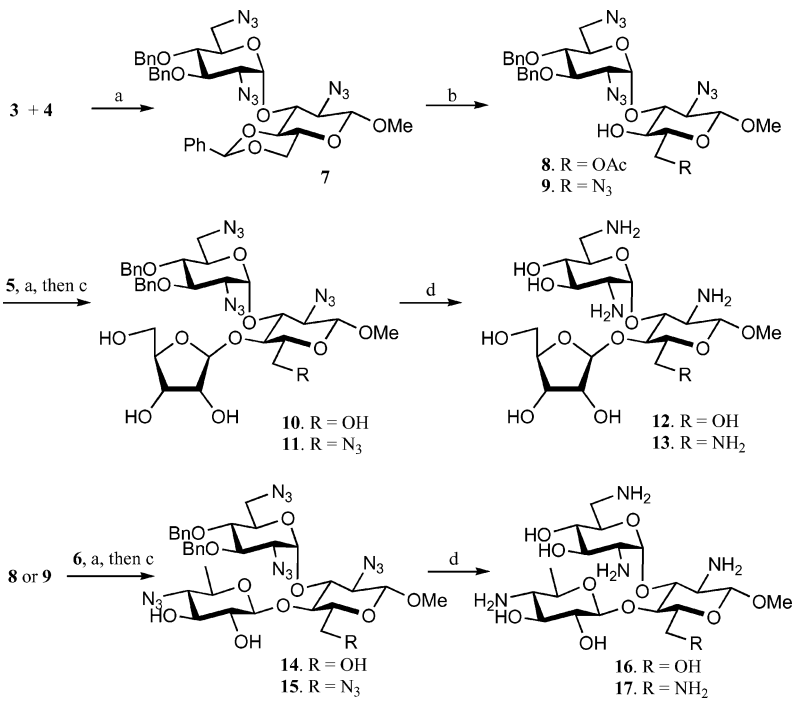
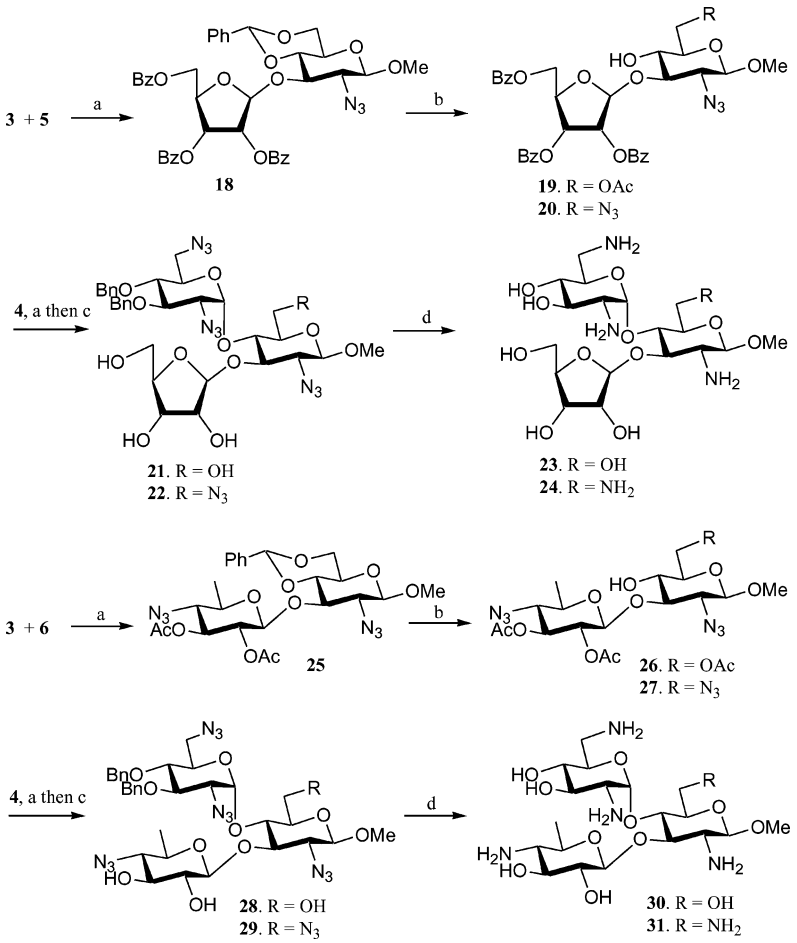


Fig. 2 Glycosyl acceptors and donors for the preparation of a library of trisaccharides.

Thus, a TMSOTf (trimethylsilyl trifluoromethane sulfonate) catalyzed glycosylation[20] of glycosyl acceptor **3** with trichloroacetimidate **4** in diethyl ether and dichloromethane afforded disaccharide **7** as only the α-anomer in a yield of 72% (Scheme 1). Removal of the benzylidene acetal of **7** using aqueous acetic acid followed by selective acetylation of the C-6 hydroxyl with acetyl chloride in pyridine afforded the acetate **8** in a good overall yield. Alternatively, the C-6 azido derivative **9** could be obtained by a regioselective tosylation of the intermediate diol with 4-toluenesulfonyl chloride (TsCl) in pyridine followed by the displacement of the tosyl group with sodium azide in DMF at 85 °C. Next, glycosyl acceptors **8** and **9** were coupled with glycosyl donors **5** and **6** using a catalytic amount of TMSOTf to give the corresponding trisaccharides, which were deacetylated with sodium methoxide in methanol to give compounds **10**, **11**, **14** and **15**, respectively. In each case, only the β-glycoside was formed due to neighboring group participation of the ester-protecting group



Scheme 1 *Reagents and conditions*: (a) TMSOTf, DCM, 4Å MS; (b) 90% AcOH–H_2O then for **8**, AcCl, Pyr, 0 °C and for **9**, TsCl, Pyr, 0 °C followed by NaN_3, DMF, 90 °C; (c) NaOMe, MeOH; (d) Pd/C, H_2, Pyr then $Pd(OH)_2$, H_2, AcOH–H_2O, 10 : 1, v/v.



Scheme 2 *Reagents and conditions*: (a) TMSOTf, DCM, 4Å MS; (b) 90% AcOH–H_2O then for **19**/**26**, AcCl, Pyr, 0 °C and for **20**/**27**, TsCl, Pyr, 0 °C followed by NaN_3, DMF, 90 °C; (c) NaOMe, MeOH; (d) Pd/C, H_2, Pyr then $Pd(OH)_2$, H_2, AcOH–H_2O, 10 : 1, v/v.

at C-2 of glycosyl donors **5** and **6**. Finally, trisaccharides **10**, **11**, **14** and **15** were deprotected by a three-step protocol involving treatment with sodium methoxide in methanol to remove the acetyl and benzoyl esters, followed by catalytic hydrogenation over Pd/C in pyridine, resulting in the selective reduction of the azido groups to amines and, finally, a second catalytic hydrogenation using $Pd(OH)_2$[45] in a mixture of acetic acid and water to remove the benzyl ethers resulting in the formation of the target compounds **12**, **13**, **16** and **17**. Impure compounds were obtained when the reduction of the azido and benzyl ethers was performed by a one step-procedure.

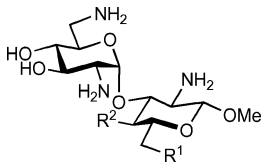
Next, attention was focused on the preparation of the β(1–3)-modified trisaccharides **23**, **24**, **30** and **31**. Thus, the key disaccharides **18** and **25** were obtained in good yield as only β-anomers by coupling glycosyl acceptor **3** with glycosyl donors **5** and **6**, respectively, using TMSOTf as the promoter (Scheme 2). Disaccharides **18** and **25** could be converted into C-6 acetates **19** and **26**, respectively by a two-step procedure involving the removal of the benzylidene acetal followed by regioselective acetylation of the more reactive primary C-6 hydroxyl using acetyl chloride in pyridine. Alternatively, tosylation of the C-6 hydroxyl of the intermediate diol followed by displacement of the resulting tosylates with sodium azide afforded azido derivatives **20** and **27**. Next, disaccharide acceptors **19**, **20**, **26** and **27** were coupled with glycosyl donor **4** using TMSOTf as the activator in a mixture of diethyl ether and dichloromethane to give, after deacetylation with sodium methoxide in methanol, trisaccharides **21**, **22**, **28** and **29**, respectively, as only α-anomers in good yield. The derivatives were deprotected using a similar three-step protocol as described above to afford target compounds **23**, **24**, **30** and **31**.

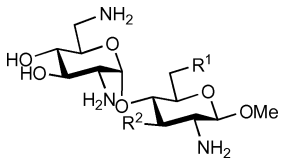
Binding of the trisaccharides to rRNA

High-resolution mass spectrometry (HRMS) has considerable potential for the monitoring of complex formations between small molecules and RNA fragments.[46,47] The mild electrospray ionization (ESI) process effectively transfers both free and complexed RNAs into the gas phase, resulting in a representation of the relative distribution of bound and free RNA present in a solution. Therefore, dissociation constants can be determined by measuring ion abundances 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 K_d values for the binding of several aminoglycoside antibiotics to rRNA fragments.[48]

ESI-FT-ICR mass spectra were acquired under noncompetitive binding conditions from 0.5 μM mixtures of untagged 16S and mass-tagged 18S RNA with 0.75, 2.5, 7.5, and 25 μM

Table 1 Binding data of compounds **12**, **13**, **16**, **17**, **23**, **24**, **30** and **31** with the 16S rRNA fragment





R^1	R^2		K_d/μmol		K_d/μmol
OH	β-D-ribose	**12**	n.b.	**23**	n.b.
NH_2	β-D-ribose	**13**	n.b.	**24**	43
OH	4-NH_2-6-deoxy-β-D-Glu	**16**	115	**30**	22
NH_2	4-NH_2-6-deoxy-β-D-Glu	**17**	281	**31**	2

n.b.: no binding observed.

concentrations of trisaccharides **12**, **13**, **16**, **17**, **23**, **24**, **30** and **31**. The relative ion abundances were used to determine dissociation constants and the resulting values are listed in Table 1. Similar values were obtained for the 16S and 18S RNA fragments, indicating no selectivity for bacterial over eukaryotic rRNA fragments.

Previously, a K_d value of 7 μM was determined for the binding of neamine to 16S RNA. For the α(1–4) linked disaccharide **1**, a similar affinity (K_d = 11 μM) was measured whereas the α(1–3) linked disaccharide **2** displayed a somewhat reduced binding (K_d = 40 μM). Compounds **16** and **17**, which are derived from the α(1–3) linked disaccharide **2** and contain an additional 4,6-dideoxy-4-aminoglucopyranoside at C-3, showed binding in the high micromolar range. The affinity of these trisaccharides was substantially lower than that of the parent disaccharide, indicating that the 4,6-dideoxy-4-aminoglucopyranoside makes unfavorable interactions with the rRNA fragment. Apparently, unfavorable interactions are more severe when a β-riboside is installed since compounds **12** and **13** showed no appreciable binding.

Compounds **23**, **24**, **30** and **31**, which are derived from disaccharide **1**, showed a more interesting structure–activity relationship. In this series, the attachment of a β-riboside to C-3 (compounds **23** and **24**) led to either a reduction or abolishment of binding. However, derivative **31**, which contains a 4,6-dideoxy-4-aminoglucopyranoside at C-3, displayed a significantly more favorable K_d than the parent compound **1** or neamine, indicating that the additional monosaccharide makes favorable interactions with the 16S RNA fragment. Compound **30**, which contains a hydroxyl at C-6, showed a somewhat reduced affinity. This observation indicates that the amino group at C-6 of **31** contributes to binding. The K_d values of compounds **16** and **17** differ only marginally, suggesting that for the α(1–3) linked series the C-6 amine does not contribute to binding with rRNA. Thus, the structure–activity relationship of compounds **16**, **17**, **30** and **31** implies that the α(1–3) and α(1–4) linked compounds bind differently with the RNA target. Furthermore, the observation that compound **31** has the most favorable binding is concurrent with our previous modeling study, which showed that the amino groups of the α(1–4) linked compound **1** overlay better with those of neamine compared to those of the α(1–3) linked derivative **2**. The finding that all β-ribosides bind with poor affinity is, however, surprising because neomycin-based antibiotics contain this monosaccharide.

The results described in this paper show that amino-containing oligosaccharides can bind with high affinity to RNA fragments. Small structural changes in the oligosaccharides resulted in dramatic differences in affinity indicating that the spatial arrangements of the amino groups of the saccharide result in specific interactions with the RNA structure. A unique property of RNA is that it can fold into a well defined three-dimensional structure. Due to this well defined shape, small molecules such as the saccharides described in this paper can target specific RNA domains, which may result in a modulation of biological activities.[4,21,49–52] It is to be expected that the screening of a large library of amino-containing oligosaccharides may result in compounds that bind with very high affinities to a target RNA fragment. Such compounds could provide leads for the development of novel antibiotics.

Experimental

General procedures

Chemicals were purchased from Aldrich and Fluka and used without further purification. Molecular sieves (AW-300, Aldrich) were activated in a microwave oven (1.5 min, 3 times) and further dried *in vacuo*. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. All reactions were performed under anhydrous conditions under an atmosphere of argon. Reactions were monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) or by charring with 5% sulfuric acid in methanol. Flash chromatography was performed on silica gel (Merck, 70–230 mesh). Iatrobeads (60 μm) were purchased from Bioscan. ^{1}H NMR (1D, 2D) and ^{13}C NMR were recorded on a Varian Merc 300 spectrometer and on Varian 500 and 600 MHz spectrometers equipped with Sun workstations. For ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$, chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H, δ 7.24; ^{13}C, δ 77.0) as an internal standard for protected compounds. The negative ion matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) was recorded on a VOYAGER-DE Applied Biosystem using dihydrobenzoic acid as a matrix. High-resolution mass spectra were obtained using a Voyager delayed extraction STR with 2,5-dihydroxybenzoic acid as an internal calibration matrix. Binding constants for the 16S A-site model RNA were determined as described previously,[16] using 33% aqueous isopropanol

containing 100 mM NH_4OAc, 0.5 μM 16S target RNA, 0.5 μM 18S control RNA, and ligand concentrations of 0.75, 2.5, 7.5, and 25 μM.

General procedure for glycosylations

Glycosylations were performed with 1.0 equivalent of glycosyl acceptor and 1.0–1.5 equivalents of the trichloroacetimidate donor which were dissolved in a selected solvent with crushed, activated AW 3 Å molecular sieves (1.5 times the total amount of glycosyl acceptor and donor). TMSOTf (0.10 equivalent) was added by syringe to a stirred and cooled mixture at an appropriate temperature (−60 °C). The progress of the reactions was monitored by TLC using a mixture of hexane and EtOAc. The temperature of the reaction was slowly raised to approximately −10 °C over 1–1.5 hours. Solid $NaHCO_3$ was then added while stirring was continued for 15 minutes at room temperature prior to filtration and concentration of the filtrate. The crude material was dissolved in a small amount of toluene and applied to a column of silica gel which was eluted with a gradient of EtOAc in hexane.

General procedure for hydrolysis of the 4,6-*O*-benzylidene acetals

The disaccharide was dissolved in 90% aqueous AcOH and the solution was stirred at 80–85 °C for 3 hours. The mixture was co-evaporated with toluene under reduced pressure and the residue was purified by silica gel column chromatography using a mixture of hexane and EtOAc as an eluent.

General procedure for acetylation of the C-6 hydroxyls

Acetyl chloride (1.1–2.0 equivalents) was added to a solution of the starting disaccharide in dry pyridine and the mixture was stirred at room temperature for 2 hours. The reaction was quenched by the addition of CH_3OH, diluted with CH_2Cl_2 and washed with a saturated solution of $NaHCO_3$, water and brine. The organic phase was dried ($MgSO_4$) and concentrated and the residue was co-evaporated with toluene. The crude material was purified by silica gel chromatography using a mixture of hexane and EtOAc as the eluent.

General procedure for introduction of the C-6 azido substituent

4-Toluenesulfonyl chloride (1.5–2.0 equivalents) was added to the solution of a disaccharide in dry pyridine and the mixture was stirred at room temperature for 4 hours. The reaction was quenched by the addition of CH_3OH, diluted with CH_2Cl_2 and washed with a saturated solution of $NaHCO_3$, water and brine. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure and the residue was co-evaporated with toluene. The resulting residue was directly used in the next step. NaN_3 (7.0 equivalents) was added to the crude tosylate in DMF and the mixture was stirred at 80–90 °C for 2–3 hours. After cooling and the addition of DCM, the organic phase was washed with water (3 times) and brine. The crude material was purified by silica gel chromatography using a mixture of hexane and EtOAc as the eluent.

General procedure for removal of the acetyl and benzoyl groups of the trisaccharides

A solution of trisaccharide in dry CH_3OH was brought to pH 12–13 by careful addition of a solution of 0.5 M CH_3ONa in CH_3OH at room temperature. After completion of the reaction (TLC hexane–EtOAc, 2 : 1–1 : 2, v/v), the solution was neutralized with Dowex 50 (H^+), filtered, and the solvents were evaporated and the residue purified by silica gel chromatography using a mixture of hexane and EtOAc as the eluent.

General procedure for the deprotection of trisaccharides

Pd/C (10%, 1.5 times the weight of the starting material) was added to a solution of the protected azido trisaccharides in pyridine under an atmosphere of Ar. After evacuation, the flask was placed under an atmosphere of H_2. The reaction was stirred for 18 hours until TLC analyses (hexane–EtOAc, 1 : 1, v/v, $CHCl_3$–CH_3OH, 9 : 1, v/v, and iPrOH–28% NH_4OH, 95 : 5, v/v) indicated 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 co-evaporated with toluene. The residue was dried *in vacuo* for several hours. Matrix-assisted time-of-flight (MALDI-TOF) MS and NMR spectroscopy confirmed the reduction of the azido groups. $Pd(OH)_2$ (Degussa type, Aldrich, 2.0 times the weight of the starting material) was added to the above obtained material and dissolved in a mixture of AcOH and H_2O (10 : 1, v/v, 2–5 mL) under Ar. The mixture was placed under an atmosphere of H_2 and stirred overnight. TLC analyses (iPrOH–28% NH_4OH, 95 : 5, v/v and iPrOH–H_2O–28% NH_4OH, 30 : 10 : 5, v/v or 30 : 20 : 10, v/v) 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 co-evaporated with toluene and the residue was dried *in vacuo* for several hours. The recovered materials were passed through a small amount of Iatrobeads and slowly eluted with a mixture of iPrOH and 28% NH_4OH. Fractions containing the products were collected and concentrated *in vacuo*. The products were brought to pH 4.5 with AcOH and freeze dried.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (7). A TMSOTf (6 μL) catalyzed glycosylation of **3** (60 mg, 0.196 mmol) with **4** (90 mg, 0.163 mmol) in a mixture of Et_2O and CH_2Cl_2 (4 : 1, v/v, 2.5 ml) was performed as described in the general procedure section. Chromatography of the product over silica gel using a mixture of hexane and EtOAc (9 : 1, v/v) as an eluent gave **7** (76 mg, 67%). $[\alpha]^{25}_D$ +30.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.10 (m, 15H, aromatics), 5.60 (s, 1H, PhCH), 5.40 (d, 1H, $J_{1',2'} = 4.0$ Hz, H-1′), 4.94–4.84 (m, 3H, $PhCH_2$), 4.62 (d, 1H, $J_2 = 11.3$ Hz, $PhCH_2$), 4.42–4.34 (m, 1H, H-6a), 4.38 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.24 (m, 1H, H-5′), 4.10 (dd, 1H, $J_{3',4'} = 10.2$, $J_{2',3'} = 9.5$ Hz, H-3′), 3.84–3.71 (m, 3H, H-3, H-6b, H-6a′), 3.61 (s, 3H, OCH_3), 3.56–3.34 (m, 5H, H-2, H-4, H-4′, H-5, H-6b′), 3.26 (dd, $J_{1',2'} = 3.8$ Hz, $J_{2',3'} = 9.5$ Hz, 1H, H-2′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8, 137.7, 136.8, 129.1, 128.5, 128.4, 128.3, 128.0 (x2), 127.8, 125.9, 103.9 (C-1), 101.5 (PhCH), 98.2 (C-1′), 81.4, 79.5, 78.6, 75.7, 75.3, 75.2, 70.8, 68.4, 65.9, 64.8 (C-2), 63.0 (C-2′), 57.5 (OCH_3), 50.9 (C-6′).

ESI HRMS: m/z 722.2759 [M + Na^+]. Calcd for $C_{34}H_{37}N_9O_8$ 722.2765.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2-azido-2-deoxy-6-*O*-acetyl-β-D-glucopyranoside (8). Removal of the benzylidene acetal of **7** (210.0 mg, 0.30 mmol) followed by selective acetylation of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (3 : 1, v/v) as an eluent gave **8** (31 mg, 87%). $[\alpha]^{25}_D$ +43.9 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.10 (m, 10H, aromatics), 5.20 (d, 1H, $J_{1',2'} = 3.5$ Hz, H-1′), 4.94–4.84 (m, 3H, $PhCH_2$), 4.63 (d, 1H, $J_2 = 11.0$ Hz, $PhCH_2$), 4.41 (dd, 1H, $J_{5,6a} = 4.5$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.37 (dd, 1H, $J_{5,6b} = 1.8$ Hz, H-6b), 4.27 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.13 (t, 1H, $J_{3,4} = J_{4,5} =$ 9.5 Hz, H-4), 4.01 (t, 1H, $J_{3',4'} = J_{2',3'} = 9.5$ Hz, H-3′), 3.83 (d, 1H, $J_{4,OH} = 3.0$ Hz, OH), 3.70–3.50 (m, 4H, H-2′, H-3, H-4′, H-6a′), 3.60 (s, 3H, OCH_3), 3.50–3.38 (m, 3H, H-5, H-5′, H-6b′), 3.28 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.0$ Hz, H-2), 2.12 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6 (CO), 137.7 (x2), 128.8, 128.7, 128.3 (x2), 128.2, 128.1, 103.6 (C-1), 99.7 (C-1′), 83.6, 80.9 (C-3′), 78.9, 75.9, 75.5, 73.5, 71.4 (C-4), 70.9, 64.8, 64.5, 64.4 (C-2), 63.1 (C-2′), 57.6 (OCH_3), 51.1 (C-6′), 21.1 (CH_3CO). ESI HRMS: m/z 676.2551 [M + Na^+]. Calcd for $C_{29}H_{35}N_9O_9$ 676.2558.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′6′-dideoxy-α-D-glucopyranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (9). Removal of the benzylidene acetal of **7** (210.0 mg, 0.30 mmol) followed by selective tosylation and introduction of the azido substituent of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (6 : 1, v/v) as an eluent gave **9** (15 mg, 79%). $[\alpha]^{25}_D$ +26.4 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H, aromatics), 5.07 (d, 1H, $J_{1',2'} = 4.0$ Hz, H-1′), 4.96–4.85 (m, 3H, $PhCH_2$), 4.62 (d, 1H, $J_2 = 11.8$ Hz, $PhCH_2$), 4.29 (d, 1H, $J_{1,2} = 7.4$ Hz, H-1), 4.08 (m, 1H, H-5′), 4.01 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3′), 3.84 (d, 1H, $J_{4,OH} = 3.0$ Hz, OH), 3.65–3.41 (m, 8H, H-2′, H-3, H-4, H-5, H-6a, H-6b, H-6a′, H-6b′), 3.55(s, 3H, OCH_3), 3.30–3.22 (m, 2H, H-2, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6, 137.5, 128.7, 128.4, 128.3, 128.0, 127.9, 103.4 (C-1), 100.1 (C-1′), 85.2, 81.1, 78.9, 75.9, 75.5, 74.9, 71.7, 71.6, 64.8, 64.7, 57.4 (OCH_3), 51.5 (C-6), 51.0 (C-6′). ESI HRMS: m/z 659.2523 [M + Na^+]. Calcd for $C_{27}H_{32}N_{12}O_7$ 659.2517.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(β-D-ribofuranosyl)-2-azido-2-deoxy-β-D-glucopyranoside (10). A TMSOTf (3 μL) catalyzed glycosylation of **8** (15 mg, 0.023 mmol) with **5** (21 mg, 0.035 mmol) was performed in CH_2Cl_2 (2.0 mL) to afford an intermediate trisaccharide, which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: m/z 1120.38 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 1 : 2–1 : 4, v/v) providing **10** (7.0 mg, 73%). $[\alpha]^{25}_D$ +10.5 (c 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.15 (m, 10H, aromatics), 5.72 (d, 1H, $J_{1',2'} =$ 3.0 Hz, H-1′), 5.29 (d, 1H, $J_{1'',2''} = 2.5$ Hz, H-1″), 4.96–4.85 (m, 3H, $PhCH_2$), 4.61 (d, 1H, $J_2 = 11.4$ Hz, $PhCH_2$), 4.27 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.20–3.90 (m, 8H, H-2″, H-3, H-3″, H-4′, H-5a″, H-5b″, H-6a, H-6b), 3.82 (d, 1H, $J = 3.5$ Hz, OH), 3.60–3.48 (m, 2H, H-3′, H-4), 3.46 (s, 3H, OCH_3), 3.45–3.25 (m, 5H, H-2, H-2′, H-5, H-6a′, H-6b′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6 (x2), 128.5, 128.1, 128.0 (x2), 127.9, 109.2 (C-1″), 103.3 (C-1), 96.8 (C-1′), 84.1, 79.6, 78.5, 77.7, 76.3, 75.5 (C-2″), 75.1 (C-2), 74.8, 70.9, 70.4, 64.9 (C-2′), 63.5, 62.7, 60.8, 57.4 (OCH_3), 53.4, 50.9 (C-6′). ESI HRMS: m/z 766.2881 [M + Na^+]. Calcd for $C_{32}H_{41}N_9O_{12}$ 766.2875.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(β-D-ribofuranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (11). A TMSOTf (3 μL) catalyzed glycosylation of **9** (16 mg, 0.025 mmol) with **5** (22.7 mg, 0.037 mmol) was performed in CH_2Cl_2 (2.0 mL) to afford an intermediate trisaccharide, which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: m/z 1103.37 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 1 : 1–1 : 2, v/v) providing **11** (4.5 mg, 65%). $[\alpha]^{25}_D$ +25.2 (c 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.15 (m, 10H, aromatics), 5.70 (d, 1H, $J_{1',2'} = 4.0$ Hz, H-1′), 4.95–4.85 (m, 3H, $PhCH_2$), 4.79 (d, 1H, $J_{1'',2''} = 7.0$ Hz, H-1″), 4.62 (d, 1H, $J_2 = 11.5$ Hz, $PhCH_2$), 4.31 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.16–3.82 (m, 4H, H-2″, H-3, H-3″, H-4′), 3.74–3.48 (m, 7H, H-3′, H-3″, H-4, H-5a″, H-5b″, H-6a, H-6b), 3.62 (s, 3H, OCH_3), 3.45–3.25 (m, 5H, H-2, H-2′, H-5, H-6a′, H-6b′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9 (x2), 128.7, 128.3, 128.2, 128.0, 103.3 (C-1), 100.0 (C-1″), 96.8 (C-1′), 79.9, 78.7, 77.4, 75.8, 75.2 (x2), 74.9, 71.2, 71.1, 69.9, 67.4, 65.2, 64.4, 63.9, 57.3 (OCH_3), 51.3 (C-6), 51.1 (C-6′). ESI HRMS: m/z 791.2933 [M + Na^+]. Calcd for $C_{32}H_{40}N_{12}O_{11}$ 791.2940.

Methyl 3-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(β-D-ribo)-2-amino-2-deoxy-β-D-glucopyranoside (12). Trisaccharide **10** (7.0 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **12** (2.0 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.93 (d, 1H, $J_{1',2'} = 4.0$ Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''} = 2.5$ Hz, H-1″), 4.35 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.01–3.92 (m, 2H, H-2″, H-5′), 3.90–3.82 (m, 3H, H-3, H-3′, H-4″), 3.80–3.72 (m, 2H, H-4′, H-6b), 3.53 (dd, 1H, $J_{5,6a} = 2.0$ Hz, $J_{6a,6b} = 11.8$ Hz, H-6a), 3.46 (s, 3H, OCH_3), 3.40–3.25 (m, 6H, H-2′, H-3″, H-4, H-5a″, H-5b″, H-6b′), 3.14 (dd, 1H, $J_{5,6a'} = 1.8$ Hz, $J_{6a',6b'} = 12.5$ Hz, H-6a′), 2.84 (dd, 1H, $J_{1,2} = 8.5$ Hz, $J_{2,3} = 13.5$ Hz, H-2), 1.82 (s, 9H, CH_3CO_2). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.2 (CH_3CO_2), 109.5 (C-1″), 101.9 (C-1), 94.5 (C-1′), 83.6, 80.0, 77.5, 75.0 (C-2″), 74.7, 70.9, 69.3 (C-5′), 68.4, 61.9 (C-4′), 61.8 (C-6), 59.5, 59.3, 57.2 (OCH_3), 55.0, 44.6 (C-2), 40.0 (C-6′), 22.6 (CH_3CO_2).

Methyl 3-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(β-D-ribofuranosyl)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside (13). Trisaccharide **11** (12.2 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **13** (4.9 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.46 (d, 1H, $J_{1',2'} = 2.4$ Hz, H-1′), 4.55 (d, 1H, $J_{1'',2''} = 7.8$ Hz, H-1″), 4.24 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.01 (m, 1H, H-5′), 3.83–3.62 (m, 6H,

H-3, H-3′, H-3″, H-4′, H-4″, H-6b), 3.60–3.48 (m, 3H, H-2″, H-4, H-6a), 3.40 (s, 3H, OCH_3), 3.38–3.20 (m, 3H, H-2′, H-5b″, H-6b′), 3.19–3.04 (m, 3H, H-5, H-5a″, H-6a′), 2.73 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 1.76 (s, 12H, CH_3CO_2). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.2 (CH_3CO_2), 102.7 (C-1), 101.2 (C-1″), 96.1 (C-1′), 79.7, 79.0, 71.2, 70.7, 70.6, 70.2, 69.3, 69.1, 66.2, 63.3 (C-2″), 63.2, 57.5 (OCH_3), 55.5 (C-2), 53.7 (C-2′), 44.3, 40.1 (C-6), 40.0 (C-6′), 22.7 (CH_3CO_2).

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(4″-azido-4″,6″-dideoxy-β-D-glucopyranosyl)-2-azido-2-deoxy-β-D-glucopyranoside (14). A TMSOTf (5 μL) catalyzed glycosylation of **8** (31 mg, 0.047 mmol) with **6** (24 mg, 0.056 mmol) was performed in CH_2Cl_2 (2.0 mL) to afford a trisaccharide (MALDI-TOF: *m/z* 931.34 [M + Na^+]) which was de-*O*-acetylated as described. The crude material was purified by silica gel chromatography (hexane–EtOAc, 3 : 1–2 : 1, v/v) providing **14** (11.0 mg, 75%). $[\alpha]^{25}_D$ +47.8 (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H, aromatics), 5.81 (d, 1H, $J_{1′,2′} = 3.5$ Hz, H-1′), 4.95–4.85 (m, 3H, $PhCH_2$), 4.63 (d, 1H, $J_2 = 11.5$ Hz, $PhCH_2$), 4.60 (d, 1H, $J_{1″,2″} = 8.0$ Hz, H-1″), 4.32 (m, 1H, H-5′), 4.29 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.15 (t, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.06 (t, 1H, $J_{3′,4′} = J_{4′,5′} = 9.0$ Hz, H-4′), 3.97 (t, 1H, $J_{2″,3″} = J_{3″,4″} = 7.5$ Hz, H-3″), 3.74 (t, 1H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.60 (s, 3H, OCH_3), 3.58–3.44 (m, 4H, H-3′, H-5″, H-6b, H-6b′), 3.43–3.30 (m, 6H, H-2′, H-2″, H-4″, H-5, H-6a, H-6a′), 3.12 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 1.44 (d, 3H, $J = 6.5$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 137.8, 128.7, 128.3, 128.2, 128.0, 103.1 (C-1), 101.6 (C-1″), 96.4 (C-1′), 79.9, 78.9, 77.4, 75.7, 75.6, 75.5, 75.2 (x2), 75.0, 71.5, 71.1, 67.4, 65.3 (C-4″), 63.4 (C-2), 60.9 (C-2′), 57.6 (OCH_3), 51.2 (C-6′), 18.2 (CH_3). ESI HRMS: *m/z* 805.3082 [M + Na^+]. Calcd for $C_{33}H_{42}N_{12}O_{11}$ 805.3096.

Methyl 3-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(4″-azido-4″,6″-dideoxy-β-D-glucopyranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (15). A TMSOTf (5 μL) catalyzed glycosylation of **9** (15 mg, 0.024 mmol) with **6** (15 mg, 0.036 mmol) was performed in CH_2Cl_2 (2.0 mL) to afford a trisaccharide (MALDI-TOF: *m/z* 914.34 [M + Na^+]) which was de-*O*-acetylated as described. The crude material was purified by silica gel chromatography (hexane–EtOAc, 1 : 1–2 : 3, v/v) providing **15** (8.0 mg, 68%). $[\alpha]^{25}_D$ +18.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H, aromatics), 5.76 (d, 1H, $J_{1′,2′} = 3.5$ Hz, H-1′), 4.95–4.85 (m, 3H, $PhCH_2$), 4.62 (d, 1H, $J_2 = 11.5$ Hz, $PhCH_2$), 4.41 (d, 1H, $J_{1″,2″} = 8.0$ Hz, H-1″), 4.30 (m, 1H, H-5′), 4.28 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.10–4.01 (m, 2H, H-4, H-4′), 3.74–3.68 (m, 2H, H-3, H-3′), 3.60 (s, 3H, OCH_3), 3.58–3.46 (m, 3H, H-5″, H-6b, H-6b′), 3.43–3.29 (m, 6H, H-2′, H-2″, H-4″, H-5, H-6a, H-6a′), 3.11 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 1.46 (d, 3H, $J = 6.5$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 137.8, 128.7, 128.3, 128.2, 128.0, 102.8 (C-1), 100.7 (C-1″), 96.5 (C-1′), 79.5, 78.7 (C-4), 77.7 (C-4′), 76.9, 76.7, 75.5, 75.4, 75.0, 74.7, 71.6 (C-5′), 71.1, 67.5 (C-2), 65.0, 63.1, 57.1 (OCH_3), 53.6, 51.0 (C-6′), 50.7 (C-6), 18.0 (CH_3). ESI HRMS: *m/z* 830.3174 [M + Na^+]. Calcd for $C_{33}H_{41}N_{15}O_{10}$ 830.3161.

Methyl 3-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(4″-amino-4″,6″-dideoxy-β-D-glucopyranosyl)-2-amino-2-deoxy-β-D-glucopyranoside (16). Trisaccharide **14** (7.0 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **16** (2.0 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.50 (d, 1H, $J_{1′,2′} = 3.5$ Hz, H-1′), 4.60 (d, 1H, $J_{1″,2″} = 8.5$ Hz, H-1″), 4.34 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.10 (m, 1H, H-5′), 4.02–3.95 (m, 3H, H-4, H-4′, H-5″), 3.90–3.74 (m, 3H, H-3, H-3′, H-6b), 3.72–3.50 (m, 3H, H-3″, H-5, H-6a), 3.52 (s, 3H, OCH_3), 3.45–3.33 (m, 3H, H-2′, H-2″, H-6b′), 3.22 (dd, 1H, $J_{5,6a′} = 2.0$ Hz, $J_{6a′,6b′} = 12.5$ Hz, H-6a′), 2.97 (t, 1H, $J_{3″,4″} = J_{4″,5″} = 9.0$ Hz, H-4″), 2.82 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 9.0$ Hz, H-2), 1.87 (s, 12H, CH_3CO_2), 1.38 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.3 (CH_3CO_2), 103.2 (C-1), 102.1 (C-1″), 96.9 (C-1′), 81.5, 75.8, 74.9, 73.7, 72.0, 70.9, 70.1, 69.2, 59.4, 57.6 (OCH_3), 56.9 (C-2), 56.0 (C-4″), 54.1 (C-2′), 40.1 (C-6′), 23.2 (CH_3CO_2), 16.8 (CH_3).

Methyl 3-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-4-*O*-(4″-amino-4″,6″-dideoxy-β-D-glucopyranosyl)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside (17). Trisaccharide **15** (15.0 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **17** (6.6 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.38 (d, 1H, $J_{1′,2′} = 3.5$ Hz, H-1′), 4.42 (d, 1H, $J_{1″,2″} = 8.0$ Hz, H-1″), 4.28 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.07 (m, 1H, H-5′), 3.95–3.70 (m, 3H, H-4, H-4′, H-5″), 3.65–3.43 (m, 3H, H-3, H-3′, H-6b), 3.51 (s, 3H, OCH_3), 3.40–3.28 (m, 5H, H-2′, H-3″, H-5, H-6a, H-6b′), 3.19 (dd, 1H, $J_{5,6a′} = 2.0$ Hz, $J_{6a′,6b′} = 12.5$ Hz, H-6a′), 3.15 (dd, 1H, $J_{1″,2″} = 8.0$ Hz, $J_{2″,3″} = 9.8$ Hz, H-2″), 3.01 (t, 1H, $J_{3″,4″} = J_{4″,5″} = 9.5$ Hz, H-4″), 2.92 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 1.80 (s, 15H, CH_3CO_2), 1.29 (d, 3H, $J = 6.5$ Hz, CH_3). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.3 (CH_3CO_2), 103.2 (C-1), 102.5 (C-1″), 97.0 (C-1′), 81.5, 78.6, 73.6, 71.8, 71.1, 70.7, 70.0 (C-5′), 69.2, 69.0, 57.7 (OCH_3), 56.7 (C-4′), 55.8 (C-2), 54.0, 40.0 (C-6), 39.8 (C-6′), 22.7 (CH_3CO_2), 16.7 (CH_3).

Methyl 3-*O*-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glycopyranoside (18). A TMSOTf (6 μL) catalyzed glycosylation of **3** (49 mg, 0.160 mmol) with **5** (115 mg, 0.160 mmol) was performed in CH_2Cl_2 (2.0 mL) as described in the general procedures section. Chromatography of the product over silica gel using a mixture of hexane and EtOAc (5 : 1, v/v) as an eluent gave **18** (116 mg, 97%). $[\alpha]^{25}_D$ +38.7 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20–7.80 (m, 6H, PhCO), 7.60–7.20 (m, 14H, aromatics), 5.80 (t, 1H, $J_{2′,3′} = J_{3′,4′} = 7.2$ Hz, H-3′), 5.62 (s, 1H, PhCH), 5.58 (dd, 1H, $J_{1′,2′} = 1.8$ Hz, $J_{2′,3′} = 7.2$ Hz, H-2′), 5.50 (m, 1H, H-3), 5.31 (d, 1H, $J_{1′,2′} = 1.8$ Hz, H-1′), 4.55 (m, 1H, H-4′), 4.39 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.37 (m, 1H, H-6a), 4.15 (m, 1H, H-5a′), 3.90–3.64 (m, 3H, H-4, H-5b′, H-6b), 3.61 (s, 3H, OCH_3), 3.59–3.38 (m, 2H, H-2, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.5, 166.2, 165.5 (PhCO), 137.0, 133.4, 133.3, 133.2, 130.3, 130.2, 130.0, 129.6, 128.6, 128.5 (x2), 126.3, 104.1 (C-1), 101.9 (PhCH), 99.0 (C-1′), 79.3, 76.6, 68.8, 67.9, 67.1, 66.8, 66.2, 61.8, 60.7 (C-2), 57.8 (OCH_3). ESI HRMS: *m/z* 774.2385 [M + Na^+]. Calcd for $C_{40}H_{37}N_3O_{12}$ 774.2377.

Methyl 3-*O*-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-azido-2-deoxy-6-*O*-acetyl-β-D-glucopyranoside (19). Removal of the benzylidene acetal of **18** (60.0 mg, 0.08 mmol) followed by selective acetylation of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (2 : 1, v/v) as an eluent gave **19** (28 mg, 97%). $[\alpha]^{25}_{D}$ +50.2 (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–7.80 (m, 6H, PhCO), 7.60–7.20 (m, 9H, PhCO), 6.01 (s, 1H, H-1′), 5.48–5.35 (m, 2H, H-2′,3′), 5.20 (dd, 1H, $J_{3,4}$ = 1.8 Hz, $J_{2,3}$ = 7.5 Hz, H-3), 4.34–4.20 (m, 1H, H-4′), 4.21 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.15 (m, 1H, H-5a′), 3.50 (s, 3H, OCH_3), 3.48–3.41 (m, 4H, H-4, H-5b′, H-6a, H-6b), 3.29–3.22 (m, 2H, H-2, H-5), 2.08 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (CH_3CO), 165.5, 165.4, 165.3, 133.7, 133.6, 133.5, 130.1, 130.0, 129.9, 129.6, 129.3, 128.8, 128.6 (x2), 103.3 (C-1), 100.7 (C-1′), 85.5, 75.4, 70.1, 69.7, 68.6, 67.4, 64.9, 62.5 (C-2), 57.4 (OCH_3), 20.9 (CH_3CO). ESI HRMS: *m/z* 728.2159 [M + Na^+]. Calcd for $C_{35}H_{35}N_3O_{13}$ 728.2170.

Methyl 3-*O*-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (20). Removal of the benzylidene acetal of **18** (60.0 mg, 0.08 mmol) followed by selective tosylation and azido substitution of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (3 : 1, v/v) as an eluent gave **20** (18.2 mg, 68%). $[\alpha]^{25}_{D}$ + 19.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20–7.80 (m, 6H, PhCO), 7.60–7.20 (m, 9H, PhCO), 6.03 (s, 1H, H-1′), 5.47–5.36 (m, 2H, H-2′, H-3′), 5.18 (dd, 1H, $J_{3,4}$ = 1.8 Hz, $J_{2,3}$ = 8.1 Hz, H-3), 4.35–4.22 (m, 1H, H-4′), 4.20 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.10 (m, 1H, H-5a′), 3.49 (s, 3H, OCH_3), 3.46–3.39 (m, 4H, H-4, H-5b′, H-6a, H-6b), 3.30–3.25 (m, 2H, H-2, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.5, 165.4, 165.3, 133.7, 133.6, 133.5, 130.1, 130.0, 129.9, 129.6, 129.3, 128.8, 128.6 (x2), 103.3 (C-1), 100.7 (C-1′), 85.4, 75.6, 70.1, 69.6, 68.6, 67.2, 64.8, 62.4 (C-2), 57.3 (OCH_3), 51.7 (C-6). ESI HRMS: *m/z* 711.2136 [M + Na^+]. Calcd for $C_{33}H_{32}N_6O_{11}$ 711.2129.

Methyl 3-*O*-(β-D-ribofuranosyl)-4-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2-azido-2-deoxy-β-D-glucopyranoside (21). A TMSOTf (6 μL) catalyzed glycosylation of **19** (28 mg, 0.040 mmol) with **4** (24 mg, 0.044 mmol) was performed in a mixture of Et_2O–CH_2Cl_2 (4 : 1, v/v, 2.5 ml) and afforded an intermediate trisaccharide which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: *m/z* 1121.08 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 1 : 1–2 : 3, v/v) providing **21** (6.0 mg, 69%). $[\alpha]^{25}_{D}$ +16.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$): δ 7.40–7.15 (m, 10H, aromatics), 5.82 (d, 1H, $J_{1′,2′}$ = 3.0 Hz, H-1′), 5.19 (d, 1H, $J_{1″,2″}$ = 5.5 Hz, H-1″), 4.95–4.84 (m, 3H, $PhCH_2$), 4.61 (d, 1H, J_2 = 11.0 Hz, $PhCH_2$), 4.31 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.20–3.96 (m, 2H, H-4″, H-5′), 3.95–3.72 (m, 8H, H-2″, H-3, H-3″, H-4′, H-5a″, H-5b″, H-6a, H-6b), 3.62 (s, 3H, OCH_3), 3.58–3.51 (m, 2H, H-3′, H-4), 3.47–3.35 (m, 5H, H-2, H-2′, H-5, H-6a′, H-6b′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8, 137.7, 128.8, 128.3, 128.2 (x2), 103.4 (C-1), 100.3 (C-1″), 96.3 (C-1′), 80.9, 79.9, 78.5, 77.4, 75.7, 75.4, 74.7, 71.4, 69.6, 68.6, 68.1, 65.9, 64.4, 63.6 (C-2′), 61.6 (C-2), 57.6 (OCH_3), 51.4 (C-6′). ESI HRMS: *m/z* 766.2887 [M + Na^+]. Calcd for $C_{32}H_{41}N_9O_{12}$ 766.2875.

Methyl 3-*O*-(β-D-ribofuranosyl)-4-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (22). A TMSOTf (5 μL) catalyzed glycosylation of **20** (18.2 mg, 0.026 mmol) with **4** (16 mg, 0.031 mmol) was performed in a mixture of Et_2O–CH_2Cl_2 (4 : 1, v/v, 2.5 ml) and afforded an intermediate trisaccharide, which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: *m/z* 1103.37 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 2 : 1–1 : 1, v/v) providing **22** (6.6 mg, 62%). $[\alpha]^{25}_{D}$ +41.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.15 (m, 10H, aromatics), 5.82 (d, 1H, $J_{1′,2′}$ = 3.5 Hz, H-1′), 5.17 (d, 1H, $J_{1″,2″}$ = 5.5 Hz, H-1″), 4.95–4.84 (m, 3H, $PhCH_2$), 4.61 (d, 1H, J_2 = 11.5 Hz, $PhCH_2$), 4.30 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.11 (s, 1H, OH), 4.02–3.82 (m, 3H, H-4″, H-5′, H-6a), 3.80–3.64 (m, 5H, H-2″, H-3, H-3″, H-4′, H-6b), 3.63 (s, 3H, OCH_3), 3.60–3.41 (m, 7H, H-2, H-3′, H-4, H-5, H-5a″, H-5b″, H-6a′), 3.40–3.35 (m, 2H, H-2′, H-6b′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7, 137.5, 128.8, 128.7, 128.4, 128.2, 103.1 (C-1), 100.4 (C-1″), 96.3 (C-1′), 80.6, 79.8, 78.4, 77.4, 75.7, 75.5, 74.3, 71.8, 71.4, 71.3, 68.7, 68.1, 65.8, 64.4 (C-2′), 63.5 (C-2), 57.3 (OCH_3), 51.6 (C-6), 51.3 (C-6′). ESI HRMS: *m/z* 791.2934 [M + Na^+]. Calcd for $C_{32}H_{40}N_{12}O_{11}$ 791.2943.

Methyl 3-*O*-(β-D-ribofuranosyl)-4-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-2-amino-2-deoxy-β-D-glucopyranoside (23). Trisaccharide **21** (17.5 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **23** (7.2 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.68 (d, 1H, $J_{1′,2′}$ = 3.5 Hz, H-1′), 4.95 (d, 1H, $J_{1″,2″}$ = 8.5 Hz, H-1″), 4.37 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.01–3.92 (m, 2H, H-2″, H-5′), 3.92–3.72 (m, 7H, H-3, H-3′, H-4′, H-4″, H-5a″, H-5b″, H-6b), 3.71–3.60 (m, 2H, H-6a, H-6b′), 3.54 (s, 3H, OCH_3), 3.40–3.25 (m, 3H, H-2′, H-3″, H-5), 3.20–3.08 (m, 2H, H-4, H-6a′), 2.85 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 1.82 (s, 9H, CH_3CO_2). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.6 (CH_3CO_2), 102.4 (C-1″), 101.8 (C-1), 96.1 (C-1′), 84.1, 74.4, 72.6, 71.0, 70.9, 70.8, 69.8, 69.4, 66.1, 63.3, 59.8, 57.6 (OCH_3), 57.1, 53.9, 40.2 (C-6′), 23.4 (CH_3CO_2).

Methyl 3-*O*-(β-D-ribofuranosyl)-4-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside (24). Trisaccharide **22** (8.4 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (10 : 1, v/v, 2.2 mL) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **24** (4.8 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.60 (d, 1H, $J_{1′,2′}$ = 3.5 Hz, H-1′), 4.95 (d, 1H, $J_{1″,2″}$ = 7.5 Hz, H-1″), 4.37 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.98–3.86 (m, 2H, H-2″, H-5′), 3.85–3.70 (m, 7H, H-3, H-3′, H-4′, H-4″, H-5a″, H-5b″, H-6b), 3.70–3.61 (m, 2H, H-6a, H-6b′), 3.54 (s, 3H, OCH_3), 3.45–3.08 (m, 5H, H-2′, H-3″, H-4, H-5, H-6a′), 2.88 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 1.74 (s, 12H, CH_3CO_2). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.6 (CH_3CO_2), 102.8 (C-1), 101.7 (C-1″), 97.0 (C-1′), 83.5, 76.8, 70.8,

70.7, 70.6, 70.5, 69.8, 69.6, 66.1, 63.3, 57.7 (OCH_3), 56.9, 53.8, 40.9 (C-6), 40.1 (C-6′), 23.4 (CH_3CO_2).

Methyl 3-*O*-(4′-azido-2′,3′-di-*O*-acetyl-4′,6′-dideoxy-β-D-glucopyranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glycopyranoside (25). A TMSOTf (25 μL) catalyzed glycosylation of **3** (213 mg, 0.760 mmol) with **6** (378 mg, 0.912 mmol) was performed in CH_2Cl_2 (4.0 mL) as described in the general procedures section. Chromatography of the product over silica gel using a mixture of hexane and EtOAc (4 : 1, v/v) as an eluent gave **25** (394 mg, 92%). $[\alpha]^{25}_D$ +29.4 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50–7.20 (m, 5H, aromatics), 5.52 (s, 1H, PhCH), 5.05 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.3$ Hz, H-3′), 4.93 (dd, 1H, $J_{1',2'} = 8.1$ Hz, $J_{2',3'} = 9.6$ Hz, H-2′), 4.68 (d, 1H, $J_{1',2'} = 8.1$ Hz, H-1′), 4.35–4.30 (m, 1H, H-5), 4.26 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 3.77 (dd, 1H, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 3.64–3.58 (m, 2H, H-4, H-6a), 3.57 (s, 3H, OCH_3), 3.40–3.08 (m, 4H, H-3, H-4′, H-5′, H-6b), 2.04 (m, 6H, CH_3CO), 1.18 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 170.0 (CH_3CO), 137.1, 129.4, 128.4, 126.3, 103.8 (C-1), 101.6 (PhCH), 101.0 (C-1′), 79.8, 79.6, 73.9 (C-3′), 72.5 (C-5′), 70.9 (C-2′), 68.6 (C-5), 66.5, 66.2 (C-2), 65.6 (C-4′), 57.7 (OCH_3), 20.9, 20.8 (2 x CH_3CO), 18.3 (CH_3). ESI HRMS: *m/z* 585.2017 [M + Na^+]. Calcd for $C_{24}H_{30}N_6O_{10}$ 585.2023.

Methyl 3-*O*-(4′-azido-2′,3′-di-*O*-acetyl-4′,6′-dideoxy-β-D-glucopyranosyl)-2-azido-2-deoxy-6-*O*-acetyl-β-D-glucopyranoside (26). Removal of the benzylidene acetal of **25** (91 mg, 0.162 mmol) followed by selective acetylation of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (2 : 1, v/v) as an eluent gave **26** (33 mg, 87%). $[\alpha]^{25}_D$ +30.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.18 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.3$ Hz, H-3′), 4.96 (dd, 1H, $J_{1',2'} = 8.1$ Hz, $J_{2',3'} = 9.2$ Hz, H-2′), 4.58 (d, 1H, $J_{1',2'} = 8.1$ Hz, H-1′), 4.43 (dd, 1H, $J_{5,6a} = 2.7$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.39 (dd, 1H, $J_{5,6b} = 5.1$ Hz, $J_{6a,6b} = 12.4$ Hz, H-6b), 4.18 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 3.73 (bs, 1H, OH), 3.55 (s, 3H, OCH_3), 3.52–3.35 (m, 3H, H-3, H-4, H-5), 3.34–3.08 (m, 3H, H-2, H-4′, H-5′), 2.04 (m, 6H, CH_3CO), 1.18 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 170.1, 169.9 (3 x CH_3CO), 103.3 (C-1), 101.6 (C-1′), 85.6, 73.6, 73.5 (C-3′), 71.7 (C-2′), 71.3, 68.8, 65.4 (C-2), 65.2 (C-4′), 63.2 (C-6), 57.5 (OCH_3), 21.0, 20.8, 20.7 (3 x CH_3CO), 18.3 (CH_3). ESI HRMS: *m/z* 539.1827 [M + Na^+]. Calcd for $C_{19}H_{28}N_6O_{11}$ 539.1816.

Methyl 3-*O*-(4′-azido-2′,3′-di-*O*-acetyl-4′,6′-dideoxy-β-D-glucopyranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (27). Removal of the benzylidene acetal of **25** (91 mg, 0.162 mmol) followed by selective tosylation and azido substitution of the C-6 hydroxyl was performed as described in the general procedures section. Chromatography over silica gel using a mixture of hexane and EtOAc (4 : 1, v/v) as an eluent gave **27** (64 mg, 67%). $[\alpha]^{25}_D$ + 42.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.15 (t, 1H, $J_{2',3'} = 9.6$ Hz, H-3′), 4.94 (dd, 1H, $J_{1',2'} = 8.4$ Hz, $J_{2',3'} = 9.6$ Hz, H-2′), 4.58 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1′), 4.23 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 3.76 (bs, 1H, OH), 3.57 (s, 3H, OCH_3), 3.49–3.38 (m, 5H, H-3, H-4, H-5, H-5′, H-6a), 3.36–3.23 (m, 2H, H-4′, H-6b), 3.18 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.0$ Hz, H-2), 2.04 (m, 6H, CH_3CO), 1.18 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 169.9 (2 x CH_3CO), 103.2 (C-1), 101.6 (C-1′), 85.4, 75.6, 73.7 (C-3′), 71.6, 71.3, 69.8, 65.4 (C-2), 65.2 (C-4′), 57.4 (OCH_3), 51.6 (C-6), 20.9, 20.7 (2 x CH_3CO), 18.3 (CH_3). ESI HRMS: *m/z* 522.1764 [M + Na^+]. Calcd for $C_{17}H_{25}N_9O_9$ 522.1775.

Methyl 3-*O*-(4″-azido-4″,6″-dideoxy-β-D-glucopyranosyl)-4-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2-azido-2-deoxy-β-D-glucopyranoside (28). A TMSOTf (14 μL) catalyzed glycosylation of **26** (33 mg, 0.064 mmol) with **4** (55 mg, 0.096 mmol) was performed in a mixture of Et_2O–CH_2Cl_2 (2 : 1, v/v, 3.0 ml) and afforded an intermediate trisaccharide, which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: *m/z* 931.34 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 3 : 1–2 : 1, v/v) providing **28** (20.0 mg, 67%). $[\alpha]^{25}_D$ +11.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H, aromatics), 5.90 (d, 1H, $J_{1',2'} = 3.6$ Hz, H-1′), 4.86 (d, 1H, $J_{1'',2''} = 8.4$ Hz, H-1″), 4.84–4.81 (m, 3H, $PhCH_2$), 4.60 (d, 1H, $J_2 = 10.8$ Hz, $PhCH_2$), 4.28 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.04 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.97–3.70 (m, 5H, H-3′, H-4′, H-5′, H-6a, H-6b), 3.65–3.48 (m, 4H, H-3″, H-5a″, H-5b″, H-6a′), 3.58 (s, 3H, OCH_3), 3.46–3.20 (m, 6H, H-2, H-2′, H-2″, H-4, H-5, H-6b′), 3.10–3.06 (m, 2H, H-4″, OH), 1.39 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8, 137.7, 128.7 (x2), 128.3, 128.2, 103.3 (C-1), 101.5 (C-1″), 96.4 (C-1′), 79.8, 79.6, 78.7, 75.6, 75.5, 75.4, 74.9, 74.7 (C-3″), 71.4, 71.3, 69.5, 67.4, 66.4 (C-4″), 63.2, 61.5, 57.7 (OCH_3), 51.3 (C-6′), 18.3 (CH_3). ESI HRMS: *m/z* 805.3088 [M + Na^+]. Calcd for $C_{33}H_{42}N_{12}O_{11}$ 805.3096.

Methyl 3-*O*-(4″-azido-4″,6″-dideoxy-β-D-glucopyranosyl)-4-*O*-(2′,6′-diazido-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α-D-glucopyranosyl)-2,6-diazido-2,6-dideoxy-β-D-glucopyranoside (29). A TMSOTf (7 μL) catalyzed glycosylation of **27** (16 mg, 0.032 mmol) with **4** (22 mg, 0.038 mmol) was performed in a mixture of Et_2O–CH_2Cl_2 (2 : 1, v/v, 3.0 ml) and afforded an intermediate trisaccharide, which contained a small amount of impurity after silica gel column chromatography (MALDI-TOF: *m/z* 914.34 [M + Na^+]). The *O*-acetyl protecting group was removed by a standard procedure. The crude material was purified by silica gel chromatography (hexane–EtOAc, 3 : 1–1 : 1, v/v) providing **29** (8.5 mg, 81%). $[\alpha]^{25}_D$ +37.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H, aromatics), 5.89 (d, 1H, $J_{1',2'} = 3.6$ Hz, H-1′), 4.85 (d, 1H, $J_{1'',2''} = 8.4$ Hz, H-1″), 4.84–4.81 (m, 3H, $PhCH_2$), 4.59 (d, 1H, $J_2 = 10.8$ Hz, $PhCH_2$), 4.27 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 3.95–3.70 (m, 3H, H-3, H-3′, H-6a), 3.70–3.51 (m, 6H, H-3″, H-4′, H-5′, H-5a″, H-6b, H-6a′), 3.58 (s, 3H, OCH_3), 3.50–3.30 (m, 6H, H-2, H-2″, H-4, H-5, H-5b″, H-6b′), 3.24 (dd, 1H, $J_{1',2'} = 3.6$ Hz, $J_{2',3'} = 10.2$ Hz, H-2′), 3.10 (t, 1H, $J_{3'',4''} = J_{4'',5''} = 9.6$ Hz, H-4″), 1.38 (d, 3H, $J = 6.0$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6, 137.5, 128.8, 128.7, 128.3, 128.2, 102.9 (C-1), 101.4 (C-1″), 96.3 (C-1′), 79.6 (C-6a), 79.3 (C-3′), 78.6 (C-3), 77.4, 75.6, 75.5, 75.4, 74.8, 74.3, 71.7, 71.4, 71.3, 67.3, 66.3 (C-4″), 63.1 (C-2′), 57.3 (OCH_3), 51.6 (C-6), 51.3 (C-6′), 18.3 (CH_3). ESI HRMS: *m/z* 830.3155 [M + Na^+]. Calcd for $C_{33}H_{41}N_{15}O_{10}$ 830.3161.

Methyl 3-*O*-(4″-amino-4″,6″-dideoxy-β-D-glucopyranosyl)-4-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-2-amino-2-deoxy-β-D-glucopyranoside (30). Trisaccharide **28** (6.1 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate

was further hydrogenated in AcOH and H_2O (2.2 mL, 10 : 1, v/v) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **30** (1.3 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.58 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.83 (d, 1H, $J_{1'',2''}$ = 7.0 Hz, H-1″), 4.41 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.08 (t, 1H, $J_{2,3} = J_{3,4}$ = 11.5 Hz, H-3), 3.98 (m, 1H, H-6a), 3.92–3.70 (m, 4H, H-3′, H-3″, H-4′, H-6b), 3.65–3.20 (m, 6H, H-2′, H-2″, H-4, H-5, H-5′, H-6a′), 3.49 (s, 3H, OCH_3), 3.15–3.10 (m, 2H, H-5″, H-6b′), 3.04 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 11.5 Hz, H-2), 2.92 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 8.0 Hz, H-4″), 1.82 (s, 12H, CH_3CO_2), 1.30 (d, 3H, J = 5.0 Hz, CH_3). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.3 (CH_3CO_2), 101.3 (C-1), 101.0 (C-1″), 96.3 (C-1′), 81.9, 74.3, 73.7, 72.6, 72.0, 70.7, 69.6, 69.0, 59.6, 57.3 (OCH_3), 56.2 (C-2), 56.0 (C-2′), 53.7, 40.3 (C-6′), 22.8 (CH_3CO_2), 16.5 (CH_3).

Methyl 3-*O*-(4″-amino-4″,6″-dideoxy-β-D-glucopyranosyl)-4-*O*-(2′,6′-diamino-2′,6′-dideoxy-α-D-glucopyranosyl)-2,6-diamino-2,6-dideoxy-β-D-glucopyranoside (31). Trisaccharide **29** (8.5 mg) was hydrogenated in pyridine (3.0 mL) in the presence of Pd/C (20 mg) as described in the general procedures section. The intermediate was further hydrogenated in AcOH and H_2O (2.2 mL, 10 : 1, v/v) in the presence of $Pd(OH)_2$ (20 mg) to give trisaccharide **31** (3.0 mg): ^{1}H NMR (D_2O, 500 MHz) (acetate salt): δ 5.46 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.78 (d, 1H, $J_{1'',2''}$ = 7.0 Hz, H-1″), 4.34 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.09 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.0 Hz, H-3), 3.85–3.64 (m, 3H, H-3′, H-3″, H-6a), 3.56–3.32 (m, 4H, H-4, H-5′, H-5″, H-6b), 3.46 (s, 3H, OCH_3), 3.30–3.10 (m, 4H, H-2′, H-2″, H-5, H-6a′), 3.16 (dd, 1H, $J_{5',6b'}$ = 7.5 Hz, $J_{6a',6b'}$ = 8.0 Hz, H-6b′), 3.01 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 2.85 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 7.5 Hz, H-4″), 1.82 (s, 10H, CH_3CO_2), 1.30 (d, 3H, J = 5.0 Hz, CH_3). ^{13}C NMR (75 MHz, D_2O) (acetate salt) δ 181.3 (CH_3CO_2), 101.6 (C-1), 101.2 (C-1″), 96.9 (C-1′), 81.5, 75.8, 73.9, 72.8, 71.4, 70.7, 70.3, 57.9, 57.5 (OCH_3), 56.9 (C-2′), 55.5 (C-2), 41.9 (C-6), 41.3 (C-6′), 23.8 (CH_3CO_2), 17.3 (CH_3).

Acknowledgements

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

References

1 F. Walter, Q. Vicens and E. Westhof, *Curr. Opin. Chem. Biol.*, 1999, **3**, 694–704.
2 T. Hermann, *Angew. Chem., Int. Ed.*, 2000, **39**, 1891–1905.
3 R. Schroeder, C. Waldsich and H. Wank, *EMBO J.*, 2000, **19**, 1–9.
4 S. J. Sucheck and C. H. Wong, *Curr. Opin. Chem. Biol.*, 2000, **4**, 678–686.
5 M. P. Mingeot-Leclercq, Y. Glupczynski and P. M. Tulkens, *Antimicrob. Agents Chemother.*, 1999, **43**, 727–737.
6 S. B. Vakulenko and S. Mobashery, *Clin. Microbiol. Rev.*, 2003, **16**, 430–450.
7 M. I. Recht, D. Fourmy, S. C. Blanchard, K. D. Dahlquist and J. D. Puglisi, *J. Mol. Biol.*, 1996, **262**, 421–436.
8 D. Fourmy, M. I. Recht and J. D. Puglisi, *J. Mol. Biol.*, 1998, **277**, 347–362.
9 D. Fourmy, S. Yoshizawa and J. D. Puglisi, *J. Mol. Biol.*, 1998, **277**, 333–345.
10 S. R. Lynch and J. D. Puglisi, *J. Mol. Biol.*, 2001, **306**, 1037–1058.
11 L. C. Jiang and D. J. Patel, *Nat. Struct. Biol.*, 1998, **5**, 769–774.
12 A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly and V. Ramakrishnan, *Nature*, 2000, **407**, 340–348.
13 Q. Vicens and E. Westhof, *Structure*, 2001, **9**, 647–658.
14 Q. Vicens and E. Westhof, *Chem. Biol.*, 2002, **9**, 747–755.
15 Q. Vicens and E. Westhof, *J. Mol. Biol.*, 2003, **326**, 1175–1188.
16 M. Inoue, M. Nonoyama, R. Okamoto and T. Ida, *Drugs Exp. Clin. Res.*, 1994, **20**, 233–239.
17 S. Fujimura, Y. Tokue, H. Takahashi, T. Nukiwa, K. Hisamichi, T. Mikami and A. Watanabe, *J. Antimicrob. Chemother.*, 1998, **41**, 495–497.
18 J. Roestamadji, I. Grapsas and S. Mobashery, *J. Am. Chem. Soc.*, 1995, **117**, 11060–11069.
19 J. Haddad, S. Vakulenko and S. Mobashery, *J. Am. Chem. Soc.*, 1999, **121**, 11922–11923.
20 R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21–123.
21 Y. Tor, *ChemBioChem*, 2003, **4**, 998–1007.
22 C. H. Chou, C. S. Wu, C. H. Chen, L. D. Lu, S. S. Kulkarni, C. H. Wong and S. C. Hung, *Org. Lett.*, 2004, **6**, 585–588.
23 S. Jana and J. K. Deb, *Curr. Drug Targets*, 2005, **6**, 353–361.
24 G. F. Busscher, F. Rutjes and F. L. van Delft, *Chem. Rev.*, 2005, **105**, 775–791.
25 M. Fridman, V. Belakhov, L. V. Lee, F. S. Liang, C. H. Wong and T. Baasov, *Angew. Chem., Int. Ed.*, 2005, **44**, 447–452.
26 E. Riguet, J. Desire, C. Bailly and J. L. Decout, *Tetrahedron*, 2004, **60**, 8053–8064.
27 J. Li, J. H. Wang, P. G. Czyryca, H. W. Chang, T. W. Orsak, R. Evanson and C. W. T. Chang, *Org. Lett.*, 2004, **6**, 1381–1384.
28 X. J. Liu, J. R. Thomas and P. J. Hergenrother, *J. Am. Chem. Soc.*, 2004, **126**, 9196–9197.
29 S. L. Yao, P. W. M. Sgarbi, K. A. Marby, D. Rabuka, S. M. O'Hare, M. L. Cheng, M. Bairi, C. Y. Hu, S. B. Hwang, C. K. Hwang, Y. Ichikawa, P. Sears and S. J. Sucheck, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3733–3738.
30 S. H. L. Verhelst, M. E. Magnee, T. Wennekes, W. Wiedenhof, G. A. van der Marel, H. S. Overkleeft, C. A. A. van Boeckel and J. H. van Boom, *Eur. J. Org. Chem.*, 2004, 2404–2410.
31 M. C. Bryan and C. H. Wong, *Tetrahedron Lett.*, 2004, **45**, 3639–3642.
32 M. Fridman, V. Belakhov, S. Yaron and T. Baasov, *Org. Lett.*, 2003, **5**, 3575–3578.
33 P. H. Seeberger, M. Baumann, G. T. Zhang, T. Kanemitsu, E. E. Swayze, S. A. Hofstadler and R. H. Griffey, *Synlett*, 2003, 1323–1326.
34 J. B. H. Tok and L. R. Bi, *Curr. Top. Med. Chem.*, 2003, **3**, 1001–1019.
35 S. Hanessian, M. Tremblay and E. E. Swayze, *Tetrahedron*, 2003, **59**, 983–993.
36 K. B. Simonsen, B. K. Ayida, D. Vourloumis, M. Takahashi, G. C. Winters, S. Barluenga, S. Qamar, S. Shandrick, Q. Zhao and T. Hermann, *ChemBioChem*, 2002, **3**, 1223–1228.
37 D. Vourloumis, M. Takahashi, G. C. Winters, K. B. Simonsen, B. K. Ayida, S. Barluenga, S. Qamar, S. Shandrick, Q. Zhao and T. Hermann, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3367–3372.
38 X. S. Ye and L. H. Zhang, *Curr. Med. Chem.*, 2002, **9**, 929–939.
39 Y. L. Ding, S. A. Hofstadler, E. E. Swayze and R. H. Griffey, *Org. Lett.*, 2001, **3**, 1621–1623.
40 A. P. Venot, E. E. Swayze, R. H. Griffey and G. J. Boons, *ChemBioChem*, 2004, **5**, 1228–1236.
41 C. W. T. Chang, Y. Hui, B. Elchert, J. H. Wang, J. Li and R. Rai, *Org. Lett.*, 2002, **4**, 4603–4606.
42 H. G. Fletcher and R. K. Ness, *J. Am. Chem. Soc.*, 1954, **76**, 760–763.
43 I. Chiumachado, J. C. Castropalomino, O. Madrazoalonso, C. Lopeteguipalacios and V. Verezbencomo, *J. Carbohydr. Chem.*, 1995, **14**, 551–561.
44 B. Elchert, J. Li, J. Wang, Y. Hui, R. Rai, R. Ptak, P. Ward, J. Takemoto, M. Bensaci and C. W. T. Chang, *J. Org. Chem.*, 2004, **69**, 1513–1523.
45 W. A. Greenberg, E. S. Priestley, P. S. Sears, P. B. Alper, C. Rosenbohm, M. Hendrix, S. C. Hung and C. H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 6527–6541.
46 R. H. Griffey, S. A. Hofstadler, K. A. Sannes-Lowery, D. J. Ecker and S. T. Crooke, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 10129–10133.
47 L. L. Cummins, S. Chen, L. B. Blyn, K. A. Sannes-Lowery, J. J. Drader, R. H. Griffey and S. A. Hofstadler, *J. Nat. Prod.*, 2003, **66**, 1186–1190.
48 K. A. Sannes-Lowery, R. H. Griffey and S. A. Hofstadler, *Anal. Biochem.*, 2000, **280**, 264–271.
49 W. D. Wilson and K. Li, *Curr. Med. Chem.*, 2000, **7**, 73–98.
50 Q. Vicens and E. Westhof, *ChemBioChem*, 2003, **4**, 1018–1023.
51 T. Hermann and E. Westhof, *Comb. Chem. High Throughput Screening*, 2000, **3**, 219–234.
52 D. J. Ecker and R. H. Griffey, *Drug Discovery Today*, 1999, **4**, 420–429.