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Synthesis of a tetrasaccharide substrate of heparanase

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Dedicated to Professor Yongzheng Hui on the occasion of his 70th birthday

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ABSTRACT

A tetrasaccharide, corresponding to the heparan sulfate heparanase substrate, namely β-D-GlcA(2S)-(1→4)-α-D-GlcN(NS,6S)-(1→4)-β-D-GlcA-(1→4)-α-D-GlcN(NS,6S)-OMe, was synthesized in a convergent manner via coupling of a pair of the disaccharide building blocks as a key step.

1. Introduction

Heparan sulfate proteoglycans (HSPGs), consisting of a central protein core covalently linked to heparan sulfate (HS) polysaccharide chains, make up a significant proportion of the cell basement membranes and the extracellular matrix (ECM), which is composed of a network of macromolecules that serve to maintain tissue and cellular architecture.[1,2] Heparanase is a mammalian *endo*-β-D-glucuronidase that specifically cleaves HS of the HSPGs in the ECM and basal membrane, and is preferentially expressed in many tumor cells.[3] Digestion of the ECM or basal membrane by heparanase releases HS-bound growth factors that promote angiogenesis, tumor growth, progression, invasion, and metastasis. Therefore, heparanase has become an emerging target for the development of novel anti-cancer drugs.[4,5] Nevertheless, the substrate specificity of heparanase has only recently been started to be understood,[3b,6] by employing purified recombinant human heparanase and a series of the structurally defined HS fragments.[7] The tetrasaccharide fragment ΔHexUA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S) (Fig. 1) turns out to be the minimum sequence well recognized by heparanase. The availability of a good heparanase substrate would lead to the establishment of a quantitative assay of the enzyme activity thus facilitating the development of therapeutic strategies targeting this important enzyme. Herein, we report the synthesis of a tetrasaccharide, GlcA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S)-OMe (**1**, Fig. 1), where the replacement of the non-reducing end ΔHexUA with GlcA, and the installation of an anomeric α-OMe at the reducing end, would facilitate the synthesis as well as the stability of the substrate.

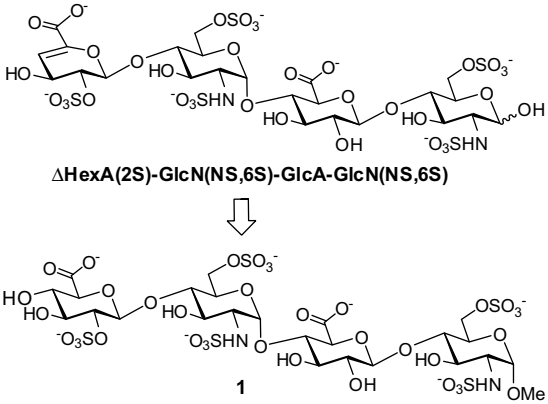

Figure 1. The minimum substrate of heparanase and its analog **1**.

2. Results and discussion

Referring to the previous protecting-group strategies for heparin synthesis,[8] we envisioned the fully protected tetrasaccharide

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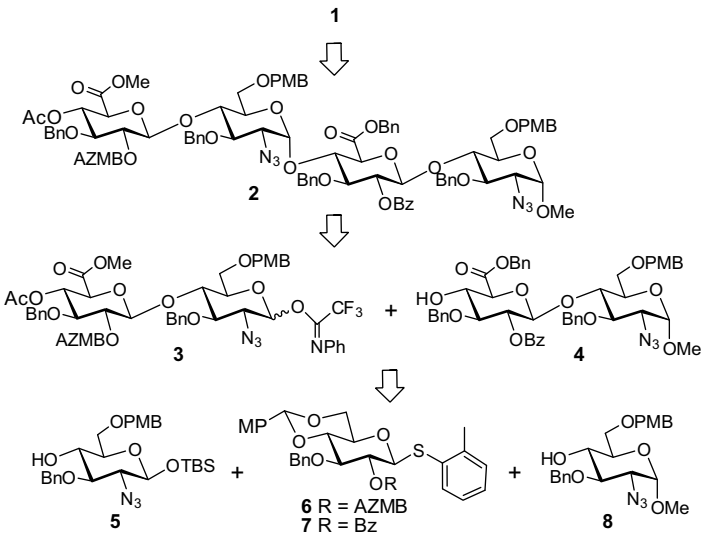
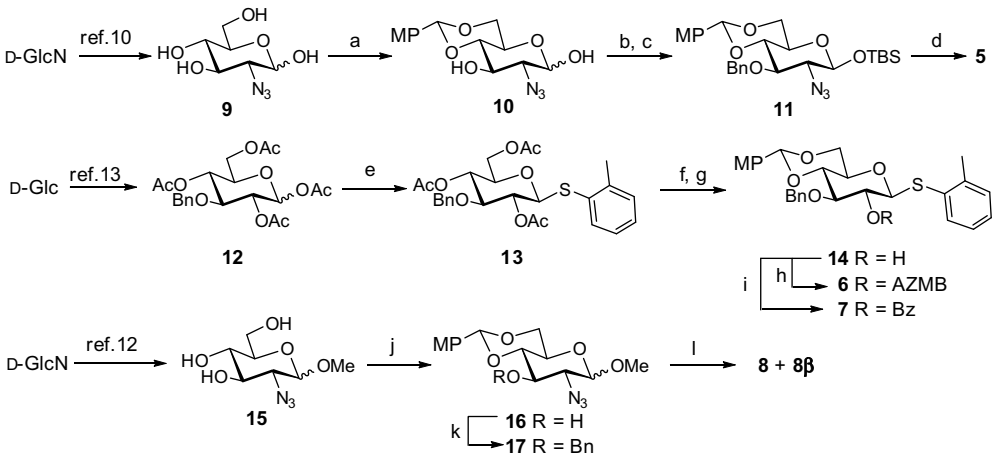


Scheme 1. Retrosynthetic plan for tetrasaccharide **1**.

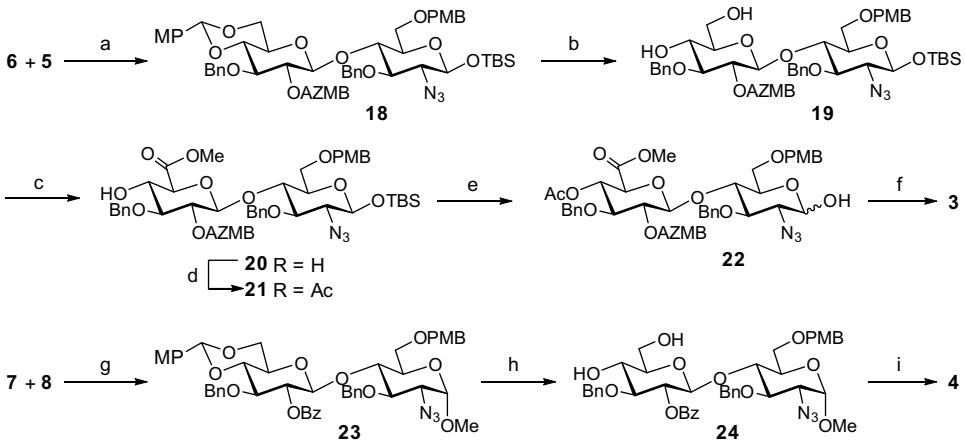
2 as an advanced precursor to the target molecule **1** (Scheme 1). Benzyl, acetyl, and benzoyl groups were used as permanent protecting groups for the hydroxyl groups; the carboxylic acid groups were blocked with methyl and/or benzyl groups; *p*-methoxybenzyl (PMB) and 2-(azidomethyl)benzoyl (AZMB)[9] groups were used for the temporary protection of the hydroxyl groups destined for sulfonation; and an azide was employed as the precursor to the sulfonated 2-amino groups of the D-GlcN residues. Considering the pseudo-symmetry of tetrasaccharide **2**, we planned to adopt a convergent 2+2 coupling of the disaccharide donor **3** and acceptor **4**. For the preparation of the disaccharides (**3** and **4**), GlcN and Glc derivatives **5**–**8** were required.

The synthesis of the GlcN and Glc derivatives **5**–**8** is depicted in Scheme 2. The 4-OH free GlcN derivatives **5** and **8** were both prepared from D-glucosamine hydrochloride in five steps (16% and 17% yields, respectively). Thus, 2-azido-2-deoxy-D-glucopyranose **9**, resulting from the diazotransfer reaction of D-glucosamine hydrochloride with triflyl azide,[10] was treated with *p*-MeOPhCH(OMe)$_2$ in the presence of *p*-TsOH in DMF to give 1,3-diol **10** (68% from GlcN). Diol **10** was subjected to selective protection with TBSCl in the presence of imidazole at low temperature (−15 °C, CH_2Cl_2) followed with BnBr and NaH in THF, providing the 3-*O*-Bn-1-β-*O*-TBS ether **11** regio- and stereoselectively in a good 69% yield. Regioselective opening of the 4,6-*O*-*p*-methoxybenzylidene acetal in **11** with $NaBH_3CN$ and TFA in a mixed solvent of CH_2Cl_2 and THF led to the desired 6-*O*-PMB derivative **5** (88% yield).[11] Treatment of the crude **9** with HCl-containing methanol gave the methyl glycoside **15** (70% for two steps),[12] which was subjected to the 4,6-*O*-*p*-methoxybenzylidene acetal formation (*p*-MePhCH(OMe)$_2$, *p*-TsOH·H_2O, DMF, 40 °C) to provide **16** (75%). Protection of the remaining 3-OH with a benzyl group (BnBr, NaH, $Bu_4N^+I^-$, THF) led to **17** (76%). Regioselective opening of the 4,6-*O*-*p*-methoxybenzylidene acetal in **17** ($NaBH_3CN$, TFA, CH_2Cl_2, THF) furnished the desired 6-*O*-PMB derivative **8**; at this stage, the two anomers (43% and 36% yields, respectively) were able to be separated by column chromatography on silica gel in nearly equal amount.

The *o*-methylphenyl thioglycosides **6** and **7** were synthesized from 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-D-glucopyranoside **12**,[13]



Scheme 2. Synthesis of the monosaccharide building blocks **5**–**8**. Reagents and conditions: (a) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH, DMF, 40 °C, 68%; (b) TBSCl, imidazole, CH_2Cl_2, −15 °C; (c) NaH, BnBr, THF, 0 °C to rt, 69% (for 2 steps); (d) $NaBH_3CN$, CF_3CO_2H, 4 Å MS, THF, CH_2Cl_2, rt, 88%; (e) *o*-methylbenzenethiol, BF_3·Et_2O, CH_2Cl_2, 0 °C to rt, 44%; (f) NaOMe, MeOH, rt, 99%; (g) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH·H_2O, CH_2Cl_2, 40 °C, 90%; (h) AZMBCl, DMAP, CH_2Cl_2, rt, 93%; (i) BzCl, Et_3N, DMAP, CH_2Cl_2, rt, 88%; (j) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH·H_2O, DMF, 40 °C, 75%; (k) BnBr, NaH, $Bu_4N^+I^-$, THF, 0 °C to rt, 76%; (l) $NaBH_3CN$, CF_3CO_2H, 4 Å MS, THF, CH_2Cl_2, rt, 43% (for **8**), 36% (for **8β**).



Scheme 3. Synthesis of the disaccharide building blocks **3** and **4**. Reagents and conditions: (a) BSP, 3 Å MS, TTBP, Tf_2O, CH_2Cl_2, −60 to −40 °C to rt, 87%; (b) AcOH, H_2O (70%), 70 °C, 93%; (c) (i) TEMPO, BAIB, CH_2Cl_2, H_2O, rt; (ii) CH_2N_2, Et_2O, rt, 88% (for 2 steps); (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 95%; (e) TBAF, AcOH, THF, −15 °C to rt, 86%; (f) ClC(=NPh)CF_3, K_2CO_3, acetone, rt, 97%; (g) BSP, 3 Å MS, TTBP, Tf_2O, CH_2Cl_2, −60 to −40 °C to rt, 90%; (h) HOAc–H_2O (70%), 70 °C, 89%; (i) TEMPO, BAIB, CH_2Cl_2, H_2O, rt; (ii) BnBr, $KHCO_3$, DMF, rt, 88% (for 2 steps).

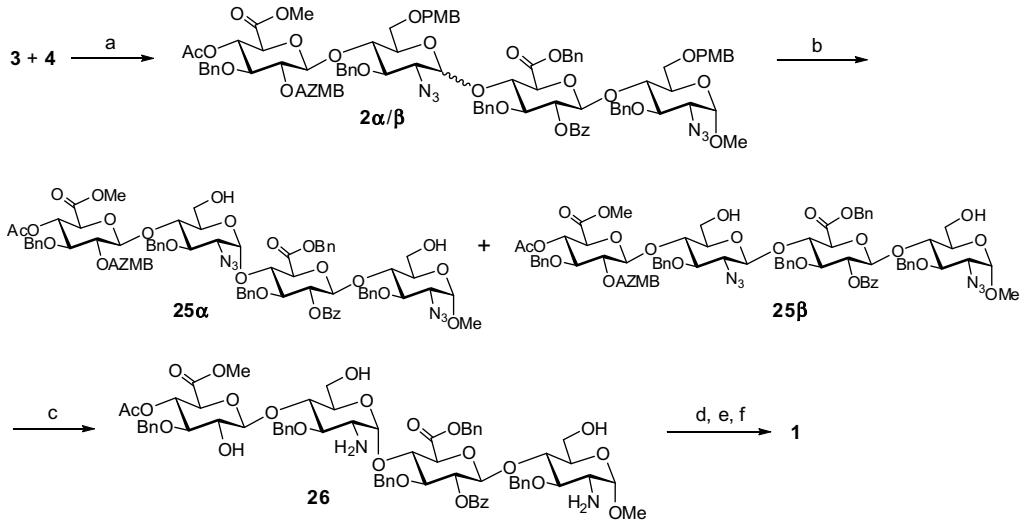
which was readily prepared from D-glucose in four steps and 51% overall yield (Scheme 3). Treatment of **12** with *o*-methylbenzenethiol in the presence of $BF_3{\cdot}Et_2O$ provided the β-thioglycoside **13** in a moderate 44% yield. Removal of the three *O*-acetyl groups in **13** (NaOMe, MeOH), followed by protection of the 4,6-di-OH with *p*-methoxybenzylidene provided **14** in 90% yield. Installation of an AZMB or a Bz group (AZMBCl or BzCl, DMAP, CH_2Cl_2) at the remaining 2-OH furnished the desired thioglycosides **6** and **7**.

The desired GlcA-(1→4)-GlcN disaccharide donor **3** and acceptor **4** were synthesized as shown in Scheme 3. Glycosidic coupling of thioglucoside **6** with *tert*-butyldimethylsilyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside **5** under the action of BSP (1-benzenesulfinyl-piperidine)–Tf_2O in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in CH_2Cl_2 provided the β-linked disaccharide **18** in a satisfactory 87% yield (H-1′, 4.55 ppm, J = 8.4 Hz).[14] Removal of the 4,6-*O*-benzylidene group in **18** with 70% HOAc gave diol **19** (93%). The resulting primary hydroxyl group was then selectively oxidized with the combination of a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) and a slight excess of [bis(acetoxy)iodo]benzene (BAIB) in a biphasic dichloromethane–water solvent system,[15] leading to the corresponding glucuronic acid derivative, which was subsequently treated with CH_2N_2 to provide the methyl uronate **20** in an excellent 88% yield. The remaining 4′-OH was protected with an acetate to give **21**. Cleavage of the 1-*O*-TBS group was achieved with tetrabutylammonium fluoride (TBAF) in the presence of acetic acid, providing lactol **22**, which was readily converted into the desired glycosyl trifluoroacetimidate **3** (ClC(=NPh)CF_3, K_2CO_3, acetone, 97%).[16]

Under similar conditions for the coupling of **6** and **5** (BSP, TTBP, Tf_2O, CH_2Cl_2), glycosylation of methyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranoside **8** with thioglycoside **7** provided the desired β-linked disaccharide **23** in an excellent 90% yield (H-1′, 4.42 ppm, J = 7.8 Hz). Similar transformations as for **18**→**20**, that is, removal of the 4′,6′-*O*-benzylidene group, selective oxidation of the resulting 6′-OH with TEMPO–BAIB, and benzyl ester formation (BnBr, $KHCO_3$, DMF) furnished the desired disaccharide acceptor **4** in good yield (78%, three steps).

With the disaccharide trifluoroacetimidate **3** and disaccharide acceptor **4** available, we then investigated the glycosylation reaction between donor **3** and acceptor **4** under conventional conditions,[17] using CH_2Cl_2 as solvent and TMSOTf as the promoter at −20 °C. However, the reaction was found to be sluggish and when the donor was completely hydrolyzed, the desired tetrasaccharide **2α/β** was isolated in only trace amounts, with the acceptor being partly recovered (Scheme 4, Table 1, entry 1). Changing the promoter to TBSOTf, we were able to obtain the tetrasaccharide in a moderate 36% yield in favor of the α anomer (α:β = 9:1) (Table 1, entry 2). Toluene has been found to be the better solvent in some condensations between a glucosyl donor and oligosaccharide acceptors bearing a 4-OH uronate moiety.[18] Indeed, the present condensation in toluene gave the tetrasaccharide **2α/β** in a better 58% yield, albeit the α:β selectivity was only moderate (4:6 α/β) (Table 1, entry 3). Lowering the temperature from −20 °C to −40 °C, we were pleased to find that the α/β ratio was improved while the yield remained good (Table 1, entry 4). We also tried homogeneous α- and β-glycosyl trifluoroacetimidates **3α** and **3β** (and the corresponding disaccharide trichloroacetimidate as well (results not shown)) as donors in the glycosylation reaction with acceptor **4** under similar conditions as for entry 3. Similar results both in yield and stereoselectivity were attained (Table 1, entries 5 and 6). It has long been known that under S_N1 conditions the effect of ethers on the oxycarbenium intermediate generally results in the formation of the thermodynamically more stable glycoside anomer, due to stabilization of the epimeric oxonium anomer by the reverse anomeric effect.[19] We thus explored the reactions with ether or THF as solvent, but the coupling yields were much lower (Table 1, entries 7 and 8).

The PMB groups in **2α/β** were selectively removed with dichlorodicyanobenzoquinone (DDQ)[20] to afford cleanly the desired α-anomer **25α** and the β-anomer **25β** via column chromatography. Subsequent reduction of the azido groups in **25α** was realized with PPh_3 in the presence of silica gel in a mixed solvent of tetrahydrofuran and water,[21] the AZMB group was simultaneously removed, furnishing tetrasaccharide **26** in 86% yield. The unprotected hydroxyl groups and amino groups by simultaneous O- and N-sulfonation afforded a mixture of partially sulfonated tetrasaccharides. On the basis of this finding, the deprotection–sulfonation sequence was altered. Thus, O-sulfonation of **26** was achieved by treatment with $SO_3{\cdot}Et_3N$ in DMF at 55 °C, followed by N-sulfonation using $SO_3{\cdot}$pyridine in triethylamine–pyridine,[22] affording the completely sulfonated tetrasaccharide, which was unambiguously confirmed by ESI-MS analyses. The sulfonated tetrasaccharide was directly subjected to saponification to remove the Ac, Bz, methyl, and benzyl groups, followed by hydrogenolytic cleavage of the benzyl groups. The target tetrasaccharide **1** was successfully obtained



Scheme 4. Synthesis of the target tetrasaccharide **1**. Reagents and conditions: (a) TBSOTf (0.2 equiv), 4 Å MS, toluene, −40 °C, 71%; (b) DDQ, CH_2Cl_2, H_2O, rt, 43% (for **25α**), 39% (for **25β**); (c) PPh_3, THF, silica gel, H_2O, rt, 86%; (d) (i) SO_3·Et_3N, DMF, 55 °C; (ii) SO_3·pyridine, Et_3N, pyridine, rt; (e) 2 N NaOH, MeOH, H_2O, rt; (f) H_2, Pd–C (10%), EtOH, rt, 36 h, 22% (for 3 steps).

Table 1
Glycosylation of disaccharide **4** with disaccharide trifluoroacetimidate **3**[a]

Entry	Donor	Promoter	Solvent	Temperature (°C)	Yield[b] (%)	α/β[c]
1	**3**	TMSOTf	CH_2Cl_2	−20	Trace	
2	**3**	TBSOTf	CH_2Cl_2	−20	36	9:1
3	**3**	TBSOTf	Toluene	−20	58	4:6
4	**3**	TBSOTf	Toluene	−40	71	6:4
5	**3α**	TBSOTf	Toluene	−40	68	6:4
6	**3β**	TBSOTf	Toluene	−40	65	6:4
7	**3**	TBSOTf	Et_2O	−20	11	
8	**3**	TBSOTf	THF	−20	Trace	

[a] Compound **3** (1.0 equiv), **4** (1.0 equiv), promoter (0.2 equiv), 4 Å molecular sieves.
[b] Isolated yields.
[c] α/β ratio was determined by ^{1}H NMR spectrometry.

after ion-exchange with Dowex 50WX4-Na^+ resin, desalting with Sephadex G-15, and lyophilization in 22% yield (for three steps).

In summary, tetrasaccharide **1**, corresponding to the heparan sulfate sequence heparanase substrate, namely β-D-GlcA(2S)-(1→4)-α-D-GlcN(NS,6S)-(1→4)-β-D-GlcA-(1→4)-α-D-GlcN(NS,6S)-OMe, has been synthesized in a convergent manner via coupling of a pair of the disaccharide building blocks as the key step. The present synthesis requires a total of 43 steps, with the longest linear sequence of 21 steps and in 0.5% overall yield from D-glucose. However, the 2+2 glycosylation in building the GlcN-α-(1→4)-GlcA linkage demands further improvement.

3. Experimental

3.1. General methods[23]

Solvents were purified in the usual way. Thin layer chromatography (TLC) was performed on precoated plates of Silica Gel HF254 (0.5 mm, Yantai, China). Flash column chromatography was performed on Silica Gel H (10–40 μm, Yantai, China). Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. NMR spectra were recorded on a Bruker AM 300 spectrometer with Me_4Si as the internal standard. *J* values were given in Hertz. Mass spectra were obtained using HP5989A or a VG Quatro mass spectrometer. Elemental analyses were performed on a Perkin–Elmer Model 2400 instrument.

3.1.1. 2-Azido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α/β-D-glucopyranose (10)

Crude **9**, prepared from D-glucosamine hydrochloride (50.0 g, 0.23 mol),[10] was dissolved in DMF (150 mL), and *p*-TsOH (2.0 g) and *p*-methoxybenzaldehyde dimethyl acetal (47.0 mL, 0.28 mol) were added. After stirring at 40 °C under reduced pressure for 8 h, the mixture was concentrated in vacuo. The obtained anomers were purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to afford **10** (19.8 g, 68%) as a white solid. R_f = 0.52 (1:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, CD_3OD): δ 7.43–7.38 (m, 2H), 6.90–6.86 (m, 2H), 5.52 (s, 1H), 5.20 (d, 0.5H, *J* = 3.3 Hz), 4.88 (s, 2H), 4.62 (d, 0.5H, *J* = 8.1 Hz), 4.22 (dd, 0.5H, *J* = 10.8, 5.4 Hz), 4.15 (dd, 0.5H, *J* = 9.9, 5.1 Hz), 4.07 (t, 0.5H, *J* = 9.9 Hz), 4.01–3.93 (m, 0.5H), 3.78 (s, 3H), 3.71 (t, 0.5H, *J* = 10.2 Hz), 3.58 (t, 0.5H, *J* = 9.3 Hz), 3.47 (t, 1H, *J* = 9.0 Hz), 3.30 (br, 1H), 3.23–3.16 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 161.6, 131.5, 131.4, 128.9, 128.9, 114.4, 103.1, 103.0, 97.9, 94.1, 83.5, 82.3, 73.2, 70.3, 70.0, 69.6, 67.7, 65.8, 63.8, 55.7; ESI-MS: 324.2 $[M+H]^+$; Anal. Calcd for $C_{14}H_{17}N_3O_6$: C, 52.01; H, 5.30; N, 13.00. Found: C, 57.76; H, 5.30; N, 12.77; IR (thin film in NaCl) ν_{max} = 2124, 1246, 1089, 985 cm^{-1}.

3.1.2. *tert*-Butyldimethylsilyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (11)

To a cooled solution of compound **10** (13.0 g, 0.040 mol) and imidazole (6.8 g, 0.10 mol) in dry CH_2Cl_2 (40.0 mL) at −15 °C, TBSCl

(6.7 g, 0.044 mol) was added. After stirring for 2.5 h, the reaction mixture was quenched by the addition of H_2O (40.0 mL). The organic phase was separated and the remaining aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with brine, dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (8:1 petroleum ether–EtOAc) to give *tert*-butyldimethylsilyl 2-azido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[24] (14.2 g, 81%) as a white solid. To a cooled solution of the above product (11.6 g, 0.027 mol) in dry THF (100.0 mL) at 0 °C, NaH (60% dispersion in mineral oil, 2.13 g, 0.053 mol) was added. After the mixture had been stirred for 1 h, benzyl bromide (4.8 mL, 0.040 mol) was added. Stirring continued at room temperature for 24 h, the reaction mixture was quenched by the addition of CH_3OH (2.5 mL). The resulting mixture was filtered through a pad of Celite and the filtrate concentrated in vacuo. The residue was purified by silica gel column chromatography (25:1 petroleum ether–EtOAc) to afford **11** (12.1 g, 85%) as a white solid. R_f = 0.82 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −89.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–7.25 (m, 7H), 6.90 (d, 2H, *J* = 8.7 Hz), 5.52 (s, 1H), 4.89 (d, 1H, *J* = 11.1 Hz), 4.78 (d, 1H, *J* = 11.3 Hz), 4.58 (d, 1H, *J* = 7.5 Hz), 4.27 (dd, 1H, *J* = 11.8, 5.1 Hz), 3.81 (s, 3H), 3.77–3.66 (m, 2H, *J* = 9.9, 5.1 Hz), 3.50 (t, 1H, *J* = 9.3 Hz), 3.41–3.33 (m, 2H), 0.93 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.0, 138.0, 129.6, 128.3, 128.1, 127.8, 127.3, 113.6, 101.3, 97.4, 81.53 78.8, 74.8, 68.7, 68.5, 66.3, 55.3, 25.5, 17.9, −4.4, −5.2; Anal. Calcd for $C_{27}H_{37}N_3O_6Si$: C, 61.46; H, 7.07; N, 7.96. Found: C, 61.71; H, 7.15; N, 7.66; IR (thin film in NaCl) ν_{max} = 2111, 1614, 1515, 1252, 1078 cm^{-1}.

3.1.3. *tert*-Butyldimethylsilyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (5)

A mixture of compound **11** (200 mg, 0.38 mmol), $NaBH_3CN$ (238 mg, 3.8 mmol), and freshly activated 4 Å molecular sieves (500 mg) in dry THF (3.8 mL) and CH_2Cl_2 (3.8 mL) was stirred at room temperature under argon for 40 min. Then CF_3CO_2H (0.55 mL, 7.6 mmol) was added dropwise. After stirring 9 h at room temperature, the reaction mixture was quenched by addition of a satd aq $NaHCO_3$ (50.0 mL). The mixture was filtered through a pad of Celite. The organic phase was separated and the remaining aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with brine, dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (8:1 petroleum ether–EtOAc) to give monosaccharide **5** (176 mg, 88%) as a colorless syrup. R_f = 0.56 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −31.1 (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.30 (m, 5H), 7.23 (d, 2H, *J* = 8.7 Hz), 6.87 (d, 2H, *J* = 8.4 Hz), 4.90 (d, 1H, *J* = 11.1 Hz), 4.77 (d, 1H, *J* = 11.1 Hz), 4.53–4.49 (m, 3H), 3.79 (s, 3H), 3.68–3.59 (m, 3H), 3.42–3.37 (m, 1H), 3.33–3.18 (m, 2H), 2.72 (br, 1H), 0.93 (d, 9H, *J* = 7.8 Hz), 0.17 (s, 3H), 0.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 138.2, 129.7, 129.3, 128.5, 128.0, 127.9, 113.8, 97.2, 82.2, 74.9, 73.8, 73.3, 72.1, 70.1, 68.0, 55.2, 25.6, 17.9, −4.3, −5.3; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{27}H_{39}N_3O_6SiNa$, 552.2506; found, 552.2504; IR (thin film in NaCl) ν_{max} = 2111, 1614, 1515, 1251, 1075 cm^{-1}.

3.1.4. 2-Methylphenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-β-D-glucopyranoside (13)

To a solution of **12** (5.1 g, 12.0 mmol) and 2-methylbenzenethiol (2.8 mL, 23.0 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C was added $BF_3{\cdot}Et_2O$ (1.7 mL, 13.0 mmol). After stirring at rt for 24 h, the reaction mixture was quenched by the addition of Et_3N (1.5 mL). The mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (5:1 petroleum ether–EtOAc) to give **13** (2.56 g, 44%) as a yellow solid. R_f = 0.60 (5:2 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −12.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, 1H, *J* = 6.9 Hz), 7.32–7.13 (m, 8H), 5.16 (d, 1H, *J* = 9.6 Hz), 5.09 (d, 1H, *J* = 9.6 Hz), 4.65–4.57 (m, 3H), 4.21–4.09 (m, 2H), 3.72 (t, 1H, *J* = 9.3 Hz), 3.63–3.58 (m, 1H), 2.39 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 169.3, 169.2, 139.8, 137.6, 132.8, 132.2, 130.2, 128.4, 128.0, 127.8, 127.7, 126.5, 86.7, 81.4, 75.9, 74.1, 71.3, 69.6, 62.5, 20.9, 20.8, 20.7; Anal. Calcd for $C_{26}H_{30}O_8S$: C, 62.14; H, 6.02. Found: C, 61.79; H, 5.91; IR (thin film in NaCl) ν_{max} = 1741, 1234, 1040 cm^{-1}.

3.1.5. 2-Methylphenyl 3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-1-thio-β-D-glucopyranoside (14)

To a solution of **13** (1.0 g, 2.0 mmol) in CH_3OH (20 mL) at room temperature was added CH_3ONa (46 mg, 2.0 mmol). After stirring at room temperature for 5 h, the reaction mixture was quenched by the addition of H^+-ion-exchange resin (1.5 g). The mixture was filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give 2-methylphenyl 3-*O*-benzyl-1-thio-β-D-glucopyranoside (750 mg, 99%) as a white solid. R_f = 0.40 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{25}$ −85.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62–7.12 (m, 9H), 4.96–4.84 (m, 5H), 4.65 (d, 1H, *J* = 8.7 Hz), 3.88–3.83 (m, 1H), 3.69–3.64 (m, 1H), 3.50–3.39 (m, 2H), 3.37–3.30 (m, 2H), 2.41 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 140.4, 139.9, 135.2, 132.2, 131.1, 129.2, 129.1, 128.5, 128.1, 127.7, 89.5, 88.0, 82.0, 76.4, 74.3, 71.2, 62.8, 21.0; Anal. Calcd for $C_{20}H_{24}O_5S$: C, 63.81; H, 6.43. Found: C, 63.61; H, 6.51; IR (thin film in NaCl) ν_{max} = 3306, 1123, 1037, 745 cm^{-1}. The same procedure described for the preparation of compound **10** (from compound **9**) was employed for the preparation of **14** from 2-methylphenyl 3-*O*-benzyl-1-thio-β-D-glucopyranoside. Purification by silica gel column chromatography (6:1:1 petroleum ether–EtOAc–CH_2Cl_2) gave monosaccharide **14** (1.0 g, 90%) as a white solid. R_f = 0.80 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −40.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, 1H, *J* = 7.2 Hz), 7.38–7.16 (m, 10H), 6.90 (d, 1H, *J* = 8.7 Hz), 5.52 (s, 1H), 4.94 (d, 1H, *J* = 11.7 Hz), 4.78 (d, 1H, *J* = 11.7 Hz), 4.63 (d, 1H, *J* = 9.3 Hz), 4.33 (dd, 2H, *J* = 10.5, 5.1 Hz), 3.80 (s, 3H), 3.75 (t, 1H, *J* = 10.2 Hz), 3.68–3.45 (m, 4H), 2.64 (br, 1H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 160.0, 140.3, 138.1, 133.1, 131.3, 130.4, 129.6, 128.4, 128.2, 128.1, 127.8, 127.3, 126.6, 113.5, 101.2, 88.5, 81.6, 80.9, 74.7, 72.5, 70.5, 68.5, 55.2, 21.018; Anal. Calcd for $C_{28}H_{30}O_6S$: C, 67.99; H, 6.11. Found: C, 68.24; H, 6.16; IR (thin film in NaCl) ν_{max} = 3294, 1517, 1251, 1070 cm^{-1}.

3.1.6. 2-Methylphenyl 2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-1-thio-β-D-glucopyranoside (6)

2-(Azidomethyl)benzoic acid (286 mg, 1.6 mmol) was dissolved in $CHCl_3$ (2.0 mL), and thionyl chloride (0.36 mL, 4.8 mmol) was added. The reaction mixture was heated to reflux for 5 h, cooled, and concentrated in vacuo using a base trap. The resulting residue was coevaporated with toluene (3 × 15 mL) to yield the crude 2-(azidomethyl)benzoyl chloride. A solution of compound **14** (266 mg, 0.54 mmol) in dry CH_2Cl_2 (2.5 mL) was stirred at room temperature, and DMAP (330 mg, 2.7 mmol) was added, followed by a solution of the crude 2-(methylazido)benzoyl (AZMB) chloride in CH_2Cl_2 (1.0 mL). After stirring at room temperature under argon for 14 h, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed twice with satd aq $NaHCO_3$ and once with water. The organic layer was dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (12:1:2 petroleum ether–EtOAc–CH_2Cl_2) to give compound **6** (326 mg, 93%) as a white solid. R_f = 0.45 (4:1

petroleum ether–EtOAc); $[\alpha]_D^{25}$ +44.6 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, 1H, *J* = 7.8 Hz), 7.59–7.37 (m, 6H), 7.16–7.11(m, 8H), 6.91 (d, 2H, *J* = 9.0 Hz), 5.58 (s, 1H), 5.36 (t, 1H, *J* = 10.2 Hz), 4.88–4.82 (m, 2H), 4.76 (d, 2H, *J* = 6.3 Hz), 4.65 (d, 1H, *J* = 12.0 Hz), 4.37 (dd, 1H, *J* = 10.5, 5.1 Hz), 3.90–3.83 (m, 3H), 3.80 (s, 3H), 3.56–3.50 (m, 1H), 2.25 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.9, 160.0, 139.9, 137.8, 137.7, 132.9, 132.3, 132.3, 130.9, 130.3, 129.5, 129.3, 128.1, 128.1, 127.9, 127.8, 127.6, 127.3, 126.6, 113.6, 101.2, 87.2, 81.4, 79.4, 74.2, 71.9, 70.5, 68.4, 55.2, 52.8, 20.8; Anal. Calcd for $C_{35}H_{34}O_7S$: C, 66.14; H, 5.40; N, 6.43. Found: C, 66.15; H, 5.52; N, 6.44; IR (thin film in NaCl) ν_{max} = 2104, 1726, 1517, 1249 cm^{-1}.

3.1.7. 2-Methylphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-1-thio-β-D-glucopyranoside (7)

A solution of compound **14** (1.0 g, 2.0 mmol) and DMAP (50 mg) in dry CH_2Cl_2 (10.0 mL) was stirred at room temperature, and Et_3N (1.5 mL, 10.2 mmol) was added, followed by addition of BzCl (0.5 mL, 4.1 mmol). After stirring under argon for 12 h, the reaction mixture was diluted with CH_2Cl_2 (100 mL), and washed twice with satd aq $NaHCO_3$ and once with water. The organic layer was dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (10:1 petroleum ether–EtOAc) to give compound **7** (1.1 g, 88%) as a white solid. R_f = 0.35 (5:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ +51.3 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, 2H, *J* = 7.5 Hz), 7.60 (t, 1H, *J* = 7.2 Hz), 7.48–7.43 (m, 5H), 7.23–7.08 (m, 8H), 6.91 (d, 2H, *J* = 9.0 Hz), 5.57 (s, 1H), 5.41–5.35 (m, 1H), 4.81 (d, 1H, *J* = 7.5 Hz), 4.80 (d, 1H, *J* = 11.7 Hz), 4.67 (d, 1H, *J* = 11.7 Hz), 4.36 (dd, 1H, *J* = 10.5, 4.8 Hz), 3.88–3.82 (m, 3H), 3.80 (s, 3H), 3.56–3.50 (m, 1H), 2.23 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.0, 160.0, 140.1, 137.7, 133.2, 132.7, 132.5, 130.3, 129.9, 129.6, 128.3, 128.1, 128.0, 127.5, 127.3, 126.5, 113.6, 110.2, 87.5, 81.3, 79.2, 74.1, 72.0, 70.5, 68.5, 55.2, 20.8; Anal. Calcd for $C_{35}H_{34}O_7S$: C, 70.21; H, 5.72. Found: C, 69.78; H, 5.65; IR (thin film in NaCl) ν_{max} = 1723, 1294, 1097 cm^{-1}.

3.1.8. Methyl 2-azido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α/β-D-gluco-pyranose (16)

The same procedure described for the preparation of compound **10** (from compound **9**) was employed for the preparation of **16** from **15**. Purification by silica gel column chromatography (4:1 petroleum ether–EtOAc) gave compound **16** (2.6 g, 75%) as a white solid. R_f = 0.40 (3:1 petroleum ether–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): 7.43–7.39 (m, 2H), 6.90 (d, 2H, *J* = 7.2 Hz), 5.50 (s, 1H), 4.80 (d, 0.4H, *J* = 3.0 Hz), 4.36–425 (m, 1.7H), 4.18 (t, 0.5H, *J* = 9.3 Hz), 3.92–3.63 (m, 1.8H), 3.81 (s, 3.0H), 3.60 (s, 1.8H), 3.51 (d, 0.9H, *J* = 9.0 Hz), 3.45 (s, 1.5H), 3.43–3.31 (m, 1.6H), 2.78 (br s, 0.9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.35, 129.2, 127.5, 127.5, 103.4, 102.0, 101.8, 99.3, 81.7, 80.5, 72.0, 69.0, 68.7, 68.4, 66.3, 66.1, 63.2, 62.2, 57.4, 55.4, 55.2; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{15}H_{20}N_3O_6$, 338.1352; found, 338.1354.

3.1.9. Methyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α/β-D-glucopyranose (17)

The same procedure described for the preparation of compound **11** (from compound **10**) was employed for the preparation of **17** from **16**. Purification by silica gel column chromatography (10:1 petroleum ether–EtOAc) gave compound **17** (881 mg, 76%) as a white solid. R_f = 0.50 (5:1 petroleum ether–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): 7.43–7.27 (m, 7H), 6.90 (d, 2H, *J* = 8.7 Hz), 5.54 (s, 1H), 4.95–4.89 (m, 1H), 4.80–4.77 (m, 1.5H), 4.36–4.24 (m, 1.5H), 4.05 (t, 0.5H, *J* = 10.2 Hz), 3.82 (s, 3H), 3.87–3.66 (m, 2.5H), 3.59–3.53 (m, 1.8H), 3.48–3.37 (m, 2.2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.0, 137.7, 129.5, 128.3, 128.2, 127.9, 127.2, 113.6, 103.3, 101.2, 81.5, 79.0, 74.9, 68.4, 66.1, 66.0, 57.4, 55.2; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{22}H_{25}N_3O_6Na$, 452.1743; found, 450.1637.

3.1.10. Methyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (8) and methyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (8β)

The same procedure described for the preparation of compound **5** (from compound **11**) was employed for the preparation of **8** and **8β** from **17**. Purification by silica gel column chromatography (7:1 petroleum ether–EtOAc) gave methyl glycoside acceptor **8** (203 mg, 43%) and compound **8β** (170 mg, 36%) as colorless syrups. Compound **8**: R_f = 0.35 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{22}$ +51.1 (*c* 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): 7.39–7.23 (m, 7H), 6.87 (d, 2H, *J* = 9.0 Hz), 4.91 (d, 1H, *J* = 8.1 Hz), 4.82–4.78 (m, 2H), 4.50 (AB, d, 2H, *J* = 11.7 Hz), 3.80 (s, 3H), 3.83–3.62 (m, 5H), 3.42 (s, 3H), 3.37 (dd, 1H, *J* = 9.9, 3.6 Hz), 2.51 (d, 1H, *J* = 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 139.0, 129.7, 129.3, 128.5, 128.1, 127.9, 113.8, 98.7, 79.9, 75.0, 73.3, 72.2, 69.8, 69.4, 62.9, 55.2, 55.2; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{22}H_{27}N_3O_6Na$, 452.1798; found, 452.1791; IR (cm^{-1}) ν_{max} = 2112, 1613, 1515, 1250, 1075. Compound **8β**: R_f = 0.33 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −42.5 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): 7.41–7.24 (m, 7H), 6.89 (d, 2H, *J* = 8.4 Hz), 4.91 (d, 1H, *J* = 11.4 Hz), 4.80 (d, 1H, *J* = 11.4 Hz), 4.51 (AB, d, 2H, *J* = 11.4 Hz), 4.18 (d, 1H, *J* = 7.8 Hz), 3.80 (s, 3H), 3.70 (d, 2H, *J* = 4.5 Hz), 3.61 (t, 1H, *J* = 9.3 Hz), 3.56 (s, 3H), 3.43–3.24 (m, 3H), 2.77 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 138.0, 129.6, 129.4, 128.5, 128.1, 128.0, 113.8, 102.9, 82.5, 75.1, 73.7, 73.4, 72.3, 69.8, 65.6, 57.1, 55.2; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{22}H_{27}N_3O_6Na$, 452.1798; found, 452.1781; IR (thin film in NaCl) ν_{max} = 2112, 1613, 1515, 1250, 1075 cm^{-1}.

3.1.11. *tert*-Butyldimethylsilyl (2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranosyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (18)

A solution of compound **6** (200 mg, 0.31 mmol), BSP (70 mg, 0.34 mmol), and TTBP (152 mg, 0.61 mmol) in dry CH_2Cl_2 (3.0 mL) was stirred for 30 min under argon over activated 3 Å molecular sieves (300 mg). The mixture was cooled to −60 °C, and Tf_2O (5 μL, 0.037 mmol) was added. The temperature was raised to −40 °C and stirring continued for 15 min. Then a solution of the compound **5** (103 mg, 0.19 mmol) in dry CH_2Cl_2 (0.5 mL) was added, and the resulting mixture was allowed to warm slowly to room temperature. The reaction mixture was quenched by the addition of Et_3N (0.5 mL) and the mixture filtered through a pad of Celite. The filtrate was concentrated and purified by silica gel column chromatography (10:1 petroleum ether–EtOAc) to afford disaccharide **18** (180 mg, 87%) as a colorless syrup. R_f = 0.55 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{25}$ −4.2 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, 1H, *J* = 8.4 Hz), 7.63–7.52 (m, 2H), 7.44–7.30 (m, 8H), 7.20–7.11 (m, 7H), 6.93–6.87 (m, 4H), 5.49 (s, 1H), 5.18 (t, 1H, *J* = 8.1 Hz), 4.94 (d, 1H, *J* = 3.0 Hz), 4.90 (s, 1H), 4.84 (d, 1H, *J* = 12.0 Hz), 4.75 (d, 1H, *J* = 10.8 Hz), 4.68–4.53 (m, 4H), 4.38 (d, 1H, *J* = 6.0 Hz), 4.38 (d, 1H, *J* = 12.0 Hz), 4.17 (dd, 1H, *J* = 10.2, 4.2 Hz), 4.00–3.94 (m, 1H), 3.96 (s, 3H), 3.83 (s, 3H), 3.70 (d, 1H, *J* = 9.0 Hz), 3.66 (d, 1H, *J* = 9.6 Hz), 3.55 (dd, 1H, *J* = 11.7, 2.7 Hz), 3.48 (t, 1H, *J* = 10.2 Hz), 3.37 (d, 1H, *J* = 11.4 Hz), 3.30–3.25 (m, 3H), 3.15 (br d, 1H, *J* = 9.3 Hz), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.5, 156.0, 159.3, 138.5, 138.2, 137.9, 133.0, 130.6, 130.0, 129.8, 129.6, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.3, 113.8, 113.5, 101.1, 100.4, 96.9, 81.7, 80.6, 78.4, 76.1, 75.1, 74.6, 74.1, 73.7, 73.0, 68.4, 68.1, 67.1, 66.0, 55.2, 55.2, 52.8, 25.5, 17.9, −4.4, −5.3; Anal.

Calcd for $C_{56}H_{66}N_6O_{13}Si$: C, 63.50; H, 6.28; N, 7.93. Found: C, 63.52; H, 6.41; N, 7.73; IR (thin film in NaCl) ν_{max} = 2932, 2860, 2110, 1733, 1614, 1516, 1251, 1072 cm^{-1}.

3.1.12. *tert*-Butyldimethylsilyl (2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (19)

A solution of compound **18** (120 mg, 0.11 mmol) in 70% aqueous HOAc (6.0 mL) was heated at 70 °C for 30 min and then concentrated. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to provide **19** (99 mg, 93%) as a colorless syrup. R_f = 0.50 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ −0.36 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 1H, *J* = 8.4 Hz), 7.63–7.52 (m, 2H), 7.42–7.30 (m, 6H), 7.26–7.16 (m, 7H), 6.93 (d, 2H, *J* = 8.4 Hz), 5.14 (t, 1H, *J* = 8.4 Hz), 4.94 (d, 1H, *J* = 9.0 Hz), 4.90 (d, 1H, *J* = 5.4 Hz), 4.76 (d, 1H, *J* = 10.8 Hz), 4.69–4.61 (m, 4H), 4.55 (d, 1H, *J* = 7.8 Hz), 4.40–4.38 (m, 1H), 4.25 (d, 1H, *J* = 11.7 Hz), 3.94 (br t, 1H, *J* = 9.3 Hz), 3.80 (s, 3H), 3.68–3.55 (m, 3H), 3.48–3.36 (m, 3H), 3.30–3.27 (m, 2H), 3.24–3.13 (m, 2H), 2.60 (br s, 1H), 1.87 (br s, 1H), 0.90 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.4, 159.3, 138.4, 137.9, 133.2, 130.5, 129.9, 129.9, 129.8, 128.4, 128.4, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 113.9, 99.8, 96.9, 83.0, 80.6, 75.8, 75.2, 75.1, 74.8, 74.5, 73.7, 73.1, 70.8, 68.2, 67.1, 62.0, 55.2, 52.9, 25.5, 17.9, −4.4, −5.3; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{48}H_{60}N_6O_{12}SiNa$, 963.3936; found, 963.3938; IR (thin film in NaCl) ν_{max} = 2931, 2860, 2111, 1731, 1252, 1068 cm^{-1}.

3.1.13. *tert*-Butyldimethylsilyl (methyl 2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (20)

Compound **19** (140 mg, 0.15 mmol) was dissolved in CH_2Cl_2–water (2:1 v/v, 2.1 mL), and TEMPO (5 mg, 0.03 mmol) and BAIB (120 mg, 0.37 mmol) were added subsequently. After the biphasic mixture was stirred vigorously for 1 h, the reaction mixture was quenched by the addition of satd aq $NaHSO_3$ (5.0 mL). The layers were separated and the aqueous layer was acidified with 1 M aqueous HCl, and extracted three times with CH_2Cl_2 (100 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, and then filtered and concentrated in vacuo to yield the corresponding crude glucuronic acid. The crude glucuronic acid was dissolved in ether (10 mL) and treated with a solution of diazomethane in ether (~0.3 M, 2.0 mL). After stirring for 10 min, the yellow solution was treated with a few drops of acetic acid until the mixture turned colorless. The solvents were evaporated and the residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to afford disaccharide **20** (126 mg, 88%) as a white solid. R_f = 0.40 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{25}$ +1.1 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, 1H, *J* = 7.8 Hz), 7.63–7.52 (m, 2H), 7.41–7.20 (m, 8H), 7.14 (br s, 5H), 6.91 (d, 2H, *J* = 8.4 Hz), 5.14 (t, 1H, *J* = 9.0 Hz), 4.99 (d, 1H, *J* = 11.4 Hz), 4.91 (d, 1H, *J* = 14.7 Hz), 4.83 (d, 1H, *J* = 11.4 Hz), 4.72 (d, 1H, *J* = 11.1 Hz), 4.66–4.56 (m, 4H), 4.39 (br d, 1H, *J* = 6.6 Hz), 4.19 (d, 1H, *J* = 12.0 Hz), 4.03–3.95 (m, 2H), 3.81 (s, 3H), 3.68–3.55 (m, 2H), 3.60 (s, 3H), 3.46 (t, 1H, *J* = 8.7 Hz), 3.41 (br d, 1H, *J* = 6.6 Hz), 3.31–3.29 (m, 2H), 3.15 (br s, 2H), 0.90 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.6, 164.4, 159.3, 138.7, 138.3, 137.9, 133.1, 130.6, 129.9, 129.7, 128.2, 128.1, 127.9, 127.7, 127.6, 127.3, 113.9, 100.1, 97.0, 81.2, 80.7, 76.2, 74.8, 74.6, 74.4, 73.8, 73.0, 72.9, 72.4, 68.1, 67.1, 55.2, 52.8, 52.7, 25.5, 17.9, −4.4, −5.3; Anal. Calcd for $C_{49}H_{60}N_6O_{13}Si$: C, 60.73; H, 6.24; N, 8.67. Found: C, 60.35; H, 6.32; N, 8.45; IR (thin film in NaCl) ν_{max} = 2955, 2932, 2860, 2111, 1733, 1252, 1071 cm^{-1}.

3.1.14. *tert*-Butyldimethylsilyl (methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (21)

To a solution of compound **20** (126 mg, 0.13 mmol) and DMAP (4 mg) in dry CH_2Cl_2 (2.0 mL), Et_3N (0.4 mL) was added followed by addition of Ac_2O (0.2 mL). The reaction mixture was stirred at room temperature under argon for 2 h and then concentrated. The residue was purified by silica gel column chromatography (4:1 petroleum ether–EtOAc) providing **21** (125 mg, 95%) as a colorless syrup. R_f = 0.75 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{28}$ −7.3 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (d, 1H, *J* = 8.1 Hz), 7.61–7.55 (m, 2H), 7.43–6.93 (m, 13H), 6.91 (d, 2H, *J* = 8.1 Hz), 5.28–5.20 (m, 2H), 5.06 (d, 1H, *J* = 11.7 Hz), 4.90 (d, 1H, *J* = 14.7 Hz), 4.74 (d, 1H, *J* = 11.7 Hz), 4.69 (d, 1H, *J* = 8.1 Hz), 4.65–4.49 (m, 4H), 4.38 (br d, 1H, *J* = 7.2 Hz), 4.19 (d, 1H, *J* = 11.4 Hz), 3.96 (t, 1H, *J* = 9.0 Hz), 3.82 (s, 3H), 3.74 (d, 1H, *J* = 10.2 Hz), 3.67–3.52 (m, 2H), 3.56 (s, 3H), 3.40–3.25 (m, 3H), 3.16 (br d, 1H, *J* = 9.6 Hz), 2.00 (s, 3H), 0.90 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3, 167.3, 164.1, 159.3, 138.6, 138.4, 137.4, 133.2, 130.5, 130.1, 129.8, 129.7, 128.2, 128.2, 127.9, 127.8, 127.7, 127.5, 127.5, 127.3, 113.9, 100.0, 97.0, 80.7, 79.7, 76.7, 75.0, 74.4, 74.1, 73.0, 72.8, 72.8, 71.2, 68.3, 67.2, 55.2, 52.8, 52.7, 25.5, 20.6, 17.9, −4.4, −5.3; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{51}H_{62}N_6O_{14}SiNa$, 1033.3991; found, 1033.4021; IR (thin film in NaCl) ν_{max} = 2950, 2932, 2111, 1760, 1251, 1075 cm^{-1}.

3.1.15. (Methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α/β-D-glucopyranose (22)

To a cooled solution of compound **21** (125 mg, 0.12 mmol) in THF (3.0 mL) at −15 °C, HOAc (9 μL, 0.15 mmol) was added followed by addition of TBAF (1 M, 0.15 mL). The temperature was raised to room temperature and stirring continued for 3 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed twice with satd aq $NaHCO_3$, dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (1.8:1 petroleum ether–EtOAc) to provide **22** (95 mg, 86%) as a white solid (α/β, 3:2). R_f = 0.50 (1:1 petroleum ether–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): 7.78–7.74 (m, 1H), 7.61 (t, 1H, *J* = 7.8 Hz), 7.53 (br d, 1H, *J* = 7.5 Hz), 7.46–7.41 (m, 3H), 7.39–7.32 (m, 2H), 7.30–7.22 (m, 3H), 7.18–7.09 (m, 5H), 6.96 (t, 2H, *J* = 8.1 Hz), 5.26–5.18 (m, 3H), 5.09 (d, 0.4H, *J* = 11.1 Hz), 4.90 (dd, 1H, *J* = 14.7, 3.9 Hz), 4.74–4.67 (m, 2H), 4.65 (br s, 1H), 4.58 (br s, 1H), 4.55–4.37 (m, 2H), 4.13 (dd, 1H, *J* = 12.0, 2.4 Hz), 3.95 (AB, d, 1H, *J* = 9.6 Hz), 3.89–3.82 (m, 3.6H), 3.76 (br d, 1H, *J* = 9.6 Hz), 3.69 (dd, 1H, *J* = 9.9, 0.9 Hz), 3.65–3.48 (m, 5H), 3.41–3.25 (m, 2H), 3.20–3.13 (m, 1H), 2.01 (d, 3H, 4.90 *J* = 1.8 Hz); HRMS (ESI) *m*/*z* calcd: for $C_{45}H_{48}N_6O_{14}Na$, 919.3126; found, 919.3115; IR (thin film in NaCl) ν_{max} = 3447, 2955, 2110, 1760, 1734, 1514, 1250, 1076 cm^{-1}.

3.1.16. (Methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (3α) and (Methyl 4-*O*-acetyl-*O*-(2-(azidomethyl)-benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (3β)

To a solution of compound **22** (163 mg, 0.18 mmol) and K_2CO_3 (30 mg, 0.22 mmol) in acetone (2.0 mL), $ClCNPhCF_3$ (57 mg, 0.27 mmol) was added. The reaction mixture was stirred at room temperature under argon for 8 h and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc, 1% Et_3N, v/v) to provide

disaccharide trifluoroacetimidate **3** (188 mg, 97%) as a colorless syrup (α/β, 3:2). Compound **3α**: R_f = 0.80 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +35.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, 1H, *J* = 8.1 Hz), 7.62 (dt, 1H, *J* = 8.4, 0.9 Hz), 7.53 (br d, 1H, *J* = 7.2 Hz), 7.48–7.34 (m, 5H), 7.30–7.23 (m, 5H), 7.19–7.05 (m, 6H), 6.98 (br d, 2H, *J* = 8.7 Hz), 6.76 (br d, 2H, *J* = 7.5 Hz), 5.29–5.21 (m, 3H), 4.90 (d, 1H, *J* = 14.1 Hz), 4.73–4.64 (m, 3H), 4.90–4.60 (m, 3H), 4.18 (d, 1H, *J* = 12.0 Hz), 4.10 (t, 1H, *J* = 9.0 Hz), 3.88 (t, 1H, *J* = 10.2 Hz), 3.84 (s, 3H), 3.72 (d, 1H, *J* = 9.9 Hz), 3.66–3.51 (m, 4H), 3.54 (s, 3H), 3.36 (br d, 1H, *J* = 9.6 Hz), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 167.2, 164.3, 159.9, 143.2, 138.4, 138.2, 137.5, 133.2, 130.4, 130.1, 129.8, 128.7, 128.3, 128.2, 128.1, 127.9, 127.8, 127.5, 127.4, 124.4, 119.3, 114.2, 100.0, 93.7, 79.8, 78.0, 76.2, 75.6, 74.2, 73.2, 73.0, 72.8, 72.8, 71.2, 66.5, 62.3, 55.2, 52.8, 52.6, 20.6. Compound **3β**: R_f = +0.85 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +25.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 1H, *J* = 7.5 Hz), 7.61 (t, 1H, *J* = 7.2 Hz), 7.52 (d, 1H, *J* = 7.2 Hz), 7.47–7.35 (m, 5H), 7.32–7.20 (m, 5H), 7.17–7.00 (m, 6H), 6.96 (d, 2H, *J* = 8.7 Hz), 6.75 (d, 2H, *J* = 7.5 Hz), 5.25–5.12 (m, 3H), 4.90 (d, 1H, *J* = 15.0 Hz), 4.73 (br d, 2H, *J* = 11.4 Hz), 4.64 (t, 1H, *J* = 6.9 Hz), 4.59–4.48 (m, 3H), 4.21 (d, 1H, *J* = 12.0 Hz), 4.06 (t, 1H, *J* = 9.6 Hz), 3.83 (s, 3H), 3.71 (d, 1H, *J* = 10.2 Hz), 3.58–3.52 (m, 3H), 3.57 (s, 3H), 3.42 (br d, 2H, *J* = 10.2 Hz), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 167.6, 167.2, 164.4, 164.1, 161.7, 159.6, 147.4, 143.1, 138.8, 138.6, 138.2, 137.5, 134.2, 133.3, 130.4, 130.0, 129.8, 128.7, 128.2, 127.9, 127.7, 127.5, 127.4, 124.4, 119.2, 115.6, 114.1, 99.8, 95.6, 80.7, 79.7, 75.8, 75.4, 75.2, 74.2, 73.1, 72. 9, 72.8, 71.3, 70.7, 66.5, 64.8, 55.2, 52.9, 52.6, 20.6, 16.4.

3.1.17. Methyl (2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranosyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (23)

The same procedure described for the preparation of compound **18** (from compounds **6** and **5**) was employed for the preparation of **23** from **7** and **8**. Purification by silica gel column chromatography (4:1 petroleum ether–EtOAc) gave disaccharide **23** (190 mg, 90%) as a white solid. R_f = 0.40 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +34.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (d, 2H, *J* = 8.4 Hz), 7.63 (t, 1H, *J* = 7.5 Hz), 7.51–7.25 (m, 11H), 7.10 (br s, 5H), 6.92 (d, 4H, *J* = 8.7 Hz), 5.46 (s, 1H), 5.20 (t, 1H, *J* = 9.0 Hz), 5.00 (d, 1H, *J* = 10.5 Hz), 4.79 (d, 1H, *J* = 12.3 Hz), 4.73–4.69 (m, 3H), 4.60 (d, 1H, *J* = 12.0 Hz), 4.42 (d, 1H, *J* = 7.8 Hz), 4.20 (d, 1H, *J* = 13.2 Hz), 4.15 (dd, 1H, *J* = 10.5, 4.8 Hz), 3.98 (t, 1H, *J* = 9.6 Hz), 3.85–3.79 (m, 1H), 3.83 (s, 3H), 3.70 (s, 3H), 3.69–3.65 (m, 2H), 3.53 (t, 1H, *J* = 9.3 Hz), 3.44–3.31 (m, 4H), 3.29 (s, 3H), 3.16–3.14 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.7, 160.0, 159.5, 138.4, 137.9, 133.3, 130.0, 129.7, 129.7, 129.6, 129.5, 128.4, 128.1, 128.2, 127.9, 127.7, 127.5, 127.5, 127.3, 114.0, 113.5, 101.1, 100.5, 98.5, 81.6, 78.3, 78.0, 76.4, 75.1, 74.2, 73.7, 73.1, 70.0, 68.4, 66.6, 66.0, 62.8, 55.3, 55.2, 55.2; Anal. Calcd for $C_{50}H_{53}N_3O_{13}$: C, 66.43; H, 5.91; N, 4.65. Found: C, 66.37; H, 6.06; N, 4.47; IR (thin film in NaCl) ν_{max} = 2110, 1720, 1517, 1251, 1098 cm^{-1}.

3.1.18. Methyl (2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (24)

The same procedure described for the preparation of compound **19** (from compound **18**) was employed for the preparation of **24** from **23**. Purification by silica gel column chromatography (3:2 petroleum ether–EtOAc) gave disaccharide **25** (122 mg, 89%) as a white solid. R_f = 0.40 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +48.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 2H, *J* = 8.7 Hz), 7.62 (t, 1H, *J* = 7.5 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 7.41–7.29 (m, 7H), 7.22–7.15 (m, 5H), 6.99 (d, 2H, *J* = 8.7 Hz), 5.15 (t, 1H, *J* = 9.3 Hz), 4.99 (d, 1H, *J* = 11.1 Hz), 4.78–4.72 (m, 3H), 4.59 (AB, d, 2H, *J* = 11.4 Hz), 4.39 (d, 1H, *J* = 7.8 Hz), 4.25 (d, 1H, *J* = 12.0 Hz), 3.94 (t, 1H, *J* = 9.3 Hz), 3.82 (t, 1H, *J* = 9.9 Hz), 3.80 (s, 3H), 3.64 (t, 2H, *J* = 11.1 Hz), 3.50 (t, 1H, *J* = 9.3 Hz), 3.42–3.26 (m, 5H), 3.30 (s, 3H), 3.21–3.15 (m, 1H), 3.29 (d, 1H, *J* = 3.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 159.5, 138.1, 137.8, 133.3, 130.3, 129.6, 129.4, 128.4, 127.7, 127.6, 127.5, 126.9, 114.1, 99.8, 98.5, 82.9, 78.0, 75.4, 75.1, 74.9, 73.8, 73.2, 70.7, 69.9, 66.5, 62.9, 62.0, 55.3, 55.2; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{42}H_{47}N_3O_{12}Na$, 808.3057; found, 808.3041; IR (thin film in NaCl) ν_{max} = 2919, 2110, 1731, 1515, 1267, 1044 cm^{-1}.

3.1.19. Methyl (benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (4)

Compound **24** (203 mg, 0.26 mmol) was dissolved in CH_2Cl_2–water (2:1 v/v, 4.5 mL), subsequently TEMPO (8 mg, 0.05 mmol) and BAIB (209 mg, 0.65 mmol) were added. After the biphasic mixture was stirred vigorously for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHSO_3$ (5.0 mL). The layers were separated and the aqueous layer was acidified with 1 M aqueous HCl, and extracted thrice with CH_2Cl_2 (100 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, and then filtered and concentrated in vacuo, yielding the corresponding crude glucuronic acid. The crude glucuronic acid was dissolved in dry DMF (5.0 mL), and $KHCO_3$ (100 mg, 1.0 mmol) was added, followed by addition of benzyl bromide (59 μL, 0.5 mmol). After stirring vigorously for 10 h, the reaction mixture was treated with a few drops of acetic acid. The solvents were evaporated and the residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to afford disaccharide **4** (202 mg, 88%) as a colorless syrup. R_f = 0.40 (2:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +55.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (d, 2H, *J* = 7.8 Hz), 7.62 (t, 1H, *J* = 6.9 Hz), 7.49 (t, 2H, *J* = 7.5 Hz), 7.40–7.26 (m, 7H), 7.15 (s, 5H), 6.98 (d, 2H, *J* = 8.4 Hz), 5.17 (t, 1H, *J* = 9.0 Hz), 5.10 (d, 1H, *J* = 11.1 Hz), 4.78–4.70 (m, 3H), 4.63 (t, 2H, *J* = 9.6 Hz), 4.43 (d, 1H, *J* = 8.1 Hz), 4.21 (d, 1H, *J* = 11.7 Hz), 4.02 (t, 1H, *J* = 9.3 Hz), 3.95 (t, 1H, *J* = 9.3 Hz), 3.84–3.78 (m, 1H), 3.82 (s, 3H), 3.67 (t, 2H, *J* = 9.9 Hz), 3.56 (s, 3H), 3.44–3.31 (m, 4H), 3.28 (s, 3H), 3.07 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.5, 164.7, 159.6, 138.6, 138.0, 133.3, 130.2, 129.7, 129.6, 128.5, 128.2, 128.1, 128.0, 127.7, 127.6, 127.3, 114.2, 100.3, 98.8, 81.3, 78.0, 75.1, 74.7, 74.0, 73.2, 73.0, 72.3, 69.9, 66.8, 62.8, 55.3, 55.2, 52.6, 29.7; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{43}H_{47}N_3O_{13}Na$, 836.3007; found, 836.3019; IR (thin film in NaCl) ν_{max} = 3497, 2918, 2109, 1720, 1515, 1139, 1073 cm^{-1}.

3.1.20. Methyl (methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α/β-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (2α/β)

A mixture of the disaccharide trifluoroacetimidate **3** (60 mg, 0.056 mmol) and disaccharide acceptor **4** (50 mg, 0.056 mmol) in dry toluene (2.0 mL) was stirred in the presence of freshly activated powdered 4 Å molecular sieves (150 mg) for 30 min at room temperature under argon. After cooling to −40 °C, TBSOTf (0.05 M, 0.24 mL) was added dropwise. The reaction mixture was stirred at −40 °C until TLC indicated complete conversion of the donor **3** and then was quenched by the addition of Et_3N (0.2 mL). The resulting mixture was filtered through a pad of Celite and concentrated. The residue was purified by silica gel column chromatography (5:2 petroleum ether–EtOAc) to provide tetrasaccharide **2α/β** (70 mg,

71%) as a colorless syrup (α/β, 3:2). R_f = 0.35 (2:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87–7.71 (m, 6.1H), 7.65–7.57 (m, 5.5H), 7.50–7.36 (m, 19.1H), 7.32–7.24 (m, 18.4H), 7.22–7.11 (m, 16.5H), 7.08–6.88 (m, 17.4H), 5.47 (d, 0.8H, *J* = 4.2 Hz), 5.27–5.19 (m, 5.7H) 5.13–5.03 (m, 4.4H), 4.95 (t, 2.5H, *J* = 8.7 Hz), 4.89–4.80 (m, 2.4H), 4.76 (s, 2.2H), 4.73–4.65 (m, 6.0H), 4.61–4.49 (m, 9.4H), 4.47–4.34 (m, 5.9H), 4.24–4.14 (6.5H), 4.11–3.85 (m, 7.7H), 3.78–3.69 (m, 17.6H), 3.66–3.63 (m, 2.4H), 3.56 (s, 7.0H), 3.63–3.26 (m, 20.8H), 3.11 (t, 1.1H, *J* = 4.8 Hz), 2.82 (d, 1.0H, *J* = 9.6 Hz), 2.03 (s, 2.5H), 2.01 (s, 3.0H); HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{94}H_{97}N_9O_{26}Na$, 1790.6442; found, 1790.6429; IR (thin film in NaCl) ν_{max} = 2937, 2111, 1735, 1611, 1515, 1456, 1364, 1251, 1073, 1037 cm^{-1}.

3.1.21. Methyl (methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (25β) and methyl (methyl 4-*O*-acetyl-2-*O*-(2-(azidomethyl)benzoyl)-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (25α)

To a solution of the tetrasaccharide **2α/β** (51 mg, 0.029 mmol) in CH_2Cl_2 (2.0 mL), H_2O (0.2 mL) was added followed by addition of DDQ (33 mg, 0.14 mmol). After stirring at room temperature for 4 h, the reaction mixture was diluted with CH_2Cl_2 (80 mL), and washed twice with satd aq $NaHCO_3$ and once with water. The organic layer was dried with Na_2SO_4, and then filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (5:2 petroleum ether–EtOAc) to give compound **25β** (17 mg, 39%) and compound **25α** (19 mg, 43%) as colorless syrups. Compound **25β**: R_f = 0.15 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ +3.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (d, 2H, *J* = 7.5 Hz), 7.84 (d, 1H, *J* = 7.8 Hz), 7.61 (t, 1H, *J* = 7.6 Hz), 7.56–7.54 (m, 2H), 7.45–7.35 (m, 10H), 7.32–7.19 (m, 11H), 7.14–7.08 (m, 11H), 5.31–5.27 (m, 3H), 5.08–5.04 (m, 3H), 4.88–4.83 (m, 3H), 4.76–4.59 (m, 8H), 4.55–4.52 (m, 2H), 4.17 (t, 1H, *J* = 7.8 Hz), 4.08 (d, 1H, *J* = 7.2 Hz), 4.04 (d, 1H, *J* = 9.6 Hz), 3.92–3.83 (m, 4H), 3.75–3.71 (m, 3H), 3.75–3.53 (m, 1H), 3.56 (s, 3H), 3.41 (d, 1H, *J* = 7.8 Hz), 3.36–3.24 (m, 3H), 3.27 (s, 3H), 3.19–3.11 (m, 2H), 2.69 (d, 1H, *J* = 10.0 Hz), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3, 167.3, 164.8, 164.2, 138,6, 138.5, 138.4, 133.4, 137.3, 134.7, 133.4, 130.6, 129.9, 129.7, 129.4, 129.0, 128.8, 128.6, 128.4, 128.3, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 127.5, 127.3, 126.8, 101.2, 101.0, 100.3, 98.8, 80.4, 79.8, 79.4, 78.0, 77.8, 76.1, 75.3, 75.2, 74.7, 74.6, 74.6, 74.1, 73.2, 73.1, 72.9, 71.4, 70.7, 67.7, 66.3, 63.3, 60.4, 60.1, 55.3, 53.0, 52.7, 20.6; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{78}H_{81}N_9O_{24}Na$, 1550.5292; found, 1550.5256; IR (thin film in NaCl) ν_{max} = 2928, 2112, 1734, 1456, 1265, 1072 cm^{-1}. Compound **25α**: R_f = +0.25 (1:1 petroleum ether–EtOAc); $[\alpha]_D^{26}$ 31.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (d, 2H, *J* = 7.3 Hz), 7.94 (d, 1H, *J* = 7.8 Hz), 7.58 (t, 1H, *J* = 7.5 Hz), 7.54 (t, 1H, *J* = 7.4 Hz), 7.47–7.41 (m, 6H), 7.36–7.33 (m, 4H), 7.29–7.04 (m, 8H), 7.15–7.06 (m, 12H), 5.37–5.29 (m, 4H), 5.20 (d, 1H, *J* = 11.2 Hz), 4.97 (d, 1H, *J* = 9.9 Hz), 4.90–4.88 (m, 2H), 4.72 (d, 1H, *J* = 10.5 Hz), 4.64–4.58 (m, 7H), 4.58 (s, 1H), 4.52–4.46 (m, 2H), 4.24 (t, 1H, *J* = 7.8 Hz), 3.97 (t, 2H, *J* = 9.5 Hz), 3.94–3.76 (m, 6H), 3.73–3.63 (m, 3H), 3.58 (br d, 1H, *J* = 6.6 Hz), 3.53 (s, 3H), 3.38 (dd, 2H, *J* = 11.1, 4.8 Hz), 3.26 (s, 3H), 3.21–3.19 (m, 2H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3, 167.5, 167.3, 164.8, 164.3, 138.8, 138.4, 138.2, 137.3, 137.2, 134.4, 133.5, 133.4, 130.6, 129.7, 129.4, 129.3, 128.7, 128.7, 128.6, 128.5, 128.3, 128.2, 128.2, 127.9, 127.8, 127.8, 127.7, 127.4, 127.1, 100.8, 100.7, 98.7, 97.5, 82.3, 79.7, 77.8, 77.6, 75.5, 75.3, 74.9, 74.6, 74.2, 73.8, 73.1, 73.0, 71.4, 70.7, 67.3, 63.2, 63.0, 60.3, 60.0, 55.3, 52.9, 52.6, 29.7, 20.6; HRMS (MALDI/DHB): calcd for $C_{78}H_{81}N_9O_{24}Na$, 1550.5292; found, 1550.5267; IR (thin film in NaCl) ν_{max} = 2927, 2110, 1736, 1264, 1071 cm^{-1}.

3.1.22. Methyl (methyl 4-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-(2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (26)

Compound **25α** (19 mg, 0.012 mmol), PPh_3 (36 mg, 0.120 mmol), and silica gel (5 mg) were dissolved in THF–water (9:1, 2.0 mL). After stirring at room temperature for 37 h, the reaction mixture was concentrated. The residue was eluted from a Sephadex LH-20 chromatography with MeOH–CH_2Cl_2 1:1 to afford **26** (14 mg, 86%) as a colorless syrup. R_f = 0.40 (10:1, MeOH–CH_2Cl_2); $[\alpha]_D^{25}$ +41.9 (*c*1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, 2H, *J* = 7.2 Hz), 7.60 (t, 1H, *J* = 7.5 Hz), 7.47 (t, 2H, *J* = 7.8 Hz), 7.34–7.24 (m, 20H), 7.14 (s, 5H), 5.42 (t, 1H, *J* = 8.4 Hz), 5.24 (d, 1H, *J* = 3.6 Hz), 5.12–4.96 (m, 6H), 4.80–4.72 (m, 3H), 4.68–4.51 (m, 5H), 4.34 (t, 1H, *J* = 7.8 Hz), 4.18 (d, 1H, *J* = 8.4 Hz), 3.96–3.70 (m, 7H), 3.63–3.58 (m, 4H), 3.55 (s, 3H), 3.44–3.32 (m, 3H), 3.21 (s, 3H), 2.68–2.62 (m, 2H), 1.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 167.9, 167.6, 164.8, 139.1, 138.9, 138.2, 137.3, 134.9, 133.5, 129.7, 129.4, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 127.9, 127.9, 127.7, 127.6, 127.5, 103.4, 100.8, 99.9, 98.6, 82.2, 81.9, 81.3, 77.9, 77.5, 75.5, 75.4, 75.4, 75.3, 74.8, 74.4, 74.2, 73.9, 73.0, 72.2, 71.0, 67.7, 61.0, 60.5, 56.0, 55.7, 55.2, 52.5, 29.7, 20.6; HRMS (MALDI/DHB) *m*/*z*: calcd for $C_{37}H_{43}NO_{13}Na$, 1339.5050; found, 1339.5027; IR (thin film in NaCl) ν_{max} = 2927, 1744, 1265, 1027, 699 cm^{-1}.

3.1.23. Methyl (2-*O*-sulfo-β-D-glucopyranosyluronate)-(1→4)-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronate)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranoside (1)

$SO_3{\cdot}NEt_3$ (30 mg, 0.165 mmol) was added to a solution of **26** (14 mg, 0011 mmol) in anhydrous DMF (1.0 mL). After stirring for 24 h at 55 °C under an argon atmosphere, the reaction mixture was quenched with Et_3N (0.2 mL) and diluted with MeOH (1.0 mL) and CH_2Cl_2 (1.0 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH–CH_2Cl_2 (1:1). The fraction that contained the sulfonated tetrasaccharide was pooled and evaporated to dryness. The residue was dissolved in anhydrous pyridine (1 mL), after which Et_3N (0.2 mL) was added followed by addition of $SO_3{\cdot}$ Py (18 mg, 0.11 mmol). After stirring for 2 h at room temperature under an argon atmosphere, the reaction mixture was diluted with MeOH (1.0 mL) and CH_2Cl_2 (1.0 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH–CH_2Cl_2 1:1. The fractions that contained the sulfonated tetrasaccharide were pooled and evaporated to dryness to yield the crude sulfonated tetrasaccharide. ESI-MS: (*m*/*z*): calcd for $C_{70}H_{77}N_2O_{35}S_4$, 544.4; found, 544.7 $[M-SO_3-3H^+]^{3-}$; *m*/*z* calcd for $C_{70}H_{77}N_2O_{38}S_5$, 571.1; found, 571.5 $[M-3H^+]^{3-}$; *m*/*z* calcd for $C_{70}H_{78}N_2O_{35}S_4$, 817.2; found, 817.2 $[M-SO_3-2H^+]^{2-}$; *m*/*z* calcd for $C_{70}H_{78}N_2O_{38}S_5$, 857.1; found, 857.3 $[M-2H^+]^{2-}$.

The crude sulfonated tetrasaccharide was dissolved in MeOH–water (9:1 v/v, 2.0 mL) and treated with a 2 M aqueous solution of NaOH (0.5 mL). After stirring at room temperature for 48 h, the reaction mixture was quenched by addition of H^+-ion-exchange resin (1.5 g). The filtrate that contained sulfonated tetrasaccharide was hydrogenated in the presence of 10% Pd–C. After 36 h, the suspension was filtered, concentrated, and eluted from a column of Dowex 50WX4-Na^+ with H_2O. The fractions that contained

sulfonated tetrasaccharide were pooled and concentrated. The residue was diluted with H_2O. The solution was layered on the top of a Sephadex G-15 chromatography column that was eluted with 0.5 M aqueous solution of NaCl. The fractions that contained the sulfonated tetrasaccharide were pooled and concentrated. The residue was diluted with H_2O. The solution was layered on the top of a Sephadex G-15 chromatography column that was eluted with H_2O. The fractions that contained the sulfonated tetrasaccharide were pooled and evaporated to dryness to yield tetrasaccharide **1** (3 mg, 22%) as a white solid. ^{1}H NMR (400 MHz, D_2O): δ 5.74 (d, 1H, J = 3.5 Hz), 5.04 (d, 1H, J = 3.0 Hz), 4.62 (dd, 2H, J = 8.1, 2.6 Hz), 4.49 (d, 1H, J = 10.7 Hz), 4.42 (br s, 2H), 4.15–4.05 (m, 2H), 4.04–3.96 (m, 3H), 3.94–3.84 (m, 8H), 3.82–3.77 (m, 2H), 3.76—3.70 (m, 3H), 3.69–3.56 (m, 5H), 3.51 (s, 3H), 3.47–3.35 (m, 5H); ^{13}C NMR (75 MHz, D_2O): δ 173.8, 165.3, 104.9, 104.7, 99.0, 98.0, 80.7, 79.5, 78.7, 78.6, 78.3, 77.8, 76.0, 75.7, 74.7, 74.5, 71.9, 71.5, 71.3, 70.8, 68.8, 68.3, 65.2, 58.1, 56.3; ESI-MS: m/z calcd for $C_{25}H_{40}N_2O_{36}S_5$, 552.0; found, 551.9 $[M-2H^+]^{2-}$, m/z calcd for $C_{25}H_{35}N_2O_{36}S_5Na_4$, 397.0; found, 397.4 $[M+4Na^+-7H^+]^{3-}$.

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